

Novel behavioral and pharmacological characterization of spontaneous stereotypy in the deer mouse

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Hierdie verhandeling word opgedra aan:

My vrou Ansa en twee pragtige kinders, Ewan en Alke

Julle is en sal altyd die grootste liefdes van my lewe bly. Jou man en julle pappa waardeer die ondersteuning, liefde en geduld onsettend baie! Sonder julle is my wêreld nie 'n wêreld nie. Sonder julle is ek niks.

* * *

Henk Oosthuizen

(22 Januarie 1986 – 1 Februarie 2015)

Ek sal jou tot aan die einde van tyd mis.

* * *

My beste en kosbare vriend, Sarel Brand

As ek terugkyk oor die tyd sedert 2008, lê die pad wat ons al saam gestap het uitgestrek binne-in 'n stralekrans van helder geluk. Op 1 Februarie 2015 het alles skielik verander en is ons gedwing om deur ongekaarte emosies te navigeer en die yslikheid van die leegte wat uit ons vriendskap gekerf is, te konfronteer.

Ek weet dit is soms, maar net soms, nog moeilik om anker in 'n nuwe lewe neer te stryk, maar ek wil hê jy moet weet dat daar vervulling in stilte is en kalmte in geduld.

Ek sal vir altyd langs jou staan.

*“And just like them old stars, I see that you've come so far –
to be right where you are. How old is your soul?
And when you're needing your space, to do some navigating –
I'll be here patiently waiting, to see what you find.”*
– Jason Mraz

* * *

“To have memories, happy or sorrowful, is a blessing, for it shows we have lived our lives without reservation.”

— Tan Twan Eng, *The Gift of Rain*

* * *

“And once the storm is over, you won’t remember how you made it through, how you managed to survive. You won’t even be sure whether the storm is really over. But one thing is certain. When you come out of the storm, you won’t be the same person who walked in. That’s what this storm’s all about.”

— Haruki Murakami

Abstract

Obsessive-compulsive disorder (OCD) is a psychiatric condition that is characterized by two main symptom cohorts, namely recurrent inappropriate thoughts (obsessions) and seemingly purposeless repetitive motor actions (compulsions). Furthermore, OCD is a clinically *heterogeneous* condition, presenting with different within- and between patient symptom phenotypes. Although OCD is classified as an obsessive-compulsive (OC) spectrum disorder, while anxiety is very often a co-presenting symptom, it is debated whether anxiety plays a role in its pathology. However, central to the diagnosis of OCD is the time-consuming nature of symptoms that interferes with the normal *social* and *occupational* routines of patients. Moreover, 70% of cases, irrespective of the symptom cohort diagnosed, respond to chronic but not sub-chronic high dose treatment with the selective serotonin reuptake inhibitors (SSRIs), e.g. escitalopram.

Validated animal models play a crucial role in acquiring new knowledge pertaining to the pathology and pharmacology of psychiatric illness, and OCD is no exception. The current study continues the validation process of the deer mouse (*Peromyscus maniculatus bairdii*) model of OCD and builds on previous work done in our laboratory. Deer mice that are bred and housed in confinement naturally develop two main forms of stereotypical behavior, namely vertical jumping and pattern running. Furthermore, based on the intensity and time-consuming nature of these behaviors, it can be categorized into levels of severity, namely high (H) and non- (N)-stereotypic cohorts. The seemingly purposeless and repetitive nature of these behaviors mimics the compulsions that characterize human OCD and constitutes the basis for face validity of the model. However, given the heterogeneous nature of OCD, the main focus of the current investigation was aimed at broadening the face validity of deer mouse behavior by 1) characterizing the social interactivity between animals from the same and different cohorts, 2) establishing if novelty-induced anxiety (*viz.* neophobia) may play a role in the manifestation of H stereotypy, 3) determining whether deer mouse stereotypy is representative of multiple OC phenotypes, and 4) if such differences do exist, are they responsive to chronic escitalopram treatment.

As patients with OCD demonstrate altered social competence in relationships with normal peers, we characterized the within and between cohort social behavior of H and N deer mice. However, in an attempt to observe the social interactions of H and N animals towards one another in the presence of an animal from a different cohort, we developed a novel three-

animal social interaction paradigm. As such, we determined that treatment-naïve H deer mice display more within cohort interaction, compared to N controls. Furthermore, H animals interact more with one another in the presence of an N animal while N animals also group together in the presence of an H animal. Moreover, chronic treatment with oral escitalopram significantly increased the sociability of H animals towards one another and towards N animals, while the social interactivity of N animals remained unaltered.

In an attempt to establish if neophobia-related anxiety may be associated with the expression of H stereotypy, we subjected animals to the marble-burying (MB) test. The test is based on the theory that neophobia will be associated with increased burying behavior on the first, but not subsequent MB trials. As such, data from the current investigation demonstrates that *all* deer mice display inherent burying behavior that is not sensitive to chronic treatment with escitalopram and therefore fails to demonstrate a neophobia-like component underlying H stereotypy. This result is in line with literature proposing that MB resembles normal and investigative, rather than pathological behavior.

As previous studies propose both *aberrant* MB and nest-building (NB) behavior to resemble OC behavior in different putative animal models of OCD, we aimed to determine whether such behavior may be expressed by deer mice, thereby resembling symptom heterogeneity in the deer mouse model of OCD. Although we identified aberrant (*viz.* high) MB behavior in 11% of animals from *both* stereotypical cohorts, chronic escitalopram treatment failed to attenuate this behavior. However, 30% of all animals of both cohorts, displayed unique large nest building (LNB) behavior. Furthermore, chronic treatment with escitalopram significantly affected LNB, decreasing the average daily and total nesting scores to levels akin to that expressed by the larger group. Although we could not demonstrate an OC construct underlying MB, we were able to provide evidence for the putative face and predictive validity of aberrant NB in deer mice. The latter supports the notion that deer mouse stereotypy is indeed heterogeneous with multiple symptom presentation.

Taken together, the results from the current study strengthens the face and predictive validity of the deer mouse model of OCD and confirm the model's status as a prominent, useful and robust animal model of OCD. Not only is altered and treatment sensitive sociability implicated in the behavior of H animals, but the model also provides a useful pre-clinical platform to investigate the heterogeneous nature of OCD and its response to treatment, as well as future explorative studies into its neurobiology.

Keywords: obsessive-compulsive disorder (OCD), deer mouse, social interaction, neophobia, marble-burying, nest-building, selective serotonin reuptake inhibitor (SSRI), escitalopram.

Opsomming

Obsessiewe-kompulsiewe siekte (OKS) is 'n psigiatrisie toestand wat deur veral twee simptomegroepe gekenmerk word, nl. herhalende, onvanpaste gedagtes (obsessies) en herhalende motoriese bewegings wat op die oog af doelloos voorkom (kompulsies). Verder is die toestand klinies *heterogeen* en presenteer dit met verskillende simptomegroepe-endofenotipes, beide in 'n enkele, maar ook tussen verskillende individue. Alhoewel OKS geklassifiseer word as 'n obsessiewe-kompulsiewe (OK)-spektrumtoestand, is daar nog nie konsensus dat ang 'n rol in die patologie daarvan speel nie. Die tydwende aard van simptome wat die normale *sosiale* en *beroepsroetine* van die pasiënt beïnvloed, speel egter 'n integrale rol by die diagnose van OKS. Ongeag van die simptomegroepe wat gediagnoseer is, reageer 70% van pasiënte boonop slegs op kroniese (maar nie sub-kroniese), hoë-dosis behandeling met die selektiewe serotonien heropnameremmers (SSHRs), bv. esitalopram.

Die huidige studie handel oor die validering van die deermuis- (*Peromyscus maniculatus bairdii*) model van OKS en bou voort op vorige studies wat in ons laboratorium uitgevoer is. Deermuise wat in aanhouding geteel en gehuisves word, ontwikkel natuurlikerwyse twee vorme van stereotipiese gedrag, nl. vertikale spronge en hardlooppatrone. Hierdie gedrag kan op grond van die ernstigheidsgraad volgens intensiteit en die tydwende aard daarvan, geklassifiseer word, nl. hoë- (H) en geen- (G) stereotipiese gedrag. Die oënskynlike doellose en herhalende wyse waarop hierdie gedrag vertoon word, boots die kompulsies van menslike OKS na en vorm die basis van die model se validering op grond van sigwaarde. Gegewe die heterogene aard van OKS, was die fokus van die huidige studie hoofsaaklik om die validering van die model op grond van sigwaarde, te verbreed deur 1) die sosiale interaksie tussen diere van dieselfde of verskillende groeperings te karakteriseer, 2) vas te stel of vreemdheid-geïnduseerde ang (nl. neofobie) moontlik 'n rol in die manifestering van H-gedrag mag speel, 3) te bepaal of die stereotipiese gedrag van deermuise verteenwoordigend kan wees van verskeie OK-endofenotipes en 4) vas te stel of, sou sodanige verskille bestaan, dit sensitiwiteit toon vir kroniese behandeling met esitalopram.

Gegewe dat pasiënte met OKS veranderde sosiale vaardighede in verhoudings met normale portuurgroepe vertoon, het ons die sosiale gedrag van H- en G-deermuise onderskeidelik ten opsigte van mekaar, asook ten opsigte van diere van 'n verskillende groepering, gekarakteriseer. Verder, in 'n poging om die sosiale interaksies van H- en G-diere onderling, maar ook in die teenwoordigheid van 'n dier van 'n verskillende groepering waar te neem, het ons 'n

nuwe sosiale interaksieparadigma ontwikkel. Ons kon as sodanig vasstel dat, in teenstelling met die gedrag van G-kontrole diere, behandelingsvrye H-deermuise meer interaksie met ander H-muise toon. Verder interageer H-diere meer met mekaar in die teenwoordigheid van 'n G-dier, terwyl laasgenoemde in die teenwoordigheid van 'n H-dier ook verkies om bymekaar te groepeer. Boonop is die sosiale interaksie tussen H-diere onderling en met G-diere beduidend verhoog deur kroniese behandeling met orale esitalopram, terwyl die sosiale interaktiwiteit van G-diere onveranderd gebly het.

In 'n poging om vas te stel of neofobie-verwante angste geassosieer kan word met die uitdrukking van H-gedrag, het ons diere blootgestel aan die albaster-begrawe (AB)-toets. Die toets is gebaseer op die teorie dat neofobie gepaard sal gaan met hoër begrawegedrag tydens die eerste, maar nie daaropvolgende, AB-proewe. Data uit die huidige studie toon egter aan dat *alle* deermuise inherente begrawegedrag vertoon wat nie sensitief is vir kroniese behandeling met esitalopram nie en skiet die resultate as sodanig tekort om 'n neofobie-verwante komponent onderliggend aan H-stereotipiese gedrag aan te toon. Hierdie resultaat is in ooreenstemming met bewyse uit vorige studies dat AB normale en ondersoekende, eerder as patologiese gedrag voorstel.

Synde dat vorige studies m.b.t. verskeie voorlopige modelle van OKS beide *afwykende* AB en nesbou (NB)-gedrag as verteenwoordigend van OK-gedrag voorstel, het ons gepoog om te bepaal of sodanige gedrag deur deermuise uitgedruk word en daardeur heterogene simptoompresentering in die deermuismodel van OKS kan voorstel. Ten spyte daarvan dat ons afwykende (nl. hoë) AB-gedrag in 11% van diere uit *beide* stereotipiese groepe geïdentifiseer het, kon kroniese esitalopram-behandeling nie daarin slaag om dit te onderdruk nie. 30% van alle diere uit beide stereotipiese groeperings het egter unieke groot-nesbou (GNB)-gedrag vertoon. Verder het kroniese behandeling met esitalopram 'n beduidende effek op GNB gehad, soveel so dat die gemiddelde daaglikse en totale nesboutellings verminder het tot vlakke soortgelyk aan dié van die groter groep. Alhoewel ons nie 'n OK-konsep onderliggend aan AB-gedrag kon aantoon nie, word bewys gelewer vir die voorlopige geldigheid van afwykende NB-gedrag op grond van sig- en voorspelbaarheidskaarte en daarom ook vir 'n heterogene diere-model van OKS.

Alles in ag genome, versterk die huidige studie die geldigheid van die deermuismodel vir OKS op grond van sig- en voorspelbaarheidskaarte en word die status van die model as 'n prom-

inente, bruikbare en kragtige dieremodel van OKS, bevestig. Nie net is veranderde en behandelings sensitiewe sosiale gedrag van H-stereotipiese diere aangetoon nie, maar dien die model ook as 'n voorgestelde pre-kliniese raamwerk vir die bestudering van die heterogene aard van OKS asook die reaksie daarvan op behandeling.

Sleutelwoorde: obsessiewe-kompulsiewe siekte (OKS), deermuis, sosiale interaksie, neofobie, albaster-begrawe, nesbou, selektiewe serotonien heropnameremmer (SSHR), esitalopram.

Congress Proceedings

The results of the current investigation were presented as follows (presenting author is underlined):

- (a) DE WET WOLMARANS, SCHAUN KORFF, MARIANNE GÜLDENPFENNIG, DAN J STEIN, BRIAN H HARVEY (2015). The deer mouse model of obsessive compulsive disorder (OCD): A platform for research in neurobiology, behavior and drug discovery. International Behavioral Neuroscience Society (IBNS) Congress, Victoria, British Columbia, Canada (2 – 7 June 2015)
- (b) DE WET WOLMARANS, DAN J STEIN, BRIAN H HARVEY (2015). Social behavior in deer mice as a novel interactive paradigm of relevance for obsessive-compulsive disorder (OCD) (poster presentation). European College of Neuropsychopharmacology Congress, Amsterdam, The Netherlands (29 August – 1 September, 2015)

* * *

Previous work by the author on the deer mouse model of OCD, but not related to the current investigation, have been presented as follows:

- a) DE WET WOLMARANS, DAN J STEIN, BRIAN H HARVEY (2009). Animal models of obsessive-compulsive disorder: where are we and where are we going? Biological Psychiatry Congress, Kleinmond, South Africa (28 – 31 May 2009)
- b) DE WET WOLMARANS, DAN J STEIN, BRIAN H HARVEY (2009). Current established and putative animal models of obsessive-compulsive disorder: a systematic review. International Conference on Pharmaceutical and Pharmacological Sciences, Potchefstroom, South Africa (23 – 26 September)
- c) DE WET WOLMARANS, LINDA BRAND, DAN J STEIN, BRIAN H HARVEY (2011). Natural stereotypy in deer mice and its association with frontal-cortical and striatal serotonin transporter (SERT) density: implications for a putative animal model of OCD. International Conference on Pharmaceutical and Pharmacological Sciences, Umhlanga, South Africa (25 – 27 September)

Publications

Previous work by the author on the deer mouse model of OCD, but not related to the current investigation, have been published as follows:

- (a) GÜLDENPFENNIG, M., WOLMARANS, D., DU PREEZ, J.L., STEIN, D.J., HARVEY, B.H. (2011). Cortico-striatal oxidative status, dopamine turnover and relation with stereotypy in the deer mouse. *Physiology and Behaviour*, 103:404-411
- (b) WOLMARANS, D., BRAND, L., STEIN, D.J., HARVEY, B.H. (2013). Reappraisal of spontaneous stereotypy in the deer mouse as an animal model of obsessive-compulsive disorder (OCD): response to chronic escitalopram treatment and baseline serotonin transporter (SERT) density. *Behavioural Brain Research*, 256:545-553

* * *

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“It is a curious thing, but perhaps those who are best suited to power are those who have never sought it. Those who, like you, have leadership thrust upon them, and take up the mantle because they must, may find to their own surprise that they wear it well.”

— J.K. Rowling

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* * *

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I Introduction

I.1 Thesis Layout

The current thesis is compiled in article format, as prescribed and approved by North-West University. As such, the main body of the thesis is presented as three manuscripts that are in submission to international, peer reviewed neuroscience journals.

However, Chapter 1 provides a concise description of the project problem statement, study questions, aims, expected outcomes and a framework of the study layout. Chapter 2 comprises the literature background supporting the current project, while chapters 3, 4 and 5 will contain the key findings of the investigation in three separate manuscripts. These manuscripts have been prepared according to the 'Instructions to Authors' provided by each journal (indicated at the beginning of each chapter) and will be presented as such. Chapter 6 summarizes the key findings of the project and concludes the study as a whole. The addendums contain 'Instructions to Authors' from the different journals, letters of permission of co-authors for subjecting manuscripts A – C for assessment purposes, and confirmations of article submissions. Furthermore, one manuscript describing previous work by the candidate into the deer mouse model of OCD and that was central in the planning of the current investigation, is also provided. This is provided for the benefit of the reader only, and is not subject to examination.

As two of the three prepared manuscripts (chapters 3 and 4) were prepared according to the guidelines of the American Psychological Association (APA), 6th ed., the referencing style of chapters 1, 2 and 6 is applied in the same manner. References in chapter 5 were prepared according to the Sage Harvard referencing style and will be presented as such.

Except for Manuscript C that has been written in UK English, the thesis is presented in US English as this was the prescribed language for Manuscripts A and B.

* * *

1.2 Problem Statement

Animal models of human psychiatric conditions are pivotal instruments that aid in elucidating the neurobiological mechanisms underlying human disorders as well as provide a suitable framework for the development and pre-clinical evaluation of new treatment strategies. The current study follows on previous work undertaken in our laboratory (Güldenpfennig, Wolmarans, du Preez, Stein, & Harvey, 2011; Korff, J. Stein, & H. Harvey, 2008; Korff, Stein, & Harvey, 2009; Wolmarans, Brand, Stein, & Harvey, 2013) and concerns the validation of spontaneous stereotypy in the deer mouse (*Peromyscus maniculatus bairdii*) as an animal model of obsessive-compulsive disorder (OCD).

In most patients, OCD is characterized by two main symptoms, namely recurrent and intrusive thoughts (obsessions) and rigid repetition of certain motor actions (compulsions) (American Psychiatric Association, 2013). OCD demonstrates high comorbidity with a group of conditions collectively known as the obsessive-compulsive (OC) spectrum disorders (Bartz & Hollander, 2006; Nestadt et al., 2001), which includes trichotillomania, compulsive gambling, anorexia and body dysmorphic disorder. Consequently, OCD has recently been classified by the American Psychiatric Association as an (OC) spectrum disorder as opposed to its previous status as an anxiety disorder (American Psychiatric Association, 2000). Moreover, albeit having an OC nature, OCD may be comorbid with impaired social competence manifesting as social phobia (SP), otherwise known as social anxiety disorder (SAD) (Kim, Reynolds, & Alfano, 2012), and poor social adjustment (Rosa et al., 2012).

OCD is clinically heterogeneous with five major OC symptom dimensions being described, viz. 1) contamination obsessions and washing compulsions, 2) responsibility for harm obsessions and checking compulsions, 3) symmetry obsessions and ordering compulsions, 4) unacceptable thoughts (e.g., sexual, religious, or aggressive in nature) and neutralizing compulsions (e.g., thought suppression), and 5) concerns about waste and hoarding compulsions (Abramovitch & Cooperman, 2015; Markarian et al., 2010). Irrespective of the symptom cohort identified, a diagnosis of OCD is also based on the time-consuming nature of symptoms that may interfere to varying degrees with the normal occupational or social functioning of the patient (American Psychiatric Association, 2013). As such, time spent executing OC behavior is routinely assessed with rating scales that equate severity with the degree of resistance to, or control over these behaviors, e.g. the Yale-Brown obsessive-compulsive scale (Y-BOCS; (Goodman et al., 1989)), the Florida obsessive-compulsive inventory (FOCI; (Storch

et al., 2007)), the dimensional obsessive-compulsive scale (DOCS; (Abramowitz *et al.*, 2010)) and the Clark-Beck obsessive-compulsive inventory (CB-OCI; (Clark, Beck, Antony, Swinson, & Steer, 2005)).

The prevalence of different OCD phenotypes has sparked debate as to whether or not OCD is related to anxiety (Bartz & Hollander, 2006; Nestadt *et al.*, 2001; Stein, 2002; Tynes, White, & Steketee, 1990). Indeed, the DSM-V (American Psychiatric Association, 2013) clearly stipulates that OCD can be diagnosed in a patient without the presence of obsessive and intrusive thoughts. However, irrespective of the symptom cohort diagnosed, up to 70% of OCD cases respond preferentially to high dose, chronic treatment with the selective serotonin reuptake inhibitors (SSRIs) (El Mansari & Blier, 2006; Fineberg & Craig, 2007), with the same algorithm being followed in the treatment of anxiety and phobia (Baldwin, Brandish, & Meron, 2008). In treatment-refractive OCD, augmentation strategies with low dose antipsychotics may be followed (El Mansari & Blier, 2006).

Suitable animal models of OCD are necessary to understand the complex neurobiological mechanisms underlying OC behavior. That obsessions may play as prominent a role as compulsive-like repetition in the symptomology of OCD, complicates the development of animal models since cognitive abnormalities such as recurrent thoughts and obsessions are impossible to demonstrate in animals. However, by associating compulsive-like repetition of certain motor actions in animals with the fundamental constructs of OCD, certain conclusions can be made that may have direct relevance to the human disorder. Thus, by associating motor stereotypy with other behavioral abnormalities also observed in human OCD as well as demonstrating a favorable response to chronic, but not sub-chronic high dose SSRIs, certain repetitive behaviors in an animal can be distinguished from such behaviors without a confounding cognitive association (Barnard, Young, Pearson, Geddes, & O'Brien, 2002; Langen, Durston, Kas, van Engeland, & Staal, 2011; Rasmussen *et al.*, 1994). This allows the model to distinguish OCD-like behavior from other illnesses such as autism, Tourette's syndrome and Parkinson's disease.

Much has already been done to validate spontaneous stereotypy in the deer mouse as an animal model for OCD (Güldenpfennig *et al.*, 2011; Korff *et al.*, 2008; Korff *et al.*, 2009; Wolmarans *et al.*, 2013) and as such the model is highly regarded as one of the foremost naturalistic animal models of OCD currently under investigation (Hoffman, 2011). In short, deer

mouse stereotypy can be categorized within two main behavioral topographies, namely repetitive vertical jumping and pattern running that occur naturally and varies in intensity, viz. high (H) and non (N)-stereotypical behavior (Wolmarans *et al.*, 2013). These time consuming behaviors mimic the rigid repetitive motor actions observed in human OCD, and form the basis for the face validity of the model. Korff and colleagues (2008) demonstrated that chronic (21-day) intraperitoneal treatment with 10 and 20 mg/kg/day fluoxetine significantly decreased the expression of spontaneous stereotypy in stereotypical deer mice. In a follow-up study (Wolmarans *et al.*, 2013) it has been demonstrated that the response of deer mouse stereotypy is selective to chronic, *but not sub-chronic* treatment with high dose SSRIs. The results of these two investigations form the basis of the robust predictive validity of the model. The model is also founded in construct as evidence for an increase in cyclic adenosine monophosphate (cAMP) and a decrease in phosphodiesterase-4 (PDE₄) expression in stereotypical animals as opposed to non-stereotypical animals has been presented (Korff *et al.*, 2009). As SSRIs are known to exert adaptive changes in this second messenger system via indirect actions on serotonin (5-hydroxytryptamine; 5HT) 1_{A/B/D} and 5HT_{2C} receptors (Barnes & Sharp, 1999; Bergqvist, Bouchard, & Blier, 1999), this observation links the pathology of deer mouse stereotypy to altered serotonergic signaling. In support of this, Wolmarans *et al.* (2013) presented further evidence that high stereotypical deer mice present with a significant decrease in striatal SERT expression compared to the non-stereotypical controls. The latter results are in line with that demonstrated in patients with OCD (Hesse *et al.*, 2005; Reimold *et al.*, 2007; Zitterl *et al.*, 2008), while SERT is the biological target for the SSRI's. Taken together with the findings of Korff and colleagues (2009), these contributions support the construct validity of the model.

Based on the aforementioned strengths of the deer mouse model of OCD, the current study aims to broaden the validity of the model in an effort to further establish spontaneous stereotypy in the deer mouse as a robust and reliable animal model of OCD. Given the heterogeneous symptomology of OCD, the primary focus of the current investigation will be to characterize deer mouse behavior with respect to its face resemblance of the diverse phenotypical manifestations of OCD. As such, four clinical aspects of OCD will be addressed viz. 1) altered sociability, 2) comorbidity with anxiety, and 3) heterogeneous OC symptomology.

* * *

1.3 Study Questions

The current study was designed to systematically address the face and predictive validities of the model, and is based on the following study questions:

STUDY QUESTION	APPLICABLE LITERATURE
1) <u>MANUSCRIPT A (Chapter 3)</u> : As is evident in OCD, do H animals present with altered social behavior compared to N controls?	(Berrocal et al., 2006; Kim et al., 2012; Rosa et al., 2012)
2) <u>MANUSCRIPT B (Chapter 4)</u> : As is debated in OCD, can an association between H behavior and anxiety-like (viz. neophobic behavior in this scenario) behavior be demonstrated in these animals using marble-burying as a putative screening test for neophobia?	(Bartz & Hollander, 2006; Njung'e & Handley, 1991)
3) <u>MANUSCRIPTS B and C (Chapters 4 and 5)</u> : As is evident in OCD, can H behavior be associated with different forms of compulsive-like behavior, or can deer mouse behavior be representative of various OC phenotypes not related to stereotypy, as assessed using <i>aberrant</i> expression of marble-burying and nest-building as screening tests for compulsive-like behavior?	(Abramowitz et al., 2010; Angoa-Pérez, Kane, Briggs, Francescutti, & Kuhn, 2013; Li, Morrow, & Witkin, 2006)
4) <u>MANUSCRIPTS A – C (Chapters 3 – 5)</u> : Should any differences in such behaviors as described above be demonstrated, are these responsive to chronic escitalopram treatment?	(Baldwin et al., 2008; Fineberg & Craig, 2007; Hoffman, 2011; Nicolas, Kolb, & Prinssen, 2006)

* * *

I.4 Project Aims

To address study question *one*, we aimed to:

- develop a novel social interaction challenge (SIC) that will enable us to investigate the group social behavior of N and H animals both within and between cohorts. Performing a SIC between three, and not two animals in different combinations of cohorts, will shed light on the social dynamics between animals of different behavioral cohorts and how the presence of an animal from either a H or N cohort will affect the behavior of an animal from a different stereotypical cohort.

To address study question *two*, the aim was to:

- apply a repeated-exposure marble-burying (MB) paradigm to assess whether deer mice habituate to burying behavior (*viz.* is MB in the deer mouse related to neophobic or inherent behavior?).

To address study question *three*, we aimed to:

- characterize *aberrant* MB and nest building (NB) behavior, and
- assess these behaviors to establish whether the compulsive-like expression of motor behavior by H deer mice can be associated with altered MB and NB when compared to N controls (*viz.* is H behavior associated with different forms of OC behavior?) or if *aberrant* MB and NB behavior may represent unique OC phenotypes in deer mouse behavior not related to stereotypy.

To determine the outcome of study question *four*, we aimed to:

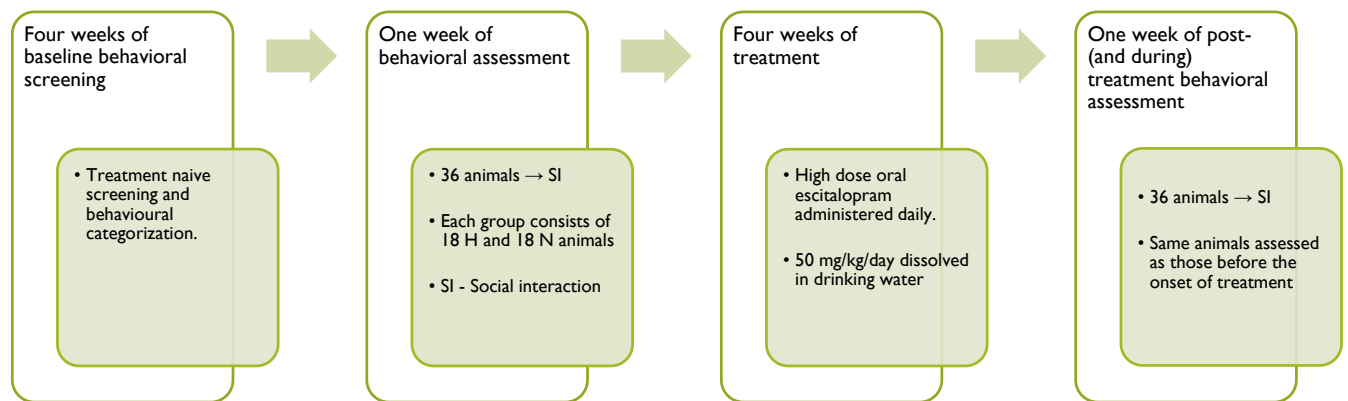
- treat all animals in the current investigation with chronic (four-week) oral escitalopram (50 mg/kg/day) and, as such treatment is effective in attenuating deer mouse stereotypy, to determine whether the same is observed with respect to possible abnormal manifestations of the abovementioned behaviors.

* * *

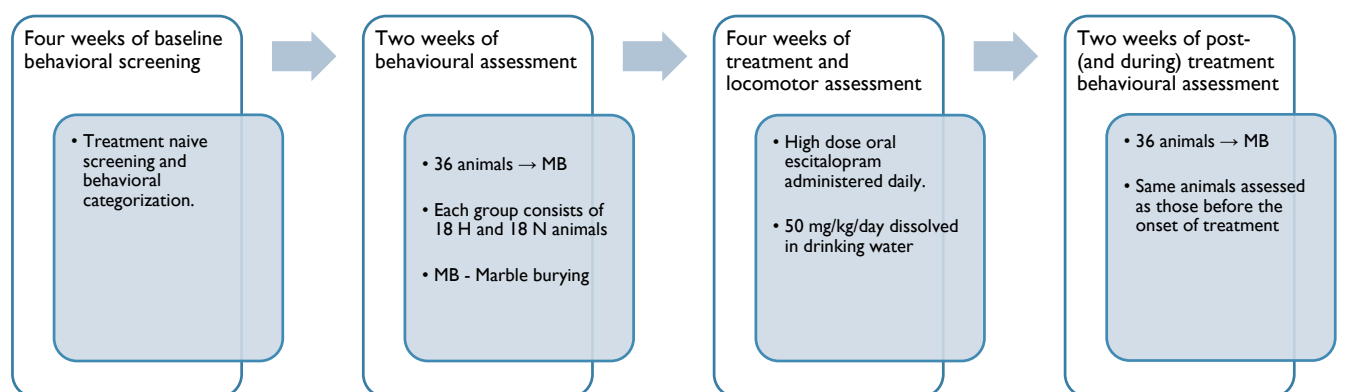
1.5 Project Layout

To address the study questions laid out above, the current investigation was subdivided into three main sections each employing 60 – 70 deer mice randomly divided between H and N animals, and using 50 mg/kg/day escitalopram as described in our earlier work (Wolmarans *et al.*, 2013). The same selection criteria used previously (Wolmarans *et al.*, 2013), but adapted for the current investigation, was applied to select animals for behavioral assessment, whereby the grey margin of animals (not clearly classified as either H or N) are excluded from behavioral assessment.

