A pharmacological and behavioural investigation of anxiety in the deer mouse model of obsessive-compulsive disorder

M Prinsloo

orcid.org/0000-0002-2115-6106

Dissertation accepted in fulfilment of the requirements for the degree *Master of Science in Pharmacology* at the North-West University

Supervisor: Dr PD Wolmarans

Co-supervisor: Prof BH Harvey

Graduation: May 2021

Student number: 24876739

A pharmacological and behavioural investigation of anxiety in the deer mouse model of obsessive-compulsive disorder

Michelle Prinsloo

(B.Pharm.)

Dissertation submitted in fulfilment of the requirements for the degree

Master of Science

in

Pharmacology

at the

North-West University (Potchefstroom Campus)

SUPERVISOR: Dr De Wet Wolmarans

CO-SUPERVISOR: Prof Brian H Harvey

POTCHEFSTROOM, SOUTH AFRICA

2020

* * *

Jeremiah 29:11

"For I know the plans I have for you," declares the Lord, "plans to prosper you and not to harm you, plans to give you hope and a future."

Proverbs 8:11

"For wisdom is more precious than rubies, and nothing you desire can compare with her"

* * *

ACKNOWLEDGEMENTS

"Be strong and of good courage, do not fear nor be afraid of them, for the Lord your God, He is the One who goes with you. He will not leave you nor forsake you." – Deuteronomy 31:6

* * *

This work is not mine alone, and without the phenomenal people in my life I wouldn't have composed this work. My sincere gratitude goes to the following:

- To my Lord - thank you for being with me in every step of the way, during the difficult times and the great times. All that I have, and all that I am is thanks to you Lord, and I will always be eternally grateful for all your blessings showered upon me. To you all the glory of this work and everything in my life.
- My parents, Gerhard and Julie - thank you so much for your continued support. Though we couldn't see each other that often due to the pandemic, you were there for me in every step of the way, motivating me and giving support when needed. Thank you for allowing me to pursue my goals and completing my masters. Everything I have accomplished these past 25 years is due to your continued love, support and guidance, your sacrifices that you've made so that I can accomplish my goals and dreams. I am grateful for evermore, as you made me the person I am today. Love you so much.
- To my love, BJ Engelbrecht - thank you for taking this challenge with me and doing our masters' degree together. Thank you for always being on my side for the past 5 and a half years. Your unconditional love, support and guidance has kept my head above water, motivated me to be the best version of myself, and to always do everything at the best of my abilities. The Lord has blessed me with you in my life, and I'm grateful that we've experienced so much in life together, and I look forward to many wonderful times ahead. I love you deeply.
- My other parents – Pieter and Adri – thank you for your encouragement, support, prays and love during the past years. You mean the world to me and I love you so.
- My dearest friends – Monique, Jaco, Crystal, Marize, Jo-hanne and Odette – thank you for your support, encouragement, and love during the past few years. Thank you for the amazing friendship that we have and that it grows stronger as the time passes. You mean the world to me.
- To my fellow friends, Masters and Doctoral students at Pharmacology - Ané, Monique, Jaco, Crystal, Geoffrey, Juandré, Jo-Anne, Larissa, Stoffel, Maret, Mari-louise, Vasti and Alexio -

thank you for all the fun times that we've shared, all the difficult times that we've went through together, and the encouragement and wisdom shared. Though the pandemic has made it hard for us to socialize, we still made it work. You made the journey worthwhile and I appreciate you all so much.

- Prof Brian Harvey, thank you for your hard work, advice, dedication, guidance and brilliant insights given with this study. I couldn't have asked for a better co-supervisor.
- To the vivarium personnel – thank you for the guidance and help with the deer mice, and that you're always available for advice and assistance. I appreciate your input and time extremely.
- Dr Geoffrey de Brouwer – thank you for the time and effort you spent in the vivarium during the pandemic and lockdown when I couldn't continue with my experiments. Thank you for your assistance and support during the writing of my dissertation. I will always be grateful for your guidance, time, advice and friendship.
- Dr De Wet Wolmarans – words cannot begin to describe how grateful I am of you. Thank you for choosing me as your student and giving me the opportunity to complete my masters. Thank you for your continued guidance, support, encouragement, and patience with me during the past 2 years. You've inspired me and allowed me to grow as a person, to become stronger and believing in myself. You're a formidable mentor and study leader, and I will always remember your insights, our conversations and life lessons given. You inspire me to be a dedicated, brilliant researcher in the future.

CONGRESS PROCEEDINGS

- PRINSLOO, M., DE BROUWER, G., SEEDAT, S., STEIN, D.J., HARVEY, B.H., WOLMARANS, P.D (2020). Deer mouse nesting behaviour in a novel anxiogenic environment and its response to serotonergic and benzodiazepine intervention: pre-clinical insights into compulsive-like behaviour and risk-taking. Presented at the Southern African Neuroscience Society (SANS) Online Symposium, 20th November 2020.

ABSTRACT

Obsessive-compulsive disorder (OCD)[1] is a chronic, debilitating psychiatric disorder with a global lifetime prevalence rate of 2.0%-2.9% and which generally manifests in early adulthood. The condition is characterised by two broad symptom groups, i.e., obsessions (disturbing and intrusive thoughts) and compulsions (persistent, repetitive, and overt behavioural routines). Although selective serotonin reuptake inhibitors (SSRIs)[2] are currently used as the first line pharmacotherapeutic intervention for OCD, only 40%-60% of patients respond to treatment. The adjunctive treatment options for SSRI-refractory OCD include low dose anti-dopaminergic drugs, i.e. quetiapine or risperidone. Still, only 30% of SSRI-refractory patients respond to these interventions.

Conceptually, compulsions can be viewed as an active coping mechanism recruited to attenuate the level of distress caused by the relevant obsession. In that regard, compulsions are 'goal-directed', although the goal in this instance does not speak to a realistic, attainable endpoint. It is unclear whether a sense of mounting anxiety provokes anxiolytic compulsive routines or whether patients struggle to refrain from engaging in excessive behaviours that in turn evoke a sense of anxiety. Importantly, OCD patients tend to overestimate and inflate the actual significance of the consequences which they feel may potentially arise should they not act on the obsession. Given the overly repetitive and often uncontrolled expression of compulsions, such behaviours are also appraised against the background of impulsivity, which exists on the opposite end of a continuum of excessive and inappropriate behaviours. Impulsive features are often described in OCD, pointing to a shared neurobiological and psychopathological architecture.

Over the past decade our laboratory has carried out several studies to characterise, validate, develop, and scrutinise the naturalistic, persistent behavioural phenotypes expressed by deer mice (Peromyscus maniculatus bairdii) as a model of compulsive-like behavioural persistence. Spontaneous stereotypy, large nest building (LNB)[3] and high marble-burying (HMB)[4] are three behaviourally heterogeneous and persistent behaviours expressed by deer mice. Though previous studies indicated that chronic, high dose escitalopram (50 mg/kg/day) attenuates LNB behaviour and spontaneous stereotypy, HMB remains treatment-refractory to such intervention.

While LNB behaviour is persistent and repetitive, the phenotype has not yet been studied in terms of the potential role that anxiety may play in its manifestation. In fact, since the immediate and long-

[1] obsessive-compulsive disorder
[2] selective serotonin reuptake inhibitors
[3] large nest building
[4] high marble burying

term temporal relationship between compulsive symptom manifestation and anxiety is not fully understood, it is unknown if anxiogenic manipulation of an environment, i.e. assessment of nesting behaviour under anxiogenic circumstances, will exacerbate the expression of LNB[1], or whether the expression of LNB is a more predetermined and inflexible behavioural phenotype. As such, this work sought to determine whether the expression of normal (NNB)[2] and LNB behaviour would adapt differently when assessed in conditions where mice would have to overcome their natural fear of open spaces to indulge in excessive nesting behaviour. We also aimed to determine how such behaviour, as measured under the aforementioned circumstances, would respond to known anti-compulsive and anxiolytic interventions.

Deer mice (182, both sexes, aged 10 – 12 weeks at the onset of investigation) were screened for nest building behaviour, and subsequently divided into two primary behavioural cohorts i.e. NNB and LNB. Each cohort was then further subdivided into three (3) drug exposure groups [n = 10 per cohort per exposure group; all drugs administered via the drinking water; control, chronic escitalopram (50 mg/kg/day), and sub-acute lorazepam (2 mg/kg/day)]. Following selection and grouping, water (control) or escitalopram were administered for 28 days as well as during the four days of post-exposure testing. Lorazepam, as a sub-acute intervention, was only administered during the four nights of behavioural testing. Thus, mice in this group also received regular drinking water during the 28-day exposure period. After 28 days of control or escitalopram exposure, the primary behavioural investigation commenced. To this end, mice were placed into novel mirror chambers, which consisted of a safe, enclosed dark space with an internal nesting material hopper as well as a white-floored, mirror-walled open space containing an external nesting material hopper. The internal hopper contained enough material to construct a nest of typical size (as determined by analyses of home cage nesting behaviour), while an excess of material was available in the external hopper. Thus, to engage in LNB behaviour, mice had to enter the open, aversive space. The nesting behaviour of each mouse that was included in the drug (or control) exposure phase was assessed in this manner for four consecutive nights, all while being videotaped for assessment of ambulatory activity in the open space. To test the unincentivized exploration of the mirror chamber, two additional groups, i.e. one NNB and one LNB group (n = 8, both untreated) were assessed in the mirror chamber for four consecutive nights. However, no nesting material was available. Prior to commencement of this work, the study was approved by the AnimCare Research Ethics Committee of the North-West University (approval number: NWU-00574-19-A5).

[1] large nest building
[2] normal nest buidling

Abstract

The main findings of this study were the following: 1) LNB[1] behaviour expressed by deer mice is an inflated, but goal-directed behavioural phenotype which remains stable, irrespective of the context in which it is assessed, 2) LNB mice find an open field arena to be less aversive compared to the behaviour or their NNB[2] expressing counterparts, although this apparent unrestrained exploration of the open space is reduced when mice are able to indulge in the expression of LNB, and 3) escitalopram and lorazepam reduced the nesting behaviour of LNB mice, while differentially affecting the open field behaviours of NNB and LNB expressing mice.

Since LNB animals presented with an inflated motivational drive to engage in nesting behaviour, irrespective of the potential negative outcomes, we have been able to confirm the excessive, persistent, and seemingly purposeless nature of LNB. LNB animals were also more likely to explore an environment associated with potential danger, and although it could be explained as differences in the underlying anxiety-state in NNB vs. LNB animals, there might be another explanation, i.e. that LNB mice exhibit impulsive-like, risk-taking behaviour. This may be true, since the expression of LNB not only responded to chronic escitalopram exposure, but also to sub-acute lorazepam; this taking into consideration that the same drug interventions differentially affected ambulatory activity in the open field.

In conclusion, we confirmed that an anxiogenic environment does not deter LNB behaviour in deer mice and that excessive nest building is an inherent and stable behavioural phenotype that is displayed by LNB animals, irrespective of anxiety-related contextual circumstance. The potentially impulsive-like, risk-taking behaviour observed in LNB animals when they are unable to carry out compulsive-like nest building, is drastically attenuated with the introduction of nesting material, i.e. when being able to express LNB behaviour. In this case it appears that engaging in excessive nesting, distracts from impulsive-like exploration of the open field area. However, future studies are needed to further elucidate the full extent of the relationships between compulsive-, anxiety- and impulsive-like traits in LNB expressing deer mice.

Keywords: obsessive-compulsive disorder; anxiety; escitalopram; lorazepam; impulsive; compulsive; risk-taking; deer mice

[1] large nest building
[2] normal nest building

TABLE OF CONTENTS

ACKNOWLEDGEMENTS II

CONGRESS PROCEEDINGS I

ABSTRACT I

1 INTRODUCTION 1

1.1 DISSERTATION LAYOUT 1

1.2 PROBLEM STATEMENT 2

1.3 HYPOTHESIS 5

1.4 STUDY AIMS AND OBJECTIVES 6

1.4.1 Project layout 7

1.5 ETHICAL APPROVAL 8

1.6 EXPECTED OUTCOMES 8

1.6.1 Baseline nest-building assessment 8

1.6.2 Post-exposure anxiety and nest building assessment 8

1.7 REFERENCES 10

2 LITERATURE REVIEW 16

2.1 OBSESSIVE-COMPULSIVE DISORDER IN THE CLINIC 16

2.1.1 Epidemiology and diagnostic description 16

2.1.2 Symptom conceptualisation 16

2.1.3 Neurobiology 19

2.1.3.1 A brief overview of the neuroanatomical architecture of OCD 19

2.1.3.2 Key concepts pertaining to dopaminergic and serotonergic signalling 20

2.1.4 The pharmacological treatment of OCD 21

2.2 PERSPECTIVES ON THE CURRENT WORK 22

2.2.1 The impulsivity-compulsivity spectrum: from risk to risk aversion? 22

2.2.2 The matter of context, anxiety, and compulsivity 24

2.2.3 Modelling OCD in the deer mouse 25

2.2.3.1 General background 25

2.2.3.2 Status of the deer mouse model of OCD 26

2.2.4 Nest building behaviour – from a life in the wild to cage life 27

2.2.4.1 Interrogating the relationship between anxiety and nest building in deer mice – conceptual background to the current study 30

2.3 REFERENCES 32

3 SCIENTIFIC MANUSCRIPT 46

ABSTRACT 50

KEYWORDS 50

3.1 INTRODUCTION 51

3.2 MATERIALS AND METHODS 53

3.2.1 Animals 53

3.2.2 Drugs 54

3.2.3 Baseline nest-building analysis and behavioural categorisation 55

3.2.4 Mirrored open field assessment 56

3.2.4.1 Apparatus 56

3.2.4.2 Procedure 56

3.2.5 Statistical analysis 57

3.3 RESULTS 58

3.3.1 Nesting behaviour 58

3.3.1.1 Baseline selection of NNB and LNB animals 58

3.3.1.2 Baseline (home cage) vs open field nesting behaviour 58

3.3.1.3 Open field nesting scores 58

3.3.1.4 Time-based adaptation in open field nesting scores 59

3.3.2 Open field behaviour 59

3.3.2.1 Open field behaviour of NNB and LNB animals with and without access to cotton wool 59

3.3.2.2 Open-field behaviour of control- and drug-exposed NNB and LNB animals with access to cotton wool 60

3.4 DISCUSSION 60

3.4.1 Nesting and open field behaviour of control exposed NNB and LNB expressing animals 61

3.4.2 The effects of drug treatment on nesting expression and open field activity 64

3.5 CONCLUSION 65

FUNDING AND DISCLOSURE 65

CRediT AUTHORSHIP CONTRIBUTION STATEMENT 66

3.6 REFERENCES 67

FIGURES 77

4 CONCLUSION 83

4.1 SUMMARY OF EXPECTED VS ACTUAL OUTCOMES 88
4.2 STUDY SHORTCOMINGS AND FUTURE DIRECTIONS 89
4.3 REFERENCES 90

ADDENDUM A 94

LETTERS OF PERMISSION FROM CO-AUTHORS TO SUBMIT CHAPTER 3 FOR EXAMINATION PURPOSES 94

ADDENDUM B 98

ADDITIONAL DETAILS PERTAINING TO THE METHODOLOGY FOLLOWED 98
LAYOUT OF GROUPS FOR THE BEHAVIOURAL INVESTIGATION 99
DAILY ROUTINE DURING THE BEHAVIOURAL INVESTIGATION 100
ADDITIONAL INFORMATION PERTAINING TO ANIMAL HUSBANDRY, HOUSING, AND CARE 101
ADDITIONAL INFORMATION PERTAINING TO DRUG ADMINISTRATION 101
SUPPLEMENTARY IMAGES 103
REFERENCES 104

1 INTRODUCTION

1.1 Dissertation Layout

The current dissertation was prepared in article format according to the requirements of the North-West University (NWU)[1], South Africa. As such, the main scientific body is presented in the form of a journal article that will be submitted for publication in an international, peer-reviewed journal, i.e. Progress in Neuropsychopharmacology and Biological Psychiatry.

The complete dissertation consists of four chapters. Chapter 1 provides a concise summary of the work presented, including a brief review of relevant literature, the problem statement, study aims and questions, working hypothesis, and experimental layout. Chapter 2 comprises a more detailed literature review which forms the theoretical basis of the investigation as it is presented in Chapter 3, while the latter comprises the main scientific body of the study, including the methodology, results, and a discussion of the major findings. Last, Chapter 4 provides a broad summary of the complete dissertation, including factors for consideration in future studies.

Two addenda are also included. These contain the letters of permission of all co-authors to include Chapter 3 for examination purposes (Addendum A) and supplementary methods, materials, and results that complement the findings presented in Chapter 3 (Addendum B).

Since Chapter 3 was prepared in accordance with the "Instructions to Authors" prescribed by Progress in Neuropsychopharmacology and Biological Psychiatry, this dissertation is written in United Kingdom English, while all references are formatted according to the journal instructions.

[1] North-West University

1.2 Problem Statement

Obsessive-compulsive disorder (OCD)[1] is a disabling psychiatric disorder (Abramowitz et al., 2009) with a global one-year prevalence rate estimated at 2.9% (Abramowitz, 2006). OCD typically manifests by the age of 25, with men often demonstrating an earlier age of onset (Abramowitz, 2006). OCD is a clinically heterogeneous disorder that is typically characterised by a combination of obsessions and/or compulsions (Abramowitz et al., 2009; Markarian et al., 2010). However, these symptoms manifest according to five main symptom subtypes, i.e. 1) contamination obsessions and washing compulsions, 2) symmetry obsessions and counting and/or ordering compulsions, 3) intrusive, repugnant obsessions associated with mental rituals acts, 4) safety/security obsessions and excessive checking behaviour, and 5) hoarding obsessions associated with collecting compulsions (Abramowitz, 2006; APA, 2013). As opposed to its former classification as an anxiety disorder (APA, 2013), OCD is now regarded as the principal disorder within the 'new' obsessive-compulsive and related disorders (OCRD)[2] group (DSM-V)[3] (APA, 2013). That said, patients experience significant distress and anxiety more often than not (Abramowitz, 2006; Abramowitz et al., 2009). Importantly, such anxiety is generally experienced along the conceptual boundaries of the obsessive-compulsive (OC)[4] phenotype diagnosed, while the enactment of theme-specific compulsive rituals is believed to attenuate the level of anxiety experienced, albeit only for a brief period of time (APA, 2013; Fineberg et al., 2015). For example, safety and security obsessions are often associated with increased levels of anxiety in terms of feeling unsafe. This in turn is believed to drive excessive locking and checking routines, but not other forms of compulsive behaviour (APA, 2013; Lack, 2012). Nevertheless, the extent to which contextual anxiety may play a role to trigger, bolster and maintain compulsive symptomology, remains disputed.

On a neurobiological level, OCD is believed to be associated with altered cortico-striatal-thalamic-cortical (CSTC)[5] circuit activity (Ahmari et al., 2013; Van der Straten et al., 2017; Zhang et al., 2019). The CSTC circuit comprises three distinct brain regions, i.e. the prefrontal cortex, striatum, and the thalamus (Harrison et al., 2013; Wood and Ahmari, 2015) that are organised in a manner that enables voluntary, goal-directed action to be planned, executed and terminated. The CSTC circuit has been demonstrated to show hyperactivity in OCD, which is thought to result in excessive and outcome-insensitive task engagement (Ji and Anticevic, 2018; Peters et al., 2016). The two major

[1] obsessive-compulsive disorder
[2] obsessive-compulsive and related disorders
[3] Diagnostic and Statistical Manual of Mental Disorders
[4] obsessive-compulsive
[5] cortico-striatal-thalamic-cortical

neurotransmitter systems implicated in the etiopathology of OCD[1] are the serotonergic and dopaminergic systems (Hesse et al., 2005) and it is generally proposed that a hyposerotonergic state enables excessive dopaminergic neurotransmission to prevail. While a pro-dopaminergic state is broadly associated with excessive behavioural engagement, serotonin is believed to act as the biobehavioural opponent of dopamine (Cools et al., 2011; Kranz et al., 2010; Tops et al., 2009). This theory is proposed to explain the fact that OCD demonstrates therapeutic response, albeit variably so, to selective serotonin reuptake inhibitor (SSRI)[2] treatment (Fineberg et al., 2010; Fineberg et al., 2013; Szechtman et al., 2020). In this regard, chronic (8-weeks and longer) and high dose treatment with selective serotonin reuptake inhibitors (SSRIs), e.g. fluoxetine and escitalopram, is necessary to yield a therapeutic response (Albert et al., 2013; Fineberg, 2004; Fineberg et al., 2015; Korff and Harvey, 2006; Korff et al., 2008). However, treatment resistance remains a clinical dilemma (Fineberg et al., 2013; Marazziti and Consoli, 2010).

On a neurocognitive level, OCD is variably characterised by altered performance across multiple cognitive domains, including set-shifting (Fontenelle et al., 2001; Goodwin and Sher, 1992), behavioural inhibition (Coles et al., 2006), impulse control (Fontenelle et al., 2005) and reward-feedback processing (Figee et al., 2011). However, not all patients present with impairments across all of these domains and an accumulating body of neuropsychological research is pointing to highly specific patterns of cognitive performance that associate with different phenotypes of compulsive symptomology (Brady et al., 2010; Exner et al., 2014; McKay, 2006; Melli et al., 2015; Raines et al., 2015).

As alluded to earlier, anxiety is believed to play a central role in the manifestation of compulsivity, a symptom that is often directed at engendering a sense of ease in the sufferer (Abramowitz and Jacoby, 2015). However, based on patient self-reports and symptom cluster analyses (Abramovitch et al., 2013; Jacoby et al., 2014; Sookman et al., 2005), this also is not always true. This finding begs the question of whether anxiety plays a unique etiopathological or modulatory role in some phenotypes of OCD only. Indeed, it will be valuable to study the relationship between anxiogenic manipulation and the manifestation of naturalistic repetitive behaviours, since a better understanding of such interactions may contribute to an improvement in the current treatment approaches. This is true, since known anxiolytics, e.g. the benzodiazepines, are not effective in the treatment of OCD and while it is known that patients do not necessarily present with generalised anxiety, it is possible that anxiety with respect to specific themes may play a role in the extent to which compulsivity is expressed

[1] obsessive-compulsive disorder
[2] selective serotonin reuptake inhibitor

(Abramowitz, 2006; (APA, 2013). The possibility that anxiogenic scenarios may modify the expression of compulsive-like behaviours in an animal model, will be the major focus of this work.

The deer mouse (Peromyscus maniculatus bairdii) model of compulsive-like behavioural persistence is a useful naturalistic animal model that presents with spontaneous and behaviourally heterogeneous compulsive-like repetition (Scheepers et al., 2018), i.e. repetitive motor actions that are performed without a clear and useful behavioural outcome. Such behaviours include spontaneous motor stereotypy (high stereotypy (HS)[1]; observed in 40-45% of the population; (Wolmarans et al., 2013)), excessively large nest building (LNB[2]; expressed by 30% of the population; (Wolmarans et al., 2016b)) and high marble-burying (HMB[3]; expressed by 11 – 15% of the population; (Wolmarans et al., 2016a)). All three behaviours are equally persistent and manifest in deer mice of both sexes from the age of 10 – 12 weeks. However, while LNB and HS respond to chronic, high dose escitalopram intervention, HMB remains overly refractory to monotherapeutic SSRIs (Wolmarans et al., 2016a). Considering the known, but poorly understood role of dopamine in the etiopathology of OCD[4], it is interesting that HMB responds to pro-dopaminergic intervention, either alone or in combination with escitalopram (De Brouwer et al., 2020); this stands in direct contrast to the well-accepted role of anti-dopaminergic augmentation strategies employed to improve the therapeutic response in SSRI[5]-refractory cases (Dold et al., 2015; Fineberg et al., 2015; Szechtman et al., 2020). These behaviours can further be distinguished based on function, since LNB and HMB represent clear goal-directed behaviours, while HS has no evident behavioural endpoint. Hence, the model could potentially be applied to investigate compulsive-like phenotypes that differ based on form, function and behavioural outcome (APA, 2013; Scheepers et al., 2018; Wolmarans et al., 2013) (for a detailed review of the model, which has been under development in our laboratory for nearly two decades, please refer to (Scheepers et al., 2018)). Here, we will apply naturalistic nest building in deer mice (Wolmarans et al., 2016b) as a model framework in which to assess whether an anxiety-provoking context will alter the expression of this seemingly safety-seeking behaviour.

[1] high stereotype
[2] large nest building
[3] high marble burying
[4] obsessive-compulsive disorder
[5] selective serotonin reuptake inhibitor

1.3 Hypothesis

Nest building behaviour in laboratory-housed deer mice is likely driven by an inherent need to construct a safe habitat that is suitable for breeding, nursing, and to provide adequate protection from predation and other dangers (Jirkof, 2014; Smithers, 1983). However, since all deer mice are housed under identical circumstances, we hypothesize that persistent engagement in LNB[1] behaviour represents a compulsive-like phenotype which is inflexible and overly dissociated from a clear goal and outcome (De Brouwer et al., 2020) and that such behaviour would persist in an anxiogenic scenario to which animals must gain access to retrieve nesting material, even if it is entirely optional to do so. On the other hand, we hypothesize that deer mice engaging in normal nest building (NNB)[2] behaviour, will either adapt their behaviour according to the context in which it is measured, i.e. expressing bolstered nesting behaviour when assessed under anxiogenic circumstances, or choose to avoid voluntary exposure to such an anxiogenic context, entirely. It therefore stands to reason that chronic high dose escitalopram, being a known anxiolytic and anti-compulsive intervention, will attenuate the nest building expression of both NNB (if bolstered due to anxiety) and LNB-expressing animals (representing a compulsive-like phenotype) as assessed in a novel anxiogenic environment. At the same time, escitalopram will increase the time that all animals voluntarily spend in such an environment. On the other hand, lorazepam — a benzodiazepine with anxiolytic, but not anti-compulsive properties — will only attenuate the potentially bolstered nesting behaviour of NNB-expressing animals as assessed under anxiogenic circumstances, while the nesting behaviour of LNB animals will remain insensitive to such intervention. Last, we expect that lorazepam, like escitalopram, will also increase the time that mice of both cohorts spend in an anxiogenic environment, irrespective of its effects on nesting behaviour.

[1] large nest building
[2] normal nest building

1.4 Study Aims and Objectives

The present work first aims to determine whether the expression of NNB[1] and LNB[2] behaviour differ in terms of its potential association with anxiety and how such behaviours will adapt as a function of contextual manipulation, i.e. assessment of nesting behaviour under normal home cage and anxiogenic circumstances. Secondly, we aim to determine how said behaviours will respond to a known anti-compulsive and anxiolytic intervention, i.e. chronic, high-dose escitalopram, as well as to lorazepam, an anxiolytic benzodiazepine. These aims will be realised by addressing the following research objectives:

I. Randomly selecting 182 deer mice of both sexes (aged 10 – 12 weeks at the onset of experimentation) to be screened for baseline nest-building behaviour over 7 consecutive days;
II. Broadly selecting 38 NNB and 38 LNB deer mice based on the data obtained in (I) which will further be divided into four main experimental groups (which include one sub-group per phenotype) as follows:
    i. NNB and LNB nesting control groups (n = 10 per behavioural cohort); exposed to normal tap water only for 32 days;
    ii. NNB and LNB nesting escitalopram groups (n = 10 per behavioural cohort); exposed to oral escitalopram (50 mg/kg/day; De Brouwer et al. (2020)) for 32 days;
    iii. NNB and LNB nesting lorazepam groups (n = 10 per behavioural cohort); exposed to lorazepam (2 mg/kg/day; (Chesley et al., 1991; Fahey et al., 2006; Hadžiabdic et al., 2012; Loring et al., 2012)) for 4 days only; and
    iv. an NNB and LNB no-nesting material control group (n = 8 per behavioural cohort); exposed to normal tap water only for 32 days.
III. Reassessing nest-building behaviour of all mice included in (i) – (iii) over 4 consecutive nights in a novel anxiogenic environment, i.e. a white-floored and mirrored open field, after 28 days of uninterrupted treatment which would continue during the 4 days of assessment according to the respective exposure groups (i.e. 32 or 4 days of continuous exposure); and
IV. Determining the extent and manner to which all mice included in (i) – (iv) voluntarily ambulate in an anxiogenic environment, i.e. a white-floored and mirrored open field, after 28 days of uninterrupted water and escitalopram exposure and during 4 days of additional water, escitalopram and lorazepam exposure, dependent on the respective exposure group.

[1] normal nest building
[2] large nest building

1.4.1 Project layout

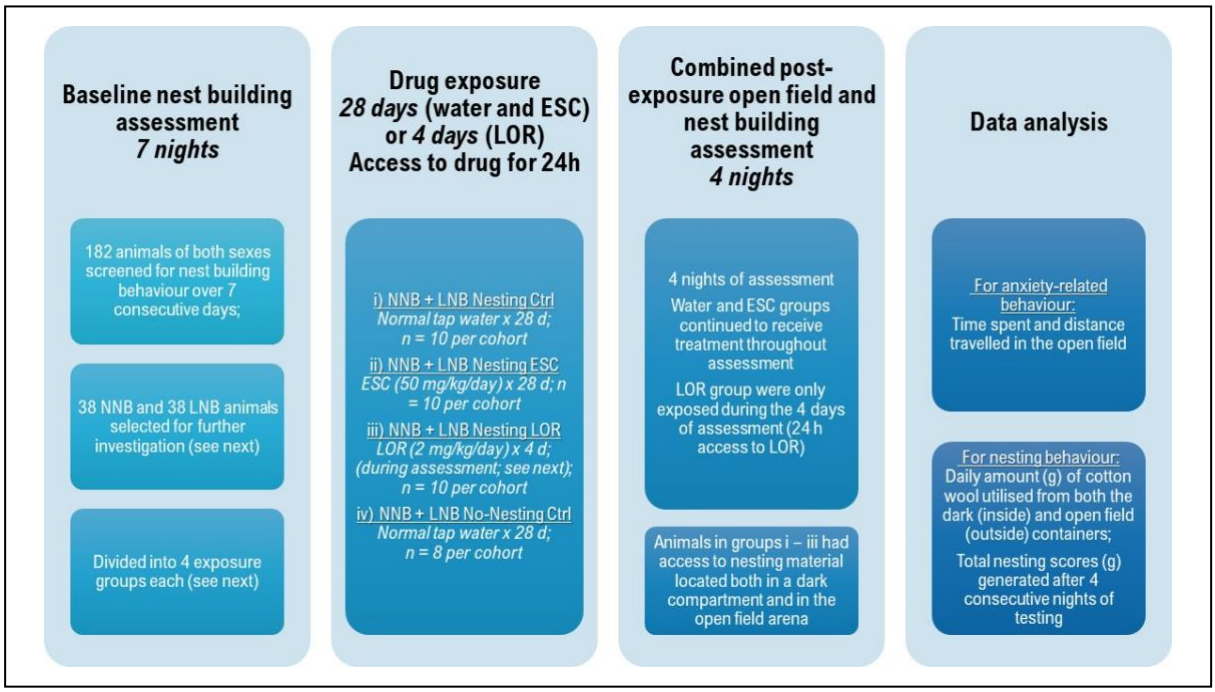

FIGURE 1-1 – *Schematic Representation of the study layout*

ESC: escitalopram; LNB: large nest-building; LOR: lorazepam; NNB: normal nest-building

* * *

Since this dissertation is drafted in article format as prescribed by the NWU[1], a complete and detailed description of the methodology followed, is provided in Chapter 3, with additional materials and methods that are not of direct relevance for the journal article that constitutes Chapter 3, provided in Addendum C.

