Nest building and marble burying: Dopaminergic phenotyping of the deer mouse model of obsessive-compulsive disorder (OCD)

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In Memory of Arina Fick (van der Merwe),

Ouma Sannie Coetzee,

Oom Johan “Smiles” Coetzee

Preface

I can do all things through Him who gives me strength.

Philippians 4:13

I find myself at the end of what was both the best, and the worst, two years of my life. Reaching this point was a monumental undertaking, one that I would not trade for the world. During my experiences over the past two years I met amazing people and made friends I will keep for the rest of my life; for the first time I felt like I was among likeminded people and that I had found a path worth pursuing. Though there were many ups and downs, unexpected crises, and great personal losses, this meant the world to me and would not have been possible without help.

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of rock climbing. You are a gentleman, which is a rare quality, and I know you will become a rock-star in the world of neuroscience.

My fellow postgraduates, Johané Gericke, Heslie Loots, Carmen Pieters, and Cailin van Staden, Mandi Hamman, Joné Pienaar, and Isma Scheepers: thank you for the past two years, for your friendship and support, for the laughs and the tears, the mad shenanigans, the late nights, the ducks, and an unforgettable time. Without you, this would have been a wholly different experience. You accepted me with all my flaws, I love you all, and wish you only the best in the years to come.

Prof Brand, thank you for giving me this chance, thank you for your warm heart and kind words. Thank you for all Prof's support over the past two years, for always being available to listen and to share your wealth of knowledge, and thank you for getting me interested in the central nervous system!

Prof Harvey, thank you for your insight and assistance on my study, it is truly humbling to have an international expert provide their input. Thank you for or the valuable notes and changes on this dissertation, as well as for the lifetime opportunity of attending the Biological Psychiatry Congress this year; it motivated me to further pursue a career in research, and was a mind-blowing experience to hear the greatest minds in psychiatry speak.

Prof Stein, though I have not met you in person, thank you for the honour of collaborating on my study. Your input was valuable and very much appreciated, and it means a lot that such a highly esteemed scientist in the field of OCD had collaborated on this study.

Thank you to all the staff at the vivarium for the care of the animals, Mrs Antoinette Fick in particular.

And last, but certainly not least (which is why I left this one for last): Dr De Wet Wolmarans, I owe everything to you. Thank you for giving me this opportunity of a lifetime, for taking me on as a student and taking a chance on me. It means more than I can put into words, you changed my life. At the end of 2017 I was lost and frightened, unsure of the future and what I wanted to do with my life, you have not only saved me, you also gave me a future worth getting excited for. Thank you for your never-ending support, care, and wisdom, for putting in more hours in a day than is humanly possible, and for your enormous heart. Dr, you are a genius and I can never hope to be your equal, but it is truly an honour to learn at the master's feet, your enthusiasm is contagious, and you always have a solution if a crisis pops up. Thank you, a thousand times, for being the best mentor I could have asked for and God bless you.

Congress proceedings

The results of the current investigation were presented at the Biological Psychiatry Congress (SANS Symposium), Century City, Cape Town, September 2019. The presenting author is underlined:

a) *ANE LOMBAARD, DAN J. STEIN, BRIAN H. HARVEY, DE WET WOLMARANS (2019): Large nest building and high marble burying in the deer mouse (Peromyscus maniculatus bairdii) and their response to serotonergic, anti-dopaminergic and combination intervention. Poster.*

Publications

Additional work by the candidate that contributed to the conceptualization of this dissertation (Addendum B):

GEOFFREY DE BROUWER[1], ARINA FICK[1], ANÉ LOMBAARD[1], DAN J STEIN[2,3], BRIAN H HARVEY[1,2], DE WET WOLMARANS[1] (2019) Large nest building and high marble-burying: two compulsive-like phenotypes expressed by deer mice (Peromyscus maniculatus bairdii) are distinctly regulated by serotonergic and dopaminergic intervention. To be submitted for publishing online in the Journal of Psychopharmacology.

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Abstract

Obsessive-compulsive disorder (OCD[1]) is a chronic, debilitating psychiatric condition that affects 3% of the global population and is characterized by obsessions, and compulsions. 40 – 60% of patients remain treatment-resistant to first-line treatment with selective serotonin reuptake inhibitors (SSRI's[2]). There is evidence to indicate that the different phenotypes of OCD differ in their underlying neurobiology, specifically with regards to differences in dysfunction of the cortico-striatal-thalamo-cortical (CSTC[3]) circuit, which could explain the varying treatment resistance in patients. The CSTC-circuit is well documented to play a key role in the epidemiology of OCD. There are only a handful of pre-clinical studies that attempt to investigate the neural underpinnings of OCD, likely due to the lack of animal models that are representative of symptom heterogeneous obsessive-compulsive-like behaviour. In this regard, the different naturalistic compulsive-like phenotypes exhibited by deer mice, large nest building (LNB[4]) and high marble burying (HMB[5]), have proved useful for the purpose of this investigation. These behaviours are equally persistent, repetitive and seemingly purposeless, and are thus reminiscent of OCD symptomology. Moreover, LNB demonstrates therapeutic response to chronic high dose (50 mg/kg/day) oral SSRI treatment, while HMB remains refractory to such intervention.

Therefore, the current investigation aimed to establish whether HMB and LNB may be founded within unique neurobiological processes as explored by means of pharmacological manipulation. Specifically, we hypothesized that LNB will be largely unresponsive to anti-dopaminergic and combination treatment, whereas HMB will respond to anti-dopaminergic treatment alone or in combination with escitalopram. Thus, the purpose of this study was to explore the differences in treatment-response of the two aforementioned compulsive-like behavioural phenotypes expressed by deer mice, i.e. LNB and HMB, by means of pharmacological intervention with either an SSRI alone, i.e. escitalopram, a low-dose anti-dopaminergic drug, i.e. flupentixol, and a combination of the two.

160 deer mice, male and female, were initially screened for marble burying behaviour by conducting the marble burying test (MBT[6]). Briefly, marble-burying cages consisted of nine glass marbles placed at equal distances from one another on a 5 cm layer of coarse river

[1] obsessive-compulsive disorder
[2] selective serotonin reuptake inhibitors
[3] cortico-striatal-thalamo-cortical
[4] large nest building
[5] high marble burying
[6] marble burying test

sand. A two-zone paradigm was followed, i.e. the marbles were placed in one half of the cage only. The screening consisted of a 30-minute session per mouse over three consecutive nights. Manual scoring of the marble directed behaviour (MDB[1]) took place post-screening. Next, all animals were screened for nest building behaviour by providing them with an excess of pre-weighed cotton wool every day for 7 consecutive days. On each morning, the built nests were removed and the remaining cotton wool weighed.

Animals were assigned to either of the two cohorts (LNB[2]/HMB[3]). HMB was defined based on the total number of marbles buried and the consistency of burying behaviour over three nights. LNB was defined based on the total quantity of cotton wool used over 7 days and the consistency of nest sizes over the separate trials. Following initial screening, the cohorts were divided into four treatment groups (n=6 per group), namely water, escitalopram (50 mg/kg/day), flupentixol (0,9 mg/kg/day), or a combination of the two drugs. The animals were treated for 28 days, after which post-treatment screening took place in the respective cohorts as described above.

Our results demonstrate a significant post-treatment reduction in the *number of marbles* buried in the control group only ($p = 0.007$; d = 2.5). However, with respect to MDB, there was an overall statistically significant effect of time on the behavioural response observed following four weeks of treatment ($p = 0.0001$). Hence, although escitalopram seemed to reduce the MDB exhibited by HMB animals, this effect was masked by the influence of time-based adaptation. In contrast, while the LNB behaviour of control-treated animals exacerbated over time ($p = 0.025$), escitalopram reduced the average total nest size over time ($p = 0.2$; d = 2.0), while such reduction in nest size was less robust for the combination treatment ($p = 0.98$; d = 0.5). Flupentixol alone had no effect.

While the LNB results were expected, the observations in HMB expressing animals would indicate that HMB may be representative of a highly treatment-resistant behavioural phenotype that should be interrogated in terms of its underlying neurobiology. Indeed, future exploration of HMB may potentially provide significant insight into the mechanisms underlying treatment-resistant persistent behaviours. LNB, on the other hand, appears to represent the classic, serotonergic model of OCD[4]. This confirms our hypothesis that LNB and HMB differ in underlying neurobiology.

[1] marble direct behaviour
[2] large nest building
[3] high marble burying
[4] obsessive-compulsive disorder

Keywords: Obsessive-compulsive disorder; marble burying; nest building; animal model; escitalopram; flupentixol; deer mouse model

Solemn declaration: I, Ané Lombaard (25087657), herewith declare that this dissertation is my own work and that no part thereof has been copied from other sources.

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1. Introduction

1.1 Dissertation approach and layout

This dissertation has been prepared in article format according to the requirements of the North-West University (NWU[1]). This implies that the main body of the work is presented in the form of a journal article that will be submitted for publication following input from the co-authors. The journal for which the work is intended is *Journal of Psychopharmacology*.

The complete dissertation will however consist of four chapters. Chapter 1 provides a brief literature background, the problem statement, working hypothesis and experimental layout. Chapter 2 comprises a review of applicable literature, while Chapter 3 contains the proposed journal article. Chapter 4 concludes the dissertation with a brief overall summary of the literature, the methods followed and the main findings of the current investigation. The addendums contain A) the letters of permission of all co-authors to submit Chapter 3 for examination purposes, and B) the complete and combined manuscript comprising the work of both A. Lombaard (this dissertation) and A. Fick (2018), with the assistance of Geoffrey de Brouwer.

The scientific manuscript (Chapter 3) was prepared in accordance with the "Instructions to Authors" provided by the Journal of Psychopharmacology (link provided at the beginning of Chapter 3).

[1] North West University

1.2 Problem statement

Obsessive-compulsive disorder (OCD[1]) is prevalent in 1.2 – 2.3% of the world's population (Ruscio et al., 2010) and is ranked among the top ten most debilitating psychiatric conditions (Veale and Roberts, 2014). OCD severely impairs the social and occupational functioning, and normal daily routines of patients. The condition is time consuming (rituals can take up an hour or more a day), while often causing significant anxiety (Ruscio et al., 2010). Furthermore, patients present with an incidence of comorbid conditions such as depression and anxiety, while often also reporting suicidal ideation (Angelakis et al., 2015, Ruscio et al., 2010). Broadly speaking, five different phenotypes of OCD symptomology have been identified, i.e. symptoms related to themes of contamination/washing (C/W[2]), safety/checking (S/C[3]), obsessive thoughts, symmetry/ordering (S/O[4]), and hoarding (Abramowitz et al., 2009, Markarian et al., 2010, Mataix-Cols et al., 2004, Mataix-Cols et al., 2005).

Only 40 – 60% of patients respond favourably to current first-line pharmacotherapeutic strategies, i.e. selective serotonin reuptake inhibitors (SSRIs[5]; Husted and Shapira, 2004, Brakoulias and Tsalamanios, 2017), while cognitive behavioural therapy (CBT[6]) has reported up to 83% successful alleviation of symptoms (Abramowitz, 1996). Strategies for treatment refractory OCD include increasing the dose of the SSRI used, switching to another SSRI, or augmenting SSRI treatment with a low-dose anti-dopaminergic intervention (Albert et al., 2013, Atmaca, 2016). As of yet, the specific details concerning the aetiology of OCD remain unknown, although progress has been made in this regard.

Evidence from several clinical studies suggest that dysfunctions in the cortical-striatal-thalamo-cortical (CSTC[7]) circuitry may be associated with obsessive-compulsive symptomology (Figee et al., 2011, Husted and Shapira, 2004, Husted et al., 2006, Stein et al., 2000). In brief, the CSTC circuitry can be regarded as a pivotal control unit for a vast array of cognitive processes (Calabresi et al., 2014, Lobo and Nestler, 2011, Lüscher and Malenka, 2011, Nestler, 2013, van Huijstee and Mansvelder, 2015). Two of the neurotransmitters intimately involved in the functioning of the CSTC circuit are dopamine and serotonin. The role of serotonin in OCD is well established due to the varying treatment response observed following the chronic administration of SSRIs; in fact, OCD is often regarded as a condition of hypo-serotonergic signalling; however, its exact role in the pathology of OCD remains

[1] obsessive-compulsive disorder
[2] contamination/washing
[3] safety/checking
[4] symmetry/ordering
[5] selective serotonin reuptake inhibitors
[6] cognitive behavioural therapy
[7] cortical-striatal-thalamo-cortical

unknown (Murphy et al., 2004, Murphy Dennis et al., 2013, Nonnis Marzano et al., 2008, Sinopoli et al., 2017). A large body of evidence also indicates that dopamine may play a key role in the propagation of obsessive-compulsive symptomology. Indeed, recent evidence supports the hypothesis that different phenotypes of OCD[1] present with unique neurobiological signatures (Figee et al., 2011, Mataix-Cols et al., 2004, Rauch et al., 2007). For example, patients with the C/W[2], but not the S/C[3] phenotype has been shown to present with attenuated reward-anticipatory dopaminergic signalling in the ventral striatum, while these individuals also seem to act in a more impulsive manner; such findings are indicative of the unique involvement of dopamine in these two symptom cohorts (Figee et al., 2011). As this idea forms the basis of the current work, a closer look at the neurobiological mechanisms underlying reward feedback processing is necessary. Briefly, two theories have been proposed which attempt to explain how individuals recruit neurobiological mechanisms to process external feedback, i.e. the theory of phasic dopaminergic release (Schultz et al., 1993, Schultz et al., 1997, Schultz, 2002, Schultz, 2007) and the theory of dopaminergic and serotonergic opponency (Daw et al., 2002, Cools et al., 2011, Boureau and Dayan, 2010). While the former explains reward and punishment learning on the basis of phasic increases and decreases in dopaminergic signalling respectively (Schultz et al., 1993, Schultz et al., 1997, Schultz, 2002, Schultz, 2007), the latter suggests that while dopamine is responsible for the coding of reward, serotonin likely acts as an opponent system by facilitating punishment learning (Daw et al., 2002). That said, while these two concepts are essentially congruent with respect to suggesting a dichotomous role for dopamine in reward and punishment learning, it has also been found that neither of these learning processes can optimally transpire in the absence of adequate serotonergic input (Palminteri et al., 2012). Conversely, while serotonin may be regarded as the functional opponent of dopamine, it can only fulfil this role in the relative absence of dopaminergic signalling. Therefore, that mono-therapeutic SSRIs[4] are often effective in the treatment of OCD can possibly be ascribed to an already reduced dopaminergic tone in OCD patients diagnosed with generally responsive phenotypes OCD. On the other hand, it stands to reason that dopamine can only adequately facilitate reward learning properly in combination with simultaneous serotonin release. It is in this principle that the current study is founded. In fact, it is likely that patients presenting with different obsessive-compulsive symptom phenotypes may indeed also present with unique dopaminergic processes underlying such symptomology. To date, this has been difficult to investigate in

[1] obsessive-compulsive disorder
[2] contamination/washing
[3] safety/checking
[4] selective serotonin reuptake inhibitor

animal models, likely due to the fact that most available models are only representative of a single, often SSRI sensitive compulsive-like phenotype (Alonso et al., 2015).

In this regard, the naturalistic repetitive and persistent behaviours expressed by subpopulations of the deer mouse *(Peromyscus maniculatus bairdii)* colony bred and housed in the Vivarium of the North-West University (NWU[1]), i.e. high stereotypy (HS[2]; expressed by 45% of the population), large nest building (LNB[3]; expressed by 30% of the population) and high marble burying (HMB[4]; expressed by 11 – 15% of the population) are all reminiscent of the compulsive-like symptoms of patients suffering from OCD[5] (Güldenpfennig et al., 2011, Wolmarans et al., 2016a, Wolmarans et al., 2016b). Moreover, while all of these behaviours are expressed irrespective of sex and seem to be equally purposeless under normal laboratory conditions, they are also expressed in an ebbing and flowing nature over the course of several assessment trials. Interestingly, while HS and LNB respond to chronic high-dose treatment with the SSRI[6], escitalopram (Wolmarans et al., 2016a, Wolmarans de et al., 2013), HMB seems to lack a therapeutic reaction to such intervention (Wolmarans et al., 2016b). Therefore, the present work aims to investigate whether two phenotypically different compulsive-like phenotypes expressed by deer mice, i.e. LNB and HMB, are associated with unique neurobiological footprints as inferred by means of pharmacological interventions that target the serotonergic and dopaminergic systems. Indeed, considering the literature summarised above, it is likely that HMB, being SSRI refractory, and LNB will respond differently to drugs targeting the dopaminergic system, either administered alone or in combination with escitalopram. If so, such observations should point to LNB and HMB being associated with distinct neurobiological footprints and provide a window onto our understanding of the neurocognitive mechanisms underlying treatment resistant OCD.

[1] North-West University
[2] high stereotypy
[3] large nest building
[4] high marble burying
[5] obsessive-compulsive disorder
[6] selective serotonin reuptake inhibitors

1.3 Study Hypothesis and Objectives

1.3.1 Hypothesis

We hypothesise that two phenotypically distinct compulsive-like behaviours expressed by deer mice, i.e. SSRI[1] sensitive LNB[2], and SSRI resistant HMB[3], will not only be confirmed to respond distinctly to chronic (28-day) high-dose oral escitalopram (50 mg/kg/day, Wolmarans et al., 2013), but that said behaviours can be distinguished on a neurobiological level based on the involvement of the dopaminergic system. More specifically, we hypothesise that as LNB demonstrates robust clinical response to SSRI monotherapy, it will remain unresponsive to low-dose (0.9 mg/kg/day) treatment with the dopamine $D_{1/2}$ receptor antagonist, flupentixol, administered either alone or in combination with escitalopram. On the other hand, as HMB seems to resemble an SSRI resistant phenotype, we hypothesize that such behaviour will respond to a combination of escitalopram and flupentixol, but not to flupentixol alone, thereby being representative of moderately treatment resistant OCD[4] which ultimately responds to SSRI and anti-dopaminergic augmentation therapy.

* * *

A note on the context in which this dissertation is presented: The work presented in this dissertation forms part of a larger project which was designed as a broad pharmacological interrogation of the role of the dopaminergic system, and by implication also the role of reward-related processes, underlying the expression of different compulsive-like phenotypes. As such, to achieve the outcomes of the larger investigation, which also included treatment groups that comprised the use of a dopaminergic potentiator, i.e. the monoamine oxidase B inhibitor, rasagiline, administered either alone or in combination, this project was divided into two separate phases which were conducted in parallel. Further, the findings of these two phases will be (have been) disseminated in two separate dissertations by two separate candidates. For examination purposes, the objectives of the full investigation will be provided here. However, for the perusal of the examiners, indications of the specific objectives addressed in each of the phases will be provided.

* * *

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

1.3.2 Study Objectives

From the literature summarized above and considering that the deer mouse model of OCD[1] may be a useful preclinical model for investigating symptom heterogeneous compulsive-like behaviour, this project aims to shed light on the neurobiology and mechanisms that may underlie different obsessive-compulsive phenotypes. More specifically we will:

1. Characterize the behaviour of deer mice with respect to its resemblance of symptom heterogeneous OCD, with special emphasis on identifying either HMB[2] or LNB[3] expressing subjects within the normal deer mouse population housed in the Vivarium of the NWU[4]; and

2. Employ distinct, chronic pharmacological interventions, administered via drinking water to determine whether such behaviours may indeed be associated with unique dopaminergic dysfunctions as shown in patients with different phenotypes of OCD. In the larger study, these interventions will aim to either bolster or inhibit dopaminergic responses alone or in combination with high dose SSRI[5] intervention in both behavioural cohorts, and will be structured as follows (*n* = 6 for all treatment groups in both behavioural cohorts):

    i. Escitalopram alone (50 mg/kg/day x 28 days, Wolmarans et al., 2013); **Findings reported in both the dissertations of **A. Fick (2018)** and **A. Lombaard (2019)***

    ii. Rasagiline alone (5 mg/kg/day x 28 days, Eigeldinger-Berthou et al., 2012); **Findings reported in the dissertation of **A. Fick (2018)***

    iii. Combined escitalopram (50 mg/kg/day) and rasagiline (5 mg/kg/day, Eigeldinger-Berthou et al., 2012) for 28 days; **Findings reported in the dissertation of **A. Fick (2018)***

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

iv. Flupentixol alone (0.9 mg/kg/day x 28 days, Murugaiah et al., 1983)
Findings reported in the dissertation of **A. Lombaard (2019)*

v. Combined escitalopram (50 mg/kg/day, Wolmarans et al., 2013) and flupentixol (0.9 mg/kg/day, Murugaiah et al., 1983) for 28 days;
Findings reported in the dissertation of **A. Lombaard (2019)*

vi. Normal water – control in all cohorts
*Findings reported in both the dissertations of **A. Fick (2018)** and **A. Lombaard (2019)**.*

* * *

A note regarding the choice to exclude animals expressing normal behaviour as an additional control group from the current study: The main focus of the current investigation was to assess whether aberrant compulsive-like behaviours, purportedly representing different obsessive-compulsive phenotypes, respond differentially to interventions that either bolster or inhibit dopaminergic signalling compared to its response to the relevant control treatments. As such, we did not include a normal behavioural control, as the only reason to do so would be to validate HMB[1] and LNB[2] as accurate frameworks in which to study OC-like behaviours. As this has been concluded before (Wolmarans et al., 2016a, Wolmarans et al., 2016b), it was decided that normal behaviour exhibiting subjects were not included in the current study design.

* * *

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

1.4 Project Layout

From this point forward, only those aspects of the study that are relevant for this dissertation, will be explained.

Please refer to Infogram 1 for a detailed summary of the study layout and procedures followed. Taking into account that only 11 – 15% of the deer mouse colony housed at the NWU[1] express HMB[2], all animals (160 in the initially screened group; 10 – 12 weeks of age at onset of experiments; both sexes) were screened for HMB behaviour (Wolmarans et al., 2016b). Further, to exclude the likelihood of a single animal presenting with both HMB and LNB[3], all animals were subsequently screened for nesting behaviour. Animals that presented with both or neither of the phenotypes, were excluded from further investigation and were either euthanized or used in studies not related to the current work. HMB and LNB expressing animals were then randomly divided into the four treatment groups (*n* = 6 per group) on the basis of behavioural expression only and treated for 28 days, where after the relevant behavioural analyses were repeated to determine the effect of the respective treatments.

[1] North-West University
[2] high marble burying
[3] large nest building

1.4.1 Detailed Study Layout

(A) Experimental pool selection:

- HMB[1] is expressed in approximately 11-15% and LNB[2] in 30% of the population.
- Male and female mice (160) sourced at random from the breeding colony maintained at the vivarium of the NWU
- Age: 10 – 12 weeks.
- Housing: single animal per cage from the onset of screening for HMB.
- Caging: individual ventilated cages, standard approved bedding (corncob), paper nesting material, food and water *ad lib*.
- Twelve-hour light/dark (06h00/18h00).
- Cleaning: once weekly.

(B) Screening for HMB:

- 3 MB[3] assessments per animal (see methodology section, Manuscript A; Chapter 3, and Addendum B).
- Light phase: dark i.e. after 18h00.
- Behavioural test cages: as home cages.
- Bedding: river sand.
- Cleaning: daily cleaning and autoclaving of burying substrate that have been reused.
- Screening protocol: 30 min test sessions, 1 day apart.

(C) Screening for LNB:

- 7 NB[4] trials per animal (see methodology section, Manuscript A; Chapter 3, and Addendum B).
- Light phase: nest building throughout dark phase, assessment during light phase ± 13h00
- Behavioural test cages: as home cages.
- Bedding: standard laboratory bedding (corncob).
- Nesting material: non-odorized cotton wool, no standard paper.
- Cleaning: once weekly; nests removed, and remaining cotton wool weighed daily
- Screening protocol: daily for 8 days, making for 7 nights of nesting assessment.

(D) Treatment phase:

- Only the 24 animals expressing HMB and LNB respectively of the initial pool of 160 are used. The rest are either used in other investigations as per ethically approved protocol, or euthanized.
- Both the HMB and LNB cohorts will undergo the following 28-day treatments (*n* = 6 per treatment group per behavioural cohort).
- Water
- Escitalopram 50 mg/kg/day
- Flupentixol 0.9 mg/kg/day
- Escitalopram 50 mg/kg/day + flupentixol 0.9 mg/kg/day

(E) Post-treatment behavioural testing:

- Repetition of (B) and (C) in treated animals

Infogram 1-1 Detailed project layout and summary of methods

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

1.5 Expected Outcomes

We expect both phases of the current project to contribute to elucidating the underlying neurocognitive constructs of phenotypically heterogeneous compulsive-like behaviours. Specifically, with respect to the aspects of work that are disseminated in the current dissertation, we expect that:

- Deer mice can be separated into cohorts expressing aberrant HMB[1] and LNB[2] behaviours;
- HMB, being SSRI[3] treatment resistant, will respond to a combination of chronic (28 day) escitalopram (50 mg/kg/day) and flupentixol (0.9 mg/kg/day), but not to either drug alone, thereby pointing to HMB being representative of clinical OCD that is sensitive to SSRI-anti-dopaminergic augmentation therapy; and
- LNB, being SSRI treatment sensitive, will respond to chronic (28 day) escitalopram, but not to flupentixol administered alone or in combination with escitalopram, thereby being representative of classic SSRI sensitive OCD.

[1] high marble burying
[2] large nest building
[3] selective serotonin reuptake inhibitors

1.6 Ethical Approval

The current investigation has been approved by the AnimCare Research Ethics Committee (NHREC reg. number AREC-130913-015) of the NWU[1] (approval number NWU-00262-16-A5) and has been completed by the researcher, Miss A. Lombaard, under supervision of the project supervisor, Dr P.D. Wolmarans. In accordance with the ethical approval procedure, we aimed to follow the ARRIVE-guidelines for animal experimentation as closely as possible by continuously refining the experimental protocol and reducing the sample sizes to the lowest number of animals per treatment group that were sufficient to address the research questions (Kilkenny et al., 2010).