Section 1 – 70 animals: 10 weeks (Study questions 1 and 4)

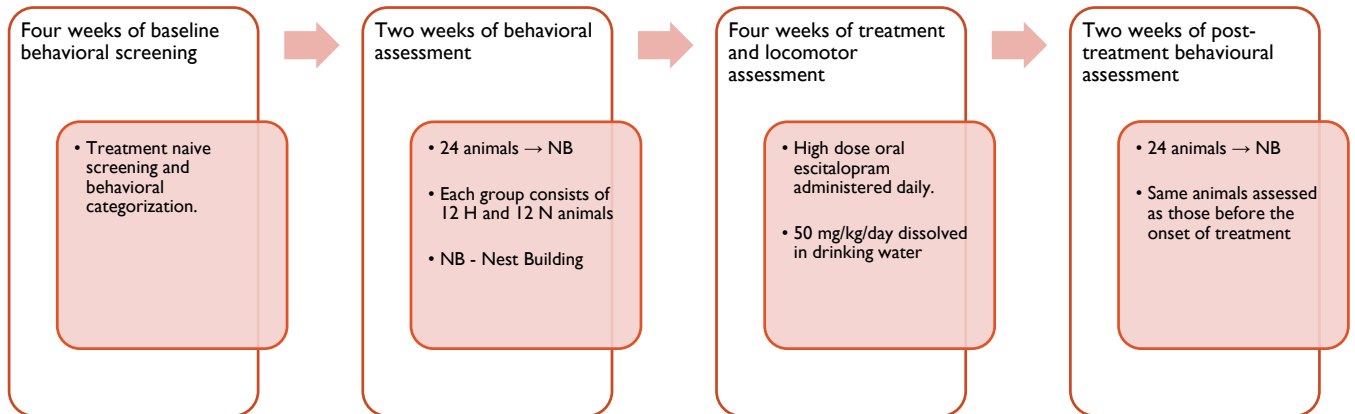


Section 2 – 70 animals: 12 weeks (Study questions 2, 3 and 4)



Section 3 follows on the next page .../.

Section 3 – 60 animals: 12 weeks (Study questions 3 and 4)



* * *

I.6 Expected results

STUDY QUESTION	EXPECTED OUTCOME
1)	It is expected that H animals will present with altered sociability towards one another and towards N animals, compared to N controls.
2)	Should anxiety be comorbid with OC behavior in the deer mouse, it is expected that H and not N animals will present with neophobia related to first exposure to glass marbles. However, should anxiety not underlie OC behavior in deer mice, H animals will bury glass marbles without habituation over all trials.
3)	It is hypothesized that H, and not N deer mice can demonstrate aberrant MB and NB behavior, implicating different forms of OC behavior characterizing H stereotypical behavior. Should such aberrant MB and NB be additional OC phenotypes in deer mouse behavior in general, it is hypothesized such behavior will manifest in animals of both stereotypical cohorts.
4)	Should aforementioned behavioral abnormalities be demonstrated, it is expected to respond to chronic high dose oral escitalopram.

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* * *

2 Literature Review

2.1 OCD in the Clinical Environment

2.1.1 The classification and diagnosis of OCD

By historical definition OCD is a debilitating psychiatric condition characterized by intrusive and disturbing thoughts (obsessions) that leads to mounting anxiety together with repetitive stereotypical behavior aimed at relieving the anxiety caused by the obsession (Stein, 2002). Strictly and according to this definition, a patient has to present with both obsessions and compulsions before OCD can be diagnosed, although as explained later, this diagnostic criterion has changed over the past three decades (American Psychiatric Association, 2013). OCD has a lifetime prevalence of between 2.5% and 3% in the general population, making it the fourth most common psychiatric disorder (Pittenger, Krystal, & Coric, 2006).

OCD is currently classified as an OC spectrum disorder by the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association, 2013). Whether it is appropriate not to categorize OCD with anxiety disorders such as phobias, post-traumatic stress disorder (PTSD), and generalized anxiety disorder, is a question that has been much debated during the past two decades (Bartz & Hollander, 2006). This debate had its origin partly in the realization that obsessions do not necessarily always translate into anxiety and that certain compulsions are in many cases not a direct consequence of either obsessions or the anxiety caused by a certain obsession. Interestingly, the DSM-V stated criteria for diagnosing OCD clearly state that either obsessions or compulsions, or a combination of both, may justify the diagnosis of OCD. Although patients can be diagnosed with OCD, irrespective of whether obsessions and compulsions, or only one of the two, are present, the DSM-V sets certain restrictions to the criteria for diagnosing obsessions and compulsions and eventually OCD. Examples of such restrictions are that the patient must realize that the obsessions or compulsions are senseless or unreasonable, that the obsessions and compulsions must be time consuming and impair the normal day to day functionality of the patient, and that the obsessions or compulsions cannot be attributed to any other mental or physical condition, or be the direct or indirect consequence of drug usage or abuse (American Psychiatric Association, 2013).

Although more than 95% of OCD patients report both obsessions and compulsions (Foa & Kozak, 1995; Goodman *et al.*, 1989), the fact that only obsessions or compulsions can be present in a patient with OCD changes the general assumption that anxiety always plays a

central role in the pathogenesis of the condition. Compulsions that manifest without the patient expressing either obsessions or anxiety can now be diagnosed as OCD. This diagnostic separation between the two symptoms has important implications with respect to modeling the condition in animals, as imitating the obsessive symptoms of the condition in animal models has proven to be especially problematic.

As will be explained in paragraph 2.1.2, OCD is a condition that presents itself in different forms and subtypes with symptoms representing a number of other conditions, from anxiety related psychiatric conditions to impulse-control disorders. Thus, demonstrating the comorbidity of OCD with other psychiatric and motor conditions may aid in the better understanding of the etiology and pathophysiology of the condition and ultimately the improvement of current treatment strategies for patients diagnosed with OCD (Bartz & Hollander, 2006).

2.1.2 The symptoms of OCD and its comorbidity with other conditions

Markarian and colleagues (2010) conclude that at least five main subtypes of OCD can be identified. These are highlighted in the Table 2-1:

Obsessions	Compulsions
Concerns about contamination	Excessive washing
Concerns about harming oneself or others	Checking and praying
Concerns about symmetry and order	Ordering, arranging and counting
Obsessions only (<i>mainly of sexual, religious or aggressive nature</i>)	No compulsions prevalent
Concerns about waste	Collecting and hoarding

TABLE 2-1: Common obsessions and compulsions in patients diagnosed with OCD

Of the above five subtypes, the most prevalent obsessions are concerns about contamination (55%), followed by inappropriate aggressive and sexual thoughts (50% and 32% respectively), and concerns about symmetry and order (36%). The most common compulsions are ritualistic checking (80%), cleaning and decontamination rituals (46%) and counting (21%) (Abramowitz *et al.*, 2010). From these statistics, it is also evident that many patients present with symptoms that span across the different subtypes of OCD, a fact that further complicates the diagnosis of the condition.

In a systematic review by Husted and Shapira (2006), the authors investigated the possible role of disgust in OCD. Disgust normally involves the evaluation of objects and events for

their possible role in contamination. Normal individuals have the ability to discount any fears of contamination if it remains below a certain level (Husted *et al.*, 2006), and thus the conclusion could be made that the normal process of fear of contamination and its subsequent extinction may be dysfunctional in patients with OCD. In addition, it could likely be concluded that OCD patients expressing concerns about contamination and subsequently engage in washing rituals, may express a lower threshold for experiencing disgust and fail to perceive a decline in the contagiousness of a contaminated object, hence the expression of washing rituals. Since the earliest definitions that suggested disgust to be the expression of revulsion against taste and other sensory stimuli (Darwin, 1965), the definition has subsequently evolved to include revulsion at the oral incorporation of contaminants (Rozin & Fallon, 1987), as well as disgust arising from abstract concerns like personal appearance, religion, and sexual thought (Rozin, Lowery, Imada, & Haidt, 1999). The authors, as well as findings from fMRI studies by Mataix-Cols and colleagues (2003) furthermore concluded that the same neurocircuitry implicated in OCD, mainly the cortico-striatal-thalamic-cortical (CSTC) circuit (see section 2.2), is also activated in the response to disgust as well as during the expression of washing and checking symptoms, providing further evidence for a possible role of disgust in the etiology of OCD.

While disgust have been associated with contamination obsessions and washing rituals, harm avoidant OCD is usually associated with a cognitive distortion presenting as inflated responsibility (Salkovskis, 1985). As feelings of inflated responsibility are related to the prevention of harm or other negative outcomes, patients overestimate normal threats, resulting in excessive preventative behavior, i.e. compulsive checking. With respect to order/symmetry OCD, patients report high degrees of being intolerant to uncertainty (Sarawgi *et al.*, 2013). Furthermore, intolerance of uncertainty also correlates strongly with GAD, SAD, and panic disorder, possibly implicating a shared psycho-etiology underlying certain phenotypes of OCD and anxiety disorders (Sarawgi *et al.*, 2013).

When reviewing the comorbidity of OCD with other mood and anxiety disorders, the general finding is that patients diagnosed with OCD exhibit a higher prevalence rate for major depressive disorder (MDD), social phobia (SP), panic disorder, and generalized anxiety disorder, compared to the general population (LaSalle *et al.*, 2004; Nestadt *et al.*, 2001). Indeed, adults with OCD report higher rates of unemployment, marital discord and financial instability (Barlow, 2002) compared to healthy controls, while children with OCD present with an impaired

ability for both making and keeping friends (Kim, Reynolds, & Alfano, 2012; Piacentini, Bergman, Keller, & McCracken, 2003). Interestingly, SP and OCD share common clinical ground in that both conditions follow a course of varying symptom intensity, are characterized by severe occupational infringement and respond preferentially to selective serotonin reuptake inhibitors (SSRI) (Assunção *et al.*, 2012). Furthermore, patients with comorbid OCD and social impairment demonstrate greater OC symptom severity and a higher rate of treatment resistance compared to patients diagnosed with OCD alone (Alarcon, Libb, & Spitler, 1993). In fact, high Y-BOCS (Yale-Brown Obsessive-Compulsive Scale) scores associated with poor social functioning are predictive of poor treatment outcome (Stewart, Yen, Stack, & Jenike, 2006) while it is postulated that increased OC symptom severity may worsen social impairment and vice versa (Rosa *et al.*, 2012). Given the clinical consequences of comorbid OCD and poor social functioning, it is interesting to note that although youth with OCD have fewer friendships and struggle to maintain these relationships, they do not perceive themselves different from normal controls with respect to having a best friend or being able to nurture these relationships once they are formed (Piacentini *et al.*, 2003). In fact it is argued that youth with OCD, instead of being unable to socialize per se, may only be more socially isolative in scenarios where normal peers may observe their behavior (Piacentini *et al.*, 2003). Interestingly, Denys and co-workers (2004) found that with respect to MDD, OCD typically precedes depression, a finding that suggests that depression does not have an etiological relationship with OCD, but rather results from OCD. Whether or not the same relationship exists between OCD and anxiety disorders is still not clear (Denys *et al.*, 2004). Furthermore, it is especially interesting to note at this stage that OCD responds exclusively to drugs that potentially inhibit the synaptic reuptake of serotonin, such as the selective serotonin reuptake inhibitors (SSRIs), also the first line drug choice for patients with MDD. However, the traditional anxiolytics, for instance the benzodiazepines, have no clinical effect in patients with OCD, nor do drugs that target the noradrenergic system (Fineberg & Craig, 2007). Consequently, demonstrating comorbidity of OCD with anxiety disorders may not necessarily be an indication that OCD is an anxiety disorder, but simply that patients with OCD are more prone to develop other anxiety disorders, compared to the general population. Indeed, that anxiety is often a co-morbid symptom in patients with MDD reemphasizes this fact.

As alluded to earlier, OCD shares many characteristics with a cluster of conditions called the obsessive-compulsive (OC) spectrum of disorders (Bartz & Hollander, 2006). Although these

conditions cannot be classified as OCD, they present with a distinctly similar range of characteristics that are also found in OCD. Obsessive thinking or compulsive behavior, though somewhat different in presentation to the typical phenomenology of OCD, is also central to the nature of these conditions. The OC spectrum of disorders can be classified into three main clusters (Bartz & Hollander, 2006): 1) body image/body sensitization/body weight concern disorders; 2) impulse control disorders; and 3) neurological disorders with repetitive behaviors. Like in OCD, the first cluster of disorders are characterized by intense intrusive and anxiety provoking thoughts and include conditions like bulimia nervosa, anorexia and hypochondriasis. The second cluster is characterized by impulsivity, such as compulsive gambling, but unlike in OCD the compulsive behavior is associated with pleasure. The third cluster includes syndromes like Tourette's syndrome and autism and can be classified as pure neurological disturbances that present with, among others, stereotypical motor behavior. Generally, patients primarily diagnosed with OCD also have higher lifetime prevalence rates for OC spectrum disorders compared to the general population (Denys *et al.*, 2004; du Toit, van Kradenburg, Niehaus, & Stein, 2001). Moreover, patients primarily diagnosed with an OC spectrum disorder also have higher prevalence rates for OCD, a relationship that is not consistently shown in comorbidity studies of OCD and mood and anxiety disorders (Bartz & Hollander, 2006; Gunstad & Phillips, 2003; Thornton & Russell, 1997).

2.1.3 The treatment of OCD

It has been widely demonstrated that OCD responds best to drugs that selectively targets serotonergic, but not noradrenergic neurotransmission, especially in the frontal cortex, striatum and thalamus (Fineberg & Craig, 2007; Grados & Riddle, 2001; Jenike, 1993; Stein, 2002; Vythilingum, Cartwright, & Hollander, 2000) (see section 2.2 for a review on the neurobiology of OCD). Clomipramine, a tricyclic antidepressant (TAD) that is particularly effective in inhibiting the presynaptic reuptake of serotonin, was the first drug shown to be consistently effective in the treatment of OCD. This is in direct contrast with desipramine, a TAD mainly inhibiting the presynaptic reuptake of noradrenalin, which has no demonstrable clinical efficacy in OCD (Fineberg & Craig, 2007). However, the development of the SSRIs was an important advance in the treatment of OCD, as these drugs have a better safety and tolerability profile than clomipramine. To date, however, no study has been able to present proof that SSRIs have greater therapeutic effect in treating OCD than clomipramine, although the lack of serious side-effects with the SSRIs (for example cardiotoxicity as seen with clomipramine treatment), may favor the prescribing of SSRIs over clomipramine (Fineberg & Craig, 2007).

Two general traits characterize the treatment of OCD with the SSRIs: 1) Unlike in depression, OCD responds optimally to SSRI treatment only after 6 to 8 weeks of treatment, and 2) a better response can usually be achieved with initial SSRI doses higher than that prescribed for the treatment of depression (Fineberg & Craig, 2007). Although it has been shown that some patients with OCD do in fact respond to SSRI doses corresponding to the doses used in depression, relapse using low dose SSRI treatment is common. Moreover, subsequent reinstatement of treatment after such a relapse is associated with a poorer clinical outcome (Maina, Albert, & Bogetto, 2001).

Resistance to SSRI therapy is a major clinical challenge. Even after long-term treatment, approximately 30% of patients remain unresponsive to monotherapy with the SSRIs (Fineberg & Craig, 2007). The treatment of refractory OCD is difficult, with some authors advocating for an increased dose and a longer duration of treatment (Bejerot & Bodlund, 1998), while others advise switching treatment to another SSRI (Fineberg, Nigam, & Sivakumaran, 2006). A third strategy that may prove to be especially useful in patients that have shown a partial response to a SSRI after 10 – 12 weeks, is to augment the SSRI therapy with a low dose antipsychotic (Erzegovesi, Guglielmo, Siliprandi, & Bellodi, 2005; Hollander, Rossi, Sood, & Pallanti, 2003; McDougale, Epperson, Pelton, Wasylink, & Price, 2000). With respect to the latter, it is interesting to note that no clinically significant difference with respect to efficacy has been observed between typical antipsychotics, such as haloperidol, and atypical antipsychotics such as clozapine or risperidone (Fineberg & Craig, 2007).

Although glutamate and gamma-amino butyric acid (GABA) are major role players in the functioning of the CSTC circuit, less work has been done in targeting these systems in the treatment of OCD. Nevertheless, GABAergic (Oulis *et al.*, 2009) as well as glutamatergic agents (Coric *et al.*, 2005; Lafleur *et al.*, 2006; Stewart *et al.*, 2010) have demonstrated their possible usefulness in treating OCD. Because of their acknowledged importance in OCD neurocircuitry, the targeting of these two neurotransmitters and their receptors for the treatment of OCD are under continuous investigation (El Mansari & Blier, 2006).

It is recommended that from the time of diagnosis, treatment should be initiated with a SSRI as first choice and titrated relatively quickly to high doses until remission of symptoms. Furthermore, treatment should be continued for at least 12 weeks before any change in the regime is considered. Once stabilized, treatment should not be interrupted for at least a year (Fineberg & Craig, 2007; Maina *et al.*, 2001).

2.2 The Neurobiology of OCD: Serotonin and a Proposed Dysfunction in the CSTC Circuitry

As the present investigation mainly focuses on the face and predictive validities of the deer mouse model of OCD, the neurocircuitry of OC behavior will only be discussed in brief.

2.2.1 Dysfunctional cognitive processes and brain areas involved in OCD

It is clear that in most cases OCD is characterized by both cognitive and behavioral abnormalities (Markarian *et al.*, 2010). Different hypotheses have been put forward to explain the symptomology of OCD. However, whether patients diagnosed with OCD express a lower threshold for disgust (Husted *et al.*, 2006) and subsequently present with compulsions like ritualistic hand washing, or simply are not sensitive to the reward of task completion (e.g. ritualistic door locking due to obsessions about security), it is clear that an abnormal regulation of goal-directed behavior is central to the symptomology of OCD. Thus, it is evident that the brain areas implicated in OCD would be among others, those that translate cognitive planning and experiences into motor behavior, and subsequently mediate goal-directed (*viz.* reward-related) behavior. These brain areas include the prefrontal cortex, striatum and thalamic nuclei that communicate with each other via different pathways (Den Heuvel *et al.*, 2010; Evans, Lewis, & lobst, 2004; Nambu *et al.*, 2000). The term 'CSTC circuit' (cortico-striatal-thalamic-cortical circuit) (Figure 2-1) denotes the functional organization of these structures (Stocco, Lebiere, & Anderson, 2010) and are organized in such a manner that the cortex innervates the striatum, which subsequently influences other parts of the basal ganglia and ultimately exerts feedback via the thalamus to the cortex. Consisting of direct (*behaviorally activating*) and indirect (*behaviorally inactivating*) pathways, the CSTC circuit is fundamental in the planning, execution and termination of complex motor behavior and reward based learning – two major processes that are hypothesized to be dysfunctional in patients with OCD (Stocco *et al.*, 2010). Given the persistent inability of most OCD patients to find closure after the 'reward of task completion', it is not surprising to find that both the anterior cingulate cortex (ACC) and the orbito-frontal cortex (OFC) are hyperactive in OCD patients as numerous functional neuroimaging studies have demonstrated (Maia, Cooney, & Peterson, 2008; Maltby, Tolin, Worhunsky, O'Keefe, & Kiehl, 2005; Markarian *et al.*, 2010; Saxena & Rauch, 2000; Shim *et al.*, 2009). The fact that the ACC is vital in the control of cognitive and behavioral processes and mediates certain executive functions, justifies the hypothesis that in patients with OCD, an overactive ACC maybe plays a role in the instigation of compulsive behavior. The fact that no 'reward after task completion' is established in most OCD patients

could suffice to explain the hyperactivity in the OFC. Considering the co-morbidity between OCD and SAD, collective evidence from imaging studies in patients with SAD also demonstrate dysfunction of CSTC circuitry, striatal lesions as well as hyperactivity in the ACC (Engel, Bandelow, Gruber, & Wedekind, 2009).

Furthermore, it is believed that there is a bias in favor of the direct thalamus-activating pathway over the indirect thalamus-inhibiting pathway in the basal ganglia of OCD patients compared to healthy controls (Saxena & Rauch, 2000) (Figure 2-1). This not only results in an overactive OFC, but also increases the activities of both the caudate nucleus and the thalamus. The subsequent hyperactivity in the CSTC circuit as a whole, is believed to be central to the pathological presentation of OCD (Bartz & Hollander, 2006; Saxena & Rauch, 2000).

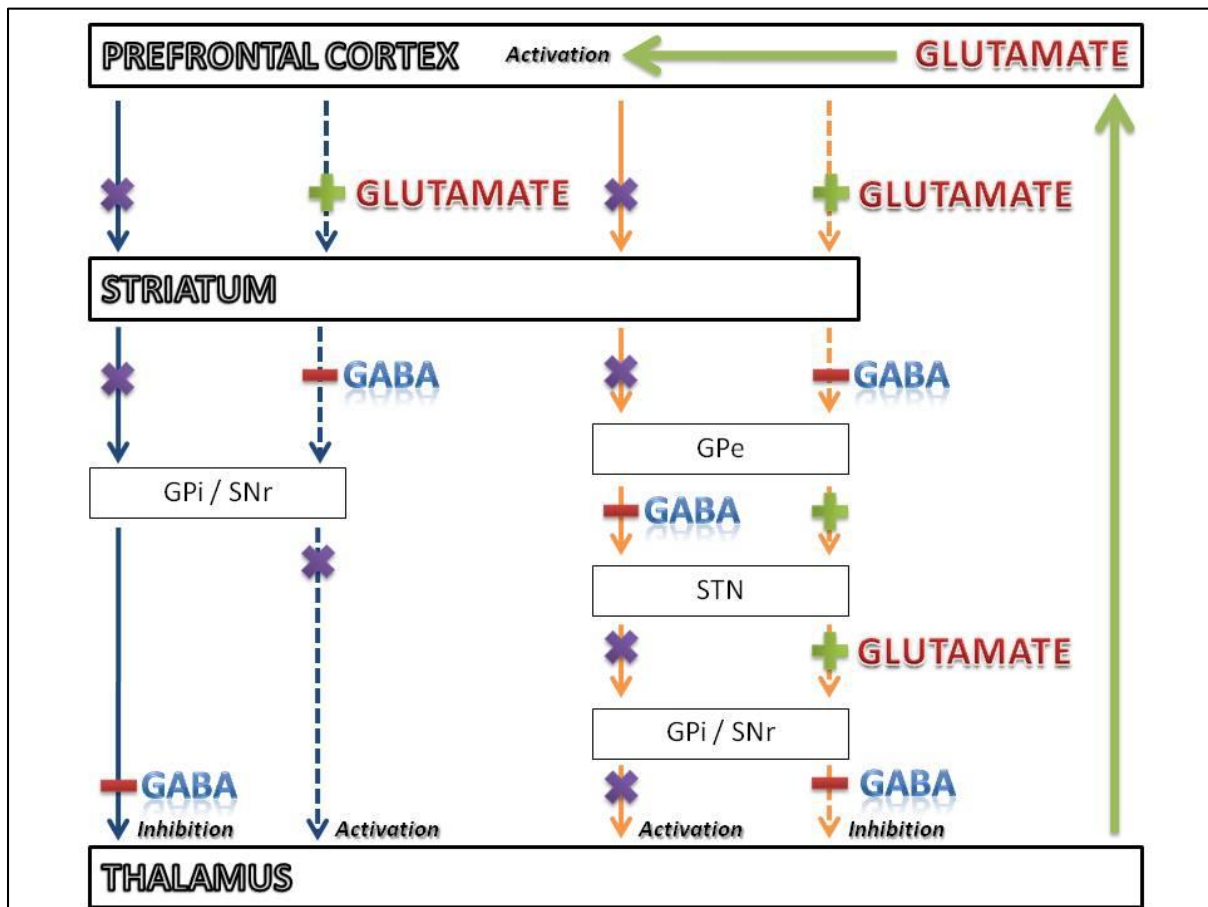


FIGURE 2-1: The CSTC circuit implicated in OCD

Blue lines, direct pathway; Orange lines, indirect pathway; Solid lines, no cortical activation of pathways; Dotted lines, cortically activated pathways; Purple crosses, no considerable neurotransmitter release; Red minus signs, GABAergic inhibition; Green plus signs, lack of target inhibition / glutamatergic activation; GPi / SNr, globus pallidus interna / substantia nigra pars reticulata; GPe, globus pallidus externa; STN, subthalamic nucleus; GABA, gamma-amino butyric acid.

2.2.2 An involvement of serotonin and dopamine in reward processing

Certain neurotransmitters have been identified as playing a central role in the pathogenesis of OCD, of which the excitatory amino acid glutamate, the inhibitory neurotransmitter GABA, and the monoamines dopamine and serotonin are the most well studied (El Mansari & Blier, 2006; Markarian *et al.*, 2010; Pittenger *et al.*, 2006; Sareen *et al.*, 2004).

The term 'reward' denotes any form of satisfactory feedback, from the pleasant taste of foods and liquids or the response to a pleasant or aversive experience, to the successful completion of a certain task (Schultz, 2002). For the sake of simplicity, it can be assumed that any motor action driven to accomplish a certain task is initiated by the anticipation of a reward that should result from the successful completion of that specific action. For example, the locking of a door is initiated by the anticipated reward of a secure environment. Since this action had been highly prioritized (Schultz, 2002), it promotes certain actions to be taken, which in this case could be identifying the correct key, inserting it into the lock and turning the key. Importantly though, under normal conditions, the repetitive experience of any given reward should mediate a process called reward-based learning or 'reward conditioning'. As explained later in this paragraph, this process prevents further reward-seeking behavior and is pivotal to the normal day-to-day functioning of any human being (Schultz, 2002).

The anticipation and appraisal of reward is closely correlated with dopaminergic signaling in the brain (Ljungberg, Apicella, & Schultz, 1991; Mirenowicz & Schultz, 1994; Schultz, Apicella, & Ljungberg, 1993). During the initial anticipation of a novel reward, roughly 75% of the dopaminergic neurons in the basal ganglia are activated (Ljungberg *et al.*, 1991; Mirenowicz & Schultz, 1994). Over time, with repetitive exposure to the same reward, the brain becomes conditioned to the reward through the complex association thereof with different environmental, physiological, and circumstantial factors. Reward conditioning enables the brain to evaluate future confrontations with the same set of factors it was conditioned to, and to associate it with an applicable reward, even before the reward has been presented (Romo & Schultz, 1990). Once the reward is presented, the subsequent reaction of the brain can manifest as either one of the following: 1) if the reward is greater than that anticipated or totally unpredicted, dopaminergic signaling is elicited; 2) if the reward has been predicted in full, no dopamine response is elicited, and 3) if the anticipated reward was omitted a suppression in dopaminergic signaling is induced (Schultz, Dayan, & Montague, 1997).

Following dopamine response after actual exposure to the reward, the basal ganglia codes a 'reward prediction error' – a term used to represent the difference between the predicted and actual reward (Schultz *et al.*, 1997). The reward prediction error is pivotal to the process of reward-based learning, as it is used to compute the changes that must be implemented in future to either keep experiencing the same reward in the case of a positive error, or experiencing a better reward in the case of a negative error. It can be postulated that the lack of a significant dopaminergic response after the manifestation of a fully predicted reward, may account in part for finding closure after task completion, as the lack of a dopamine response will not induce further reward-seeking behavior. Patients diagnosed with OCD clearly do not find closure after task completion. As they constantly exhibit repetitive compulsive behavior of some nature, it can be hypothesized that, concerning the processes of reward appraisal and reward-based learning, a dysfunctional reward system (*viz.* hyperactive dopaminergic transmission) may be central to the pathology of OCD (Husted *et al.*, 2006).

The fact that behavioral inhibition has been associated with serotonergic neurotransmission (Cools, Roberts, & Robbins, 2008; Daw, Kakade, & Dayan, 2002) provides a possible explanation for the ameliorative effects observed when treating OCD with drugs enhancing serotonergic functioning. As excessive dopaminergic signaling is a major role player in the pathology of OCD, a number of authors propose a role for the serotonergic system to act as the behavioral opponent of dopamine (Cools *et al.*, 2008; Daw *et al.*, 2002; Deakin *et al.*, 1991; Fletcher & Korth, 1999). In a comprehensive review of the opponent interactions between serotonin and dopamine (Daw *et al.*, 2002), the authors use the term 'opponency' to describe a situation in which more than one system code for different affective events. As such, it is known that the dopaminergic system codes for rewarding stimuli and that it is crucial for reward-based learning, while serotonin is activated during the experience of aversive stimuli (Daw *et al.*, 2002; Fletcher, 1995; Fletcher & Korth, 1999; Fletcher, Korth, & Chambers, 1999; Kapur & Remington, 1996). Indeed, work undertaken in our laboratory using an animal model of posttraumatic stress disorder has clearly demonstrated the central role for serotonin in the bio-behavioral response to aversive stimuli (Harvey, Naciti, Brand, & Stein, 2003; Harvey, Brand, Jeeva, & Stein, 2006), as well as its response to serotonergic agents (Harvey, Naciti, Brand, & Stein, 2004; Uys *et al.*, 2006). Also, in a number of studies undertaken by Fletcher and colleagues (Fletcher, Ming, & Higgins, 1993; Fletcher, 1995; Fletcher & Korth, 1999), the authors have broadly shown that enhancing serotonergic signaling suppresses both condi-

tioned behaviors (such as lever pressing for food) and unconditioned behaviors (such as feeding) which is normally associated with an activation of dopaminergic signaling. Consequently, when antagonizing serotonin or agonizing dopamine, the opposite effect is mediated. This also corresponds with data demonstrating that serotonin antagonizes the effects of dopamine in the substantia nigra and striatum (Kapur & Remington, 1996).

Daw *et al* (2002) hypothesizes that if dopamine is responsible for approach behavior, motor excitement, and reward processing, serotonin will be associated with avoidance behavior, motor suppression, and the processing of aversive or punishing stimuli. In different terms, it can be understood that the balance between reward seeking behavior and aversive reactions is related to the balance between dopaminergic and serotonergic signaling respectively (Daw *et al.*, 2002; Deakin *et al.*, 1991). However, in direct contrast to this hypothesis, it has been postulated that patients with depression express a bias in favor of the processing of negative, instead of positive stimuli as a result of the relative lack of sufficient serotonergic signaling demonstrated in this condition (Cools *et al.*, 2008; Murphy, Smith, Cowen, Robbins, & Sahakian, 2002). Thus, serotonin may be involved in the mediation of contrary behaviors, namely the preservation of a normal mood (euthymia) and the suppression of reward-based behavioral responses. Deakin and colleagues (1991) attempted to resolve this paradox by explaining that the serotonergic projections from the dorsal and median raphe nuclei respectively, are responsible for different cognitive and behavioral functions. The authors explained this by implicating the serotonergic projection from the dorsal raphe nuclei to the amygdala and basal ganglia to be the opponent of dopamine functioning, while the projection from the median raphe nuclei is postulated to play a role in the pathology of mood disorders such as depression. Bearing in mind that OCD are often diagnosed with co-morbid SAD, data demonstrating decreased striatal D₂ (resulting in disinhibition of behavior) and dorsal raphe 5HT_{1A} (resulting in increased serotonergic neurotransmission) receptor expression in patients with SAD, supports this hypothesis (Engel *et al.*, 2009).