[1] North-West University

1.5 Ethical Approval

The current investigation has been approved by the AnimCare Research Ethics Committee (NHREC[1] reg. nr.: AREC[2]-130913-015) of the NWU[3] (approval number NWU-00574-19-A5). The ARRIVE[4]-guidelines for animal experimentation was adhered to as closely as possible (Percie du Sert et al., 2020).

1.6 Expected Outcomes

1.6.1 Baseline nest-building assessment

We expect differences in deer mouse nesting behaviour to result in spontaneous variance in the total between-subject nesting scores generated after 7 consecutive nights of nest-building analysis as well as in the extent to which the between-day nesting scores vary. Based on this data, we expect approximately 25 – 30% of deer mice to present with LNB[5] behaviour (Wolmarans et al., 2016b).

1.6.2 Post-exposure anxiety and nest building assessment

First, we expect the LNB phenotype, being persistent and excessive against the background of a standard laboratory context, to be inflexible and insensitive to contextual manipulation, i.e. when being assessed in a novel anxiogenic environment (a white-floored and mirrored open field). We further expect LNB animals to show preoccupation with satisfying an inherent motivational drive to nest to such an extent, that they would voluntarily ambulate within the open field to gain access to excessive quantities of nesting material. On the other hand, since we hypothesise NNB[6] to be governed by adequate and normal processes of action-outcome control, we expect the nesting behaviour of NNB-expressing animals to adjust according to manipulation of the context. In other words, we expect NNB animals to either build larger nests when assessed in the white-floored and mirrored open field, or to avoid unnecessary exposure to the open field entirely.

With respect to the response of deer mouse nesting behaviour to the various drug exposures employed, we expect the following. First, since we hypothesise LNB to be compulsive-like in nature, we expect such behaviour only to respond to chronic high-dose escitalopram, a known anti-compulsive agent, but not to normal water or the anxiolytic benzodiazepine, lorazepam, thereby

[1] National Health Research Ethics Council
[2] Animal Research Ethics Committee
[3] North-West University
[4] Animal Research: Reporting of *In Vivo* Experiments
[5] large nest building
[6] normal nest building

differentiating such behaviour from an anxiety-related coping response. Second, since we believe NNB[1]-expressing animals to bolster their building activity when assessed under anxiogenic circumstances, i.e. building nests of a larger size due to being assessed in an anxiogenic environment, we expect such behaviour, if evident, to abate as a function of both chronic, high-dose escitalopram and lorazepam, but not normal water intervention; in this case, escitalopram would also be effective, since it is known to be both an anxiolytic and an anti-compulsive therapy.

Last, we expect both escitalopram and lorazepam, but not normal water to increase the time spent and the distance travelled in the open field arena, irrespective of their effects on nesting expression. We expect this finding to hold true, since voluntary ambulation in an anxiogenic space should be entirely dependent on the level of anxiety experienced. Since both drugs are known to be anxiolytic, we believe all animals exposed to such interventions will demonstrate reduced levels of open field aversion, compared to animals exposed to normal tap water only.

In summary, we expect this research to deliver valuable insights into the expression of LNB[2] behaviour as observed in deer mice and its potential association with (or dissociation from) contextual anxiety. We further believe that our findings will contribute to a better understanding of the continuum which organises normal context-driven behaviours and its so-called abnormal analogues, which is understood here as compulsive-like behaviour.

[1] normal nest building
[2] large nest building

1.7 References

Abramovitch, A., Abramowitz, J.S., Mittelman, A., 2013. The neuropsychology of adult obsessive-compulsive disorder: a meta-analysis. Clin Psychol Rev 33(8), 1163-1171.

Abramowitz, J.S., 2006. Understanding and Treating Obsessive-Compulsive Disorder. Lawrence Erlbaum Associates, Mahwah, New Jersey.

Abramowitz, J.S., Jacoby, R.J., 2015. Obsessive-compulsive and related disorders: a critical review of the new diagnostic class. Annual review of clinical psychology 11, 165-186.

Abramowitz, J.S., Taylor, S., McKay, D., 2009. Obsessive-compulsive disorder. The Lancet 374(9688), 491-499.

Ahmari, S.E., Spellman, T., Douglass, N.L., Kheirbek, M.A., Simpson, H.B., Deisseroth, K., Gordon, J.A., Hen, R., 2013. Repeated cortico-striatal stimulation generates persistent OCD-like behavior. Science 340(6137), 1234-1239.

Albert, U., Aguglia, A., Bramante, S., Bogetto, F., Maina, G., 2013. Treatment-resistant obsessive-compulsive disorder (OCD): current knowledge and open questions. Clin Neuropsychiatry 10(1), 19-30.

APA (American Psychiatric Association)., 2013. Diagnostic and statistical manual of mental disorders (DSM-5®). American Psychiatric Pub.

Brady, R.E., Adams, T.G., Lohr, J.M., 2010. Disgust in contamination-based obsessive-compulsive disorder: A review and model. Expert Review of Neurotherapeutics 10(8), 1295-1305.

Chesley, S., Lumpkin, M., Schatzki, A., Galpern, W., Greenblatt, D., Shader, R.I., Miller, L., 1991. Prenatal exposure to benzodiazepine—I. Prenatal exposure to lorazepam in mice alters open-field activity and GABAA receptor function. Neuropharmacology 30(1), 53-58.

Coles, M.E., Schofield, C.A., Pietrefesa, A.S., 2006. Behavioral inhibition and obsessive–compulsive disorder. Journal of anxiety disorders 20(8), 1118-1132.

Cools, R., Nakamura, K., Daw, N.D., 2011. Serotonin and dopamine: unifying affective, activational, and decision functions. Neuropsychopharmacology 36(1), 98-113.

De Brouwer, G., Fick, A., Lombaard, A., Stein, D.J., Harvey, B.H., Wolmarans, D.W., 2020. Large nest building and high marble-burying: Two compulsive-like phenotypes expressed by deer mice (Peromyscus maniculatus bairdii) and their unique response to serotoninergic and dopamine modulating intervention. Behavioural Brain Research 393, 112794.

Dold, M., Aigner, M., Lanzenberger, R., Kasper, S., 2015. Antipsychotic Augmentation of Serotonin Reuptake Inhibitors in Treatment-Resistant Obsessive-Compulsive Disorder: An Update Meta-Analysis of Double-Blind, Randomized, Placebo-Controlled Trials. Int J Neuropsychopharmacol 18(9).

Exner, C., Zetsche, U., Lincoln, T.M., Rief, W., 2014. Imminent Danger? Probabilistic classification learning of threat-related information in obsessive-compulsive disorder. Behavior Therapy 45(2), 157-167.

Fahey, J.M., Pritchard, G.A., Reddi, J.M., Pratt, J.S., Grassi, J.M., Shader, R.I., Greenblatt, D.J., 2006. The effect of chronic lorazepam administration in aging mice. Brain Res 1118(1), 13-24.

Figee, M., Vink, M., de Geus, F., Vulink, N., Veltman, D.J., Westenberg, H., Denys, D., 2011. Dysfunctional reward circuitry in obsessive-compulsive disorder. Biological psychiatry 69(9), 867-874.

Fineberg, N.A., 2004. Pharmacological treatment for obsessive-compulsive disorder. Psychiatry 3(6), 72-76.

Fineberg, N.A., Potenza, M.N., Chamberlain, S.R., Berlin, H.A., Menzies, L., Bechara, A., Sahakian, B.J., Robbins, T.W., Bullmore, E.T., Hollander, E., 2010. Probing compulsive and impulsive behaviors, from animal models to endophenotypes: a narrative review. Neuropsychopharmacology 35(3), 591-604.

Fineberg, N.A., Reghunandanan, S., Brown, A., Pampaloni, I., 2013. Pharmacotherapy of obsessive-compulsive disorder: evidence-based treatment and beyond. Australian & New Zealand Journal of Psychiatry 47(2), 121-141.

Fineberg, N.A., Reghunandanan, S., Simpson, H.B., Phillips, K.A., Richter, M.A., Matthews, K., Stein, D.J., Sareen, J., Brown, A., Sookman, D., 2015. Obsessive–compulsive disorder (OCD): Practical strategies for pharmacological and somatic treatment in adults. Psychiatry Research 227(1), 114-125.

Fontenelle, L., Marques, C., Engelhardt, E., Versiani, M., 2001. Impaired set-shifting ability and therapeutic response in obsessive-compulsive disorder. The Journal of neuropsychiatry and clinical neurosciences 13(4), 508-510.

Fontenelle, L.F., Mendlowicz, M.V., Versiani, M., 2005. Impulse control disorders in patients with obsessive–compulsive disorder. Psychiatry and clinical neurosciences 59(1), 30-37.

Goodwin, A.H., Sher, K.J., 1992. Deficits in set-shifting ability in nonclinical compulsive checkers. Journal of Psychopathology and Behavioral Assessment 14(1), 81-92.

Hadžiabdic, J., Elezovic, A., Imamovic, B., Bečic, E., 2012. The improvement of lorazepam solubility by cosolvency, micellization and complexation. Jordan Journal of Pharmaceutical Sciences 5(2).

Harrison, B.J., Pujol, J., Cardoner, N., Deus, J., Alonso, P., Lopez-Sola, M., Contreras-Rodriguez, O., Real, E., Segalas, C., Blanco-Hinojo, L., Menchon, J.M., Soriano-Mas, C., 2013. Brain corticostriatal systems and the major clinical symptom dimensions of obsessive-compulsive disorder. Biol Psychiatry 73(4), 321-328.

Hesse, S., Muller, U., Lincke, T., Barthel, H., Villmann, T., Angermeyer, M.C., Sabri, O., Stengler-Wenzke, K., 2005. Serotonin and dopamine transporter imaging in patients with obsessive-compulsive disorder. Psychiatry Res 140(1), 63-72.

Jacoby, R.J., Leonard, R.C., Riemann, B.C., Abramowitz, J.S., 2014. Predictors of quality of life and functional impairment in Obsessive–Compulsive Disorder. Comprehensive psychiatry 55(5), 1195-1202.

Ji, J.L., Anticevic, A., 2018. Functional MRI in Psychiatric Disorders. Functional MRI. Basic Principles and Emerging Clinical Applications in Anesthesiology and the Neurological Sciences, 91-118.

Jirkof, P., 2014. Burrowing and nest building behavior as indicators of well-being in mice. Measuring Behavior 234, 139-146.

Kilkenny, C., Browne, W.J., Cuthill, I.C., Emerson, M., Altman, D.G., 2014. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. Animals 4(1), 35-44.

Korff, S., Harvey, B.H., 2006. Animal models of obsessive-compulsive disorder: rationale to understanding psychobiology and pharmacology. Psychiatr Clin North Am 29(2), 371-390.

Korff, S., Stein, D.J., Harvey, B.H., 2008. Stereotypic behaviour in the deer mouse: pharmacological validation and relevance for obsessive compulsive disorder. Prog Neuropsychopharmacol Biol Psychiatry 32(2), 348-355.

Kranz, G., Kasper, S., Lanzenberger, R., 2010. Reward and the serotonergic system. Neuroscience 166(4), 1023-1035.

Lack, C.W., 2012. Obsessive-compulsive disorder: Evidence-based treatments and future directions for research. World J Psychiatry 2(6), 86-90.

Loring, D.W., Marino, S.E., Parfitt, D., Finney, G.R., Meador, K.J., 2012. Acute lorazepam effects on neurocognitive performance. Epilepsy Behav 25(3), 329-333.

Marazziti, D., Consoli, G., 2010. Treatment strategies for obsessive-compulsive disorder. Expert opinion on pharmacotherapy 11(3), 331-343.

Markarian, Y., Larson, M.J., Aldea, M.A., Baldwin, S.A., Good, D., Berkeljon, A., Murphy, T.K., Storch, E.A., McKay, D., 2010. Multiple pathways to functional impairment in obsessive–compulsive disorder. Clinical psychology review 30(1), 78-88.

McKay, D., 2006. Treating disgust reactions in contamination-based obsessive–compulsive disorder. Journal of behavior therapy and experimental psychiatry 37(1), 53-59.

Melli, G., Chiorri, C., Carraresi, C., Stopani, E., Bulli, F., 2015. The two dimensions of contamination fear in obsessive-compulsive disorder: Harm avoidance and disgust avoidance. Journal of Obsessive-Compulsive and Related Disorders 6, 124-131.

Percie du Sert, N., Hurst, V., Ahluwalia, A., Alam, S., Avey, M.T., Baker, M., Browne, W.J., Clark, A., Cuthill, I.C., Dirnagl, U., 2020. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. Journal of Cerebral Blood Flow & Metabolism 40(9), 1769-1777.

Peters, S.K., Dunlop, K., Downar, J., 2016. Cortico-striatal-thalamic loop circuits of the salience network: a central pathway in psychiatric disease and treatment. Frontiers in systems neuroscience 10, 104.

Raines, A.M., Allan, N.P., Oglesby, M.E., Short, N.A., Schmidt, N.B., 2015. Examination of the relations between obsessive–compulsive symptom dimensions and fear and distress disorder symptoms. Journal of affective disorders 183, 253-257.

Scheepers, I.M., Wolmarans, D.W., Stein, D.J., Harvey, B.H., 2018. Peromyscus maniculatus bairdii as a naturalistic mammalian model of obsessive-compulsive disorder: current status and future challenges. Metabolic brain disease 33(2), 443-455.

Smithers, R.H.N., 1983. XXIII. Families cricetidae and muridae, rats and mice, The Mammals of the Southern-African Subregion. University of Pretoria, Pretoria, South Africa, pp. 220-220 - 296.

Sookman, D., Abramowitz, J.S., Calamari, J.E., Wilhelm, S., McKay, D., 2005. Subtypes of obsessive-compulsive disorder: Implications for specialized cognitive behavior therapy. Behavior Therapy 36(4), 393-400.

Szechtman, H., Harvey, B.H., Woody, E.Z., Hoffman, K.L., 2020. The Psychopharmacology of Obsessive-Compulsive Disorder: A Preclinical Roadmap. Pharmacol Rev 72(1), 80-151.

Tops, M., Russo, S., Boksem, M.A., Tucker, D.M., 2009. Serotonin: modulator of a drive to withdraw. Brain and cognition 71(3), 427-436.

Van der Straten, A., Denys, D., Van Wingen, G., 2017. Impact of treatment on resting cerebral blood flow and metabolism in obsessive compulsive disorder: a meta-analysis. Scientific Reports 7(1), 1-8.

Wolmarans, D.W., Brand, L., Stein, D.J., Harvey, B.H., 2013. Reappraisal of spontaneous stereotypy in the deer mouse as an animal model of obsessive-compulsive disorder (OCD): Response to escitalopram treatment and basal serotonin transporter (SERT) density. Behavioural Brain Research 256, 545-553.

Wolmarans, D.W., Stein, D.J., Harvey, B.H., 2016a. Of mice and marbles: Novel perspectives on burying behavior as a screening test for psychiatric illness. Cogn Affect Behav Neurosci 16(3), 551-560.

Wolmarans, D.W., Stein, D.J., Harvey, B.H., 2016b. Excessive nest building is a unique behavioural phenotype in the deer mouse model of obsessive–compulsive disorder. Journal of Psychopharmacology 30(9), 867-874.

Wood, J., Ahmari, S.E., 2015. A framework for understanding the emerging role of corticolimbic-ventral striatal networks in OCD-associated repetitive behaviors. Frontiers in systems neuroscience 9, 171.

Zhang, H., Wang, B., Li, K., Wang, X., Li, X., Zhu, J., Zhao, Q., Yang, Y., Lv, L., Zhang, M., 2019. Altered functional connectivity between cerebellum and cortico-striato-thalamo-cortical circuit in Obsessive-Compulsive Disorder. Frontiers in psychiatry 10, 522.

2 LITERATURE REVIEW

2.1 Obsessive-compulsive disorder in the clinic

2.1.1 Epidemiology and diagnostic description

Obsessive-compulsive disorder (OCD)[1] is a psychiatric disorder which usually manifests in early adulthood or late adolescence and is characterised by two broad symptom groups, i.e. obsessions (intrusive and disturbing thoughts) and compulsions (repetitive, persistent, and overly rigid covert or overt behavioural routines) (APA, 2013). It is a severe and debilitating condition that causes significant distress and anxiety in patients which purportedly lead them to engage in obsessive-compulsive thoughts and behaviours (Abramowitz, 2006; Abramowitz et al., 2009; Fineberg et al., 2015). With a global lifetime prevalence rate between 2.0% - 2.9%, OCD is diagnosed in both men and women with a slight predominance in women (Abramovitch et al., 2013; Labad et al., 2008) and men generally demonstrating an earlier age of onset. Although OCD is no longer classified as an anxiety disorder (APA, 2013), it is noteworthy that anxiety plays a major role in obsessive-compulsive symptom manifestation, with stress likely having a significant role in the extent to which symptoms wax and wane (Abramowitz, 2006; Katz et al., 2018). Since the publication of the DSM-V[2], the condition has been classified as the principal condition within the 'Obsessive-Compulsive and Related Disorders (OCRD)[3]' group (APA, 2013; Krzyszkowiak et al., 2019). Though no definitive precipitant to the symptom onset of OCD has been described, some evidence points to traumatic or stressful experiences playing a potential role (Abramowitz, 2006; Robinson and Freeston, 2014).

2.1.2 Symptom conceptualisation

As alluded to above, patients broadly present with two significant symptom clusters, either one of which or both can be sufficient to make an accurate diagnosis. Apart from obsessions and compulsions being the primary symptoms of OCD, it is important to note that OCD is a phenotypically heterogeneous disorder (Abramowitz, 2006; Blakey et al., 2019). Obsessions and compulsions generally cluster within one or more specific themes (**Figure 2-1**), with the most common themes being contamination/washing, safety/checking, symmetry/ordering, repugnant intrusive thoughts/mental routines, and hoarding (Raines et al., 2015).

[1] obsessive-compulsive disorder
[2] diagnostic and statistical manual of mental disorders, 5th edition
[3] obsessive-compulsive and related disorders

FIGURE 2-1 - *Classification of obsessions and related compulsive symptomology*
Adapted from Abramovitch and Cooperman (2015); Abramowitz et al. (2009)

Further, patients demonstrate highly specific cognitive deficits with respect to the symptom phenotype diagnosed, e.g. rigidly engaging in 'neutralising' cleansing routines following exposure to mild contamination. In other words, OCD[1] patients do not present with broad and overarching cognitive disability (Olley et al., 2007) but are rather affected with respect to highly particular facets of daily life such as cleaning. Since the phenotypic content of obsessions and compulsions include concerns that most individuals experience on a day-to-day basis, e.g. concerns about contamination or safety, certain diagnostic criteria must be met before these can be attributed as manifestations of OCD. Symptoms must be time-consuming (taking up more than one hour per day), significantly interfere with the normal daily social and occupational functioning of the individual, and not be attributable to any other known condition or the use of any substance (APA, 2013). Importantly, OCD patients vary in the degree to which they demonstrate insight into the meaning of their symptoms; most patients demonstrate good to fair insight into the futility of their symptom-specific convictions and behaviours (Catapano et al., 2010; Jakubovski et al., 2011). Insight is important, since OCD can be distinguished from delusions based on the fact that patients with delusions believe their symptoms to be borne from true and actual circumstances (APA, 2013).

With respect to the proposed origins of obsessions, two types of obsessional origins have been proposed (Lee and Kwon, 2003). Reactive obsessions refer to doubts and thoughts evoked by external stimuli or conditions that seemingly carry a risk or that evoke a sense of unease that patients

[1] obsessive-compulsive disorder

find distressing. In response, patients engage in neutralising routines to prevent what they believe will be actual negative repercussions (Abramowitz, 2006). Some examples include concerns about accidents, contamination, making mistakes, and asymmetry. On the other hand, invasive thoughts, urges, or images that are borne within, but that could be modified by external factors, are called autogenous obsessions (Abramowitz, 2006; Lee and Kwon, 2003). Examples in this category are inappropriate aggressive or sexual impulses or invasive blasphemous or indecent thoughts. These irrational thoughts are often overwhelming and are firmly resisted because they are experienced as highly revolting or otherwise disquieting (Abramowitz, 2006; Lee and Kwon, 2003). That said, three components set clinical obsessive-compulsive symptoms aside from other psychomotor repetitive phenomena (Abramowitz, 2006). First, obsessions typically invade the mind as undesirable, uncontrollable, and often also what is perceived to be highly improper, mental images. Second, the content of such obsessions is conflicting with the overall beliefs of the patient, i.e. being egodystonic. Last, patients experience obsessions as something to be controlled, avoided or neutralised, thereby demonstrating subjective resistance to the symptomology (Abramovitch and Cooperman, 2015; Abramowitz, 2006). Although compulsive behaviours are likely the most prominent mechanism that patients with OCD[1] enact to neutralise the intrusive nature of said obsessions, other mechanisms brought into play include distraction, thought replacement, scenario prevention, thought suppression, and justification of actions (Abramovitch and Cooperman, 2015; Abramowitz, 2006; Freeston and Ladouceur, 1997).

Drawing a line that bridges obsessions on the one side, with compulsions on the other, while also considering the phenomenology of OCD summarised above, compulsions can be regarded as active coping strategies recruited to mitigate the level of distress caused by the relevant obsession (Abramowitz, 2006; Abramowitz et al., 2009; APA, 2013). Not to be confused with repetitive, mechanical, habitual behaviours as observed in certain disorders such as Tourette's syndrome, compulsions are rather more goal-directed, albeit the 'goal' not being a realistic endpoint, since it appears OCD patients are perhaps inhibited in their ability to reach a sense of goal accomplishment (Nielen et al., 2009). Considering that compulsions are not directed at mitigating an actual risk, the very act of performing compulsions contributes to their senseless repetition. Stated differently, compulsive behaviour is self-reinforcing in that continuous engagement in the same routines without an actual and tangible outcome, contributes to its own execution (Nielen et al., 2009). Once having completed a specific compulsive routine, the intrusive thoughts quickly return to haunt the individual, triggering another compulsive bout (Abramowitz, 2006; Abramowitz et al., 2009). It is the close

[1] obsessive-compulsive disorder

relationship between what is likely anxiety-provoking obsessions and anxiety-reducing compulsions which sets OCD[1] aside from most other disorders that also present with repetitive psychomotor symptomology (Abramovitch and Cooperman, 2015; Abramowitz, 2006). Though compulsive rituals are executed to reduce anxiety in the short term, in the long term they can be regarded as maladaptive responses (Abramowitz, 2006). Indeed, compulsivity hinders patients from learning that their obsessional fears are unrealistic. The obsessional fear is thus enhanced by fostering the concept that compulsive rituals are mandatory and useful to avoid catastrophe (Abramowitz, 2006). However, that OCD can be diagnosed in the absence of anxiety or obsessions, somewhat clouds our understanding of the condition and hence investigations into the relationship between compulsion-provoking (or contextual) anxiety, is continuing (Nadeau et al., 2013; Nestadt et al., 2001; Raines et al., 2015). This research construct will also be a core focus of the current work.

2.1.3 Neurobiology

2.1.3.1 A brief overview of the neuroanatomical architecture of OCD

Although the complete neurobiological architecture of OCD has not yet been elucidated, this topic has been studied extensively during the past few decades. Irrespective of study or patient cohort, undisputed evidence points to the involvement of dysfunctional cortico-striatal-thalamic-cortical (CSTC)[2] circuitry in the manifestation of OCD. The CSTC circuit comprises four distinct brain regions that are associated with obsessive-compulsive (OC)[3] symptomology, i.e. the prefrontal cortex (notably the orbitofrontal cortex (OFC)[4] and the anterior cingulate cortex (ACC)[5]), the striatum, and the thalamus (Harrison et al., 2013; Milad and Rauch, 2012). Collectively, the different structures of the CSTC circuit are responsible for the planning, execution, valuation, and termination of complex motor programmes. As also alluded to earlier, it is often proposed that patients with OCD fail to value the rewarding and behaviour-terminating properties of adequate task completion. More specifically, the CSTC circuitry is believed to be hyperactive in patients with OCD, thereby facilitating persistent behavioural routines (Kathmann et al., 2005; Rossi et al., 2005; van den Heuvel et al., 2005).

Two pathways gate the relay of signals from the cortex via the striatum to the thalamus, viz. the behaviourally activating direct and behaviourally inactivating indirect pathway. When the frontal cortex is overly active in the absence of adequate thalamic feedback control, both thought processes and

[1] obsessive-compulsive disorder
[2] cortico-striatal-thalamic-cortical
[3] obsessive-compulsive
[4] orbitofrontal cortex
[5] anterior cingulate cortex

motor behaviour can become excessive (Alptekin et al., 2001; Korff and Harvey, 2006). Insufficient thalamic-cortical control is in turn ascribed to a bias in favour of the direct pathway over the indirect pathway within the striatum (Burguiere et al., 2015). However, a causal relationship between such over-activation and OCD[1] has not yet been established; at best, it can be said that an association between OCD and CSTC[2] overactivation exists. The two major neurotransmitter systems that have up to date been implicated in the etiopathology of OCD, are the serotonergic and dopaminergic systems (Hesse et al., 2005; Pauls et al., 2014; Wood et al., 2018). Still, the glutamatergic and gamma-aminobutyric acid (GABA)[3]-ergic systems have recently also gained interest (Grados et al., 2015). More specifically, as serotonin is traditionally regarded as a motivationally inhibiting neurotransmitter (Daw et al., 2002), it is hypothesized that OCD patients present with deficits in serotonergic signalling.

2.1.3.2 Key concepts pertaining to dopaminergic and serotonergic signalling

Dopamine is an important neuromodulator in the CSTC circuit and acts as a gating mechanism for motor routines. Activity in the direct and indirect pathways can be differentially modulated by the activation of dopamine D_1 and D_2 receptors in the striatum (Frank and Claus, 2006) and other regions of the CSTC circuit (e.g. OFC)[4] (Winter et al., 2009). Whereas the direct pathway expresses D_1 receptors, the indirect pathway expresses D_2 receptors (Groenewegen, 2003; Nambu, 2008; Stocco et al., 2010). Importantly, activation of D_1 receptors results in direct pathway activation. However, if D_2 receptors are activated, the behaviourally inactivating indirect pathway, is inactivated, hence facilitating a net activating signal to be relayed to the thalamus (Groenewegen, 2003; Nambu, 2008; Stocco et al., 2010). Importantly, within the context of OCD, an expectation of a potential outcome, e.g. the locking of a door which will make one feel safe, would trigger a spike in dopamine release that will activate the direct pathway and inactivate the indirect pathway, thereby facilitating a behavioural response. However, following the completion of such a task, i.e. having valued the actual outcome, dopamine secretion should decrease, thereby resulting in the inactivation of the direct pathway and the activation of the indirect pathway. It has previously been suggested that OCD patients may present with deficits in feedback learning because they might suffer from what can be conceptualised as a hyperdopaminergic state (Koo et al., 2010; Nikolaus et al., 2010; Westenberg et al., 2007). In other words, irrespective of the fact that a certain behavioural routine has been executed,

[1] obsessive-compulsive disorder
[2] cortico-striatal-thalamic-cortical
[3] gamma-aminobutyric acid
[4] orbitofrontal cortex

the necessary dopamine reductions that would normally facilitate behavioural inhibition, may not be present or sufficient.

Serotonin is another crucial role player in the CSTC[1] circuit, which is known to be involved in a diverse range of functions e.g. modulating behaviour, motor functions, sleep, mood, cognition, eating patterns and reproduction (Simpson et al., 2011; Sinopoli et al., 2017; Van Der Wee et al., 2004). Specifically, serotonin has been prominently associated with behavioural inhibition (Cools et al., 2011; Kranz et al., 2010), hence being generally proposed as the behavioural opponent of dopamine (Cools et al., 2011; Kranz et al., 2010; Tops et al., 2009). As referred to above and considering the first-line pharmacotherapeutic use of highly selective serotonin-reuptake inhibitors (SSRIs)[2] (Fineberg et al., 2011), OCD[3] can also be conceptualised as a condition characterised by an underlying hyposerotonergic state (Koo et al., 2010; Nikolaus et al., 2010; Westenberg et al., 2007). The most notable serotonin receptor subtypes identified to play a role in OCD are the serotonin (5HT)[4] $_{1A/2A/1B/2C}$ receptor subtypes as well as modulations that affect the expression and function of the serotonin transporter (SERT)[5] (Brakoulias and Stockings, 2019; Murphy et al., 2008; Sinopoli et al., 2017). That said, the serotonergic system is complex. The global and diffuse distribution of serotonin receptor subtypes complicates conclusions on their role in specific behavioural anomalies such as those that characterise OCD.

2.1.4 The pharmacological treatment of OCD

Successful pharmacological treatment of OCD was first documented in patients with comorbid depression, since clomipramine, a serotonergic tricyclic antidepressant with moderate noradrenergic activity, was used to treat the "obsessional" symptoms sometimes observed in depressed patients (Ananth, 1986; Philpott, 1976). The use of clomipramine within this context not only sparked research into the use of serotonergic agents in patients with comorbid depression and OCD, but also for the treatment of patients with OCD as a primary diagnosis. From 1990 onwards, many studies have established the overall, albeit suboptimal and varying efficacy of clomipramine for the treatment of OCD (Fineberg and Craig, 2007; Marazziti et al., 2008). Although tertiary tricyclic antidepressants like clomipramine also show moderate noradrenergic activity, it is the serotonergic component of their actions that has been shown to result in obsessive-compulsive symptom attenuation, since secondary amines with a prominent noradrenergic effect, e.g. desipramine, are ineffective (Fineberg and Craig,

[1] cortico-striatal-thalamic-cortical
[2] selective serotonin reuptake inhibitors
[3] obsessive-compulsive disorder
[4] serotonin
[5] serotonin transporter

2007; Pallanti et al., 2002; Piccinelli et al., 1995). In later years, selective serotonin reuptake inhibitors (SSRIs)[1] that have little or no effect on dopamine or norepinephrine reuptake was proven clinically adequate, with a success rate of 40 – 60% (Katzman et al., 2014) reported. SSRIs, including escitalopram, fluoxetine, sertraline, fluvoxamine, and paroxetine, are now regarded as the first line pharmacotherapeutic intervention for OCD[2] (Fineberg et al., 2015; Katzman et al., 2014). With respect to the use of SSRIs in OCD, some important considerations need to be kept in mind. First, symptom attenuation requires between 8 – 12 weeks of uninterrupted treatment with higher-than-normal dosages (Szechtman et al., 2020). Second, SSRIs are not exclusively used for the treatment of OCD, but are also employed to treat a spectrum of psychiatric disorders, i.e. depression, generalised anxiety disorder, panic disorder, and post-traumatic stress disorder (Katzman et al., 2014; Szechtman et al., 2020). Third, a significant percentage of patients remain refractory to the effects of SSRIs, regardless of the dose, duration of treatment or the specific drug used (Katzman et al., 2014; Szechtman et al., 2020). Therefore, and considering that some patients only respond to psychotherapeutic interventions (Aardema and O'Connor, 2007; Blakey et al., 2019; McLean et al., 2015), it is likely that the etiopathology of OCD is multifactorial, involving several psychobiological processes. That said, some progress has been made in terms of the treatment of SSRI-refractory OCD. The use of low- dose neuroleptics, e.g. the anti-dopaminergic agents, quetiapine, risperidone, olanzapine, and haloperidol, has especially shown promise (Dold et al., 2015; Fineberg et al., 2015).