All animals were bred and housed at the Vivarium (SAVC reg. number FR15/13458; SANAS GLP compliance number G0019) of the NWU, Potchefstroom campus. All procedures performed were done so in accordance with the code of ethics and complied with national legislation (South African National Standard for the Care and Use of Animals for Scientific Purposes; SANS 10386:2008).

[1] North-West University

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2 Literature Review

2.1 OCD in the clinic

2.1.1 Clinical symptomology and epidemiology

Obsessive-compulsive disorder (OCD[1]) is a chronic, clinically heterogeneous psychiatric condition. Originally classified as an anxiety disorder in the DSM[2]-IV; it has since been reclassified as the archetype disorder in a newly described class of disorders, i.e. "Obsessive-Compulsive and Related Disorders" (OCRDs[3]) in the DSM-V (American Psychiatric Association, 2013). The Yale-Brown Obsessive-Compulsive Scale (Y-BOCS[4]) serves as a diagnostic aid, as it provides a score based on a clinician rated 10-item scale accessing the severity of obsessions and compulsions; each item is rated from 0 (no symptoms) to 4 (severe symptoms) for a final score ranging from 0 to 40 (Goodman et al., 1989). OCD is characterised by the occurrence of both obsessions and compulsions where obsessions refer to involuntary, intrusive thoughts related to a number of topics, including contamination, images of a disturbing or disgusting nature, or unwanted urges such as harming others (American Psychiatric Association, 2013). On the other hand, compulsions are either overt or covert ritualistic behavioural patterns, usually aimed at reducing the level of distress caused by the experience of obsessions. Such routines are broadly related to the underlying obsessive theme, (Table 2-1) and often include excessive handwashing, grooming, checking, and praying (American Psychiatric Association, 2013). Importantly, obsessions can be separated from delusions based on the level of insight patients demonstrate; individuals suffering from OCD are fully aware that their symptoms and experiences are irrational, abnormal and disruptive; however, they are incapable of stopping and/or preventing engagement in such behaviour (American Psychiatric Association, 2013).

Data on the prevalence of OCD varies due to discrepancies recorded in different countries that result from differences in survey methodology and because of differences pertaining to the diagnosis of the condition (Ruscio et al., 2010, Wahl et al., 2010, Weissman, 1998). For example, diagnostic discrepancies can be accounted for by an underestimation of the severity of the condition (Ruscio et al., 2010, Wahl et al., 2010), resulting in OCD being overlooked by psychiatrists in up to 70% of patients (Wahl et al., 2010). It has been proposed that a major contributor to this dilemma is the high rates of diagnostic comorbidity observed in patients with

[1] obsessive-compulsive disorder
[2] Diagnostic and Statistical Manual of Mental Disorders
[3] obsessive-compulsive and related disorders
[4] Yale-Brown Obsessive-Compulsive Scale

OCD[1]. Indeed, 76% of patients also present with anxiety disorders, 63% with mood disorders, 56% with impulse control disorders, and 39% with substance use disorders (Ruscio et al., 2010). That said, the lifetime prevalence of OCD is widely accepted to be 2.3%, while a 12-month prevalence rate of 1.2% has been reported (Ruscio et al., 2010). The mean age of onset for OCD is 19 years, while early-onset OCD, i.e. before the age of 10 years, is more prevalent in males (Mathis et al., 2011). Otherwise, there appears to be no discrimination between sex in terms of symptom prevalence (Abramowitz et al., 2009, Pittenger et al., 2006).

The classic picture of OCD is representative of five broad phenotypes of obsessive-compulsive symptomology (Table 2-1). However, since the publication of the DSM[2]-V, hoarding has been awarded unique disorder status, albeit still categorized with the other obsessive-compulsive and related disorders, *viz.* OCD, trichotillomania, excoriation and body dysmorphic disorder (American Psychiatric Association, 2013). Of these, it has been shown that the contamination/washing (C/W[3]) phenotype is the most common (55%), followed by obsessive thoughts (especially of an aggressive (50%) and sexual (32%) theme), symmetry/ordering (S/O[4], 36%), and safety/checking (S/C[5], 34%) (Abramowitz et al., 2003, Markarian et al., 2010, Rasmussen and Tsuang, 1986).

Obsessive Theme	**Compulsive Rituals**
Contamination	Cleaning, handwashing, or grooming
Symmetry and order	Arranging and ordering objects, counting rituals
Being responsible for harming oneself or others	Checking, locking
Unwanted thoughts that can contradict the individual's beliefs and morals, such as urges to physically harm loved ones, to perform indecent sexual acts, obsessed with losing salvation	Repetitive praying, thinking "good thoughts", even self-punishment
Fears of losing objects	Collection compulsions and hoarding; however, see DSM-V

Table 2-1: Classification of obsessions and associated compulsive symptoms
(Adapted from Abramowitz et al., 2009, American Psychiatric Association, 2013)

Although patients have been shown to present with more than one of these symptom subtypes at a specific time, the phenotypic presentation of OCD remains relatively stable over time (Mataix-Cols et al., 2008). In other words, once a symptom (or symptoms) manifests, it is unlikely to be replaced by another symptom. Nevertheless, symptom phenotypes often seem to differ based on its persistence and patterns of comorbidity, prompting recent research into

[1] obsessive-compulsive disorder
[2] Diagnostic and Statistical Manual of Mental Disorders
[3] contamination/washing
[4] symmetry/ordering
[5] safety/checking

potential psychobiological differences underlying their expression. For example, Kichuk et al. (2013) reported that the symmetry and ordering phenotype, as opposed to symptoms of the intrusive thought phenotype, were less likely to wax and wane. Also, patients diagnosed with the S/C[1] phenotype appear to be at greatest risk for presenting with comorbid psychiatric conditions (Fullana et al., 2010), particularly anxiety disorders (Hasler et al., 2005), while those suffering from obsessions related to violence are more likely to present with comorbid post-traumatic stress disorder (PTSD[2], Hasler et al., 2005).

Importantly, not all compulsive rituals are related to an underlying obsession, and neither do all patients experience anxiety (Figee et al., 2016). In some cases, patients first present with a tendency toward engaging in compulsive behaviours, which are then followed by experiences of obsessive thoughts (Robbins et al., 2012). Also, compulsions can first manifest as inflated harm avoidance routines that gradually evolve to impulsive, habitual behavioural routines (Kashyap et al., 2012).

As most of the phenotypical content of OCD[3] is related to everyday themes, an accurate diagnosis of OCD depends on a number of criteria. First, either obsessions or compulsions, or as is most often the case, a combination of both must be present. Second, these symptoms must be time-consuming, taking up more than one hour per day, and must impair the normal functioning of the individual (American Psychiatric Association, 2013). Third, symptoms must not be aetiologically related to any other Axis I or II disorder and fourth, patients must demonstrate insight into the futility and irrationality of their symptoms (Rasmussen and Eisen, 1994).

2.1.2 Neurobiology

2.1.2.1 A neurobiology founded in cortico-striatal-thalamo-cortical involvement

Although the aetiology of OCD remains largely unknown, an abundance of evidence points to aberrant serotonergic and dopaminergic signalling, most notably within the cortico-striatal-thalamo-cortical (CSTC[4]) circuitry (Figee et al., 2011, Husted and Shapira, 2004, Husted et al., 2006, Stein et al., 2000). Importantly, although a causal relationship has not yet been established, patients with OCD invariably present with dysfunction in the brain structures that collectively constitute the CSTC pathways, i.e. the prefrontal cortex, striatum and thalamus (Figee et al., 2011, Husted and Shapira, 2004; Husted et al., 2006; Stein et al., 2000). Broken

[1] safety/checking
[2] post-traumatic stress disorder
[3] obsessive-compulsive disorder
[4] cortico-striatal-thalamo-cortical

down into its different components, the brain structures making up the CSTC[1]-circuit regulate cognitive planning and goal-directed motor behaviour (Stocco et al., 2010). In short, the prefrontal cortex is responsible for the top-down control of many higher order executive tasks, such as learning (Pasupathy and Miller, 2005, Antzoulatos and Miller, 2011), memory (Warden and Miller, 2010), categorising information (Antzoulatos and Miller, 2011, Freedman et al., 2001), cognitive flexibility (Clarke et al., 2004, Gruber et al., 2010, Rygula et al., 2010), strategic planning and inhibitory control (Chudasama et al., 2003, Dalley et al., 2011). In turn, the striatum, relaying signalling from the prefrontal cortex to the thalamus, controls neuroplasticity and voluntary actions (Calabresi et al., 2014, Lobo and Nestler, 2011, Lüscher and Malenka, 2011, Nestler, 2013, van Huijstee and Mansvelder, 2015). Divided into the dorsal and ventral striatum, it is responsible for the execution and regulation of goal-directed and habitual behaviour (Berke and Hyman, 2000, Everitt and Robbins, 2005, Pennartz et al., 2011) and decision making and reward-related behaviour (Schultz, 2007), respectively. Last, the thalamus mediates motivation and emotional drive, as well as planning for the expression of goal-directed behaviour (Haber and Calzavara, 2009, Jones, 2012). Taken together, dysfunctions in the aforementioned structures have all been associated with different neurocognitive processes underlying OCD[2], e.g. dysfunctional reward-based learning, behavioural disinhibition, and misinterpretation of perceived threats, all of which tie in with the cognitive theories that attempt to explain the condition (see paragraph 2.1.3). Whether the aetiopathology of OCD is limited to dysfunction in a specific component of the CSTC-circuit, or whether it is caused by deficits in the entirety of the CSTC-circuit, remains to be confirmed.

Nevertheless, the classic model of the CSTC points to two distinct relay circuits that propagate signalling via the basal ganglia, *viz.* the direct and indirect pathways. These have defined, opposing roles with regards to the functional processes that govern locomotion and motor responses, decision making, and motivation (Bateup et al., 2010, Valjent et al., 2009, Lobo and Nestler, 2011, Smith et al., 2013, Volkow et al., 2013). More specifically, the activation of the direct pathway is associated with the execution of prior cortically planned motor routines, while behavioural inhibition is associated with indirect pathway activity (Figure 2-1). Indeed, a functional bias in favour of the direct over the indirect pathway has been shown in individuals with OCD (Kravitz et al., 2012, Markarian et al., 2010, Rauch et al., 2007), which is believed to contribute to the excessive mental and behavioural routines observed in the condition (Abramowitz et al., 2009). The functional organization of the two pathways can briefly be described as follows (Figures 2-1 and 2-2):

[1] cortico-striatal-thalamo-cortical
[2] obsessive-compulsive disorder

- ***The direct pathway:*** In the dorsal striatum, cortical activation of medium spiny neurons causes a release of glutamate, which subsequently activate the substantia nigra pars reticulata (SNr[1]) and the globus pallidus interna (GPi[2]). However, this pathway, that predominantly expresses dopamine D_1 receptors, is tonically inactivated by gamma-aminobutyric acid (GABA[3])-ergic signalling to the SNr. Therefore, upon activation, medium spiny neurons from the ventral part of the striatum that also express GABA[4] receptors, apply an inhibitory tone on the said GABAergic neurons of the SNr[5], ultimately leading to disinhibition of the direct pathway and its glutamatergic projections to the thalamus, initiating the thalamo-cortical execution of complex cognitive and motor functions. In other words, a planned functional routine is relayed via the direct pathway through the basal ganglia and thalamus, and ultimately executed via activation of the prefrontal cortex, thereby closing the direct CSTC[6] loop. It is the execution of such plans, that ultimately leads to a sense of task completion and the inactivation of the direct pathway. This is, among others, an important construct that has been shown to be dysfunctional in patients with OCD[7].
- ***The indirect pathway:*** In contrast, the dorsal striatum also activates medium spiny neurons that project *indirectly* to the SNr via the globus pallidus externa (GPe[8]) and the sub-thalamo nuclei (STN[9]). This pathway, that primarily expresses D_2 receptors, is also, if not activated, tonically inhibited. Upon activation, the indirect pathway inhibits the GABAergic neurons of the GPe, resulting in disinhibition of its glutamatergic projections to the STN. Said activation of excitatory projections from the STN in turn activates the inhibitory GABAergic neurons projecting to the SNr, thereby causing net inhibition of the both the thalamus and the prefrontal cortex, preventing the execution of planned behaviours.

From the above, two important aspects become evident. First, it is clear that a bias in favour of the behaviourally activating direct pathway over the indirect pathway could potentially be associated with the persistent, repetitive and inflexible engagement in the compulsive-like routines observed in patients with OCD. Second, upon cortical activation of the striatum, both the direct and indirect pathways are activated simultaneously. Therefore, if the former facilitates the execution of behavioural routines, and the latter inhibits the same processes,

[1] substantia nigra pars reticulata
[2] globus pallidus interna
[3] gamma aminobutyric acid
[4] gamma aminobutyric acid
[5] substantia nigra pars reticulata
[6] cortico-striatal-thalamo-cortical
[7] obsessive-compulsive disorder
[8] globus pallidus externa
[9] sub-thalamo nuclei

our neurobiology is faced with a conundrum. Indeed, how are planned routines then executed? The answer to this question lies in the differential expression of D_1 and D_2 receptors in the respective pathways, a concept that we will return to in paragraph 2.1.2.2 below.

Not only is the CSTC-circuitry vital for the planning and execution of voluntary behaviours, it also plays a significant role in the processing of and responding to rewarding (direct pathway) and punishing (indirect pathway) feedback (Yager et al., 2015); dysfunctions in both of these processes have also been implicated in OCD[1], as will be highlighted later.

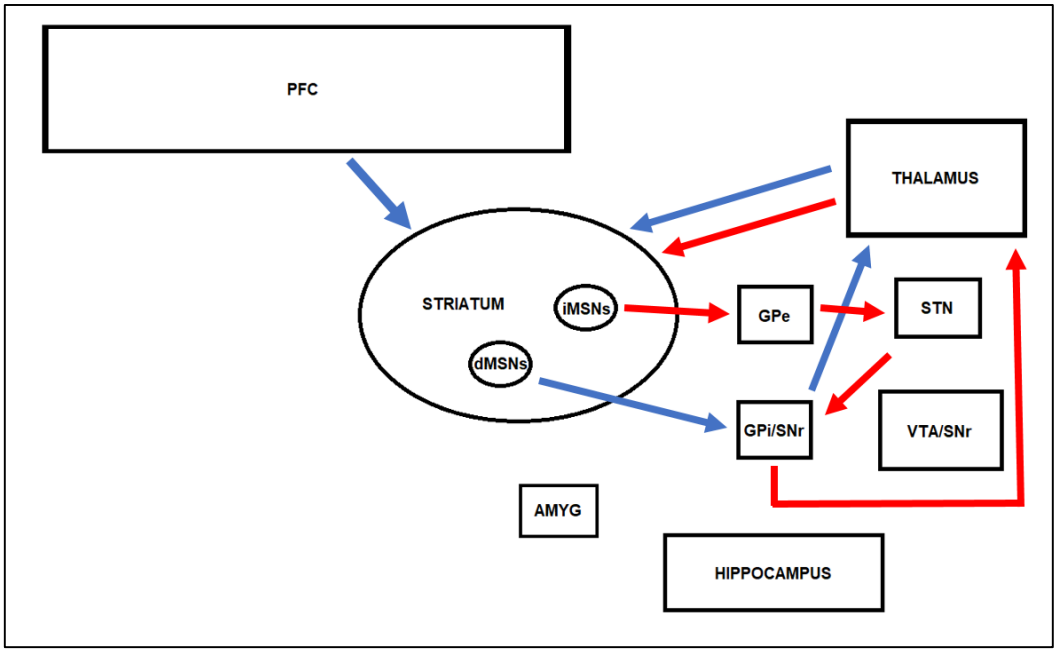

Figure 2-1: Simplified schematic of the direct and indirect pathways

Blue line: direct pathway; red line: indirect pathway; dMSN and iMSN: direct pathway and indirect pathway medium spiny neurons respectively; PFC: prefrontal cortex; GPe: globus pallidus externa; GPi: globus pallidus interna; SNr: substantia nigra pars reticulata; STN: subthalamo nuclei; VTA: ventral tegmental area; AMYG: amygdala.
Adapted from Yäger et al. (2015)

[1] obsessive-compulsive disorder

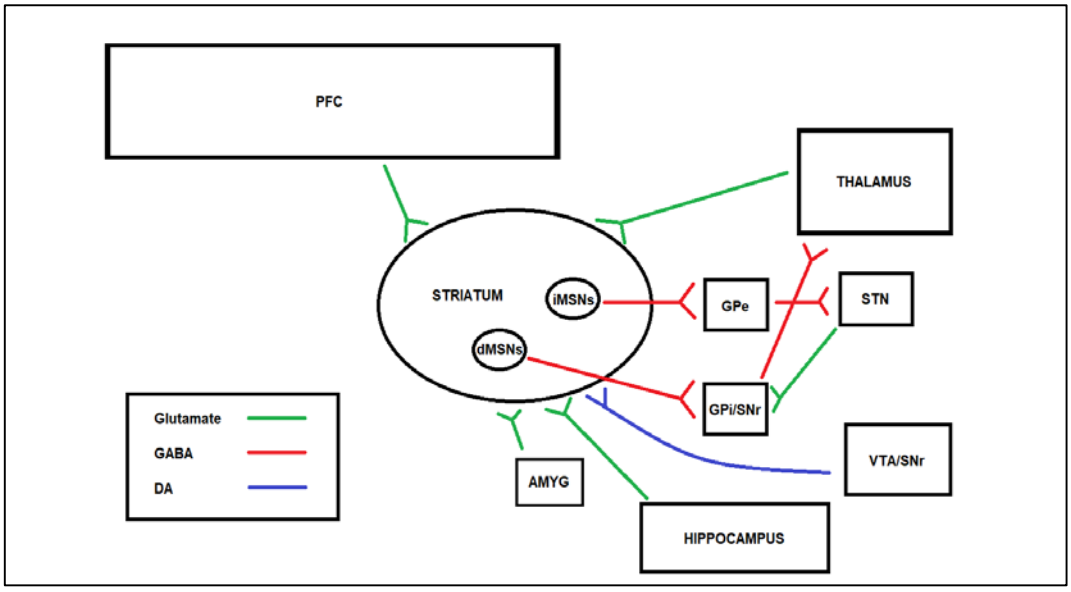

Figure 2-2: Simplified schematic of striatal inputs and outputs

Green line: glutamatergic inputs; blue line: dopaminergic inputs; red line: inhibitory projections; dMSN and iMSN: direct and indirect pathway medium spiny neurons respectively; PFC: prefrontal cortex; GPe: globus pallidus externa; GPi: globus pallidus interna; SNr: substantia nigra pars reticulata; STN: subthalamo nuclei; VTA: ventral tegmental area; AMYG: amygdala.
Adapted from Yäger *et al.* (2015)

2.1.2.2 Cortico-striatal interplay between dopamine and serotonin

As alluded to earlier, OCD[1] is invariably characterized by aberrant dopaminergic and serotonergic processing in the CSTC[2] circuitry. In this paragraph, a brief overview of the functional interaction between these two neurotransmitters will be provided.

Dopamine and serotonin, two fundamental neurotransmitters within the central nervous system, are generally accepted to function as opposing role players in behavioural regulation (Daw et al., 2002, Cools et al., 2008, den Ouden et al., 2013). Whereas dopamine is classically regarded to be responsible for behavioural engagement (Calabresi et al., 2014), serotonin has been shown to play a significant role in avoidance behaviour and impulse inhibition (Cools et al., 2008, Palminteri et al., 2012, Attar et al., 2012). The dopaminergic system is organized into three major signalling clusters, i.e. the mesolimbic-cortical, nigro-striatal and tuberoinfundibular pathways (Fuxe et al., 1974, Rodrigues et al., 2011). With respect to psychiatric illness, the mesolimbic-cortical and nigro-striatal pathways, linking the limbic system with the frontal cortex, and the substantia nigra pars compacta (SNc[3]) with the dorsal striatum respectively, are especially important. Considering the earlier mentioned conundrum pertaining to the two opposing signals, i.e. 'go / no-go' generated upon

[1] obsessive-compulsive disorder
[2] cortico-striatal-thalamo-cortical
[3] substantia nigra pars compacta

simultaneous cortical activation of the direct and indirect pathways of the striatum respectively, dopamine acts as a vital switch that if secreted in tandem with striatal activation, temporarily shuts down signalling in the indirect pathway, shunting the executive balance towards direct pathway activation (Hernández-López et al., 1997, Hernández-López et al., 2000, Goto and Grace, 2005). This function of dopamine is intricately linked with its activity on the D_1 expressing neurons of the direct pathway and its simultaneous stimulation of the D_2 receptors of the indirect pathway. In short, the expectation of a specific, generally beneficial, outcome results in dopamine being released within the nigrostriatal pathway which synapses with both the direct and indirect pathways of the striatum (Yager et al., 2015). However, upon stimulation, the D_2 receptors of the indirect pathway, being inhibitory G-protein coupled proteins, inhibit neuronal firing, whereas the excitatory D_1 G-protein coupled receptors propagate firing of the neurons in the direct pathway (Hernández-López et al., 1997). Therefore, during the enactment of planned motor and cognitive routines, simultaneous stimulation of the direct and indirect pathways causes behavioural conflict, which are momentarily counteracted by the phasic release of dopamine. As such, dopamine is regarded to be the primary neurotransmitter involved in behavioural engagement.

In contrast, the actions of serotonin are arguably more complex and diverse. Not only does serotonin exert its actions via seven different receptor classes (5-HT[1]$_{1\text{-}7}$), each with its own subtypes, but it also influences brain functions over a global and vast range of neurocognitive domains, including mood and executive behaviour, eating patterns, cognitive planning ability, sleep architecture, reproduction, and general motor coordination (Vanhoutte, 1990, Murphy et al., 2008, Murphy and Lesch, 2008, Murphy et al., 2004). In contrast to the relatively region-specific distribution of dopaminergic neurons, serotonin projections originate in the nuclei of the brainstem, i.e. the dorsal raphe nuclei (DRN[2]) and median raphe nuclei (MRN[3]; Boureau and Dayan, 2010) and broadly project to the remainder of the central nervous system, including the cortex, amygdala, striatum, thalamus, periaqueductal grey matter and hypothalamus (from the DRN), as well as the septal nuclei, hippocampus, and hypothalamus (from the MRN; Azmitia and Segal, 1978, O'Hearn and Molliver, 1984, Geyer et al., 1976). With respect to its interaction with dopamine, serotonin is generally regarded to be the functional antagonist of dopaminergic processes (Daw et al., 2002, Palminteri et al., 2012, Goddard et al., 2008), although the actions of serotonin and its interactions with dopamine are much more complex (Boureau and Dayan, 2010). The opponency theory (Daw et al., 2002) explains fundamental aspects of compulsivity and its response to serotonergic

[1] 5-hydroxytryptamine / serotonin
[2] dorsal raphe nucleus
[3] median raphe nucleus

pharmacotherapy. Indeed, in line with the neurobiological theories of OCD[1], treatment with selective serotonin reuptake inhibitors (SSRIs[2]) yield promising, albeit varying, therapeutic outcomes (Albert et al., 2018, Husted and Shapira, 2004). Against the background of its interactions with the dopaminergic system and CSTC[3] signalling, the major serotonergic receptors that play important modulatory roles are the 5-$HT_{1/2}$ receptor subclasses (Sinopoli et al., 2017).

Briefly, following its release from the basal nuclei, serotonin regulates dopaminergic functioning via several mechanisms (Esposito et al., 2008, Azmitia and Segal, 1978, Beart and McDonald, 1982, Geyer et al., 1976, Hervé et al., 1987, Parent, 1981, Egerton et al., 2008, De Deurwaerdère et al., 2004, Higgins and Fletcher, 2003, Lavoie and Parent, 1990, Spoont, 1992, Harrison et al., 1997, Nedergaard et al., 1988, Di Giovanni et al., 2010, Di Giovanni et al., 2008, Cools et al., 2010). First, serotonin reduces the bursting rate of dopaminergic cells (Di Giovanni et al., 1999). Second, it modulates the relative balance between regional dopamine concentrations (De Deurwaerdère and Spampinato, 1999). Third, serotonin is responsible for synaptic pruning of the projections that control dopamine release (Bortolozzi et al., 2005). In order to provide a better understanding of these processes, a brief overview of each of the predominant serotonin receptors within the central nervous system that are implicated in the pathogenesis of OCD, will be provided.

The 5-HT_1 receptor subtype

The 5-HT_{1A} receptor occurs in high densities within in the prefrontal cortex (PFC[4]; De Almeida and Mengod, 2008) and hippocampus (Akimova et al., 2009), while the basal ganglia and thalamus present with lower densities (Akimova et al., 2009). The receptor is an important role player in the manifestation of aggression, anxiety and impulsivity (Akimova et al, 2009), of which the latter two are associated with OCD. When activated, the 5-HT_{1A} receptor can stimulate dopamine release within the PFC (Sakaue et al., 2000, Calabresi et al., 2014, Yager et al., 2015). With respect to OCD, 5-HT_{1A} receptor involvement has been shown in both clinical (Goddard et al., 2008) and pre-clinical studies (Ichimaru et al., 1995), where 5-HT_{1A} *stimulation* has been shown to propagate and bolster compulsive-like persistence.

5-$HT_{1B/D}$ receptors are expressed in higher levels within the striatum and frontal cortex compared to the rest of the central nervous system and are known to act as autoreceptors that inhibit serotonin release, thus contributing to its anxiolytic effects (Pytliak et al., 2011).