Taken from the above, an abnormal reward appraisal system may be central to the pathology of OCD. As OCD patients generally do not find closure after task completion, it is hypothesized that the dopaminergic response following the presentation of a given reward, is always of the same magnitude and that reward based learning therefore does not occur. Moreover, an increase in serotonergic neurotransmission elicited by SSRIs may act as the behavioral opponent of said hyperactive dopaminergic transmission, resulting in the attenuation of OC symptoms.

2.3 Designing Animal Models of OCD

In short, as explained in chapter 1 and paragraph 2.1, OCD is characterized not only by compulsions but also in most cases by disturbances in cognition of which obsessions can be included. In the following paragraphs, it will become clear that although perseverative motor repetition, which may represent human compulsions, is a common behavioral phenotype in animals, it is difficult to associate this behavior with cognitive deficits. In animal models of OCD this association is very important, as it differentiates normal repetitive stereotypy from compulsive-like motor repetition.

When considering the development of a suitable animal model of OCD, certain important questions need to be borne in mind: First, what are the core symptoms of the condition that the putative animal model is required to display? Second, which treatment options should be considered once the appropriate symptomology has been described? Third, which neurobiological targets should be scrutinized for similarities between the animal model and the human condition? In essence, these three fundamental questions constitute the foundation of behavioral research in animals and can otherwise be conceptualized as face, predictive and construct validity, respectively (van der Staay, 2006). Subsequently, the importance of each of these validation criteria as they apply to the development of a suitable animal model of OCD will be discussed.

2.3.1 Repetitive behavior – corner stone for establishing face validity for OCD

OCD patients typically experience constant recurrent thoughts or compulsive-like repetition of certain actions and as such repetitive (and perseverant) behavior is central to the manifestation of OCD symptomology. Although the repetition of behavioral sequences forms part of normal human behavior, under certain conditions and pathological states it may become abnormally frequent, rigid and resistant to change, purposeless, and relatively mono-dimensional – the four principles that collectively define the term stereotypy (Garner & Mason, 2002; Langen, Durston, Kas, van Engeland, & Staal, 2011). For example, locking doors and brushing teeth are normal daily behavior of a ritualistic nature, but in patients with OCD it may become a pathological manifestation resulting in major functional impairment. Applying the four principles of stereotypy to strengthen the face validity of a given animal model of OCD seems to be uncomplicated, as compulsive-like repetition of certain behavioral sets is relatively easy to model in animals. Consequently, all the animal models of OCD currently at our disposal are based foremost on the demonstration of some form of stereotypical behavior

(Albelda & Joel, 2012a; Alonso, López-Solà, Real, Segalàs, & Menchón, 2015; Fineberg *et al.*, 2010; Joel, J. Stein, & Schreiber, 2008) (refer to paragraph 2.3.4 for a more detailed discussion on animal models for OCD). However, great care is to be taken if stereotypical behavior forms the only strength of a putative animal model of OCD, as pathological motor repetition is a diagnostic criterion for a number of human disorders as well as a common behavioral manifestation in many different animal species housed under confined circumstances (Eilam, Zor, Szechtman, & Hermesh, 2006). In addition, if motor stereotypy can be described as lower order repetition, most OCD patients also present with higher order repetitions such as recurrent thoughts or obsessions (Abramowitz, Franklin, Schwartz, & Furr, 2003). Although it is just as important as the demonstration of motor stereotypy, these types of repetitions involve cognitive abnormalities and are very difficult to model, as this by implication would suggest that an animal is obsessive. An animal model of OCD therefore needs to distinguish between the stereotypy expressed in OCD patients, and the various forms of stereotypy observed in other disorders. In addition, in order to utilize repetitive animal behavior as a means of establishing face validation in animal models of OCD, the development and manifestation of stereotypy in animals needs to be explored (Eilam *et al.*, 2006).

2.3.1.1 Stereotypy in humans – a common symptom of comorbid conditions

Motor stereotypy is a diagnostic criterion for a number of human conditions and, although the basal ganglia is involved in the pathogenesis of most forms of stereotypy (Garner & Mason, 2002; Haber & Calzavara, 2009; Langen, Kas, Staal, van Engeland, & Durston, 2011; Maia *et al.*, 2008), the various conditions respond differently to the available treatment options. As a result of this, a number of studies have attempted to elucidate the exact role of the basal ganglia in the etiology of the repetitive behavior observed in conditions such as OCD, Tourette's syndrome, trichotillomania, and autism. Since then, three major functional loops linking the cortex and basal ganglia have been described (Groenewegen, Van Den Heuvel, Cath, Voorn, & Veltman, 2003; Langen *et al.*, 2011). In short they are termed the sensorimotor loop (linking the sensory and motor cortices with the putamen), the associative loop (linking the dorsolateral prefrontal cortex with the basal ganglia), and the limbic loop (comprising the anterior cingulate and orbitofrontal cortices). The main function of each loop is believed to be the control or regulation of goal directed behavior (sensorimotor), cognitive functioning (associative) and motivational behavior (limbic) (Langen *et al.*, 2011). If any success is to be gained during the development of an animal model of OCD, the differences between the neurobiological and behavioral presentation of the stereotypies observed in some of the most

prevalent conditions associated with motor repetition, need to be understood. Therefore, in the following few paragraphs a brief discussion on the stereotypies associated with Tourette's syndrome, autism and OCD respectively will be given.

Tourette's syndrome is characterized by verbal and motor tics (American Psychiatric Association, 2013). It mostly develops between 5 and 7 years of age, peaks in severity at age 12, and then gradually ameliorates during puberty. An inverse relationship has been demonstrated between symptom severity and the volume of the caudate nucleus and, although the condition is postulated to be of a hyper-dopaminergic nature due to its favorable response to dopaminergic antagonists, it can also be treated with SSRIs (Langen *et al.*, 2011; Makki, Behen, Bhatt, Wilson, & Chugani, 2008; Rasmussen *et al.*, 1994). Caudate involvement implicates the limbic loop and distinguishes the stereotypy observed in Tourette's from pure motor repetition.

Patients with autism present with three main symptoms, namely stereotypy, non-stereotypical repetitive behavior, and restricted interests (American Psychiatric Association, 2013). A number of studies have associated autism with abnormalities of the anterior cingulate and posterior parietal cortices (Shafritz, Dichter, Baranek, & Belger, 2008) and it has been demonstrated that patients with autism make more mistakes in experimental tasks and fail to distinguish between correct and incorrect responses compared to healthy control subjects (Thakkar *et al.*, 2008). Although the repetitive behavior expressed in autistic patients respond to some degree to SSRIs (Kolevzon, Mathewson, & Hollander, 2006), dopamine antagonists are still the mainstay of therapy (Barnard, Young, Pearson, Geddes, & O'Brien, 2002).

Stereotypical behavior in most patients with OCD involves motor and cognitive repetition (Abramowitz *et al.*, 2003). As reviewed in section 2.2, OCD has been associated with increased activity in the ACC and OFC, and interestingly also in the caudate nucleus (Maia *et al.*, 2008; Maltby *et al.*, 2005; Markarian *et al.*, 2010; Saxena & Rauch, 2000). The anterior cingulate and orbitofrontal cortices functions as the cortical inputs of the limbic loop, while the caudate functions as the striatal entry point for the associative loop, implicating a possible role for the cross-talk between these two major pathways in the pathology of OCD (Figure 2-2). A number of studies using positron emission tomography (PET) have demonstrated decreased midbrain levels of the SERT in patients with OCD compared to healthy controls (Hesse *et al.*, 2005; Reimold *et al.*, 2007; Zitterl *et al.*, 2008). Although studies done by Simpson (Simpson *et al.*, 2003) and Pogarell (Pogarell *et al.*, 2003) could not replicate the latter

findings, they did demonstrate that pre-treatment SERT density positively correlated with response to treatment with clomipramine, although this was inversely related to the severity of OCD symptomology (Zitterl *et al.*, 2008). In summary, it seems that the repetitive behavior of OCD is unique in that it involves motor and cognitive repetition and responds mostly to monotherapy with the SSRIs and not atypical antipsychotics. Subsequently, these two parameters are the principle targets under consideration in an animal model of OCD.

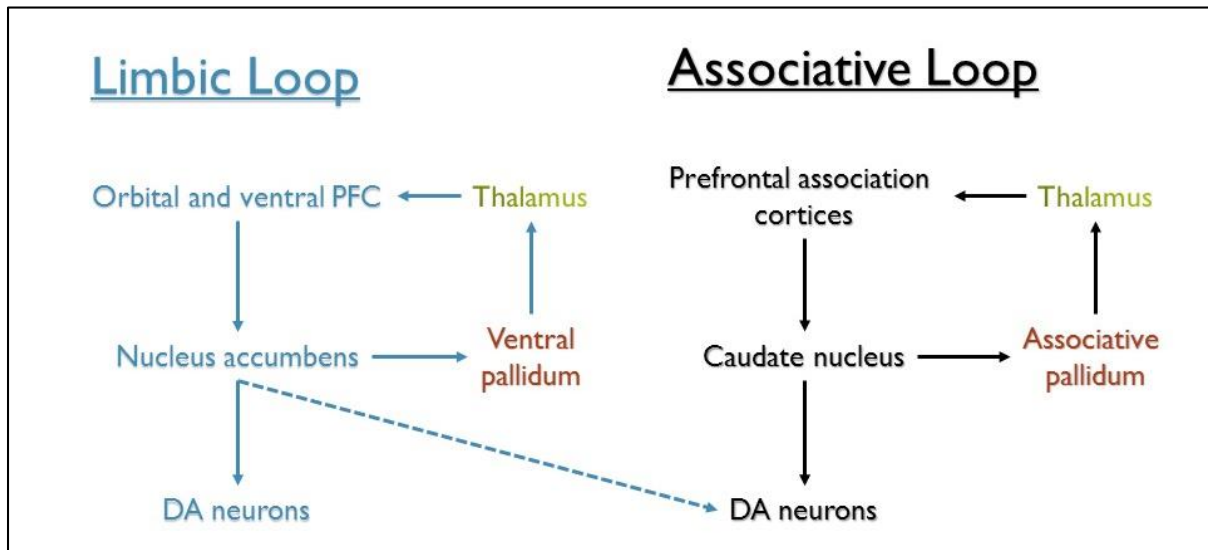


FIGURE 2-2: The limbic and associative CSTC loops

Dotted arrow indicates dopaminergic cross-talk between the limbic and associative loops.

Modified from Mannella, Gurney, & Baldassarre, 2013

2.3.1.2 Stereotypy in animals – normal biology or abnormal pathology?

Motor stereotypy is a common manifestation in many animal species and usually develop when wild animals are bred or kept in restricted and/or environmentally deprived habitats (Eilam *et al.*, 2006). However, it can also be induced by behavioral training and through pharmacological or genetic manipulation of the neuronal pathways implicated in repetitive behavior, especially the cortico-striatal circuitry (Berridge, Aldridge, Houchard, & Zhuang, 2005; Chou-Green, Holscher, Dallman, & Akana, 2003; Egashira *et al.*, 2008; Joel & Avisar, 2001; Klavir, Flash, Winter, & Joel, 2009; Langen *et al.*, 2011; Szechtman, Sulis, & Eilam, 1998; Tsaltas *et al.*, 2005).

Many different theories have been put forward to explain the development of stereotypy resulting from environmental deprivation or so called cage stereotypy, including that a restrictive environment only allows a certain behavioral pattern to be executed (Ridley, 1994) or that the stress resulting from confinement induces abnormal repetitive motor behavior (Langen *et al.*, 2011). Cage stereotypy mostly manifests as stationary behavior, in other words

motor repetitions that are being executed only in a few preferred locations, and although it seems senseless (as do the compulsions in human OCD), it is not necessarily the case. In fact a number of studies have reported that while most OCD patients realize the futility of their actions, preventing the completion of these motor routines sometimes results in acute anxiety (Eilam *et al.*, 2006; Robbins, Gillan, Smith, de Wit, & Ersche, 2012). It can thus be argued that at least in some patients, repetitive behavior plays an anxiolytic role. Still, most forms of cage stereotypy resemble one another through their rigid repetition of various motor routines. The question thus arises how can these behaviors be distinguished from one another? Referring back to paragraph 2.3.1.1, different forms of stereotypy are central to the diagnosis of each human condition. While rigid motor patterns without any evident cognitive influence forms the core of stereotypical behavior in conditions such as Tourette's syndrome, tics and autism, compulsive-like stereotypy is exhibited by OCD patients. Eilam and colleagues (2006) propose that compulsive-like repetitions can be distinguished from pure motor stereotypy based on the idea that compulsions are characterized by flexibility and thoughtfulness. They explain that in most cases, patients with OCD will not execute the compulsive behavioral routine if the environmental paradigm triggering it is not present (therefore the behavior is regarded as flexible) and that the compulsions are mostly the result of obsessions or inappropriate thoughts. These two proposed characteristics of compulsions could be employed to distinguish between rigid motor patterns and compulsive-like repetitive behavior, both of which manifest as motor stereotypy.

The genetic manipulation of animals resulting in stereotypy, or the pharmacological modulation of certain receptors implicated in the pathology of stereotypy, provides one major advantage over cage stereotypy in that it induces repetitive behavior as a direct consequence of the relevant manipulation. Thus, the roles of individual pathways and receptors in the development of stereotypy can be investigated and thus have great importance from a construct point of view. In other words, they can be used to study certain defined neurobiological underpinnings believed to mediate the presenting stereotypy. A major disadvantage of these models is that, as a direct consequence of this predetermined construct, they disregard the role of crosstalk between the different neuronal pathways and neurotransmitters in the development of OCD.

To summarize, stereotypical behavior in itself may strengthen the face validity of an animal model of OCD, provided that it can be associated with either the demonstration of flexibility

or thoughtfulness, or linked with good predictive and construct validity which could substantiate the type of stereotypy being expressed.

2.3.2 A favorable response to SSRI – the mainstay of predictive validity

Although all the current animal models of OCD demonstrate various forms of repetitive behavior, it is evident that these behaviors need to be associated with good predictive and construct validity, as stereotypy in itself is a common behavioral manifestation in animals. As described in section 2.1, for an animal model of OCD to be credible, it must demonstrate selective response to the SSRIs, either as monotherapy, or in a combination with low-dose antipsychotics (Fineberg & Craig, 2007; Grados & Riddle, 2001). In addition, the model must be unresponsive to treatment strategies that have no clinical effect in human OCD, for instance drugs targeting the noradrenergic system and the benzodiazepines (Grados & Riddle, 2001).

Various treatment regimens have been utilized in the different animal models of OCD, including acute, sub-chronic and chronic administration of the SSRIs, either as monotherapy or in a combination with drugs from different classes. However, as explained in paragraph 2.1.3, patients with OCD preferentially respond to high dose SSRIs and only after chronic administration. The predictive validity of an animal model of OCD can thus be strengthened if it is demonstrated that chronic, but not acute or sub-chronic, and high dose (but not nominal or antidepressant doses) treatment with the SSRIs is successful in alleviating stereotypy, while drugs ineffective in human OCD exert no response.

2.3.3 Establishing the construct of OCD in animals

The criteria that must be met to establish construct validity of an animal model of OCD can be summarized from section 2.2. In short, the CSTC circuitry is fundamental in the pathology of OCD and must therefore be implicated in the animal model. As patients with OCD mostly respond selectively to the SSRIs, serotonin is regarded as a major role player, whether direct or indirect. Post-synaptic 5HT_{2C} receptor activation is thought to be the major mediator of the anti-compulsive effects of the SSRIs, while the down regulation of 5HT_{1B/D} receptors is hypothesized to be linked to an increase in serotonergic neurotransmission following chronic treatment with the SSRIs (El Mansari & Blier, 2006). Although the presynaptic 5HT_{1A} receptors in the raphe nuclei may suppress the serotonergic outflow to the cortex, it is believed that 5HT_{1A} antagonism will only quicken the onset of action of the SSRIs and that the down

regulation of the 5HT_{1A} receptors during chronic SSRI-treatment is not a contributing factor to the efficacy of the SSRIs (Ceglia *et al.*, 2004).

As a bias in favor of the activity of the direct pathway (expressing D₁ receptors) through the basal ganglia over that of the indirect pathway (expressing D₂ receptors) (Figure 2-1) is postulated in the pathology of OCD, it would be interesting to evaluate the behavioral effects following the administration of various selective dopaminergic stimulating and blocking agents in established and putative animal models of OCD.

Although a number of other factors, such as an altered oxidative status (Güldenpfennig, Wolmarans, du Preez, Stein, & Harvey, 2011), increased glutamatergic signaling (Pittenger *et al.*, 2006), increased nitric oxide levels (Atmaca, Tezcan, Kuloglu, & Ustundag, 2005), the circadian effects of the female hormonal cycle (Abramowitz *et al.*, 2003), and increased growth hormone levels (Kluge *et al.*, 2006) to name but a few, may contribute to the symptomology of OCD, these are likely to be secondary to changes in serotonin and/or dopamine. Furthermore, most of these markers or mediators of disease are altered in a wide variety of conditions (Brand, Möller, & Harvey, 2015) and therefore cannot be used to establish the construct validity of an animal model of OCD. However, these markers can be used to strengthen an already solid foundation built from a combination of good face, predictive and construct validity.

Taken together, the construct validity of an animal model of OCD could be strengthened if the stereotypy can be linked to a dysfunctional CSTC circuit, abnormal serotonergic and dopaminergic signaling, differences in the relevant serotonin receptor densities and the activity of their respective second messengers compared to healthy controls. Lastly, construct validity is strengthened if changes in stereotypical behavior pre- and post-treatment can be correlated with altered serotonergic and possibly dopaminergic signaling.

* * *

2.4 A Short Review of Current Animal Models of OCD

Although repetitive behavior that may imitate human motor compulsions may be quite straightforward to demonstrate in animals, demonstrating its association with an underlying condition that may represent human OCD is extremely difficult. Considering paragraphs 2.3.1 – 2.3.3, it is evident that motor stereotypy, as exhibited by many animal species, needs to be linked to a favorable response to chronic high dose SSRIs or a combination of chronic SSRIs and low-dose antipsychotics. Moreover, this needs to have its origin in either an obsessive or thoughtful origin, and serotonergic and dopaminergic abnormalities in the prefrontal cortex and basal ganglia, or both. Although many animal models of OCD have been proposed during the past 35 years (Alonso *et al.*, 2015), it is difficult to discriminate between established and putative models, since none of them can as yet be regarded as definitive. Each model however, reflects some characteristics of the condition that may be helpful towards novel drug discovery for the treatment of human OCD. A number of key models will be presented below with the exception of genetic models, such as *Sapap3*, *Slitrk5*, *DICT-7*, *Hoxb8lox* and other mouse models (Alonso *et al.*, 2015), that are beyond the scope of this work.

2.4.1 Animal models based on pharmacological manipulation

2.4.1.1 *8-OH-DPAT-induced decrease in spontaneous alternation*

Spontaneous alternation can be explained as the natural tendency of rats to explore novel places in any given environment and without bias towards any given location (Albelda & Joel, 2012b). In this model, originally developed by Yadin and colleagues (1991), the authors demonstrated that administration of the 5HT_{1A} agonist, 8-OH-DPAT to rats was associated with a compulsive-like decrease in spontaneous alternating behavior that provides the foundation for the face validity of the model. A number of studies have also demonstrated that sub-chronic and chronic administration of fluoxetine and clomipramine, but not desipramine, prevented this decrease in spontaneous alternation (Fernández-Guasti, Ulloa, & Nicolini, 2003; Yadin *et al.*, 1991), thus strengthening the predictive validity of the model. Although the decrease in spontaneous alternation is brought about by targeting the serotonergic system – a fact that may provide the model with some degree of construct validity – this behavior is also common in conditions such as Parkinson's disease, autism and schizophrenia (Albelda, Bar-On, & Joel, 2010; Langen *et al.*, 2011), which may somewhat lessen the face validity of the model.

2.4.1.2 *Quinpirole-induced compulsive checking*

In this model developed by Szechtman, Sulis and Eilam (1998), compulsive checking behavior was induced in rats by the administration of the $D_{2/3}$ receptor agonist, quinpirole. Compulsive checking was defined with the demonstration that animals treated with quinpirole preferentially stopped at two specific locations in a given environment compared to the non-specific exploratory behavior of saline treated rats. The authors distinguished the compulsive checking behavior induced by quinpirole from motor stereotypy by implicating a role for thoughtfulness. They demonstrated that in addition to the compulsive checking at the two preferred locations in the environment, rats treated with quinpirole demonstrated shorter return times to these locations compared to other locations in the cage and performed a certain set of behaviors at each location. These observations markedly increase the strength of the model's face validity, as it not only demonstrates repetitive behavior, but also links the repetition with cognitive planning. However, chronic clomipramine only partially attenuated the compulsive checking (Szechtman *et al.*, 1998) while no trial with an SSRI has yet been performed. In addition, the model has not been subjected to challenge with drugs known to be ineffective in human OCD, which diminishes its overall predictive validity. While some construct validity may be provided by the implication of D_2 receptor involvement, it must be stressed that this model may represent only a part of the clinical picture of OCD, as the behavior results from dopaminergic modulation and therefore does not account for the crosstalk between dopamine and serotonin. In line with this, recent data failed to demonstrate involvement of $5HT_{2A/C}$ receptors in the attenuation of checking behavior by mCPP (Tucci, Dvorkin-Gheva, Johnson, Wong, & Szechtman, 2015), supporting findings that clomipramine only partially attenuates the compulsive checking induced by quinpirole.

2.4.2 An animal model based on behavioral training

2.4.2.1 *The signal attenuation model*

This model involves the training of rats to press a lever for reward, in this case food. The presentation of the reward is accompanied by a cue that signals a successful lever press, upon which the food is introduced. Subsequently, the rewarding property of the signal is attenuated by repeatedly presenting the cue without the reward. In short, rats keep pressing the lever in a compulsive manner, even if no reward is gained from their actions. This model has been developed by Joel and Avisar (2001) based on the theory that a dysfunctional reward-based learning system may be central to the pathogenesis of OCD and thus demonstrates excellent face and some construct validity. Not only is compulsive-like lever pressing demonstrated,

but it is also linked with a dysfunction in reward-based learning that distinguishes lever pressing from rigid motor stereotypy. Furthermore, that the dopaminergic, serotonergic and glutamatergic systems have been implicated in the model further strengthens the construct validity of the model. The authors argue that the model has a solid predictive foundation, as acute treatment with an SSRI, but not with desipramine or diazepam, have been found to attenuate compulsive lever pressing. However, this aspect somewhat diminishes the predictive validity of the model since human OCD only responds after chronic and high dose SSRI treatment (Fineberg & Craig, 2007).

2.4.3 Animal models based on the natural expression of compulsive behavior

2.4.3.1 *Marble-burying in mice and rats*

Although some studies employ marble-burying (MB) as a measure of avoidance-related anxiety (viz. neophobia) (Nicolas, Kolb, & Prinssen, 2006), findings that rodents often do not habituate to the harmless and non-reactive nature of marbles and that MB is also expressed in the absence of anxiety (Thomas *et al.*, 2009), suggest a behavior that is more compatible with inherent rather than defensive or neophobic burying. Furthermore, an abundance of literature demonstrates that rats and mice will often persist in burying harmless objects (Londei, Valentini, & G. Leone, 1998; Thomas *et al.*, 2009) and that this seemingly non-functional repetitive behavior may reflect the behavioral symptoms of OCD. Considering the roles of reward and task completion in the pathology of OCD, Londei and colleagues (1998) postulated that the burying of marbles by rodents begins as a normal investigation of novel objects. As marbles do not have reactionary properties, the authors postulate that a lack of perceptible feedback, that should induce a sense of task-completion, induces compulsive burying. This hypothesis, which implicates a role for thoughtfulness, combined with the compulsive-like burying of marbles, strengthens the face validity of the model. However, it has also been argued that the non-reactive nature of harmless stimuli such as glass marbles may elicit a form of anxiety in the animal (Diamant, Croiset, De Zwart, & De Wied, 1991) and it remains to be investigated whether this form of burying is entirely devoid of a stress-related construct. Indeed, OCD may have a bimodal nature in which seemingly purposeless compulsions may induce anxiety (related to obsessions) or vice versa (Robbins *et al.*, 2012). Apart from being a screening-test for putative agents used in the treatment of OCD (Egashira *et al.*, 2008; Gyertyan, 1995; Millan, Girardon, Mullot, Brocco, & Dekeyne, 2002), MB has also been used to model treatment response relating to impulsiveness (Schneider & Popik, 2007), autism

(Angoa-Pérez, Kane, Briggs, Francescutti, & Kuhn, 2013) and co-morbid behavioral and psychological symptoms of dementia (Torres-Lista, López-Pousa, & Giménez-Llort, 2015).

Interestingly, neophobic and inherent burying often respond to the same pharmacological agents (De Boer & Koolhaas, 2003; Nicolas *et al.*, 2006). All three forms of burying has shown sensitivity for traditional anxiolytics i.e., benzodiazepines (Nicolas *et al.*, 2006), various classes of noradrenergic and serotonergic antidepressants i.e., desipramine, imipramine, citalopram, paroxetine and fluvoxamine (Abe, Nakai, Tabata, Saito, & Egawa, 1998; Broekkamp, Rijk, Joly-Gelouin, & Lloyd, 1986; Millan *et al.*, 2002). Haloperidol (Nicolas *et al.*, 2006) and chlorpromazine (Bruins Slot, Bardin, Auclair, Depoortere, & Newman-Tancredi, 2008) *viz.* traditional antipsychotics, have also been demonstrated to attenuate burying behavior. This being said, the predictive validity of burying behavior as a screening test for anxiety and/or compulsive-like repetition is undermined by contradictory results e.g. response to anxiogenic drugs (Nicolas *et al.*, 2006), and that it demonstrates sensitivity to a number of miscellaneous compounds not related to the treatment of either anxiety or compulsivity (de Almeida, de Carvalho, Silva, de Sousa, & de Freitas, 2014; Honda, Kawaura, Soeda, Shirasaki, & Takahama, 2011; Krass, Rünkorg, Wegener, & Volke, 2010; Uday, Pravinkumar, Manish, & Sudhir, 2007). Moreover, previous pharmacological studies have used mostly acute single-dose treatments ranging from 30 – 90 minutes before recording burying behavior (Nicolas *et al.*, 2006; Uday *et al.*, 2007), this in spite of certain classes of drugs, e.g., antidepressants, being clinically effective in the treatment of anxiety and compulsions only following chronic administration (Fineberg & Craig, 2007; Huh, Goebert, Takeshita, Lu, & Kang, 2011).

For a discussion of the methodology followed in the MB test, refer to chapter 4 (Manuscript B).

2.4.3.2 *Nest-building in mice and rabbits*

In the wild, mice and other rodents build nests for protection against the elements and predators and to provide a nursery to breed and raise young (Smithers, 1983). Under laboratory conditions, mice build nests to shelter from harsh lighting, low ambient temperatures and human handling (Jirkof, 2014). As such, motivation for nesting is high and nest building (NB) behavior is routinely assessed as a measure of general well-being of laboratory-housed mice. Although nesting forms part of the normal behavioral repertoire of mice, between-species differences in nest size, structure and locale is profound (Gaskill *et al.*, 2013). However, NB

can also demonstrate within-species variance, while it has been suggested that large nest building (LNB) behavior, in at least mice and rabbits, may represent compulsiveness (Greene-Schloesser *et al.*, 2011; Hoffman & Rueda Morales, 2009; Li, Morrow, & Witkin, 2006).

Albeit being a *normal* behavioral manifestation in rodents, a previous investigation into the nesting behavior of house mice (*Mus musculus*), demonstrated *aberrant* NB by categorizing animals into BIG and SMALL NB cohorts (Greene-Schloesser *et al.*, 2011). The BIG cohort is regarded as being compulsive while the SMALL cohort is defined as the control group. The authors demonstrated a 40-fold difference between the nest sizes of the BIG and SMALL cohorts, with the behavior of the BIG cohort possibly correlated with the clinical symptoms of hoarding and concerns about security often observed in human OCD. Furthermore, it has also been demonstrated that mice from the BIG cohort bury more marbles than mice from the SMALL cohort, thus constituting a link between NB behavior and MB, another proposed animal model of OCD (see above). These findings provide the basis for the face validity of the model. The authors also provided evidence that the SSRI fluoxetine and the SRI clomipramine, but not the NRI desipramine, attenuated NB behavior of the BIG cohort. Also, NB behavior does not return to baseline values during the four weeks post-treatment, a finding that correlates with studies done in OCD patients that demonstrated the long lasting behavioral effects of chronic SSRI-treatment in patients with OCD following cessation of drug administration (Delgado & Moreno, 1998). Subsequently the model demonstrates good predictive validity. However, the model has to date not been subjected to testing with respect to its construct validity for OCD.

A recent series of studies have investigated nest building behavior in rabbits as a model of OCD (Hoffman & Rueda Morales, 2009; Hoffman & Rueda Morales, 2012). This model is relevant to understanding compulsions related to feelings of incompleteness, "just right" sensations, and the perception of task completion and has provided some interesting evidence in support of its face validity for OCD. However, this model still requires investigation with regard to predictive and construct validity and will not be discussed further at this time.

In summary, NB may be a novel animal model of OCD, providing that the CSTC circuitry and other biological markers associated with OCD can be implicated in its underlying biology. For a detailed discussion of the methodology followed for the assessment of NB, refer to Chapter 5 (Manuscript C).

2.5 A Review of Spontaneous Stereotypy in the Deer Mouse: 1999 – 2014

Since stereotypical behavior in the deer mouse (*Peromyscus maniculatus bairdii*) was first studied in 1999 by Powell and colleagues at the University of Florida (Powell, Newman, Pendergast, & Lewis, 1999), much work has been done to investigate the pathogenesis and underlying pathology of these behaviors. Deer mice exhibit three main topographies of stereotypy when housed under normal laboratory conditions, namely repetitive jumping, pattern running and backward somersaulting (Hadley, Hadley, Ephraim, Yang, & Lewis, 2006; Korff, J. Stein, & H. Harvey, 2008). These three behavioral phenotypes have since been under investigation as putative animal models of stereotypical movement disorder (Hadley *et al.*, 2006; Powell *et al.*, 1999; Presti & Lewis, 2005), and more recently, OCD (Güldenpfennig *et al.*, 2011; Korff, Stein, & Harvey, 2009; Wolmarans, Brand, Stein, & Harvey, 2013). In this section, the history of the model will briefly be reviewed, from its initial development as an animal model of stereotypical movement disorder to the more current investigations concerning OCD. Furthermore, the validation status of the deer mouse model of OCD will be evaluated, focusing on the model's current strengths and shortcomings.

2.5.1 Major sequential developments in the characterization of deer mouse stereotypy

Powell and colleagues was the first group to conceptualize the behavior expressed in deer mice housed in confinement as being abnormal and of a stereotyped nature (Powell *et al.*, 1999). The main purpose at that time was to evaluate the influence of environmental enrichment on the development of stereotypy and therefore animals were not categorized based on the degree of stereotypy they expressed, but rather according to the percentage time each animal spent exhibiting stereotypy. In addition, the authors demonstrated that 62% of the animals housed in laboratory cages developed stereotypy, a number that has changed somewhat during the course of the model's development due to the implementation of newer assessment protocols. Important to note though, is that not all animals develop this behavioral trait, a fact that has not changed over the past years.