An aspect of great importance to the current study is the fact that traditional anxiolytics, e.g. the benzodiazepines and the 5-HT$_{1A}$[3] receptor dualist, buspirone, is generally ineffective in the treatment of OCD. This is especially perplexing considering the well-established relationship between anxiety and the expression of compulsive routines (Bartz and Hollander, 2006).

2.2 Perspectives on the current work

2.2.1 The impulsivity-compulsivity spectrum: from risk to risk aversion?

Generally, impulse control disorders (ICDs)[4] and OCD[5] are believed to resemble the opposite ends of a psychomotor continuum, with the former being excessive in terms of risk-taking or risk-seeking features, and the latter in terms of harm-avoidance (Fineberg et al., 2010; Fontenelle et al., 2011; Kashyap et al., 2012). Impulsivity can possibly be defined as susceptibility towards unplanned, rapid

[1] selective serotonin reuptake inhibitors
[2] obsessive-compulsive disorder
[3] serotonin
[4] impulse control disorders
[5] obsessive-compulsive disorder

reactions to external or internal stimuli, without the necessary consideration for the possible negative consequences (Fineberg et al., 2010; Frydman et al., 2020). It can also be seen as 'giving in to urges', motivational states or impulses. Considering the motivational construct of compulsive behaviours, these can be regarded as excessive behaviours aimed at mitigating a specific, but unrealistic and inflated risk (Abramovitch and Cooperman, 2015; Abramowitz, 2006; APA, 2013). That said, studies have shown that there are impulsive features within OCD (Ettelt et al., 2007; Summerfeldt et al., 2004), leading to the thought that compulsivity and impulsivity may share their neurobiological and psychopathological architecture (Fontenelle et al., 2005; Fontenelle et al., 2011). For example, while OCD patients often experience a form of anxiolytic reward through their compulsive engagement, these behaviours, e.g. symmetry-orientated or hoarding compulsions, may resemble impulsive actions. Also, some ICD patients also display repetitive behaviours aimed at reducing anxiety, i.e. skin picking or trichotillomania, thus aligning the condition with the phenomenology of OCD (Ferrão et al., 2009; Fontenelle et al., 2011). Further, there are indications that impulsivity in OCD patients becomes more distinguished as the severity of OCD increases (Kashyap et al., 2012).

Impulsive and compulsive behaviours are related on many levels which have been difficult to untangle in the past few decades. According to Fineberg et al. (2010), impulsivity and compulsivity possibly portray orthogonal components of the same construct, rather than being polar opposites, and that they may be present in different disorders. Some of these disorders include mood disorders, e.g. depression or bipolar disorder, kleptomania, pathological gambling, trichotillomania, anxiety disorders, e.g. panic disorder or social phobia, attention-deficit/hyperactivity disorder (ADHD)[1], eating disorders and Tourette's syndrome (Del Casale et al., 2019; Fineberg et al., 2018; Fineberg et al., 2010; Greenberg et al., 2017). Interestingly, there is a possibility that these conditions share certain pathophysiological constructs, since several of these disorders are likely to co-exist, either within the family or within the same individual (Fineberg et al., 2010). However, in contrast to the known anti-compulsive effect of SSRIs[2] (Katzman et al., 2014; Szechtman et al., 2020), impulsivity has previously been correlated with increased serotonin release (Fletcher et al., 2007; Hennig et al., 2020; Talpos et al., 2006; Winstanley et al., 2004), i.e. decreasing in response to serotonin depletion. While 5-HT$_{2A}$[3] antagonists show promise as anti-impulsive agents, psychostimulant drugs, e.g. amphetamine and methylphenidate, have been found to increase impulsive behaviour through dopaminergic neurotransmission (Dellu-Hagedorn et al., 2018; Pattij and Vanderschuren, 2008, 2020). Studies also

[1] attention deficit hyperactivity disorder [2] selective serotonin reuptake inhibitors [3] serotonin

indicate that anti-glutamatergic neurotransmission exacerbate impulsive behaviour via the non-selective antagonism of the N-methyl-D-aspartate (NMDA)[1] receptor (Higgins et al., 2003; Higgins et al., 2016; Mirjana et al., 2004). This is especially interesting, since the GABA[2]-ergic benzodiazepine drug class, which normally inhibits neurotransmission in several brain areas, i.e. the substantia nigra, hippocampus, hypothalamus and the cerebral cortex (Trevor, 2017), can also cause paradoxical, i.e. disinhibited, behavioural reactions which are associated with the opposite effects of the desired outcomes, i.e. increased levels of hyperactivity, aggression, and anxiety (Bond, 1998; Panes et al., 2020; Paton, 2002). Although behavioural disinhibition is not exclusively related to the use of benzodiazepines and considering that the mechanisms underlying said disinhibition is not yet fully understood, it suffices to say that it may promote impulsive like behaviours which are characterised by significant risk for which the individual may not afford the necessary consideration.

2.2.2 The matter of context, anxiety, and compulsivity

Following from the above, the overall inefficacy of benzodiazepines in the treatment of OCD[3] (Robbins et al., 2019) orientates psychopharmacological research in OCD towards an interesting question. If obsessions and anxiety in OCD patients are related, what role if any, does anxiety play in the symptom manifestation of the condition? This is a highly debated question in both psychology and psychiatry (Abramovitch et al., 2013; Abramowitz, 2006; Abramowitz and Jacoby, 2015). Indeed, it is unclear whether mounting anxiety triggers seemingly anxiolytic compulsive routines, or whether the mere fact that patients are unsuccessful to refrain from engaging in excessive behaviours evokes a sense of anxiety. That benzodiazepines are ineffective in treating compulsions, would suggest the latter to be more in line with our current understanding. However, this remains to be established.

To gain a deeper understanding of the role that anxiety may play in the pathogenesis of OCD[4], we must afford some attention to the concept of context. As previously mentioned, obsessions can be associated with severe distress and anxiety, purportedly motivating patients to engage in what they might feel to be anxiolytic, compulsive routines (Abramowitz, 2006; APA, 2013). Two things are important here. First, if any anxiolytic effect is to be experienced, it is short-lived and likely contributes to the reinforcement of compulsive behaviour (Abramovitch et al., 2013). Two, OCD patients are not overly and generally anxious. Rather, patient self-reports indicate that they often feel distressed with respect to certain highly specific themes, e.g. fearing contamination or self-harm directly related to

[1] N-methyl-D-aspartate
[2] gamma-aminobutyric acid
[3] obsessive-compulsive disorder
[4] obsessive-compulsive disorder

their specific OCD symptom dimension (Nadeau et al., 2013; Overduin and Furnham, 2012; Raines et al., 2015; Schaefer et al., 2014). In other words, fearing probable death after touching a 'contaminated' surface, should not cause drastic concern in patients suffering from symmetry/ordering OCD. As such, it can be argued that in order to expand our understanding of OCD, 'contextual anxiety', i.e. anxiety that is specifically related to the context of the obsessive-compulsive thematic content, should be regarded as a hallmark trait of OCD that may provide some insight if appropriately studied. However, since OCD patients overestimate and inflate the actual significance of what they feel would be serious consequences should they not engage in anxiolytic (or neutralising) compulsions (Belloch et al., 2011; Exner et al., 2014), it is mostly unknown whether such anxiety is borne from within, or whether actual external anxiogenic stimuli have an influence on the severity and persistence of their symptom presentation. This question is often indirectly interrogated in sessions of exposure and response prevention (ERP)[1], wherein patients are exposed to the actual content of their obsessions, i.e. contaminated surfaces, but being prevented from engaging in neutralising behavioural routines (Blakey et al., 2019). While such therapy is aimed at gradually attuning patients to the fact that no actual harm befalls them if they do not engage in neutralising routines, the technique itself is associated with significant distress (Bram and Björgvinsson, 2004; McKay, 2006; Ong et al., 2016). Nevertheless, even ERP remains ineffective in a significant number of patients, which begs the question of whether anxiety have a differential effect on obsessive-compulsive symptomology in some individuals, compared to others.

If one considers that OCD generally does not respond to benzodiazepines, but that benzodiazepines are oftentimes used to alleviate acute flares of anxiety in patients with OCD (Starcevic et al., 2016), it remains to be established what role contextual anxiety may play in the etiopathology and manifestation of OCD[2] as it presents in different patients. This question will form a primary focus in this work.

2.2.3 Modelling OCD in the deer mouse

2.2.3.1 General background

To expand our understanding of the etiopathology, neurobiology and phenotypic presentation of psychiatric disorders, animal models are essential. However, the extent to which this knowledge base can be broadened and deepened, is entirely dependent on the accuracy with which a specific animal model can emulate the phenotypic presentation, neurobiological foundation and treatment response (or non-response) of the clinical condition. The validation criteria that define each of these are known

[1] exposure and response prevention
[2] obsessive-compulsive disorder

as face, construct, and predictive validity respectively (Fineberg et al., 2010; van der Staay, 2006; Willner, 1986). However, it is also true that while a specific model might be valid and robust, it is vital that interrogations of such models be applied in a methodologically sound manner which speaks to the research questions asked. In other words, the application of such models should conform to high methodologically valid standards (De Brouwer et al., 2019; de Brouwer and Wolmarans, 2018). This is paramount, since the inappropriate application of laboratory methods without cognisance of its adequacy to deliver accurate answers pertaining to the research question asked, is counterproductive. This is especially important, since animal models of neuropsychiatric conditions are increasingly critiqued for their overall lack of translational contribution to clinical advancements (Stanford, 2020); in our view, such criticism is entirely valid.

Over the past 13 years, much work has been done in our laboratory to develop, characterise, validate and scrutinise the naturalistic, persistent behavioural phenotypes expressed by deer mice (Peromyscus maniculatus bairdii) against the background of compulsive-like behaviour (de Brouwer and Wolmarans, 2018; Güldenpfennig et al., 2011; Korff et al., 2008, 2009; Scheepers et al., 2018; Wolmarans et al., 2013; Wolmarans et al., 2016a, 2016b). Moreover, a significant body of work in the model focused on the development and application of contextually relevant research methodologies to answer the respective questions asked in the most appropriate manner (de Brouwer et al., 2020b). One of these, i.e. the assessment of compulsive-like large nest building by deer mice, will not only be applied here, but will yet again be expanded and applied within a novel context to investigate anxiety as it may associate (or dissociate) with such behaviour.

2.2.3.2 Status of the deer mouse model of OCD

Spontaneous stereotypy, expressed by 45% of the laboratory-housed deer mouse population, was initially investigated as a model of disorders characterised by stereotypical movements (Presti et al., 2002; Presti et al., 2004). However, based on the seemingly purposeless and waxing and waning nature of said movements, i.e. vertical jumping, somersaulting and patterned running, the species became of significant interest as a potential model of compulsive-like persistence (Korff and Harvey, 2006; Korff et al., 2008; Wolmarans et al., 2013). Later, the deer mouse was investigated as a potential model of naturalistic, phenotypically heterogeneous compulsive-like behaviour with the characterisation of large nest building (LNB)[1] behaviour (expressed by 30% of all laboratory-housed deer mice; Wolmarans et al. (2016b)), and high marble burying (HMB)[2], that affects only 11 - 15% of

[1] large nest building
[2] high marble burying

the population (Wolmarans et al., 2016a). Although differing in phenotypical presentation, all three phenotypes develop spontaneously in both sexes by the age of 10 – 12 weeks. They are further equally persistent and repetitive. Whereas stereotypy and LNB respond to chronic (28-day), oral exposure to a high-dose (50mg/kg/day) of the SSRI[1], escitalopram (Wolmarans et al., 2013; Wolmarans et al., 2016b), HMB remains treatment-refractory (de Brouwer and Wolmarans, 2018; Wolmarans et al., 2016a); this despite the fact that such behaviour is just as persistent, repetitive and seemingly purposeless. Moreover, contrary to the current treatment guidelines, HMB responds to a combination of pro-dopaminergic intervention, e.g. the monoamine oxidase B inhibitor, rasagiline, and escitalopram, likely pointing to a unique role for dopamine in the presentation of this phenotype (de Brouwer et al., 2020a).

Being a naturalistic model, deer mice present research with a significant advantage in that the neurodevelopmental origins and trajectory of compulsive-like behavioural phenotypes can be studied. Further, since no external influences are needed to trigger, strengthen, or modulate the aforementioned behaviours, the model lends itself to the study of how specific interventions and manipulations can lead to behavioural adaptation. Being the only truly naturalistic model of OCD[2], the deer mouse can potentially contribute to a unique and well-researched, albeit pre-clinical understanding of the processes that may underlie compulsivity; this would not be possible in pharmacological or genetic models.

Since we will focus on naturalistic LNB[3] and its relationship with contextual anxiety, a detailed review of the model falls beyond the scope of the current literature review. For this, please refer to (Scheepers et al., 2018).

2.2.4 Nest building behaviour – from a life in the wild to cage life

From an evolutionary perspective, nest building is seen as an intrinsic component of the normal behavioural repertoire of many animal species, including fish, birds, and small mammals, e.g. rodents (Deméré et al., 2002). In fact, to build adequate and well-constructed nests is vital for several reasons. In the wild, egg-laying animals and small mammals' nest for protection against severe weather conditions, i.e. direct sunlight, strong air and water currents, to keep safe from predators (Deméré et al., 2002), and to provide a suitable environment for breeding and nursing. Interestingly, nest building behaviour can also be a form of communication, especially in birds where the nesting behaviour of

[1] selective serotonin reuptake inhibitor
[2] obsessive-compulsive disorder
[3] large nest building

males is indicative of life experience, genetic quality, parental ability, virility and overall dominance (Jose et al., 1998). Competition between different species or between different animals of the same species also plays a significant role in the architecture, distribution and overall success of nests as breeding habitats (Beintema and Muskens, 1987; Collias, 1964; Smithers, 1983; Wolff, 1994; Wolton, 1985). Further, environmental and seasonal fluctuation causes animals to adapt their nesting behaviour, since different climates are associated with temporal changes in the availability of various nesting materials or since nesting behaviour itself may be responsive to changes in temperature (Rajendram et al., 1987). However, what remains indisputable is that rather than not building nests, most nesting animals, including rodents, present with an innate motivational drive to nest, irrespective of the circumstances (Guillette et al., 2016; Schaefer, 1976). In other words, nesting behaviour can be understood as a necessary form of coping behaviour, which although innate, is modifiable by circumstantial change.

Against this background, one would expect all laboratory-housed animals of the same species to uniformly express nest building behaviour which could at most differ between sexes (Deacon, 2012; Jirkof, 2014). This would be a valid and logical conclusion, since all intra-species subjects of a laboratory-housed rodent population are bred, reared, and maintained under identical conditions. Interestingly, this is not the case. A robust body of research demonstrated over time that vast inter-individual variance characterises the nest building expression of different animals of the same species (Greene-Schloesser et al., 2011; Hess et al., 2008; Jirkof, 2014; Sherwin, 1997; Wolmarans et al., 2016). Further, larger nesting scores do not cluster based on sex, pointing to the likelihood that instead of being modulated by the factors referred to above as well as other evolutionary influences, nesting behaviour as expressed in the laboratory is subject to manipulation by other psychobiological processes (de Brouwer et al., 2020a). However, an important question should be asked here: What is 'normal'? This question should be considered carefully, since 'abnormal' behaviour is a subjective concept, especially in naturalistic models that do not make use of any behaviour-modifying stimuli. One can argue that against the background of OCD[1], persistence and repetition should be key. For example, if animals engage in a single bout of 'large' nesting behaviour over the course of several repeated assessments, such a subject will likely not be considered to express 'compulsive-like' behaviour. Also, the behaviour should be perceived as being excessive and time-consuming, in that measured against the nesting score distribution of the larger population, compulsive-like behaviour will be characterised by nesting scores that are consistently higher than the average scores generated by the majority of subjects in the study population (de Brouwer et al., 2020b; Wolmarans et al., 2016);

[1] obsessive-compulsive disorder

especially since there is no apparent function for or advantage of larger than 'normal' nests in the laboratory.

From a methodological point of view, such conclusions are drawn only after repetitive testing, usually over seven consecutive days during which mice have access to nesting material for at least 22 hours in every 24-hour cycle (Wolmarans et al., 2016b). This enables researchers to characterise both total weekly nesting scores and the between-day variance in the daily nesting scores generated. Since a detailed description of the methods followed is provided in Chapter 3, it suffices to say that as a starting point, enough animals are selected for initial screening so that a sufficient number (depending on the study design) of LNB[1]-expressing animals (generally 30% of the population) would be identified. Each home cage is then supplied with an excess of pre-weighed cotton wool every day for seven consecutive days. Every subsequent day (at the same time), the remaining cotton wool, i.e. the wool not used for nesting, is weighed, the between-day difference calculated, and the cage supplied with new pre-weighed cotton wool (Wolmarans et al., 2016b). After seven days, the total nesting scores (in grams) are calculated by adding the daily nesting scores and the between-day variances are calculated to identify the LNB[2] compulsive-like phenotype within the normal population (Wolmarans et al., 2016b). Therefore, both parameters are applied to broadly categorise NNB[3] and LNB expressing animals (**Figure 2-2**).

[1] large nest building
[2] large nest building
[3] normal nest building

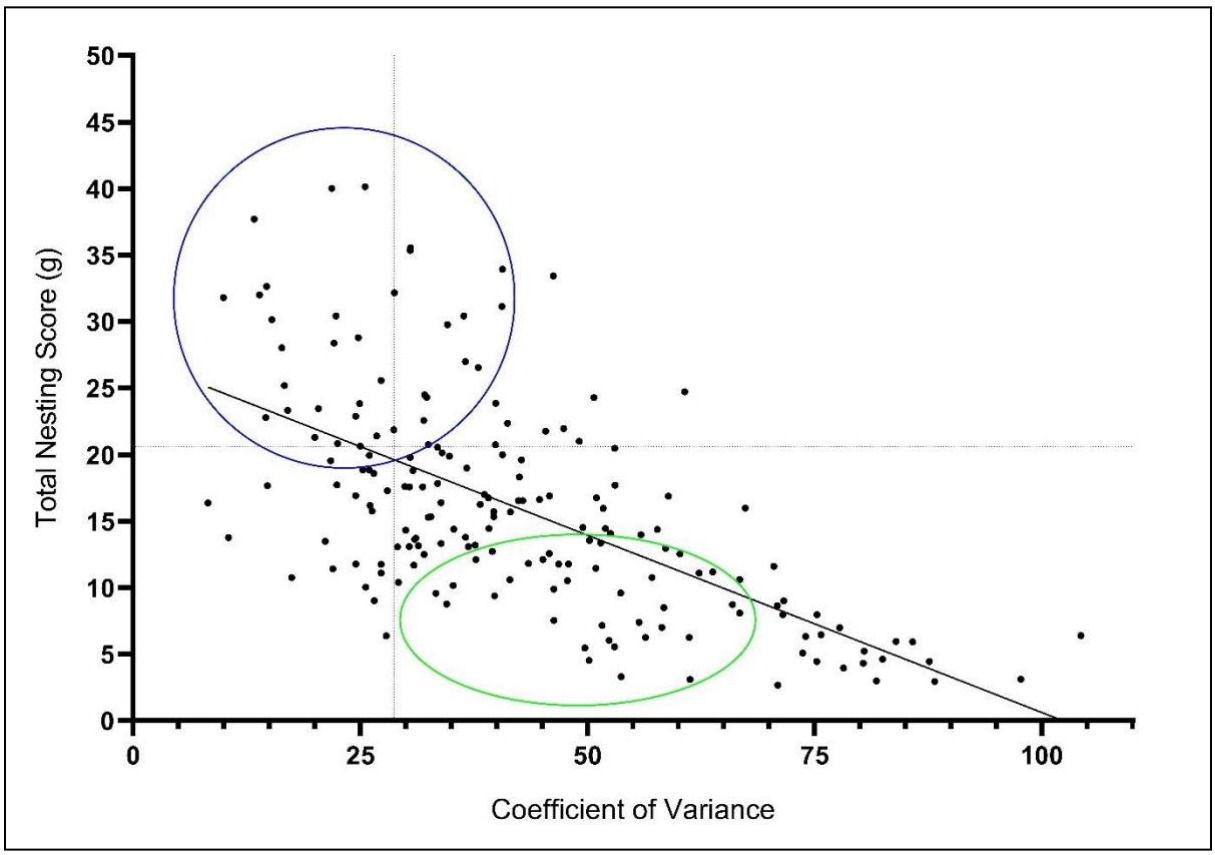

FIGURE 2-2 - *Total nesting scores plotted against the respective coefficients of variance with respect to the daily nesting scores generated by all animals included in this study. Large nesting (LNB) animals enclosed in the blue oval; normal nesting (NNB) animals enclosed in the green oval; For a full description of the methods, statistics and selection procedure, please refer to Chapter 3.*

2.2.4.1 *Interrogating the relationship between anxiety and nest building in deer mice – conceptual background to the current study*

As alluded to above, it is clear that nests as built in the wild are necessary for the survival of individual animals, offspring and a species as a whole (Deméré et al., 2002; Guillette et al., 2016; Schaefer et al., 2014). Further, considering the nature of the aforementioned factors that have an influence on nesting expression, it can be argued that natural nest building behaviour is primarily founded within a safety-driven construct (Clough, 1982; Latham and Mason, 2004). In this investigation, we will interrogate how the nesting behaviour of deer mice adapts to a safety-related modification of the nesting environment, i.e. when assessed in an 'unsafe' and anxiogenic (Kliethermes et al., 2003; Lockie et al., 2017), mirrored (Fuss et al., 2013; Kliethermes et al., 2003; Reddy and Kulkarni, 1997; Walf et al., 2009) and white-floored (Carola et al., 2002; Crawley and Goodwin, 1980; Pich and Samanin, 1989; Smythe et al., 1996) open field. Considering that 30% of deer mice engage in LNB[1]

[1] large nest building

behaviour which is not only persistent and repetitive, but also SSRI[1]-sensitive, we aim to investigate if such behaviour is dissociated from actual context and external goal-orientated feedback. In our view, it is likely that LNB behaviour, which serves no apparent purpose in the laboratory, is not driven by ethologically relevant processes; rather, it may be an artefact of an aberrant underlying psychobiological process. In contrast, we aim to determine whether NNB[2] behaviour can be juxtaposed as a natural and innate process which is subject to contextual and environmental manipulation as assessed under 'unsafe' and anxiogenic circumstances. Since naturalistic nesting behaviour in the wild is likely aimed at ensuring a safe environment, we hypothesise that NNB behaviour may be bolstered in an anxiogenic space.

Studying this hypothesis will be useful for three primary reasons. First, anxiety and OCD[3] interact on several levels and although OCD is overly insensitive to anxiolytic treatment in the long run, anxiolytics do show promise when employed to alleviate acute flares of obsession-related anxiety in patients with OCD (Impey et al., 2020; McGowan, 2020). It will therefore be valuable to determine the extent to which LNB is related to acute anxiety. Second, by differentiating LNB from NNB behaviour on a phenotypical level, important insights can be gained with respect to nesting behaviour in the model organism. The most important of these would be to confirm whether NNB and LNB behaviour represent a 'normal' and an 'abnormal' phenotype, respectively. Also, it would clarify whether LNB behaviour should be applied as a framework in which to investigate different aspects of an anxiety-driven coping response or as an inflexible, but goal-directed and persistent behaviour as we have previously proposed it to be. Last, given the evolutionary function of nesting behaviour, investigations into NNB and LNB behaviour as expressed by deer mice, may bring us closer to a translationally significant model, since it would be possible to test several hypotheses pertaining to the manner in which innate behaviours evolve to become 'abnormal' (Fineberg et al., 2010; Robbins et al., 2012; Robbins et al., 2019).

[1] selective serotonin reuptake inhibitor
[2] normal nest building
[3] obsessive-compulsive disorder

2.3 References

Aardema, F., O'Connor, K., 2007. The menace within: Obsessions and the self. Journal of Cognitive Psychotherapy 21(3), 182-197.

Abramovitch, A., Abramowitz, J.S., Mittelman, A., 2013. The neuropsychology of adult obsessive-compulsive disorder: a meta-analysis. Clin Psychol Rev 33(8), 1163-1171.

Abramovitch, A., Cooperman, A., 2015. The cognitive neuropsychology of obsessive-compulsive disorder: A critical review. Journal of Obsessive-Compulsive and Related Disorders 5, 24-36.

Abramowitz, J.S., 2006. Understanding and Treating Obsessive-Compulsive Disorder. Lawrence Erlbaum Associates, Mahwah, New Jersey.

Abramowitz, J.S., Jacoby, R.J., 2015. Obsessive-compulsive and related disorders: a critical review of the new diagnostic class. Annual review of clinical psychology 11, 165-186.

Abramowitz, J.S., Taylor, S., McKay, D., 2009. Obsessive-compulsive disorder. The Lancet 374(9688), 491-499.

Alptekin, K., Degirmenci, B., Kivircik, B., Durak, H., Yemez, B., Derebek, E., Tunca, Z., 2001. Tc-99m HMPAO brain perfusion SPECT in drug-free obsessive-compulsive patients without depression. Psychiatry Research: Neuroimaging Section 107 Volume 107, 51-56.

Ananth, J., 1986. Clomipramine: an antiobsessive drug. The Canadian Journal of Psychiatry 31(3), 253-258.

APA (American Psychiatric Association)., 2013. Diagnostic and statistical manual of mental disorders (DSM-5®). American Psychiatric Pub.

Bartz, J.A., Hollander, E., 2006. Is obsessive–compulsive disorder an anxiety disorder? Progress in Neuro-Psychopharmacology and Biological Psychiatry 30(3), 338-352.

Beintema, A., Muskens, G., 1987. Nesting success of birds breeding in Dutch agricultural grasslands. Journal of Applied Ecology, 743-758.

Belloch, A., Cabedo, E., Carrió, C., Fernández-Alvarez, H., García, F., Larsson, C., 2011. Group versus individual cognitive treatment for Obsessive-Compulsive Disorder: Changes in non-OCD symptoms and cognitions at post-treatment and one-year follow-up. Psychiatry research 187(1–2), 174-179.

Blakey, S.M., Abramowitz, J.S., Leonard, R.C., Riemann, B.C., 2019. Does Exposure and Response Prevention Behaviorally Activate Patients With Obsessive-Compulsive Disorder? A Preliminary Test. Behavior Therapy 50, 214-224.

Bond, A.J., 1998. Drug-induced behavioural disinhibition. CNS drugs 9(1), 41-57.

Brakoulias, V., Stockings, E., 2019. A systematic review of the use of risperidone, paliperidone and aripiprazole as augmenting agents for obsessive-compulsive disorder. Expert opinion on pharmacotherapy 20(1), 47-53.

Bram, A., Björgvinsson, T., 2004. A psychodynamic clinician's foray into cognitive-behavioral therapy utilizing exposure-response prevention for obsessive-compulsive disorder. American journal of psychotherapy 58(3), 304-320.

Burguiere, E., Monteiro, P., Mallet, L., Feng, G., Graybiel, A.M., 2015. Striatal circuits, habits, and implications for obsessive-compulsive disorder. Curr Opin Neurobiol 30, 59-65.

Carola, V., D'Olimpio, F., Brunamonti, E., Mangia, F., Renzi, P., 2002. Evaluation of the elevated plus-maze and open-field tests for the assessment of anxiety-related behaviour in inbred mice. Behavioural Brain Research 134, 49-57.

Catapano, F., Perris, F., Fabrazzo, M., Cioffi, V., Giacco, D., De Santis, V., Maj, M., 2010. Obsessive–compulsive disorder with poor insight: a three-year prospective study. Progress in Neuro-Psychopharmacology and Biological Psychiatry 34(2), 323-330.

Clough, G., 1982. Environmental effects on animals used in biomedical research. Biological Reviews 57(3), 487-523.

Collias, N.E., 1964. The evolution of nests and nest-building in birds. American Zoologist, 175-190.

Cools, R., Nakamura, K., Daw, N.D., 2011. Serotonin and dopamine: unifying affective, activational, and decision functions. Neuropsychopharmacology 36(1), 98-113.

Crawley, J., Goodwin, F.K., 1980. Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. Pharmacology Biochemistry and Behavior 13(2), 167-170.

Daw, N.D., Kakade, S., Dayan, P., 2002. Opponent interactions between serotonin and dopamine. Neural networks 15(4-6), 603-616.

De Brouwer, G., Fick, A., Harvey, B.H., Wolmarans, D.W., 2019. A critical inquiry into marble-burying as a preclinical screening paradigm of relevance for anxiety and obsessive–compulsive disorder: Mapping the way forward. Cognitive, Affective, & Behavioral Neuroscience 19(1), 1-39.

de Brouwer, G., Fick, A., Lombaard, A., Stein, D.J., Harvey, B.H., Wolmarans, D.W., 2020a. Large nest building and high marble-burying: two compulsive-like phenotypes expressed by deer mice (Peromyscus maniculatus bairdii) and their unique response to serotoninergic and dopamine modulating intervention. Behavioural Brain Research, 112794.

de Brouwer, G., Harvey, B.H., Wolmarans, D.W., 2020b. Naturalistic operant responses in deer mice (Peromyscus maniculatus bairdii) and its response to outcome manipulation and serotonergic intervention. Behavioural Pharmacology 31(4), 343-358.

de Brouwer, G., Wolmarans, D.W., 2018. Back to basics: A methodological perspective on marble-burying behavior as a screening test for psychiatric illness. Behavioural Processes 157, 590-600.

Deacon, R., 2012. Assessing Burrowing, Nest Construction, and Hoarding in Mice. Journal of Visualized Experiments : JoVE(59), 2607.