[1] obsessive-compulsive disorder
[2] selective serotonin reuptake inhibitors
[3] cortico-striatal-thalamo-cortical
[4] prefrontal cortex

Although their significance for OCD[1] remains uncertain, both clinical (Hollander et al., 1992, Pigott et al., 1991, Khanna et al., 2001) and pre-clinical studies (Shanahan et al., 2009) point to the exacerbation of persistent behaviours upon modulation of this receptor subclass. However, no clear role for either its activation or inhibition has been shown.

The 5-HT$_2$ receptor subtype

With respect to the 5-HT$_2$ receptor subclass, the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors are of most importance in OCD. 5-HT$_{2A}$ receptors demonstrate the greatest expression in the basal ganglia (Pytliak et al., 2011) and its activation has been shown to result in the effects associated with the use of psychedelics such as lysergic acid diethylamide (LSD[2]), and the positive symptoms of schizophrenia, namely sensory hallucinations (Kometer et al., 2012, Nichols, 2004), delusions, and other symptoms such as anxiety and increased appetite (Pytliak et al., 2011). Further, considering OCD, activation of 5-HT$_{2A}$ receptors leads to impaired decision making as well as impaired response inhibition, while their inhibition decreases anxiety and promotes cognitive flexibility (Aznar and Hervig, 2016).

In contrast, the 5-HT$_{2C}$ receptor is expressed in high concentrations throughout most brain regions, including the structures of the CSTC[3] circuit (Mengod, 2011, Sharma et al., 1997, Clemett et al., 2000, Barbon et al., 2011, Finnema et al., 2014). Although its precise neurobiological role has not yet been elucidated, the 5-HT$_{2C}$ receptor plays a significant role in the regulation of mood, anxiety, sleep, appetite and sexual behaviour (Pytliak et al., 2011, Heisler et al., 2007) and is thus heavily implicated in psychiatric conditions such as major depressive disorder and schizophrenia. That said, its involvement in OCD is especially interesting. Indeed, pharmacological studies in patients (Zohar et al., 1987, Ramasubbu et al., 2000) and animals (Finnema et al., 2014, Chou-Green et al., 2003) point to a regulatory role of the 5-HT$_{2C}$ receptor in the propagation of compulsive routines, where 5-HT$_{2C}$ receptor modulation has been shown to exacerbate symptoms; again, this may either be a consequence of inhibition or activation. Indeed, that SSRIs[4] only demonstrate therapeutic effect in OCD after chronic administration, points to the long-term neuromodulation of 5-HT$_{2C}$ receptor expression (Atmaca, 2016, Bloch et al., 2010) underlying the therapeutic response.

Taken from the above, it is clear that dysregulated dopaminergic and serotonergic processes could be a major construct of obsessive-compulsive symptomology. It can be postulated that OCD is borne from dysfunctional crosstalk between the dopaminergic and serotonergic

[1] obsessive-compulsive disorder
[2] lysergic acid diethylamide
[3] cortical-striatal-thalamo-cortical
[4] selective serotonin reuptake inhibitors

systems, a theory largely congruent with the therapeutic efficacy of SSRIs[1] and low-dose anti-dopaminergic interventions in the treatment of OCD[2].

2.1.2.3 Weighing the consequences: learning from reward and punishment

The way in which humans and animals adapt in the wake of changing circumstances fundamentally relies on learning from beneficial (rewarding) or negative (punishing) outcomes. Indeed, while our pursuit of rewarding, satisfying, expected or even just optimal outcomes plays an important role to ensure our longevity, our ability to avoid aversive, punishing, harmful and potentially dangerous scenarios is equally important for our survival. Against this background, it can be hypothesized that the ability to adequately complete tasks that are seemingly related to one's survival, e.g. locking of doors, decontamination routines, making sure that no harm befalls oneself or others, and engaging in religious rituals to such an extent that one can believe eternal damnation is highly unlikely, is rewarding and will therefore be associated with phasic dopaminergic signalling which will prone one to engage in constant reward-seeking behaviours (Boureau and Dayan, 2010). That said, continuous and repetitive experiences of the same outcome over time results in a blunted dopaminergic response, thereby preventing excessive engagement in the same routines (Schultz, 2007). On the other hand, experiences of negative outcomes, i.e. being robbed after not properly locking up a home, or becoming ill after drinking contaminated water, should induce preventative behavioural routines when faced with future similar scenarios (Jung et al., 2011). Remarkably, optimal reward and punishment learning, like the planning and execution of complex motor and cognitive routines, also depends on the functional interplay between dopamine and serotonin. While the exact dopaminergic and serotonergic mechanisms involved in reward and punishment feedback processing are complex (for example, see Boureau et al., 2011), a robust body of evidence points to phasic dopaminergic increases during the anticipation and experience of reward (Reynolds et al., 2001, Tang et al., 2001, Calabresi et al., 2007, Kreitzer and Malenka, 2007, Cohen and Frank, 2009, Gerfen and Surmeier, 2011, Hong and Hikosaka, 2011). In contrast, depressions in striatal dopamine release coupled with simultaneous increases in serotoninergic signalling are observed during the processing of punishing feedback (Palminteri et al., 2012, Nakamura et al., 2008, Tanaka et al., 2009). In light of the above, OCD was previously proposed to be a condition of dysfunctional reward processing (Figee et al., 2011). Specifically, it is believed that individuals suffering from OCD fail to consolidate a sense of adequate task completion, therefore persistently engaging in reward-seeking, no-task-completion routines (Wahl et al., 2008). Moreover, OCD shares certain trait

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

characteristics with addiction and substance use disorders in terms of compulsive engagement while in fact having been described as a form of behavioural addiction by some (Holden, 2001, Denys et al., 2004). In support of this theory, it has been demonstrated that some OCD[1] patients, while enacting compulsive routines, present with blunted reward-anticipatory dopaminergic responses (Figee et al., 2011, Figee et al., 2013) similar to observations in patients abusing alcohol, nicotine, and cannabis (Wrase et al., 2007, Martin-Soelch et al., 2003, Bühler et al., 2010, van Hell et al., 2010), and individuals suffering from compulsive gambling (Reuter et al., 2005, de Greck et al., 2010, Choi et al., 2012, Balodis et al., 2012). As such, a theoretical approach to intervene in these conditions would be to bolster dopaminergic activity during the transient experience of rewarding outcomes, thereby coding sufficient rewarding feedback and preventing future reward seeking behaviour. Interestingly, while such interventions are already followed in the treatment of addiction disorders (Goldstein et al., 2009, Drapier et al., 2006), anti-dopaminergic, and not dopaminergic interventions are currently employed in the treatment of OCD (see paragraph 2.1.4.2). Nevertheless, although aligning to some extent with the opponency theory describing the functional roles of dopamine and serotonin, such approach seems unsupportive of the concept of OCD being a hypo-serotonergic and hyperdopaminergic condition, as explained earlier. Suffice to say that the manifestation of abnormal, persistent behaviours is a function of the complex interplay between the two neurotransmitters and that imbalances in either direction, may ultimately give rise to the symptom presentation. This concept was demonstrated in pharmacological studies showing that the *inherent,* baseline dopaminergic tone within a specific individual, is vital in determining the outcome of treatment. Specifically, it was demonstrated that under circumstances of a low baseline dopaminergic tone, dopamine enhancers (e.g. bromocriptine) *improve* reward-based learning (Cools et al., 2009). The opposite was found to be true for patients with high baseline dopamine levels (Cools et al., 2009). This is a concept of importance in the current investigation.

Considering the above as well as the fact that treatment response remains suboptimal, (see paragraph 2.1.4.2), recent evidence has been indicative of potential differences in the underlying neurobiology of patients presenting with different obsessive-compulsive phenotypes, most notably so the C/W[2] and S/C[3] cohorts (Figee et al., 2011). Further, these differences were found to be related to unique reward and punishment processing profiles. As opposed to patients with S/C OCD, patients presenting with C/W OCD, presented with blunted activation of the ventral striatal activation during phases of reward anticipation (Figee

[1] obsessive-compulsive disorder
[2] contamination/washing
[3] safety/checking

et al., 2011). Further, C/W[1] OCD[2] also seems to be associated more with impulsive responding and a diminished ability to delay receiving rewards. Conversely, individuals diagnosed with S/C OCD engaged in significantly fewer impulsive behaviours and presented with an inflated capacity to delay reward-directed responding (Figee et al., 2011, Pinto et al., 2014), while also presenting with dysfunctional signalling in brain areas responsible for motor functioning and attention, i.e. the STN[3] and the PFC[4] (Mataix-Cols et al., 2004). Remarkably, it was also found that treatment-resistant patients are often those that present with greater deficits in reward-feedback processing (Figee et al., 2011).

Taken together, it can be hypothesized that different phenotypes of OCD are founded in unique neurocognitive constructs that may be related to underlying differences in the dopaminergic and serotonergic modulation of reward and punishment feedback processing. Further, considering the body of literature reviewed above, it is likely that such differences may partly be responsible for the varying response of patients to currently employed pharmacotherapeutic strategies (see below).

2.1.3 A brief summary of other cognitive theories

2.1.3.1 OCD as a condition of behavioural disinhibition

Contradictory to the common belief that OCD is exclusively associated with the need to be in complete control, evidence has been presented that OCD patients exhibit deficits in behavioural inhibition (Grassi, 2016), i.e. displaying impulsivity and riskier decision making compared to healthy controls (Grassi et al., 2015). This led to the Robbins hypothesis, suggesting OCD to be condition of behavioural disinhibition or behavioural addiction (Robbins and Clark, 2015). Especially counting rituals or compulsions related to the ordering of objects could be explained by behavioural disinhibition, as these actions are entirely without purpose.

2.1.3.2 Over-estimation of threat

Considering that anxiety is a prominent symptom experienced by individuals with OCD (Stein et al., 2010), it is safe to postulate that overestimation of the dangers of perceived threats could be a key contributor to the development of obsessions and compulsions. Anxiety, as is true for most other psychiatric illnesses, arises due to dysfunctional cognitive processes which are often associated with maladaptive behavioural strategies recruited to alleviate the level of

[1] contamination/washing
[2] obsessive-compulsive disorder
[3] sub-thalamo nucleus
[4] prefrontal cortex

arousal caused by the perceived threat, e.g. “If I do not lock the car ten times, someone *will* break in”; (Sookman and Pinard, 2002). However, as the execution of compulsive behaviours does not serve to prevent disconfirmation of the *alleged* threat, such routines negatively reinforce the severity of the underlying, implicitly generated experience of anxiety (Sookman and Pinard, 2002). This provides a potential cognitive basis for both the S/C[1] and the C/W[2] phenotypes.

2.1.3.3 Intolerance of uncertainty

The concept of uncertainty intolerance is closely linked to overestimation of threat, as it can be described as the need to feel in control at all times in order to feel safe (Sookman and Pinard, 2002). This purportedly leads to the execution of repetitive rituals in order to create a semblance of control. It is believed that hoarding could align with this theory as an individual might fear any possible future eventuality and thus hoards objects for the sake of “just in case” (Sookman and Pinard, 2002).

2.1.3.4 An over-reliance on habitual responses

It is well documented that OCD[3], irrespective of symptom phenotype, is characterised by cognitive inflexibility (Gruner and Pittenger, 2017). In other words, individuals with OCD are unable to deviate from repeatedly reinforced behaviours. Habitual engagement is normal in the everyday lives of most human beings. In fact, to act in a habitual manner is a pivotal construct of successful and efficient functioning, as one does not have to recruit time-consuming and energy-taxing cognitive processes to complete normal daily routine driven tasks, e.g. changing gears in a car or brushing teeth. In this sense, habitual responses streamline and facilitate the optimised execution of routine behaviours. However, if such habits transcend through all layers of our executive functioning, it becomes pathological to the extent that planned and highly goal-directed behaviours become habitual responses, irrespective of changing outcomes. Such overreliance on habitual responses has been demonstrated in patients with OCD, who in spite of their behaviour not resulting in anything near a desired outcome, continue to engage in seemingly purposeless behaviour (de Wit et al., 2012). Further, such habit-like responses are also expressed in patients who do not necessarily report anxiolytic responses during the execution of compulsive rituals, exemplifying the habitual, rather than goal-directed nature of some obsessive-compulsive phenotypes.

[1] safety/checking
[2] contamination/washing
[3] obsessive-compulsive disorder

2.1.4 The treatment of OCD

The treatment of OCD[1] is generally based on a two-pronged approach, i.e. cognitive and pharmacological therapy, either of which can be used in isolation or as a combined strategy (Wolmarans et al., 2017a). A third, less explored option pertains to the manipulation of brain function by means of electric stimuli, e.g. deep brain stimulation, or surgery (Denys et al., 2010). These are normally reserved for severe treatment refractory OCD.

2.1.4.1 Cognitive behavioural therapy

At its core, cognitive behavioural therapy (CBT[2]) relies on the psychological reprogramming of seemingly dysfunctional cognitive processes (see Hoffman et al., 2012). As such, anyone who has completed CBT training can assist patients with altering their beliefs and perceptions under controlled circumstances. Considering the well-established CBT technique, exposure and response prevention (ERP[3]), patients are gradually exposed to a contextually relevant scenario, over time increasing in intensity. For example, patients suffering from C/W[4] OCD, will gradually be exposed to scenarios in which there exists a *purported* risk of being contaminated. Such hypothetical exposures result in mounting anxiety and a pressing urge to engage in neutralizing routines. However, patients are prevented from carrying out such behaviour (Abramowitz, 1996). The ultimate purpose of this approach is to assist patients in realising that their fears are irrational and that no harm can actually befall them if they fail to engage in compulsive neutralizing routines. In other words, patients learn over time that compulsive behaviour, although seemingly functional in their view, is fruitless and counterproductive. Although treatment outcomes depend on a number of different factors, e.g. symptom severity, the length and intensity of therapeutic sessions and patient commitment, success rates in terms of symptom alleviation of up to 83% have been reported (Abramowitz, 1996). Moreover, although treatment resistance remains a realistic dilemma (Sookman and Steketee, 2010), it has also been shown that CBT results in longer lasting therapeutic benefit after treatment cessation, compared to pharmacotherapeutic strategies (Tundo et al., 2007).

2.1.4.2 Pharmacotherapy

OCD generally demonstrates therapeutic response to drugs that enhance serotonergic neurotransmission, i.e. the serotonin reuptake inhibitors (SRIs[5]), e.g. clomipramine, and

[1] obsessive-compulsive disorder
[2] cognitive behavioural therapy
[3] exposure and response prevention
[4] contamination/washing
[5] serotonin reuptake inhibitors

SSRIs[1], e.g. fluoxetine and escitalopram (Brakoulias and Tsalamanios, 2017, Atmaca, 2016). Although the exact neurobiological mechanisms underlying the aetiopathology of OCD[2] remains unclear, early observations that patients with OCD responded to clomipramine (Thoren et al., 1980) triggered work into the potential serotonergic processes that may be involved. In line with the hypo-serotonergic theory of OCD, bolstered serotonergic signalling induced by *chronic* (12 – 16 weeks and longer) *high dose* (up to three times the doses normally prescribed in major depression) SRI[3] or SSRI administration, delivers satisfactory outcomes in at least 40 – 60% of patients (Husted and Shapira, 2004, Brakoulias and Tsalamanios, 2017). In this regard, therapeutic response is considered as being successful if a greater than 25% reduction in the pre-treatment Y-BOCS[4] score (Brakoulias and Tsalamanios, 2017) is reached. Although analogous in therapeutic efficacy, SSRIs are preferred due to their improved safety profile (Brakoulias and Tsalamanios, 2017, Atmaca, 2016). That said, treatment resistance remains a clinical obstacle with the remaining 40 – 60% of patients, depending on survey, continuing to present with compulsive symptomology. Briefly, moderate treatment resistant OCD is defined as the failure of at least two adequate therapeutic trials of SRIs / SSRIs to yield therapeutic response (Goodman et al., 1993). In such cases, either of two treatment approaches are normally considered, i.e. increasing the dose or switching to another SRI / SSRI, or augmenting the SRI / SSRI with low-dose anti-dopaminergic therapy, e.g. risperidone, haloperidol, clozapine or flupentixol (Millan et al., 2015, Bloch et al., 2006, Bloch et al., 2010). Augmentation therapy specifically has been shown useful in up to one third of patients with first-line treatment resistant OCD (Albert et al., 2013, Bloch et al., 2010). However, treatment refractory OCD is defined as treatment failure after at least three SRI/ SSRI trials, the augmentation thereof with at least two sequentially introduced atypical antipsychotics, and CBT[5] in conjunction with SRI / SSRI treatment (Husted and Shapira, 2004). In these instances, surgery remains as the only other option.

2.1.4.3 Is treatment non-response related to the obsessive-compulsive phenotype diagnosed?

As alluded to earlier, an increasing body of evidence points to the possibility of unique psychobiological processes underlying different phenotypes of OCD (Landeros-Weisenberger et al., 2010, Cavallini et al., 2002, Mataix-Cols, 2006). Further, such differences are likely founded within both the neurobiological and cognitive constructs, highlighting a need to look closer at the aetiopathological mechanisms associated with obsessive-compulsive phenotypes. This seems increasingly necessary for our efforts to develop improved anti-

[1] selective serotonin reuptake inhibitors
[2] obsessive-compulsive disorder
[3] serotonin reuptake inhibitor
[4] Yale-Brown obsessive-compulsive scale
[5] cognitive behavioural therapy

compulsive therapeutic strategies. During the past three decades, evidence in support of such neurocognitive differences underlying different obsessive-compulsive phenotypes has increasingly been presented in the form of pharmacological treatment data. For example, hoarding compulsions prove to be the most resistant to SSRI[1] intervention, the theory being that such behaviour may primarily be founded on dopaminergic mechanisms (Stein et al., 2007, Mataix-Cols et al., 1999). On the other hand, obsessive thoughts, S/C[2], and S/O[3] phenotypes have generally been shown to respond more favourably to SSRIs (Starcevic and Brakoulias, 2008, Mataix-Cols et al., 1999, Abramowitz et al., 2003, Mataix-Cols et al., 2002, Rufer et al., 2006, Erzegovesi et al., 2001, Shetti et al., 2005, Landeros-Weisenberger et al., 2010). That said, patients with S/O OCD[4] have been found to respond only after a longer period of treatment than that necessary to yield therapeutic effect in other sensitive phenotypes (Alonso et al., 2010), while these individuals also present with the highest risk for suicide among all patients with OCD (Albert et al., 2004, Garyfallos et al., 2010, Pinto et al., 2006, Eisen et al., 2006). Last, patients presenting with C/W[5] OCD seem to parallel those with hoarding disorder in general demonstrating poor treatment response to standard SSRI treatment (Starcevic and Brakoulias, 2008, Stein et al., 2007, Shetti et al., 2005), potentially indicating that this phenotype also differ from SSRI-sensitive phenotypes on a neurobiological level.

Taken together, it is evident that our understanding of both the psychobiological processes underlying OCD, and the factors contributing to treatment resistance, must be expanded. To this extent, preclinical research is vital, while accurate animal models that may elucidate aspects of symptom heterogeneous OCD, would be valuable.

2.2 Modelling OCD in animals

For decades, animals have been employed to investigate the basic foundations of human conditions (Hajar, 2011), particularly as screening tools for novel treatment strategies and as paradigms in which to divulge aetiopathological mechanisms (d'Angelo et al., 2014). As it is very difficult to confirm the presence of obsessive thoughts in animals, preclinical models will likely never be able to simulate clinical OCD in its entirety. Indeed, almost all current animal models rely on the demonstration of observable and measurable repetitive behavioural routines that are seemingly reminiscent of compulsive rituals in humans (d'Angelo et al., 2014). That said, animal models that have been validated on the basis of meeting certain

[1] selective serotonin reuptake inhibitor
[2] safety/checking
[3] symmetry/ordering
[4] obsessive-compulsive disorder
[5] contamination/washing

critical criteria, are not only deemed useful, but can provide significant insight into the human condition. More specifically, animal models of psychiatric illness should be valid in terms of their face, construct and predictive resemblance of the modelled condition (Willner, 1984, Willner, 1986). Briefly, face validity refers to symptomological and phenotypic overlap between the animal and the human condition (Willner, 1984, Willner, 1986). A model founded on robust construct validity, shares near analogous psychobiological involvement with the clinical condition (Willner, 1984, Willner, 1986). Last, predictive validity describes the level of overlap in treatment response (and non-response) between the animal model and the human disorder (Willner, 1984, Willner, 1986). Further, we previously described a fourth criterion to be met, i.e. methodological validity, which speaks to the conceptual and procedural integrity of the methods followed and reported (Wolmarans et al., 2017b). This applies to both the accuracy and suitability of the methods followed, but also to comparisons of between-laboratory findings.

Animal models of OCD[1] are broadly clustered into three different categories, i.e. behavioural, pharmacological and genetic models. Behavioural models include naturalistic persistent deer mouse behaviour (Wolmarans et al., 2013, Powell et al., 1999), excessive lever pressing in rats (Joel and Avisar, 2001), and large nest building in rodents (Greene-Schloesser et al., 2011, Hoffman and Morales, 2009, Wolmarans et al., 2016b), while genetic models have been described based on selective manipulation of the 5-HT_{2C} receptor as well as knockout of the *Hoxb8* (Greer and Capecchi, 2002, Chen et al., 2010), *SAPAP3* (Welch et al., 2007) and *Slitrk5* genes (Shmelkov et al., 2010). Further, the underlying constructs of behavioural and genetic models may overlap if the naturalistic genetic trigger of abnormal behaviours can successfully be carried over from one generation to a next via, inter alia, inbreeding protocols. However, pharmacological models rely on the direct manipulation of specific neurobiological targets. While these models, e.g. quinpirole-induced compulsive checking (Szechtman et al., 1998) and 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT[2]) induced reductions in spontaneous alternation (Yadin et al., 1991), may provide significant perspectives on some of the potential causes of OCD, they are often restrictive in terms of highlighting the mechanisms underlying the natural and chronological manifestation of persistent behaviour. Therefore, toward a better understanding of the aetiology and naturalistic mechanisms that may play a role in the manifestation of OCD, spontaneous behavioural models are useful. As the current investigation will focus on two functionally unique, but equally persistent and repetitive naturalistic phenotypes expressed by deer mice (*Peromyscus maniculatus bairdii*), i.e. large

[1] obsessive-compulsive disorder
[2] 8-hydroxy-2-(di-n-propylamino)-tetralin

nest building (LNB[1]) and high marble burying (HMB[2]), the deer mouse model of OCD[3], as well as said two behavioural phenotypes will be afforded closer attention, henceforth. For an extensive review on other animal models of OCD, please see (d'Angelo et al., 2014).

2.2.1 The deer mouse (Peromyscus maniculatus bairdii) model of OCD

Naturalistic persistent behaviour expressed by deer mice bred and housed in confinement is a validated preclinical framework in which to study the spontaneous and naturalistic manifestation of compulsive-like behavioural persistence (Scheepers et al., 2018, Wolmarans et al., 2013). As a strict outbreeding protocol has been followed since the establishment of the first in-house colony at the North-West University (NWU[4]), the mice used in our laboratory can be regarded as a true wild-type strain (Joyner et al., 1998, Scheepers et al., 2018), providing for a genetically diverse study population. At the time of its initial characterisation (Korff et al., 2008), the face validity of the model was founded upon similarities between the spontaneous manifestation of high stereotypical (HS[5]) behaviour that was observed in 40% of deer mice irrespective of sex, i.e. pattern running, backwards somersaulting and vertical jumping (Korff et al., 2008). Moreover, as is true for clinical OCD, deer mouse stereotypy is expressed in a waxing and waning cycle over the course of a single dark cycle, a pattern that repeats over several successive nights of screening (Wolmarans et al., 2013). Importantly, these behaviours serve no apparent functional purpose and can therefore be regarded as being reminiscent of the seeming functional futility of clinical compulsion. Further, HS also presents with good construct and predictive validity, demonstrating perturbations in both oxidative stress (Güldenpfennig et al., 2011) and serotonergic pathways (Wolmarans et al., 2013) as well as therapeutic response to chronic, but not sub-chronic, high dose (50 mg/kg/day) oral escitalopram treatment (Wolmarans et al., 2013). In later work, investigations into the potential expression of other phenotypically distinct, but equally persistent and naturalistic behaviours in the model, revealed two additional compulsive-like phenotypes, *viz.* LNB and HMB (please refer to paragraphs 2.2.2 and 2.2.3 below for a conceptual description of LNB and HMB against the background of compulsivity). Although these still remain to be elucidated on a neurobiological level (to which extent the current work is being performed), epidemiological and pharmacological treatment data revealed significant and interesting findings. First, as is true for HS, both LNB and HMB are spontaneously expressed by animals of both sexes (Wolmarans et al., 2016b, Wolmarans et al., 2016c). Second, while 30% of

[1] large nest building
[2] high marble burying
[3] obsessive-compulsive disorder
[4] North-West University
[5] high stereotypy

animals engage in LNB[1] (Wolmarans et al., 2016a), only 11 – 15% of animals develop HMB[2] (Wolmarans et al., 2016b). Last, as opposed to LNB that (like HS[3]) responds to chronic high dose oral escitalopram (Wolmarans et al., 2016a), HMB seems to represent more of an SSRI[4] resistant phenotype (Wolmarans et al., 2016b). As such, the deer mouse model may provide a useful avenue for the aetiopathological exploration of symptom heterogeneous compulsive-like behaviour and the potential mechanisms that may underlie treatment-resistance.

Important: As the current dissertation is written in article format, this section of the literature review will also provide some methodological background to the work presented in Chapter 3. As the methods followed in Chapter 3 have largely been based on previous work in our laboratory, a full dissection of the methodology followed as reported in Chapter 3 fell outside the scope of the journal paper submitted to *Journal of Psychopharmacology.* As such, although it is beyond the scope of the current literature review to contain a detailed review of methods, this section will briefly provide some foundation for the work performed as reported in Chapter 3.