At that time, the group did not make use of automated screening and the behavioral categorization was done by means of visual observation (Powell *et al.*, 1999). Due to the time constraints imposed by this method of screening, the authors observed each animal twice weekly for only 5 minutes at a time. Since then the same group has broadened their investigations with the purpose of developing and validating an animal model of stereotypical movement disorder (Presti & Lewis, 2005).

In 2004 the same group introduced automated screening which opened up the possibility of evaluating the behavior over longer periods of time. This resulted in the first quantitative assessment of stereotypy scores with the measurement unit being expressed as counts per hour (Presti, Watson, Kennedy, Yang, & Lewis, 2004). The authors classified the animals into one of two cohorts, viz. low-stereotypical (L, less than 300 vertical beam interruptions per hour), and high-stereotypical (H, more than 1000 vertical beam interruptions per hour) based on the mean stereotypy score calculated following behavioral screening during an eighteen hour-long session over two days (Presti & Lewis, 2005). Although this classification system excluded animals that executed 300 to 1000 vertical movements per hour, the authors intended to determine whether any differences in the striatal opioid content between the L and H cohorts could be demonstrated. It was therefore crucial to exclude the 'grey' middle population of animals from the group, a rationale that was exploited in the current study as well. In addition, due to the practical constraints imposed by the methodology of striatal microdialysis, the pattern running and backward somersaulting topographies were excluded from the study as these types of behavior interfered with the normal functioning of the microdialysis equipment.

In 2005, the validation of deer mouse stereotypy as a putative animal model of OCD was initiated, with Korff and colleagues (2008) being the first group to comprehensively describe the face and predictive validity of deer mouse stereotypy as a putative animal model of OCD. As pattern running and backward somersaulting were included in this study, the authors realized that the original classification system developed by Presti and colleagues was insufficient to meet the purposes of the study at that time. The main reason for this was that backward somersaulting resulted in more beams being broken per stereotypical movement than was the case with vertical jumping. Subsequently the authors re-classified the behavior expressed by deer mice into three cohorts, viz. non-stereotypical behavior (N, less than 1000 beam interruptions per hour), low-stereotypical behavior (L, 1000 – 2000 beam interruptions per hour), and high-stereotypical behavior (H, more than 2000 beam interruptions per hour) (Korff *et al.*, 2008). Important to note is that during this study, as well as two subsequent studies from this group (Güldenpfennig *et al.*, 2011; Korff *et al.*, 2009), the animals were classified into either one of the cohorts using a mean stereotypy score calculated from the data obtained during three individual one-hour long behavioral screening sessions, each one week apart to exclude the influence of handling stress. With the acquisition of more advanced tracking apparatus and software, this methodology as well as the classification criteria was

reappraised to provide for an accurate categorization process that more appropriately resembles the clinical diagnosis of OCD (American Psychiatric Association, 2013; Wolmarans *et al.*, 2013).

2.5.2 The current validity of the deer mouse model of OCD

In the first article published on deer mouse stereotypy and its relevance to OCD, Korff and colleagues (2008) demonstrated that deer mouse stereotypy is heterogeneous within a given population of animals. As such, 45% of the animals were classified as H, 41% as L, and 16% as N. Without elaborating on the importance of the diversity of stereotypy expressed, the authors compared the differences in symptom severity in human OCD to the heterogenic distribution of the stereotypy scores of deer mice. Indeed, it must be stressed that these behaviors develop naturally and that a number of deer mice do not exhibit stereotypy at all. In a follow-up investigation, 12-hour screening during the dark cycle was implemented and demonstrated the expression of stereotypy by individual animals to be time-consuming and varying in nature (Wolmarans *et al.*, 2013). Although the N cohort of animals is small compared to the cohorts exhibiting either high or low stereotypical behavior, it can be postulated that a genetic basis for the expression of stereotypy may exist and that the proposed influences of confinement stress (Langen *et al.*, 2011) and the restricting role of the environment on normal behavioral motor patterns (Ridley, 1994) may only be triggering factors for the development of stereotypy and not necessarily the etiological origins thereof.

Although Powell presented evidence for an ameliorative effect of environmental enrichment on the expression of deer mouse stereotypy (Powell *et al.*, 1999), which is in line with the hypothesis of Ridley (1994), animals in both standard and enriched cages did however develop stereotypical behavior. The difference was that a smaller percentage of animals in enriched cages expressed stereotypy, also developing it at a later stage and displaying mostly pattern running instead of vertical jumping or somersaulting (Powell *et al.*, 1999). In addition, the average number of stereotypical movements expressed in animals housed in enriched cages was less than the amounts expressed in their fellow subjects housed in the standard cages. These findings were replicated in a study done by Hadley and colleagues (2006). This observation, combined with the evidence that a proportion of animals do not develop any form of stereotypy, would suggest that the environment these animals are kept in is not the determining factor in the expression of stereotypy and that it may only play a modulatory role in the extent to which stereotypy is exhibited. As such, the face validity of the model is based on the demonstration of time consuming (Wolmarans *et al.*, 2013) rigid repetition of certain

motor patterns of varying intensity and of a heterogenic nature across a given population and that it is not entirely dependent on environment, but may have a genetic basis. In addition, the demonstration that deer mouse stereotypy cannot be prevented by environmental enrichment, but only alleviated to a certain extent, is in line with the hypothesis that compulsions can be distinguished from rigid motor patterns on the basis of thoughtfulness and flexibility (Eilam *et al.*, 2006). Although this behavior does not disappear in total, it can be influenced by environmental factors and thus can be regarded as flexible. This hypothesis further strengthens the face validity of the deer mouse model for OCD.

* * *

Evidence in support of the predictive validity of the model has also been well-established. Korff and colleagues (2008) demonstrated that chronic high dose treatment with the SSRI fluoxetine, but not the NRI desipramine, decreased the amount of stereotypy exhibited by H and L animals without affecting general locomotor activity. As chronic and high dose treatment with the SSRIs is the mainstay of treatment for human OCD, these findings contribute positively to the predictive validation of deer mouse stereotypy as an animal model of OCD. Furthermore, it has later been demonstrated that stereotypy is sensitive to chronic but not sub-chronic treatment with an SSRI (Wolmarans *et al.*, 2013). Based on the hypothesis that a bias in favor of the direct pathway through the basal ganglia over that of the indirect pathway is central to the pathology of OCD, Korff and colleagues (2008) also challenged deer mice with sub-acute quinpirole at a dosage of 5 mg/kg/day. Quinpirole, a non-selective $D_{2/3}$ agonist, should theoretically have inactivated the indirect pathway, leading to an exacerbation of stereotypy. Indeed, Szechtman and colleagues (1998) used quinpirole to induce what they termed 'compulsive checking' in rats, another putative animal model of OCD (see paragraph 2.4.1). However, in the Korff study, quinpirole attenuated the spontaneous stereotypy exhibited by deer mice. Given the number of conflicting reports regarding the effects of dopamine releasers like amphetamine and selective dopaminergic agents in the treatment of OCD (Denys, van der Wee, Janssen, De Geus, & Westenberg, 2004), the fact that quinpirole decreases the stereotypical behavior in deer mice implicates a role for dopamine in the pathology of deer mouse stereotypy and thus strengthens the construct of the model, albeit only with regards to the neurotransmitters involved. If one attempts to draw a parallel to human OCD, in contrast to what has been proposed with respect to dopamine's role in OCD, the Korff study suggests that at least the stereotypy component of the OCD spectrum of symptoms may be related to reduced dopaminergic activity within certain CSTC circuits. It should

however be reiterated that various studies have failed to demonstrate a hyperdopaminergic state in OCD (Brambilla, Perna, Bussi, & Bellodi, 2000; Pitchot, 1996). Further, while amphetamine-like drugs (dopamine releasers) are known to exacerbate obsessive-compulsive symptoms, dopamine agonists may also improve symptoms in OCD patients (Denys *et al.*, 2004). Moreover, while dopamine agonists may precipitate compulsive/stereotypic behavior in a non-pathological (induced) animal model such as the quinpirole model described by Szechtman and colleagues, they seem to suppress these behaviors in spontaneous stereotypy models (Korff *et al.*, 2008; Vandeboeck & Ödberg, 1997). These data concur that further study is not only necessary to delineate the role of dopamine in stereotypy as it pertains to OCD, but also to better understand the underlying neurobiology of induced versus natural stereotypy.

Korff and colleagues (2008) also found that the administration of m-chlorophenylpiperazine (mCPP), a non-selective 5HT_{1A/2A/2B/2C} agonist, decreased the degree of stereotypy expressed in deer mice. This is in line with the hypothesis that the stimulation of 5HT_{2C} receptors may be an important mediator of the suppressing effects of serotonin on reward-seeking behavior (Millan, Dekeyne, & Gobert, 1998). This mechanism is postulated to be central to at least some forms of OCD. However, conflicting clinical reports have been published regarding the effects of mCPP administration in patients with OCD, with some authors reporting an exacerbation of symptoms (Hollander *et al.*, 1991), while others failed to demonstrate the same (Khanna, John, & Lakshmi Reddy, 2001). As mCPP decreased the amount of stereotypy expressed in deer mice, a role for the 5HT_{1A/2C} receptors is implicated in the pathology of deer mouse stereotypy. Interestingly, mCPP also suppresses checking behavior in the quinpirole-model of OCD (Tucci *et al.*, 2013; Tucci *et al.*, 2015), thus strengthening the finding in deer mice. The finding emphasizes that further study is needed in order to better understand the regulatory role of serotonin in deer mouse stereotypy, especially with regard to receptor involvement. The crosstalk between serotonin, dopamine, glutamate, and GABA in the CSTC circuitry is complex. The manner in which stereotypy develops may shed light on the functioning of the CSTC circuitry and aid in explaining the conflicting results reported concerning dopaminergic and serotonergic drug challenges and their effect on stereotypy.

* * *

As dopamine and serotonin have been implicated in the symptomology of deer mouse stereotypy (Korff *et al.*, 2008), the validity of the model is strongly founded on this construct. It has been demonstrated that the amount of stereotypy expressed in deer mice is positively

correlated to frontal-cortical, but not striatal, cAMP levels and inversely related to PDE4-enzyme activity in the frontal cortex but not striatum (Korff *et al.*, 2009). The select involvement of the frontal cortex strongly supports a frontal cortical lesion in OCD (Evans *et al.*, 2004; Husted *et al.*, 2006; Markarian *et al.*, 2010), thus satisfying an important construct for OCD. The authors proposed that, given that an earlier study (Korff *et al.*, 2008) demonstrated reduced stereotypy in H mice following administration of a non-selective 5HT_{1A} agonist, increased stereotypy would be characterized by reduced 5HT_{1A} G_i dependent adenylate cyclase coupling and increased levels of cAMP. In both brain regions, the authors also found a significant inverse correlation between PDE₄ activity and stereotypic behavior. Since PDE₄ selectively hydrolyses cAMP, this negative correlation indicates that the increase in cAMP observed as a function of stereotypy in H mice is related to increased 5HT_{1A}-adenylate cyclase-cAMP signaling and reduced hydrolysis by PDE₄ (Korff *et al.*, 2009). Perseverative locomotor paths have been associated with the stimulation of 5HT_{1B/1D} receptors (Shanahan, Velez, Masten, & Dulawa, 2011) and the desensitization of these receptors is thought to mediate some of the ameliorative effects of the SSRIs on OCD symptomology (Blier, El Mansari, Ducharme, & Bouchard, 1996). As the desensitization of frontal-cortical 5HT_{1B/1D} auto-receptors results in the increased release of frontal-cortical serotonin (which in turn can be associated with the attenuation of stereotypy), the assumption would be that the frontal-cortical cAMP levels in H animals, should actually be lower than in the N cohort, which is not the case. The opposite observation in deer mice on the one hand can be ascribed to the use of mCPP, a relatively non-selective 5HT agonist, or due to state-dependent differences in the animal models used, such as natural versus induced stereotypy models. In line with the hypothesis implicating decreased serotonergic signaling in OCD, Wolmarans *et al.* demonstrated decreased striatal SERT density in H, but not N animals (Wolmarans *et al.*, 2013).

Studies confirming the involvement of the direct and indirect pathways in the development of deer mouse stereotypy (Presti & Lewis, 2005), together with data implicating the frontal cortex and the striatum in the pathology of these behaviors strengthens the construct validity of the model.

Recent clinical studies have revealed the presence of oxidative stress in patients with OCD (Behl, Swami, Sircar, Bhatia, & Banerjee, 2010; Chakraborty *et al.*, 2009; Selek *et al.*, 2008), as well as the therapeutic benefits of add-on treatment with the glutathione precursor, N-acetyl

cysteine (NAC) (Camfield, Sarris, & Berk, 2011). Deer mice also show disturbed redox balance in frontal cortical circuits while these deficits are correlated with severity of stereotypy (Güldenpfennig *et al.*, 2011).

In summary, deer mouse stereotypy presents with sufficient face, predictive and construct validity to constitute a solid foundation for its further development as an animal model of OCD. Nevertheless, the face validity of the model can be strengthened by studying the association of high stereotypical behavior with compulsive-like traits in the animal, such as marble-burying and nest building. Furthermore, the predictive validity of the model can be taken further by assessing the effects of augmenting SSRI treatment with low-dose antipsychotics in animals that are refractory to SSRI monotherapy, and demonstrating that acute administration of the SSRIs does not exert any ameliorative effect in H mice. The administration of selective 5HT_{2C} agonists as monotherapy and 5HT_{1A/B/D} antagonists in combination with the SSRIs may also produce valuable results. Since the pathogenesis of OCD remains a topic of much debate, specifically with respect to the brain regions and neuronal systems and neurotransmitters implicated in OCD, the construct validity of the model is at this point relatively robust. However, studies aimed at strengthening this aspect of the model by confirming a role for a dysfunctional reward-based learning system in H deer mice would be very valuable. Such a demonstration would be in line with the hypothesis that reward may play a role in the symptomatology of OCD and correlate with findings from the signal-attenuation model of Joel & Avisar (2001).

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2.6 Conclusion to Chapter 2

Like many other psychiatric conditions, OCD remains an enigma. In the clinical environment the diagnosis of the condition is challenged by many difficulties, including the high prevalence of comorbid disorders (LaSalle *et al.*, 2004; Nestadt *et al.*, 2001) and the variation in obsessive-compulsive symptomatology (Abramovitch & Cooperman, 2015). The etiological and pathological foundations of the condition have also not yet been fully elucidated (Fineberg & Craig, 2007). However, it is clear that functional abnormalities in the CSTC circuitry are central to the symptomatology of OCD (Markarian *et al.*, 2010) and that most patients respond selectively to treatment with SSRIs (Vythilingum *et al.*, 2000). Thus, the demonstration of CSTC involvement and a selective response to chronic, high dose SSRIs, at least in 70% of animal subjects,

would constitute a solid foundation for the development of an animal model of OCD. However, these cannot be the only strengths of an animal model of OCD, as many psychiatric conditions share the same characteristics. OCD is also characterized by the rigid repetition of certain motor patterns that can be distinguished from simple motor repetition on the basis of thoughtfulness and flexibility (Eilam *et al.*, 2006). Another aim in the development of a model for OCD would thus be to investigate the origins and the characteristics of the different forms of motor stereotypy expressed in these animals.

In the following chapters, the current study will be presented based on the study questions raised in chapter 1, and includes manuscripts on social behavior (chapter 3), MB behavior (chapter 4), NB behavior (chapter 5) and drug response (chapters 3 – 5).

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3 Manuscript A

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- *De Wet Wolmarans* designed the investigation in consultation with *Brian H Harvey*, performed all behavioral, pharmacological, and statistical analyses, wrote the first version of the manuscript, and edited the manuscript following input from the co-authors.
- *Dan J Stein* acted as clinical consultant with respect to experimental design and manuscript review.
- *Brian H Harvey* was study supervisor, assisted in the interpretation of results and was corresponding author in the submission of the final manuscript to *Social Neuroscience*.

IMPORTANT INFORMATION

- Instructions to the author are included in Addendum A.
- The published article is provided in PDF format in Addendum E.
- As per the instructions to the author, figures, tables, and legends are provided at the end of the manuscript.
- Both co-authors provided consent for the paper to be assessed as part of the Ph.D. thesis of *De Wet Wolmarans* (Addendum B).
- The submitted manuscript has been received by *Social Neuroscience* and is currently under review (see Addendum C for proof of submission).

Social behavior in deer mice as a novel interactive paradigm of relevance for obsessive-compulsive disorder (OCD)

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Abstract

Greater obsessive-compulsive (OC) symptom severity may be associated with poor social adjustment. Rather than possessing deficits in social skill *per se*, OCD patients may be more socially isolative in the presence of normal controls. We aimed to apply a novel social interaction challenge (SIC) to an established animal model of OCD, *viz.* the deer mouse, to assess complex social behavior in animals by investigating group sociability and its response to chronic escitalopram treatment (50mg/kg/day x 28 days), both within and between non (N)- (*viz.* normal) and high (H)- (*viz.* OCD-like) stereotypical cohorts. Using automated screening, we scored approach behavior, episodes of proximity, duration of proximity and relative net weighted movement. H animals socialized more with one another within cohort in all of the above parameters compared to the within cohort behavior of N animals. Furthermore, the social behavior of H animals towards one another, both within and between cohort demonstrated significant improvements following chronic escitalopram treatment. However, the study also demonstrates that the social interaction between H and N animals remain poor even after chronic escitalopram treatment. To conclude, findings from the current investigation support clinical data demonstrating altered sociability in patients with OCD.

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Introduction

Obsessive-compulsive disorder (OCD) has a lifetime prevalence of 1 – 3% (Stein, 2002) and is characterized by recurrent and intrusive thoughts (obsessions) and rigid repetition of certain motor actions (compulsions) (American Psychiatric Association, 2013). According to DSM-V, OCD is classified under the OC spectrum (American Psychiatric Association, 2013).

OCD is often comorbid with social anxiety disorder (SAD) (Kim, Reynolds, & Alfano, 2012) or poor social adjustment (Rosa et al., 2012). Adults with OCD report higher rates of unemployment, marital discord and financial instability (Kim et al., 2012), while children display an impaired ability for making and keeping friends (Kim et al., 2012; Piacentini, Bergman, Keller, & McCracken, 2003). Both social phobia and OCD follow a course of varying symptom intensity, are characterized by severe occupational infringement and respond preferentially to selective serotonin reuptake inhibitors (SSRI) (Baldwin, Brandish, & Meron, 2008; Lochner et al., 2003; Niederauer, Braga, De Souza, Meyer, & Volpato Cordioli, 2007), while comorbid OCD and social impairment demonstrate greater OC symptom severity and treatment resistance (Alarcon, Libb, & Spitzler, 1993; Khanna, Rajendra, & Channabasavanna, 1988) with a poor treatment outcome (Stewart et al., 2010). Finally, increased OC symptom severity may worsen social impairment and vice versa (Rosa et al., 2012). Although youth with OCD have fewer friendships and struggle to maintain these relationships, they do not perceive *themselves* as different from normal controls (Piacentini et al., 2003). Instead of being unable to socialize per se, they may only be more socially isolative in scenarios where normal peers may observe their behavior (Piacentini et al., 2003).

Spontaneous stereotypy in the deer mouse (*Peromyscus maniculatus bairdii*) is a naturalistic animal model of OCD that is founded on robust face, construct and predictive validity (Albelda & Joel, 2012; Joel, 2006; Joel, J. Stein, & Schreiber, 2008; Wang, Simpson, & Dulawa,

2009). Deer mouse stereotypy can generally be categorized into repetitive jumping and pattern running (Korff, J. Stein, & H. Harvey, 2008; Powell, Newman, Pendergast, & Lewis, 1999), behaviors that correspond with the repetitive and compulsive-like motor actions of OCD. As is true for stereotypy, compulsivity is an inappropriate and persistent behavior with no apparent goal (Bartz & Hollander, 2006). Thus, the deer mouse demonstrates varying symptom intensity as is the case in OCD (Korff et al., 2008; Wolmarans, Brand, Stein, & Harvey, 2013). As in the case of human OCD (El Mansari & Blier, 2006; Fineberg, 2004), chronic but not sub-chronic SSRI treatment decreases stereotypic behaviors while it also replaces high-stereotypical bouts with periods of normal rodent activity (Wolmarans et al., 2013). High stereotypic deer mice also present with decreased striatal serotonin transporter (SERT) density (Wolmarans et al., 2013), the latter also described in OCD (Atmaca et al., 2011; Reimold et al., 2007; Zitterl et al., 2008).

Given that the complex nature of social relationships between OCD patients and normal peers is relatively under-studied, and that it is uncertain how well these social impairments resolve following treatment, the *primary aim* of the current investigation was to apply the deer mouse model of OCD to assess the social behavior of N- (*viz.* healthy controls) and H- (*viz.* OCD-like) stereotypical deer mice. The deer mouse presents with unique species specific challenges relating to behavioral analysis that complicates experimental design, e.g. their high stereotypical nature complicates behavioral assessment of anxiety in the elevated plus maze (EPM) and open field test (OFT). Similarly, the standard social interaction challenge (SIC) for rodents (Duxon, Kennett, Lightowler, Blackburn, & Fone, 1997; Jenkins, Harte, McKibben, Elliott, & Reynolds, 2008; Kask, Nguyen, Pabst, & Von Hörsten, 2001; Möller, Du Preez, Emsley, & Harvey, 2011) is likewise complex. While the SIC is most often performed with two sex- and weight matched animals from identical behavioral and treatment backgrounds (Duxon et al., 1997; Jenkins et al., 2008; Kask et al., 2001; Möller et al., 2011; Scattoni, Martire, Cartocci, Ferrante, & Ricceri, 2013; Willey, Varlinskaya, & Spear, 2009; Wongwitdecha & Marsden, 1996), a new SIC paradigm will be developed to closely simulate the social interaction between OCD patients and normal peers.

Drawing from clinical literature relating to social behavior in patients with OCD and taking the high stereotypical nature of these animals into account, we hypothesized that 1) the behavior of animals from both the N- and H-cohorts will differ depending on the cohort structure of the group, 2) chronic escitalopram treatment will significantly influence the behavior

of H animals toward one another and toward N controls, and 3) different criteria than those published previously for evaluating the social behavior of rodents need to be applied to attain there aforementioned objectives. The *secondary aim* was to develop a novel SIC that would enable investigation of *group* social behavior of N- and H-animals both within and between cohorts, and how such behavior responds to the typical pharmacotherapy regime used in OCD, viz. chronic SSRI treatment. To this end, performing a SIC between three, and not two animals in different combinations of cohorts, will be applied to study the social dynamics between animals of different behavioral cohorts and how the presence of an animal from either a H or N cohort will affect the behavior of an animal from a different stereotypical cohort. Employing a pharmacotherapy arm (escitalopram 50mg/kg/day x 28 days) will reveal how treatment alters social competence of H deer mice as well as the behavioral cohort structure of the group. As far as we are aware, this is the first study in a translational animal model of OCD that has assessed the complicated nature of group social interaction between animals of different stereotypical classifications.

Materials and Methods

Animals

70 deer mice were obtained from the deer mouse colony maintained and housed at the vivarium of North-West University (NWU), Potchefstroom, South Africa (Ethical Approval Number - NWU-00066-10-S5). The original colony was established using breeding pairs obtained from the Peromyscus Genetic Stock Centre at the University of South Carolina. As reported previously (Korff et al., 2008) stereotypical behavior in the deer mouse is established at the age of 8 weeks and only mice between the ages of 10 and 12 weeks were chosen as experimental subjects at the onset of behavioral assessments (day 0). Mice were randomly chosen from different litters, without sex or weight bias and housed in groups of 6 same-sex animals per cage. Earlier studies have already noted that the severity of stereotypy in deer mice is independent of sex (Hadley, Hadley, Ephraim, Yang, & Lewis, 2006; Powell et al., 1999; Presti, Watson, Kennedy, Yang, & Lewis, 2004; Wolmarans et al., 2013). One week before the start of the behavioral assessments, each animal was allocated individually to an automatic climate-controlled laboratory cage (35cm (l) x 20cm (w) x 13cm (h); Techniplast® S.P.A., Varese, Italy) and maintained at 23°C on a 12-hour light/dark cycle (lights on at 06h00 and off at

18h00). Food and water were provided ad lib for the duration of the study. Cages were cleaned and new bedding material added weekly.

Initial classification of N and H animals

Before performing the SIC, animals were categorized as N or H according to a previously published protocol (Wolmarans et al., 2013). In short, all animals underwent 12-hour behavioral screening once a week for five consecutive screens. On any specific assessment day, animals were moved from their housing environment to the behavioral screening room. These areas are located on the same floor of the vivarium and are environmentally controlled with respect to temperature (23°C), relative humidity (55%), and light cycle (same as for home cages). Subsequently each animal was introduced to a behavioral test cage (21cm (w) x 21cm (l) x 35cm (h); Accuscan® Inc., Columbus, Ohio, USA) constructed from clear, translucent Plexiglas®. Bedding material was provided in quantities enough to cover the floor of the test cages, but also ensuring that it did not interrupt the scoring of behavioral data. Food was provided ad lib on the floor of the cage in the form of broken-up rodent chow pellets. Bottled water was provided through a tight-fit hole in the wall of each test cage. Cages were covered with lids that allowed uninterrupted airflow. The animals were introduced to these environments by 16h00 and habituated for at least two hours before the 12-hour behavioral assessments started at the onset of the dark cycle. Test cages were cleaned after each screen.

Behavioral assessment was performed using the Fusion® Animal Activity Monitoring System (Accuscan® Inc., Columbus, Ohio, USA), and analyzed using Fusion® software. Briefly, each testing cage presents with a grid of infra-red light beams that cross the cage roughly 2 cm above the cage floor along the X- and Y-axes (constituting a grid), while another set of beams (Z-axis) crosses the cage 10 cm above the cage floor, but only parallel to the lower Y-axis. Parallel beams are spaced 2.5 cm apart and record activity every time it is interrupted by movement. The number of vertical beam interruptions (VBI) was used as a broad measure of vertical jumping activity, while the number of clockwise and anti-clockwise cage revolutions (CR) was used to evaluate the expression of pattern running (Wolmarans et al., 2013). During the recording session, the Fusion® software generates data continuously, allowing for experimental playback and the export of behavioral reports the following day.

Following the first five behavioral screens, animals were divided into N and H groups according to our previously published protocol (Wolmarans et al., 2013). As findings from previous

studies (Wolmarans et al., 2013) demonstrated the time-consuming nature of stereotypy, we amended the protocol to include both the intensity of stereotypical bouts (average of the highest individual 30-minute stereotypy counts generated during each of the five respective trials) and the time spent executing stereotypy (number of 30-minute H stereotypical bouts generated over all five trials) as indicators of behavioral severity. The published criteria for defining vertical and horizontal H intervals are represented in Table 1. This data was plotted for both vertical (Figure 1A) and horizontal activity (cage revolutions; Figure 1B) and, as no distinction was made between the different forms of stereotypy, H animals could demonstrate stereotypical behavior in either one or both of the behavioral topographies. We subsequently included 18 animals in the H group (Figures 1A and 1B combined; subjects indicated with sex symbols) that expressed both the highest intensity of stereotypy for the longest cumulative duration of time, irrespective of whether an individual did so in only one or both of the topographies (the sum total of sex symbols from Figures 1A and 1B, i.e. 25, exceeds 18 as more than one animal displayed H behavior in both topographies). The group of N animals was simply constituted from the 18 animals that consistently generated the lowest stereotypy counts (Figures 1A and 1B combined; black dots plotted at $X = 0$).

As only 18 animals were included in each cohort following the first five behavioral screens, a grey margin of 34 animals was excluded from the experimental design. The rationale for the exclusion of a grey margin of animals is to improve sensitivity, as described elsewhere (Wolmarans et al., 2013).

The Social Interaction Challenge (SIC)

Before the SIC (performed on day 0), all animals were habituated individually to the test environment three times for 10 minutes during the dark cycle from day -3 to day -1. Briefly, the SIC was performed during the dark cycle under dim white light (40 lux) in black Plexiglas® cages (50cm x 50cm) between 19:00 and 24:00. On the night of testing, three same-sex animals were color marked (color stickers attached on the back of the animal) and simultaneously introduced to the testing cages to prevent possible resident-intruder interactions. Animals were combined in groups consisting of HHH, NNN, HHN and NNH animals, respectively (Table 2). All animals were naive with respect to social contact and each animal was only used once (thus 18 N and H animals, respectively). The SIC was performed three times before the onset of treatment (pre-treatment control) and again for three times following

four weeks of escitalopram treatment with the same animals. However, individuals introduced to the cages for the post-treatment SIC were combined differently than during the pre-treatment SIC to exclude the possible influence of familiarization. Therefore, each animal had contact with another animal only once over the course of both the pre- and post-treatment challenges.

Animals were left to socialize for 5 minutes and the number of approaches (interaction distance set as $\leq 15\text{cm}$), episodes of proximity (set as $\leq 5\text{cm}$), cumulative duration of proximity and net weighted movement with respect to one another was calculated using Ethovision XT II software (Noldus Information Technology BV, Wageningen, Netherlands;) (Table 3). Net weighted movement is an objective measure of the intensity of approach and avoidance behavior (Spruijt, Hol, & Rousseau, 1992), allowing the movement of subjects toward (*viz.* more positive values; approach behavior) or away (*viz.* more negative values; avoidance behavior) from each other to be calculated, regardless of the direction of movement. Video files (not shown) were scrutinized and cross-analyzed with the behavioral data to verify the presence of social and not aggressive behavior. Although controversy remains as to the exact nature, pathology and clinical relevance of rodent aggression (Blanchard, Wall, & Blanchard, 2003), we excluded the presence of aggressive behavior (biting, scratching, boxing, and wrestling (Kalinine et al., 2014; Lewis, Gariépy, Gendreau, Nichols, & Mailman, 1994)), through careful scrutiny of the video files.

For more information regarding Ethovision XT® II software, please visit <http://www.noldus.com>.

Drug and administration

As published previously (Wolmarans et al., 2013) escitalopram oxalate was prepared for oral administration by dissolving in the drinking water. Physical handling of the mice was kept to a minimum to prevent any possible anxiogenic effects. For the same reason, oral gavage was not considered as an appropriate dosage route.

Following the five screens and pre-treatment all animals only received tap water, with escitalopram administered orally for four weeks in the drinking water. The final concentration of the drinking solution was constituted at 20mg/100ml ensuring that each animal would receive 50 mg/kg/day (within close limits) of escitalopram. The dose calculation was based on a prior

pilot study where the average water intake per mouse was determined as 0.25 ml/g/day (data not shown), and confirmed by others.