Del Casale, A., Kotzalidis, G.D., Rapinesi, C., Girardi, P., 2019. Current Psychopharmacology of Obsessive-Compulsive Spectrum Disorders. Current neuropharmacology 17(8), 668.

Dellu-Hagedorn, F., Rivalan, M., Fitoussi, A., De Deurwaerdère, P., 2018. Inter-individual differences in the impulsive/compulsive dimension: deciphering related dopaminergic and serotonergic metabolisms at rest. Philosophical Transactions of the Royal Society B: Biological Sciences 373(1744), 20170154.

Deméré, T., Hollingsworth, B.D., Unitt, P., 2002. Nests and nest-building animals. Field Notes, 13.

Dold, M., Aigner, M., Lanzenberger, R., Kasper, S., 2015. Antipsychotic Augmentation of Serotonin Reuptake Inhibitors in Treatment-Resistant Obsessive-Compulsive Disorder: An Update Meta-Analysis of Double-Blind, Randomized, Placebo-Controlled Trials. Int J Neuropsychopharmacol 18(9).

Ettelt, S., Ruhrmann, S., Barnow, S., Buthz, F., Hochrein, A., Meyer, K., Kraft, S., Reck, C., Pukrop, R., Klosterkötter, J., 2007. Impulsiveness in obsessive–compulsive disorder: results from a family study. Acta Psychiatrica Scandinavica 115(1), 41-47.

Exner, C., Zetsche, U., Lincoln, T.M., Rief, W., 2014. Imminent Danger? Probabilistic classification learning of threat-related information in obsessive-compulsive disorder. Behavior Therapy 45(2), 157-167.

Ferrão, Y.A., Miguel, E., Stein, D.J., 2009. Tourette's syndrome, trichotillomania, and obsessive–compulsive disorder: How closely are they related? Psychiatry research 170(1), 32-42.

Fineberg, N., Chamberlain, S., Hollander, E., Boulougouris, V., Robbins, T., 2011. Translational approaches to obsessive-compulsive disorder: from animal models to clinical treatment. British journal of pharmacology 164(4), 1044-1061.

Fineberg, N.A., Apergis-Schoute, A.M., Vaghi, M.M., Banca, P., Gillan, C.M., Voon, V., Chamberlain, S.R., Cinosi, E., Reid, J., Shahper, S., 2018. Mapping compulsivity in the DSM-5 obsessive compulsive and related disorders: cognitive domains, neural circuitry, and treatment. International Journal of Neuropsychopharmacology 21(1), 42-58.

Fineberg, N.A., Craig, K.J., 2007. Pharmacological treatment for obsessive–compulsive disorder. Anxiety disorders Part 3 of 3 6(6), 234-239.

Fineberg, N.A., Potenza, M.N., Chamberlain, S.R., Berlin, H.A., Menzies, L., Bechara, A., Sahakian, B.J., Robbins, T.W., Bullmore, E.T., Hollander, E., 2010. Probing compulsive and impulsive behaviors, from animal models to endophenotypes: a narrative review. Neuropsychopharmacology 35(3), 591-604.

Fineberg, N.A., Reghunandanan, S., Simpson, H.B., Phillips, K.A., Richter, M.A., Matthews, K., Stein, D.J., Sareen, J., Brown, A., Sookman, D., 2015. Obsessive–compulsive disorder (OCD): Practical strategies for pharmacological and somatic treatment in adults. Psychiatry Research 227(1), 114-125.

Fletcher, P.J., Tampakeras, M., Sinyard, J., Higgins, G.A., 2007. Opposing effects of 5-HT 2A and 5-HT 2C receptor antagonists in the rat and mouse on premature responding in the five-choice serial reaction time test. Psychopharmacology 195(2), 223-234.

Fontenelle, L.F., Mendlowicz, M.V., Versiani, M., 2005. Impulse control disorders in patients with obsessive–compulsive disorder. Psychiatry and clinical neurosciences 59(1), 30-37.

Fontenelle, L.F., Oostermeijer, S., Harrison, B.J., Pantelis, C., Yücel, M., 2011. Obsessive-compulsive disorder, impulse control disorders and drug addiction. Drugs 71(7), 827-840.

Frank, M.J., Claus, E.D., 2006. Anatomy of a decision: striato-orbitofrontal interactions in reinforcement learning, decision making, and reversal. Psychological review 113(2), 300.

Freeston, M.H., Ladouceur, R., 1997. What Do Patients Do With Their Obsessive Thoughts? Behaviour Research and Therapy 35, 335-348.

Frydman, I., Mattos, P., de Oliveira-Souza, R., Yücel, M., Chamberlain, S.R., Moll, J., Fontenelle, L.F., 2020. Self-reported and neurocognitive impulsivity in obsessive-compulsive disorder. Comprehensive psychiatry 97, 152155.

Fuss, J., Richter, S.H., Steinle, J., Deubert, G., Hellweg, R., Gass, P., 2013. Are you real? Visual simulation of social housing by mirror image stimulation in single housed mice. Behav Brain Res 243, 191-198.

Grados, M.A., Atkins, E.B., Kovacikova, G.I., McVicar, E., 2015. A selective review of glutamate pharmacological therapy in obsessive-compulsive and related disorders. Psychol Res Behav Manag 8, 115-131.

Greenberg, E., Grant, J.E., Curley, E.E., Lochner, C., Woods, D.W., Tung, E.S., Stein, D.J., Redden, S.A., Scharf, J.M., Keuthen, N.J., 2017. Predictors of comorbid eating disorders and association with other obsessive-compulsive spectrum disorders in trichotillomania. Comprehensive Psychiatry 78, 1-8.

Greene-Schloesser, D., Van der Zee, E., Sheppard, D., Castillo, M., Gregg, K., Burrow, T., Foltz, H., Slater, M., Bult-Ito, A., 2011. Predictive validity of a non-induced mouse model of compulsive-like behavior. Behavioural brain research 221(1), 55-62.

Groenewegen, H.J., 2003. The basal ganglia and motor control. Neural plasticity 10(1-2), 107-120.

Guillette, L.M., Scott, A.C., Healy, S.D., 2016. Social learning in nest-building birds: a role for familiarity. Proceedings of the Royal Society B: Biological Sciences 283(1827), 20152685.

Güldenpfennig, M., Wolmarans, D.W., Du Preez, J.L., Stein, D.J., Harvey, B.H., 2011. Cortico-striatal oxidative status, dopamine turnover and relation with stereotypy in the deer mouse. Physiology & behavior 103(3-4), 404-411.

Harrison, B.J., Pujol, J., Cardoner, N., Deus, J., Alonso, P., Lopez-Sola, M., Contreras-Rodriguez, O., Real, E., Segalas, C., Blanco-Hinojo, L., Menchon, J.M., Soriano-Mas, C., 2013. Brain corticostriatal systems and the major clinical symptom dimensions of obsessive-compulsive disorder. Biol Psychiatry 73(4), 321-328.

Hennig, J., Netter, P., Munk, A.J., 2020. Interaction between serotonin and dopamine and impulsivity: A gene× gene-interaction approach. Personality and Individual Differences, 110014.

Hess, S.E., Rohr, S., Dufour, B.D., Gaskill, B.N., Pajor, E.A., Garner, J.P., 2008. Home improvement: C57BL/6J mice given more naturalistic nesting materials build better nests. Journal of the American Association for Laboratory Animal Science 47(6), 25-31.

Hesse, S., Muller, U., Lincke, T., Barthel, H., Villmann, T., Angermeyer, M.C., Sabri, O., Stengler-Wenzke, K., 2005. Serotonin and dopamine transporter imaging in patients with obsessive-compulsive disorder. Psychiatry Res 140(1), 63-72.

Higgins, G., Ballard, T., Huwyler, J., Kemp, J., Gill, R., 2003. Evaluation of the NR2B-selective NMDA receptor antagonist Ro 63-1908 on rodent behaviour: evidence for an involvement of NR2B NMDA receptors in response inhibition. Neuropharmacology 44(3), 324-341.

Higgins, G.A., Silenieks, L.B., MacMillan, C., Sevo, J., Zeeb, F.D., Thevarkunnel, S., 2016. Enhanced attention and impulsive action following NMDA receptor GluN2B-selective antagonist pretreatment. Behavioural brain research 311, 1-14.

Impey, B., Gordon, R.P., Baldwin, D.S., 2020. Anxiety disorders, post-traumatic stress disorder, and obsessive–compulsive disorder. Medicine.

Jakubovski, E., Pittenger, C., Torres, A.R., Fontenelle, L.F., do Rosario, M.C., Ferrão, Y.A., de Mathis, M.A., Miguel, E.C., Bloch, M.H., 2011. Dimensional correlates of poor insight in obsessive–compulsive disorder. Progress in Neuro-Psychopharmacology and Biological Psychiatry 35(7), 1677-1681.

Jirkof, P., 2014. Burrowing and nest building behavior as indicators of well-being in mice. Measuring Behavior 234, 139-146.

Jose, J., MØLler, A.P., Soler, M., 1998. Nest building, sexual selection and parental investment. Evolutionary Ecology 12(4), 427-441.

Kashyap, H., Fontenelle, L.F., Miguel, E.C., Ferrão, Y.A., Torres, A.R., Shavitt, R.G., Ferreira-Garcia, R., Do Rosário, M.C., Yücel, M., 2012. 'Impulsive compulsivity'in obsessive-compulsive disorder: A phenotypic marker of patients with poor clinical outcome. Journal of psychiatric research 46(9), 1146-1152.

Kathmann, N., Rupertseder, C., Hauke, W., Zaudig, M., 2005. Implicit sequence learning in obsessive-compulsive disorder: further support for the fronto-striatal dysfunction model. Biol Psychiatry 58(3), 239-244.

Katz, D., Laposa, J.M., Rector, N.A., 2018. Anxiety sensitivity, obsessive beliefs, and the prediction of CBT treatment outcome for OCD. International Journal of Cognitive Therapy 11(1), 31-43.

Katzman, M.A., Bleau, P., Blier, P., Chokka, P., Kjernisted, K., Van Ameringen, M., 2014. Canadian Anxiety Guidelines Initiative Group on behalf of the Anxiety Disorders Association of Canada/Association Canadienne des troubles anxieux and McGill University. Canadian clinical practice guidelines for the management of anxiety, posttraumatic stress and obsessive-compulsive disorders. BMC Psychiatry 14(Suppl 1), S1.

Kliethermes, C.L., Finn, D.A., Crabbe, J.C., 2003. Validation of a modified mirrored chamber sensitive to anxiolytics and anxiogenics in mice. Psychopharmacology (Berl) 169(2), 190-197.

Koo, M.-S., Kim, E.-J., Roh, D., Kim, C.-H., 2010. Role of dopamine in the pathophysiology and treatment of obsessive–compulsive disorder. Expert review of neurotherapeutics 10(2), 275-290.

Korff, S., Harvey, B.H., 2006. Animal models of obsessive-compulsive disorder: rationale to understanding psychobiology and pharmacology. Psychiatr Clin North Am 29(2), 371-390.

Korff, S., Stein, D.J., Harvey, B.H., 2008. Stereotypic behaviour in the deer mouse: pharmacological validation and relevance for obsessive compulsive disorder. Prog Neuropsychopharmacol Biol Psychiatry 32(2), 348-355.

Korff, S., Stein, D.J., Harvey, B.H., 2009. Cortico-striatal cyclic AMP-phosphodiesterase-4 signalling and stereotypy in the deer mouse: attenuation after chronic fluoxetine treatment. Pharmacology Biochemistry and Behavior 92(3), 514-520.

Kranz, G., Kasper, S., Lanzenberger, R., 2010. Reward and the serotonergic system. Neuroscience 166(4), 1023-1035.

Krzyszkowiak, W., Kuleta-Krzyszkowiak, M., Krzanowska, E., 2019. Treatment of obsessive-compulsive disorders (OCD) and obsessive-compulsive-related disorders (OCRD). Psychiatria polska 53(4), 825-843.

Labad, J., Menchon, J.M., Alonso, P., Segalas, C., Jimenez, S., Jaurrieta, N., Leckman, J.F., Vallejo, J., 2008. Gender differences in obsessive-compulsive symptom dimensions. Depression And Anxiety 25(10), 832-838.

Latham, N., Mason, G., 2004. From house mouse to mouse house: the behavioural biology of free-living Mus musculus and its implications in the laboratory. Applied Animal Behaviour Science 86(3-4), 261-289.

Lee, H.J., Kwon, S.M., 2003. Two Different Types Of Obsession: Autogenous Obsessions And Reactive Obsessions. Behaviour Research and Therapy 41, 11-29.

Lockie, S.H., McAuley, C.V., Rawlinson, S., Guiney, N., Andrews, Z.B., 2017. Food seeking in a risky environment: a method for evaluating risk and reward value in food seeking and consumption in mice. Frontiers in neuroscience 11, 24.

Marazziti, D., Golia, F., Consoli, G., Presta, S., Pfanner, C., Carlini, M., Mungai, F., Dell'Osso, M.C., 2008. Effectiveness of long-term augmentation with citalopram to clomipramine in treatment-resistant OCD patients. CNS spectrums 13(11), 971-976.

McGowan, O., 2020. Pharmacogenetics of anxiety disorders. Neuroscience letters 726, 134443.

McKay, D., 2006. Treating disgust reactions in contamination-based obsessive–compulsive disorder. Journal of behavior therapy and experimental psychiatry 37(1), 53-59.

McLean, C.P., Zandberg, L.J., Van Meter, P.E., Carpenter, J.K., Simpson, H.B., Foa, E.B., 2015. Exposure and response prevention helps adults with obsessive compulsive disorder who do not respond to pharmacological augmentation strategies. The Journal of clinical psychiatry 76(12), 1653.

Milad, M.R., Rauch, S.L., 2012. Obsessive-compulsive disorder: beyond segregated cortico-striatal pathways. Trends Cogn Sci 16(1), 43-51.

Mirjana, C., Baviera, M., Invernizzi, R.W., Balducci, C., 2004. The serotonin 5-HT 2A receptors antagonist M100907 prevents impairment in attentional performance by NMDA receptor blockade in the rat prefrontal cortex. Neuropsychopharmacology 29(9), 1637-1647.

Murphy, D.L., Fox, M.A., Timpano, K.R., Moya, P.R., Ren-Patterson, R., Andrews, A.M., Holmes, A., Lesch, K.-P., Wendland, J.R., 2008. How the serotonin story is being rewritten by new gene-based discoveries principally related to SLC6A4, the serotonin transporter gene, which functions to influence all cellular serotonin systems. Neuropharmacology 55(6), 932-960.

Nadeau, J.M., Lewin, A.B., Arnold, E.B., Crawford, E.A., Murphy, T.K., Storch, E.A., 2013. Clinical correlates of functional impairment in children and adolescents with obsessive–compulsive disorder. Journal of Obsessive-Compulsive and Related Disorders 2(4), 432-436.

Nambu, A., 2008. Seven problems on the basal ganglia. Current opinion in neurobiology 18(6), 595-604.

Nestadt, G., Samuels, J., Riddle, M.A., Liang, K.Y., Bienvenu, O.J., Hoehn-Saric, R., Grados, M., Cullen, B., 2001. The relationship between obsessive-compulsive disorder and anxiety and affective disorders: Results from the Johns Hopkins OCD family study. Psychological Medicine 31(3), 481-487.

Nielen, M., Den Boer, J., Smid, H., 2009. Patients with obsessive–compulsive disorder are impaired in associative learning based on external feedback. Psychological Medicine 39(9), 1519-1526.

Nikolaus, S., Antke, C., Beu, M., Müller, H.-W., 2010. Cortical GABA, striatal dopamine and midbrain serotonin as the key players in compulsive and anxiety disorders-results from in vivo imaging studies. Reviews in the Neurosciences 21(2), 119-140.

Olley, A., Malhi, G., Sachdev, P., 2007. Memory and executive functioning in obsessive-compulsive disorder: A selective review. Journal of Affective Disorders 104(1-3), 15-23.

Ong, C.W., Clyde, J.W., Bluett, E.J., Levin, M.E., Twohig, M.P., 2016. Dropout rates in exposure with response prevention for obsessive-compulsive disorder: What do the data really say? Journal of Anxiety Disorders 40, 8-17.

Overduin, M.K., Furnham, A., 2012. Assessing obsessive-compulsive disorder (OCD): A review of self-report measures. Journal of Obsessive-Compulsive and Related Disorders 1(4), 312-324.

Pallanti, S., Hollander, E., Bienstock, C., Koran, L., Leckman, J., Marazziti, D., Pato, M., Stein, D., Zohar, J., 2002. Treatment non-response in OCD: methodological issues and operational definitions. International Journal of Neuropsychopharmacology 5(2), 181-191.

Panes, A., Verdoux, H., Fourrier-Reglat, A., Berdai, D., Pariente, A., Tournier, M., 2020. Misuse of benzodiazepines: Prevalence and impact in an inpatient population with psychiatric disorders. Br J Clin Pharmacol 86(3), 601-610.

Paton, C., 2002. Benzodiazepines and disinhibition: a review. Psychiatric Bulletin 26(12), 460-462.

Pattij, T., Vanderschuren, L.J., 2008. The neuropharmacology of impulsive behaviour. Trends in pharmacological sciences 29(4), 192-199.

Pattij, T., Vanderschuren, L.J., 2020. The Neuropharmacology of Impulsive Behaviour, an Update.

Pauls, D.L., Abramovitch, A., Rauch, S.L., Geller, D.A., 2014. Obsessive–compulsive disorder: an integrative genetic and neurobiological perspective. Nature Reviews Neuroscience 15(6), 410-424.

Philpott, R., 1976. The assessment of drugs in obsessional states. British journal of clinical pharmacology 3(1 Suppl 1), 91.

Piccinelli, M., Pini, S., Bellantuono, C., Wilkinson, G., 1995. Efficacy of drug treatment in obsessive-compulsive disorder. Br J Psychiatry 166, 424-443.

Pich, E.M., Samanin, R., 1989. A two-compartment exploratory model to study anxiolytic/anxiogenic effects of drugs in the rat. Pharmacological research 21(5), 595-602.

Presti, M.F., Powell, S.B., Lewis, M.H., 2002. Dissociation between spontaneously emitted and apomorphine-induced stereotypy in Peromyscus maniculatus bairdii. Physiology & behavior 75(3), 347-353.

Presti, M.F., Watson, C.J., Kennedy, R.T., Yang, M., Lewis, M.H., 2004. Behavior-related alterations of striatal neurochemistry in a mouse model of stereotyped movement disorder. Pharmacology Biochemistry and Behavior 77(3), 501-507.

Raines, A.M., Allan, N.P., Oglesby, M.E., Short, N.A., Schmidt, N.B., 2015. Examination of the relations between obsessive–compulsive symptom dimensions and fear and distress disorder symptoms. Journal of affective disorders 183, 253-257.

Rajendram, E.A., Brain, P.F., Parmigiani, S., Mainardi, M., 1987. Effects of ambient temperature on nest construction in four species of laboratory rodents. Italian Journal of Zoology 54(1), 75-81.

Reddy, D., Kulkarni, S., 1997. Differential anxiolytic effects of neurosteroids in the mirrored chamber behavior test in mice. Brain research 752(1-2), 61-71.

Robbins, T.W., Gillan, C.M., Smith, D.G., de Wit, S., Ersche, K.D., 2012. Neurocognitive endophenotypes of impulsivity and compulsivity: towards dimensional psychiatry. Special Issue: Cognition in Neuropsychiatric Disorders 16(1), 81-91.

Robbins, T.W., Vaghi, M.M., Banca, P., 2019. Obsessive-compulsive disorder: puzzles and prospects. Neuron 102(1), 27-47.

Robinson, L.J., Freeston, M.H., 2014. Emotion and internal experience in obsessive compulsive disorder: reviewing the role of alexithymia, anxiety sensitivity and distress tolerance. Clinical Psychology Review 34(3), 256-271.

Rossi, S., Bartalini, S., Ulivelli, M., Mantovani, A., Di Muro, A., Goracci, A., Castrogiovanni, P., Battistini, N., Passero, S., 2005. Hypofunctioning of sensory gating mechanisms in patients with obsessive-compulsive disorder. Biol Psychiatry 57(1), 16-20.

Schaefer, H.S., Larson, C.L., Davidson, R.J., Coan, J.A., 2014. Brain, body, and cognition: Neural, physiological and self-report correlates of phobic and normative fear. Biological psychology 98, 59-69.

Schaefer, V., 1976. Geographic variation in the placement and structure of oriole nests. The Condor 78(4), 443-448.

Scheepers, I.M., Wolmarans, D.W., Stein, D.J., Harvey, B.H., 2018. Peromyscus maniculatus bairdii as a naturalistic mammalian model of obsessive-compulsive disorder: current status and future challenges. Metabolic brain disease 33(2), 443-455.

Sherwin, C., 1997. Observations on the prevalence of nest-building in non-breeding TO strain mice and their use of two nesting materials. Laboratory Animals 31(2), 125-132.

Simpson, H.B., Slifstein, M., Bender Jr, J., Xu, X., Hackett, E., Maher, M.J., Abi-Dargham, A., 2011. Serotonin 2A receptors in obsessive-compulsive disorder: a positron emission tomography study with [11C] MDL 100907. Biological psychiatry 70(9), 897-904.

Sinopoli, V.M., Burton, C.L., Kronenberg, S., Arnold, P.D., 2017. A review of the role of serotonin system genes in obsessive-compulsive disorder. Neuroscience & Biobehavioral Reviews 80, 372-381.

Smithers, R.H.N., 1983. XXIII. Families CRICETIDAE and MURIDAE, Rats and mice, The Mammals of the Southern-African Subregion. University of Pretoria, Pretoria, South Africa, pp. 220-220 - 296.

Smythe, J.W., Murphy, D., Bhatnagar, S., Timothy, C., Costall, B., 1996. Muscarinic antagonists are anxiogenic in rats tested in the black-white box. Pharmacology Biochemistry and Behavior 54(1), 57-63.

Stanford, S.C., 2020. Some Reasons Why Preclinical Studies of Psychiatric Disorders Fail to Translate: What Can Be Rescued from the Misunderstanding and Misuse of Animal 'Models'? *Alternatives to Laboratory Animals*, 48(3), 106-115.

Starcevic, V., Berle, D., do Rosário, M.C., Brakoulias, V., Ferrão, Y.A., Viswasam, K., Shavitt, R., Miguel, E., Fontenelle, L.F., 2016. Use of benzodiazepines in obsessive–compulsive disorder. International clinical psychopharmacology 31(1), 27-33.

Stocco, A., Lebiere, C., Anderson, J.R., 2010. Conditional routing of information to the cortex: A model of the basal ganglia's role in cognitive coordination. Psychological review 117(2), 541.

Summerfeldt, L.J., Hood, K., Antony, M.M., Richter, M.A., Swinson, R.P., 2004. Impulsivity in obsessive-compulsive disorder: comparisons with other anxiety disorders and within tic-related subgroups. Personality and Individual Differences 36(3), 539-553.

Szechtman, H., Harvey, B.H., Woody, E.Z., Hoffman, K.L., 2020. The Psychopharmacology of Obsessive-Compulsive Disorder: A Preclinical Roadmap. Pharmacol Rev 72(1), 80-151.

Talpos, J.C., Wilkinson, L.S., Robbins, T.W., 2006. A comparison of multiple 5-HT receptors in two tasks measuring impulsivity. Journal of Psychopharmacology 20(1), 47-58.

Tops, M., Russo, S., Boksem, M.A., Tucker, D.M., 2009. Serotonin: modulator of a drive to withdraw. Brain and cognition 71(3), 427-436.

Trevor, A.J., 2017. Sedative-Hypnotic Drugs, in: Katzung, B.G. (Ed.) Basic & Clinical Pharmacology, 14e. McGraw-Hill Education, New York, NY.

van den Heuvel, O.A., Veltman, D.J., Groenewegen, H.J., Cath, D.C., van Balkom, A.J.L.M., van Hartskamp, J., Barkhof, F., van Dyck, R., 2005. Frontal-Striatal Dysfunction During Planning in Obsessive-Compulsive Disorder. Archives of General Psychiatry, 62(3), pp.301-309.

van der Staay, F.J., 2006. Animal models of behavioral dysfunctions: basic concepts and classifications, and an evaluation strategy. Brain Res Rev 52(1), 131-159.

Van Der Wee, N.J., Stevens, H., Hardeman, J.A., Mandl, R.C., Denys, D.A., van Megen, H.J., Kahn, R.S., Westenberg, H.M., 2004. Enhanced dopamine transporter density in psychotropic-naive patients with obsessive-compulsive disorder shown by [123I] þ-CIT SPECT. American Journal of Psychiatry 161(12), 2201-2206.

Walf, A.A., Koonce, C., Manley, K., Frye, C.A., 2009. Proestrous compared to diestrous wildtype, but not estrogen receptor beta knockout, mice have better performance in the spontaneous alternation and object recognition tasks and reduced anxiety-like behavior in the elevated plus and mirror maze. Behav Brain Res 196(2), 254-260.

Westenberg, H.G., Fineberg, N.A., Denys, D., 2007. Neurobiology of obsessive-compulsive disorder: serotonin and beyond. CNS spectrums 12(S3), 14-27.

Willner, P., 1986. Validation criteria for animal models of human mental disorders: learned helplessness as a paradigm case. Progress in neuro-psychopharmacology and biological psychiatry 10(6), 677-690.

Winstanley, C.A., Theobald, D.E., Dalley, J.W., Glennon, J.C., Robbins, T.W., 2004. 5-HT 2A and 5-HT 2C receptor antagonists have opposing effects on a measure of impulsivity: interactions with global 5-HT depletion. Psychopharmacology 176(3-4), 376-385.

Winter, S., Dieckmann, M., Schwabe, K., 2009. Dopamine in the prefrontal cortex regulates rats behavioral flexibility to changing reward value. Behavioural brain research 198(1), 206-213.

Wolff, J.O., 1994. Reproductive success of solitarily and communally nesting white-footed mice and deer mice. Behavioral Ecology 5(2), 206-209.

Wolmarans, D.W., Brand, L., Stein, D.J., Harvey, B.H., 2013. Reappraisal of spontaneous stereotypy in the deer mouse as an animal model of obsessive-compulsive disorder (OCD): Response to escitalopram treatment and basal serotonin transporter (SERT) density. Behavioural Brain Research 256, 545-553.

Wolmarans, D.W., Stein, D.J., Harvey, B.H., 2016. Excessive nest building is a unique behavioural phenotype in the deer mouse model of obsessive-compulsive disorder. Journal of Psychopharmacology 30(9), 867-874.

Wolmarans, D.W., Stein, D.J., Harvey, B.H., 2016a. Of mice and marbles: Novel perspectives on burying behavior as a screening test for psychiatric illness. Cognitive, Affective, & Behavioral Neuroscience 16(3), 551-560.

Wolmarans, D.W., Stein, D.J., Harvey, B.H., 2016b. Excessive nest building is a unique behavioural phenotype in the deer mouse model of obsessive–compulsive disorder. Journal of Psychopharmacology 30(9), 867-874.

Wolton, R., 1985. The ranging and nesting behaviour of Wood mice, Apodemus sylvaticus (Rodentia: Muridae), as revealed by radio-tracking. Journal of Zoology 206(2), 203-222.

Wood, J., LaPalombara, Z., Ahmari, S.E., 2018. Monoamine abnormalities in the SAPAP3 knockout model of obsessive-compulsive disorder-related behaviour. Philosophical Transactions of the Royal Society B: Biological Sciences 373(1742), 20170023.

3 SCIENTIFIC MANUSCRIPT

This article will be submitted to the journal *Progress in Neuro-psychopharmacology and Biological Psychiatry* and was formatted according to the 'Guide for Authors' prescribed by this journal.

***

Guide for the authors:

https://www.elsevier.com/journals/progress-in-neuro-psychopharmacology-and-biological-psychiatry/0278-5846/guide-for-authors

***

Article Title:

Deer mouse nesting behaviour in a novel anxiogenic environment and its response to serotonergic and benzodiazepine intervention: pre-clinical insights into compulsive-like behaviour and risk-taking.

***

Author Contributions:

- *MP* has designed the study under supervision of *DWW*. She performed all the pharmacological and behavioural experiments and wrote the first version and edited versions of this manuscript.
- *DWW* and *BHH* acted as supervisor and co-supervisor of this study, respectively. They conceptualized and designed this work and were involved in every step of this investigation. They revised the first and final versions of this article and the dissertation.
- *DWW* assisted with the experiments, interpretation of the results, writing of the manuscript, and is the corresponding author of this work.
- *GdB* assisted with the experiments, data processing, interpretation of the results and the writing of all versions of the manuscript.
- *DJS* and *SS* assisted with reviewing of the manuscripts, and acted as clinical consultants on this work.

***

Important Information:

- All the co-authors in this article provided consent for assessment as part of the M.Sc. dissertation of Michelle Prinsloo (Addendum A); consent for supervisor and co-supervisor granted by implication.
- As per the author guideline, figures are provided at the end of this manuscript following the reference list.
- As per the author guideline, abbreviations are provided not as footnotes, but as a list following the title page.
- All supplementary data referred to in this manuscript, are included in Addendum B.

***

Title Page:

Deer mouse nesting behaviour in a novel anxiogenic environment and its response to serotonergic and benzodiazepine intervention: pre-clinical insights into compulsive-like behaviour and risk-taking

Michelle Prinsloo[a], Geoffrey de Brouwer[a], Soraya Seedat[b], Dan J Stein[c,d]; Brian H Harvey[a,d], De Wet Wolmarans[a]*

[a]*Center of Excellence for Pharmaceutical Sciences, Faculty of Health Sciences, North West-University, Potchefstroom, South Africa;*

[b]*Department of Psychiatry, Stellenbosch University, Tygerberg, South Africa;*

[c]*Department of Psychiatry and Neuroscience Institute, University of Cape Town, South Africa,*

[d]*MRC Unit on Risk and Resilience in Mental Disorders, Cape Town, South Africa*

*Corresponding author: De Wet Wolmarans, Centre of Excellence for Pharmaceutical Sciences, Research Sub-programme: Translational Neuroscience and Neurotherapeutics, Department of Pharmacology, Faculty of Health Sciences, Building G23, North-West University, 11 Hoffman Street, Potchefstroom, South Africa, 2520.