2.2.2 Large nest building

In the wild, rodents build nests for various evolutionary reasons (Latham and Mason, 2004, Hess et al., 2008). Various aspects, i.e. genetics (Lynch, 1980), environmental configuration (Porter and Busch, 1978) and endocrinological status (Voci and Carlson, 1973) influence nest building (NB[5]) behaviour. Although being a normal behavioural manifestation in rodents, previous investigations into the nesting behaviour of house mice (*Mus musculus*; Greene-Schloesser et al., 2011) and deer mice (Wolmarans et al., 2016a) demonstrated aberrant NB in some animals that is persistent, repetitive, and seemingly purposeless compared to normal NB behaviour. From a teleonomic perspective (Thornhill, 1996), LNB can be regarded as a maladaptation in a specific component of the normal behavioural repertoire of deer mice. Indeed, considering that mice build nests for the purposes of safety and protection, temperature regulation and to provide adequate nurseries for offspring (Jirkof, 2014), excessively large nests expressed in the laboratory serve no unique purpose. From an evolutionary perspective, nesting size and quality play a major role in mate choice and reproductive success in some species, e.g. birds (Holveck and Riebel, 2009), fish (Jamieson, 1995), and frogs (Felton et al., 2006). However, this is not true for mice, which generally

[1] large nest building
[2] high marble burying
[3] high stereotypy
[4] selective serotonin reuptake inhibitor
[5] nest building

exercise mate choice by random or on the basis of amongst others, dominance, probability of genetic success, overall health, or patterns of ultrasonic vocalization (for a detailed review, see Latham and Mason (2004). That both male and female deer mice engage in LNB[1] (Wolmarans et al., 2016a), also excludes the likelihood of sex-related differences in nesting phenotype. Therefore, it is likely that excessive NB[2] is expressed at the cost of other functions for which effort, time and energy is required, and may thus be regarded as a naturalistic maladaptation (Crespi, 2000). Therefore, taken at face value, LNB can be regarded as being reminiscent of compulsive-like behaviour, in this study hypothesized to be a different obsessive-compulsive phenotype than HMB[3]. Further, LNB responds to both SRI[4] (clomipramine) and SSRI[5] (fluoxetine and escitalopram; Greene-Schloesser et al., 2011; Wolmarans et al., 2016a) treatment, but not to the noradrenalin reuptake inhibitor (NRI[6]), desipramine (Greene-Schloesser et al., 2011). Also, recent results demonstrated reduced serotonin levels in LNB animals in brain regions implicated in OCD[7] (Winter et al., 2018, Mitra et al., 2016, Mitra et al., 2017), thereby strengthening the construct validity of LNB as a model of OCD. From a different perspective, one that addresses the construct of perfectionism, a recent series of studies have investigated NB behaviour in rabbits as a model of OCD (Hoffman and Morales, 2009, Hoffman and Morales, 2012). This model is proposed to be relevant for our understanding of compulsions related to feelings of incompleteness, "just right" sensations, and the perception of task completion and has provided some interesting evidence in support of the behavioural phenotype's face validity for OCD.

2.2.2.1 Perspectives on the current investigation: the assessment of nest building behaviour

To characterize persistence and repetition, NB analyses are normally conducted over 7 consecutive days (Wolmarans et al., 2016a). In short, an excess of pre-weighed cotton wool is placed on top of the metal grid of the animal's home cage and left for 24 hours. On every following day, the remaining cotton wool is weighed, and an excess of cotton wool again provided (Wolmarans et al., 2016a). Following completion of the 7-day trial period, the total nesting score (in grams) is calculated and applied to characterize a compulsive-like LNB phenotype within the normal deer mouse population. Indeed, we have shown previously that 20 – 30% of deer mice, irrespective of stereotypical cohort or sex, express aberrant large LNB behaviour (Wolmarans et al., 2016a). Such an approach is necessary, as NB[8] demonstrates

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

a waxing and waning nature over the course of several trials (Wolmarans et al., 2016a). As such, in order to obtain an accurate picture of the true nesting phenotype, several nights of screening are necessary.

2.2.3 High marble burying

In this section, HMB[1] will not be discussed as a unique behavioural phenotype within the deer mouse model, but rather as a separate model of OCD[2] that, although highly disputed (De Boer and Koolhaas, 2003, De Brouwer and Wolmarans, 2018), is widely applied in various laboratories.

Marble burying (MB[3]), without any clear guideline describing the requirements for its severity or persistence, was initially considered as a screening test for anxiety (Broekkamp et al., 1986); however, it has later been utilised as a measure of compulsive-like behaviour. The premise on which application of the marble burying test (MBT[4]) for psychiatric illness is founded, is twofold. First, in analyses of anxiety it is proposed that a more anxious cohort of rodents will engage in active burying behaviour following a neophobic response elicited by exposure to foreign, albeit harmless, objects in a home cage environment (Pinel and Treit, 1978, De Brouwer and Wolmarans, 2018). Second, with respect to analyses of obsessive-compulsive like behaviour, it has been hypothesized that animals engaging in MB behaviour do so without a clear goal in mind as marbles are regarded as non-anxiogenic and non-reactive (Thomas et al., 2009).

However, the face validity of MB as an animal model and screening test for either psychiatric construct must be evaluated only after considering the ethological value of burying behaviour. Indeed, digging, burrowing and burying serve analogous purposes across both natural and laboratory settings and are central to rodent survival and social structure (Ebensperger and Blumstein, 2006, Deacon, 2012). Fundamentally, burying is motivationally driven by the need to store food (Fleming and Brown, 1975, Jenkins and Breck, 1998), to control temperature (Ellison, 1995, Tracy and Walsberg, 2000), to facilitate social interaction and nurture and protect young (Denenberg et al., 1969) and to avoid predation (Ruffer, 1965, Tracy and Walsberg, 2000, Ebensperger and Blumstein, 2006). Further, such behaviours can also be regarded as a mandatory behavioural need, as they persist in cages that already contain extensive pre-burrowed networks (Sherwin et al., 2004). However, although digging and burrowing are natural and persistent under laboratory conditions, even in the offspring of

[1] high marble burying
[2] obsessive-compulsive disorder
[3] marble burying
[4] marble burying test

captive bred animals (Adams and Boice, 1981, Weber and Hoekstra, 2009), such behaviour is modifiable by a number of factors including pre-exposure to the burying substrate that results in decreased, albeit continued burrowing activity (Schultz, 1972). Further, the burying substrate itself can influence the number of burying episodes and the overall measurable digging activity (Layne and Ehrhart, 1970, Webster et al., 1981). Overall digging behaviour can further be influenced by genetics, even in closely related species, which can exhibit notably different burrow architecture and digging activity (Layne and Ehrhart, 1970, Webster et al., 1981, Dudek et al., 1983, Weber and Hoekstra, 2009). Considering that common standard housing conditions in most rodent housing facilities constitute only a thin layer of any given form of bedding material, e.g. corn cob (Jimenez-Gomez et al., 2011), wood chips (Deacon, 2012), or paper (Mason et al., 2006), digging and burrowing cannot be readily expressed. It can therefore be expected that these behaviours will be expressed to a greater extent following the provision of ample bedding or burrowing substrate (Adams and Boice, 1981, Webster et al., 1981).

Taking into account previous hypotheses that rodents engage in MB[1] as a means of defence, it must be considered that defensive burying is itself a mechanism to avoid realistic and tangible threat. Therefore, to distinguish the behavioural phenotypes akin to defensive burying from that observed in response to harmless objects, Pinel and Treit (1978) introduced a shock prod paradigm and observed that a rat will present with a strong tendency to bury a shock prod if confronted with such objects in its familiar surroundings. Various other objects have also been identified as aversive stimuli e.g., electrified shock prods (Pinel and Treit, 1978), mouse traps (Terlecki et al., 1979), flash bulbs (Terlecki et al., 1979, Gray et al., 1981), noxious smells (Silverman, 1978), pepper sauce and other unpleasant liquids (Wilkie et al., 1979, Poling et al., 1981). Interestingly, however, later studies also found that rodents bury harmless and non-fear-provoking objects, i.e. glass marbles (Broekkamp et al., 1986) and rat chow pellets (Poling et al., 1981, Londei et al., 1998), thus undermining the value of introducing marbles in home cages as a "neophobic" stimulus. Rather, burying behaviour may be dependent on the emotional state of a subject rather than be propagated by a clear outcome in mind (Londei et al., 1998). In fact, it has been shown that different levels of aggression (Koolhaas et al., 1999) or stress could influence burying behaviour (Londei et al., 1998). This is evidenced by findings that compared defensive burying responses in rats and hamsters during exposure to a shock prod (Whillans and Shettleworth, 1981), demonstrating that even though hamsters associate shock with the presence of a prod, they do not engage in burying behaviour similar to that expressed by rats (Whillans and Shettleworth, 1981).

[1] marble burying

Therefore, if burying behaviour is natural, which criteria must be met if MB[1] is to be applied as a screening tool for anxiety- and/compulsive-like behaviour? First, anxiety (or neophobia as in the case of the MBT[2]) and compulsions differ in meaningful ways, foremost of which is that neophobic responses should abate over time, while compulsive behaviour should persist (De Brouwer and Wolmarans, 2018). Indeed, with respect to anxiety-like behaviours related to novelty in other behavioural paradigms, such behaviours have been shown to abate as a function of repeated exposure (Savy et al., 2015) or even over the course of a single continuous test session (Choleris et al., 2001). On the other hand, considering that compulsions are mostly directed towards reducing the distress caused by persistent intrusive thoughts related to specific scenarios, MB behaviour should be appreciated based on an animal's persistent, repetitive and behaviourally inflexible preoccupation and direct interaction with marbles (Njung'e and Handley, 1991, Wolmarans et al., 2017a, Wolmarans et al., 2016b). However, as MB is often only applied during a single session, neither of these criteria is met, undermining the face validity of the model with respect to both conditions.

As the MBT is mostly employed as a rapid screening tool to establish whether an animal may be anxious or engaging in compulsive behaviour, no study has yet investigated the construct validity of the model for either condition per se. However, some insight into the neurobiological basis of MB behaviour can be found in pharmacological treatment data, of which numerous results have been published. A brief overview of these findings indicate that MB responds to several interventions that include agents which target the noradrenergic, dopaminergic, serotonergic, cholinergic, glutamatergic, and GABA[3]-ergic systems (for a complete review of these and other pharmacological treatment findings, refer to (De Brouwer and Wolmarans, 2018). Further, several miscellaneous receptors have also been targeted, including neurokinin, imidazoline, calcium, and endocannabinoid receptors, while genetic studies have also considered a number of putative anxiety-linked receptor targets (Lähdesmäki et al., 2002, Egashira et al., 2008, Gavioli et al., 2007, Tasan et al., 2009, Angoa-Pérez et al., 2013). Taking this into account, it must be considered that if MB activity is triggered and driven by a possible anxiogenic and/or obsessive-compulsive-like construct, the test itself as applied in preclinical literature presents with poor construct and predictive validity as a screening test for either behaviour. Indeed, neither clinical anxiety nor OCD[4] demonstrates response to many of these interventions.

[1] marble burying
[2] marble burying test
[3] gamma aminobutyric acid
[4] obsessive-compulsive disorder

2.2.3.1 A perspective on the current investigation: methodological aspects of the marble burying setup

Zone configuration

The MBT[1] can be performed in a one zone (marbles spaced evenly on the burying substrate throughout the test cage) or a two zone (marbles spaced evenly on one side of the testing arena only) paradigm (Handley, 1991, Gyertyan, 1995, Takeuchi et al., 2002, Nicolas et al., 2006, Slot et al., 2008, Thomas et al., 2009, Badgujar and Surana, 2010, Kinsey et al., 2011, Prajapati et al., 2011, Nardo et al., 2014). However, as a one zone paradigm does not allow the animal to avoid exposure to marbles and therefore is insensitive to behaviours driven by voluntary engagement, the current investigation will employ a two zone paradigm only. Following placement of the marbles, the mouse is introduced to the experimental cage and left to interact with the marbles for 30 minutes. Thereafter, the number of marbles buried is counted by observers that are unfamiliar with the cohort to which the tested individuals belong (Harasawa et al., 2006, Angoa-Pérez et al., 2013, De Almeida and Mengod, 2008, Wolmarans et al., 2016b). Importantly, different investigators apply different criteria to determine if a marble is buried (or covered). Whereas some studies refer to buried marbles as those covered up to two-thirds of its size in bedding material (Shimazaki et al., 2004, Slot et al., 2008, Badgujar and Surana, 2010, Shimada et al., 2011), others apply a 50% (Kinsey et al., 2011) or fully (Torres-Lista et al., 2015) covered criterion.

Burying Substrates

Substrates commonly used include corn cob (Thomas et al., 2009, Jimenez-Gomez et al., 2011, Angoa-Pérez et al., 2013), sawdust (Harasawa et al., 2006, Slot et al., 2008, Krass et al., 2010, Dixit et al., 2014), wood chips (Londei et al., 1998, Saadat et al., 2006, Llaneza and Frye, 2009, Thomas et al., 2009), wood shavings (Poling et al., 1981), river sand (De Brouwer and Wolmarans, 2018) and Sani-chips© (Young et al., 2006, Thomas et al., 2009, Kinsey et al., 2011). However, the choice of an appropriate burying substrate is important for several reasons. Due to the sparse and light nature of burying substrates such as sawdust (pine, weighed and calculated at 0.17 g/cm^3; De Brouwer and Wolmarans, 2018) and wood shavings (pine, weighed and calculated at 0.07 g/cm$^{3;}$ De Brouwer and Wolmarans, 2018), marbles simply placed gently on the surface of these substrates may appear from the outset to be covered to a depth of at least two-thirds of its size when compared to denser substrates (De Brouwer and Wolmarans, 2018). Further, as most investigations do not report the use of video tracking, endpoint quantification of the number of marbles buried may be a caveat in the interpretation of data. In contrast, denser substrates with a higher mass per volume ratio, e.g.

[1] marble burying test

corncob (weighed and calculated at 0.38 g/cm^3; De Brouwer and Wolmarans, 2018) or river sand (weighed and calculated at 1.65 g/cm^3; De Brouwer and Wolmarans, 2018), are generally more resistant to the effects of normal exploration and therefore may be better suited as appropriate substrates in which to carry out the MBT[1] . In fact, in substrates such as sawdust and wood shavings, marbles of a greater mass settle to the bottom of the testing arena quicker compared to marbles of a lower mass; these substrates are therefore subject to disturbance by any routine movement of the animals during the test session (De Brouwer and Wolmarans, 2018). For this reason, the current investigation made use of river sand as the burying substrate of choice.

Marbles

Standard glass marbles, 13 mm in diameter, were used. Marbles were disinfected with 90% surgical alcohol to remove scent traces and avoid unnecessary neophobic triggers.

Habituation before exposure

The novelty of burying substrates may trigger natural exploratory activity in the form of digging and burrowing and may therefore influence the number of marbles being covered (Gyertyan, 1995, Thomas et al., 2009). Therefore, to exclude the possible effects of novel cage exploration on burying outcomes, it is important to consider adequate habituation in burying substrates before the onset of behavioural analysis. This may be more applicable for anxiety rather than compulsivity-related studies, because in compulsivity studies, animals should be exposed over the course of repetitive trials, instead of a single trial (Njung'e and Handley, 1991, Gyertyan, 1995, Thomas et al., 2009, Wolmarans et al., 2016b, Taylor et al., 2017, De Brouwer and Wolmarans, 2018). Hence, habituation is introduced coincidentally in the experimental design. In fact, it has previously been shown that when the test is repeated on up to five consecutive days with the same subjects, no significant differences in burying activity are observed in some studies (Poling et al., 1981, Njung'e and Handley, 1991, Gyertyan, 1995, Thomas et al., 2009, Wolmarans et al., 2016b). However, with investigations into anxiety-like behaviour, it is important to exclude the possible effects of other novelty factors, e.g. burying substrate on burying performance (Casarotto et al., 2010, Umathe et al., 2008, Gawali et al., 2016, Taylor et al., 2017). With respect to the current investigation, we attempted to exclude the influence of a possible neophobic trigger underlying the burying response, and therefore habituated all individuals in the burying substrate for at least 24 hours prior to the first test.

[1] marble burying test

2.2.4 The relevance of high marble burying and large nest building as different obsessive-compulsive phenotypes in the current investigation

Considering the literature background presented here, little progress has been made in addressing the poor treatment response of patients with OCD[1]. Recent findings relating to the neurocognitive constructs underlying different obsessive-compulsive phenotypes (Figee et al., 2011) provide some indication that a novel understanding of OCD may be necessary. The fact that HMB[2] expressed by an 11 – 15% minority of deer mice seems just as persistent and purposeless as LNB[3] or HS[4], but that it does not respond to serotonergic intervention, may indicate that aberrant burying behaviour may be a unique compulsive-like phenotype within the deer mouse model of OCD[5], especially one that is SSRI[6] treatment resistant. Therefore, based on the matters of persistence, repetition and seemingly purposelessness, the current investigation sets out from the perspective that HMB and LNB are two different phenotypes of the same condition that may lend itself toward providing a valuable framework in which to investigate unique phenotypes of the same condition and hence studying selective mechanisms and treatment interventions. Further, we propose HMB to resemble treatment resistant compulsive-like behaviour founded on the unique involvement of the dopaminergic system (since it is unresponsive to SSRI intervention). Moreover, we hypothesize that LNB, based on its response to SSRI intervention, will resemble a compulsive-like phenotype akin to the classic picture of OCD, i.e. a condition of hypo-serotonergic signalling. Moreover, we propose that HMB and LNB will diverge on the basis of their unique response to anti-dopaminergic intervention administered either alone or in combination with escitalopram, as alluded to earlier.

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

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3 Journal Article

Large nest building and high marble burying in the deer mouse *(Peromyscus maniculatus bairdii)* and its response to serotonergic, anti-dopaminergic and combination intervention

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Author Contributions:

- *Ané Lombaard* assisted in the conceptualization of the study together with *De Wet Wolmarans*, performed the behavioral analyses, processed the data, interpreted the statistical analyses and assisted in the writing of the manuscript.
- All authors contributed equally to this work.

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- Instructions to the author can be accessed here: https://journals.sagepub.com/author-instructions/JOP
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Abstract

Background: Obsessive-compulsive disorder (OCD) is a debilitating psychiatric condition. 40 – 60% of patients become treatment resistant to standard treatment with selective serotonin reuptake inhibitors (SSRIs), in which case augmentation therapy with atypical low-dose anti-dopaminergic drugs are often employed. There is mounting evidence that the different phenotypes of OCD differ in their underlying neurobiology. Yet, there is no concrete model to explain the aetiology of symptom heterogeneous OCD.

Aims: The aim of this study was to explore the potential differences in treatment-response of two spontaneous compulsive-like behavioural phenotypes expressed by deer mice, i.e. SSRI-responsive large nest building (LNB), and SSRI-refractory high marble burying (HMB), by means of pharmacological intervention with either an SSRI alone, i.e. escitalopram, a low-dose antidopaminergic drug, i.e. flupentixol, and a combination of the two.

Methods: Animals exhibiting HMB (over three consecutive 30-minute sessions separated by 24 hours) or LNB (over seven consecutive nights) respectively were selected for chronic treatment with escitalopram (50 mg/kg/day), flupentixol (0.9 mg/kg/day), or a combination of the two at the aforementioned doses for 28 days (n = 6 animals per treatment group). Behavioural testing was repeated post-treatment.

Results: HMB, although demonstrating time-dependent adaptation, remained resistant to all of the interventions used in this investigation. However, LNB responded equally well to escitalopram alone or the combination thereof with flupentixol ($p < 0.0001$) but showed no significant response to flupentixol alone.

Conclusion: The data reported here confirm previous findings from this laboratory that identified HMB as a relatively treatment-resistant compulsive-like phenotype, while LNB may be reminiscent of treatment-responsive OCD.

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Keywords:

Obsessive-compulsive disorder; marble burying; nest building; animal model; escitalopram; flupentixol; deer mouse model

Introduction

Obsessive-compulsive disorder (OCD) is a chronic, debilitating psychiatric condition that affects 1 – 2% of the global population and is characterised by obsessions, i.e. intrusive, unwanted thoughts, and/or compulsions, i.e. persistent, repetitive and time-consuming behavioural routines (American Psychiatric Association, 2013). In general, five broad phenotypes of OCD symptomology have been identified, i.e. contamination obsessions and washing compulsions, safety obsessions and checking compulsions, obsessions about symmetry and ordering compulsions, covert intrusive thoughts with repugnant themes, i.e. violent or sexual acts that are associated with ritualistic, self-directed acts, e.g. praying, and fears of losing objects and collecting compulsions (Abramowitz et al., 2009, Markarian et al., 2010, Mataix-Cols et al., 2004, Mataix-Cols et al., 2005, Denys et al., 2004a, McKay et al., 2004).

Selective serotonin reuptake inhibitors (SSRIs) are currently considered as the first line pharmacological intervention for OCD (Koran and Simpson, 2013); however, a significant number of patients remain treatment-resistant (Husted and Shapira, 2004, Brakoulias and Tsalamanios, 2017, Hudak et al., 2012). In such cases, adjunctive therapy with low-dose atypical anti-dopaminergic drugs has been reported to attenuate symptoms in some SSRI refractory cases (Kim et al., 2018) and is a promising avenue for pre-clinical investigation. With this in mind, an abundance of evidence point to the likelihood that different phenotypes of OCD differ in their underlying neurobiology (Harrison et al., 2013, Hashimoto et al., 2011, Karadag et al., 2011, Miguel et al., 2005), cognitive neuropsychology (Denys et al., 2004b, Fontenelle et al., 2005, Martoni et al., 2015, McKay et al., 2004, Rufer et al., 2006) and treatment response (Miguel et al., 2005, Stein et al., 2007, Rufer et al., 2006), i.e. the hoarding phenotype often proves to be the most treatment resistant (Stein et al., 2007, Rufer et al., 2006). Moreover, recent findings also highlighted potential differences in reward-feedback processing associated with different phenotypes of obsessive-compulsive symptomology, potentially implicating unique contributions of serotonergic and dopaminergic signalling in the manifestation of different symptom dimensions (Figee et al., 2011). However, these findings must yet be corroborated (Cavedini et al., 2006) as only a few investigations that probed potential associations between obsessive-compulsive symptomology and reward processing follow a symptom subtype specific approach (Jung et al., 2011).

On a neurobiological level, perturbations in cortico-striatal-thalamo-cortical (CSTC) circuitry are well documented to be implicated in OCD (Husted et al., 2006, Stocco et al., 2010). Further, the mechanisms underlying the clinical efficacy of SSRI intervention remain elusive; however, considering theories that explain behavioural interactions between dopamine and

serotonin (Daw et al., 2002, Balasubramani et al., 2015, Rogers, 2010), the clinical efficacy of SSRI intervention in patients with OCD could potentially be ascribed to its inhibition of dopaminergic activity (Palminteri et al., 2012, Goddard et al., 2008). Considering the less than optimal treatment response of OCD to SSRI intervention, as well as contradictory findings with respect to anti-dopaminergic treatment efficacy both in the clinic (Albelda and Joel, 2012, Millan et al., 2015, Bloch et al., 2010) and in preclinical models (Honda et al., 2011), it may be possible that unique bio-behavioural processes that potentially underlie the expression of obsessive-compulsive symptoms may contribute to the rate of treatment response recorded.

With respect to the current investigation and considering the literature reviewed above, there is a lack of preclinical studies that attempt to explore the neural underpinnings of different OCD phenotypes; this is likely due to the lack of animal models that are representative of symptom heterogeneous obsessive-compulsive like behaviour (Scheepers et al., 2018). In this regard, the different naturalistic compulsive-like phenotypes exhibited by deer mice (*Peromyscus maniculatus bairdii*), i.e. large nest-building (LNB; Wolmarans et al., 2016a) and high marble-burying (HMB; Wolmarans et al., 2016b), may present a useful avenue for exploration. Indeed, these behaviours are equally persistent, repetitive (although demonstrating a waxing and waning nature that is reminiscent of OCD symptomology; Abramowitz et al. 2015), and seemingly purposeless (Scheepers et al., 2018). However, whereas spontaneous stereotypy (Wolmarans et al., 2013) and LNB (Wolmarans et al., 2016a) demonstrate therapeutic response to chronic high dose (50 mg/kg/day) oral treatment with the SSRI, escitalopram, we have shown previously that HMB responds to a lesser extent to such intervention (Wolmarans et al., 2016b). Therefore, the current investigation aimed to establish whether HMB and LNB may be founded on unique neurobiological underpinnings that underlie specific distinctive responses to high-dose escitalopram, low-dose flupentixol, a $D_{1/2}$ antagonist, or a combination of both. More specifically, we hypothesise that as LNB already demonstrates adequate response to SSRI intervention, such behaviour will be less sensitive to anti-dopaminergic and combination intervention. However, since HMB is less responsive to SSRI treatment alone, we believe it possible that such behaviour represents a compulsive-like phenotype more founded within dopaminergic perturbations. As such, we expect intervention with anti-dopaminergic treatment as used in clinically SSRI refractory OCD, either alone or in combination with escitalopram, to modulate such behaviour in a unique manner, compared to the response of LNB following the same intervention.