Statistics

Due to social behavior of animals demonstrating notable variance, data are not distributed normally (Shapiro-Wilk tests have been run for each data set. Significance was $p < 0.05$ for all sets). As such, Mann-Whitney analysis was applied to comparisons of the between-group treatment-naïve social behavior of H and N deer mice (Figure 2) and the within-group responses to treatment (Figures 3i and 4i). To compare the treatment-naïve social behavior of N and H animals in mixed paradigms with the behavior of N and H animals in the NNN and HHH control groups respectively (Figure 2), Kruskal-Wallis ANOVA was applied. Furthermore, Kruskal-Wallis ANOVA was also applied to compare the statistical significance of endpoint differences in the average response to treatment (Figures 3i and 4i) and the percentage change from baseline (Figures 3ii and 4ii) in the behavior of N and H animals in mixed paradigms, with the NNN and HHH groups as respective controls. Statistical significance was set at $p < 0.05$. All statistical analysis was followed by Cohen's d calculations to establish the practical significance of effect magnitude. Cohen's d is an effect size used to indicate the standardised difference between two means. Effect sizes are considered large at values between 0.8 and 1.29 and very large at values higher than 1.3. Statistical analysis was performed with IBM® SPSS® Statistics 22.0 and GraphPad Prism® 6.01 software under the guidance of the statistical consultation service of North-West University, Potchefstroom.

Results

Considering that no form of aggressive behavior was observed during scrutiny of video files (not shown), the results of the SIC will be discussed under three headings viz. Figure 2, Figure 3, and Figure 4. For a detailed description of the different groups and the interactions measured, see Table 2. Table 3 provides a description of the various parameters measured. Figure 2 presents findings regarding the social behavior of treatment-naïve N and H animals while Figures 3i and 3ii presents comparisons between the pre- and post-treatment manifestation of social behavior of N animals. Figures 4i and 4ii presents the same comparisons as Figure 3, but with respect to social behavior of H animals. Descriptive statistics for all analyses are provided in Tables 4 – 6.

Figure 2 (Tables 4 and 5) – Baseline social behavior of N and H animals

Animals in the HHH group demonstrated significantly higher treatment-naïve approaches (**2A**, 18.5 ± 3.1 , $**p = 0.009$) as well as episodes of proximity between one another (**2B**, 16.2 ± 3.3 , $*p = 0.04$), both characterized by large effect sizes ($^{\#}d = 0.95$ and $^{\#}d = 1.0$ respectively), when compared to the behavior of animals in the NNN group (**2A**, 8.5 ± 1.8 and **2B**, 7.7 ± 2.4 respectively). These data are validated by the trends toward increased duration of proximity (**2C**, 9.9 ± 2.7 , $^{\#}d = 0.92$) and net weighted movement (**2D**, 4.0 ± 1.4 , $^{\#}d = 1.03$) observed between animals in the HHH group compared to that of the NNN group (**2C**, 4.3 ± 1.3 and **2D**, 0.5 ± 0.4 respectively), indicating that H subjects approached, rather than avoided one another throughout the 5 minute challenge.

Although H animals in the HHN group demonstrated a trend towards an increased number of episodes spent together in the presence of an N animal (**2B**, HH[N]; 12.0 ± 2.6 , $^{\#}d = 1.2$) compared to the same behavior of N animals in the presence of an H animal (**2B**, NN[H]; 5.0 ± 4.0), this was not demonstrated in any of the other parameters (**2A**, **C** and **D**; HH[N] vs. NN[H]). However, in contrast to the behavior of N animals toward an H animal (Figure 2A – D, [NN]H), large (**2A** and **B**) and very large (**2C**) effect sizes were observed with respect to the behavior of H animals toward an N animal ([HH]N) vs. which [NN]H (**2A**: 16.7 ± 7.0 vs. 4.3 ± 1.7 , $^{\#}d = 1.15$; **2B**: 13.7 ± 4.1 vs. 5.2 ± 2.0 , $^{\#}d = 1.13$; **2C**: 8.5 ± 2.9 vs. 2.2 ± 1.0 , $^{\#\#}d = 1.32$).

Considering comparisons of the behavior of N and H animals in mixed groups (viz. NNH and HHN) with their behavior toward one another in the respective NNN and HHH control groups (Table 5), N animals demonstrated indistinguishable social behavior toward one another (NN[H]) and toward an H animal ([NN]H) (Figure 2A – D). However, although no statistical significance could be established, large negative effect sizes were observed in the behavior of H animals in the mixed group toward one another with respect to duration of proximity (**2C**, HH[N], 5.5 ± 1.2 , $^{\ast}d = -0.86$) and toward an N animal with respect to net weighted movement (**2D**, [HH]N, 0.035 ± 1.61 , $^{\ast}d = -1.03$) compared to the behavior of H animals in the HHH group (**2C**, 9.9 ± 2.7 and **2D**, 3.4 ± 1.4 respectively).

Figure 3 (Table 6) – Pre- and post-treatment social behavior of N animals

Chronic treatment with escitalopram significantly increased the number of approaches with a large effect size between animals in the NNN grouping (**3i A**, 22.0 ± 4.3 vs. 8.5 ± 1.8 , $^{\#}d = 1.03$) but failed to influence either episodes and duration of proximity or net weighted movement within the same group (**3i B – D**). Furthermore, although the behavior of N animals toward one another in a mixed group remained unchanged, (Figure 3i A – D, NN[H]), N animals demonstrated trends with large effect sizes toward increased approach behavior and duration of proximity with respect to social interaction with H animals (**3i A**, [NN]H, 14.7 ± 5.7 vs. 4.3 ± 1.7 , $^{\#}d = 1.15$ and **3i C**, [NN]H, 5.7 ± 2.1 vs. 2.1 ± 0.9 , $^{\#}d = 0.9$) following treatment. Interestingly, Cohen's d calculations revealed post-treatment differences with large negative effect sizes with respect to the number of approaches towards one another (**3i A**, NN[H], 9.0 ± 1.6 , $^{\ast}d = -1.16$) and duration of proximity between one another (**3i C**, NN[H], 2.7 ± 0.7 , $^{\ast}d = -1.23$) in the behavior of N animals in the NNH group, compared to animals in the NNN group (**3i A**, 22 ± 4.3 and **3i C**, 9.0 ± 3.0 respectively). However, taking the net weighted movement into account, a trend towards a more positive post-treatment value was observed in the behavior of N animals toward one another in the NNH group, compared to the NNN controls (**3i D**, NN[H], 8.6 ± 7.4 vs. 0.2 ± 0.5 , $^{\ast}d = 0.82$).

Considering the percentage change from average baseline with respect to the number of approaches, episodes of proximity and duration of proximity, chronic escitalopram treatment had minimal effect on magnitudes of change, with the exception being a noteworthy increase in the episodes of proximity between N animals in the NNH group compared to the behavior of animals in the NNN control group (Figure **3ii B**, NN[H], 233 ± 120.2 vs. 50 ± 38.6 , $^{\ast}d = 1.13$).

Figure 4 (Table 6) – Pre- and post-treatment social behavior of H animals

Chronic escitalopram induced meaningful changes in the behavior of H animals in the HHH grouping, with a significant increase demonstrating a very large effect size in the number of approaches observed (**4i A**, 49.9 ± 4.6 vs. 18.50 ± 3.1 , $**** p < 0.0001$, $^{\#\#}d = 1.92$). Furthermore, albeit narrowly missing statistical significance, H animals within the HHH group also demonstrated trends with large effect sizes toward increased duration of proximity (**4i C**, 17.6 ± 2.4 vs. 9.9 ± 2.7 , $p = 0.06$, $^{\#}d = 1.0$) and net weighted movement (**4i D**, 15.0 ± 4.3 vs. 4.0 ± 1.4 ; $p = 0.18$, $^{\#}d = 0.91$) following treatment. Moreover, escitalopram treatment tended

to increase the number of approaches and duration of proximity between H animals in the presence of an N animal (HH[N]; **4i A**, 38.8 ± 10.9 vs. 13.2 ± 1.2 , $^{##}d = 1.62$ and **4i C**, 13.7 ± 5.7 vs. 5.5 ± 1.2 , $^{##}d = 1.38$). However, comparisons between the post-treatment behavior of H animals toward one another in the HHH and HHN groups respectively, revealed noteworthy differences displaying large negative effect sizes with respect to the number of episodes of proximity, and net-weighted movement (HH[N]; **4i B**, 10.3 ± 4.1 vs. 24.1 ± 5.7 , $^{*}d = -1.14$ and **4i D**, 2.6 ± 3.3 vs. 15.0 ± 4.3 , $^{*}d = -0.94$).

In contrast to the increased post-treatment socialising between H animals, the social interaction of H animals toward an N animal ([HH]N; **4i A – D**) remained poor, even after four weeks of chronic treatment with escitalopram. As such, H animals approached N animals less often (**4i A**, 23.8 ± 8.5 vs. 49.9 ± 4.6 , $p = 0.06$, $^{**}d = -1.3$), spent fewer episodes of proximity near N animals (**4i B**, 7.5 ± 2.8 vs. 24.1 ± 5.7 , $p = 0.09$, $^{**}d = -1.39$), spent less time in the vicinity of N animals (**4i C**, 8.1 ± 2.6 vs. 17.6 ± 2.4 , $p = 0.08$, $^{**}d = -1.4$) and generated a more negative net weighted movement interacting with N animals (**4i D**, 1.0 ± 1.4 , vs. 15.0 ± 4.3 , $p = 0.31$, $^{**}d = -1.3$) compared to each other in the HHH control group. Furthermore, percentage change from average baseline behavior showed meaningful differences with large (**4ii A**) and very large (**4ii B, C**) negative effect sizes observed in the behavior of H animals toward N animals, compared to the behavior of H animals in the HHH control group ([HH]N; **4ii A**, 76.5 ± 63.0 vs. 185.1 ± 23.33 , $^{*}d = -0.81$; **4ii B**, $(-)48.3 \pm 19.4$ vs. 50.56 ± 35.5 , $^{**}d = -1.3$, and **4ii C**, $(-)10.5 \pm 29.4$ vs. 158.3 ± 34.7 , $^{**}d = -1.91$).

Discussion

The most important findings from this study are 1) the demonstration that distinct differences between the treatment-naïve behavior are observed in N and H animals within and between cohorts (*Figure 2*), 2) that a robust treatment-related increase in the interaction of H animals with one another in both the HHH and HHN groups is observed and 3) poor pre- and post-treatment social interaction of N animals toward H animals in the NNH group (*Figure 3i*).

OCD is a multifactorial condition that not only involves the experience of recurrent intrusive thoughts and/or expression of seemingly purposeless and repetitive behavior (Bartz & Hollander, 2006), but is also characterized by a number of cognitive (Belloch et al., 2011; Dietrich, Emrich, Becker, & Münte, 1996) and functional impairments (Jacoby, Leonard, Riemann, & Abramowitz, 2014; Nadeau et al., 2013; Storch et al., 2014). Furthermore various clinical

investigations have found distinct disturbances in the social behavior of OC patients compared to normal controls (Khanna et al., 1988; Rosa et al., 2012; Storch et al., 2006). Although many aspects of OCD-related changes in social behavior is still unknown, deficits manifest as altered social stability with respect to patient-parent, patient-sibling and patient-peer relationships (Rosa et al., 2012), an increased incidence of co-morbid conditions such as social phobia (Assunção et al., 2012) and poor social adjustment (Khanna et al., 1988; Rosa et al., 2012), and higher rates of occupational impairment such as unemployment and financial instability (Kim et al., 2012). Furthermore, co-morbid diagnosis of OCD and conditions of social impairment are characterized by increased OC symptom severity and poor treatment response (Rosa et al., 2012).

Given the complex nature of OC-related social behavior, the aim of the current investigation was to explore social behavior of animals in a validated animal model of OCD, to establish how these behaviors respond to a typical drug treatment regime applicable to OCD, and how these correlate with that noted in the human condition. In an attempt to do this, we set out to develop a novel SIC capable of assessing within and between cohort social interactions that is of relevance in disorders such as OCD where the extent of social impairment is influenced by the proximity of peer groups. Since the study set out to investigate the group social behavior of same-cohort animals in the presence of an animal from another cohort, a typical two-animal SIC could not be followed. A novel SIC paradigm was therefore developed whereby the social behavior of three animals could be investigated simultaneously. Different group structures (viz. NNN, HHH, NNH and HHN) enabled us to investigate the social behavior of N (viz. normal) and H (viz. OCD-like) animals in all possible within and between cohort combinations (for a description of the various interactions measured, see Table 2).

In the light of H animals demonstrating increased treatment-naïve sociability within cohort compared to N controls (*Figure 2*), our hypothesis that H animals would interact with one another in a distinctly different manner, may hold sway. Furthermore, treatment-naïve H animals in the HHN mixed group demonstrated trends of being more sociable toward one another (*Figure 2B*; HH[N]) and toward N animals (*Figure 2A, B and C*; [HH]N) compared to the interaction of N animals with each other or with an H counterpart (*Figure 2 A – C*; NN[H] and [NN]H respectively). However, given the social impairments often described in OCD patients, it would have been reasonable to anticipate that H animals would tend to act more isolative than sociable compared to N animals. Nevertheless, these findings support those

described in earlier clinical investigations (Piacentini et al., 2003) which have concluded that OCD-patients, rather than lacking social skill per se, are in fact capable of forming social bonds in terms of friendship. Furthermore, our findings with respect to the interactions of N animals with an H counterpart, showing that H animals tend to isolate themselves within a normal peer group, are congruent with clinical data that have reported that children and adolescents with OCD are more susceptible to peer victimization and marginalization than non-psychiatric controls (Storch et al., 2006).

Concerning the influence of chronic escitalopram treatment on the social interaction of N and H animals respectively, we anticipated that the behavior of N animals (*viz.* normal controls) would not be altered. Although N animals demonstrated increased approach behavior within-cohort following chronic treatment (*Figure 3i A*; NNN), no significant differences or large magnitudes of effect were displayed in any of the other measured variables. As such, we interpret the change in approach behavior to be investigative rather than social in nature. Considering the behavior of N animals toward one another in the presence of an H animal, social cohesion remained poor even after chronic escitalopram treatment (*Figure 3i A – C*; NN[H]). However, as shown in the post-treatment difference between the net weighted movement scores of N animals interacting with one another in the NNN and NNH cohorts respectively (*Figure 3i D*), the presence of an H animal in the cage resulted in an increased tendency of N animals to group together. Furthermore, increased approach behavior and duration of proximity was observed with respect to the interaction of N animals with an H animal following drug treatment (*Figure 3i A, C*; [NN]H), suggesting that SSRI treatment tended to restore the above-mentioned marginalisation. Whether this finding results from an increase in investigative behavior noted in N animals, or from the possibility of H animals being more approachable following treatment (see below), is unclear, but it is nonetheless of interest how an SSRI may modify inter-group socialisation between N and H animals. These data are of particular interest as for the first time we have support in an animal model that spontaneous marginalisation of OCD patients by their normal peer group does indeed occur (Storch et al., 2006).

The baseline social behavior of H animals was significantly altered by four-week escitalopram administration in that H animals demonstrated increased post-treatment sociability toward one another in both the HHH and HHN paradigms (*Figure 4i A, C and D*). However, the

magnitude of difference between the post-treatment sociability of H animals toward one another within these two groups demonstrate large effect sizes (*Figure 4i B – D*), suggesting that the presence of an N animal in the cage moderated the effects of treatment in H animals to some extent. Although clinical data regarding this phenomenon is limited, at least one case report documented that social phobia towards healthy peers experienced by a patient with OCD only responded to a *combination* of the SSRI paroxetine (high dose, 60 mg/day) and the atypical antipsychotic, risperidone (low dose, 1 mg/day) (Yamauchi et al., 2004). This is consistent with our findings that show a more substantial increase in the social interactivity of H animals within-cohort, compared to the interaction of H animals in the presence of an N animal. Nevertheless, that escitalopram treatment resulted in substantial trends toward increased social interactivity between H animals in the HHN paradigm, irrespective of the presence of an N animal (*Figure 4i A, C*), is in agreement with how SSRI's may improve OCD symptoms, viz. by reducing social anxiety and improving social interaction with peers (Baldwin et al., 2008).

Chronic escitalopram treatment failed to influence the behavior of H animals toward N subjects. In fact, instead of improving the social relationships between H and N animals, escitalopram favorably altered the social interaction between animals of the H cohort only, as can be seen in *Figures 4i A and C*. This finding is particularly interesting in that it implies that the SSRI selectively modified social behaviour in H animals in relation to themselves, but not toward normal peers. In fact, the social interaction of H animals with an N animal within the HHN group, and not between one another, seems to have deteriorated following treatment (*Figure 4ii, A – C*). It can be argued that as OCD patients respond preferentially to chronic high-dose SSRI treatment, an associated improvement in co-morbid social deficits, including those involving interactions toward normal peers, could have ensued. However, our data agrees with clinical evidence in that the social competence of H deer mice did indeed improve, albeit toward one another. We have demonstrated previously that although chronic escitalopram treatment decreases the number of high stereotypical bouts, H animals persist in the expression of repetitive motor stereotypy (Wolmarans et al., 2013). OCD patients have been known to hide their symptoms from normal peers (Piacentini et al., 2003), irrespective of how often they occur. We therefore argue that, since H deer mice may still express OC bouts following drug treatment, a decrease in stereotypical bouts associated with an increase in social competence corresponds with clinical findings and that chronic escitalopram treatment dramatically improves the symptomology of H animals.

Limitations in the study include the complicated design of the SIC, although at the same time this approach has proven to be a valuable instrument with which to assess the complex nature of social structures. Nevertheless, the need for various comparisons to be made could have resulted in some comparisons narrowly missing statistical significance. Given the natural between-animal variance in social behavior, it is possible that a larger sample pool could have resulted in more robust differences. Secondly, behavioral analysis with deer mice is problematic given the excessive species-specific expression of motor stereotypy. Social behavior, as is traditionally defined (Jenkins et al., 2008; Kask et al., 2001; Möller et al., 2011), is mostly absent in a SIC performed in deer mice and hence a combination of different behavioral measures and a novel means of investigation is required to distinguish social behavior from other forms of interaction (eg. aggression). However, we believe that the combination of approach behavior, episodes of proximity, duration of proximity and net weighted movement, co-analyzed with video data, provides a robust and reliable means of assessing social behavior in deer mice.

Conclusion

As far as we are aware, this is the first investigation in an animal model of OCD where the inter-group sociability of H (*viz.* OCD-like) and N (*viz.* normal) controls has been compared. This is the first study to explore the manifestation of *group* social interactions between rodents of the same and different behavioral cohorts and how this behavior can be manipulated by a typical drug treatment regimen employed in human OCD. Furthermore, it describes how social impairment may modify response to treatment, in particular how the presence of an N animal may abrogate SSRI response on social behaviour in H animals. The study has demonstrated that distinct differences exist between treatment-naïve behavior of N and H animals within and between cohorts, that the sociability of H animals toward one another (both within and between cohorts) improves following chronic escitalopram treatment, while the interaction of H animals with N animals remains poor. Data from the current investigation is congruent with clinical evidence relating to the social behavior of OCD patients and their social experiences in the presence of healthy peers. However, our findings also suggest that greater focus on social behavior of OCD patients is needed; while they may experience increased social function after treatment with SSRIs, it is also possible that enduring social impairments require rigorous assessment and targeting. Not only do the results of the current investigation highlight the relevance, usefulness and impact of the deer mouse model of OCD,

but also contribute to our understanding of the complex social behavior between animals of different behavioral cohorts. Finally, the model sheds new light on how more complex social interaction paradigms may be relevant to patients with OCD.

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Conflicts of interest

The authors declare that over the past three years, Dan Stein has received research grants and/or consultancy honoraria from AMBRF®, Biocodex®, Cipla®, Lundbeck®, National Responsible Gambling Foundation, Novartis®, Servier®, and Sun®. In the past three years, Brian Harvey has participated in advisory boards and received honoraria from Servier®, and has received research funding from Servier® and Lundbeck®. The authors declare that, except for income from the primary employer and research funding to BHH from the South African Medical Research Council, and the above-mentioned exceptions, no financial support or compensation has been received from any individual or corporate entity over the past three years for research or professional services, and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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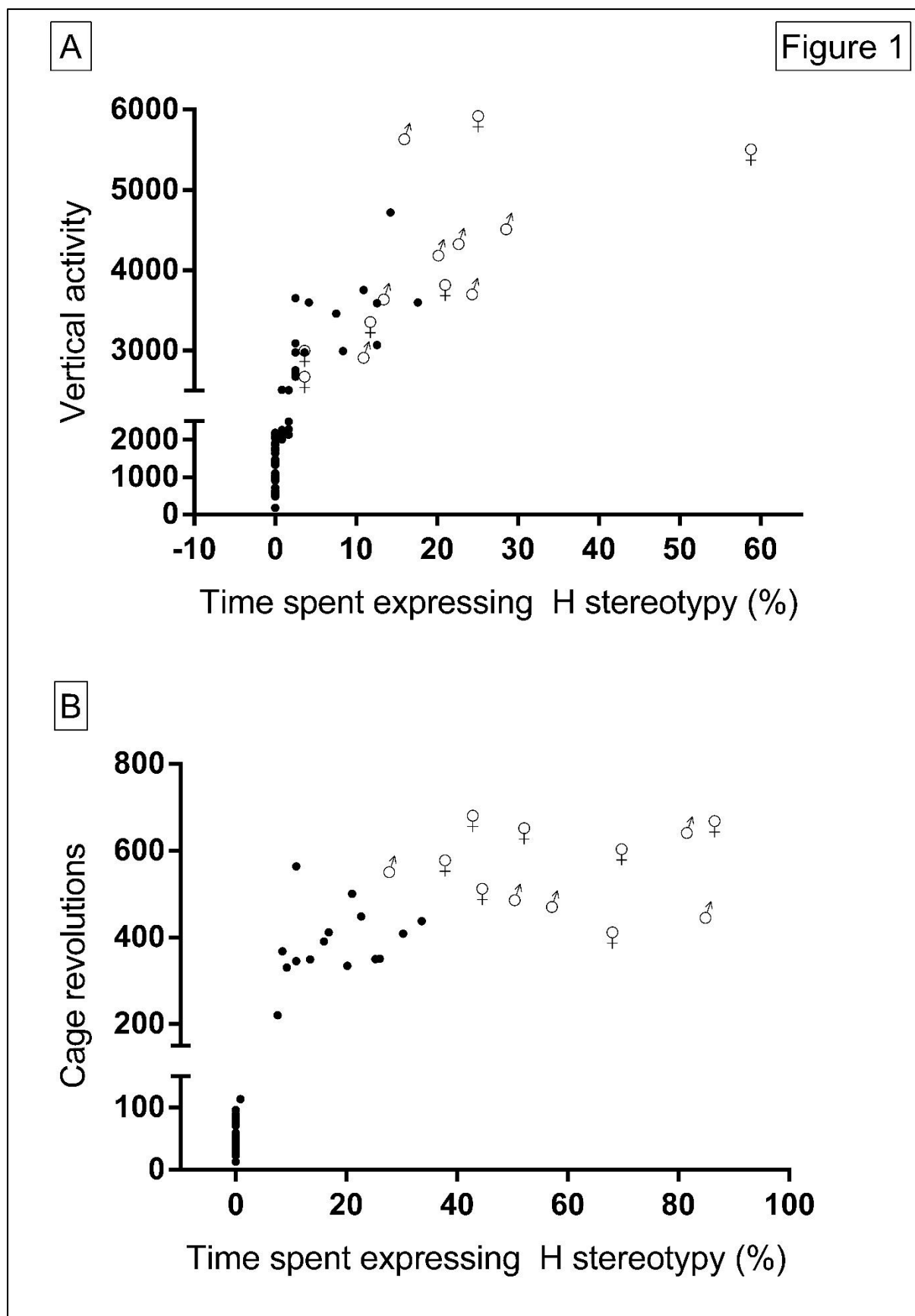
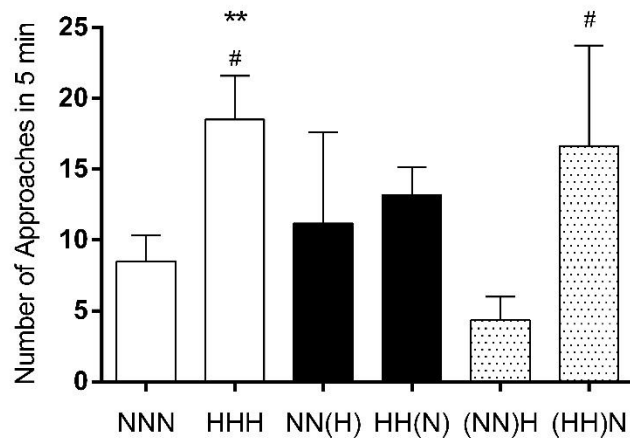
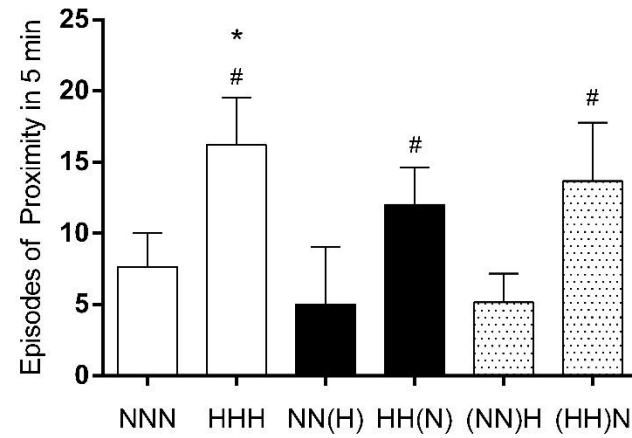


Figure 2

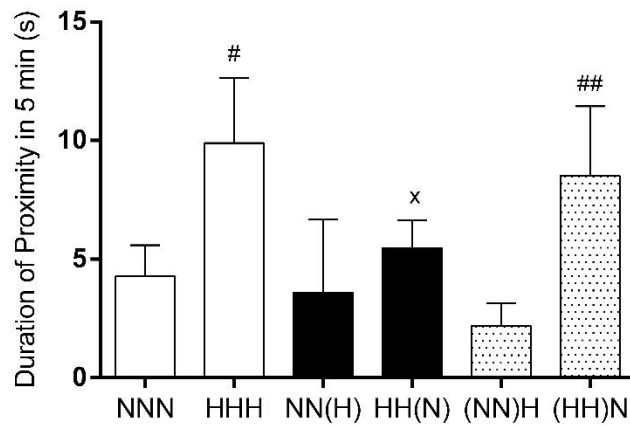
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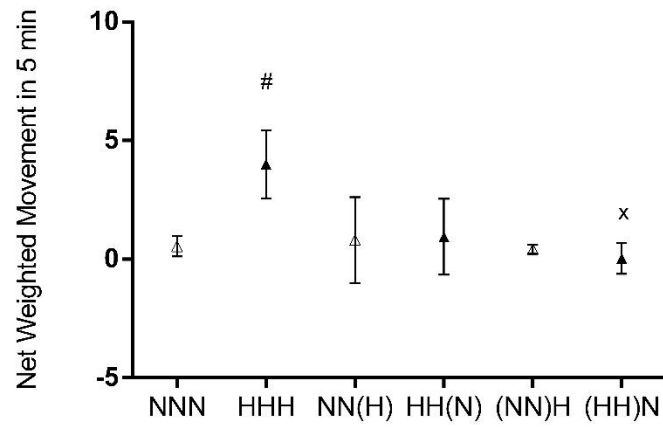


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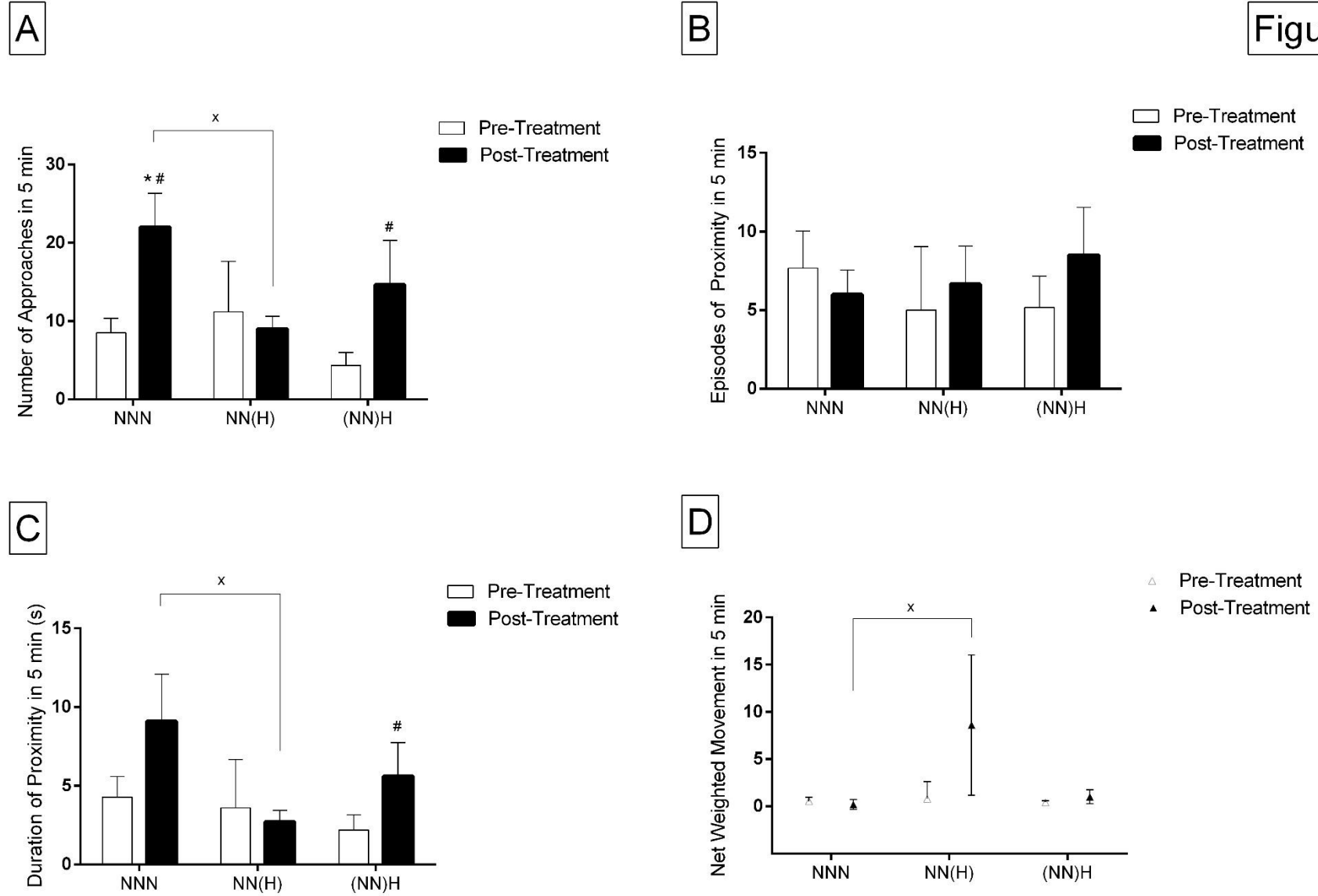