Email: dewet.wolmarans@nwu.ac.za; **Tel:** +27 (018) 299 2230

Abbreviations:

1) OCD: obsessive-compulsive disorder
2) SRI: serotonin reuptake inhibitor
3) SSRI: selective serotonin reuptake inhibitor
4) LNB: large nest building
5) NNB: normal nest building
6) COV: coefficients of variance
7) 2-way ANOVA: two-way analysis of variance
8) 2-way RM ANOVA: two-way repeated measures analysis of variance

Abstract

Obsessive-compulsive disorder (OCD) is characterized by the excessive performance of specific behaviours which may be related to underlying distress and anxiety. By applying naturalistic large nest building (LNB) behaviour in deer mice *(Peromyscus maniculatus bairdii)*, a validated model of compulsive-like behavioural persistence, we aimed to examine the potential relationship between an anxiety-provoking context and nesting expression. LNB and normal nesting (NNB; as a behavioural control) expressing mice were subdivided into three drug exposure groups per cohort, i.e. normal water (28 days), chronic escitalopram (50 mg/kg/day, 28 days) and sub-acute lorazepam (2 mg/kg/day; 4 days). A fourth group (n = 8 per cohort), only tested for anxiety-like behaviour, also only received water. After treatment, mice were individually placed inside large white-floored and mirror-walled open field arenas which contained a separate dark, enclosed area to allow voluntary exposure to the aversive open space for 4 consecutive nights of testing. Each area contained a cotton wool hopper i.e. internal and external hoppers. Our findings demonstrate that LNB expressed by deer mice is an inflated, but goal-directed behavioural phenotype which remains stable, irrespective of the context in which it is assessed. Further, LNB mice find an open field arena to be less aversive compared to the behaviour of their NNB expressing counterparts, and escitalopram and lorazepam differentially affect the nesting and open field behaviour of NNB and LNB expressing mice.

Keywords

anxiety; deer mouse; escitalopram; lorazepam; nest building; obsessive-compulsive disorder.

3.1 Introduction

Obsessive-compulsive disorder (OCD) is a debilitating psychiatric condition that causes significant distress and impairment (Abramowitz, 2006; Abramowitz et al., 2009; Blakey et al., 2019). With a global lifetime prevalence of nearly 3%, OCD is diagnosed in both men and women and usually manifests by early adolescence or early adulthood (Abramovitch et al., 2013; Labad et al., 2008). OCD is diagnosed based on obsessions (intrusive and unwanted thoughts or ideas) or compulsions (repetitive and persistent behaviours or mental acts). OCD is a phenotypically heterogeneous disorder (Abramowitz, 2006; Blakey et al., 2019) with obsessions and compulsions spanning several dimensions, i.e. contamination/washing, safety/checking, symmetry/ordering, repugnant intrusive thoughts/mental routines and related compulsions (Raines et al., 2015; Rowsell and Francis, 2015).

Although no longer classified as an anxiety disorder (APA, 2013), anxiety is believed to play a major role in obsessive-compulsive symptom manifestation, with stress having a significant role on the extent to which symptoms wax and wane (Abramowitz, 2006; Abramowitz et al., 2009; Hedman et al., 2017; Riesel et al., 2019). Further, OCD demonstrates a significant degree of comorbidity (Brakoulias et al., 2017) and share some overlap in terms of treatment with anxiety disorders (Gordon et al., 2016). Although no definitive precipitant to obsessive-compulsive symptom onset has been described, some evidence points to increased anxiety or stressful experiences playing a potential role (Abramowitz, 2006; Fineberg et al., 2015; Hedman et al., 2017; Szechtman et al., 2020). Further, the relationship between anxiety and OCD is complex (Abramovitch and Cooperman, 2015; Abramowitz, 2006). While compulsive behaviour causes significant distress in most individuals (APA, 2013), inflated anxiety with respect to specific themes can also be associated with compulsive expression (Sassaroli et al., 2015). Compulsions may be viewed as a behavioural coping strategy to reduce the level of distress (Abramowitz et al., 2009; APA, 2013); however, it can also be regarded as a form of neurocognitive disinhibition (Fineberg et al., 2018; Parkes et al., 2019). Indeed, there has been interest in the so-called 'impulsive-compulsive' continuum (Belin-Rauscent et al., 2016; Chamberlain et al., 2016; Mick and Hollander, 2006). As opposed to the proposed risk-mitigating nature of compulsions, impulsivity is seen as unplanned, rapid behavioural responses aimed at gaining a reward without due consideration of the potential risks of expressing such behaviour (Fineberg et al., 2010; Frydman et al., 2020). Thus, although impulsivity and compulsivity are both characterised by inadequate behavioural control, they can be regarded as different dimensions of a continuum of maladaptive behaviours. A study by Kashyap et al., (2012) indicated that obsessive-compulsive and impulsive symptom severity to be positively correlated while another study by Belin et al., (2008) points to impulsivity as a predictor of compulsive symptom manifestation. Animal models of OCD

have not, however, paid a great deal of attention to either stress induced stereotypy, or to the compulsivity-impulsivity continuum.

The first-line pharmacological treatment of OCD typically comprises 8- to 12-week, high dose serotonin reuptake inhibitor (SRI) or selective serotonin reuptake inhibitor (SSRI) intervention, which is successful in 40 – 60% of patients (Dougherty et al., 2018). In treatment refractory cases, a number of approaches can be followed, e.g. increasing the SRI/SSRI dose, switching to a different SSRI, or augmenting SSRI therapy with low-dose neuroleptic (anti-dopaminergic) drugs, e.g. quetiapine, risperidone, olanzapine and haloperidol (Albert et al., 2018; Dold et al., 2015; Fineberg et al., 2015). Considering the proposed neuropsychological association between anxiety and OCD, it is important to note that anxiolytic drugs such as the benzodiazepines, e.g. clonazepam and alprazolam, are not effective for the treatment of the condition (Atmaca, 2016; Robbins et al., 2019). Rather, these and other anxiolytics may be of value to treat comorbid obsessive-compulsive symptoms which present with aberrant motor manifestations, e.g. catatonia (Makhinson et al., 2012), and anxiety (Bandelow, 2008; Hollander et al., 2003). Therefore, the psychobiological association between anxiety and OCD is likely complex and a better understanding may be fruitful in our attempts to improve current treatments.

Animal models are useful to expand our understanding of human psychiatric disorders (Alonso et al., 2015; Anderzhanova et al., 2017), but translational studies regarding OCD remain challenging (Alonso et al., 2015; Eilam et al., 2012). While behaviours that resemble compulsive-like behavioural engagement, i.e. being persistent, repetitive, and excessively directed at goal, are relatively simple to observe and quantify, modelling the affective components of the disorder is challenging (Fineberg et al., 2010). Our laboratory has investigated different naturalistic and persistent behavioural phenotypes expressed by deer mice (Peromyscus maniculatus bairdii) (de Brouwer et al., 2018; De Brouwer et al., 2019; de Brouwer et al., 2020a; De Brouwer et al., 2020b; Güldenpfennig et al., 2011; Korff et al., 2008, 2009; Wolmarans et al., 2013; Wolmarans et al., 2016a, 2016b; Wolmarans et al., 2017). One such behaviour, i.e. large nest building behaviour (LNB; Wolmarans et al. (2016b)), is expressed by 30-35% of deer mice of both sexes by the age of 10 weeks. Nest building is an intrinsic component of the normal behavioural repertoire of many animal species, including mice (Deméré et al., 2002). In fact, to build adequate and well-constructed nests is vital for several reasons, e.g. protection against severe weather conditions and predators (Deméré et al., 2002) and providing a suitable environment for breeding and nursing (Smithers, 1983). Furthermore, nesting behaviour, although innate, is sensitive to external feedback and modifiable by changes in circumstance. Against this background, it could be expected that all laboratory-housed animals of the same species would build nests

uniformly, at most differing between sexes (Deacon, 2012; Jirkof, 2014). This would be a valid conclusion, since all animals of a laboratory-housed rodent species are bred, reared, and maintained under identical conditions. Interestingly, this is not the case. Yet, a robust body of research has demonstrated that the nest-building expression of different animals of the same species, vary significantly (Greene-Schloesser et al., 2011; Hess et al., 2008; Jirkof, 2014; Sherwin, 1997; (Wolmarans et al., 2016b). Further, larger nesting scores normally do not cluster based on sex, pointing to the likelihood that instead of being modulated by the factors referred to above as well as other evolutionary influences, laboratory nesting behaviour may be subject to influences by other psychobiological processes (de Brouwer et al., 2020a). In deer mice, LNB is persistent and repetitive (de Brouwer et al., 2020a; Wolmarans et al., 2016b) and decreases with administration of chronic, high-dose escitalopram, alone or in combination with the low-dose anti-dopaminergic agent, flupentixol (de Brouwer et al., 2020a).

Since rodent nesting behaviour has clear functional starting and ending points (Hoffman and Rueda Morales, 2009), is subject to outcome-feedback manipulation (Lustberg et al., 2020) and that it manifests without external intervention (Wolmarans et al., 2016b), lends it well to the study of how such excessive behaviour may relate to or interact with different psychobiological processes. Here, we aim to study how normal nesting behaviour (NNB) and LNB respectively, respond to changes in a safety-related context, i.e. assessment in a mirrored open field. Specifically, we hypothesise that since LNB is maladaptive, excessive, and inflexible, irrespective of psychobiology, such behaviour would not increase in an anxiogenic context, as opposed to the behaviour of NNB expressing mice. We further hypothesise that LNB, but not NNB expressing mice, would overcome their natural fears of an actual, but entirely avoidable, anxiogenic context, to persist in such behavioural expression. We also expect that should LNB be associated with an elevation in anxiety, such behaviour will respond to both high chronic, high-dose escitalopram and sub-acute lorazepam intervention.

3.2 Materials and methods

3.2.1 Animals

Since only 20 – 30% of deer mice express LNB behaviour (Wolmarans et al., 2013; Wolmarans et al., 2016b), an initial pool of 182 deer mice (*Peromyscus maniculatus bairdii*) of both sexes, aged 10-12 weeks at the onset of experimentation, were included in this investigation. Animals were bred and supplied by the North-West University (NWU) vivarium, Potchefstroom, South Africa (SAVC registration number: FR15/13458; SANAS GLP compliance number: G0019; AAALAC accreditation file:

1717). Experimental procedures were carried out in the same facility (ethical approval number: NWU-00574-19-A5; AnimCare Research Ethics Committee, NWU) and complied with the South African National Standard (SANS) for the Care and Use of Animals for Scientific Purposes (SANS 10386:2008). Since nest building behaviour in deer mice is fully developed by the age of 10 weeks, mice were group-housed in same sex cages [35 cm (l) x 20 cm (w) x 13 (h) cm; Techniplast® S.P.A., Varese, Italy; 4 – 6 animals per cage] until one day prior to the onset of the first baseline nest-building analysis, from which point onwards animals were single housed in identical cages for the remainder of the study. Cages were automatically climate-controlled and kept at an ambient temperature of 23°C with a relative humidity of 50% on a 12-hour light/dark cycle (06:00/18:00). Food and water were provided ad libitum throughout the investigation, while cages were cleaned and fresh corncob supplied weekly between 8:00 and 10:00 (Wolmarans et al., 2016a). Except during periods of nest building experimentation, mice were supplied with paper towel and a small white polyvinyl chloride pipe [10 cm (l); Ø = 4 cm] as a form of environmental enrichment.

3.2.2 Drugs

Escitalopram oxalate (Lundbeck A/S, Denmark; 50 mg/kg/day; (de Brouwer et al., 2018; Wolmarans et al., 2013; Wolmarans et al., 2016a, 2016b)) and lorazepam (Aspen Pharmacare, South Africa; 2 mg/kg/day; (Chesley et al., 1991; Fahey et al., 2006; Hadžiabdic et al., 2012)) were prepared for chronic (28-day) and sub-acute (4-day) oral administration via the drinking water, respectively (see below for dosing regimen). The concentrations of the drugs in solution were calculated based on the average daily fluid consumption of deer mice (0.25 ml/g/day; de Brouwer et al. (2020a); de Brouwer et al. (2020b); Wolmarans de et al. (2013)) to deliver the desired daily dosages. Water intake of drug-exposed mice was measured daily to confirm drug intake (refer supplementary data; Addendum B). Oral drug administration via drinking water, as opposed to intraperitoneal injection or oral gavage, is the preferred administration route in our laboratory given the long (28-day) duration of chronic drug administration in the model and the relative anxiogenic potential of the other administration routes. Following the initial, drug-naïve nest building screen (see paragraph 3.3.3), escitalopram was administered for 28 days before the onset of behavioural testing (see paragraph 3.3.4.2) as well as throughout the 4 experimental days of testing. Lorazepam was only administered during the 4 experimental days of behavioural testing. This dosing regimen was instituted since the therapeutic mechanism of SSRIs require long term administration to reach a therapeutic effect (Goddard et al., 2008), whereas benzodiazepines are favoured for their rapid onset of action, often being used in short-term dosing regimens to manage anxiety (Starcevic, 2014). Since testing was conducted for 4

consecutive nights over the full 12-hour dark cycle, escitalopram and lorazepam were supplied in excess in both the experimental and home cages of mice during this time. Control animals received tap water only for the duration of the study.

3.2.3 Baseline nest-building analysis and behavioural categorisation

To separate mice into the NNB and LNB cohorts, a baseline nesting screen was performed for all the initially included 182 deer mice. Since deer mouse nesting behaviour is characterised by between-day variance (de Brouwer et al., 2020a; Wolmarans et al., 2016), the classification of compulsive-like LNB is based on a 7-day screening procedure. Every day between 10:00 and 12:00, each home cage was supplied with an excess of pre-weighed cotton wool in the roof of the home cage (Wolmarans et al., 2016b). Every subsequent day, between 10:00 and 12:00, the cotton wool that was not utilised was weighed and the daily difference calculated. Built nests were removed and discarded every day. Mice therefore had access to nesting material for at least 22 hours of every day which included the complete dark cycle and first hours after the lights have been switched on (Jirkof, 2014). At the end of the 7-day nest-building screen, the total nesting score in grams for each mouse was calculated by adding together the daily quantities of cotton wool used (Wolmarans et al., 2016b). LNB behaviour was defined as nesting scores that broadly clustered within the upper quartile (75th percentile) of the total nesting score distribution (paragraph 3.4.1.1) and the lower quartile (25th percentile) of distribution with respect to the coefficients of variance calculated based on the daily nesting scores. Conversely, NNB animals were identified as those subjects, which consistently built nests of which the scores broadly varied between the 25th and 50th percentile of the total nesting score distribution, but which varied the least in doing so. The average of the total nesting scores generated by the selected NNB animals was used to determine the quantity of cotton wool to be placed in the dark compartment of the open field (paragraph 3.3.4.2), i.e. $\approx$ 1.5 g. Food and water were available as normal during these 7 days, but no paper towel was supplied. Following the first baseline nest building screen, 38 animals of each nesting cohort were selected and randomly allocated to four different experimental groups. Two groups of each behavioural cohort (n = 10 per group) received either normal water (control) or escitalopram for 28 days prior to the 4 days of post-exposure nesting and open field testing (paragraph 3.3.4). One group of each cohort (n = 10 per group) received lorazepam during the 4 days of nesting and open field testing only. Last, 8 animals of each cohort received only normal water for 28 days, prior to being tested for open field behaviour only. The remaining 106 mice not selected for inclusion in this work, were either used for other related investigations, or euthanized.

3.2.4 Mirrored open field assessment

3.2.4.1 Apparatus

The mirrored open field consisted of an open-roof square box [50 cm (l) x 50 cm (w) x 30 cm (h); **Figure 3-1**] constructed from black Plexiglas®. In one corner of the box, an enclosed, dark chamber [17 cm (l) x 17 cm (w) x 30 cm (l)] was constructed with a 5 cm x 5 cm opening through which mice could gain access (**Figure 3-1**). To evoke anxiety and bolster the aversive character of the open field area, a white Plexiglas® floor and mirrored walls were used. All walls were mirrored, except for the area directly adjacent and opposite to the dark compartment. White floors and mirrors exacerbate the aversive nature of an open-field arena (Carola et al., 2002; Crawley and Goodwin, 1980; Pich and Samanin, 1989; Smythe et al., 1996) and induce avoidance behaviour in mice (Fuss et al., 2013; Kliethermes et al., 2003; Reddy and Kulkarni, 1997; Walf et al., 2009). However, in this case, entry into the open field area was entirely optional, since mice could freely move between the black-floored and dark compartment and the open field. Further, food and water or drug solutions, depending on the group assessed, were provided ad libitum immediately next to the exit of the dark box, i.e. food and water was available without mice having to access the white-floored open field or interacting with mirror reflection (**Figure 3-1**). Two steel mesh nesting material hoppers were also included in each box, one of which was located within the dark compartment, and the other in the opposite corner of the open field arena. In other words, mice had access to some cotton wool in the dark compartment without the need to enter the open field. However, to acquire more wool, animals had to enter and cross the open field. Digital video cameras were mounted above all arenas for the recording of trials and to facilitate automated behavioural tracking.

3.2.4.2 Procedure

From the 29th day of control or escitalopram, and on the first day of lorazepam exposure, all the selected NNB and LNB animals were tested for open field and nesting behaviour for 12 hours over 4 consecutive dark cycles, i.e. each animal was tested four times over consecutive dark cycles. For all groups, except for the two groups assessed in the absence of nesting material, cotton wool was supplied both in the dark compartment (average of 1.5 g per mouse; paragraph 3.3.3) and the open field nesting hoppers (excess; > 6 g). The starting weight of the wool in each of the hoppers were weighed and recorded before each trial. Mice were introduced to the dark compartments every day at 18:00 and left to freely explore the entire arena under dim red light. At 07:00 on each of the subsequent days, animals were returned to their home cages for the remainder of the day, before being returned to the mirrored open field at 18:00. All subjects were euthanized following the fourth

and last trial. Every morning, following trial completion, photographs were taken of the arena (refer supplementary data; Addendum B), built nests discarded, arenas cleaned, and new pre-weighed cotton wool supplied. Since all sessions were video recorded for the entire 12-hour dark cycle, the ambulatory activity could be scored with Ethovision® XT 14 software (Noldus® Information Technologies, Wageningen, The Netherlands). Ambulation in the open field, i.e. time spent (h) and total distance travelled (m) in the arena was quantified. Further, nest locations were recorded and the remaining cotton wool in each of the hoppers weighed. To separate goal-directed retrieval of cotton wool from other forms of voluntary ambulation in the open field, one group of each behavioural cohort was assessed as described above, but in the absence of cotton wool; however, the empty nesting hoppers were still inserted.

3.2.5 Statistical analysis

All statistical analyses were performed with GraphPad® Prism® version 8.4 software (GraphPad®, San Diego, California). A Spearman's rank-order correlation was used to assess the relationship between the individual total nesting scores generated by the 182 initially included deer mice and the coefficients of variance (COV) calculated with respect to the daily nesting scores. Descriptive statistics were applied to broadly determine the 75th percentile of the individual total nesting score distribution and the lower 25th percentile of the COV distribution. These values were used to broadly identify 38 NNB and LNB animals, respectively. Two-way analysis of variance (2-way ANOVA) was applied to analyse the differences between the average total home cage (last 4 days of screening) and 4-day open field nesting scores of control-exposed NNB and LNB animals, as well as differences between the average inside (wool utilised from the hopper within the black compartment), outside (wool utilised from the hopper in the open field) and total nesting scores generated by NNB and LNB animals in the different exposure groups over the 4 days of testing. Differences between the total time spent (h) and distances travelled (m) in the open field were analysed in the same manner. Where data sets were pooled based on interaction results, student's t-tests (or Mann-Whitney tests in the case of non- Gaussian distribution) or ordinary one-way ANOVA (or Kruskal-Wallis tests in the case of non-Gaussian distribution) were performed. Last, to determine whether deer mouse nesting behaviour would adapt as a function of potential habituation to the open field, 2-way repeated measures ANOVA (2-way RM ANOVA) was applied. Nesting and locomotion scores were set as the respective dependent factors, while arena (home cage / open field), drug exposure, and behavioural phenotype were set as independent factors, depending on the analysis. Post-hoc pairwise comparisons between the different groups were performed by means of Tukey's (within phenotype, between exposure comparisons) or

Sidak's (within exposure, between phenotype comparisons) multiple comparisons tests. Statistical significance was set at $p < 0.05$ for all analyses. Cohen's d calculations were used to determine the magnitude of noteworthy differences between relevant groups. Only large effect sizes ($d \leq 0.8$) were shown (Ellis, 2010; Nakagawa and Cuthill, 2007).

3.3 Results

3.3.1 Nesting behaviour

3.3.1.1 Baseline selection of NNB and LNB animals

Spearman's rank-order correlation revealed a significant and strong negative linear relationship between the individual total nesting scores generated by deer mice and the COVs calculated from the individual daily nesting scores [$r(180) = -0.63$; 95CI: -0.71 - -0.52; $p < 0.0001$]. The 38 selected NNB (enclosed oval) and LNB (enclosed circle) animals are represented in **Figure 3-2A**.

3.3.1.2 Baseline (home cage) vs open field nesting behaviour

The average individual total nesting scores generated by NNB and LNB animals within the home cages (last 4 days of the 7-day nest building screen; baseline) and the open field arena are represented in **Figure 3-2B**. Although the nesting scores of NNB mice trended towards increasing in the open field, ($d = 0.87$; **Figure 3-2Bi**), 2-way ANOVA did not reveal a statistically significant interaction between arena and phenotype [$F(1,18) = 0.0035$; $p = 0.95$; **Figure 3-2Bi**]. However, a significant main effect of phenotype was observed [$F(1,18) = 55.96$; $p < 0.0001$]. As such, data for both arenas were pooled per phenotype and a two-tailed Mann-Whitney U-test was performed (**Figure 3-2Bii**). A significant increase in the nesting behaviour of LNB compared to NNB animals was shown (4.53 g vs. 1.71 g; $U = 30$; $p < 0.0001$).

3.3.1.3 Open field nesting scores

The average inside, outside, and total 4-day nesting scores of NNB and LNB animals are represented in **Figures 3-3 A-C**. With respect to the average 4-day inside nesting scores generated by NNB and LNB animals respectively (**Figure 3-3A**), no significant interaction between phenotype and drug exposure was found [$F(2,54) = 2.24$; $p = 0.116$]. Further, neither phenotype nor drug exposure had a significant main effect on the inside nesting scores. Significant two-way interactions between phenotype and exposure were revealed for both the outside [$F(2,54) = 9.86$; $p = 0.0002$; **Figure 3-3B**] and total [$F(2,53) = 10.49$; $p = 0.0001$; **Figure 3-3C**] nesting scores. Also, drug exposure, but not phenotype, had a significant main effect on both outside and total nesting scores [**Figure 3-3B**:

F(2,54) = 6.671; *p* = 0.0026; **Figure 3-3C**: *F*(2,53) = 5.967; *p* = 0.0046]. With respect to outside nesting scores (**Figure 3-3B**), the behaviour of NNB animals was unaffected by drug exposure. However, significant and meaningful reductions were noted in the outside nesting scores of escitalopram (0.86 ± 0.6 g; 95CI: 1.33 – 4.12; *p* < 0.0001; *d* = 2.09) and lorazepam (0.84 ± 0.9 g; 95CI: 1.34 – 4.13; *p* < 0.0001; *d* = 1.89) exposed animals, compared to control exposed LNB animals (3.6 ± 2.0 g). The same pattern of findings was observed for the total nesting scores. Here, the scores of control exposed LNB animals (5.14 ± 2.04 g) were significantly higher than both the escitalopram (2.13 ± 0.67 g; 95CI: 1.52 – 4.5; *p* < 0.0001; *d* = 2.22) and lorazepam exposed
(2.2 ± 1.1 g; 95CI: 1.46 – 4.44; *p* < 0.0001; *d* = 1.85) LNB animals.

3.3.1.4 Time-based adaptation in open field nesting scores

The average total nesting scores generated by control exposed NNB and LNB animals in the open field over the four respective dark cycles, are represented in **Figure 3-4**. 2-way RM ANOVA did not reveal an interaction between time (trial) and phenotype [*F*(3.54) = 1.46; *p* = 0.24]. Further, a significant main effect of phenotype [*F*(1,18) = 13.56; *p* = 0.0017], but not time [*F*(2.3, 40.7) = 0.14; *p* = 0.89), was observed.

3.3.2 Open field behaviour

3.3.2.1 Open field behaviour of NNB and LNB animals with and without access to cotton wool

The open field behaviour of control exposed NNB and LNB animals in arenas with (control; Ctrl) and without cotton wool (no wool control; NW Ctrl), is represented in **Figure 3-5**. 2-way ANOVA revealed statistically significant interactions between arena setup and phenotype for both the total time spent [*F*(1,31) = 13.34; *p* = 0.0009; **Figure 3-5A**] and distance travelled [*F*(1,31) = 23.19; *p* < 0.0001; **Figure 3-5B**] in the open field. Also, significant main effects of arena setup (**Figure 3-5A**: *F*(1,31) = 4.83; *p* = 0.036; **Figure 3-5B**: *F*(1,31) = 7.87; *p* = 0.009) and phenotype (**Figure 3-5A**: *F*(1,31) = 6.89; *p* = 0.013; **Figure 3-5B**: *F*(1,31) = 4.72; p = 0.038) were observed. Post-hoc comparisons revealed significant decreases in both the time spent in the open field (**Figure 3-5A**: 4.5 ± 1.7 h vs. 2.1 ± 0.98 h; 95CI: 0.98 — 3.67; *p* = 0.0006; *d* = 1.74) as well as the total distance travelled (**Figure 3-5B**: 1559.4 ± 906.3 m vs. 244.7 ± 120.6 m; 95CI: 730.6 — 1899.0; *p* < 0.0001; *d* = 2.56) by LNB, but not
NNB animals as assessed in the no-wool and wool-containing arenas, respectively. Regarding the no wool setup, LNB mice spent significantly more time (**Figure 3-5A**: 1.96 ± 0.8 h vs. 4.5± 1.7 h; 95CI: -3.91 — -1.08; *p* = 0.0005) and travelled significantly further inside the open field space (**Figure 3-5B**:

1559.4 ± 906.3 m vs. 354.1 ± 197.8 m; 95CI: -1819.0 — -591.9; $p < 0.0001$), than NNB mice assessed in the same manner.

3.3.2.2 Open-field behaviour of control- and drug-exposed NNB and LNB animals with access to cotton wool

No significant interaction between drug exposure and phenotype was observed with respect to the time spent in the open field by NNB and LNB animals respectively [$F(2,51) = 0.713$; $p = 0.495$], but a significant main effect of drug-exposure [$F(2,51) = 10.16$; $p = 0.0002$] was noted (**Figure 3-6A**). As such, data across both phenotypes were pooled for each exposure group and analysed by means of ordinary one-way ANOVA [$F(2.55) = 8.76$; $p = 0.0005$]. Tukey's multiple comparisons post-hoc test revealed statistically significant reduction in the time spent in the open field by escitalopram exposed (1.2 ± 0.55 h) vs. control exposed (2.3 ± 1.1 h; 95CI: 0.48 – 1.79; $p = 0.0003$; $d = 1.40$; **Figure 3-6A**) and lorazepam exposed mice (1.9 ± 0.8 h; 95CI: -1.34 – -0.28; $p = 0.039$; $d = 1.02$; **Figure 3-6A**).

With respect to the total distance travelled (**Figure 3-6B**), a statistically significant interaction between drug exposure and phenotype was seen [$F(2,51) = 6.109$; $p = 0.0042$]. Further, significant main effects of drug exposure [$F(2,51) = 5.793$; $p = 0.0054$] and phenotype [$F(2,51) = 4.852$; $p = 0.0322$] were observed. Post-hoc comparisons revealed that control exposed NNB animals assessed in the presence of cotton wool, travelled significantly further in the open field than their LNB counterparts (700.7 ± 532.6 m vs. 211.9 ± 65.15 m; 95CI: 196.3 — 781.3; $p = 0.0004$; $d = 1.64$). Significant reductions were also observed between the distances travelled by control-exposed NNB mice (700.7 ± 532.6 m) and NNB mice exposed to escitalopram (170.2 ± 41.7 m; 95CI: 252.1 — 808.9; $p < 0.0001$; $d = 1.85$) and lorazepam (372.8 ± 192.95 m; 95CI: 41.9 — 613.9; $p = 0.021$, $d = 0.90$).

3.4 Discussion

The main findings of this work are that 1) LNB expressed by deer mice is an inflated, but goal-directed behavioural phenotype which remains stable, irrespective of the context in which it is assessed, 2) LNB mice find an open field arena to be less aversive compared to the behaviour of their NNB expressing counterparts, and 3) escitalopram and lorazepam differentially affect the nesting and open field behaviour of NNB and LNB expressing mice.

OCD is characterised by intrusive thoughts and repetitive behavioural symptoms that cause significant distress and discomfort and interfere with the normal day-to-day functioning of patients (APA, 2013). Compulsive symptoms, e.g. excessive washing or checking, are purposeless in terms of functional outcome, since it is expressed in response to implicit and inflated concerns about the potential, but

unrealistic negative consequences that may arise from failure to engage in neutralising rituals (Abramowitz, 2006; Fineberg et al., 2015; Szechtman et al., 2020). While most patients suffering from OCD experience some degree of distress, compulsivity that occurs in the absence of an underlying obsession or feelings of distress, is also sufficient for a clinical diagnosis. With respect to the current investigation, it has been shown that some OCD patients demonstrate increased vigilance and attentional bias towards every day scenarios that align with the thematic content of their symptoms (Chamberlain et al., 2005; Exner et al., 2014; Wang and Zhanjiang, 2017). These findings point to a potential role for implicit, but context-related anxiety in the manifestation of compulsivity (Hinds et al., 2012). However, that anxiolytic pharmacotherapy is generally ineffective for the long-term management of the condition, highlights a need to better understand the underlying relationships between context-related anxiogenic manipulation and the expression of compulsive behaviour (Nadeau et al., 2013; Nestadt et al., 2001; Raines et al., 2015). This may be of particular value to interpret certain obsessive-compulsive phenotypes where the symptoms are aimed at avoiding outcomes which are believed to be potentially harmful, e.g. in safety/checking or contamination/washing OCD (Melli et al., 2015).

Deer mouse behaviour is representative of naturalistic, phenotypically heterogeneous persistent behavioural expression that resembles compulsive symptomology (Scheepers et al., 2018). Here, we applied NNB and LNB to investigate the potential relationship between the expression of nesting behaviour and safety-related anxiety under circumstances of contextual modification, i.e. assessment in an anxiogenic open-field. We further set out to determine whether NNB and LNB expressing mice would differ in terms of their open field behaviour. Last we aimed to establish how the nesting and open field behaviour or NNB and LNB expressing animals would respond to the anti-compulsive and anxiolytic drug, escitalopram, and the anxiolytic, lorazepam.