Materials and methods

Animals

Since only 11 – 15% (Wolmarans de et al., 2016) and 30% (Wolmarans et al., 2016a) of deer mice, irrespective of sex, express HMB and LNB respectively, a total of 160 deer mice, aged 10 – 12 weeks at the onset of investigation, were initially acquired from the deer mouse colony housed and maintained at the Vivarium of North-West University, Potchefstroom, South Africa (ethics approval number: NWU-00262-16-A5; AnimCare Research Ethics Committee, registration number: AREC-130913-015; South African National Standard (SANS) for the Care and Use of Animals for Scientific Purposes; SANS 10386:2008). Animals not selected for inclusion in either of the behavioural groups, i.e. HMB and LNB, were excluded from further investigation and used for studies unrelated to this work. The original colony was obtained from the *Peromyscus* Genetic Stock Centre at the University of South Carolina. Mice were randomly selected without litter, weight or sex bias. Previous investigations confirmed that both LNB and HMB are equally expressed across both sexes (De Brouwer and Wolmarans, 2018, Wolmarans et al., 2016a, Wolmarans et al., 2016b). Animals were housed in groups of four to six same-sex animals per cage until one week prior to the first behavioural assessment, from which point onwards animals were housed individually. Cages (35 cm (l) × 20 cm (w) × 13 cm (h); Techniplast SPA, Varese, Italy) were individually ventilated and climate-controlled and maintained at 23°C on a 12-hour light/dark cycle (with lights switched on at 06h00 and off at 18h00). Food, water, and drug solutions where applicable, were provided *ad libitum* for the duration of the study. Cages were cleaned and bedding changed weekly. Prior to behavioural experimentation, bedding consisted of ground corncob (particle ø 3 – 4 mm) and nesting material was provided in the form of unscented, white paper towel.

Drugs

Following the last pre-treatment behavioural screen for nesting and marble burying (MB) activity, all animals received chronic (28-day) treatment with either 1) normal drinking water, 2) high-dose escitalopram oxalate (50 mg/kg/day; Wolmarans et al., 2013), 3) low-dose flupentixol (0.9 mg/kg/day; Murugaiah et al., 1983b), or 4) a combination of escitalopram oxalate and flupentixol. The dose of escitalopram employed in this investigation was chosen based on prior studies in our laboratory (Wolmarans et al., 2016a, Wolmarans et al., 2016b). To mimic the treatment response of clinical OCD, a low dose of flupentixol (0.9 mg/kg/day) was chosen based on extensive previous investigations that employed oral drug administration via drinking water (Murugaiah et al., 1982, Murugaiah et al., 1983a, Jenner and

Marsden, 1987). Following selection (see paragraphs 2.3 and 2.4), both cohorts were divided into the four treatment groups (*n* = 6 per group). All drugs (H. Lundbeck® A/S, Ottiliavej, Denmark) were prepared for oral administration via drinking water; fresh drug solutions were prepared every second day (Wolmarans et al., 2016a, Wolmarans et al., 2016b). Due to the potential influence of anxiety on the behavioural expression of laboratory animals (Drude et al., 2011), physical handling was kept to a minimum. Drug concentrations were calculated based on the average water intake of deer mice (0.25 mg/kg/day) as previously determined in our laboratory (Wolmarans et al., 2013) and confirmed by others (Aschhoff et al., 2000). Further, as animals were housed alone from the beginning of experimentation, fluid intake could be monitored; drug addition to drinking solutions did not alter the average water consumption (data not shown).

Behavioural tests

General background

As alluded to earlier, the aim of this investigation was to study potential associations between different behavioural phenotypes and treatment response. Therefore, clear separation between animals that expressed either of the cohorts, *but not both*, was needed. As such, all 160 animals were first screened for HMB activity (paragraph 2.3.2). Following the selection of the 24 HMB expressing animals, these, along with the other 136 mice from the original pool of 160 were screened for LNB (paragraph 2.3.2). Although none of the HMB-expressing individuals also engaged in LNB, animals that did not express either of the behaviours were also excluded from further study. Further, as the present investigation sought to characterise the treatment response of compulsive-like cohorts, we have not included non-compulsive-like animals. The normal behavioural controls for LNB and HMB, as well as their responses to chronic high-dose oral escitalopram (50 mg/kg/day) have been described before (Wolmarans et al., 2016a, Wolmarans et al., 2016b).

The marble burying test (MBT)

[Figure 1A: Correlation Total MB Score vs. Coefficient of Variance Flupentixol]

The marble burying test (MBT) was carried out according to a previously published method with minor modifications (De Brouwer and Wolmarans, 2018). Briefly, MB cages (exact replicas of the home cages) were prepared with a 5-cm thick layer of course river sand on which nine glass marbles (ø=15 mm) were arranged in a 3 x 3 grid equidistant to one another in *one half* of the cage only, thereby constituting a two-zone MBT setup. This allowed mice to willingly and voluntarily engage with marbles while also ensuring that novelty-induced

avoidance behaviour could be identified. The use of course river sand, as opposed to sawdust or husk as often reported (Egashira et al., 2008a, Krass et al., 2010), prevents marbles being unintentionally covered due to routine cage locomotion (De Brouwer and Wolmarans, 2018). Since deer mice are nocturnal animals, all marble-burying assessments were carried out from 1 hour after onset of the dark cycle under dim red light (40 lux).

On the morning of the day prior to the first MB assessment, i.e. 36 hours before the first MB assessment (pre-treatment screen) and again after four weeks of treatment (post-treatment screen), mice were habituated to the burying substrate by replacing the corncob home cage bedding with coarse river sand; this was necessary to prevent novelty induced digging during the first MB trial (Njung'e and Handley, 1991, Gyertyan, 1995). Subsequently, each animal was assessed for MB activity over three nights in three separate 30-minute trials (one per night), separated by 24 hours. This process was repeated before and after treatment. At any given time of assessment, animals were removed from their home cages and introduced into pre-prepared MB cages. Due to the highly active nature of deer mice, cages were covered with clear Plexiglass® covers to prevent animals from leaving the cages. Mice were subsequently left to explore and interact with marbles for 30 minutes in the absence of human observers, while being videotaped for later analysis. After each 30-minute session, animals were returned to their respective home cages which still contained the same burying substrate and left for 24 hours. This procedure was repeated twice over the next two days, until all animals underwent three separate pre- and post-treatment screens.

To identify the 24 HMB expressing animals, we modified previously published methods to some extent; to highlight the conceptual differences between the methodology followed here and the methodology followed elsewhere, as well as to appraise the findings reported in this investigation against the background of previously reported literature, kindly refer to Broekkamp et al., 1986, Egashira et al., 2008a, Egashira et al., 2008b, Umathe et al., 2008, and Thomas et al., 2009. The number of marbles covered in burying substrate to a depth at least two-thirds of the marble size (Wolmarans et al., 2016b) was quantified after the completion of each 30-minute screening session. This was done by two experimentally blind observers whose scores were averaged. Importantly, after the first three pre-treatment MB assessments had been completed, the 24 HMB-expressing animals were selected based on 1) the average number of marbles covered *and* 2) the persistence of burying behaviour over three trials as evinced by lower coefficients of variance with respect to the between daily burying scores (Wolmarans et al., 2016b; **Figure 1A**). Only the 24 animals selected for HMB during the pre-treatment phase were retested in the MBT after 28 days of receiving their respective treatment.

That said, with respect to employing MB as a screening test for anti-compulsive drug action, we have argued previously (De Brouwer and Wolmarans, 2018) that quantification of the number of marbles 'buried', as is most often reported (Broekkamp et al., 1986, Egashira et al., 2008a, Egashira et al., 2008b, Umathe et al., 2008), is inadequate, inappropriate and counter-productive for highlighting seemingly purposeless compulsive-like persistence. Instead, compulsive-like behaviour as emulated in the MBT should be characterised by behavioural persistence, repetition and voluntary, goal-directed interaction. Therefore, considering the non-reactive nature of glass marbles, we subsequently also characterised *preoccupied interaction* with marbles, *viz.* marble-directed behavioural episodes (MDB) in the 24 selected HMB expressing animals. Indeed, such behaviour speaks more to the compulsive-like actions of mice (De Brouwer et al., 2019). MDB were scored as 1) sniffing and licking of marbles, 2) rolling or touching marbles with forepaws, 3) standing on marbles, and 4) any movement directed in the immediate vicinity of a marble that was aimed at covering the marble, each adding a single count toward the MDB score. As such, in the 24 selected HMB expressing animals, the MBT was evaluated pre- and post-treatment according to both criteria, i.e. the number of marbles buried *and* the number of MDB (De Brouwer et al., 2019).

Nest-building analysis

[Figure 1B: Correlation Total Nesting Score vs. Coefficient of Variance Flupentixol]

Following the last pre-treatment MBT, the 24 animals selected for HMB (to exclude the possibility of some of these engaging both phenotypes) as well as the other 136 mice of the original pool underwent screening for nest building (NB) activity over the course of seven consecutive days (Wolmarans et al., 2016a). Each day, home cages were prepared with an excess of pre-weighed non-scented hospital grade cosmetic cotton wool placed above the steel grid within the roof of the home cage (Wolmarans et al., 2016a). Every subsequent day between 13h00 and 14h00, built nests were removed, discarded and the unused cotton wool weighed (Wolmarans et al., 2016a). Mice were therefore left to utilize the cotton wool for approximately 23 hours of every day. As NB analyses were conducted in the home cages, food and water were available *ad libitum* for the duration of the experimentation. Animals did not have access to any other form of nesting material during this time. As with marble-burying, classification of LNB (**Figure 1B**) was based on 1) total nesting score generated over seven days, and 2) the behavioural variance over the course of seven days as reflected by the coefficients of variance with respect to the daily nesting scores. As such, only animals that consistently built the largest nests were included in the LNB cohort (**Figure 1B**; Wolmarans et al., 2016a). Further, only the 24 identified LNB animals were retested for NB activity after 28-days of treatment.

Statistical analyses

Statistical analyses were performed with GraphPad Prism® 8 (GraphPad Software, San Diego, USA) under the guidance of the Statistical Consultation Service of NWU, Potchefstroom. Linear regression analyses and column statistics (25th and 75th quartiles) were applied to identify HMB- and LNB-expressing subjects. Pre-and post-treatment expression of HMB and LNB, as well as locomotor activity in the case of the HMB were analysed and compared by means of two-way repeated measures analysis of variance (2-way RM-ANOVA) followed by Bonferroni post-hoc comparisons (Wolmarans et al., 2016a, Wolmarans et al., 2016b). Behavioural expression (MB and NB scores respectively) was set as between subject factor and time and treatment as within subject factors. Statistical significance was set at $p < 0.05$ for all analyses. All statistical analyses were followed by pairwise calculations of Cohen's *d* effect size to determine the practical significance of differences. A large effect size was considered as $d > 0.8$, and a very large effect size as $d > 1.3$.

Results

Marble burying

A total of 24 HMB expressing animals were selected from the initial pool of 160 (**Figure 1A**).

Number of marbles buried

[Figure 2A: Marble burying – Number of marbles buried]
Time and treatment failed to interact significantly with respect to the number of marbles buried (F [3, 20] = 1.61; p = 0.218). However, time had an overall significant main effect on the burying scores (F [1, 2] = 19.63; p = 0,0003). Post-hoc comparisons revealed a significant post-treatment reduction in the number of marbles buried in the control group only (**Figure 2A**: 1.2 ± 0.59 vs. 3.7 ± 1.47; p = 0.007; CI: 0.63 – 4.49; d = 2.5), while reductions characterized by large effect sizes were also observed in the escitalopram (2.0 ± 2.10 vs. 3.7 ± 0.89; p = 0.094; d = 1.2) and in the combination treated groups (1.2 ±1.29 vs. 2.8 ± 0.48; p = 0.154; d = 1.8). Flupentixol alone failed to elicit any response.

Number of marble-directed interactions (MDB)

[Figure 2B: Marble burying – Marble directed behaviour]
Again, no significant interaction between time and treatment was demonstrated with respect to the number of MDBs (F [3, 20] = 1.48; p = 0.251); however, time on its own was a significant contributing factor to the observed changes in behaviour (F [1, 20] = 23.24; p = 0.0001). In

this instance, post-hoc analyses revealed a statistically significant reduction in the number of post-treatment MDBs observed in the escitalopram-treated group compared (**Figure 2B**: 19.0 ± 5.57 vs. 30.4 ± 5.92; p = 0.003; CI: 3.58 – 19.2; d = 2.0). No significant pre- vs. post-treatment differences were demonstrated for any of the other groups. However, all other treatment groups presented with reductions of practical significance in MDB after four weeks of treatment (Control: 22.4 ± 4.77 vs. 26.1 ± 2.58, p = 0.85; d = 1.0; Flupentixol: 22.0 ± 9.05 vs. 29.6 ± 8.54, p = 0.056; d = 0.9; Combination: 4.7 ± 5.59 vs. 15.6 ± 6.59, p = 0.451; d = 0.8).

Nest-building

[Figure 3: Nest-building]

A total of 24 LNB expressing deer mice that did not also engage in HMB, were selected from the initial pool of 160 deer mice. A statistically significant two-way interaction was demonstrated between time and treatment with respect to the pre- and post-treatment nesting scores of LNB animals (F [3, 20] = 5.05; p = 0.009). Subsequent pairwise analyses revealed that the average natural expression of LNB was exacerbated over time in the control group (**Figure 3**: 34.1 ± 12.05 g vs. 19.8 ± 3.86 g; p = 0.025; CI: 1.5 g – 27.01 g; d = 1.8). Although not statistically significant, such exacerbation was not only prevented, but reversed by the administration of escitalopram (**Figure 3**: 14.9 ± 6.66 vs. 24.5 ± 2.80, p = 0.2; d = 2.0). This response was blunted by the addition of flupentixol (**Figure 3**: 16.5 ± 12.86 g vs. 22.1 ± 5.90g, p = 0.982; d = 0.5). Significant post-treatment differences in nesting scores of animals treated with escitalopram (14.9 ± 6.66 g, p = 0.004; d =1.0) and the combination (16.5 ± 7.57, p = 0.01, d = 0.3) were observed compared to control (34.1 ± 12.05 g). Flupentixol on its own had no effect on nesting behaviour.

Locomotor activity

[Figure 4: Marble-burying locomotor activity]

A two-way RM ANOVA was run to determine whether time or treatment had a significant impact on the general locomotion of the mice included in the MB groups. No significant interaction between time and treatment was observed (**Figure 4**; F [3,8] = 0.31, p = 0.818). Further, neither time (**Figure 4**; F [1,8] = 1.05, p = 0.335) nor treatment (**Figure 4**; F [3,8] = 1.28, p = 0.345) alone had any significant effect on locomotor activity.

Discussion

The present work aimed to establish whether different phenotypes of compulsive-like behaviours, previously shown to differ in their response to serotonergic intervention (Wolmarans et al., 2016a, Wolmarans et al., 2016b), can be separated based on the involvement of the dopaminergic system. Based on the main findings of this investigation, our results are congruent with previous findings in demonstrating that escitalopram significantly attenuated large NB behaviour. Although the same response seemed true for MB activity, burying behaviour adapted as a function of time, and not treatment. Further, flupentixol, neither on its own, nor in combination with escitalopram, had any additional effect on either of the phenotypes.

There is a distinct need for a better understanding of the mechanisms underlying obsessive-compulsive symptomology, especially considering the burden of treatment resistance in OCD (Husted and Shapira, 2004, Brakoulias and Tsalamanios, 2017). This is an important consideration as the development of novel pharmacological alternatives ultimately depends on studying underlying neurobiological processes. In this regard, aberrations in the CSTC circuitry are well known to be associated with OCD (Figee et al., 2011, Husted and Shapira, 2004, Husted et al., 2006, Stein et al., 2000). Although a causal relationship has not been established, it is believed that hypo-serotonergic signalling within these brain areas relative to an overactive D_1 receptor expressing pathway may be a neurobiological correlate of obsessive-compulsive symptom propagation (Murphy et al., 2004, Murphy Dennis et al., 2013, Nonnis Marzano et al., 2008, Sinopoli et al., 2017, Millan et al., 2015, Blier and de Montigny, 1998, Stahl, 1998). Further, deficits in goal-directed action motivation (Pinto et al., 2014, Gillan et al., 2014) and reward-feedback processing, also regulated by dopaminergic signalling within the CSTC circuitry, have also been implicated in OCD (Bayer and Glimcher, 2005, Palminteri et al., 2012). In line with the above, current approaches to treatment are conceptually supportive of the opponency theory that describes the functional relationship between serotonin and dopamine (Daw et al., 2002). Whereas serotonin is generally accepted to abrogate/inhibit action, avoidance and safety-seeking, dopamine is considered to motivate approach and reward-seeking behaviour (Palminteri et al., 2012). Nevertheless, not all patients respond to the current treatments (Hudak et al., 2012, Miguel et al., 2005, Stein et al., 2007, Rufer et al., 2006) and the fact that certain phenotypes of OCD seem to be characterized by higher rates of treatment-resistance than others (Leckman et al., 2009, Stein et al., 2007, Rufer et al., 2006), may be indicative of unique perturbations in the crosstalk between serotonergic and dopaminergic signalling in these patients. This may potentially be different from what is generally accepted to be the case in obsessive-compulsive theory. However, although some neuropsychological research has been indicative of such a possibility

(Landeros-Weisenberger et al., 2010, Cavallini et al., 2002, Mataix-Cols, 2006), in-depth empirical research in this area remains sparse.

To this extent, animal models of OCD are generally restrictive. Usually they are representative of a single behavioural phenotype, i.e. LNB (Hoffman and Morales, 2009) or compulsive-like behavioural persistence, i.e. high operant responses (Joel and Avisar, 2001), or restrictive ambulatory repertoires (Yadin et al., 1991). This makes investigations into symptom heterogeneous behaviours difficult. Further, most models respond optimally to both serotonergic and anti-dopaminergic intervention (Szechtman et al., 1998, Fineberg et al., 2011), a factor that is to some degree counterproductive for investigations into treatment-resistance. Also, in the case of genetic or pharmacological models, the observed symptomology is inherently linked to the neurobiological actions of the causal intervention (Szechtman et al., 2017, Chou-Green et al., 2003, Yadin et al., 1991) and thus predictive for the treatment response. Therefore, to further our understanding of the mechanisms underlying treatment resistance, studies of naturalistic, compulsive-like behaviours, whether in a single or different species or models, may be informative. As alluded to before, naturalistic persistent behaviours expressed by deer mice may provide a useful avenue to investigate potential neurobiological differences underlying equally compulsive-like, but unique behavioural phenotypes. Not only do mice of both sexes develop different forms of compulsive-like behaviours, i.e. LNB (Wolmarans et al., 2016a) and HMB (Wolmarans et al., 2016b), they do so variably with 30% and 11 – 15% of animals presenting with each of the phenotypes, respectively (see Scheepers et al. 2017 for an extensive review and a description of such behaviours against the background of OCD). More importantly, while LNB demonstrates robust response to high dose chronic escitalopram intervention, HMB seems less responsive. As such, the present work aimed to explore whether HMB, as opposed to LNB, may be responsive to anti-dopaminergic augmentation, thereby pointing to a unique neurobiological involvement in HMB compared to LNB.

Considering our findings with respect to MB activity, neither of the drug interventions elicited any effect. In fact, although a significant difference was demonstrated with respect to the pre- and post-treatment burying scores of control-treated animals (**Figure 2A**) and the marble directed interactions of escitalopram-treated animals (**Figure 2B**), time and not treatment had an overall main effect on the behaviours observed, indicating that over time, HMB animals potentially became less interested to interact with the objects. This finding, observed in the absence of any significant changes in the general locomotor activity of the animals (**Figure 4**), is in line with our previous data that demonstrated escitalopram to be ineffective in attenuating MB behaviour (Wolmarans et al., 2016b). That no change in either of the behavioural measures could be demonstrated in response to escitalopram intervention is significant,

indicating the insensitivity of HMB to serotonergic intervention. Indeed, this is true as both parameters are regarded as robust measures of burying activity. While most investigations that apply the MBT as a measure of compulsive-like behaviour only quantify the number of marbles 'buried' (De Brouwer and Wolmarans, 2018), such an approach is inaccurate for a number of reasons (see (De Brouwer and Wolmarans, 2018) for review). Suffice to say that appraisals of MB activity from an OCD perspective should be based on observations of a persistent, recurrent and goal-directed preoccupation with the objects. In fact, marbles coincidentally become covered during normal cage exploration, *viz.* digging and burrowing (Thomas et al., 2009). Therefore, only focusing on the number of marbles buried yields a false impression of the underlying burying phenotype.

In line with previous research (Wolmarans et al., 2016a), our data confirm an association between LNB and hypo-serotonergic signalling. While we could not demonstrate a significant difference between the pre-and post-treatment nesting scores of animals treated with high-dose escitalopram, the results must be regarded against the background of the significant increase in nesting scores observed in control-treated animals (**Figure 3**). Such behavioural drift in the behaviour of control treated animals was unexpected. However, it has been shown before that social isolation of animals not only facilitates the development of restricted behavioural phenotypes (Presti-Torres et al., 2012), but also induces cage anxiety (Olsson and Westlund, 2007). Although the potential influence of anxiety underlying LNB behaviour in deer mice has not been investigated before, it is likely that prolonged isolation may partly be responsible for the exacerbating drift in LNB behaviour. That said, isolating animals is a necessary aspect of the experimental design and could unfortunately not be prevented. Further, considering that a clear pre-treatment separation in nesting scores is evident prior to social isolation, and that LNB is expressed in a waxing and waning nature between different nights, i.e. not to the same degree during each night, suggest that LNB expressing animals are not overly anxious. Consequently, we conclude that such behaviour is, irrespective of the underlying trigger, a persistent, repetitive compulsive-like phenotype. Nevertheless, further study is warranted to establish whether anxiety plays a specific role in the promulgation of LNB, thereby providing a useful avenue for investigating the potential influence of anxiety on the expression of a compulsive-like phenotype. In fact, against this background, high dose escitalopram prevented the exacerbation of nesting behaviour (Figure 3), a response that parallels the clinical efficacy of chronic SSRIs in the treatment of compulsivity *and* anxiety.

While our findings are in line with previous observations, a core focus of this work was to establish whether HMB, as a potential treatment resistant phenotype, would respond to SSRI augmentation with low dose anti-dopaminergic drug intervention. As such approaches are often applied in clinically treatment refractory OCD, such an observation would support the

notion that HMB represents a treatment-resistant compulsive-like phenotype. Our data failed to demonstrate this and merits further investigation into alternative neurobiological theories to explain the manifestation of HMB. While the unresponsiveness of LNB to flupentixol augmentation relative to its modification by escitalopram alone could have been expected, neither flupentixol alone nor its combination with escitalopram, elicited any demonstrable effect on burying behaviour. Therefore, our data would indicate that HMB is not sensitive to either of the treatment interventions employed here, and hence is not related to the classic hypo-serotonergic, hyper-dopaminergic profile of OCD. Importantly, the hoarding phenotype is known to respond poorly to pharmacological drug intervention. Considering the potential similarity in symptomology between the burying phenotype and clinical hoarding symptoms, it is possible that MB behaviour as expressed by deer mice may be representative of a treatment-resistant hoarding-like phenotype. This remains to be investigated.

Conclusion

The present work demonstrates the unique response of two phenotypically different spontaneous compulsive-like phenotypes in deer mice, i.e. LNB and HMB, to serotonergic drug intervention. While LNB seems to be representative of the classic hypo-serotonergic picture of OCD, HMB remains resistant to such intervention. Further, anti-dopaminergic treatment alone or in combination with escitalopram failed to elicit any response in any of the two behavioural phenotypes. While this was expected with respect to LNB, our observations in HMB expressing animals would indicate that HMB represents a highly treatment-resistant behavioural phenotype that should be interrogated in terms of its underlying neurobiology. Indeed, future exploration of HMB and alternative treatment strategies may potentially provide significant insight into the mechanisms underlying treatment-resistant persistent behaviours.