Figure 3ii

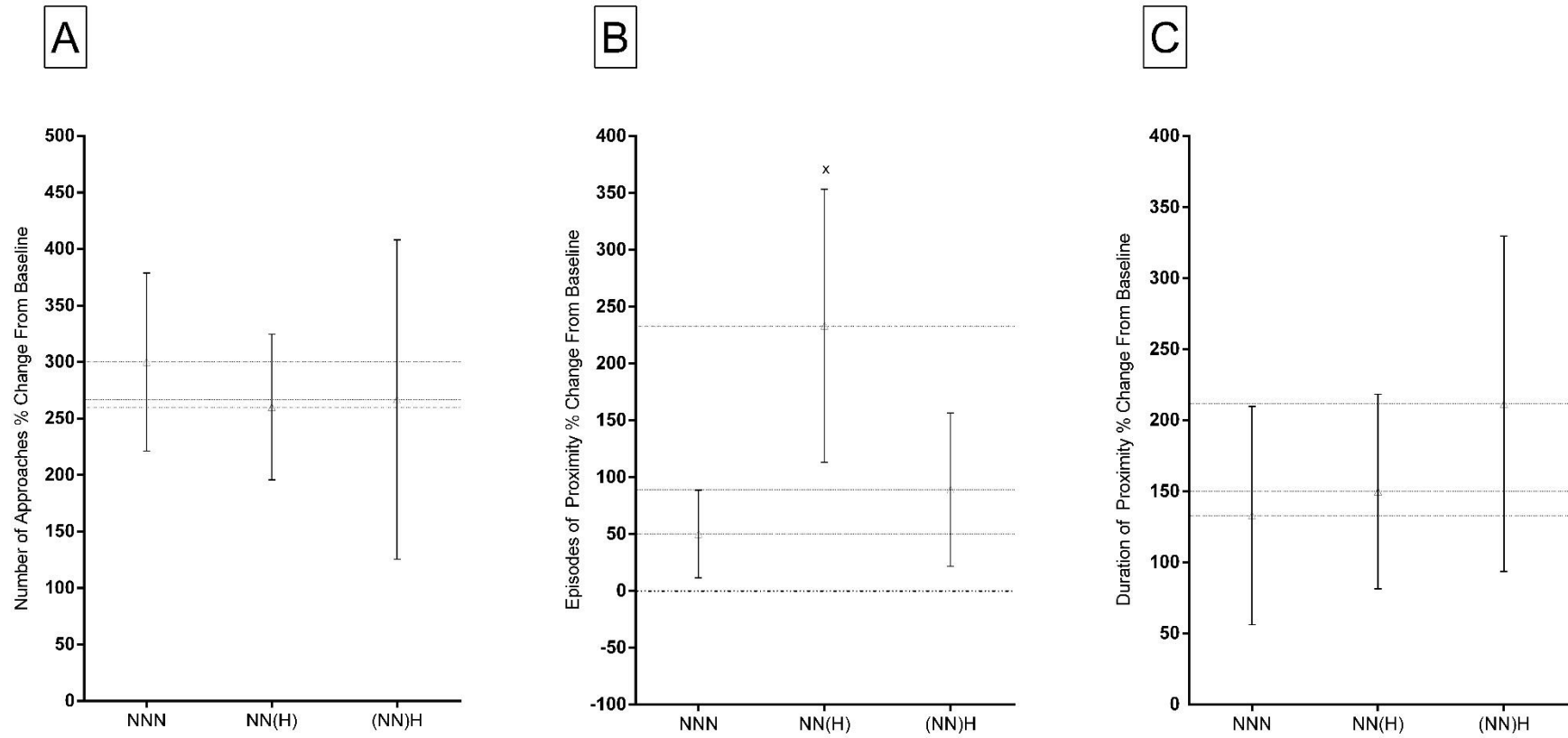


Figure 4i

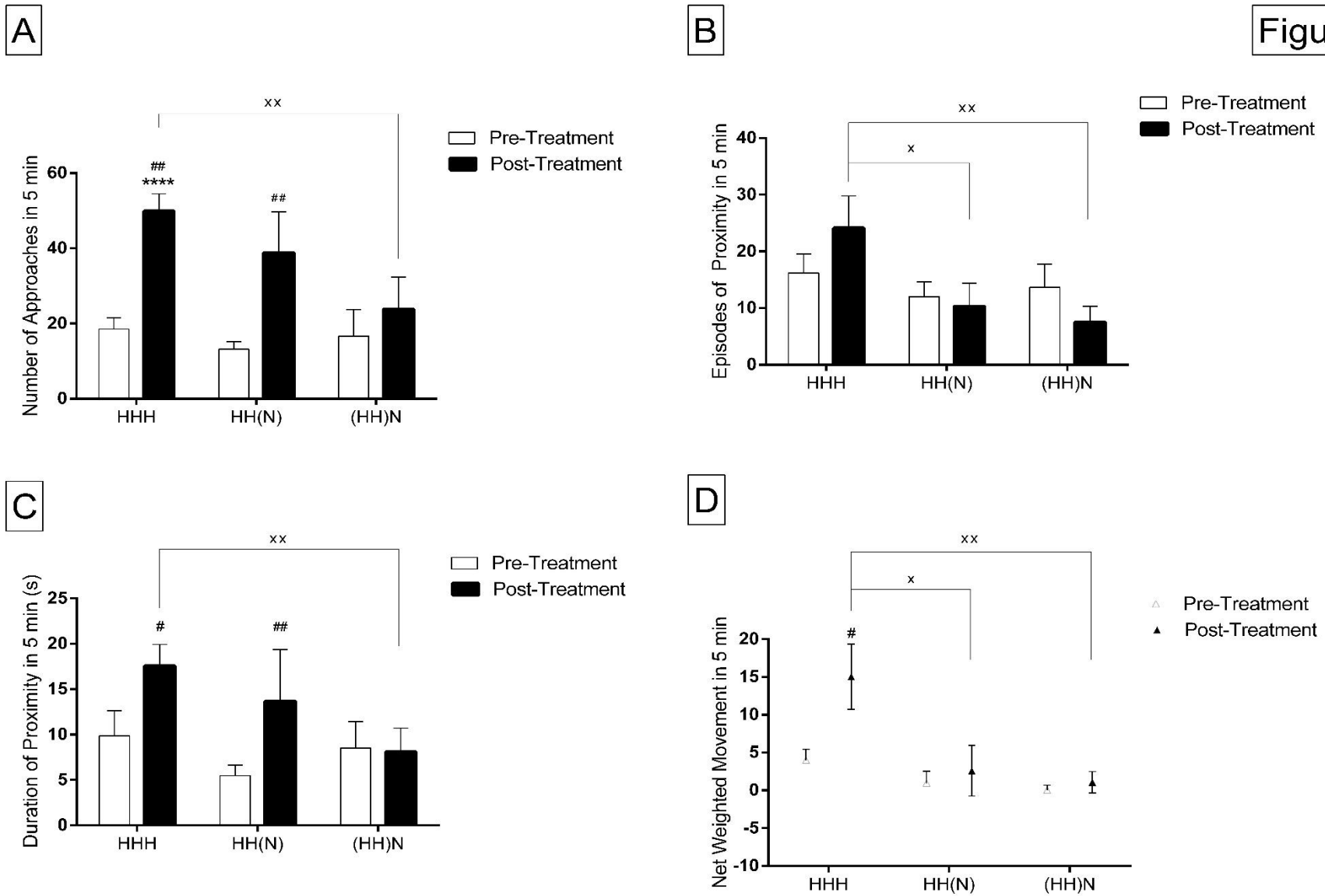


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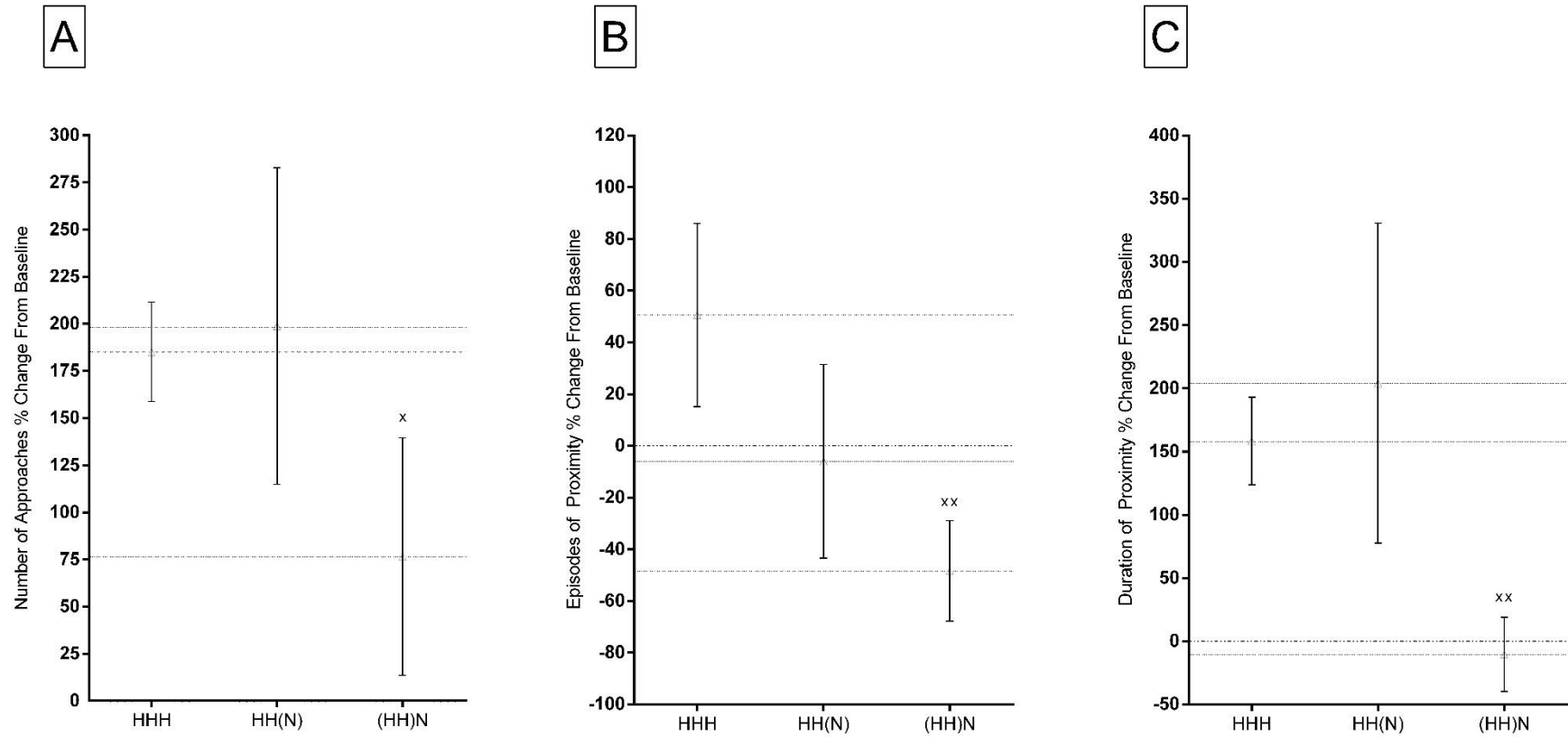


Table I

Stereotypy Intensity	Vertical beam inter- ruptions / 30 min	Cage revolutions / 30 min
N	< 500	< 150
H	> 2000	> 200

Criteria for defining N and H intervals of stereotypical activity with respect to vertical activity and cage revolutions

Table 2

Group Name <i>Each performed three times before and after treatment</i>	Description of group structure and interactions measured <i>(n) values as obtained during one assessment</i>
HHH / NNN	• Three H or N animals grouped together • Number of approaches measured in both directions between respective animals (viz. a-b, a-c; b-a, b-c; c-a, c-b; $n = 6$) • Number of episodes of proximity between all subjects measured (viz. a-b, a-c, b-c; $n = 3$) • Cumulative duration of proximity (in seconds) measured between all subjects (viz. a-b, a-c, b-c; $n = 3$) • Net weighted movement calculated between respective animals in both directions (viz. a-b, a-c; b-a, b-c; c-a, c-b; $n = 6$)
HHN	• Two H animals and one N animal grouped together • <u>HH</u>[N]: Social behavior measured between two H animals only but with an N animal in the cage. Number of approaches in both directions ($n = 2$), number of episodes of proximity ($n = 1$), duration (sec) of proximity ($n = 1$) and net weighted movement in both directions ($n = 2$). • [HH]N: Social behavior of the two H animals measured towards the N animal. Number of approaches of each H animal towards the N animal ($n = 2$), number of episodes of proximity ($n = 2$), duration of proximity ($n = 2$) and net weighted movement of each H animal towards / away from the N animal ($n = 2$)
NNH	• Two N animals and one H animal grouped together • <u>NN</u>[H]: Social behavior measured between two N animals only but with an H animal in the cage. Number of approaches in both directions ($n = 2$), number of episodes of proximity ($n = 1$), duration of proximity (s; $n = 1$) and net weighted movement in both directions ($n = 2$). • [NN]H: Social behavior of the two N animals measured towards the H animal. Number of approaches of each N animal towards the H animal ($n = 2$), number of episodes of proximity ($n = 2$), duration of proximity ($n = 2$) and net weighted movement of each N animal towards / away from the H animal ($n = 2$)

Description of group structures in the social interaction paradigm and the nature of interactions measured

Table 3

Behavior measured by Ethovision®	Description / Definition
Number of approaches (viz. moving to)	The state is 'Moving to' when the focal subject (Actor) is moving towards another subject (Receiver). Interaction distance set as $\leq 15\text{cm}$.
Number of episodes and cumulative duration of proximity (s)	A discrete variable with two possible states, 'In proximity' and 'Not in proximity': • The state is 'In Proximity' when the distance between the selected body points of the focal subject (Actor) and the body points of another subject (Receiver) is lower than a user-defined 'In proximity' threshold (set as $\leq 5\text{cm}$). • The state is 'Not in proximity' when the distance between the selected body points of the Actor and the body points of another subject Receiver is greater than 5cm.
Net weighted movement	The signed, distance-weighted change in distance between two subjects from one sample to the next. Net weighted movement is weighted by the distance between two subjects. Changes in positions of subjects which are at a large distance from each other have a lower weight, so they can be distinguished from movements at close distance, which have a different biological meaning. Unlike 'Relative movement', this is a continuous variable (in distance units). The 'Net weighted movement' is positive if the subject (Actor) is getting closer to another subject (Receiver), negative in the other case.

Definitions and descriptions of the social interaction parameters measured

Table 4

			Pre-treatment comparisons between N and H behavior (Figure 2, Mann-Whitney U test)		
			<i>p</i>	Mann-Whitney <i>U</i>	Cohen's <i>d</i>
Number of Approaches	NNN vs HHH	<i>n</i> = 18	** 0.009	80.5	# 0.95
	NN(H) vs HH(N)	<i>n</i> = 6	0.37	12.0	0.19
	(NN)H vs (HH)N	<i>n</i> = 9	0.14	8.5	# 1.15
Episodes of Proximity	NNN vs HHH	<i>n</i> = 9	* 0.04	17.0	# 1.00
	NN(H) vs HH(N)	<i>n</i> = 3	0.4	2.0	# 1.20
	(NN)H vs (HH)N	<i>n</i> = 6	0.17	9.0	# 1.13
Duration of Proximity	NNN vs HHH	<i>n</i> = 9	0.11	22.0	# 0.92
	NN(H) vs HH(N)	<i>n</i> = 3	0.7	3.0	0.51
	(NN)H vs (HH)N	<i>n</i> = 6	0.13	8.0	## 1.32
Net Weighted Movement	NNN vs HHH	<i>n</i> = 18	0.18	119.0	# 1.03
	NN(H) vs HH(N)	<i>n</i> = 6	0.9	17.0	0.03
	(NN)H vs (HH)N	<i>n</i> = 6	0.47	13.0	0.38

Descriptive statistics of pairwise between-group social behavior of treatment naive N and H animals

Statistical significance and large effect sizes as determined by Cohen's *d* calculations are provided in bold script.
(*n*-values are provided as a total of three repetitions)

Table 5

			Pre-treatment comparisons of N and H behavior in mixed paradigms with NNN and HHH controls respectively (Figure 2, Kruskal-Wallis ANOVA)	
			<i>p</i>	Cohen's <i>d</i>
Number of Approaches (Control <i>n</i> = 18)	NN(H)	<i>n</i> = 6	> 0.99	0.22
	HH(N)		0.93	(-) 0.60
	(NN)H	<i>n</i> = 6	0.58	(-) 0.71
	(HH)N		0.95	(-) 0.12
Episodes of Proximity (Control <i>n</i> = 9)	NN(H)	<i>n</i> = 3	0.66	(-) 0.37
	HH(N)		> 0.99	(-) 0.58
	(NN)H	<i>n</i> = 6	> 0.99	(-) 0.42
	(HH)N		> 0.99	(-) 0.26
Duration of Proximity (Control <i>n</i> = 9)	NN(H)	<i>n</i> = 3	0.85	(-) 0.15
	HH(N)		> 0.99	× (-) 0.86
	(NN)H	<i>n</i> = 6	0.55	(-) 0.67
	(HH)N		> 0.99	(-) 0.18
Net Weighted Movement (Control <i>n</i> = 18)	NN(H)	<i>n</i> = 6	> 0.99	(-) 0.08
	HH(N)		0.63	(-) 0.61
	(NN)H	<i>n</i> = 6	> 0.99	(-) 0.10
	(HH)N		0.32	× (-) 1.03

Descriptive statistics of comparisons between the behavior of N and H animals in mixed paradigms (NNH and HHN) with the behavior of animals within the respective NNN and HHH control groups
 Statistical significance and large effect sizes as determined by Cohen's *d* calculations, are provided in bold script.
 (*n*-values are provided as a total of three repetitions)

Table 6

		Pre- vs post-treatment within group comparisons (Figures 3i and 4i, Mann-Whitney U test)				Endpoint comparisons of N and H behavior in mixed paradigms with NNN and HHH controls respectively (Figures 3i and 4i, Kruskal-Wallis ANOVA)		Endpoint comparisons: % change from baseline in the behavior of N and H animals in mixed para- digms compared to NNN and HHH controls re- spectively (Figures 3ii and 4ii, Kruskal-Wallis ANOVA)	
		<i>n</i>	<i>p</i>	Mann-Whitney <i>U</i>	Cohen's <i>d</i>	<i>p</i>	Cohen's <i>d</i>	<i>p</i>	Cohen's <i>d</i>
Number of Approaches	NNN	18	* 0.02	86.5	# 1.03	Control		Control	
	NN(H)	6	0.42	12.5	(-) 0.20	0.28	× (-) 1.16	> 0.99	(-) 0.16
	(NN)H	6	0.36	12.0	# 1.15	> 0.99	(-) 0.45	> 0.99	(-) 0.09
Episodes of Proximity	NNN	6	> 0.99	40.5	(-) 0.30	Control		Control	
	NN(H)	3	0.80	3.5	0.30	> 0.99	0.15	0.50	× 1.13
	(NN)H	6	0.44	13.0	0.54	0.89	0.41	> 0.99	0.27
Duration of Proximity	NNN	9	0.16	24.0	0.74	Control		Control	
	NN(H)	3	0.70	3.0	(-) 0.27	0.42	× (-) 1.23	> 0.99	0.09
	(NN)H	6	0.38	12.0	# 0.90	> 0.99	(-) 0.47	> 0.99	0.30
Net Weighted Movement	NNN	18	0.76	152.0	(-) 0.17	Control			
	NN(H)	6	0.45	13.0	0.70	0.61	× 0.82		
	(NN)H	6	0.81	16.0	0.52	> 0.99	0.40		
Number of Approaches	HHH	18	**** < 0.0001	38.5	## 1.92	Control		Control	
	HH(N)	6	0.37	12.0	## 1.62	0.86	(-) 0.47	> 0.99	0.09
	(HH)N	6	0.57	14.0	0.37	0.06	×× (-) 1.30	0.35	× (-) 0.81
Episodes of Proximity	HHH	6	0.45	31.5	0.58	Control		Control	
	HH(N)	3	0.90	4.0	(-) 0.39	0.56	× (-) 1.14	> 0.99	(-) 0.66
	(HH)N	6	0.33	11.5	(-) 0.72	0.09	×× (-) 1.39	0.17	×× (-) 1.30
Duration of Proximity	HHH	9	0.06	19.0	# 1.00	Control		Control	
	HH(N)	3	0.70	3.0	## 1.38	> 0.99	× (-) 0.46	> 0.99	0.28
	(HH)N	6	0.67	15.0	(-) 0.06	0.08	×× (-) 1.40	0.09	×× (-) 1.91
Net Weighted Movement	HHH	18	0.18	119.0	# 0.91	Control			
	HH(N)	6	> 0.99	18.0	0.27	0.19	× (-) 0.94		
	(HH)N	6	0.47	13.0	0.39	0.31	×× (-) 1.30		

Descriptive statistics of within and between-group comparisons of the pre- and posttreatment social behavior of N and H mice

Statistical significance and large effect sizes as determined by Cohen's *d* calculations, are provided in bold script. (*n*-values are provided as a total of three repetitions)

FIGURE CAPTIONS

Figure 1 – Stereotypy intensity vs. time spent expressing H stereotypy

1A: Mean of highest individual daily vertical activity scores over the first five baseline behavioral screens vs. time spent expressing H stereotypy. **1B:** mean of highest individual daily cage revolution scores over the first five baseline behavioral screens vs. time spent expressing H stereotypy.

Sex symbols indicate high stereotypical males and females, respectively.

Figure 2 – Baseline social behavior of N and H animals

For a description of the different group structures, see Table 2. Descriptive statistics are provided in Tables 4 and 5. Data is presented as mean $\pm$ SEM. (*Pairwise Mann-Whitney U-test; #Cohen's *d* effect size of pairwise comparisons; *Cohen's *d* effect size between between social behavior of N and H animals in mixed paradigms with respective NNN and HHH controls).

Figure 3 – Comparisons of pre- and post-treatment social behavior of N animals

Figure 3i: Within- and between group comparisons of pre- and post-treatment social behavior. For a description of the different group structures, see Table 2. Descriptive statistics are provided in Table 6. Data is presented as mean $\pm$ SEM. (*Pairwise Mann-Whitney U-test; #Cohen's *d* effect size of within-group comparisons; *Cohen's *d* effect size of end-point differences in the post-treatment social behavior between N animals within the NNH paradigm and those in the NNN control group).

Figure 3ii: Post-treatment percentage change from average baseline social behavior with respect to approach behavior, episodes of proximity and duration of proximity. (*Cohen's *d* effect size of end-point differences in the post-treatment social behavior between N animals within the NNH paradigm and those in the NNN control group).

Figure 4 – Comparison of pre- and post-treatment social behavior of H animals

Figure 4i: Within- and between group comparisons of pre- and post-treatment social behavior. For a description of the different group structures, see Table 2. Descriptive statistics are provided in Table 6. Data is presented as mean $\pm$ SEM. (*Pairwise Mann-Whitney U-test; #Cohen's *d* effect size of within-group comparisons; *Cohen's *d* effect size of end-point differences in the post-treatment social behavior between H animals within the HHN paradigm and those in the HHH control group).

Figure 4ii: Post-treatment percentage change from average baseline social behavior with respect to approach behavior, episodes of proximity and duration of proximity. (*Cohen's *d* effect size of end-point differences in the post-treatment social behavior between N animals within the NNH paradigm and those in the NNN control group).

* * *

4 Manuscript B

Article accepted for publication in *Cognitive, Affective and Behavioral Neuroscience* titled:

“Of mice and marbles: Novel perspectives on burying behavior as a screening test for psychiatric illness”

AUTHOR CONTRIBUTIONS

- *De Wet Wolmarans* designed the investigation in consultation with *Brian H Harvey*, performed all behavioral, pharmacological, and statistical analyses, wrote the first version of the manuscript, and edited the manuscript following input from the co-authors.
- *Dan J Stein* acted as clinical consultant with respect to experimental design and manuscript review.
- *Brian H Harvey* was study supervisor, assisted in the interpretation of results and was corresponding author for submission of the final manuscript to *Cognitive, Affective and Behavioral Neuroscience*.

IMPORTANT INFORMATION

- Instructions to the author are included in Addendum A.
- The published article is provided in PDF format in Addendum F.
- As per the instructions to the author, figures, tables, and legends are provided at the end of the manuscript.
- Both co-authors provided consent for the paper to be assessed as part of the Ph.D. thesis of *De Wet Wolmarans* (Addendum B).
- The submitted manuscript has been received by *Cognitive, Affective and Behavioral Neuroscience* and is currently under review (see Addendum C for proof of submission).

Of mice and marbles: Novel perspectives on burying behavior as a screening test for psychiatric illness

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Abstract

Burying forms part of the normal behavioral routine of rodents, albeit being species-specific. However, it is suggested that aberrant burying behavior, of which marble-burying (MB) is an example, may represent neophobic and/or compulsive-like behavior. The current investigation assessed MB in an established animal model of obsessive-compulsive disorder (OCD), namely spontaneous stereotypy in the deer mouse, to establish if high (H) stereotypy is associated with neophobia and/or another compulsive endophenotype, compared to non (N)-stereotypical controls. A three-trial, one-zone MB test was performed over three consecutive evenings before and after chronic treatment with high dose (50 mg/kg/day) oral escitalopram. Neophobia was measured based on the number of marbles buried during the first pre- and post-treatment MB trials, and compulsive-like behavior based on the number of marbles buried over all pre- and post-treatment MB trials respectively. Data from the current study supports earlier findings that burying is a normal behavioral routine (i.e. inherent burying behavior, IBB) that is expressed by all deer mice irrespective of stereotypical cohort and not associated with either neophobia or compulsiveness. Indeed, chronic escitalopram treatment, being similarly effective in clinical anxiety and OCD as well as in attenuating H behavior, failed to influence IBB. Although 11% of animals presented with a unique burying endophenotype (i.e. high burying behavior, HBB), treatment also failed to attenuate said behavior, necessitating

further investigation as to its relevance. In conclusion, MB cannot be regarded as a measure of anxiety-like or compulsive behavior in the deer mouse model of OCD.

Keywords

Marble-burying; obsessive-compulsive disorder; neophobia; deer mouse; animal model; escitalopram

Introduction

Burying, burrowing and digging form part of the normal behavioral repertoire of rodents both in the wild and the laboratory (Deacon, 2006; Smithers, 1983). These strain-specific behaviors are mostly aimed at searching for food, burying both noxious and harmless objects, and building adequate nurseries capable of protecting against predators and providing suitable environments to breed (Smithers, 1983). Evidently, burying and digging are expressed in relatively non-anxiogenic scenarios (i.e. nesting, hoarding, foraging) as well as under anxiogenic circumstances (i.e. burying of noxious objects, confronting predators).

Defensive burying (Pinel & Treit, 1978) can be defined as the process of moving loose bedding material vertically upwards and over potentially harmful stimuli or sources of aversive stimulation (i.e. shock prod). Due to the stress provoking nature of an electric shock, defensive burying has been used as a measure of avoidance-dependent anxiety in a number of previous investigations (for a review, see De Boer & Koolhaas, 2003). Another anxiety-related form of burying (or neophobic burying) relates to novelty-induced anxiety (Diamant, Croiset, De Zwart, & De Wied, 1991; Torres-Lista, López-Pousa, & Giménez-Llort, 2015) following exposure to novel, but non-reactive harmless objects. In theory, whereas animals demonstrate non-habituation towards defensive burying, neophobic burying should attenuate over time following repeated exposure to the same stimuli (Londei, Valentini, & G. Leone, 1998).

However, an abundance of literature (Egashira et al., 2008; Londei et al., 1998; Njung'e & Handley, 1991; Thomas et al., 2009) demonstrates that rodents often persist in burying harmless forms of stimuli in the absence of anxiety that, for the purpose of this paper, will be referred to as inherent burying, of which marble burying (MB) is a typical example. As such, it has been hypothesized that this represents non-functional repetitive behavior analogous to the behavioral symptoms of obsessive-compulsive disorder (OCD) (Thomas et al., 2009; Umathe, Manna, & Jain, 2012). Although some studies employ MB as a measure of avoidance-

related anxiety (Nicolas, Kolb, & Prinssen, 2006), findings relating to non-habituation to marbles in the absence of anxiety (Thomas et al., 2009), are more compatible with inherent rather than defensive or neophobic burying. Indeed, MB is a screening-test for potential OCD treatments (Egashira et al., 2008; Gyertyan, 1995; Millan, Girardon, Mullot, Brocco, & Dekeyne, 2002). However, the test lacks illness specificity and has been used to model treatment response in impulsivity (Schneider & Popik, 2007), autism (Angoa-Pérez, Kane, Briggs, Francescutti, & Kuhn, 2013) and dementia (Torres-Lista et al., 2015).

Defensive, neophobic and inherent burying often respond to the same pharmacological agents (De Boer & Koolhaas, 2003; Nicolas et al., 2006). All three forms of burying have shown sensitivity for traditional anxiolytics i.e., benzodiazepines (Nicolas et al., 2006; Njung'e & Handley, 1991), and various classes of noradrenergic and serotonergic antidepressants i.e., desipramine, imipramine, citalopram, paroxetine and fluvoxamine (Abe, Nakai, Tabata, Saito, & Egawa, 1998; Broekkamp, Rijk, Joly-Gelouin, & Lloyd, 1986; Millan et al., 2001). Haloperidol (Nicolas et al., 2006) and chlorpromazine (Bruins Slot, Bardin, Auclair, Depoortere, & Newman-Tancredi, 2008), which are traditional antipsychotics, also attenuate burying behavior. That said, the predictive validity of burying behavior as a screening test for anxiety and/or compulsive-like repetition is undermined by contradictory results e.g. response to anxiogenic drugs (Nicolas et al., 2006), and sensitivity to a number of compounds not related to the clinical treatment of either anxiety or compulsivity (de Almeida, de Carvalho, Silva, de Sousa, & de Freitas, 2014; Honda, Kawaura, Soeda, Shirasaki, & Takahama, 2011; Krass, Rünkorg, Wegener, & Volke, 2010; Uday, Pravinkumar, Manish, & Sudhir, 2007). Moreover, previous pharmacological studies have used mostly acute single-dose treatments ranging from 30 – 90 minutes before recording burying behavior (Nicolas et al., 2006; Uday et al., 2007), despite antidepressants only being effective after chronic administration (Fineberg & Craig, 2007; Huh, Goebert, Takeshita, Lu, & Kang, 2011).

As opposed to learned or pharmacologically induced perseverative behavior, spontaneous stereotypy in the deer mouse is a naturalistic animal model of OCD founded on robust face, construct and predictive validity (Güldenpfennig, Wolmarans, du Preez, Stein, & Harvey, 2011; Korff, J. Stein, & H. Harvey, 2008; Korff, Stein, & Harvey, 2009; Wolmarans, Brand, Stein, & Harvey, 2013). Deer mouse stereotypy can generally be categorized into repetitive jumping and pattern running (Korff et al., 2008; Powell, Newman, Pendergast, & Lewis, 1999), behaviors that correspond with the repetitive motor actions of OCD. Furthermore, deer mouse

stereotypy demonstrates varying symptom intensity as in OCD and employs ‘time spent executing stereotypy’ as a marker of behavioral severity and treatment outcome (Wolmarans et al., 2013). Although deer mouse stereotypy has not yet been studied with respect to anxiety-like behavior, it demonstrates response to chronic, but not sub-chronic treatment with high doses of a selective serotonin reuptake inhibitor (SSRI), which is similarly effective in the treatment of anxiety and compulsive behavior in humans (Wolmarans et al., 2013).