3.4.1 Nesting and open field behaviour of control exposed NNB and LNB expressing animals

With respect to the home cage and open field nesting scores, we demonstrate that NNB and LNB is stable and innate, irrespective of the testing environment (**Figures 3-2Bi**; **3-3, 3-4**). Although the behaviour of NNB animals showed a greater degree of adaptation in the open field, the testing arena did not have a significant impact on the nesting scores generated. This finding highlights some noteworthy aspects of deer mouse nesting behaviour. LNB expressing animals exhibit a strong, inherent drive to express nesting, irrespective of the context in which it is measured. Therefore, it can be concluded that LNB is excessive, persistent, and seemingly purposeless in terms of functional outcome. This also tends to be true for NNB expressing mice, which although trending towards

generating higher nesting scores in the open field, maintained nest building behaviour at a level akin to which was found in the home cage. While we predicted the reported open field nesting behaviour of LNB animals, we hypothesised that NNB, representing a normal behavioural phenotype, would adapt based on external anxiogenic feedback. This hypothesis seemed not to hold true. However, although it might be possible that NNB in deer mice is not subject to safety-related contexts as has been shown in other species before (Deméré et al., 2002; Guillette et al., 2016; Schaefer, 1976), this conclusion cannot be made without considering the testing procedure. Since mice were supplied with sufficient quantities of cotton wool to build nests of average 'normal' size in the dark compartment of the open field (**Figure 3-2A**), NNB mice seemed to be sensitive to the well-known aversion carried by an open space (Webster et al., 1979) and refrained from entering the open field to gain access to more nesting material. This is supported by the fact that NNB mice tested in the absence of cotton wool spent significantly less time and travelled shorter distances in the open field, compared to their LNB counterparts (**Figure 3-5**). Further, that both cohorts consistently utilised all of the cotton wool in the internal hopper (**Figure 3-3A**), but that LNB animals continued to retrieve more wool from the external hopper, suggests that LNB animals present with an inflated motivational drive to engage in nesting behaviour, irrespective of the potential negative outcomes of such behaviour. These data are congruent with our previous findings, showing that LNB expressing animals will endure more voluntary foot-shocks to gain access to nesting material (de Brouwer et al., 2020b). While it is true that excessive behaviours displayed by laboratory-housed rodents may result from non-stimulating home cage environments (Bechard et al., 2016; Powell et al., 1999; Van de Weerd et al., 1997), this does not appear to be the case for LNB expressing mice, as the expression of such behaviour remained unaffected by alterations in the testing environment applied here.

Extending previous work, our data were informative with respect to the open field behaviour of deer mice. As alluded to earlier, LNB expressing mice (selected based on home cage nesting scores) which were assessed for open field behaviour in the absence of cotton wool, spent significantly more time and travelled longer distances in the open field compared to their NNB expressing counterparts assessed in the same manner (**Figure 3-5**). This finding highlights two important aspects. First, and as alluded to earlier, the white-floored and mirrored open field (Carola et al., 2002; Crawley and Goodwin, 1980; Fuss et al., 2013; Kliethermes et al., 2003; Lockie et al., 2017; Pich and Samanin, 1989; Reddy and Kulkarni, 1997; Smythe et al., 1996; Walf et al., 2009) was found more aversive by NNB expressing animals, which spent more time in the dark compartment, compared to LNB expressing animals. Second, when assessed in a novel anxiogenic space, LNB expressing deer mice are more likely to explore an environment associated with potential danger. While this difference

could also be explained by possible differences in the levels of underlying anxiety between LNB and NNB mice, there may be a different explanation. Once the open field behaviour of LNB animals was assessed in the presence of cotton wool, these animals spent less time and travelled shorter distances in the open field (**Figure 3-5**). This drastic change would normally be interpreted as an anxiogenic response. However, considering that provision of nesting material is consistently shown to reduce housing stress in home cages (Deacon, 2012; Hess et al., 2008; Jirkof, 2014; Van de Weerd et al., 1997) and that the open field behaviour of NNB expressing animals tested with nesting material remained unaltered, is in disagreement with such a conclusion. Rather, we propose that LNB expressing mice could exhibit impulsive risk-taking behaviour. This notion is supported by our previous aforementioned findings regarding LNB mice being overly insensitive to punishing outcomes (de Brouwer et al., 2020b), but also by the fact that the provision of nesting material attenuated the expression of such excessive open field behaviour. Impulsivity can be regarded as 'giving in to urges', and is characterised by unplanned, rapid reactions to internal or external stimuli without considering the potential negative consequences of such engagement (Fineberg et al., 2010; Frydman et al., 2020). Impulsive features are often reported in OCD (Abramovitch and McKay, 2016; Boisseau et al., 2012; Ettelt et al., 2007; Kashyap et al., 2012) and have been implicated as a predictor of compulsive symptom manifestation (Belin et al., 2008). This points to some degree of psychobiological overlap between the two traits (Fontenelle et al., 2011). In this regard, our finding showing reduced open field ambulation in LNB, but not NNB expressing animals following the introduction of nesting material to the arena (**Figure 3-5**), is noteworthy. Here, we show that by the simple introduction of nesting material to the open field arena, the behaviour of LNB animals becomes focused and directed at obtaining cotton wool. This is evinced in LNB expressing subjects generating significantly higher nesting scores during these trials (**Figure 3-3C**), compared to NNB expressing animals, although both cohorts spent equal time in the open field (**Figure 3-5A**). Clinical data explaining the temporal relationship between impulsive and compulsive symptom expression are scant. However, recent research pointed to positive correlations between impulsive and compulsive symptom severity (Chamberlain et al., 2018) which may inform the differences in the nesting and open field behaviour of NNB and LNB expressing animals, reported here. Nevertheless, further research is required to elaborate on this finding. Last, our data must also be considered against the broad background of animal behaviour, as animal species differ in terms of exploration and avoidance of novel and potentially anxiogenic spaces (Carter et al., 2013; Walsh and Cummins, 1976). Given that both NNB and LNB animals explored the open field, albeit to different degrees, it is likely that deer mice are not overly anxious. However, that NNB expressing animals showed increased aversion to the open field,

supportis our perspective that LNB expressing mice are less sensitive to such a context and more prone to voluntary risk-taking.

3.4.2 The effects of drug treatment on nesting expression and open field activity

Last, we aimed to discern between the effects of general anxiety and compulsive-like nesting behaviour on open field ambulation by means of pharmacological intervention. Specifically, chronic high dose intervention with the known anxiolytic and anti-compulsive agent, escitalopram and sub-acute intervention with the anxio-selective benzodiazepine, lorazepam, was used to determine how the nesting and open field behaviour of NNB and LNB expressing animals would respond. In this regard, two important observations were made. First, both interventions markedly attenuated the open field nesting scores of LNB, but not NNB expressing animals (**Figure 3-3C**). Second, escitalopram, but not lorazepam reduced the time spent in the open field by animals of both cohorts (**Figure 3-6A**), while both escitalopram and lorazepam reduced the total distance travelled by NNB expressing animals in the open field (**Figure 3-6B**). While the latter finding may seem counterintuitive, it is important to note that this result can potentially be attributed to increased pre-exposure stereotypical running activity by NNB mice, a behaviour which is shown by some deer mice (Presti et al., 2002) and which may be a potential artefact of underlying anxiety (Péter et al., 2017) in laboratory housed rodents. The resulting effect of drug treatment on the distance travelled by NNB expressing animals may therefore likely be attributable to the anxiolytic effects of both drugs. We considered that motor inhibition could have played a role in this result; however, the lack of change in the distances travelled by LNB mice, refutes such a conclusion. In fact, in the LNB expressing cohort, escitalopram, but not lorazepam, reduced the time spent in the open field (**Figure 3-6A**), while simultaneously having a marked attenuating influence on the nesting expression. In comparison, lorazepam attenuated the nesting scores without altering the open field behaviour, compared to control-exposed animals. While our data with respect to the effect of escitalopram on nest building expression agree with previous reports (De Brouwer et al., 2020b; Wolmarans et al., 2016b), the effect of lorazepam needs further elucidation. Although lorazepam seemed to have affected excessive nesting behaviour per se, it also maintained the open field behaviour of LNB expressing animals at levels akin to that displayed by control-exposed animals. However, in contrast to control-exposed LNB expressing animals that engaged in large nesting behaviour while ambulating in the open field, the lorazepam-exposed LNB animals did not engage to the same extent in nesting (**Figure 3-3C**). One explanation for this would be supportive of the idea that LNB animals may present with an underlying impulsive trait. Lorazepam has previously been shown to cause behavioural disinhibition in some

individuals (Bond, 1998; Panes et al., 2020; Paton, 2002) and impulsive task-engagement in others (Loring et al., 2012). Conversely, SSRIs are moderately effective for the treatment of compulsivity, and have shown some promise in the treatment of impulsivity (Hollander and Rosen, 2000; Silveira et al., 2020; Wolff and Leander, 2002). Collectively, the treatment data presented here point to potential underlying psychobiological differences that typify NNB and LNB behaviour and while changes in the behaviour of NNB expressing animals might align more with the modification of naturalistic anxiety, adaptations in the nesting and open-field behaviour of LNB animals, are likely related to modification of processes which might play a role in the impulsive-compulsive continuum. Further, that NNB remained entirely unresponsive to drug interventions which had marked effects on their open field activity, supports our conclusion that NNB, other than LNB, is normal, inherent and devoid of psychobiological interference.

3.5 Conclusion

The present work indicates that NNB and LNB are stable, innate behavioural phenotypes that are displayed, irrespective of the context in which it is assessed. We further demonstrate that LNB expressing animals find an anxiogenic open field environment less aversive than NNB expressing animals, a finding which might point to an underlying impulsive trait in these animals. Last, we show here that escitalopram and lorazepam differentially affect nesting and open field behaviour in NNB and LNB expressing deer mice, indicating that these phenotypes might be associated with differences in underlying psychobiology. In conclusion, this work is informative with respect to the nature and manifestation of excessive and persistent large nest building in deer mice. Continued studies of naturalistic NNB and LNB behaviour in this species might be useful for our understanding of behavioural manifestations and interactions relating to compulsive, impulsive and anxious behavioural phenotypes.

Funding and Disclosure

The present work was jointly funded by institutional research funding awarded to DWW and BHH and grants awarded to BHH by the Medical Research Council of South Africa.

CRediT Authorship Contribution Statement

MP: Methodology, Investigation, Writing – Original Draft; GdB: Investigation, Writing – Original Draft, Writing – Review & Editing; SS: Supervision, Writing – Review & Editing; DJS: Writing – Review & Editing; BHH: Supervision, Writing – Review & Editing, Funding Acquisition; DWW: Conceptualization, Methodology, Validation, Investigation, Formal analysis, Supervision, Resources, Writing – Original Draft, Writing – Review & Editing, Funding Acquisition.

3.6 References

Abramovitch, A., Abramowitz, J.S., Mittelman, A., 2013. The neuropsychology of adult obsessive–compulsive disorder: a meta-analysis. Clinical psychology review 33(8), 1163-1171.

Abramovitch, A., Cooperman, A., 2015. The cognitive neuropsychology of obsessive-compulsive disorder: A critical review. Journal of Obsessive-Compulsive and Related Disorders 5, 24-36.

Abramovitch, A., McKay, D., 2016. Behavioral impulsivity in obsessive–compulsive disorder. Journal of behavioral addictions 5(3), 395-397.

Abramowitz, J.S., 2006. Understanding and Treating Obsessive-Compulsive Disorder. Lawrence Erlbaum Associates, Mahwah, New Jersey.

Abramowitz, J.S., Taylor, S., McKay, D., 2009. Obsessive-compulsive disorder. The Lancet 374(9688), 491-499.

Albert, U., Marazziti, D., Di Salvo, G., Solia, F., Rosso, G., Maina, G., 2018. A systematic review of evidence-based treatment strategies for obsessive-compulsive disorder resistant to first-line pharmacotherapy. Current medicinal chemistry 25(41), 5647-5661.

Alonso, P., Lopez-Sola, C., Real, E., Segalas, C., Menchon, J.M., 2015. Animal models of obsessive-compulsive disorder: utility and limitations. Neuropsychiatric Disease and Treatment 11, 1939-1955.

Anderzhanova, E., Kirmeier, T., Wotjak, C.T., 2017. Animal models in psychiatric research: The RDoC system as a new framework for endophenotype-oriented translational neuroscience. Neurobiology of stress 7, 47-56.

APA (American Psychiatric Association), 2013. Diagnostic and statistical manual of mental disorders (DSM-5®). American Psychiatric Pub.

Atmaca, M., 2016. Treatment-refractory obsessive compulsive disorder. Progress in Neuro-Psychopharmacology and Biological Psychiatry 70, 127-133.

Bandelow, B., 2008. The medical treatment of obsessive-compulsive disorder and anxiety. CNS spectrums 13(S14), 37-46.

Bechard, A.R., Cacodcar, N., King, M.A., Lewis, M.H., 2016. How does environmental enrichment reduce repetitive motor behaviors? Neuronal activation and dendritic morphology in the indirect basal ganglia pathway of a mouse model. Behavioural brain research 299, 122-131.

Belin-Rauscent, A., Daniel, M., Puaud, M., Jupp, B., Sawiak, S., Howett, D., McKenzie, C., Caprioli, D., Besson, M., Robbins, T., 2016. From impulses to maladaptive actions: the insula is a neurobiological gate for the development of compulsive behavior. Molecular psychiatry 21(4), 491-499.

Belin, D., Mar, A.C., Dalley, J.W., Robbins, T.W., Everitt, B.J., 2008. High impulsivity predicts the switch to compulsive cocaine-taking. Science 320(5881), 1352-1355.

Blakey, S.M., Abramowitz, J.S., Leonard, R.C., Riemann, B.C., 2019. Does Exposure and Response Prevention Behaviorally Activate Patients With Obsessive-Compulsive Disorder? A Preliminary Test. Behavior Therapy 50, 214-224.

Boisseau, C.L., Thompson-Brenner, H., Caldwell-Harris, C., Pratt, E., Farchione, T., Barlow, D.H., 2012. Behavioral and cognitive impulsivity in obsessive–compulsive disorder and eating disorders. Psychiatry research 200(2-3), 1062-1066.

Bond, A.J., 1998. Drug-induced behavioural disinhibition. CNS drugs 9(1), 41-57.

Brakoulias, V., Starcevic, V., Belloch, A., Brown, C., Ferrao, Y.A., Fontenelle, L.F., Lochner, C., Marazziti, D., Matsunaga, H., Miguel, E.C., Reddy, Y.C.J., do Rosario, M.C., Shavitt, R.G., Shyam Sundar, A., Stein, D.J., Torres, A.R., Viswasam, K., 2017. Comorbidity, age of onset and suicidality in obsessive–compulsive disorder (OCD): An international collaboration. Comprehensive Psychiatry 76, 79-86.

Carola, V., D'Olimpio, F., Brunamonti, E., Mangia, F., Renzi, P., 2002. Evaluation of the elevated plus-maze and open-field tests for the assessment of anxiety-related behaviour in inbred mice. Behavioural Brain Research 134, 49-57.

Carter, A.J., Feeney, W.E., Marshall, H.H., Cowlishaw, G., Heinsohn, R., 2013. Animal personality: what are behavioural ecologists measuring? Biological Reviews 88(2), 465-475.

Chamberlain, S.R., Blackwell, A.D., Fineberg, N.A., Robbins, T.W., Sahakian, B.J., 2005. The neuropsychology of obsessive compulsive disorder: the importance of failures in cognitive and

behavioural inhibition as candidate endophenotypic markers. Neuroscience & Biobehavioral Reviews 29(3), 399-419.

Chamberlain, S.R., Leppink, E.W., Redden, S.A., Grant, J.E., 2016. Are obsessive–compulsive symptoms impulsive, compulsive or both? Comprehensive psychiatry 68, 111-118.

Chamberlain, S.R., Stochl, J., Redden, S.A., Grant, J.E., 2018. Latent traits of impulsivity and compulsivity: toward dimensional psychiatry. Psychological medicine 48(5), 810-821.

Chesley, S., Lumpkin, M., Schatzki, A., Galpern, W., Greenblatt, D., Shader, R.I., Miller, L., 1991. Prenatal exposure to benzodiazepine—I. Prenatal exposure to lorazepam in mice alters open-field activity and GABAA receptor function. Neuropharmacology 30(1), 53-58.

Crawley, J., Goodwin, F.K., 1980. Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. Pharmacology Biochemistry and Behavior 13(2), 167-170.

de Brouwer, G., Fick, A., Harvey, B.H., Wolmarans, D.W., 2018. A critical inquiry into marble-burying as a preclinical screening paradigm of relevance for anxiety and obsessive–compulsive disorder: Mapping the way forward. Cognitive, Affective, & Behavioral Neuroscience 19(1), 1-39.

De Brouwer, G., Fick, A., Harvey, B.H., Wolmarans, D.W., 2019. A critical inquiry into marble-burying as a preclinical screening paradigm of relevance for anxiety and obsessive–compulsive disorder: Mapping the way forward. Cognitive, Affective, & Behavioral Neuroscience 19(1), 1-39.

de Brouwer, G., Fick, A., Lombaard, A., Stein, D.J., Harvey, B.H., Wolmarans, D.W., 2020a. Large nest building and high marble-burying: two compulsive-like phenotypes expressed by deer mice (Peromyscus maniculatus bairdii) and their unique response to serotoninergic and dopamine modulating intervention. Behavioural Brain Research, 112794.

de Brouwer, G., Harvey, B.H., Wolmarans, D.W., 2020b. Naturalistic operant responses in deer mice (Peromyscus maniculatus bairdii) and its response to outcome manipulation and serotonergic intervention. Behavioural Pharmacology 31(4), 343-358.

Deacon, R., 2012. Assessing Burrowing, Nest Construction, and Hoarding in Mice. Journal of Visualized Experiments : JoVE(59), 2607.

Deméré, T., Hollingsworth, B.D., Unitt, P., 2002. Nests and nest-building animals. Field Notes, 13.

Dold, M., Aigner, M., Lanzenberger, R., Kasper, S., 2015. Antipsychotic Augmentation of Serotonin Reuptake Inhibitors in Treatment-Resistant Obsessive-Compulsive Disorder: An Update Meta-Analysis of Double-Blind, Randomized, Placebo-Controlled Trials. Int J Neuropsychopharmacol 18(9).

Dougherty, D.D., Brennan, B.P., Stewart, S.E., Wilhelm, S., Widge, A.S., Rauch, S.L., 2018. Neuroscientifically informed formulation and treatment planning for patients with obsessive-compulsive disorder: a review. JAMA psychiatry 75(10), 1081-1087.

Eilam, D., Zor, R., Fineberg, N., Hermesh, H., 2012. Animal behavior as a conceptual framework for the study of obsessive–compulsive disorder (OCD). Behavioural brain research 231(2), 289-296.

Ellis, P.D., 2010. The essential guide to effect sizes: Statistical power, meta-analysis, and the interpretation of research results. Cambridge University Press.

Ettelt, S., Ruhrmann, S., Barnow, S., Buthz, F., Hochrein, A., Meyer, K., Kraft, S., Reck, C., Pukrop, R., Klosterkötter, J., 2007. Impulsiveness in obsessive–compulsive disorder: results from a family study. Acta Psychiatrica Scandinavica 115(1), 41-47.

Exner, C., Zetsche, U., Lincoln, T.M., Rief, W., 2014. Imminent Danger? Probabilistic Classification Learning of Threat-Related Information in Obsessive-Compulsive Disorder. Behavior Therapy 45, 157-167.

Fahey, J.M., Pritchard, G.A., Reddi, J.M., Pratt, J.S., Grassi, J.M., Shader, R.I., Greenblatt, D.J., 2006. The effect of chronic lorazepam administration in aging mice. Brain Res 1118(1), 13-24.

Fineberg, N.A., Apergis-Schoute, A.M., Vaghi, M.M., Banca, P., Gillan, C.M., Voon, V., Chamberlain, S.R., Cinosi, E., Reid, J., Shahper, S., 2018. Mapping compulsivity in the DSM-5 obsessive compulsive and related disorders: cognitive domains, neural circuitry, and treatment. International Journal of Neuropsychopharmacology 21(1), 42-58.

Fineberg, N.A., Potenza, M.N., Chamberlain, S.R., Berlin, H.A., Menzies, L., Bechara, A., Sahakian, B.J., Robbins, T.W., Bullmore, E.T., Hollander, E., 2010. Probing compulsive and impulsive behaviors, from animal models to endophenotypes: A narrative review. Neuropsychopharmacology 35(3), 591-604.

Fineberg, N.A., Reghunandanan, S., Simpson, H.B., Phillips, K.A., Richter, M.A., Matthews, K., Stein, D.J., Sareen, J., Brown, A., Sookman, D., 2015. Obsessive–compulsive disorder (OCD): Practical strategies for pharmacological and somatic treatment in adults. Psychiatry Research 227(1), 114-125.

Fontenelle, L.F., Oostermeijer, S., Harrison, B.J., Pantelis, C., Yücel, M., 2011. Obsessive-compulsive disorder, impulse control disorders and drug addiction. Drugs 71(7), 827-840.

Frydman, I., Mattos, P., de Oliveira-Souza, R., Yücel, M., Chamberlain, S.R., Moll, J., Fontenelle, L.F., 2020. Self-reported and neurocognitive impulsivity in obsessive-compulsive disorder. Comprehensive psychiatry 97, 152155.

Fuss, J., Richter, S.H., Steinle, J., Deubert, G., Hellweg, R., Gass, P., 2013. Are you real? Visual simulation of social housing by mirror image stimulation in single housed mice. Behav Brain Res 243, 191-198.

Goddard, A.W., Shekhar, A., Whiteman, A.F., McDougle, C.J., 2008. Serotoninergic mechanisms in the treatment of obsessive–compulsive disorder. Drug discovery today 13(7-8), 325-332.

Gordon, R.P., Brandish, E.K., Baldwin, D.S., 2016. Anxiety disorders, post-traumatic stress disorder, and obsessive–compulsive disorder. Medicine 44(11), 664-671.

Greene-Schloesser, D., Van der Zee, E., Sheppard, D., Castillo, M., Gregg, K., Burrow, T., Foltz, H., Slater, M., Bult-Ito, A., 2011. Predictive validity of a non-induced mouse model of compulsive-like behavior. Behavioural brain research 221(1), 55-62.

Guillette, L.M., Scott, A.C., Healy, S.D., 2016. Social learning in nest-building birds: a role for familiarity. Proceedings of the Royal Society B: Biological Sciences 283(1827), 20152685.

Güldenpfennig, M., Wolmarans, D.W., Du Preez, J.L., Stein, D.J., Harvey, B.H., 2011. Cortico-striatal oxidative status, dopamine turnover and relation with stereotypy in the deer mouse. Physiology & behavior 103(3-4), 404-411.

Hadžiabdic, J., Elezovic, A., Imamovic, B., Bečic, E., 2012. The Improvement of Lorazepam Solubility by Cosolvency, Micellization and Complexation. Jordan Journal of Pharmaceutical Sciences 5(2), 141-154.

Hedman, E., Ljótsson, B., Axelsson, E., Andersson, G., Rück, C., Andersson, E., 2017. Health anxiety in obsessive compulsive disorder and obsessive compulsive symptoms in severe health anxiety: An investigation of symptom profiles. Journal of Anxiety Disorders 45, 80-86.

Hess, S.E., Rohr, S., Dufour, B.D., Gaskill, B.N., Pajor, E.A., Garner, J.P., 2008. Home improvement: C57BL/6J mice given more naturalistic nesting materials build better nests. Journal of the American Association for Laboratory Animal Science 47(6), 25-31.

Hinds, A.L., Woody, E.Z., Van Ameringen, M., Schmidt, L.A., Szechtman, H., 2012. When too much is not enough: obsessive-compulsive disorder as a pathology of stopping, rather than starting. PLoS One 7(1), e30586.

Hoffman, K.L., Rueda Morales, R.I., 2009. Toward an understanding of the neurobiology of "just right" perceptions: Nest building in the female rabbit as a possible model for compulsive behavior and the perception of task completion. Behavioural Brain Research 204(1), 182-191.

Hollander, E., Kaplan, A., Stahl, S.M., 2003. A double-blind, placebo-controlled trial of clonazepam in obsessive-compulsive disorder. The World Journal of Biological Psychiatry 4(1), 30-34.

Hollander, E., Rosen, J., 2000. Impulsivity. Journal of Psychopharmacology 14(2_suppl1), S39-S44.

Jirkof, P., 2014. Burrowing and nest building behavior as indicators of well-being in mice. Measuring Behavior 234, 139-146.

Kashyap, H., Fontenelle, L.F., Miguel, E.C., Ferrão, Y.A., Torres, A.R., Shavitt, R.G., Ferreira-Garcia, R., Do Rosário, M.C., Yücel, M., 2012. 'Impulsive compulsivity'in obsessive-compulsive disorder: A phenotypic marker of patients with poor clinical outcome. Journal of psychiatric research 46(9), 1146-1152.

Kliethermes, C.L., Finn, D.A., Crabbe, J.C., 2003. Validation of a modified mirrored chamber sensitive to anxiolytics and anxiogenics in mice. Psychopharmacology (Berl) 169(2), 190-197.

Korff, S., Stein, D.J., Harvey, B.H., 2008. Stereotypic behaviour in the deer mouse: pharmacological validation and relevance for obsessive compulsive disorder. Prog Neuropsychopharmacol Biol Psychiatry 32(2), 348-355.

Korff, S., Stein, D.J., Harvey, B.H., 2009. Cortico-striatal cyclic AMP-phosphodiesterase-4 signalling and stereotypy in the deer mouse: attenuation after chronic fluoxetine treatment. Pharmacology Biochemistry and Behavior 92(3), 514-520.

Labad, J., Menchon, J.M., Alonso, P., Segalas, C., Jimenez, S., Jaurrieta, N., Leckman, J.F., Vallejo, J., 2008. Gender differences in obsessive–compulsive symptom dimensions. Depression and Anxiety 25(10), 832-838.

Lockie, S.H., McAuley, C.V., Rawlinson, S., Guiney, N., Andrews, Z.B., 2017. Food seeking in a risky environment: a method for evaluating risk and reward value in food seeking and consumption in mice. Frontiers in neuroscience 11, 24.

Loring, D.W., Marino, S.E., Parfitt, D., Finney, G.R., Meador, K.J., 2012. Acute lorazepam effects on neurocognitive performance. Epilepsy Behav 25(3), 329-333.

Lustberg, D., Iannitelli, A.F., Tillage, R.P., Pruitt, M., Liles, L.C., Weinshenker, D., 2020. Central norepinephrine transmission is required for stress-induced repetitive behavior in two rodent models of obsessive-compulsive disorder. Psychopharmacology, 1-15.

Makhinson, M., Furst, B.A., Shuff, M.K., Kwon, G.E., 2012. Successful treatment of co-occurring catatonia and obsessive-compulsive disorder with concurrent electroconvulsive therapy and benzodiazepine administration. The journal of ECT 28(3), e35-e36.

Melli, G., Chiorri, C., Carraresi, C., Stopani, E., Bulli, F., 2015. The two dimensions of contamination fear in obsessive-compulsive disorder: Harm avoidance and disgust avoidance. Journal of Obsessive-Compulsive and Related Disorders 6, 124-131.

Mick, T.M., Hollander, E., 2006. Impulsive-compulsive sexual behavior. CNS spectrums 11(12), 944-955.

Nadeau, J.M., Lewin, A.B., Arnold, E.B., Crawford, E.A., Murphy, T.K., Storch, E.A., 2013. Clinical correlates of functional impairment in children and adolescents with obsessive–compulsive disorder. Journal of Obsessive-Compulsive and Related Disorders 2(4), 432-436.

Nestadt, G., Samuels, J., Riddle, M.A., Liang, K., Bienvenu, O.J., Hoehn-Saric, R., Grados, M., Cullen, B., 2001. The relationship between obsessive-compulsive disorder and anxiety and affective disorders: results from the Johns Hopkins OCD Family Study. Psychological medicine 31(3), 481.

Nakagawa, S., Cuthill, I.C., 2007. Effect size, confidence interval and statistical significance: a practical guide for biologists. Biological reviews 82(4), 591-605.

Panes, A., Verdoux, H., Fourrier-Reglat, A., Berdai, D., Pariente, A., Tournier, M., 2020. Misuse of benzodiazepines: Prevalence and impact in an inpatient population with psychiatric disorders. Br J Clin Pharmacol 86(3), 601-610.

Parkes, L., Tiego, J., Aquino, K., Braganza, L., Chamberlain, S.R., Fontenelle, L.F., Harrison, B.J., Lorenzetti, V., Paton, B., Razi, A., 2019. Transdiagnostic variations in impulsivity and compulsivity in obsessive-compulsive disorder and gambling disorder correlate with effective connectivity in cortical-striatal-thalamic-cortical circuits. NeuroImage 202, 116070.

Paton, C., 2002. Benzodiazepines and disinhibition: a review. Psychiatric Bulletin 26(12), 460-462.

Péter, Z., Oliphant, M.E., Fernandez, T.V., 2017. Motor stereotypies: a pathophysiological review. Frontiers in neuroscience 11, 171.

Pich, E.M., Samanin, R., 1989. A two-compartment exploratory model to study anxiolytic/anxiogenic effects of drugs in the rat. Pharmacological research 21(5), 595-602.

Powell, S.B., Newman, H.A., Pendergast, J.F., Lewis, M.H., 1999. A Rodent Model of Spontaneous Stereotypy: Initial Characterization of Developmental, Environmental, and Neurobiological Factors. Physiology & Behavior 66(2), 355-363.

Presti, M.F., Powell, S.B., Lewis, M.H., 2002. Dissociation between spontaneously emitted and apomorphine-induced stereotypy in Peromyscus maniculatus bairdii. Physiology & behavior 75(3), 347-353.

Raines, A.M., Allan, N.P., Oglesby, M.E., Short, N.A., Schmidt, N.B., 2015. Examination of the relations between obsessive–compulsive symptom dimensions and fear and distress disorder symptoms. Journal of Affective Disorders 183, 253-257.

Reddy, D., Kulkarni, S., 1997. Differential anxiolytic effects of neurosteroids in the mirrored chamber behavior test in mice. Brain research 752(1-2), 61-71.

Riesel, A., Klawohn, J., Grützmann, R., Kaufmann, C., Heinzel, S., Bey, K., Lennertz, L., Wagner, M., Kathmann, N., 2019. Error-related brain activity as a transdiagnostic endophenotype for obsessive-compulsive disorder, anxiety and substance use disorder. Psychological medicine 49(7), 1207-1217.

Robbins, T.W., Vaghi, M.M., Banca, P., 2019. Obsessive-compulsive disorder: puzzles and prospects. Neuron 102(1), 27-47.

Rowsell, M., Francis, S.E., 2015. OCD Subtypes: Which, If Any, Are Valid? Clinical Psychology: Science and Practice 22(4), 414-435.

Sassaroli, S., Centorame, F., Caselli, G., Favaretto, E., Fiore, F., Gallucci, M., Sarracino, D., Ruggiero, G.M., Spada, M.M., Rapee, R.M., 2015. Anxiety control and metacognitive beliefs mediate the relationship between inflated responsibility and obsessive compulsive symptoms. Psychiatry Research 228(3), 560-564.

Schaefer, V., 1976. Geographic variation in the placement and structure of oriole nests. The Condor 78(4), 443-448.