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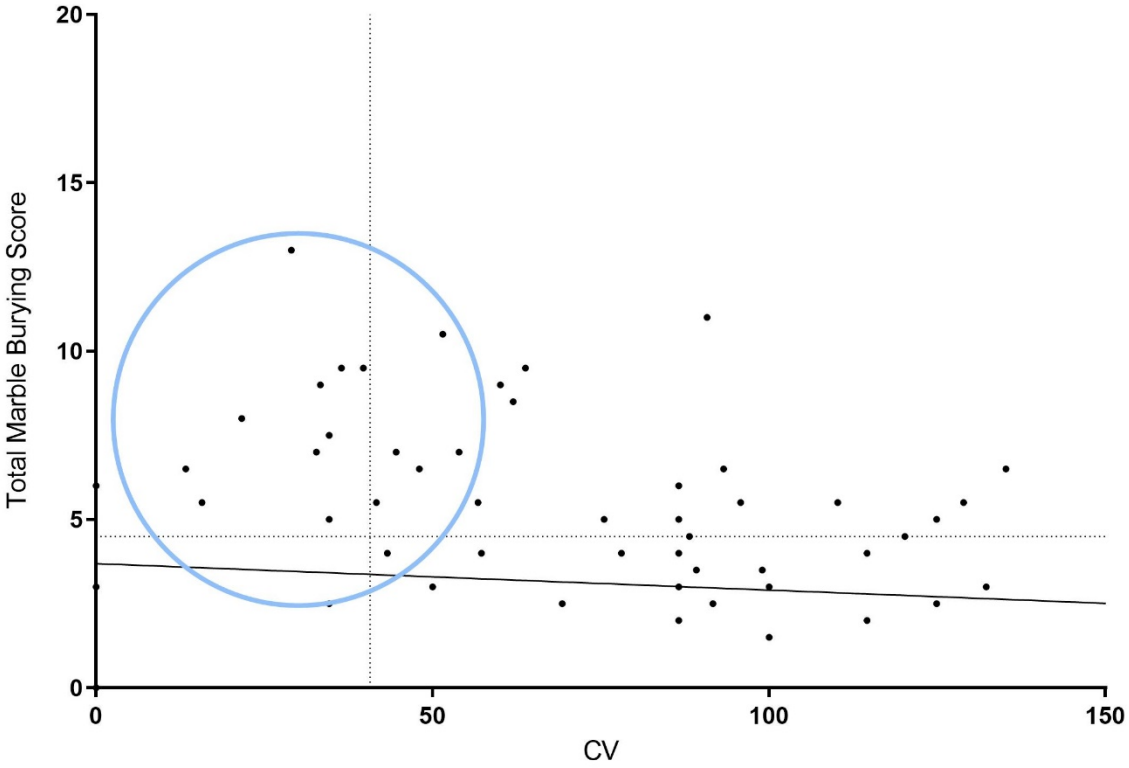

Figure 1A

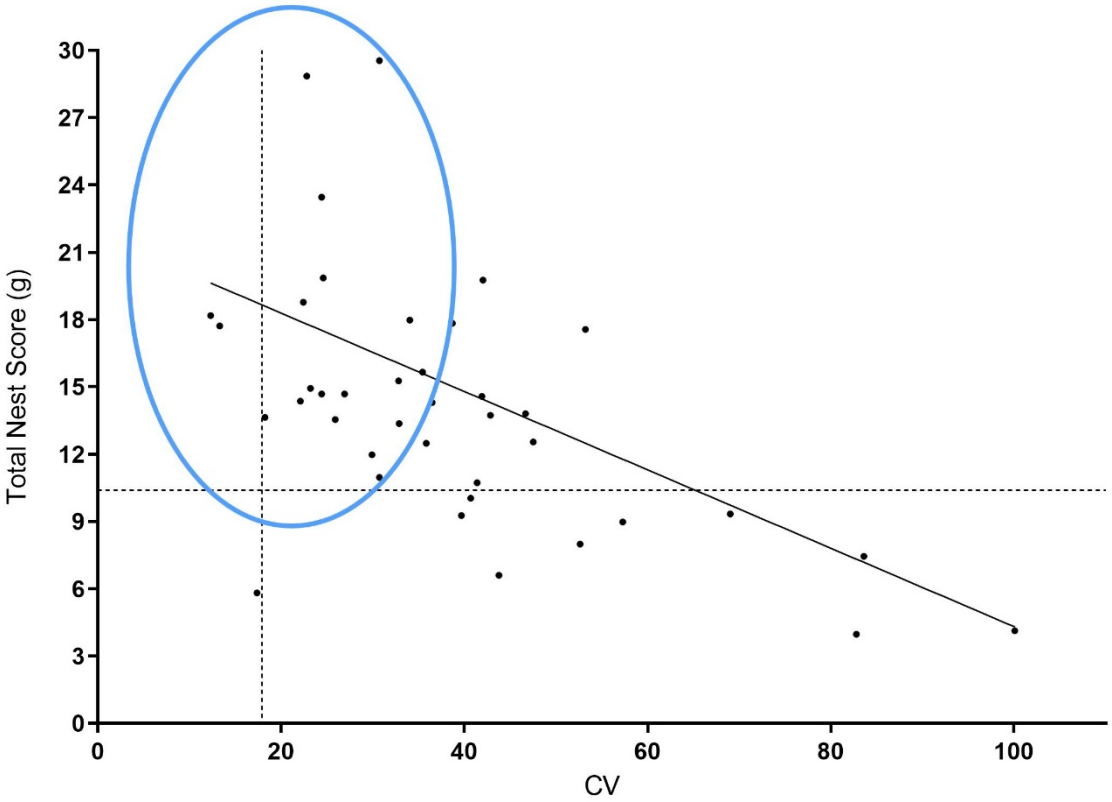

Figure 1B

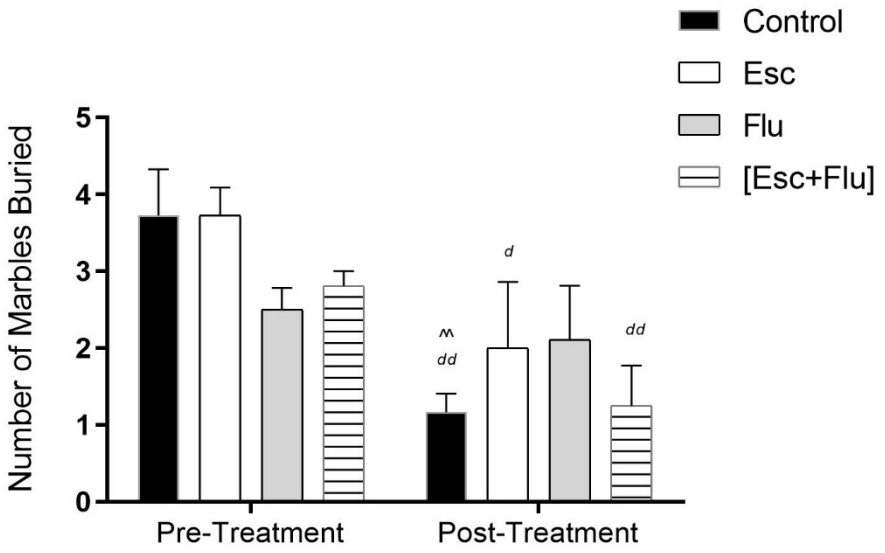

Figure 2A

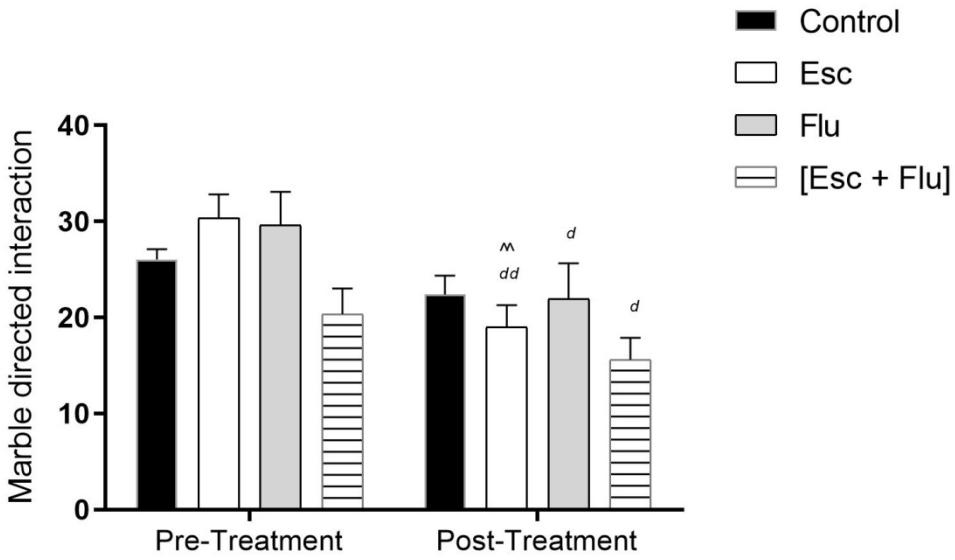

Figure 2B

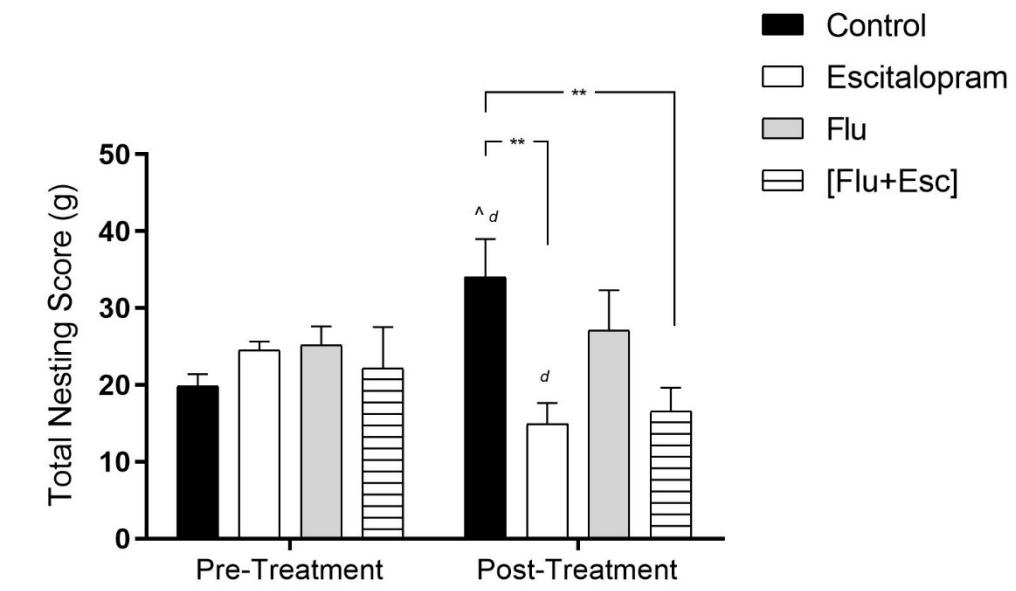

Figure 3

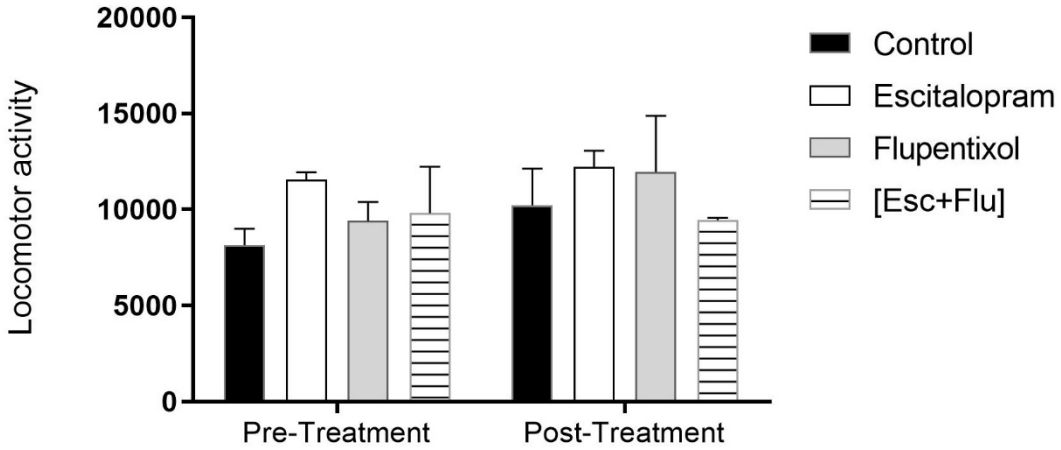

Figure 4

FIGURE CAPTIONS

Figure 1A: Plot of total marble burying scores after three nights and the coefficients of variance with respect to the daily burying activity. Data reflects all animals that were initially screened. Blue circle: HMB animals selected for treatment.

Figure 1B: Plot of total nest building scores after seven 24h periods and the coefficients of variance with respect to the daily nesting activity. Data reflect all animals that were initially screened. Blue circle: LNB animals that were selected for treatment.

Figure 2A: Average pre- vs. post-treatment number of marbles buried over three trials by high marble burying (HMB) animals. ^Pre- vs. post-treatment; within treatment groups. ^^p < 0.01; (Two-way RM-ANOVA, Bonferroni post-hoc). Cohen's effect size: 0.8 > d < 1.3 > dd. Data is mean ± SEM.

Figure 2B: Average pre- vs. post-treatment number of marble-directed interactions over three trials by high marble burying (HMB) animals. ^Pre- vs. post-treatment; within treatment groups. ^^p < 0.01 (Two-way RM-ANOVA, Bonferroni post-hoc). Cohen's effect size: 0.8 > d < 1.3 > dd. Data is mean ± SEM.

Figure 3: Average pre- vs. post-treatment total nesting scores after seven trials in large nest building (LNB) animals. ^Pre- vs. post-treatment; within treatment groups. *Pre- and post-treatment comparison between treatment groups. ^p < 0.05; **p < 0.01 (Two-way RM-ANOVA, Bonferroni post-hoc). Cohen's effect size: 0.8 > d < 1.3 > dd. Data is mean ± SEM.

Figure 4: Average pre- vs. post-treatment expression of locomotor activity in three animals of each treatment group over three trials. Two-way RM ANOVA (no significant interaction or main effects reported).

4 Conclusion

The present work firstly aimed to characterize the behaviour of deer mice with regards to its resemblance to symptom heterogeneous OCD[1]. Secondly, the present study aimed to investigate the response of these behaviours to serotonergic, anti-dopaminergic, and combination intervention. Indeed, we screened for and identified two phenotypically different persistent and compulsive-like phenotypes, namely large nest building (LNB[2], Wolmarans et al., 2016a), and high marble burying (HMB[3], Wolmarans et al., 2016b), that are spontaneously expressed by both sexes. With regards to the response to treatment, our main findings were that 1) escitalopram significantly attenuated LNB, but not HMB behaviour, and 2) flupentixol, neither on its own, nor in combination with escitalopram, had any additional effect on either of the phenotypes. In addition, we demonstrate for the first time the importance of appraising marble burying based on *marble-directed behaviour* (MDB[4]), a measure that accounts for preoccupation, instead of focusing on the *number* of marbles buried.

Up to 40 – 60% of patients suffering from OCD remain resistant to the standard first-line pharmacotherapeutic intervention, i.e. chronic, high dose treatment with selective serotonin reuptake inhibitors (SSRIs[5], Husted and Shapira, 2004, Brakoulias and Tsalamanios, 2017). Strategies to address treatment resistance include increasing the dose of the SSRI used, switching to a different serotonin reuptake inhibitor (SRI[6]) or SSRI, or augmentation therapy with a low-dose dopaminergic antagonist (Millan et al., 2015, Bloch et al., 2006, Bloch et al., 2010). In this regard, augmentation therapy has proven to be successful in up to a third of SSRI refractory patients (Albert et al., 2013, Bloch et al., 2010).

An increasing body of evidence points to different symptom phenotypes of OCD differing in their underlying neurobiological constructs (Landeros-Weisenberger et al., 2010, Cavallini et al., 2002, Mataix-Cols, 2006). Furthermore, considering patterns of treatment resistance to serotonergic interventions with or without anti-dopaminergic augmentation, it is increasingly evident that while serotonin is a crucial role player, the matter of whether patients will respond to SSRIs or not, may be founded on the unique involvement of dopaminergic processes. This idea is borne from both neurobiological (Denys et al., 2004, Hesse et al., 2005, Murray et al., 2017) and neurocognitive research (Palminteri et al., 2012, Pinto et al., 2014, Figee et al.,

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

2011). Indeed, the clinical dilemma of treatment resistance may likely be founded on the fact that patients with different phenotypes of OCD[1] have been shown to present with unique dopaminergic reward-feedback processing deficits (Palminteri et al., 2012, Figee et al., 2011, Ferreira et al., 2017). Briefly, while phasic reductions in dopamine code learning responses to aversive stimuli, phasic dopaminergic increases are responsible for coding rewarding stimuli (Schultz *et al.*, 1993; Schultz *et al.*, 1997; Schultz, 2002; Schultz, 2007; 2016). That said, adequate serotonergic neurotransmission *is also needed for both of these processes* to be facilitated (Fischer & Ullsperger, 2017). Although the construct of reward per se does not form the primary focus of the current work, the fact that distinct reward-related processes – which is intricately linked to opposing dopaminergic responses – have been shown in patients with different phenotypes of OCD, formed the foundation of the current working hypothesis. Indeed, we hypothesize that patients who respond to SSRI[2] monotherapy present with an underlying neurobiological construct already akin to low dopaminergic tone (Cools et al., 2009), but in which hypo-serotonergic signalling may drive the symptomology. On the other hand, SSRI refractory OCD could potentially be founded on the basis of an increased baseline dopaminergic tone; therefore, it may be possible that augmentation of SSRI therapy with a low-dose *anti-dopaminergic* intervention, may be beneficial (Cools et al., 2009). However, such investigations are difficult to perform in humans for a number of reasons, including diagnostic discrepancies between study populations, the extent to which patients commit and adhere to long-term treatment, and ethical considerations.

As such, and taking the brief literature reviewed here in mind, the deer mouse model of OCD may be a useful paradigm in which to study the neurobiological underpinnings of symptom heterogeneous OCD, as deer mice of both sexes present with at least three seemingly purposeless compulsive-like behavioural phenotypes (Scheepers et al., 2018) of which HMB[3] and LNB[4] are investigated here. Therefore, the aim of this work was to determine how SSRI resistant HMB and SSRI sensitive LNB would respond to escitalopram, the anti-dopaminergic agent, flupentixol, and a combination of both. We hypothesized that HMB will resemble a moderately treatment resistant compulsive-like phenotype that would, like many patients presenting with SSRI resistant OCD, respond to the augmentation of escitalopram with the anti-dopaminergic drug, flupentixol. However, since LNB already demonstrates robust response to escitalopram (Wolmarans et al., 2016a), we believed that such behaviour would be representative of classic, hypo-serotonergic OCD; therefore, we did not expect LNB to

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

benefit from either flupentixol monotherapy or combined escitalopram and flupentixol. That said, considering the data presented here, our hypothesis was only somewhat confirmed.

* * *

Considering our findings with respect to marble-burying activity, our hypothesis that this phenotype would be representative of anti-dopaminergic sensitive OCD[1], could not be confirmed. In fact, neither of the drug interventions elicited any effect. Rather, burying behaviour adapted as a function of time as HMB[2] expressing animals potentially became less interested to interact with the objects. Although this finding is in line with our previous data that demonstrated escitalopram to be ineffective in attenuating marble burying behaviour (Wolmarans et al., 2016b), it is also confirmation of the fact that HMB is not founded within a neurobiology characterized by excessive dopaminergic signalling. Importantly, our data are robust as it provides an irrefutable description and measure of burying activity. While most investigations that apply the MBT[3] as a measure of compulsive-like behaviour only quantify the number of marbles "buried" (De Brouwer and Wolmarans, 2018), such an approach is inaccurate (De Brouwer and Wolmarans, 2018). Suffice to say that appraisals of marble burying activity from an OCD perspective should be based on observations of persistent, recurrent and goal-directed preoccupation with the objects. In fact, marbles coincidentally become covered due to the normal cage exploration activity of rodents, *viz.* digging and burrowing (Thomas et al., 2009). Therefore, only focusing on the number of marbles buried, yield a false picture of the actual underlying burying phenotype.

Congruent with our previous findings (Wolmarans et al., 2016a), our data confirm an association between LNB[4] and a hypo-serotonergic state. While we could not demonstrate a significant difference between the pre-and post-treatment nesting scores of animals treated with high-dose escitalopram, the results must be regarded against the background of the significant increase in nesting scores observed in control-treated animals. In other words, as HMB seems to attenuate over time, LNB seems to demonstrate time-dependent exacerbation. Although a number of factors, including socially isolating animals during adulthood may contribute to the worsening of LNB, this is a necessary aspect of the experimental design and could unfortunately not be prevented. That said, considering that a clear pre-treatment separation in nesting scores can be made without long-term prior social isolation and that LNB is expressed in a waxing and waning nature between different nights, i.e. not to the same degree during each night, support the idea that LNB is, irrespective of the underlying trigger,

[1] obsessive-compulsive disorder
[2] high marble burying
[3] marble burying test
[4] large nest building

a persistent, repetitive compulsive-like phenotype. Nevertheless, whether isolation anxiety may play a specific role in the promulgation of LNB[1] and thereby provide a useful avenue for investigation potential influences of anxiety in the expression of a compulsive-like phenotype, should be investigated further.

* * *

The work presented here is not without shortcomings. First, considering the vast number of animals needed to identify a minority of HMB[2] expressing animals, group sizes could not be expanded beyond what has currently been used. Therefore, it is likely that given the often-large effect sizes reported in the absence of statistical significance, the statistical power of the investigation could have been improved with larger group sizes. That said, this work was aimed at a theoretical pharmacological dissection of different compulsive-like phenotypes, to which extent we believe our findings are valid. Second, the separation of animals into LNB and HMB cohorts are dependent on the appraisal of group behaviour. As no clear cut-off point can realistically be applied on such behaviours, a certain degree of conceptual error is intrinsic to the nature of this work. While we randomly allocated animals—which we felt expressed compulsive-like behaviours against the background of behaviours expressed by the larger population—to different treatment groups, we cannot confirm without doubt that such separation was sufficient to yield an accurate framework in which to study phenotype-specific neurobiological aberrancies. Third, the primary behaviours assessed here demonstrated unexpected, yet notable time-dependent adaptations. In the case of LNB, the exacerbation of nesting behaviour reported in the control-treated group may have been related to social-isolation evoked anxiety, especially since nesting constitutes a safety seeking (Jirkof, 2014) and thermoregulatory (Stewart and McAdam, 2017) activity, which can presumably be of greater importance for single-housed animals. However, we do not believe LNB to be an artefact of other forms of social isolation-induced pathologies, i.e. psychosis (Karkhanis et al., 2014), as these typically manifest as a result of isolation during important juvenile neurodevelopmental windows. Here, animals are only isolated from adulthood. Nevertheless, isolating animals is a necessary requirement of such analyses and cannot be prevented. As such, and as alluded to earlier, investigations of the explicit role of anxiety in the expression of LNB are warranted. Last, due to the extensive study design, we employed only a single dose flupentixol. This was well researched and chosen based on an exhaustive review of the literature. That said, flupentixol is a relatively old drug and the majority of findings pertaining to long-term oral administration via drinking water, were from research prior to 1990. Although the abundance of studies employed doses of up to 0.25 mg/kg/day as a low dose of flupentixol,

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

these mostly employed intraperitoneal injection or single daily oral gavage. We therefore decided to select the lowest dose used in chronic studies that employed administration methodologies similar to ours (Murugaiah et al., 1982; Murugaiah et al., 1983; Jenner and Marsden, 1987). Although a dose-response study for flupentixol can be valuable, the sensibility of such a pilot must be considered against the background of an experimental model. Indeed, an important question would be what expected outcomes are envisaged from such a study if the underlying neurobiology of the investigated excessive phenotypes have not yet been elucidated. Therefore, and considering the ethical principles that guide current animal research, we based our methods on previous research, instead.

* * *

In conclusion, the present work demonstrates the unique response of two phenotypically different spontaneous compulsive-like phenotypes, i.e. HMB[1] and LNB[2] to serotonergic drug intervention. While LNB seems to be representative of the classic hypo-serotonergic picture of OCD[3], HMB remains resistant to such intervention. Further, anti-dopaminergic treatment, either on its own or in combination with escitalopram, failed to elicit any response in any of the two behavioural phenotypes. While this was expected with respect to LNB, our observations in HMB expressing animals would indicate that HMB may be representative of a highly treatment-resistant behavioural phenotype that should be interrogated in terms of its underlying neurobiology. Indeed, future exploration of HMB may potentially provide significant insight into the mechanisms underlying treatment-resistant persistent behaviours.

[1] high marble burying
[2] large nest building
[3] obsessive-compulsive disorder

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Addendum A

Letters of permission to submit Chapter 3 for examination purposes.

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Dear Sir/Madam,

LETTER OF CONSENT: INCLUSION OF DRAFT MANUSCRIPT FOR EXAMINATION PURPOSES IN THE DISSERTATION OF ANE LOMBAARD

I, Prof Dan Stein, co-author of the manuscript titled "*Large nest building and high marble burying in the deer mouse (Peromyscus maniculatus bairdii) and its response to serotonergic, anti-dopaminergic and combination intervention*", hereby consent to this work being submitted for examination purposes as part of the M.Sc. dissertation of Miss Ané Lombaard.

Sincerely,

Dan J. Stein, FRCPC, PhD
Professor and Chair
Dept. of Psychiatry & Mental Health
University of Cape Town

Addendum B

The article in Addendum B was co-written by the candidate. This is the combined work of the Candidate, Geoffrey de Brouwer, and A. Fick (2018).

Large nest building and high marble-burying: two compulsive-like phenotypes expressed by deer mice (Peromyscus maniculatus bairdii) are distinctly regulated by serotonergic and dopaminergic intervention

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Abstract

The current investigation aimed to further dissect the deer mouse (*Peromyscus maniculatus bairdii*) model of obsessive-compulsive disorder (OCD) with respect to two compulsive-like behavioural phenotypes *viz.* large nest building (LNB) and high marble-burying (HMB) as characterized in the species. Since LNB is sensitive to chronic, high dose escitalopram intervention while HMB seems less so, we aimed to determine whether the two behaviours could be further separated based on their response to 4 weeks of serotonergic (i.e. escitalopram 50 mg/kg/day) or dopaminergic modulation, i.e. the dopamine $D_{1/2}$ receptor blocker, flupentixol (0.9 mg/kg/day) and the monoaminoxidase type B inhibitor, rasagiline (5 mg/kg/day), and their combinations with escitalopram. We found that LNB is responsive to chronic escitalopram with or without co-administration of flupentixol, while HMB remained insensitive to either of these interventions. However, HMB showed significant reduction following chronic combined escitalopram and rasagiline intervention, pointing to a unique involvement of the dopaminergic system in HMB compared to LNB. Additionally, we report for the first time that scoring preoccupied interaction with marbles over several trials is a more appropriate measure of compulsive-like behavioural persistence. In conclusion, the data presented here provide evidence that two unique, naturally occurring compulsive-like behaviours in deer mice have different neurobiological underpinnings. This has valuable implications for the pre-clinical modelling and our understanding of symptom heterogeneity and treatment resistant OCD.