As MB may reflect both defensive/neophobic (anxiety-like) and inherent (compulsive-like) burying behavior, the current investigation attempts to contribute to our understanding of its phenomenology by challenging an established animal model of OCD, namely spontaneous stereotypy in the deer mouse, with MB. The aim of the current study will be 1) to investigate whether the compulsive-like expression of motor behavior by high stereotypical (H) deer mice can be associated with unique MB behavior compared to non-stereotypical (N) controls, 2) to assess whether deer mice habituate to burying behavior and 3) whether such behavior is attenuated by chronic (four-week), high dose (50 mg/kg/day) oral escitalopram (Baldwin, Brandish, & Meron, 2008).

Materials and methods

Animals

70 deer mice (males and females) were obtained from the deer mouse colony maintained and housed at the vivarium of North-West University (NWU), Potchefstroom, South Africa (*Ethical Approval Number - NWU-00066-10-S5*). As reported previously (Powell et al., 1999) stereotypical behavior in the deer mouse is established by the age of 8 weeks and only mice between the ages of 10 and 12 weeks were chosen as experimental subjects at the onset of behavioral assessments (day 0). Mice were randomly chosen from different litters, without sex or weight bias and housed in groups of 6 same-sex animals per cage. Earlier studies have already noted that the severity of stereotypy in deer mice is independent of sex (Hadley, Hadley, Ephraim, Yang, & Lewis, 2006; Korff et al., 2008). One week before the onset of behavioral assessments, each animal was allocated individually to an automatic climate-controlled laboratory cage (35cm (l) x 20cm (w) x 13cm (h); Techniplast® S.P.A., Varese, Italy) and maintained at 23°C on a 12-hour light/dark cycle (lights on at 06h00 and off at 18h00). Food and water were provided ad lib for the duration of the study. Cages were cleaned and new bedding material added weekly.

Generating behavioral data and classification of NS and HS animals

In order to screen animals for baseline expression of stereotypy, the same protocol as published previously (Wolmarans et al., 2013) has been followed. In short, all animals underwent 12-hour behavioral screening once a week for five consecutive screens. On any specific assessment day, animals were moved from their housing environment to the behavioral screening room. These areas are located on the same floor of the vivarium and are environmentally controlled with respect to temperature (23°C), relative humidity (55%), and light cycle (same as for home cages). Subsequently each animal was introduced to a behavioral test cage (21 cm (w) x 21 cm (l) x 35cm (h); Accuscan® Inc., Columbus, Ohio, USA) constructed from clear, translucent Plexiglas®. Home cage bedding material was provided in quantities enough to cover the floor of the test cages, but also ensuring that it did not interrupt the scoring of behavioral data. Food was provided ad lib on the floor of the cage in the form of broken-up rodent chow pellets. Bottled water was provided through a tight-fit hole in the wall of each test cage. Cages were covered with lids that allowed uninterrupted airflow. The animals were introduced to these environments by 16h00 and habituated for at least two hours before the 12-hour behavioral assessments started at the onset of the dark cycle. Test cages were cleaned after each screen.

Behavioral assessment was performed using the Fusion® Animal Activity Monitoring System (Accuscan® Inc., Columbus, Ohio, USA), and analyzed using Fusion® software. Each testing cage presents with a grid of infra-red light beams that cross the cage roughly 2 cm above the cage floor along the X- and Y-axes (constituting a grid), while another set of beams (Z-axis) crosses the cage 10 cm above the cage floor, but only parallel to the lower Y-axis. Parallel beams are spaced 2.5 cm apart and record activity every time it is interrupted by movement. The number of vertical beam interruptions (VBI) was used as a measure of vertical jumping activity, while the number of clockwise and anti-clockwise cage revolutions (CR) was used to evaluate the expression of pattern running. During the recording session, the Fusion® software generates data continuously, allowing for experimental playback and the export of behavioral reports the following day.

Following the first five behavioral screens, animals were divided into N and H groups according to our previously published protocol (Wolmarans et al., 2013). As findings from previous studies demonstrated the time-consuming nature of stereotypy, we amended the protocol to include both *intensity* (number of stereotypical counts per 30 minutes) and *frequency* (number

of 30-minute HS bouts per 12 hour dark cycle or time spent executing stereotypy) as indicators of behavioral severity. The published criteria for defining vertical and horizontal H intervals are reproduced in Table 1. This data was plotted for both vertical (Figure 1A) and horizontal activity (cage revolutions; Figure 1B) and, as no distinction was made between the different forms of stereotypy, H animals could demonstrate stereotypical behavior in either one or both of the behavioral topographies. We subsequently included 18 animals in the H group (Figures 1A and 1B combined; subjects indicated with crosses) that expressed both the highest intensity of stereotypy and the highest frequency of H stereotypical bouts, irrespective of whether an individual did so in only one or both of the topographies (the sum total of crosses from Figures 1A and 1B, i.e. 25, exceeds 18 as more than one animal displayed H behavior in both topographies). The group of N animals was simply constituted from the 18 animals that consistently generated the lowest stereotypy counts (Figures 1A and 1B combined; black dots plotted at $X = 0$).

As only 18 animals were included in each cohort following the first five behavioral screens, a grey margin of 34 animals was excluded from the experimental design. The rationale for the exclusion of a grey margin of animals is to improve sensitivity, as described elsewhere (Wolmarans et al., 2013).

Marble-burying test

In the current investigation, the MB test was applied as previously published (Broekkamp et al., 1986) with respect to the setup for a one-zone paradigm, with slight modifications. Following the last baseline behavioral screen, the bedding material of the 18 N and H animals was exchanged with sawdust. Animals were habituated in these cages in the absence of marbles for at least 24 hours before the first MB test. All experiments were carried out under dim red light during the dark cycle. In order to assess MB but prevent avoidance behavior, 15 glass marbles ($\varnothing$ 1 cm) were evenly spaced on sawdust (obtained from South African Vaccine Producers, Sandringham, South Africa; average flake size 4mm, layer 5cm thick) in exact copies of the home cages. Each mouse was allocated to a marble-containing cage and allowed 30 minutes to explore. After returning the animals to their home cages, the marbles were counted. Scoring took place blindly by an observer unfamiliar with the classification status of the animals. A marble was considered buried when 2/3 or more of its size was covered with sawdust. MB was measured in the same animals during three separate trials on three consecutive evenings before treatment (T1 – 3) and again during three trials in the same animals

following four weeks of treatment (T4 – 6) (see ‘Drugs’ below). Locomotor activity was assessed separately from MB and as stereotypy and locomotor activity were simultaneously recorded by the Fusion® software, data from the last baseline screen (pre-treatment) was compared to that obtained following four weeks of treatment. Screening was performed one night before the first pre- (T1) and post- (T4) treatment MB trials.

Drugs

Escitalopram oxalate was prepared for oral administration by dissolving it in the drinking water (Wolmarans et al., 2013). Physical handling of the mice was kept to a minimum to prevent any possible anxiogenic effects. For the same reason, oral gavage was not considered as an appropriate dosage route. The final concentration of the drinking solution was constituted at 20mg/100ml ensuring that each animal would receive 50 mg/kg/day (within close limits) of escitalopram. The dose calculation was based on a prior pilot study where the average water intake per mouse was determined as 0.25 ml/g/day (data not shown), and confirmed by others (Aschhoff, Schroff, Wildenauer, & Richter, 2000).

Statistics

Statistical analysis was performed using GraphPad® Prism® 6 and IBM SPSS Statistics 22 under guidance of the Statistical Consultation Service of North-West University, Potchefstroom. Two-way analysis of variance (ANOVA) followed by Bonferroni post-tests was applied to compare the pre- and post-treatment expression of marble-burying behavior and locomotor activity both within, and between N and H cohorts. Behavioral cohorts were set as between subject (independent) factor and time and treatment as within (dependent) subject factors. The same statistics were applied to compare high burying behavior (HBB; see Discussion for definition) with inherent burying behavior (IBB). In this case, the burying cohort was set as between subject factor and time and treatment as within subject factor. HBB behavior was statistically separated from IBB based on 1) the extent to which the average HBB scores deviate from the average of scores of IBB and 2) calculations of coefficients of variance in daily burying scores (as the total average burying scores did not demonstrate a normal distribution, a Spearman’s correlation between the total average number of marbles buried and the coefficients of variance was applied)..As such, only animals that demonstrated burying behavior above the 75th percentile of the distribution, deviating from the mean with two times the standard deviation *and with* a calculated coefficient of variance below the 25th percentile of the

distribution were included in the HBB group. To compare the pre- and post-treatment locomotor activity of animals expressing HBB behavior, a two-tailed paired t-test was applied. Statistical significance was set at $p < 0.05$ for all analyses.

Results

Marble burying studies:

Figures 2A – B describe MB behavior in N and H deer mice receiving escitalopram over three different trials. An initial evaluation of the data did not demonstrate significant differences in MB between the N and H cohorts with respect to the first trial (neophobic burying behavior) either before (Figure 2A, T1) or after four weeks (Figure 2A, T4) of treatment with escitalopram. Furthermore, a Pearson's correlation between the average stereotypy count and the pre-treatment number of marbles buried failed to display a linear relationship (both sets of data were normally distributed; $r(34) = -0.06$, $p = 0.74$; data not shown). Moreover, chronic escitalopram failed to influence first trial MB behavior within both of the cohorts. The average number of marbles buried throughout all pre-treatment trials for N and H animals also did not differ, while treatment failed to influence this behavior either within or between behavioral cohorts (data not shown). Neither N nor H animals habituated towards burying behavior, irrespective of treatment, as no inter-trial differences in MB could be observed either within or between behavioral cohorts (Figure 2B, T1 – 6).

Given the apparent lack of separation between H and N animals, MB behavior of individual animals was analyzed independent of degree of stereotypy. A Spearman's correlation ran between the average number of marbles buried over all trials and the coefficients of variance calculated with respect to the daily burying behavior, revealed a strong negative association (Figure 3A, $r(34) = -0.64$, $p < 0.0001$). As such, four animals (one female, three male) were identified that consistently expressed increased MB behavior as demonstrated by burying scores within the upper 75th percentile of the distribution, deviating with more than two standard deviations from the mean IBB score and that manifest with little inter-day variance (coefficients of variance are contained within the lower 25th percentile of distribution). No interaction between burying behavior and treatment ($F(5, 204) = 0.97$, $p = 0.43$) was demonstrated, but a significant main effect of burying cohort ($F(1, 54) = 17.04$, $p < 0.0001$) was observed. This significant increase in HBB compared to the larger group was noted throughout all trials but one (Figure 3B, ** $p < 0.005$). Furthermore, taking the average pre- (T1 –

3) and post- (T4 – 6) treatment expression of burying behavior together, no interaction between burying behavior and treatment ($F(1, 212) = 0.70, p = 0.4$) was found, although again the main effect of HBB was significant ($F(1, 21) = 58.16, p < 0.0001$), with post-hoc analysis demonstrating the significance to be even more profound (Figure 3C, **** $p < 0.0001$).

Importantly, it must be emphasized that apart from the four animals expressing HBB behavior, all deer mice in the current investigation displayed IBB (Table 2). While this behavior varied in expression between individual animals, it also varied in intensity across different trials within the same animal (Figure 3D, data sheet submitted as supplementary material). In fact, apart from the consistent burying behavior of the animals expressing HBB behavior, no apparent burying pattern could be recognized in either the same animal, the same behavioral cohort or with respect to its response to treatment (Figure 2A, B, D).

Locomotor activity studies:

A significant two-way interaction was evident between cohort and treatment ($F(1, 34) = 6.319, p = 0.02$). N deer mice displayed a significant increase in baseline locomotor activity compared to H animals (Figure 4A, **** $p < 0.0001$), while chronic escitalopram treatment significantly decreased the locomotor activity of both N and H animals (Figure 4A, **** $p < 0.0001, * p = 0.02$). Regarding the four animals expressing HBB, escitalopram significantly decreased locomotor activity in HBB animals (Figure 4B, $t(3) = 3.99, * p = 0.03$).

Discussion

The major findings of the present work are 1) that H deer mice do not present with altered MB behavior compared to N animals, 2) that all deer mice exhibit a level of IBB that is not subject to habituation, 3) that a characteristically different form of burying behavior (HBB) has been identified within the species, but across different behavioral cohorts, and 4) that neither IBB nor HBB behavior is sensitive to chronic (four-week) high dose escitalopram treatment (50 mg/kg/day).

Although defensive and neophobic burying is a normal behavioral coping response in mice and as such cannot be used to model a psychiatric illness (De Boer & Koolhaas, 2003), aberrant defensive, neophobic, and inherent burying behavior, of which MB has been suggested to be an example (Broekkamp et al., 1986; Njung'e & Handley, 1991; Thomas et al., 2009), may be

useful for modeling illnesses analogous to compulsivity and anxiety. However, it is this dichotomy in its application and pharmacological sensitivity that contributes to uncertainty as to what behavioral phenotype(s) the test may in fact be measuring.

OCD responds preferentially to SSRIs, and invariably to a higher dose than the nominal antidepressant dose (El Mansari & Blier, 2006). Although stereotypy is a perseverative behavior common to a number of neuropsychiatric disorders, including OCD (Powell et al., 1999), deer mouse stereotypy is reversed by serotonergic but not noradrenergic antidepressants (Korff et al., 2008), while chronic treatment is obligatory (Wolmarans et al., 2013). Further, high stereotypy in deer mice correlates to reduced striatal SERT density (Wolmarans et al., 2013), a recognized pathological feature of OCD (Zitterl et al., 2008), while SERT is the preferential target for SSRIs (El Mansari & Blier, 2006). This being said, deer mouse stereotypy was challenged with MB as a possible screening test for anxiety (neophobia) and compulsivity. In addition, we determined the response of MB to a known anxiety and OCD treatment modality, i.e. chronic high-dose escitalopram.

The current investigation has delivered a number of fundamental observations that are relevant to MB behavior as a potential model for anxiety and compulsive-like behavior. Apart from the fact that MB failed to demonstrate significant differences between H and N animals (Figure 2A and B), it is also evident that the burying behavior of deer mice is not associated with novelty induced anxiety (neophobia, Figure 2A). Further, the non-habituation of deer mice towards marbles (Figure 2B) is consistent with that published previously (Thomas et al., 2009). Combining this observation with results demonstrating that *all* deer mice exhibit burying behavior (Table 2), supports the hypothesis that novel, but harmless and non-reactive objects are devoid of anxiogenic properties and that the MB test more appropriately resembles investigative, rather than anxious behavior (Londei et al., 1998). Furthermore, since MB behavior varies between species (Nicolas et al., 2006) and that IBB is displayed in the majority of deer mice across both behavioral cohorts (N and H), these behaviors may be a manifestation of normal, and not pathological, activity within the species, supporting the demonstration that chronic treatment with escitalopram failed to affect MB behavior in these animals (Figure 3B, C and D).

However, we found that a minority of deer mice exhibit a characteristically different phenotype of burying behavior that we refer to as high burying behavior (HBB). HBB could be statistically and behaviorally distinguished from IBB, and was expressed in only 4 of the 36

animals (2N, 2H, see Table 2, Figures 3C, D). This is based on the following: First, a strong negative correlation was demonstrated between the average number of marbles buried over all trials and the coefficients of variance calculated from the daily burying scores (Figure 3A). As such, HBB is statistically distinguishable from IBB in that 1) the average pre- and post-treatment (as well as the combined) HBB values fall within the upper quartile of burying distribution and deviate with more than twice the standard deviation from the mean IBB score (Figure 3A, Table 2), while such behavior demonstrates very little inter-trial variability (respective co-efficients of variance are contained within the lower quartile of distribution; Figure 3A). Secondly, animals expressing HBB *completely* buried the counted marbles underneath 5cm of sawdust by means of digging. As such, if face validity is considered, the phenotypical presentation of HBB behavior displayed by a small number of deer mice is characteristically different from the IBB expressed by the larger group for which the 2/3 buried criterion could be applied. Furthermore, as with IBB, HBB did not demonstrate any exclusive association with either the H or N cohort, was not a result of neophobia and did not respond to chronic high dose escitalopram treatment. Although we assumed HBB to be representative of compulsive behavior, given the strengths of the deer mouse model to emulate the bio-behavioral and treatment response characteristics of OCD, and that HBB behavior was demonstrated in both H and N animals, these findings raise the question as to what the behavioral and clinical significance of HBB may be. As such, HBB may represent a unique within-species burying phenotype that may resemble another behavioral manifestation that is related to perseverative behavior, i.e. hoarding. Indeed, this idea is supported by the delineation of hoarding disorder in DSM-5 (Morein-Zamir et al., 2014). Moreover, a recent study (Torres-Lista et al., 2015) demonstrated that only the number of completely buried, and not horizontally displaced or partly buried marbles, positively correlated with increased anxiety in the open field test, supporting our view that HBB may be related to a different neuropsychological construct, as compared to IBB.

That N deer mice display increased baseline locomotor activity compared to H animals (Figure 4A) may be attributable to the manner in which locomotor activity is scored. As H deer mice express significantly more vertical activity than N animals, they spend less time in contact with the horizontal beams (responsible for recording locomotor behavior) than N animals. While previous investigations in our laboratory have shown decreased locomotor activity following chronic escitalopram treatment in the N cohort only (Wolmarans et al., 2013), the present data demonstrates a post-treatment decrease in locomotor activity in both N and H cohorts.

Methodological differences, such as the duration of study (12 vs. 8 weeks) could be responsible, producing an age-related downward shift in locomotor activity. However, as no changes in the expression of either IBB or HBB following treatment could be demonstrated, it is unlikely that changes in locomotor activity could have contributed in any meaningful way.

In summary, the present work has broadened our understanding of the behavioral phenotypes that may be represented by MB behavior. Findings from the current investigation demonstrate that while all deer mice express MB behavior, such behavior is not subject to habituation and does not respond to chronic high dose escitalopram treatment. As such, we failed to demonstrate the relevance of MB behavior as a screening test for either neophobia or compulsive-like behavior in deer mice, and suggest that such behavior is primarily investigative in nature. Furthermore, we identified a different within-species burying phenotype (HBB) that is displayed by a small minority of deer mice and that requires further investigation. Burying behavior is a natural phenomenon inherent to most rodent species, while it requires experimental and methodological manipulation, such as pre-test restraint (Kedia & Chattarji, 2014) or correlations with other behavioral tests (Londei et al., 1998; Torres-Lista et al., 2015), to be a useful screening tool for either anxiety or compulsivity. Using a known OCD translational model has identified the inherent shortcomings of the MB test for OC behavior, although similar studies should also be undertaken in other translational models (Joel & Avisar, 2001; Szechtman et al., 2001), as well as in the promising range of genetic models currently available (Berridge, Aldridge, Houchard, & Zhuang, 2005; Chou-Green, Holscher, Dallman, & Akana, 2003; Shanahan et al., 2009). Given the inherent tendency of rodents to bury, we strongly argue against drawing conclusions relating to anxiety and compulsive-like behavior from a test that is dependent on MB behavior alone. Our findings further support the conclusion of De Boer and Koolhaas (2003) that as far as face or predictive validity is concerned, MB behavior on its own is not a valid model for either anxiety or compulsive-like behavior.

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Conflicts of interest

The authors declare that over the past three years, Dan Stein has received research grants and/or consultancy honoraria from AMBRF®, Biocodex®, Cipla®, Lundbeck®, National Responsible Gambling Foundation, Novartis®, Servier®, and Sun®. In the past three years, Brian Harvey has participated in advisory boards and received honoraria from Servier®, and has received research funding from Servier® and Lundbeck®. The authors declare that, except for income from the primary employer and research funding to BHH from the South African Medical Research Council, and the above-mentioned exceptions, no financial support or compensation has been received from any individual or corporate entity over the past three years for research or professional services, and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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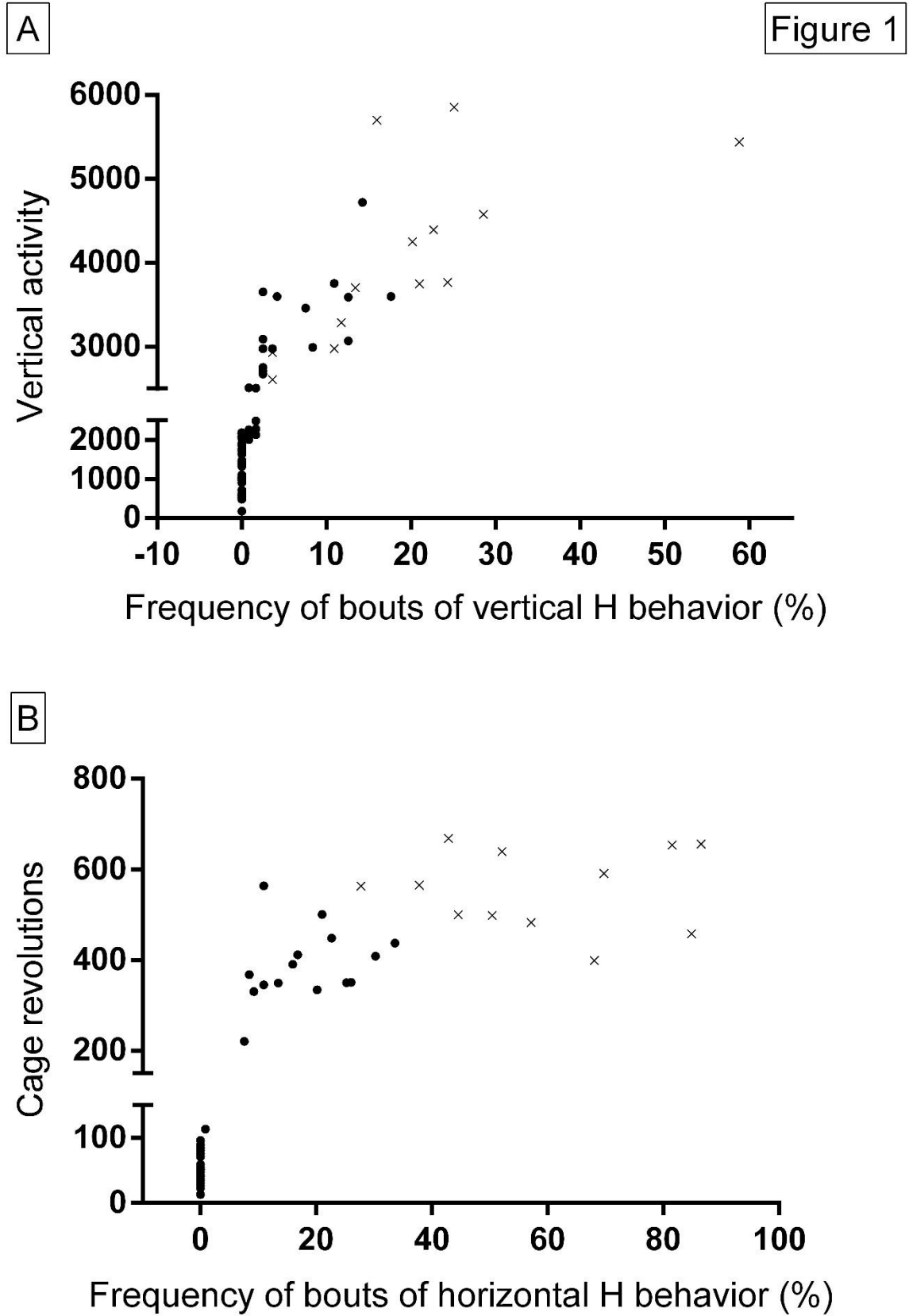


Figure 2

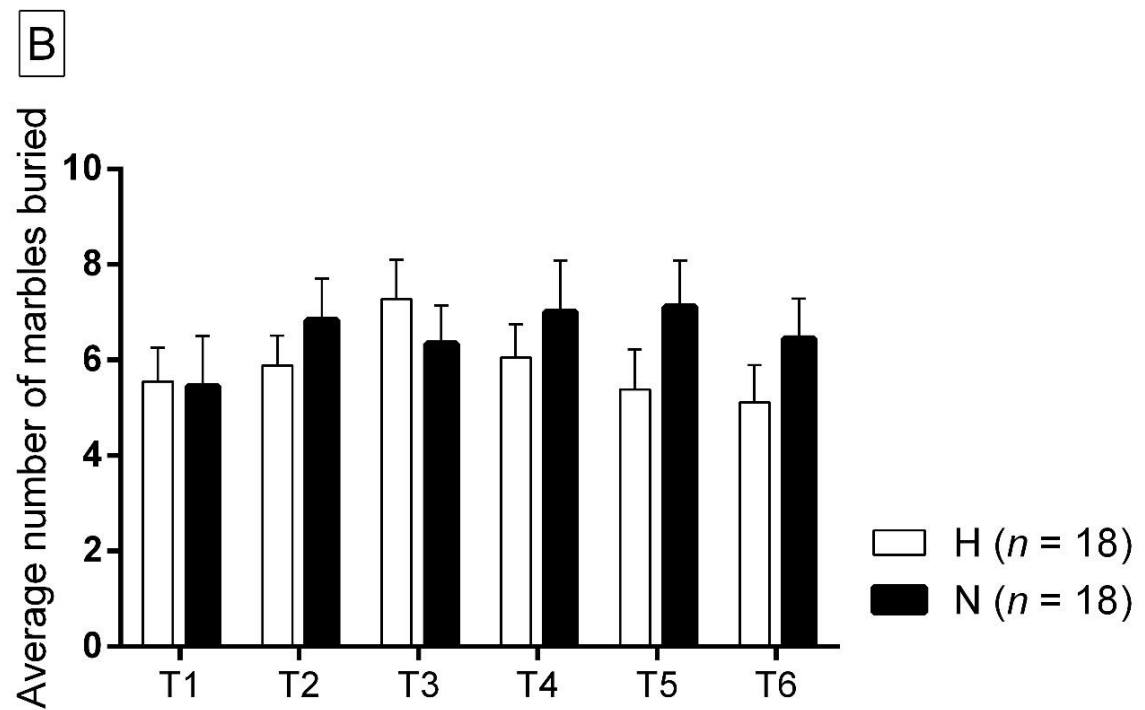
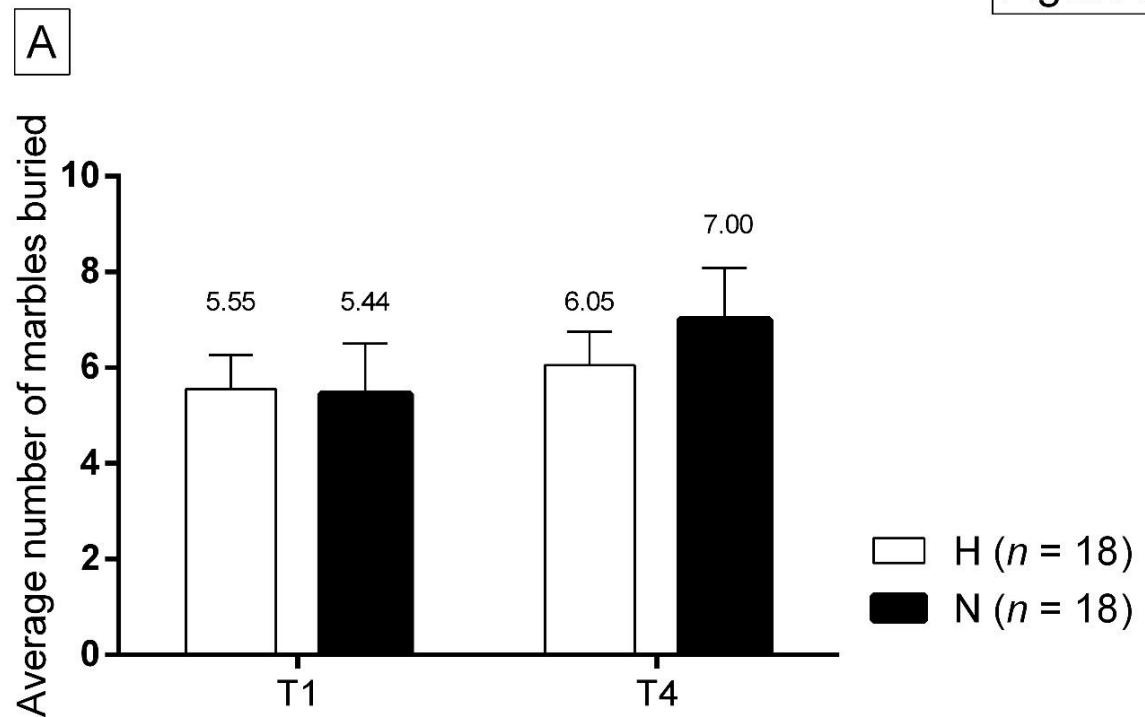
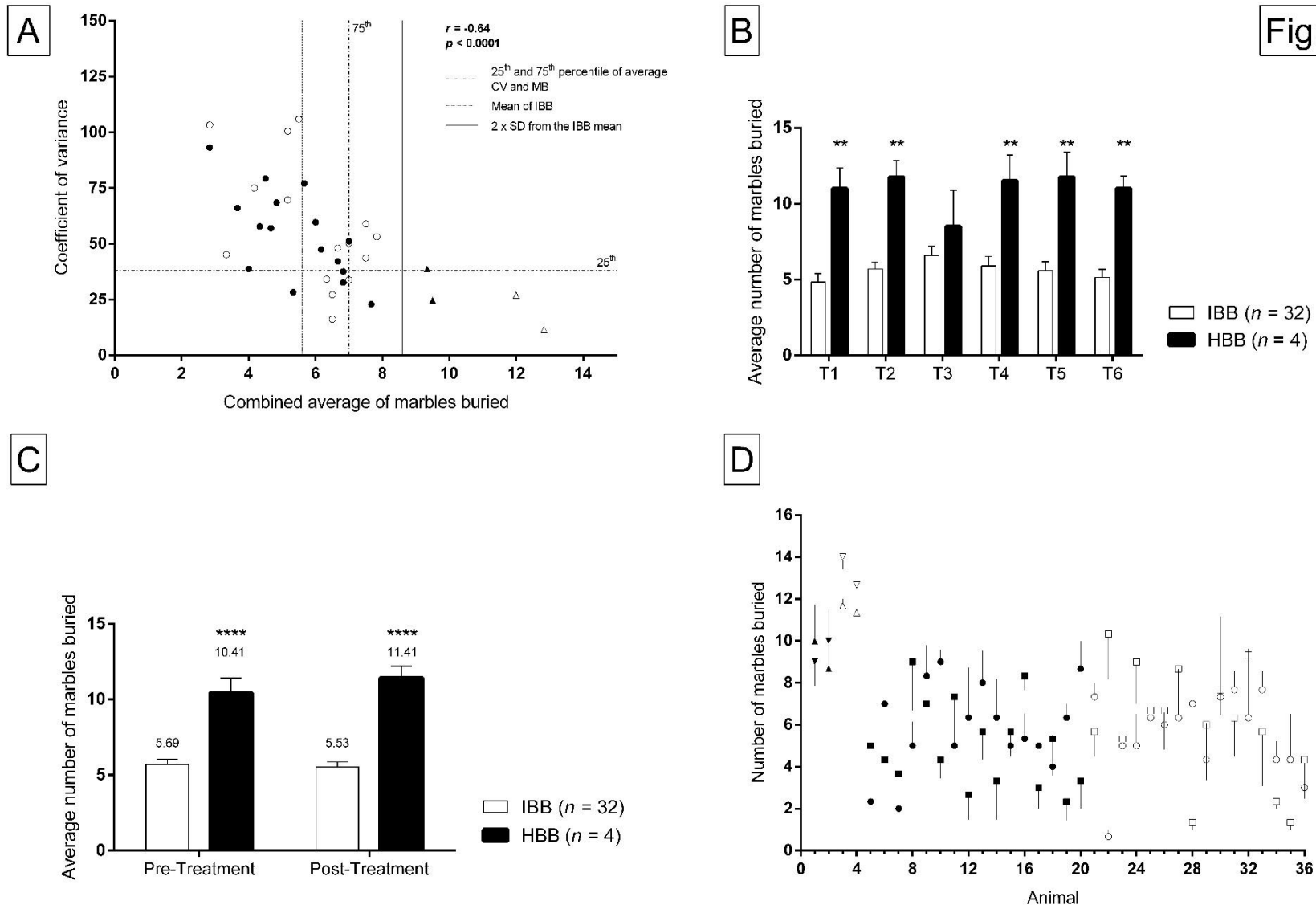