Scheepers, I.M., Stein, D.J., Harvey, B.H., 2018. Peromyscus maniculatus bairdii as a naturalistic mammalian model of obsessive-compulsive disorder: current status and future challenges. Metabolic brain disease 33(2), 443-455.

Sherwin, C., 1997. Observations on the prevalence of nest-building in non-breeding TO strain mice and their use of two nesting materials. Laboratory Animals 31(2), 125-132.

Silveira, M.M., Wittekindt, S.N., Mortazavi, L., Hathaway, B.A., Winstanley, C.A., 2020. Investigating serotonergic contributions to cognitive effort allocation, attention, and impulsive action in female rats. Journal of Psychopharmacology 34(4), 452-466.

Smithers, R.H.N., 1983. XXIII. Families CRICETIDAE and MURIDAE, Rats and mice, The Mammals of the Southern-African Subregion. University of Pretoria, Pretoria, South Africa, pp. 220-220 - 296.

Smythe, J.W., Murphy, D., Bhatnagar, S., Timothy, C., Costall, B., 1996. Muscarinic antagonists are anxiogenic in rats tested in the black-white box. Pharmacology Biochemistry and Behavior 54(1), 57-63.

Starcevic, V., 2014. The reappraisal of benzodiazepines in the treatment of anxiety and related disorders. Expert review of neurotherapeutics 14(11), 1275-1286.

Szechtman, H., Harvey, B.H., Woody, E.Z., Hoffman, K.L., 2020. The Psychopharmacology of Obsessive-Compulsive Disorder: A Preclinical Roadmap. Pharmacol Rev 72(1), 80-151.

Van de Weerd, H., Van Loo, P., Van Zutphen, L., Koolhaas, J., Baumans, V., 1997. Preferences for nesting material as environmental enrichment for laboratory mice. Laboratory animals 31(2), 133-143.

Walf, A.A., Koonce, C., Manley, K., Frye, C.A., 2009. Proestrous compared to diestrous wildtype, but not estrogen receptor beta knockout, mice have better performance in the spontaneous alternation and object recognition tasks and reduced anxiety-like behavior in the elevated plus and mirror maze. Behav Brain Res 196(2), 254-260.

Walsh, R.N., Cummins, R.A., 1976. The open-field test: a critical review. Psychological bulletin 83(3), 482.

Wang, P., Zhanjiang, L., 2017. Attentional bias on threating stimuli in patients with obsessive-compulsive disorder. Chinese Journal of Behavioral Medicine and Brain Science 26(11), 1050-1056.

Webster, D.G., Baumgardner, D.J., Dewsbury, D.A., 1979. Open-field behavior in eight taxa of muroid rodents. Bulletin of the Psychonomic Society 13(2), 90-92.

Wolff, M.C., Leander, J.D., 2002. Selective serotonin reuptake inhibitors decrease impulsive behavior as measured by an adjusting delay procedure in the pigeon. Neuropsychopharmacology 27(3), 421-429.

Wolmarans, D.W., Brand, L., Stein, D.J., Harvey, B.H., 2013. Reappraisal of spontaneous stereotypy in the deer mouse as an animal model of obsessive-compulsive disorder (OCD): Response to escitalopram treatment and basal serotonin transporter (SERT) density. Behavioural Brain Research 256, 545-553.

Wolmarans, D.W., Stein, D.J., Harvey, B.H., 2016. Excessive nest building is a unique behavioural phenotype in the deer mouse model of obsessive-compulsive disorder. Journal of Psychopharmacology 30(9), 867-874.

Wolmarans, D.W., Stein, D.J., Harvey, B.H., 2016a. Of mice and marbles: Novel perspectives on burying behavior as a screening test for psychiatric illness. Cogn Affect Behav Neurosci 16(3), 551-560.

Wolmarans, D.W., Stein, D.J., Harvey, B.H., 2017. Social behavior in deer mice as a novel interactive paradigm of relevance for obsessive-compulsive disorder (OCD). Social neuroscience 12(2), 135-149.

Figures

FIGURE 3-1 - Photographic (A) and schematic (B) representation of the mirrored open field arena. F: food hopper; W: water bottle; nesting material hopper in the open field is duplicated in the enclosed compartment

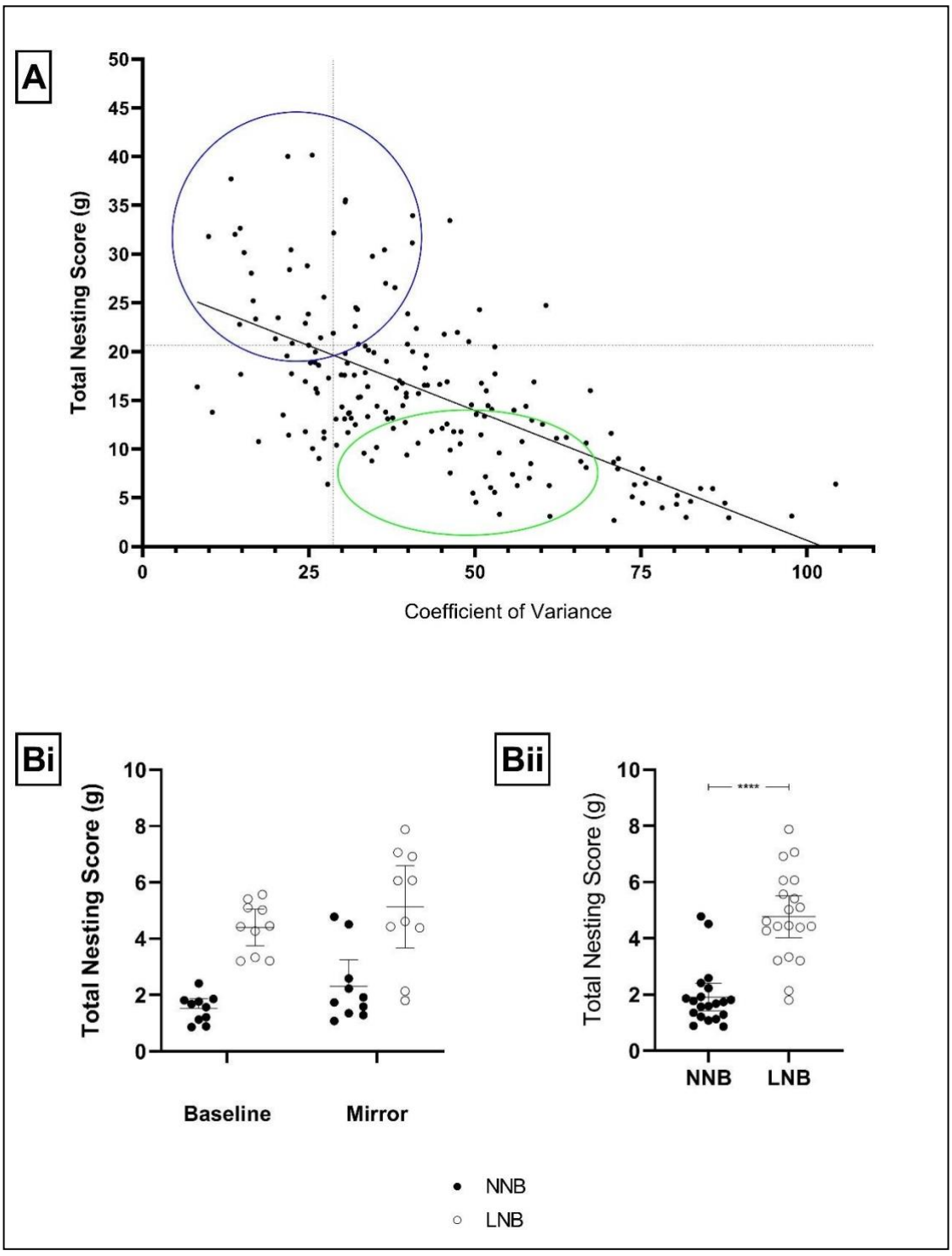

FIGURE 3-2 - (A): Spearman's correlation of the individual total nesting scores (g) plotted against the respective coefficients of variance pertaining to the inter-day nesting scores. LNB animals enclosed in the circle; NNB animals enclosed in the oval. (Bi): Individual total nesting scores (g) generated by NNB and LNB animals in the home cage (baseline; last four days of assessment) and the mirrored open field (mirror). (Bii): Pooled individual total nesting scores generated in the home cages and mirrored open field (g) of control exposed NNB and LNB expressing deer mice. LNB: large nest building behaviour; NNB: normal nest building behaviour.

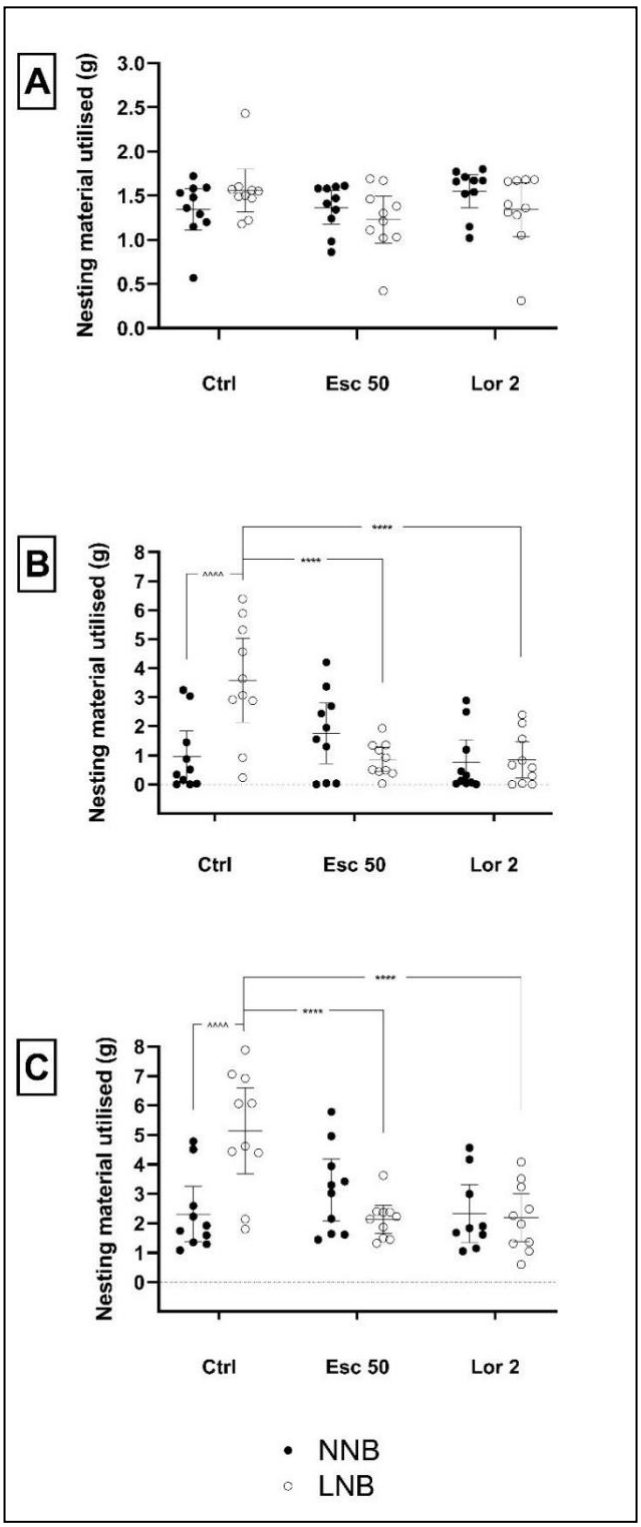

FIGURE 3-3 - Average daily weights of cotton wool utilised from the inside (A) and outside (B) nesting material dispensers by NNB and LNB expressing mice. The combined weights are depicted in (C). Error bars represent means with 95% confidence intervals. Ordinary two-way analysis of variance (ANOVA) followed by Sidak's (^) or Tukey's (*) multiple comparisons tests. Ctrl: control; Esc 50: escitalopram; LNB: large nest building behaviour; Lor 2: lorazepam; NNB: normal nest building behaviour.

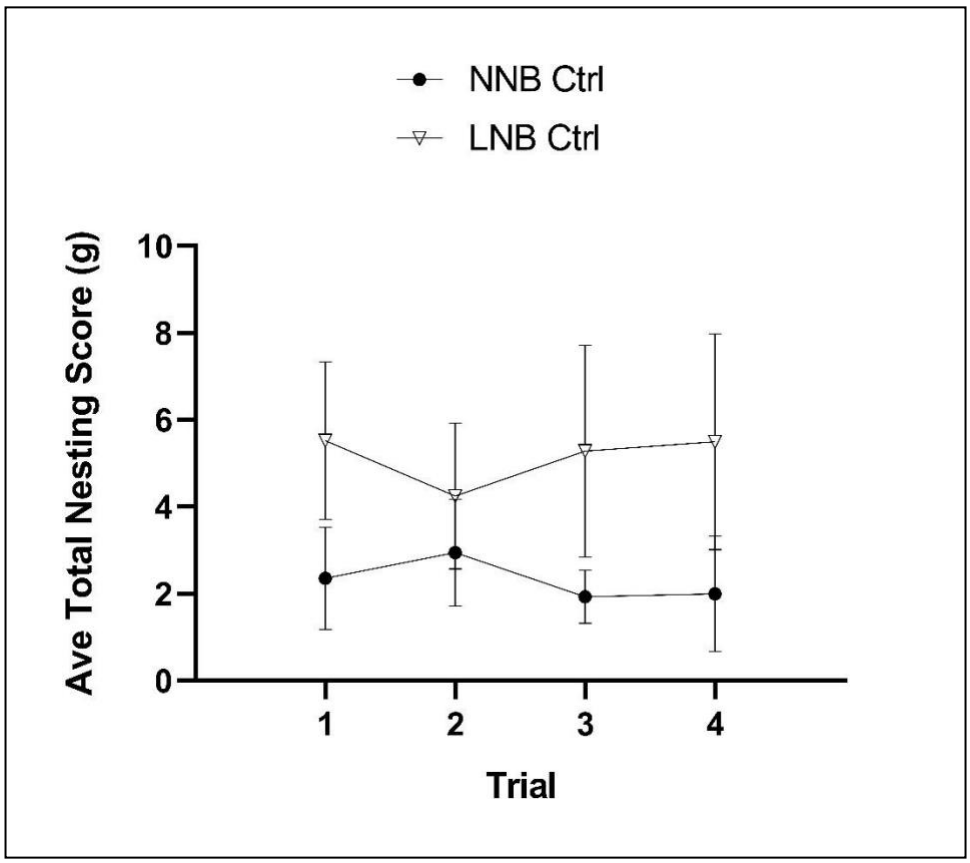

FIGURE 3-4 - Average total nesting scores (inside and outside combined) generated by control exposed NNB and LNB expressing animals over the four nights of open field assessment. Error bars represent means with 95% confidence intervals. LNB: large nest building behaviour; NNB: normal nest building behaviour.

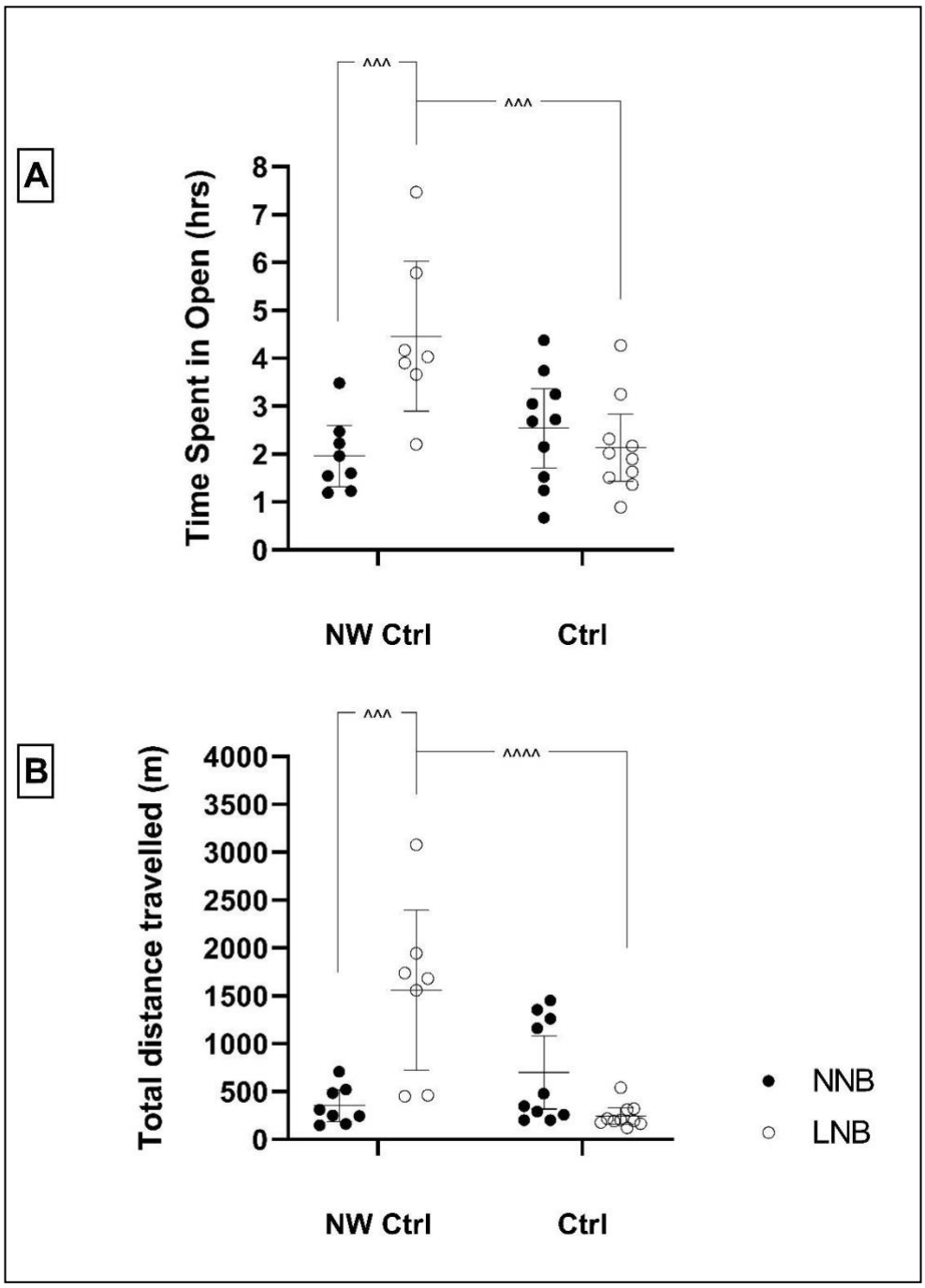

FIGURE 3-5 - Average daily time spent (A) and distance travelled in (B) the mirrored open field by control-exposed NNB and LNB expressing animals (two different groups per cohort) without (NW Ctrl) and with (Ctrl) access to cotton wool in the open field. Ctrl: control-exposed animals provided with cotton wool; LNB: large nest building behaviour; NNB: normal nest building behaviour; NW Ctrl: no-wool control-exposed animals. Ordinary two-way analysis of variance (ANOVA) followed by Sidak's (^) multiple comparisons tests.

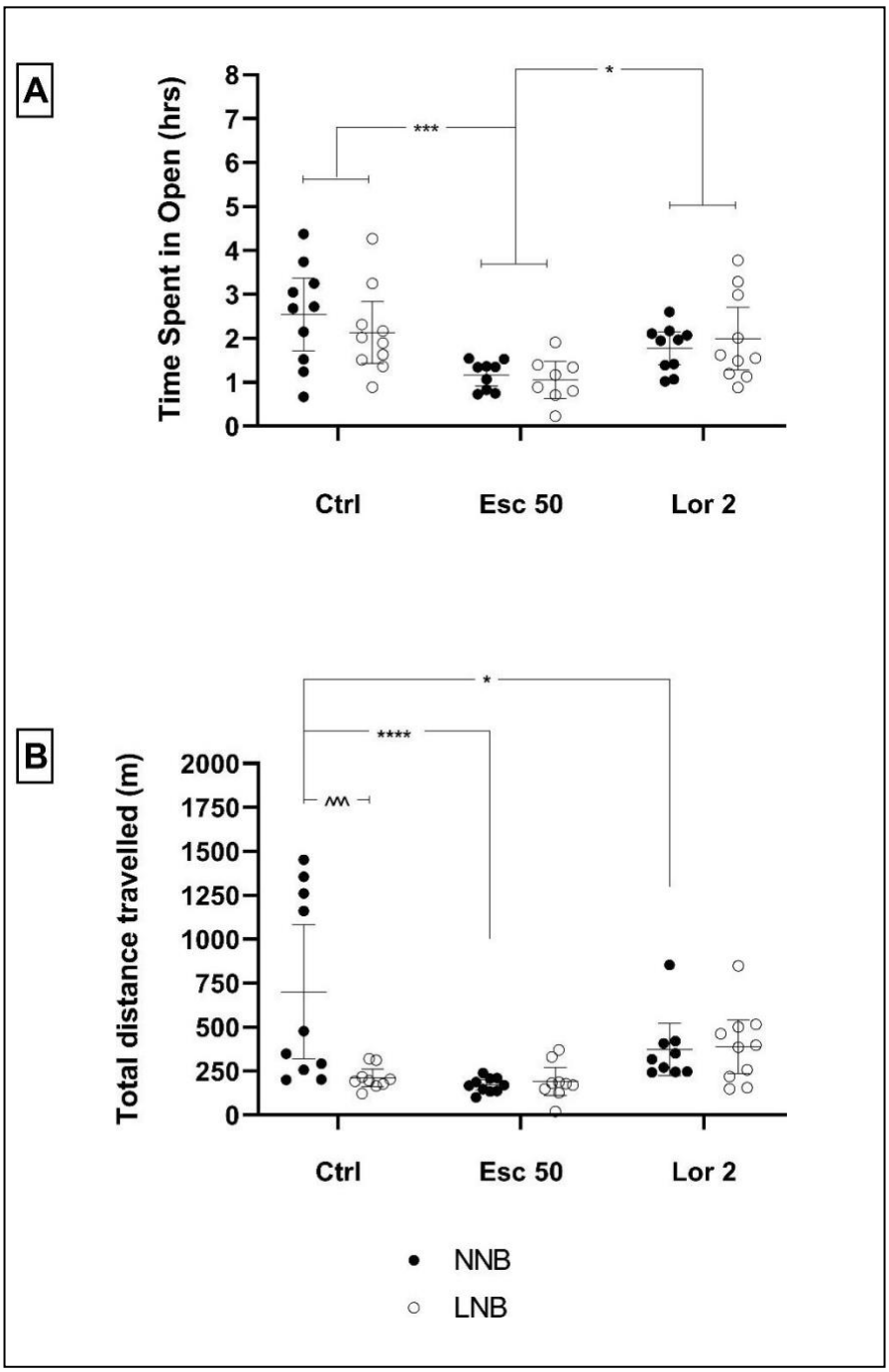

FIGURE 3-6 - Average daily time spent in (A) and distance travelled in (B) the mirrored open field by NNB and LNB expressing animals. Ordinary two-way analysis of variance (ANOVA) followed by Sidak's (^) or Tukey's (*) multiple comparisons tests. Ctrl: control; Esc 50: escitalopram; LNB: large nest building behaviour; Lor 2: lorazepam; NNB: normal nest building behaviour.

4 CONCLUSION

The aim of the current study was to determine whether normal nest building (NNB)[1] and large nest building behaviour (LNB)[2] behaviour differ in their potential association with anxiety and how such behaviours would adapt as a function of contextual manipulation, i.e. the assessment of nesting behaviour under normal home cage and novel anxiogenic circumstances. We also wanted to determine how said behaviours would respond to a known anti-compulsive and anxiolytic intervention, i.e. chronic, high-dose escitalopram, as well as to sub-acute treatment with lorazepam, an anxiolytic benzodiazepine. The main findings of this work were that 1) LNB expressed by deer mice is an inflated, but goal-directed behavioural phenotype which remains stable, irrespective of the context in which it is assessed, 2) LNB mice find an open field arena to be less aversive compared to the behaviour or their NNB expressing counterparts, and 3) escitalopram and lorazepam differentially affect the nesting and open field behaviour of NNB and LNB expressing mice.

* * *

Obsessive-compulsive disorder (OCD)[3] is a symptom heterogenous and disabling neuropsychiatric disorder that is typically characterised by a combination of obsessions and/or compulsions, with patients experiencing distress and anxiety more often than not (Abramowitz, 2006; Fineberg et al., 2015; Szechtman et al., 2020). Chronic, high dose selective serotonin reuptake inhibitor (SSRI)[4] treatment, currently constitutes the first line pharmacotherapeutic intervention for OCD (Fineberg et al., 2015; Katzman et al., 2014), though low-dose anti-dopaminergic drugs, e.g. quetiapine, risperidone, are also prescribed as adjunctive therapy for patients that remain refractory to SSRIs (Aardema and O'Connor, 2007; Blakey et al., 2019; McLean et al., 2015; Szechtman et al., 2020). Still, treatment response remains suboptimal with only 40-60% of patients demonstrating adequate symptom attenuation (Albert et al., 2018), and as such, a better understanding of OCD is needed to advance current approaches.

It is widely accepted that obsessions, being intrusive and dominating, cause severe distress and anxiety in the sufferer (Abramowitz, 2006; Abramowitz et al., 2009; APA, 2013). As a result, patients engage in so-called anxiolytic and neutralising behavioural routines (compulsions). Importantly, the anxiolytic relief experienced after engaging in compulsive rituals is short-lived, a scenario that reinforces the continued engagement in such behaviour (Gordon et al., 2016). It should also be

[1] normal nest building
[2] large nest building
[3] obsessive-compulsive disorder
[4] selective serotonin reuptake inhibitor

stressed that OCD[1] patients do not necessarily present with general anxiety; rather, feelings of distress are confined to the conceptual boundaries of certain specific themes, e.g. safety, contamination, and harm (Abramovitch et al., 2013). Further, OCD patients tend to overestimate and inflate the actual significance of what they feel could be severely negative consequences should they not engage in neutralising, or risk-mitigating behaviour (Belloch et al., 2011; Exner et al., 2014). However, it is mostly unknown whether such anxiety is always borne from within, or whether actual external anxiogenic stimuli affect the severity and persistence of obsessive-compulsive symptom presentation. This question is important, since treatment data are conflicting. For example, while, benzodiazepines, an anxio-selective drug class, are not used for the treatment of OCD, they seem useful for the alleviation of acute anxiety flares in patients with OCD (Starcevic et al., 2016). On the other hand, chronic high dose SSRI[2] intervention is known to be effective for the treatment of OCD and anxiety disorders (Albert et al., 2018). Therefore, it is likely that the relationship between anxiety and obsessive-compulsive symptom expression is complex and characterised by significant inter- individual differences with respect to how such interactions contribute to symptom expression. Here, we focused on this theme by investigating how the expression of NNB[3] and compulsive-like LNB[4] in deer mice relates to contextual, i.e. safety-related anxiety.

Laboratory housed deer mice (Peromyscus maniculatus bairdii) of both sexes express phenotypically heterogeneous repetitive, persistent, and seemingly purposeless behaviours from the age of 10 weeks. Furthermore, these behaviours, including LNB which is expressed by 30-35% of deer mice, manifest spontaneously and without external interference (de Brouwer and Wolmarans, 2018; Güldenpfennig et al., 2011; Korff et al., 2008, 2009; Scheepers et al., 2018; Wolmarans et al., 2013; Wolmarans et al., 2016a, 2016b). As such, the deer mouse model is a useful framework to study the psychobiological mechanism underlying naturalistic behavioural persistence. Regarding this work, it is important to note that nesting behaviour is a normal part of the behavioural repertoire of several animal species, including deer mice. However, in the laboratory, LNB, as opposed to NNB, serves no functional purpose and as such, form the focus of our current research. A significant body of research has already been done to describe and interrogate LNB as a framework for the study of compulsive-like behaviours. Briefly, LNB is persistent and repetitive over several consecutive assessments and manifests in a goal-directed manner which is subject to cognitive planning and outcome-feedback control (Hoffman and Rueda Morales, 2009). Further, LNB demonstrates robust clinical response to

[1] obsessive-compulsive disorder
[2] selective serotonin reuptake inhibitor
[3] normal nest building
[4] large nest building

chronic, high dose oral intervention with the SSRI[1], escitalopram, as well as to a combination of escitalopram and low dose augmentation with the anti-dopaminergic agent, flupentixol (de Brouwer et al., 2020a). From an evolutionary perspective, nesting behaviour serves a protective role (Denenberg et al., 1969; Lynch, 1980; Wolff, 1994). Here, we applied such behaviour to investigate whether the expression of NNB[2] and LNB[3] is differentially affected by manipulations of a safety-related construct, i.e. by assessing nesting activity in an anxiogenic open field arena.

* * *

In terms of our first main finding, we demonstrated LNB, and to a lesser degree NNB, to be stable and innate, irrespective of the testing environment. As such, LNB expressing animals exhibit a strong, inherent drive to express nesting, irrespective of the context in which it is measured. Therefore, LNB is excessive, persistent, and seemingly purposeless in terms of functional outcome. This also tends to be true for NNB expressing mice, which although trending towards generating higher nesting scores in the open field, maintained nesting building behaviour at a level akin to which was found in the home cage. Although it might be possible that NNB in deer mice is not subject to safety-related contexts, it should be noted that mice were supplied with sufficient quantities of cotton wool to build nests of average 'normal' size in the dark compartment of the open field and that NNB mice could in fact have been sensitive to the potential risk carried by an open space, hence having refrained from entering the open field. Contrary to this behaviour, LNB animals continued to retrieve more wool from the external hopper located in the open, anxiogenic space, suggesting that animals of this cohort present with an inflated motivational drive to engage in nesting behaviour, irrespective of the potential negative outcomes of such behaviour. This data is largely congruent with our previous findings (de Brouwer et al., 2020b).

* * *

Our data with respect to the open field behaviour of deer mice was informing. LNB expressing mice which were assessed for open field behaviour in the absence of cotton wool, spent significantly more time and travelled longer distances in the open field compared to their NNB expressing counterparts assessed in the same manner. This finding highlighted an important aspect separating the two cohorts. When assessed in a novel anxiogenic space, LNB expressing deer mice were more likely to explore an environment associated with potential danger, compared to NNB expressing mice. While this observation could be explained by possible differences in the levels of underlying anxiety between

[1] selective serotonin reuptake inhibitor
[2] normal nest building
[3] large nest building

LNB[1] and NNB[2] mice, there might be a different view, i.e. that LNB expressing mice possibly exhibit impulsive risk-taking behaviour. This idea is supported by our previous findings regarding LNB mice being overly insensitive to punishing outcomes (de Brouwer et al., 2020b), but also by the fact that the provision of nesting material attenuated the expression of such excessive open field behaviour. Clinical data explaining the temporal relationship between impulsive and compulsive symptom expression are scant. However, recent research pointed to positive correlations between impulsive and compulsive symptom severity (Chamberlain et al., 2018) which may be informative for the differences in the nesting and open field behaviour of NNB and LNB expressing animals, reported here. Nevertheless, further research is required to elaborate on this finding. Importantly, given that both NNB and LNB animals explored the open field, albeit to differing degrees, it is likely that deer mice are not overly anxious. It is clear that NNB expressing animals showed increased aversion to the open field, supports our perspective that LNB expressing mice are less sensitive to such a context and more prone to voluntary risk-taking.