Keywords:

Obsessive-compulsive disorder; marble-burying test; nest building; animal model; escitalopram; flupentixol; rasagiline; deer mouse model; treatment resistance

Introduction

Obsessive-compulsive disorder (OCD) is a neuropsychiatric disorder that affects 2-3% of the global population. The condition is characterized by obsessions, i.e. intrusive, unwanted thoughts, and/or compulsions, i.e. persistent, repetitive and time-consuming behavioural routines (Ruscio et al., 2010; American Psychiatric Association, 2013). Classically, five broad phenotypes of obsessive-compulsive (OC) symptom phenotypes have been identified, i.e. contamination obsessions with washing compulsions, safety obsessions with checking compulsions, obsessions concerning symmetry paired with ordering/organizing compulsions, covert intrusive thoughts with repugnant themes, i.e. violent or sexual acts that are associated with ritualistic, self-directed acts, e.g. praying, and fears of losing objects and hoarding compulsions (Mataix-Cols et al., 2005; Hirschtritt et al., 2017). Although excessive collecting symptoms are frequently noted in OCD (Boerema et al., 2019), fewer than 5% of OCD patients are diagnosed with hoarding as the primary obsessive compulsive-phenotype (Albert et al., 2015). In fact, hoarding had been afforded unique disorder status in the DSM-5 (American Psychiatric Association, 2013). A diagnosis of hoarding disorder differs in meaningful ways from OCD, with or without collecting compulsions. For example, hoarding-related thoughts are usually not experienced as intrusive or distressing, rather eliciting a sense of pleasure; therefore, as opposed to patients suffering from OCD, individuals with hoarding disorder do not feel overly compelled to engage in symptom-correcting behaviours (Albert et al., 2015). Further, in hoarding disorder, patients describe symptoms from an ego-syntonic perspective, rather than the ego-dystonic symptom experience of patients with OCD (Albert et al., 2012). Last, and important for the current investigation, hoarding disorder seems overly treatment refractory (Bloch et al., 2014) to the first line pharmacotherapeutic interventions for the treatment of OCD, i.e. selective serotonin reuptake inhibitors (SSRIs; Fineberg et al., 2013; Network, 2018).

That said, a considerable proportion of OCD patients also remain unresponsive to SSRI treatment (Atmaca, 2016; Hirschtritt et al., 2017). In such cases, SSRI therapy is often augmented with low-dose dopamine-2 (D_2) receptor antagonists, e.g. haloperidol, risperidone or quetiapine (Bloch et al., 2006; Fineberg et al., 2013; Veale et al., 2014). Importantly, these drugs, most notably the second generation neuroleptic compounds, e.g. risperidone, also inhibit serotonin receptors, including the 5-$HT_{1A/D}$ and 5-$HT_{2A/C}$ subtypes (Richelson and Souder, 2000), which may contribute to their efficacy as augmenting agents (Ramasubbu et al., 2000; Westenberg et al., 2007; Goddard et al., 2008).

Increasingly, evidence suggests that different subtypes of OCD vary in their underlying neurobiology (Mataix-Cols et al., 2004; Leckman et al., 2009; Thorsen et al., 2018), cognitive neuropsychology (McKay et al., 2004; Hashimoto et al., 2011; Martoni et al., 2015), and treatment response (Rufer et al., 2006; Stein et al., 2007; Grados and Riddle, 2008). There is also growing interest in whether particular Research Domain Criteria (RDoC) constructs, such as reward processing, are more relevant to particular OCD subtypes (Figee et al., 2011; Hauser et al., 2017). Indeed, recent evidence highlighted potentially unique reward-feedback processes underlying different phenotypes of obsessive-compulsive (OC) symptomology, which, considering the role of serotonin and dopamine in reward feedback processing, could implicate unique serotonergic and dopaminergic involvement in the manifestation of different symptom dimensions of OCD (Cavedini et al., 2006; Figee et al., 2011; Wong et al., 2015)

Few pre-clinical studies have explored the neurobiological underpinnings of different compulsive-like phenotypes within a specific model framework. This is likely due to the lack of animal models that are representative of naturalistic, but heterogeneous OC-like behaviour (Scheepers et al., 2018). In this regard, we have previously described three unique naturalistic compulsive-like phenotypes exhibited by deer mice (*Peromyscus maniculatus bairdii*), i.e. spontaneous stereotypy (Wolmarans et al., 2013), large nest-building (LNB; Wolmarans et al., 2016a) and high marble-burying (HMB; Wolmarans et al., 2016b). These three behaviours, which have been reviewed in depth before (Scheepers et al., 2018), are equally repetitive, persistent and seemingly purposeless. Whereas spontaneous stereotypy (Wolmarans et al., 2013) and LNB (Wolmarans et al., 2016a) demonstrate therapeutic response to chronic high dose (50 mg/kg/day) oral SSRI administration, HMB is less responsive to such intervention (Wolmarans et al., 2016b). As such and taking its phenotypic presentation in mind, we have previously proposed that HMB may provide a useful avenue for exploring hoarding-related mechanisms (Scheepers et al., 2018).

Considering the literature summarized above, it would be of interest to determine whether selective pharmacological manipulation of serotonergic and dopaminergic activity would modify the expression of LNB and HMB in unique ways. To this extent, we employed chronic, high-dose oral administration of the SSRI, escitalopram (50 mg/kg/day), alone or in combination with either a dopamine-selective $D_{1/2}$ receptor antagonist, flupentixol (0.9 mg/kg/day; Jenner and Marsden, 1987), or the dopaminergic monoamine oxidase B (MAO-B) inhibitor, rasagiline (5 mg/kg/day; Eigeldinger-Berthou et al., 2012). Specifically, we hypothesized that since LNB demonstrates robust response to SSRI intervention alone, such behaviour will not show additional response to anti-dopaminergic modulation either alone or in combination with escitalopram. In contrast, since HMB is less responsive to SSRI

intervention, we propose it to be possible that such behavior represents a compulsive-like phenotype more founded within dopaminergic perturbations, thereby demonstrating unique response to interventions targeting dopamine signaling.

Materials and methods

Animals

Since only 11 – 15% and 30% of deer mice, regardless of sex, express HMB (Wolmarans et al., 2016b; de Brouwer and Wolmarans, 2018) and LNB respectively (Wolmarans et al., 2016a), a total of 280 deer mice were initially screened for marble-burying (MB) behaviour, followed by nest building (NB) screening, allowing for the selection of 36 HMB and 36 LNB expressing subjects, respectively. Each of the respective groups were further divided into six drug groups (n = 6 per group; see below). All mice (outbred; both sexes; 10 – 12 weeks old at the onset of investigation) were obtained from the in-house deer mouse colony of the Vivarium of North-West University, Potchefstroom, South Africa. The study was approved by the relevant ethics committee prior to the onset of investigation (approval number: NWU-00262-16-A5; AnimCare Research Ethics Committee, registration number: AREC-130913-015). All steps of animal handling conformed to the South African National Standard (SANS) for the Care and Use of Animals for Scientific Purposes (SANS 10386:2008). The original breeding pairs were acquired from the *Peromyscus* Genetic Stock Centre at the University of South Carolina, USA. Animals were group-housed in same-sex groups of four to six animals per individually ventilated and climate-controlled home cage (35 cm (l) × 20 cm (w) × 13 cm (h); Techniplast SPA, Varese, Italy) until one week prior to the first behavioural assessment. From this point onwards, animals were single housed; this is necessary in assessments of NB as experiments are carried out over the course of 24 hours (Wolmarans et al., 2016a). Environmental conditions were maintained at 23°C, 50% humidity, and a 12-hour light/dark cycle (06h00/18h00). Food and water (or drug solutions where applicable) were provided *ad libitum* for the duration of the study, except during the execution of the 30-minute marble-burying tests. Cages were changed and cleaned once a week. Prior to behavioural experimentation, bedding consisted of ground corncob (particle ø 3–4 mm) and nesting material was provided in the form of unscented, white paper towel. Animals not selected for inclusion into either cohort, i.e. HMB or LNB, were excluded from further investigation and used for studies unrelated to this work.

Behavioural tests – general background

The aim of the investigation was to study associations between different behavioural phenotypes and unique drug responses. Therefore, clear separation between animals that

expressed either behaviour, *but not both*, was imperative. As such, all 280 animals were first screened for HMB activity (paragraph 2.3.1). Following the selection of the 36 HMB expressing animals they, along with an additional 144 mice from the original pool of 280, were screened for LNB (paragraph 2.3.2). Although none of the HMB-expressing individuals also engaged in LNB, animals that expressed neither of the behaviours were also excluded from further study. Further, as the present investigation sought to characterize the drug-response of compulsive-like cohorts, non-compulsive-like animals were excluded. The normal behavioural controls for LNB and HMB, as well as their responses to chronic high-dose oral escitalopram (50 mg/kg/day) have been described elsewhere (Wolmarans et al., 2016a; Wolmarans et al., 2016b; however, please refer to the supplementary data for a comparison between NNB and LNB animals).

The marble-burying test

The marble-burying test (MBT) was carried out as described previously (de Brouwer and Wolmarans, 2018). Briefly, marble-burying cages (exact replicas of the home cages) were prepared with a 5-cm thick layer of course river sand on top of which nine glass marbles (ø=15 mm) were arranged in a 3 x 3 grid equidistant to one another on *one half* of the cage only, thereby constituting a two-zone MBT setup. This allowed mice to willingly and voluntarily engage with marbles while also ensuring that novelty-induced avoidance behavior could be expressed if present and so identified. The use of course river sand, as opposed to sawdust, husk or wood chips as often reported in this test (Poling et al., 1981; Young et al., 2006; Kedia and Chattarji, 2014; Taylor et al., 2017), prevents marbles being unintentionally covered due to routine cage locomotion (de Brouwer and Wolmarans, 2018; de Brouwer et al., 2019). Since deer mice are nocturnal animals, all marble-burying assessments were carried out from 1 hour after onset of the dark cycle under dim red light (40 lux).

Thirty-six hours prior to the first marble-burying assessment before (pre-intervention screen) and again after four weeks of drug administration (post-intervention screen), mice were habituated to the burying substrate by replacing the corncob home cage bedding with the same coarse river sand used in the MBT; this was to prevent novelty induced digging (Handley, 1991; Gyertyan, 1995). Subsequently, each animal was assessed for marble-burying activity over three nights in three separate 30-minute trials (one per night), separated by 24 hours. This process was repeated before and after chronic drug administration. At the time of testing, animals were removed from their home cages and introduced into pre-prepared marble-burying cages. Due to the highly active nature of deer mice, cages were covered with clear covers to prevent animals from leaving the cages. Mice were subsequently left to explore and interact with marbles for 30 minutes in the absence of human observers, while being videotaped for later analysis. After each 30-minute session, animals were returned to their

respective home cages which still contained sand and left under standard housing conditions for approximately 24 hours until the next trial. This procedure was repeated twice over the next two days, until all animals underwent three separate pre- and post-intervention screens.

To identify the 36 HMB expressing animals, and appraise the findings reported in this investigation against the background of previously reported results (Egashira et al., 2013; Egashira et al., 2018), the number of marbles covered in burying substrate to a depth at least two-thirds of the marble size were quantified after completion of each 30-minute screening session. This was performed by two experimentally blind observers of whom the scores were averaged. Importantly, after the first three pre-intervention marble-burying assessments had been completed, the 36 HMB-expressing animals were selected based on 1) the average number of marbles covered *and* 2) the persistence of burying behaviour over three trials as evinced by lower coefficients of variation with respect to the between daily burying scores (Wolmarans et al., 2016b; **Figure 1A**). Only the 36 animals selected for HMB during the pre-intervention phase were retested in the MBT after 28 days of drug intervention.

When MB is used as a screening test for anti-compulsive drug action, quantification of the number of marbles 'buried' is most often reported (Egashira et al., 2013; Wolmarans et al., 2016b; Taylor et al., 2017; Egashira et al., 2018). However, this measure is inadequate for highlighting seemingly purposeless compulsive-like persistence (de Brouwer and Wolmarans, 2018). Thus, marble-directed behaviours (MDBs) were assessed to better establish behaviours that are indicative of *preoccupation* with the marbles (de Brouwer et al., 2019). MDBs were scored by visual inspection of video-taped trials and assigned a single count for any of the following: 1) sniffing and licking of marbles, 2) rolling or touching of marbles with forepaws, 3) standing on marbles, and 4) any movement directed in the immediate vicinity of a marble that was aimed at covering the marble. As for the number of marbles buried, scorers were blind to the experimental conditions.

Nest building analysis

Following the last pre-intervention MBT, home cages of the 36 HMB expressing subjects and an additional 144 mice were prepared with an excess of pre-weighed, non-scented hospital grade cosmetic cotton wool placed above the steel grid within the roof of the home cage. Every subsequent day between 13h00 and 14h00, built nests were removed, discarded and the unused cotton wool weighed (Wolmarans et al., 2016a). Mice were therefore left to utilize the cotton wool for approximately 23 hours of every day for 7 consecutive days. As NB analyses were conducted in the home cages, food and water were available *ad-libitum* for the duration of this screening. Animals did not have access to any additional form of nesting material during this time. As with marble-burying, classification of LNB (**Figure 1B**) was based

on 1) total nesting score generated over seven days, and 2) the behavioural variance over the course of seven days as reflected by the coefficients of variation with respect to the daily nesting scores. Only the 36 animals which consistently built the largest nests, were included in the LNB cohort (**Figure 1B**; Wolmarans et al., 2016a) and retested after 28-days of drug intervention.

Locomotor activity

As adequate locomotor ability (LMA) is an important prerequisite for behavioural expression as well as being an accurate identifier of drug-induced locomotor activation or inhibition, general locomotor behaviour was assessed by tracking the total distance moved during the execution of each MBT by means of Ethovision XT® 14 software (Noldus® Information Technologies, Wageningen, The Netherlands). Importantly, although it is possible that marble-directed preoccupation could result in falsely lowered ambulatory scores, we have previously shown that no correlation exists between the number of MDB and the total distances moved by deer mice during a 30-minute session (de Brouwer and Wolmarans, 2018).

Drug interventions and post-intervention testing

36 HMB and 36 LNB animals were selected following the first round of MB and NB assessments. Each group was divided randomly into 6 intervention groups (n = 6), each receiving chronic (28-day) administration of either 1) normal drinking water, 2) high-dose escitalopram (50 mg/kg/day; Wolmarans et al., 2013; Wolmarans et al., 2016a; Wolmarans et al., 2016b), 3) rasagiline (5 mg/kg/day; Eigeldinger-Berthou et al., 2012), 4) flupentixol (0.9 mg/kg/day; Jenner and Marsden, 1987), 5) a combination of escitalopram and rasagiline, or 6) a combination of escitalopram and flupentixol at the aforementioned doses. Higher than normally used oral doses of rasagiline were administered (Huang et al., 1999) as 13 – 15% of orally administered rasagiline promptly redistributes to the skin (Meier-Davis et al., 2012) while another 36 – 40% is eliminated during the first pass effect (Mittal et al., 2016). To mimic its application in clinical OCD, a low dose of flupentixol (0.9 mg/kg/day) was chosen based on earlier work that employed oral drug administration via drinking water (Murugaiah et al., 1982; Murugaiah et al., 1983; Jenner and Marsden, 1987). All drugs (H. Lundbeck® A/S, Ottiliavej, Denmark) were administered via the drinking water at concentrations calculated to deliver the stated doses according to the daily water intake of deer mice (0.25 mg/kg/day) as previously determined in our laboratory (Wolmarans et al., 2013) and confirmed by others (Aschhoff et al., 2000). Fresh drug solutions were prepared every second day (Wolmarans et al., 2013; Wolmarans et al., 2016a; Wolmarans et al., 2016b). As per the observations reported previously (Jenner and Marsden, 1987; Huang et al., 1999; Eigeldinger-Berthou et al., 2012;

Wolmarans et al., 2013), the addition of the drugs to the drinking water did not alter the average water consumption (data not shown). Oral drug administration by means of drinking water is the preferred route in our laboratory due to the potential influence of injection- or oral gavage-induced anxiety on the behavioural expression of deer mice, particularly when considering the lengthy 28-day drug administration period. Following 28 days of drug administration, mice were retested according to identical procedures as those explained above.

Statistical analysis

Statistical analyses were performed using GraphPad Prism® 8.01 (GraphPad® Software, San Diego, USA). As the behavioural data generated for the complete pool of 280 animals were not normally distributed (**Figure 1A**, total burying scores: $p < 0.0001$; **Figure 1B**, total NB scores: $p < 0.01$; Shapiro-Wilk test), Spearman's correlational analyses and column statistics (broad clustering within the upper 75th percentile for burying scores, MDBs and total nesting scores, and the lower 25th percentile for the respective coefficients of variation) were applied to identify HMB- and LNB-expressing subjects (Wolmarans et al., 2016a; Wolmarans et al., 2016b). Pre-and post-intervention expression of HMB and LNB, as well as LMA in the case of the HMB expressing animals were analysed and compared by means of two-way repeated measures analysis of variance (2-way RM-ANOVA) followed by Bonferroni post-hoc comparisons (Wolmarans et al., 2016a; Wolmarans et al., 2016b). Behavioural scores (of MB, MDB and NB) were set as between subject factor and time and intervention as within subject factors. Statistical significance was set as $p < 0.05$ for all analyses. All statistical analyses were followed by pairwise calculations of Cohen's *d* effect size to determine effect size. Effect sizes were considered large when $d > 0.8$, and very large when $d > 1.3$.

Results

Marble-burying and nest building screening and cohort selection

Selection of the HMB cohort

36 HMB expressing animals were selected from the initial pool of 280 animals (**Figure 1A:** $r_s(239) = -0.68$, $p = < 0.0001$). As 39 animals failed to cover a single marble over the course of the three consecutive pre-intervention trials, they were excluded from further MB analysis. Hence, **Figure 1A** is representative of 241 data points only. Subjects presenting with HMB are enclosed within the oval.

Selection of the LNB cohort

None of the 36 HMB expressing animals engaged in LNB activity as well. Thus, a total of 36 individuals with LNB were selected (**Figure 1B:** $r_s(178) = -0.51$, $p = < 0.0001$). Identified LNB expressing individuals are enclosed within the oval.

Pre- and post-intervention marble-burying comparisons

With respect to the number of marbles buried (**Figure 2A**), no significant interaction was demonstrated between time and intervention ($F[5, 30] = 2.24$; $p = 0.076$). However, time ($F[1, 30] = 46{,}78$; $p < 0.0001$), but not intervention ($F[5, 30] = 1.87$; $p = 0.13$), had an overall significant main effect on the burying scores; post-hoc comparisons: control group (1.17 ± 0.56 vs. 3.73 ± 1.47; $p = 0.003$; CI: 0.73 – 4.40; $d = 2.49$), escitalopram alone group (2.0 ± 2.1 vs. 3.73 ± 0.89; $p = 0.077$; CI: -0.11 – 3.57; $d = 1.16$), rasagiline alone group (0.44 ± 0.29 vs. 3.67 ± 1.38; $p = 0.0002$; CI: 1.38 – 5.06; $d = 3.85$), flupentixol/escitalopram group (1.25 ± 1.29 vs. 2.81 ± 0.48; $p = 0.14$; CI: -0.28 – 3.40; $d = 1.77$), and the rasagiline/escitalopram group (0.69 ± 1.02 vs. 2.16 ± 0.77; $p = 0.19$; CI: -0.8 – 3.30; $d = 1.65$) groups as well. No significant differences between the burying scores generated by mice of the different drug groups were observed (**Figure 2A**, post-intervention).

Concerning MDBs (**Figure 2B**), a significant interaction between time and intervention was demonstrated ($F[5, 30] = 3.20$; $p = 0.019$), while both time ($F[1, 30] = 47.94$; $p < 0.0001$) and intervention ($F[5, 30] = 4.22$; $p = 0.005$) had significant main effects on the results reported. Post-hoc analyses revealed statistically significant reductions in the post-intervention MDBs expressed by animals in the escitalopram alone group (19.0 ± 5.57 vs. 30.39 ± 5.92; $p = 0.0013$; CI: 3,74 – 19,04; $d = 1.98$), the flupentixol alone group (21.95 ± 9.05 vs. 29.61 ± 8.54; $p = 0.049$; CI: 0.01 - 15.32; $d = 0.87$) and the escitalopram/rasagiline group (8.39 ± 4.32 vs. 23.72 ± 5.71; $p < 0.0001$; CI: 7.68 – 22.99; $d = 3.06$). Also, reductions in MDBs of large effect sizes were also observed at 4 weeks in the control (22.39 ± 4.77 vs. 26.06 ± 2.58, $p = >0.99$; $d = 1.0$) and the escitalopram/flupentixol combination: 15.61 ± 5.69 vs. 20.33 ± 6.58, $p = 0.549$; $d = 0.8$). The main effect of intervention on the number of MDBs observed is apparent from the post-intervention differences observed between the different drug groups (**Figure 2B**, post-intervention). The combination of escitalopram and rasagiline resulted in a significantly lower number of MDBs (8.38 ± 4.32) compared to the control group (22.39 ± 4.77; $p = 0.002$; CI: 3.61 – 24.40; $d = 3.08$), the escitalopram alone group (19.0 ± 5.57; $p = 0.042$; CI: 0.22 – 21.01; $d = 2.14$), and the flupentixol alone group (21.95 ± 9.05; $p = 0.003$; CI: 3.16 – 23.96; $d = 2.03$). Further, while not reaching statistical significance, the combination of escitalopram and rasagiline was more effective than rasagiline alone (17.57 ± 4.03; $p = 0.136$; $d = 2.19$).

Pre- and post-intervention nest-building comparisons

A statistically significant two-way interaction was demonstrated between time and intervention with respect to the nesting scores of LNB animals (F[5, 30] = 4,350; p = 0.0043), while neither time (F[1, 30] = 2.461, p = 0.127), nor intervention (F[5, 30] = 1.473, p = 0.2279) had a main effect on the result (**Figure 3**). Subsequent pairwise analyses revealed the average expression of naturalistic LNB to increase over time (control: 34.06 ± 12.05g vs. 19.83 ± 3.86g; p = 0.0151; CI: -26.43g – -2.04g; d = 1.79). Although not statistically significant, such exacerbation was reversed by the administration of escitalopram as evidenced by the large effect size noted (14.92 ± 6.67g vs. 24.51 ± 2.80g, p = 0.20; d = 2.03). While the combination of escitalopram and rasagiline (28.08 ± 10.6g vs. 20.0 ± 4.7g, p = 0.426; d =1.05) somewhat reversed this result, the combination of flupentixol and escitalopram resulted in nest building scores akin to that observed in the escitalopram alone group at four weeks (16.53 ± 7.57g vs. 14.92 ± 6.67g, p > 0.99; d = 0.23). Rasagiline (27.94 ± 7.3g vs. 20.41 ± 1.98g, p = 0.547; d = 1.62) and flupentixol (27.08 ± 12.86g vs. 25.18 ± 5.9g, p > 0.99; d = 0.2) administered alone, failed to elicit any noteworthy response.

Only escitalopram alone (14.92 ± 6.67g vs. 34.06 ± 12.05g; p = 0.003; CI: 4.42g – 33.87g; d = 2.05) and the escitalopram/flupentixol combination (16.53 ± 7.57g vs. 34.06 ± 12.05g; p = 0.009; CI: 2.78g – 32.24g; d = 1.78) were able to significantly reduce LNB behaviour compared to the control group at 4 weeks.

Locomotor activity

Two-way RM ANOVA failed to reveal a significant interaction between time and intervention with respect to the total distance moved (**Figure 4**; F[5, 12] = 0.215, p = 0.95). Furthermore, neither time (F[1, 12] = 0.58, p = 0.461), nor intervention (F[5, 12] = 1.812, p = 0.185) had a significant main effect on the result observed.

Discussion

The main findings of this investigation were that LNB and HMB show unique responses to serotonergic and dopaminergic intervention in that 1) HMB is more responsive to combined escitalopram and rasagiline, compared to escitalopram or flupentixol alone, or a combination of flupentixol and escitalopram, and 2) LNB is responsive to escitalopram alone or combined with flupentixol, but not to rasagiline alone, flupentixol alone, or combined escitalopram and rasagiline.

Considering the burden of treatment resistance in OCD (Husted et al., 2006; Brakoulias and Tsalamanios, 2017), there is a need to better understand the mechanisms underlying OC symptomology. This is important, as the development of novel pharmacological alternatives ultimately depends on elucidating the underlying neurobiological processes which play a role in psychiatric disorders. While a significant number of patients do not demonstrate adequate response to the current first line interventions, e.g. SSRIs or cognitive behavioral therapy (CBT), second line interventions, e.g. augmenting SSRIs with antidopaminergic drugs, only provides relief in 40 – 60% of SSRI refractory patients (Atmaca, 2016). Further, the neurobiological mechanisms via which current pharmacotherapies elicit their effects are largely unknown. For example, anti-dopaminergic treatment is typically ineffective, and may even exacerbate OC symptomology if used as monotherapy (Lykouras et al., 2003; Westenberg et al., 2007; Kim et al., 2019), often only being effective in some patients when used in combination with SSRIs (Atmaca, 2016). This points to potential crosstalk between serotonergic and dopaminergic mechanisms in the treatment response (Lykouras et al., 2003; Westenberg et al., 2007; Kim et al., 2019). On a psychobiological level, unique involvement of goal-directed action motivation (Voon et al., 2015) and reward-feedback processing have been shown in patients with different phenotypes of OCD (Palminteri et al., 2012; Ferreira et al., 2017; Murray et al., 2019). These processes are all regulated by dopamine and serotonin (Boureau and Dayan, 2011; Schultz, 2013; Faulkner and Deakin, 2014; Gruner and Pittenger, 2017). Moreover, putative evidence points to unique dopaminergic processes in patients with hoarding disorder, i.e. resembling a state more akin to that observed in patients suffering from addiction (McLaughlin et al., 2018) and impulsivity (Suñol et al., 2019), while patients suffering from hoarding disorder broadly describe their symptoms from a pleasurable, ego-syntonic perspective; this as opposed to the ego-dystonic experiences of OCD patients. Also, hoarding disorder and hoarding symptoms in OCD demonstrate poor treatment response to SSRIs and its augmentation with low-dose anti-dopaminergic agents (Bloch et al., 2006; Fineberg et al., 2013; Veale et al., 2014), while some reports even indicate symptom attenuation after pro-dopaminergic intervention (McLaughlin et al., 2018).