* * *

With respect to our third main finding, two important observations were made. First, escitalopram and lorazepam markedly attenuated the open field nesting scores of LNB, but not NNB expressing animals. Second, escitalopram, but not lorazepam reduced the time spent in the open field by animals of both cohorts, while both escitalopram and lorazepam reduced the total distance travelled by NNB expressing animals in the open field. While the latter finding may seem counterintuitive, we believe this result to be attributed to increased stereotypical running activity, which are known to be displayed by some deer mice (Presti et al., 2002) and which may be a potential artefact of underlying anxiety (Péter et al., 2017) in laboratory housed rodents. In animals of the LNB expressing cohort, a different observation was made. Here, escitalopram, but not lorazepam, reduced the time spent in the open field, while simultaneously having a robust attenuating influence on the nesting expression. In comparison, lorazepam attenuated the nesting scores without altering the open field behaviour, compared to control-exposed animals. Our data with respect to the effect of escitalopram on nest building expression agree with previous reports (De Brouwer et al., 2020a; Wolmarans et al., 2016b); however, the effect of lorazepam needs further elucidation. Although lorazepam seemed to have affected excessive nesting behaviour per se, it maintained the open field behaviour of LNB expressing animals at levels akin to that displayed by control-exposed animals. However, as opposed to control-

[1] large nest building
[2] normal nest building

exposed LNB[1] expressing animals which engaged in large nesting behaviour while ambulating in the open field, the lorazepam-exposed LNB animals did not engage to the same extent in nesting. One explanation for this is supportive of the idea that LNB animals may present with an underlying impulsive trait. Lorazepam has previously been shown to cause behavioural disinhibition in some individuals (Bond, 1998; Panes et al., 2020; Paton, 2002) and impulsive task-engagement in others (Loring et al., 2012). Conversely, SSRIs[2] are moderately effective for the treatment of compulsivity and have shown some promise in the sub-acute treatment of impulsivity (Hollander and Rosen, 2000; Silveira et al., 2020; Wolff and Leander, 2002). Collectively, the treatment data presented here point to potential underlying psychobiological differences that typify NNB[3] and LNB. While changes in the behaviour of NNB expressing animals might more align with the modification of anxiety, adaptations in the nesting and open-field behaviour of LNB animals, are likely related with modification of behaviours which might represent the impulsive-compulsive continuum. Further, that NNB remained entirely unresponsive to drug interventions which had noteworthy effects on open field activity, supports our conclusion that NNB, other than LNB, is normal, inherent and devoid of psychobiological interference.

* * *

In conclusion, our findings confirmed that LNB behaviour in deer mice is not affected by anxiogenic manipulation and that nest building is a stable, innate behavioural phenotype. The potential impulsive-like and risk-taking behaviour, which was observed in animals of the LNB cohort, may provide a useful background for future studies that aim to investigate the relationships between anxiety, impulsivity, and compulsive-like behaviour.

* * *

[1] large nest building
[2] selective serotonin reuptake inhibitors
[3] normal nest building

4.1 Summary of expected vs actual outcomes

Expected Outcome	Actual Outcome
• 25-30% of deer mice will present with LNB[1]	✓
• LNB will be inflexible and insensitive to contextual manipulation	✓ LNB animals also presented with reduced anxiety in the open field that likely speaks to an underlying impulsive trait. Future studies are needed.
• NNB[2] behaviour will adapt to contextual manipulation	✗ NNB was not sensitive to contextual adaptation and remained stable.
• LNB in the open field will only respond to escitalopram, being an anti-compulsive intervention	✗ LNB responded to lorazepam also; however, our collective nesting-open field findings point to a likely role for impulsivity in this response.
• Inflated nesting behaviour in the open field by NNB expressing animals would respond to both escitalopram and lorazepam, being anxiolytic interventions	✗ NNB expressing animals did not present with inflated nest building activity. Further, NNB behaviour was not sensitive to either intervention.
• Escitalopram and lorazepam would increase the time spent in the open field in animals of both cohorts	✗ Escitalopram, but not lorazepam, reduced the time spent in the open field, likely due to changes in an underlying impulsive trait.

[1] large nest building
[2] normal nest building

4.2 Study shortcomings and future directions

The present work was not without shortcomings, which in our view, need to be highlighted. First, considering that only 30-35% of deer mice present with LNB[1], this work employed 10 deer mice of both sexes per phenotype per treatment group, and 8 animals in a further two control-exposed groups which were assessed in the absence of nesting material. Although our results revealed robust and significant differences in the behaviour of NNB[2] and LNB mice, individual differences in the expression of open field behaviour, i.e. the four NNB expressing animals that travelled significantly longer distances in the arena, might have been better conceptualised with increased group sizes. Further, our findings with respect to the open field behaviour of animals tested in the absence of cotton wool will better be explained with the inclusion of treatment groups. Such inclusion will be informative in terms of potential impulsive or risk-taking behaviour, that we propose here to underlie the behaviour of LNB animals. Last, we propose that investigations that specifically focus on impulsivity and risk-taking be continued in the deer mouse model, as in our view, the current data can be substantiated with investigations that employ different methodologies aimed at this research construct.

* * *

[1] large nest building
[2] normal nest building

4.3 References

Aardema, F., O'Connor, K., 2007. The menace within: Obsessions and the self. Journal of Cognitive Psychotherapy 21(3), 182-197.

Abramovitch, A., Abramowitz, J.S., Mittelman, A., 2013. The neuropsychology of adult obsessive–compulsive disorder: a meta-analysis. Clinical psychology review 33(8), 1163-1171.

Abramowitz, J.S., 2006. Understanding and Treating Obsessive-Compulsive Disorder. Lawrence Erlbaum Associates, Mahwah, New Jersey.

Abramowitz, J.S., Taylor, S., McKay, D., 2009. Obsessive-compulsive disorder. The Lancet 374(9688), 491-499.

Albert, U., Marazziti, D., Di Salvo, G., Solia, F., Rosso, G., Maina, G., 2018. A systematic review of evidence-based treatment strategies for obsessive-compulsive disorder resistant to first-line pharmacotherapy. Current medicinal chemistry 25(41), 5647-5661.

APA (American Psychiatric Association), 2013. Diagnostic and statistical manual of mental disorders (DSM-5®). American Psychiatric Pub.

Belloch, A., Cabedo, E., Carrió, C., Fernández-Alvarez, H., García, F., Larsson, C., 2011. Group versus individual cognitive treatment for Obsessive-Compulsive Disorder: changes in non-OCD symptoms and cognitions at post-treatment and one-year follow-up. Psychiatry Research 187(1-2), 174-179.

Blakey, S.M., Abramowitz, J.S., Leonard, R.C., Riemann, B.C., 2019. Does Exposure and Response Prevention Behaviorally Activate Patients With Obsessive-Compulsive Disorder? A Preliminary Test. Behavior Therapy 50, 214-224.

Bond, A.J., 1998. Drug-induced behavioural disinhibition. CNS drugs 9(1), 41-57.

Chamberlain, S.R., Stochl, J., Redden, S.A., Grant, J.E., 2018. Latent traits of impulsivity and compulsivity: toward dimensional psychiatry. Psychological medicine 48(5), 810-821.

de Brouwer, G., Fick, A., Lombaard, A., Stein, D.J., Harvey, B.H., Wolmarans, D.W., 2020a. Large nest building and high marble-burying: two compulsive-like phenotypes expressed by deer mice

(Peromyscus maniculatus bairdii) and their unique response to serotoninergic and dopamine modulating intervention. Behavioural Brain Research, 112794.

de Brouwer, G., Harvey, B.H., Wolmarans, D.W., 2020b. Naturalistic operant responses in deer mice (Peromyscus maniculatus bairdii) and its response to outcome manipulation and serotonergic intervention. Behavioural Pharmacology 31(4), 343-358.

de Brouwer, G., Wolmarans, D.W., 2018. Back to basics: A methodological perspective on marble-burying behavior as a screening test for psychiatric illness. Behavioural Processes 157, 590-600.

Denenberg, V.H., Taylor, R.E., Zarrow, M., 1969. Maternal behavior in the rat: an investigation and quantification of nest building. Behaviour 34(1), 1-16.

Exner, C., Zetsche, U., Lincoln, T.M., Rief, W., 2014. Imminent danger? Probabilistic classification learning of threat-related information in obsessive-compulsive disorder. Behavior therapy 45(2), 157-167.

Fineberg, N.A., Reghunandanan, S., Simpson, H.B., Phillips, K.A., Richter, M.A., Matthews, K., Stein, D.J., Sareen, J., Brown, A., Sookman, D., 2015. Obsessive–compulsive disorder (OCD): Practical strategies for pharmacological and somatic treatment in adults. Psychiatry Research 227(1), 114-125.

Gordon, R.P., Brandish, E.K., Baldwin, D.S., 2016. Anxiety disorders, post-traumatic stress disorder, and obsessive–compulsive disorder. Medicine 44(11), 664-671.

Güldenpfennig, M., Wolmarans, D.W., Du Preez, J.L., Stein, D.J., Harvey, B.H., 2011. Cortico-striatal oxidative status, dopamine turnover and relation with stereotypy in the deer mouse. Physiology & behavior 103(3-4), 404-411.

Hoffman, K.L., Rueda Morales, R.I., 2009. Toward an understanding of the neurobiology of "just right" perceptions: Nest building in the female rabbit as a possible model for compulsive behavior and the perception of task completion. Behavioural Brain Research 204(1), 182-191.

Hollander, E., Rosen, J., 2000. Impulsivity. Journal of Psychopharmacology 14(2_suppl1), S39-S44.

Katzman, M.A., Bleau, P., Blier, P., Chokka, P., Kjernisted, K., Van Ameringen, M., 2014. Canadian Anxiety Guidelines Initiative Group on behalf of the Anxiety Disorders Association of Canada/Association Canadienne des troubles anxieux and McGill University. Canadian clinical practice

guidelines for the management of anxiety, posttraumatic stress and obsessive-compulsive disorders. BMC Psychiatry 14 (Suppl 1), S1.

Korff, S., Stein, D.J., Harvey, B.H., 2008. Stereotypic behaviour in the deer mouse: pharmacological validation and relevance for obsessive compulsive disorder. Progress in Neuropsychopharmacology and Biological Psychiatry 32(2), 348-355.

Korff, S., Stein, D.J., Harvey, B.H., 2009. Cortico-striatal cyclic AMP-phosphodiesterase-4 signalling and stereotypy in the deer mouse: attenuation after chronic fluoxetine treatment. Pharmacology Biochemistry and Behavior 92(3), 514-520.

Loring, D.W., Marino, S.E., Parfitt, D., Finney, G.R., Meador, K.J., 2012. Acute lorazepam effects on neurocognitive performance. Epilepsy Behavior 25(3), 329-333.

Lynch, C.B., 1980. Response to divergent selection for nesting behavior in Mus musculus. Genetics 96(3), 757-765.

McLean, C.P., Zandberg, L.J., Van Meter, P.E., Carpenter, J.K., Simpson, H.B., Foa, E.B., 2015. Exposure and response prevention helps adults with obsessive compulsive disorder who do not respond to pharmacological augmentation strategies. The Journal of clinical psychiatry 76(12), 1653.

Panes, A., Verdoux, H., Fourrier-Reglat, A., Berdai, D., Pariente, A., Tournier, M., 2020. Misuse of benzodiazepines: Prevalence and impact in an inpatient population with psychiatric disorders. British Journal of Clinical Pharmacology 86(3), 601-610.

Paton, C., 2002. Benzodiazepines and disinhibition: a review. Psychiatric Bulletin 26(12), 460-462.

Péter, Z., Oliphant, M.E., Fernandez, T.V., 2017. Motor stereotypies: a pathophysiological review. Frontiers in neuroscience 11, 171.

Presti, M.F., Powell, S.B., Lewis, M.H., 2002. Dissociation between spontaneously emitted and apomorphine-induced stereotypy in Peromyscus maniculatus bairdii. Physiology & behavior 75(3), 347-353.

Scheepers, I.M., Wolmarans, D.W., Stein, D.J., Harvey, B.H., 2018. Peromyscus maniculatus bairdii as a naturalistic mammalian model of obsessive-compulsive disorder: current status and future challenges. Metabolic brain disease 33(2), 443-455.

Silveira, M.M., Wittekindt, S.N., Mortazavi, L., Hathaway, B.A., Winstanley, C.A., 2020. Investigating serotonergic contributions to cognitive effort allocation, attention, and impulsive action in female rats. Journal of Psychopharmacology 34(4), 452-466.

Starcevic, V., Berle, D., do Rosário, M.C., Brakoulias, V., Ferrão, Y.A., Viswasam, K., Shavitt, R., Miguel, E., Fontenelle, L.F., 2016. Use of benzodiazepines in obsessive–compulsive disorder. International clinical psychopharmacology 31(1), 27-33.

Szechtman, H., Harvey, B.H., Woody, E.Z., Hoffman, K.L., 2020. The Psychopharmacology of Obsessive-Compulsive Disorder: A Preclinical Roadmap. Pharmacol Rev 72(1), 80-151.

Wolff, J.O., 1994. Reproductive success of solitarily and communally nesting white-footed mice and deer mice. Behavioral Ecology 5(2), 206-209.

Wolff, M.C., Leander, J.D., 2002. Selective serotonin reuptake inhibitors decrease impulsive behavior as measured by an adjusting delay procedure in the pigeon. Neuropsychopharmacology 27(3), 421-429.

Wolmarans, D.W., Brand, L., Stein, D.J., Harvey, B.H., 2013. Reappraisal of spontaneous stereotypy in the deer mouse as an animal model of obsessive-compulsive disorder (OCD): Response to escitalopram treatment and basal serotonin transporter (SERT) density. Behavioural Brain Research 256, 545-553.

Wolmarans, D.W., Stein, D.J., Harvey, B.H., 2016a. Of mice and marbles: Novel perspectives on burying behavior as a screening test for psychiatric illness. Cognitive, Affective, & Behavioral Neuroscience 16(3), 551-560.

Wolmarans, D.W., Stein, D.J., Harvey, B.H., 2016b. Excessive nest building is a unique behavioural phenotype in the deer mouse model of obsessive–compulsive disorder. Journal of Psychopharmacology 30(9), 867-874.

ADDENDUM A

Letters of Permission from Co-Authors to Submit Chapter 3 for Examination Purposes

Department of Psychiatry and Mental Health

Professor **Dan J Stein, FRCPC, PhD, DPhil**
Head: Department of Psychiatry and Mental Health, UCT & GSH
Email: dan.stein@uct.ac.za
UCT Dept of Psychiatry and Mental Health, Groote Schuur Hospital (J-2), Anzio Rd,
Observatory 7925, Cape Town, South Africa
Phone: +27 21 4042137 Fax: +27 21 4488158
URL: http://www.health.uct.ac.za/departments/psychiatry/about/

The Director: Higher Degrees Administration
North-West University
11 Hoffman Street
Potchefstroom
2520
SOUTH AFRICA

Dear Sir/Madam,

RE: CO-AUTHOR PERMISSION TO SUBMIT CHAPTER 3 OF THIS DISSERTATION FOR EXAMINATION PURPOSES

Hereby I, Dan Stein, from University of Cape Town, a co-author of the manuscript titled *"Deer mouse nesting behaviour in a novel anxiogenic environment and its response to serotonergic and benzodiazepine intervention: pre-clinical insights into compulsive-like behaviour and risk-taking"*, which is included in Chapter 3 of this dissertation, give permission for this work to be submitted for examination purposes.

Dan J Stein

UNIVERSITEIT•STELLENBOSCH•UNIVERSITY
jou kennisvennoot • your knowledge partner

11 November 2020

The Director: Higher Degrees Administration
North-West University
11 Hoffman Street
Potchefstroom
2520
SOUTH AFRICA

Dear Sir/Madam,

RE: CO-AUTHOR PERMISSION TO SUBMIT CHAPTER 3 OF THIS DISSERTATION FOR EXAMINATION PURPOSES

Hereby I, Prof Soraya Seedat, University of Stellenbosch, a co-author of the manuscript titled *"Deer mouse nesting behaviour in a novel anxiogenic environment and its response to serotonergic and benzodiazepine intervention: pre-clinical insights into compulsive-like behaviour and risk-taking",* which is included in Chapter 3 of this dissertation, give permission for this work to be submitted for examination purposes.

Regards,

Soraya Seedat
Distinguished Professor and Executive Head of Department

Medicine and Health Sciences

Geneeskunde en Gesondheidswetenskappe Ezo

Nyango nezee Nzululwazi kwezeMpilo

Departement Psigiatrie ▪ Department of Psychiatry
Posbus / PO Box 241 • Kaapstad / Cape Town • 8000 • Suid-Afrika / South Africa
Francie van Zijl-rylaan / Drive, Tygerberg, 7505, Suid-Afrika / South Africa
Tel: +27 21 938 9227/9116 · Faks / Fax: +27 21 938 9738

NWU®
NORTH-WEST UNIVERSITY
NOORDWES-UNIVERSITEIT
YUNIBESITI YA BOKONE-BOPHIRIMA

The Director: Higher Degrees Administration
North-West University
11 Hoffman Street
Potchefstroom
2520
SOUTH AFRICA

11 November 2020

Dear Sir/Madam,

RE: CO-AUTHOR PERMISSION TO SUBMIT CHAPTER 3 OF THIS DISSERTATION FOR EXAMINATION PURPOSES

Hereby I, Dr. Geoffrey de Brouwer, North-West University, Post-doc, and co-author of the manuscript titled *"Deer mouse nesting behaviour in a novel anxiogenic environment and its response to serotonergic and benzodiazepine intervention: pre-clinical insights into compulsive-like behaviour and risk-taking"*, which is included in Chapter 3 of this dissertation, give permission for this work to be submitted for examination purposes.

Regards,

Dr G de Brouwer

North-West University, Potchefstroom, South Africa

ADDENDUM B

Additional details pertaining to the methodology followed

This addendum contains supplementary detailed insights into the methodology followed in this work.

This Addendum should therefore be read in oversight with Chapter 3.

Layout of groups for the behavioural investigation

Eight groups of deer mice (n = 10 for groups 1-6; n = 8 for groups 7 and 8; see below) were screened for nest building behaviour over 7 consecutive days. Since all drugs were administered in the drinking water and to establish and confirm drug intake, each animal subsequently received normal drinking water for 7 days and were afterwards exposed to their respective treatments for 32 days (either 28 days of escitalopram exposure and a further 4 days of open field experiments while still being exposed to escitalopram or 28 days of receiving water, with 4 days of lorazepam exposure while being assessed in the open field). Groups 7 and 8 did not receive any drug intervention (see below).

Since deer mice were bred gradually, investigations were completed in a phased manner. **Table C1** provides a summary of the dates during which different groups were selected, drug exposed and assessed in the open field.

TABLE C1 – LAYOUT OF DIFFERENT GROUPS AND DATES

Start Date	End date	Group tested	Group description
04/02/2020	31/03/2020	Group 1 (n = 8) Group 4 (n = 5)	LNB[1] Control NNB[2] Control
21/03/2020	06/05/2020	Group 1 (n = 2) Group 2 (n = 7)	LNB Control LNB Escitalopram
28/03/2020	16/05/2020	Group 4 (n = 5) Group 5 (n = 4)	NNB Control NNB Escitalopram
05/04/2020	30/05/2020	Group 5 (n = 6) Group 8 (n = 1)	NNB Escitalopram NNB Control (No Wool)
09/04/2020	03/06/2020	Group 2 (n = 3) Group 7 (n = 8)	LNB Escitalopram LNB Control (No Wool)
18/05/2020	03/07/2020	Group 3 (n = 10) Group 6 (n = 4)	LNB Lorazepam NNB Lorazepam
29/06/2020	14/08/2020	Group 6 (n = 6) Group 8 (n = 8)	NNB Lorazepam NNB Control (No Wool)

[1] large nest building
[2] normal nest building

Daily routine during the behavioural investigation

- On each morning throughout the investigation, nest building assessment, water bottle weight measurement and/or drug solution replacement was conducted.
- Thereafter the following parameters were recorded: 1) temperatures of the housing and experimental (open-field) rooms, 2) humidity of both rooms, and 3) food and water or drug availability.
- Deer mice in breeding, rearing or experimental housing cages were monitored every day to confirm health and wellbeing. For the remainder of each day, left undisturbed in their home cages.
- Once a week, cages were cleaned and fresh corncob, water and paper towel provided. Cages were cleaned with F10® SCXD Veterinary Disinfectant/Cleanser solution (Reg No Act 29 GNR 529/29990/040/150; DAFF Registration Number: G3073).
- On days during which open field assessment took place, identified mice were moved from the housing room to the experimental room at 17:30. Since the light cycle was set at 06:00/18:00, mice were habituated in the experimental room for 30 minutes prior to the onset open field assessment. Mice were introduced to the dark compartment of the open field at 18:00 and arenas covered with clear Plexiglas® to prevent animals from leaving the arenas during the night. This process was repeated four times for each mouse.
- On each morning of the days on which open field assessment took place, mice were returned to their home cages and left to rest for the remainder of the day. Built nests (if applicable) were photographed and removed, while the remaining cotton wool in both the internal and external hoppers was weighed. Arenas were cleaned and polished and prepared with new pre-weighted cotton wool (if applicable), food and water (or drug).
- Drug exposed animals received drug solutions both in the home cage and open field arenas so as not to interrupt exposure.

Additional information pertaining to animal husbandry, housing, and care

- Prior to onset of the study, animals were bred and supplied by the North-West University (NWU)[1] vivarium, Potchefstroom, South Africa (SAVC[2] registration number: FR15/13458; SANAS[3] GLP compliance number: G0019; AAALAC accreditation file: 1717).
- Mice were weaned at the age of 21 days, and group housed (4-6 same sex animals per cage; 35 cm (l) x 20 cm (w) x 13 (h) cm; Tecniplast® S.P.A., Varese, Italy), until selection for further study.
- Prior to the start of the baseline investigation, mice between the ages of 10-12 weeks were single housed in identical cages and remained housed so for the remainder of the study.
- All food (mouse pellets; Labchef; Nutritionhub (PTY) LTD. Reg nr: 2012/141960/07) were autoclaved at 90°C for 15 minutes before use.

Additional information pertaining to drug administration

- Escitalopram oxalate (50 mg/kg/day; Wolmarans et al. (2013)) and lorazepam (2 mg/kg/day; Fahey et al. (2006)) were prepared for oral administration via the drinking water.
- Escitalopram oxalate was weighed on a microbalance and added to the appropriate volume of diluent (Milli-Q→ ultrapure water), shaken and sonicated until it was fully dissolved. This process was repeated every alternative day. The final concentration of the drug solution was 32 mg escitalopram oxalate / 100 ml water. This equals 20 mg of free escitalopram base per 100 ml water.
- Lorazepam was reconstituted in drinking water from a parenteral formula. 0.8 mg lorazepam were syringed and constituted in 100 ml of water.
- Drug concentrations were based on the average fluid intake of deer mice, i.e. 0.25 ml/g/day.
- Fresh drug solutions were made every other day.
- Every day, water bottles were weighed to confirm drug intake (**Figure C-1**). Since all animals demonstrated a modest increase in fluid intake throughout all groups once drugs were introduced to drinking solutions, all animals received at least the minimum required concentration of drug per day. That said, once treatment administration started in smaller liquid volumes, leakage from water bottles increased by up to 1.2ml per day, likely due to

[1] North-West University
[2] South African Veterinary Council
[3] South African National Accreditation System

alterations in capillary properties (Lambert and Delchambre, 2005) due to the lower volume of liquid added to the water bottles.

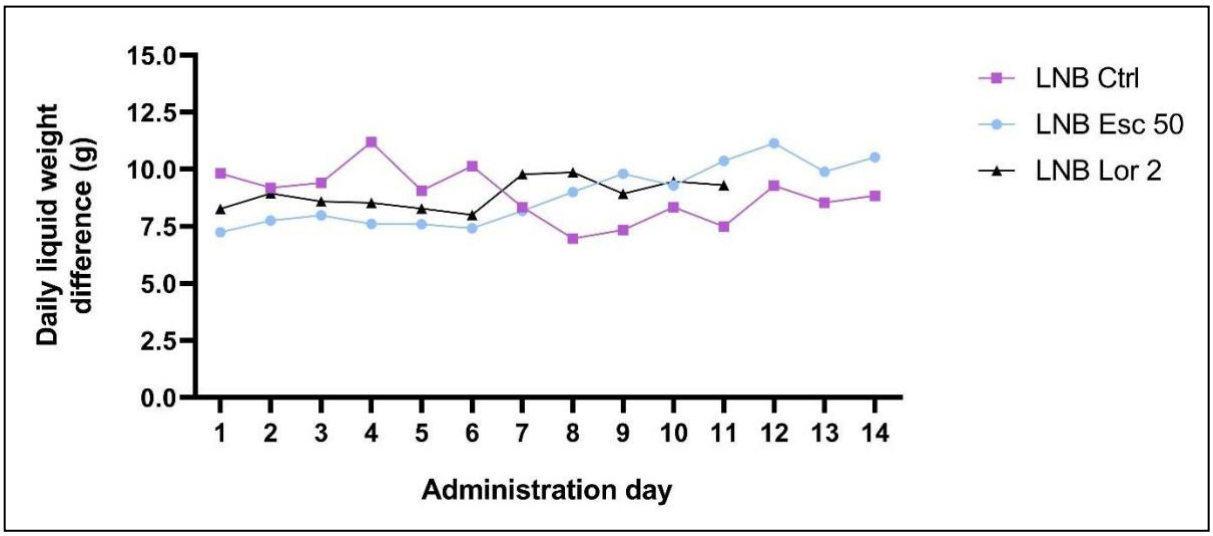

FIGURE B-1 - Fluid intake data of mice receiving either normal water (days 1 – 7, black and blue lines or days 1 – 14, purple line) and escitalopram (days 8 – 14, blue line), and lorazepam (days 8 – 11, black line). Data are representative of the mean average daily fluid intake of 10 mice per group. One-way repeated measures analysis of variance (RM-ANOVA)[1] revealed no significant differences in the average fluid intake per group. LNB: large nest building.

[1] repeated measures analysis of variance

Supplementary images

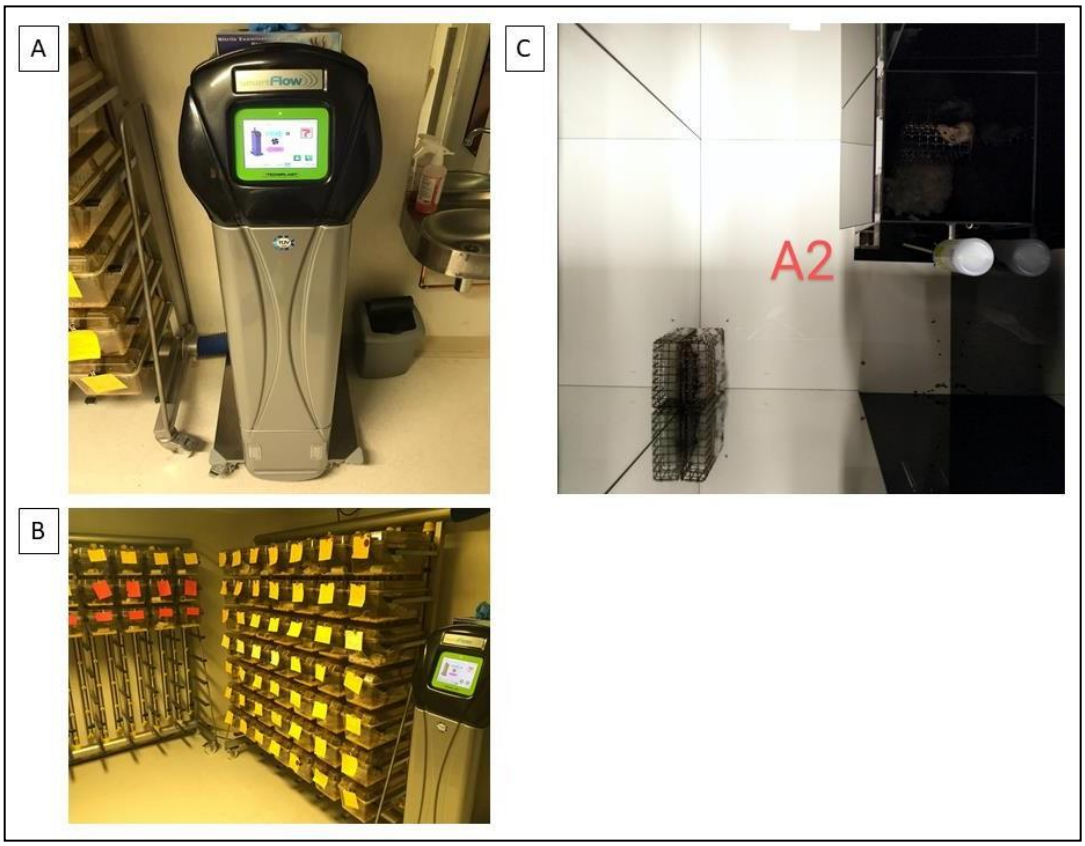

Figure B-2 - (A): Tecniplast® Smart Flow for monitoring of ventilation, humidity and temperature in the cages; (B): Deer mice housing system (cages: 35 cm (l) x 20 cm (w) x 13 (h) cm; Tecniplast® S.P.A., Varese, Italy) according to standard laboratory conditions. Each cage was marked according to the different groups (breeding pairs, group-housed or single housed) for the identification of animals; (C): The mirror chamber (after the 12-hour experimentation) with the dark box, steel mesh-grids for the provision of cotton wool, water bottle and food container

References

Fahey JM, Pritchard GA, Reddi JM, et al. (2006) The effect of chronic lorazepam administration in aging mice. Brain Res 1118(1): 13-24.

Lambert P and Delchambre A (2005) Parameters ruling capillary forces at the submillimetric scale. Langmuir 21(21): 9537-9543.

Wolmarans DW, Brand L, Stein DJ, et al. (2013) Reappraisal of spontaneous stereotypy in the deer mouse as an animal model of obsessive-compulsive disorder (OCD): Response to escitalopram treatment and basal serotonin transporter (SERT) density. Behavioural Brain Research 256(0): 545-553.

* * *

END OF DISSERTATION

* * *