Animal models of OCD are usually representative of a single behavioral phenotype based on its face similarity to OCD routines (Yadin et al., 1991; Tsaltas et al., 2005; Joel, 2006; Hoffman and Morales, 2009; Egashira et al., 2018). This complicates investigations into symptom-heterogeneous OCD (McKay et al., 2004; Nedeljkovic et al., 2009). Indeed, to further our understanding of the mechanisms underlying OCD treatment resistance and how this relates to the neurobiological constructs, studies of heterogeneous, but spontaneous and naturalistic compulsive-like behaviors within a specific model species will be a valuable extension of current research. To this extent, the persistent behavioral phenotypes expressed by deer

mice, i.e. LNB and HMB, may prove useful. Deer mice of both sexes present to a varying extent with LNB (Wolmarans et al., 2016a) and HMB (Wolmarans et al., 2016b). Whereas roughly 30% of the laboratory-housed deer mouse colony develop LNB and 45% highly stereotypical behaviors, only 11 – 15% of animals express HMB—see Scheepers et al. (2018) for an extensive review. These behaviors are equally persistent and repetitive over several successive trials, and seemingly purposeless under normal laboratory conditions (Scheepers et al., 2018). Moreover, LNB is responsive to SSRI intervention (Wolmarans et al., 2016a); HMB is not (Wolmarans et al., 2016b). These phenotypes may therefore also be founded on different neurobiological underpinnings. Against this background, the clinical utility of low-dose anti-dopaminergic augmentation for the treatment of SSRI-refractory OCD, is well established. However, considering recent literature (Figee et al., 2011; Pinto et al., 2014; Ferreira et al., 2017; McLaughlin et al., 2018; Suñol et al., 2019) and taking into account the unresponsiveness of HMB to SSRIs, we also aimed to investigate how LNB and HMB would respond to the actions of a pro-dopaminergic intervention, i.e. rasagiline, a strategy often employed in conditions characterized by impulsivity (Caye et al., 2019) and dysfunctional reward processing (Spijker and Nolen, 2010).

With respect to burying activity, our results demonstrate a time-dependent adaptation to the burying scores, without any significant main effect of intervention, nor an interaction reported between time and intervention. As such, we could not confirm an effect of drug within any of the groups after four weeks of drug administration, as all interventions, except for the flupentixol alone group, presented with substantial reductions in burying scores at 4 weeks of drug exposure. This is a noteworthy observation, especially since the locomotor behavior of all the groups remained unaltered after chronic drug administration (**Figure 4**). The *burying scores* reported here can likely be contributed to initial interest in the objects shown by most animals which abated over time. Further, we applied previously reported scoring methodology (Egashira et al., 2013; Egashira et al., 2018) and characterized a marble as ‘buried’ if two-thirds of its size was covered in burying substrate. However, this approach is arguably not sensitive enough to highlight actual ‘burying’ performance, rather being more indicative of investigative or exploratory behavior which ultimately results in the partial covering of objects (de Brouwer and Wolmarans, 2018). As such, we also scored MDBs. Here, several noteworthy observations were made. First, intervention and time interacted significantly on the findings reported, while both time and intervention had significant main effects on the MDB of HMB deer mice. Second, it is evident that the behavior of control-exposed animals remained at the same level of preoccupation with the objects as they did prior to intervention. Third, animals that were administered escitalopram alone, a combination of escitalopram and rasagiline, and flupentixol alone, demonstrated post-intervention reductions in MDB.

Importantly, while no significant differences between any of the drug groups prior to administration has been noted, the number of MDB (**Figure 2B**) differed significantly between the different groups after 4 weeks of drug administration. In fact, although escitalopram and flupentixol alone elicited significant reductions in the MDB of HMB mice, the combination of rasagiline with escitalopram proved to be the most effective intervention in this regard, with no differences demonstrated between the post-intervention control animals and any of the other groups. This indicates that the post-intervention behavior of animals receiving escitalopram and flupentixol can likely be ascribed to a time-dependent adaptation, which is not true for the combined escitalopram and rasagiline group. That no significant difference in the post-intervention MDB behavior of escitalopram exposed animals could be demonstrated compared to the control cohort, confirms our previous results (Wolmarans et al., 2016b). However, that such behavior responds to a combination of escitalopram and rasagiline, is interesting given that dopamine bolstering drugs typically result in exacerbation of compulsive-like behaviors (Aarons et al., 2012; Sevincok et al., 2014; Voon et al., 2015). This finding highlights a unique role of dopamine in this phenotype and is indicative of a specific neurobiological mechanism underlying HMB that may not necessarily be implicated in SSRI or combined SSRI and anti-dopaminergic drug sensitive phenotypes. In fact, it is likely that HMB resembles a bio-behavioral trait akin to clinical hoarding disorder. This possibility needs further investigation.

In contrast to the expression of burying activity, LNB exacerbated over time (**Figure 3**), which from an adaptational perspective, separates LNB on a phenotypical level from HMB. In fact, in contrast to the expression of burying behavior, LNB expressing animals are even more compelled to engage in excessive nesting behavior as time progresses. Although further study is required, such natural exacerbation in nesting behavior may point to an underlying anxiogenic construct. Considering the evolutionary function of nesting behavior (Jirkof, 2014; Stewart and McAdam, 2017; Lewarch and Hoekstra, 2018) and that LNB serves no functional purpose, such compulsive-like behavior may be borne from inflated fear-anxiety. This hypothesis is supported by the response of LNB to high-dose chronic escitalopram and the combination of escitalopram and flupentixol. Although pre- and post-intervention nesting scores of neither of these groups differed significantly (**Figure 3**), only escitalopram and the combination of escitalopram and flupentixol prevented the exacerbation of nesting behavior versus control animals; in fact, it reversed this trend. This is not only congruent with response in treatment-sensitive OCD but may also be supportive of anxiogenic involvement in the expression of LNB. Indeed, both chronic high dose SSRIs and low-dose anti-dopaminergic drugs are known anxiolytic treatments (Spijker and Nolen, 2010; Network Oregon ECHO, 2018), even though the clinical anti-obsessive-compulsive effect of these agents are not

exclusively related to an anxiolytic response (Baldwin et al., 2014; Hirschtritt et al., 2017). However, until the potential influence of anxiety on the expression of LNB has been adequately investigated, these findings could also be related to a neurocognitive construct other than anxiety, more in line with feedback processing theory. In fact, that both rasagiline and combined escitalopram plus rasagiline exacerbated LNB expression, suggests LNB to be representative of some inflated form of goal-directed outcome (Lim et al., 2008; Ondo and Lai, 2008), eliciting a sense of reward that if not gated adequately, becomes akin to behavioral addiction (Robbins and Clark, 2015). This has been proposed as a core neurocognitive construct of OCD (Voon et al., 2015; Gillan et al., 2016). In fact, since nesting behavior is inherently rewarding, it could likely be bolstered by either natural feedback interpretation or by increased dopaminergic activity geared towards achieving reward (Boureau and Dayan, 2011; du Hoffmann and Nicola, 2014; da Silva et al., 2018). Also, dopaminergic potentiation has been shown in pre-clinical (Presti et al., 2004; Sesia et al., 2013; Tucci et al., 2013) and clinical literature (Pessiglione et al., 2006; Voon et al., 2009; Garcia-Ruiz et al., 2014) to facilitate and fortify persistent and repetitive behaviors characterized by cognitive inflexibility.

A potential shortcoming of this work is the small sample sizes. Given the often-large effect sizes reported in the absence of statistical significance, the statistical power of the investigation could have been improved with larger group sizes. Nevertheless, taking into account the large number of animals needed to yield sufficient HMB and LNB expressing subjects, this work was aimed at a theoretical pharmacological dissection of different OC-like phenotypes; to this extent, we believe our findings are valid.

Conclusion

Our data broadly supports the notion that different behavioral OC-related phenotypes are supported by underlying differences in neurobiology. We have confirmed that LNB responds robustly to SSRI therapy, while further demonstrating that the addition of flupentixol to escitalopram parallels the response of escitalopram alone on nesting behavior. In contrast, HMB is less responsive to either escitalopram, flupentixol or their combination, only being attenuated by a combination of escitalopram and rasagiline. Therefore, HMB seems less representative of the classic hyposerotonergic, hyperdopaminergic picture of OCD (Westenberg et al., 2007), paving the way for its interrogation as a behavioral phenotype founded upon a unique neurocognitive construct. In conclusion, this work provides first-of-its-kind pharmacological evidence that LNB and HMB in deer mice, both previously proposed to be pre-clinical models of compulsivity, differ in response to pharmacotherapy. Further work is needed to determine whether these distinctions have parallels in humans.

Declarations of Interest

The authors declare that there is no conflict of interest.

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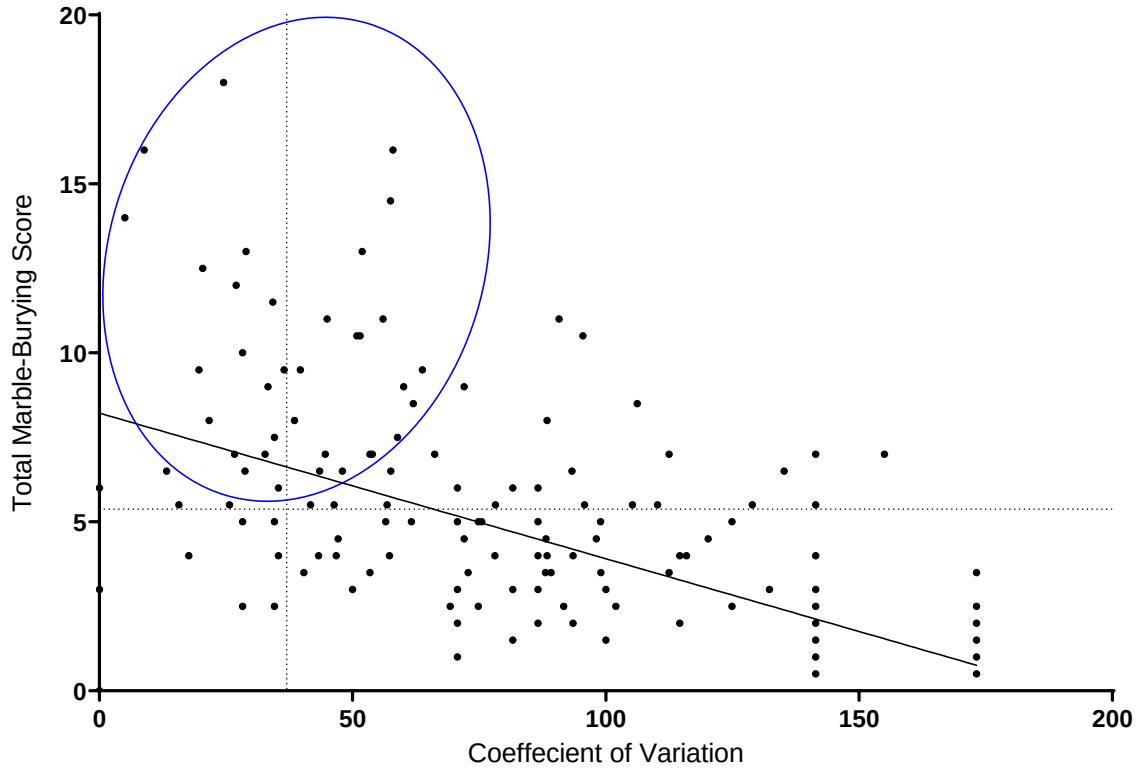
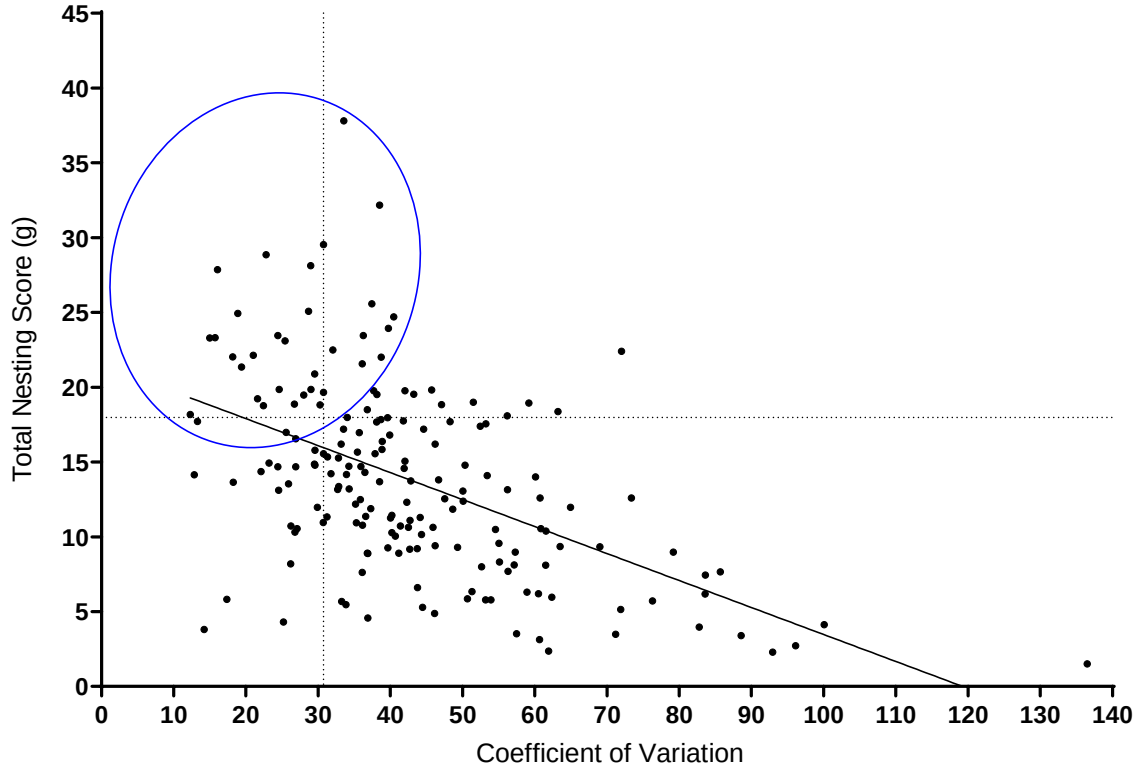
Figure 1

A


Total Marble-Burying Score
0
5
10
15
20
0
50
100
150
200
Coeffecient of Variation

B


Total Nesting Score (g)
0
5
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15
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25
30
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130
140
Coefficient of Variation

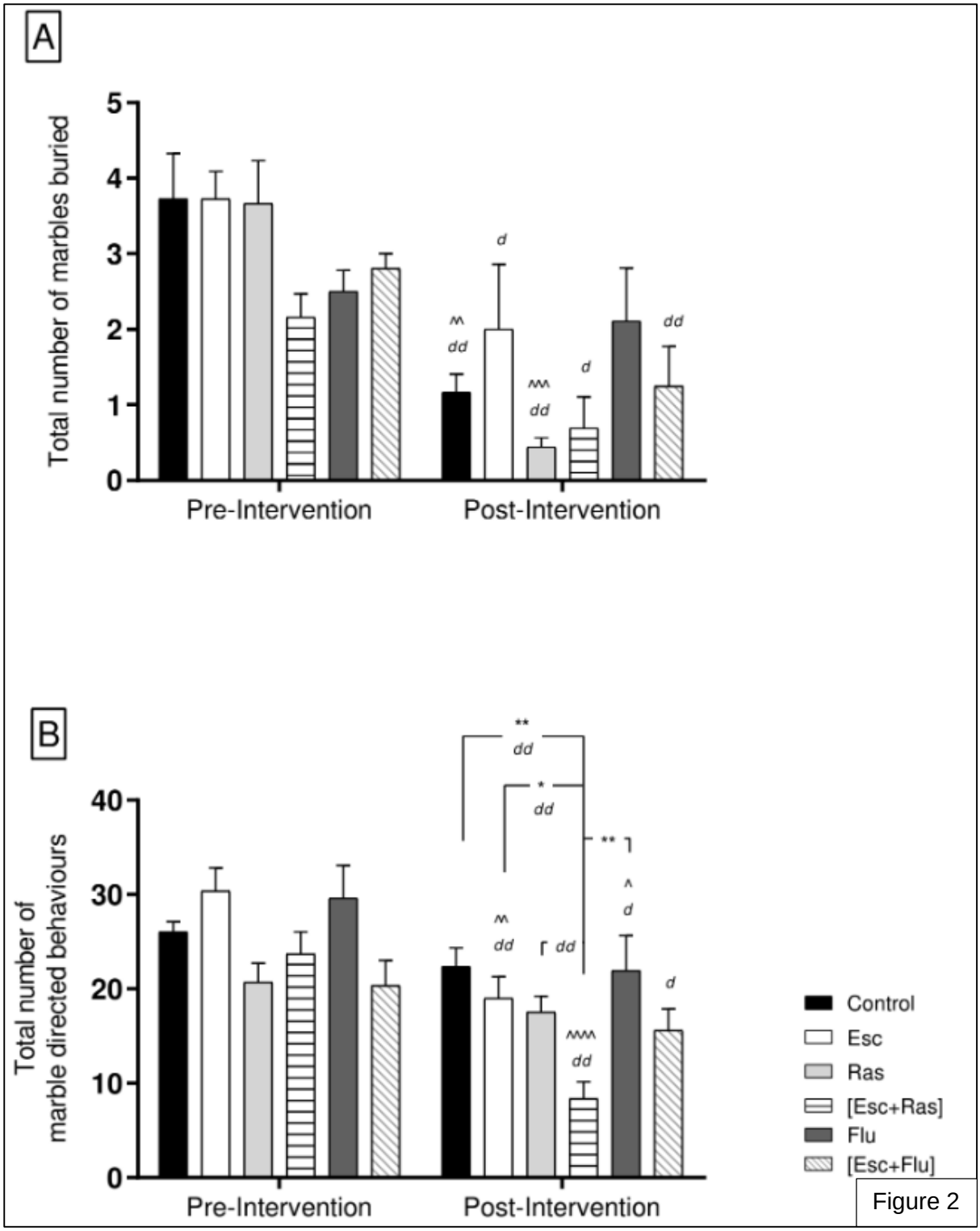
A
Total number of marbles buried
5
4
3
2
1
0
Pre-Intervention
Post-Intervention
B
Total number of marble directed behaviours
40
30
20
10
0
Pre-Intervention
Post-Intervention
Control
Esc
Ras
[Esc+Ras]
Flu
[Esc+Flu]

Figure 2

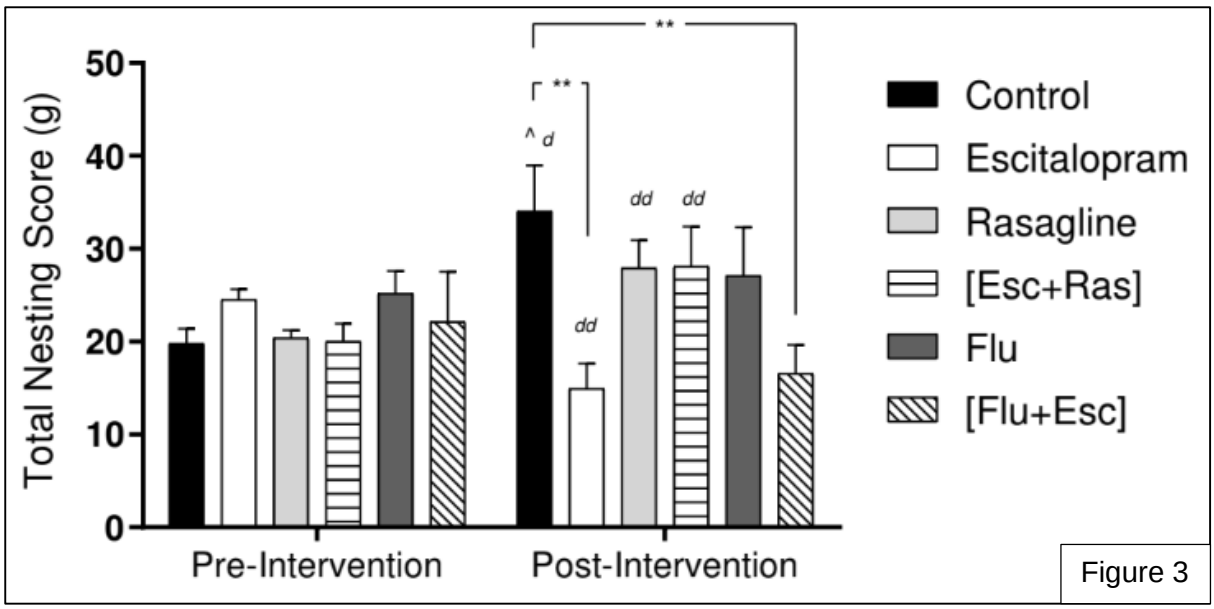

Figure 3

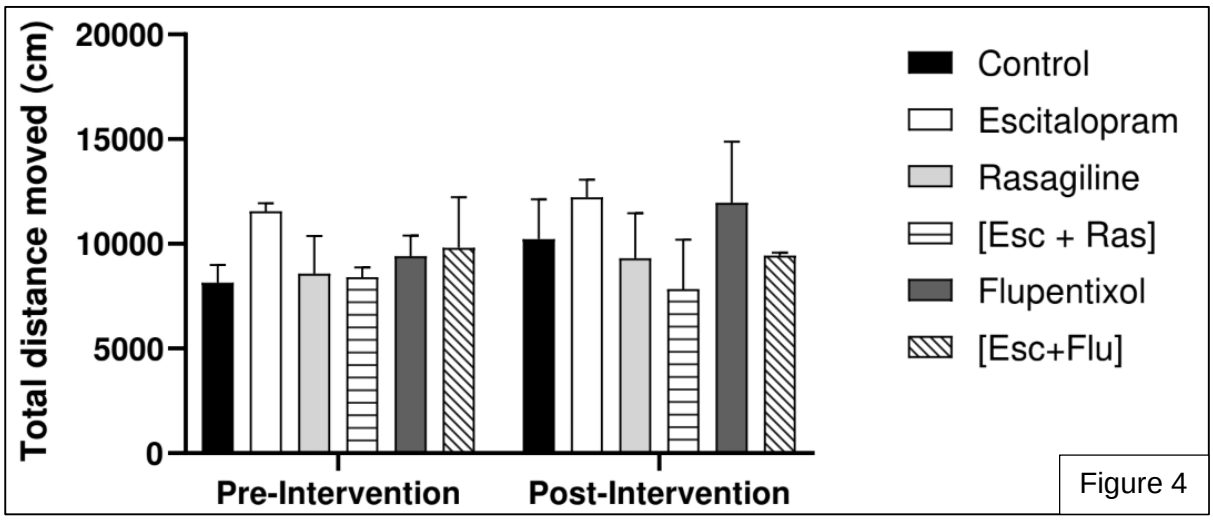

Figure 4

Figure Captions

Figure 1. Pre-selection screening data used for cohort selection. **A)** Marble-burying behavior during screening, expressed as Pearson's correlation ($r_s(239) = -0.68$, $p = < 0.0001$) between the total number of marbles buried and coefficients of variance for each individual animal over three test sessions. HMB (high marble-burying) animals were selected from the oval (highest marble-burying scores/lowest individual variance). **B)** Nest building activity during screening, expressed as Pearson's correlation ($r_s(178) = -0.51$, $p = < 0.0001$) between the total nesting score (g) and coefficients of variance for each individual animal over seven nights of screening. LNB (large nest building) animals were selected from the oval (highest total nesting score/lowest individual variance). All data represented as mean ± SEM.

Figure 2. Marble-burying data. **A)** Average pre- vs. post-intervention number of marbles buried over three pre- and three post-intervention trials by high marble-burying (HMB) animals. ^ Indicates significant pre- vs. post-intervention interactions within drug groups. ^^ $p = 0.0028$; ^^^ $p = 0.0002$ (Two-way RM-ANOVA, Bonferroni post-hoc). Cohen's effect size: $d > 0.8$; *dd* > 1.3. **B)** Average pre- vs. post-intervention number of marble-directed behaviors (MDBs) over three pre- and three post-intervention trials by high HMB animals. ^ Indicates pre- vs. post- intervention interactions within drug groups. * Indicates post-intervention interactions between drug groups. ^ $p = 0.0494$; ^^ $p = 0.0013$; ^^^^ $p < 0.0001$; * $p = 0.0416$; control vs. escitalopram & rasagiline ** $p = 0.0018$; flupentixol vs. escitalopram & rasagiline ** $p = 0.0028$ (Two-way RM-ANOVA, Bonferroni post-hoc). Cohen's effect size: $d > 0.8$; *dd* > 1.3. All data represented as mean ± SEM.

Figure 3. Average total nesting scores after seven days of pre- and seven days of post-intervention nest building screening in large nest building (LNB) animals. ^ Indicates significant pre- vs. post-intervention interactions within drug groups. * indicates post-intervention comparisons between drug groups. ^ $p = 0.0151$; control vs. escitalopram ** $p = 0.0029$; control vs. flupentixol ** $p = 0.0087$ (Two-way RM-ANOVA, Bonferroni post-hoc). Cohen's effect size: $d > 0.8$; *dd* > 1.3. Data represented as mean ± SEM.

Figure 4. Average pre- vs. post-intervention expression of motor activity in three randomly selected animals per drug group over three pre- and post-intervention trials. Two-way RM ANOVA revealed no significant interactions or main effects.