Figure 3



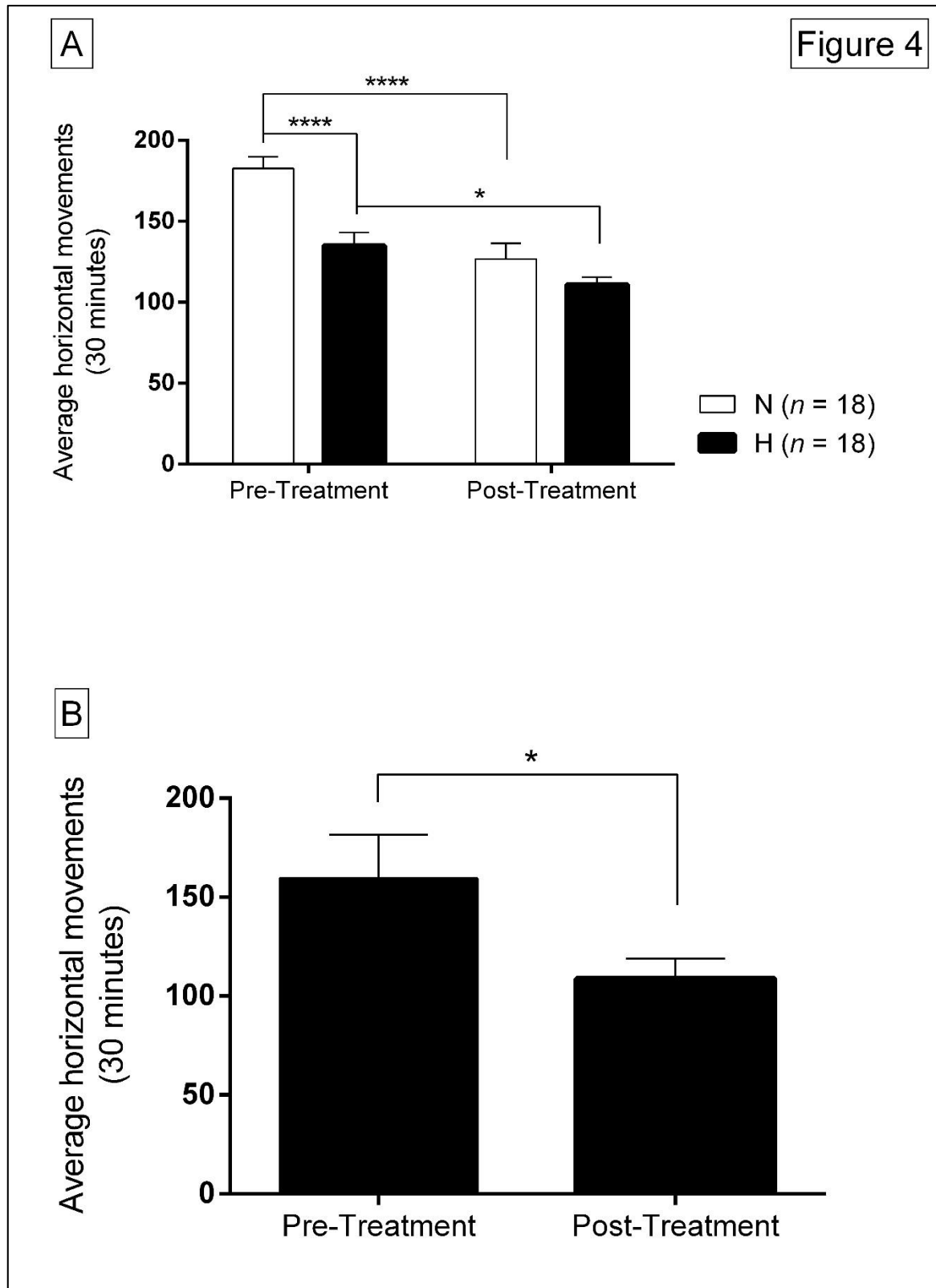


Table I

Stereotypy Intensity	Vertical beam inter- ruptions / 30 min	Cage revolutions / 30 min
N	< 500	< 150
H	> 2000	> 200

Criteria for defining N and H intervals of stereotypical activity with respect to vertical activity and cage revolutions

N: non-stereotypical; H: high stereotypical

Table 2

Animal	Behavioral Cohort	Pre-Treatment Average number of mar- bles buried	Post-Treatment Average number of mar- bles buried	Combined Average number of mar- bles buried
1	H	10.0	9.0	9.5
2	H	8.7	10.0	9.3
3	N	11.7	14.0	12.8
4	N	11.3	12.7	12.0
Average		10.4	11.4	10.9
5	H	2.3	5.0	3.7
6	H	7.0	4.3	5.7
7	H	2.0	3.7	2.8
8	H	5.0	9.0	7.0
9	H	8.3	7.0	7.7
10	H	9.0	4.3	6.7
11	H	5.0	7.3	6.2
12	H	6.3	2.7	4.5
13	H	8.0	5.7	6.8
14	H	6.3	3.3	4.8
15	H	5.0	5.7	5.3
16	H	5.3	8.3	6.8
17	H	5.0	3.0	4.0
18	N	4.0	5.3	4.7
19	N	6.3	2.3	4.3
20	N	8.7	3.3	6.0
21	N	7.3	5.7	6.5
22	N	0.7	10.3	5.5
23	N	5.0	5.3	5.2
24	N	5.0	9.0	7.0
25	N	6.3	6.7	6.5
26	N	6.0	6.7	6.3
27	N	6.3	8.7	7.5
28	N	7.0	1.3	4.2
29	N	4.3	6.0	5.2
30	N	7.3	7.7	7.5
31	N	7.7	6.3	7.0
32	N	6.3	9.3	7.8
33	N	7.7	5.7	6.7
34	N	4.3	2.3	3.3
35	N	4.3	1.3	2.8
36	N	3.0	4.3	3.7
Average		5.7	5.5	5.6
Standard Deviation		2.0	2.4	1.5

Average pre-treatment, post-treatment and combined number of marbles buried

Animals 1 – 4 express HBB. Animals 5 – 36 express IBB. Average pre- and post-treatment (and combined) marble burying values for animals 1 – 4 are more than 2 standard deviation units higher from the average of animals expressing IBB in each respective phase of the study.

FIGURE CAPTIONS**Figure 1 – Stereotypy intensity vs. frequency of high stereotypical bouts**

1A: Mean of fifteen highest individual daily vertical activity scores over the first five baseline behavioral screens vs. frequency of bouts of H activity. **1B:** Mean of fifteen highest individual daily cage revolution scores over the first five baseline behavioral screens vs. frequency of bouts of H activity. Crosses indicate H animals.

H: high stereotypical animals; N: non-stereotypical animals.

Figure 2 – Comparisons between MB behavior of H and N deer mice

2A: Comparison of the average number of marbles buried during the first pre-treatment (T1) and first post-treatment (T4) trials respectively.

2B: Inter-trial comparisons of the average number of marbles buried (T1 – T6).

H: high stereotypical animals; N: non-stereotypical animals.

Data represented as mean $\pm$ SEM

Figure 3 – Comparisons between the MB behavior of animals expressing HBB and IBB respectively

3A: Coefficients of variance with respect to daily burying scores against the combined average number of marbles buried by each individual over all trials; closed triangles: H animals expressing HBB; open triangles: N animals expressing HBB; closed dots: H animals expressing IBB; open dots: N animals expressing IBB; CV: coefficient of variance; SD: standard deviation.

3B: Inter-trial comparisons of the average number of marbles buried (T1 – T6), $**p < 0.005$.

3C: Comparison of the average number of marbles buried over the three pre-treatment (T1 – T3) and three post-treatment (T4 – T6) trials respectively, $****p < 0.0005$.

3D: Average pre- and post-treatment number of marbles buried by individual animals; closed triangles: H animals expressing HBB; open triangles: N animals expressing HBB; upward triangles: pre-treatment value; downward triangles: post-treatment value; closed squares and dots: IBB of H animals; open squares and dots: IBB of N animals; dots: pre-treatment; squares: post-treatment.

H: high stereotypical animals; N: non-stereotypical animals.

Data represented as mean $\pm$ SEM

Figure 4 – Comparisons of pre- and post-treatment locomotor activity

4A: Pre- and post-treatment locomotor activity of N and H animals, **** $p < 0.0005$, * $p < 0.05$; H: high stereotypical animals; N: non-stereotypical animals.

4B: Pre- and post-treatment locomotor activity of animals expressing HMB behavior, * $p < 0.05$

H: high stereotypical animals; N: non-stereotypical animals.

Data represented as mean $\pm$ SEM

SUPPLEMENTARY DATA (as referred to in-text)*Number of pre- and post-treatment number of marbles buried*

Group	Mouse	Gender	T1	T2	T3	T4	T5	T6	Number	Pre-Treatment (T1 - T3)			Post-Treatment (T4 - T6)			Pre-test and post-test combined		
										Average	Standard deviation	Coefficient of variance	Average	Standard deviation	Coefficient of variance	Average	Standard deviation	Coefficient of variance
H	63	F	7	10	13	11	7	9	1	10.0	3.0	30.0	9.0	2.0	22.2	9.5	2.3	24.7
H	64	M	12	11	3	7	12	11	2	8.7	4.9	56.9	10.0	2.6	26.5	9.3	3.6	38.7
N	3	M	12	11	12	15	14	13	3	11.7	0.6	4.9	14.0	1.0	7.1	12.8	1.5	11.5
N	15	M	13	15	6	13	14	11	4	11.3	4.7	41.7	12.7	1.5	12.1	12.0	3.2	26.9
H	52	F	3	2	2	5	2	8	5	2.3	0.6	24.7	5.0	3.0	60.0	3.7	2.4	66.1
H	18	F	7	4	10	1	1	11	6	7.0	3.0	42.9	4.3	5.8	133.2	5.7	4.4	77.1
H	38	F	2	3	1	8	1	2	7	2.0	1.0	50.0	3.7	3.8	103.3	2.8	2.6	93.2
H	46	F	3	5	7	9	13	5	8	5.0	2.0	40.0	9.0	4.0	44.4	7.0	3.6	51.1
H	50	F	8	6	11	7	7	7	9	8.3	2.5	30.2	7.0	0.0	0.0	7.7	1.8	22.8
H	56	F	9	10	8	4	3	6	10	9.0	1.0	11.1	4.3	1.5	35.3	6.7	2.8	42.1
H	62	F	1	5	9	8	6	8	11	5.0	4.0	80.0	7.3	1.2	15.7	6.2	2.9	47.5
H	2	M	3	5	11	5	2	1	12	6.3	4.2	65.7	2.7	2.1	78.1	4.5	3.6	79.2
H	5	M	3	10	9	7	7	3	13	8.0	2.6	33.1	5.7	2.3	40.8	6.8	2.6	37.5
H	25	M	4	5	10	7	1	2	14	6.3	3.2	50.8	3.3	3.2	96.4	4.8	3.3	68.5
H	28	M	4	5	6	8	5	4	15	5.0	1.0	20.0	5.7	2.1	36.7	5.3	1.5	28.2
H	31	M	3	6	7	9	9	7	16	5.3	2.1	39.0	8.3	1.2	13.9	6.8	2.2	32.6
H	33	M	5	5	5	2	5	2	17	5.0	0.0	0.0	3.0	1.7	57.7	4.0	1.5	38.7
H	42	M	7	3	2	8	6	2	18	4.0	2.6	66.1	5.3	3.1	57.3	4.7	2.7	57.0
H	57	M	7	5	7	1	4	2	19	6.3	1.2	18.2	2.3	1.5	65.5	4.3	2.5	57.8
H	65	M	10	6	10	2	6	2	20	8.7	2.3	26.6	3.3	2.3	69.3	6.0	3.6	59.6
N	7	F	8	6	8	4	5	8	21	7.3	1.2	15.7	5.7	2.1	36.7	6.5	1.8	27.1
N	8	F	0	1	1	13	12	6	22	0.7	0.6	86.6	10.3	3.8	36.6	5.5	5.8	105.9
N	19	F	1	0	14	3	5	8	23	5.0	7.8	156.2	5.3	2.5	47.2	5.2	5.2	100.5
N	44	F	7	6	2	5	11	11	24	5.0	2.6	52.9	9.0	3.5	38.5	7.0	3.5	50.3
N	59	F	6	7	6	7	5	8	25	6.3	0.6	9.1	6.7	1.5	22.9	6.5	1.0	16.1
N	60	F	5	7	6	9	3	8	26	6.0	1.0	16.7	6.7	3.2	48.2	6.3	2.2	34.1
N	4	M	4	11	4	11	9	6	27	6.3	4.0	63.8	8.7	2.5	29.0	7.5	3.3	43.6
N	16	M	7	7	7	2	1	1	28	7.0	0.0	0.0	1.3	0.6	43.3	4.2	3.1	75.0
N	22	M	1	5	7	11	5	2	29	4.3	3.1	70.5	6.0	4.6	76.4	5.2	3.6	69.7
N	24	M	15	3	4	10	7	6	30	7.3	6.7	90.8	7.7	2.1	27.2	7.5	4.4	58.9
N	29	M	6	8	9	4	10	5	31	7.7	1.5	19.9	6.3	3.2	50.8	7.0	2.4	33.8
N	30	M	0	11	8	11	10	7	32	6.3	5.7	89.8	9.3	2.1	22.3	7.8	4.2	53.2
N	34	M	6	9	8	1	6	10	33	7.7	1.5	19.9	5.7	4.5	79.6	6.7	3.2	48.1
N	47	F	3	6	4	3	2	2	34	4.3	1.5	35.3	2.3	0.6	24.7	3.3	1.5	45.2
N	17	M	0	6	7	1	1	2	35	4.3	3.8	87.4	1.3	0.6	43.3	2.8	2.9	103.3
N	26	M	4	4	1	3	8	2	36	3.0	1.7	57.7	4.3	3.2	74.2	3.7	2.4	66.1
Green: Animals expressing HBB behavior																		
H: High-stereotypical animals																		
N: Non-stereotypical animals																		
T1 - T3: Number of marbles buried over the three respective pre-treatment trials																		
T4 - T6: Number of marbles buried over the three respective post-treatment trials																		
Data supplements 'Discussion' and Figure 3C and 3D demonstrating IBB in all animals that manifests as inherent, but varying behavior.																		
The average number of pre-, post-, and combined marbles buried by animals highlighted in green (HBB) is more than two STD DEV units higher than the averages of animals expressing IBB (in white)																		

H: high stereotypical; N: non-stereotypical; F: female; M: male, Green Field: Animals expressing HBB; T1 – 6: Individual MB trials

5 Manuscript C

Article accepted for publication in *Journal of Psychopharmacology* titled:

“Excessive nest building is a unique behavioural phenotype in the deer mouse model of obsessive-compulsive disorder”

AUTHOR CONTRIBUTIONS

- *De Wet Wolmarans* designed the investigation in consultation with *Brian H Harvey*, performed all behavioural, pharmacological, and statistical analyses, wrote the first version of the manuscript, and edited the manuscript following input from the co-authors.
- *Dan J Stein* acted as clinical consultant with respect to experimental design and manuscript review.
- *Brian H Harvey* was study supervisor, assisted in the interpretation of results and was corresponding author for submission of the final manuscript to *Journal of Psychopharmacology*.

IMPORTANT INFORMATION

- Instructions to the author are included in Addendum A.
- The published article is provided in PDF format in Addendum G.
- As per the instructions to the author, figures, tables, and legends are provided at the end of the manuscript.
- Both co-authors provided consent for the paper to be assessed as part of the Ph.D. thesis of *De Wet Wolmarans* (Addendum B).
- The submitted manuscript has been received by *Journal of Psychopharmacology* and is currently under review (see Addendum for proof of submission).

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Excessive nest building is a unique behavioural phenotype in the deer mouse model of obsessive-compulsive disorder

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Abstract

Obsessive-compulsive disorder (OCD) is a phenotypically heterogeneous condition characterized by time-consuming intrusive thoughts and/or compulsions. Irrespective of the symptom type diagnosed, the severity of OCD is characterized by heterogeneity in symptom presentation that complicates diagnosis and treatment. Heterogeneity of symptoms would be invaluable in an animal model. Nest building (NB) behaviour forms part of the normal behavioural repertoire of rodents that demonstrate profound between-species differences. However, it has been proposed that within-species differences in NB behaviour (i.e. aberrant vs normal NB) may resemble OC-like symptoms. In an attempt to investigate whether other OC-like behaviours are present in an animal model of OCD, or if aberrant NB behaviour may represent a unique OC phenotype in such a model, the current study assessed NB behaviour in high (H, viz. OC) and non- (N, viz. normal) stereotypical deer mice. Subsequently, 12 N and H animals respectively were provided with an excess of cotton wool daily for one week prior to and following four weeks of high-dose oral escitalopram treatment (50 mg/kg/day). Data from the current investigation demonstrate daily nesting activity to be highly variable in deer mice, with stereotypy and nest-building being independent behaviours. However, we identified unique aberrant large NB behaviour (LNB) in 30% of animals from both cohorts, while such behaviour was attenuated by escitalopram to pre-treatment nesting scores of the

larger group. In summary, behavioural and drug-treatment evidence confirms that deer mouse behaviour does indeed resemble symptom heterogeneity related to OCD, and as such expands its face and predictive validity for the disorder.

Introduction

Obsessive-compulsive disorder (OCD) has a lifetime prevalence of 1 – 3% (Stein, 2002) and is characterized by recurrent intrusive thoughts (obsessions) and rigid repetition of certain behaviours (compulsions) (American Psychiatric Association, 2013). Furthermore, being clinically heterogeneous, OCD is characterized by a range of phenotypically different symptom types (Williams et al., 2013). Four major obsessive-compulsive symptom dimensions have been described viz. 1) contamination obsessions and washing compulsions, 2) responsibility for harm obsessions and checking compulsions, 3) symmetry obsessions and ordering compulsions, and 4) unacceptable thoughts (e.g., sexual, religious, or aggressive in nature) and neutralizing compulsions (e.g., thought suppression) (Abramovitch and Cooperman, 2015). Irrespective of the symptom dimensions identified, a diagnosis of OCD is based on the time-consuming nature of such symptoms and how their severity impacts the time spent on, degree of resistance to, and control over these behaviours (American Psychiatric Association, 2013).

Animal models of OCD have enjoyed a great deal of interest recently (Alonso et al., 2015; Hoffman, 2011). However, most if not all these models centre around one particular aspect of the behavioural phenomenology of OCD, such as non-goal-directed stereotypy, excessive grooming, or exaggerated nest building. Considering the heterogeneity of OCD, an animal model that typifies a range of phenotypes that may separate it from other known stereotypic disorders, i.e. autism and tics would be invaluable (Cagnin et al., 2014; Edwards et al., 2004; Ghanizadeh, 2010). Spontaneous stereotypy in the deer mouse (*Peromyscus maniculatus bairdii*) is a validated naturalistic animal model of OCD (Alonso et al., 2015; Hoffman, 2011; Wolmarans et al., 2013). Deer mouse stereotypy can be categorized into repetitive jumping and pattern running (Powell et al., 1999) that correspond with the repetitive motor actions of OCD. Furthermore, as in human OCD the model demonstrates varying symptom intensity (i.e. high (H) vs. non (N)-stereotypic), and employs ‘time spent executing stereotypy’ as a marker of behavioural severity and treatment outcome (Wolmarans et al., 2013). The predictive validity of the model is founded on its response to chronic but not sub-chronic treatment with high doses of a selective serotonin reuptake inhibitor (SSRI) (Wolmarans et al., 2013) and its lack of response to noradrenergic antidepressants (Korff et al., 2008), as are

evident in OCD (Fineberg and Craig, 2007). Concerning construct validity, neurobiology studies have described the association of H behaviour with altered cortico-striatal cyclic adenosine monophosphate (cAMP) / phosphodiesterase-4 (PDE4) signalling (Korff et al., 2009), purported to be linked to SSRI response (El Mansari and Blier, 2006; Goddard et al., 2008), and decreased striatal SERT density (Wolmarans et al., 2013), a finding described in OCD (Reimold et al., 2007; Zitterl et al., 2008).

In the wild, mice and other rodents build nests for protection against the elements and predators, and to provide a nursery to breed and raise young (Smithers, 1983). Under laboratory conditions, mice build nests to shelter from harsh lighting, low ambient temperatures and human handling (Jirkof, 2014). As such, motivation for nesting is high and nest building (NB) behaviour is routinely assessed as a measure of general well-being of laboratory-housed mice (Jirkof, 2014). Although nesting forms part of the normal behavioural repertoire of mice, between-species differences in nest size, structure and locale are profound (Gaskill et al., 2013). However, nesting behaviour can also demonstrate within-species variance, and it has been suggested that large nest building (LNB) behaviour may represent compulsiveness (Greene-Schloesser et al., 2011; Li et al., 2006). Indeed, LNB has been associated with increased marble burying behaviour (MB), a measure of compulsiveness, while chronic high-dose fluoxetine significantly attenuates BIG nest building in mice (Greene-Schloesser et al., 2011).

Considering that nesting is a normal behavioural routine in rodents, but that aberrant LNB in animal behaviour may represent a different phenotype of OCD, the current investigation assessed NB behaviour in an established animal model of OCD. The aims were to establish 1) if within-species differences in nesting behaviour can be demonstrated, irrespective of the stereotypical cohort (viz. can deer mouse behaviour in general be representative of different OC-phenotypes?), and 2) whether potential differences in nesting behaviour are sensitive to chronic treatment with high-dose oral escitalopram (50 mg/kg/day).

Materials and methods

Animals

60 deer mice were obtained from the deer mouse colony maintained and housed at the vivarium of North-West University (NWU), Potchefstroom, South Africa (Ethical Approval

Number - NWU-00066-10-S5). The original colony was established using breeding pairs obtained from the *Peromyscus* Genetic Stock Centre at the University of South Carolina. As reported previously (Powell et al., 1999) stereotypical behaviour in the deer mouse is established at the age of 8 weeks and only mice between the ages of 10 and 12 weeks were chosen as experimental subjects at the onset of behavioural assessments (day 0). Mice were randomly chosen from different litters, without sex or weight bias and housed in groups of 6 same-sex animals per cage. Earlier studies have already noted that the severity of stereotypy in deer mice is independent of sex (Hadley et al., 2006; Powell et al., 1999; Presti and Lewis, 2005; Wolmarans et al., 2013). One week before the start of the behavioural assessments, each animal was allocated individually to an automatic climate-controlled laboratory cage (35cm (l) x 20cm (w) x 13cm (h); Techniplast® S.P.A., Varese, Italy) and maintained at 23°C on a 12-hour light/dark cycle (lights on at 06h00 and off at 18h00). Food and water were provided ad lib for the duration of the study. Cages were cleaned and new bedding material added weekly.

Generating behavioural data and classification of N and H animals

Before assessing nest building behaviour, animals were categorized as N or H according to a previously published protocol (Wolmarans et al., 2013). In short, all animals underwent 12-hour behavioural screening once a week for five consecutive screens. On any specific assessment day, animals were moved from their housing environment to the behavioural screening room. These areas are located on the same floor of the vivarium and are environmentally controlled with respect to temperature (23°C), relative humidity (55%), and light cycle (same as for home cages). Subsequently each animal was introduced to a behavioural test cage (21cm (w) x 21cm (l) x 35cm (h); Accuscan® Inc., Columbus, Ohio, USA) constructed from clear, translucent Plexiglas®. Bedding material was provided in quantities enough to cover the floor of the test cages, but also ensuring that it did not interrupt the scoring of behavioural data. Food was provided ad lib on the floor of the cage in the form of broken-up rodent chow pellets. Bottled water was provided through a tight-fit hole in the wall of each test cage. Cages were covered with lids that allowed uninterrupted airflow. The animals were introduced to these environments by 16h00 and habituated for at least two hours before the 12-hour behavioural assessments started at the onset of the dark cycle. Test cages were cleaned after each screen.

Behavioural assessment was performed using the Fusion® Animal Activity Monitoring System (Accuscan® Inc., Columbus, Ohio, USA), and analysed using Fusion® software. Briefly, each

testing cage presents with a grid of infrared light beams that cross the cage roughly 2 cm above the cage floor along the X- and Y-axes (constituting a grid), while another set of beams (Z-axis) crosses the cage 10 cm above the cage floor, but only parallel to the lower Y-axis. Parallel beams are spaced 2.5 cm apart and record activity every time it is interrupted by movement. The number of vertical beam interruptions (VBI) was used as a broad measure of vertical jumping activity, while the number of clockwise and anti-clockwise cage revolutions (CR) was used to evaluate the expression of pattern running (Wolmarans et al., 2013). During the recording session, the Fusion® software generates data continuously, allowing for experimental playback and the export of behavioural reports the following day.

Following the first five behavioural screens, animals were divided into N and H groups according to our previously published protocol (Wolmarans et al., 2013). As findings from previous studies (Wolmarans et al., 2013) demonstrated the time-consuming nature of stereotypy, we amended the protocol to include both the intensity (average of the highest individual 30-minute stereotypy counts generated during the five respective trials) and frequency (percentage of 30-minute H stereotypical bouts generated over all five trials, viz. time spent executing stereotypy) as indicators of behavioural severity. The criteria for defining vertical and horizontal H intervals are represented in Table 1.

Table 1: *Criteria for defining N and H intervals of stereotypical activity with respect to vertical activity and cage revolutions*

Stereotypy Intensity	Vertical beam interruptions / 30 min	Cage revolutions / 30 min
N	< 500	< 150
H	> 2000	> 200

N: non-stereotypical; H: high stereotypical

This data was plotted for both vertical (Figure 1a) and horizontal activity (cage revolutions; Figure 1b) and, as no distinction was made between the different forms of stereotypy, H animals could demonstrate stereotypical behaviour in either one or both of the behavioural topographies. We subsequently included 12 animals in the H group (Figures 1a and 1b combined; subjects indicated with crosses) that expressed both the highest intensity of stereotypy and the highest frequency of H stereotypical bouts, irrespective of whether an individual did so in only one or both of the topographies (the sum total of crosses from Figures 1a and 1b, i.e. 16, exceeds 12 as more than one animal displayed H behaviour in both topographies). The

group of N animals was simply constituted from the 12 animals that consistently generated the lowest stereotypy counts (Figures **1a** and **1b** combined; black dots plotted at $X = 0$). As only 12 animals were included in each cohort following the first five behavioural screens, a grey margin of 36 animals was excluded from the experimental design. The rationale for the exclusion of a grey margin of animals is to improve sensitivity, as described elsewhere (Presti and Lewis, 2005).

Assessing nest building behaviour

Following the last baseline behavioural screen, nesting behaviour was assessed in the same animals for seven consecutive nights before and after chronic (four-week) treatment with oral escitalopram (50 mg/kg/day). An excess of pre-weighed standard cosmetic cotton wool (non-scented) was introduced in the roof of each home cage every day between 15h00 and 16h00. As mice generally build their nests just before dawn (Jirkof, 2014), the remaining cotton wool was removed and weighed between 13h00 and 14h00 on the following day. Daily nesting scores were expressed in grams of cotton wool utilized and added together for a total nesting score after one week (Greene-Schloesser et al., 2011). Animals did not have access to any other form of nesting material and food and water was supplied as normal.

Assessment of locomotor activity

As stereotypy and locomotor activity was simultaneously recorded by the Fusion® software, data from the last baseline screen (pre-treatment) was compared to that obtained from another screen following four weeks of treatment, performed one night before assessment of post-treatment nest building behavior.

Drugs

As previously reported (Wolmarans et al., 2013), escitalopram oxalate was prepared for oral administration by dissolving it in the drinking water. Physical handling of the mice was kept to a minimum to prevent any possible anxiogenic effects. For the same reason, oral gavage was not considered as an appropriate dosage route. The final concentration of the drinking solution was constituted at 20mg/100ml ensuring that each animal would receive 50 mg/kg/day (within close limits) of escitalopram. The dose calculation was based on a prior pilot study where the average water intake per mouse was determined as 0.25 ml/g/day (data not shown), and confirmed by others (Aschhoff et al., 2000).

Statistics

Statistical analysis was performed using GraphPad® Prism® 6 under guidance of the statistical consultation service of North-West University, Potchefstroom. Two-way analysis of variance (ANOVA) followed by post-hoc testing with Bonferroni corrections was applied to compare the pre- and post-treatment expression of nest building behaviour and locomotor activity both within and between N and H cohorts. Behavioural cohort was set as between subject (independent) factor and time and treatment as within (dependent) subject factors. The same statistics were applied to compare the response of large nest building (LNB) and normal nest-building (NNB) behaviour to treatment. In this case, the nesting cohort was set as between subject factor and time and treatment as within subject factor. Independent unpaired t-tests were applied to compare the baseline expression of normal and large nesting behaviour. Statistical significance was set at $p < 0.05$ for all analyses.

Results

Nesting behaviour in drug-naïve groups

Nest building behaviour in deer mice display within- and between animal variance with respect to daily nesting scores with no apparent differences in pre-treatment nesting behaviour noted between N and H animals (Figure 2a). Despite the lack of association between nesting and stereotypy (Figure 2a), Pearson's correlation revealed a strong negative relationship between the average total pre-treatment nesting scores and the coefficients of variance (CV) relating to the daily nesting scores within the N and H cohorts (Figure 2b: $r(24) = -0.75$, $r^2 = 0.56$, $p < 0.0001$) demonstrating that animals expressing LNB, regardless of severity of stereotypy, displayed less between-day variance, suggesting a more consistent pattern of aberrant behaviour. Subsequently, seven animals (30%; 4H, 3N) were identified that consistently displayed LNB located in the upper quartile of the total nesting score distribution (≥ 25.9 g) and the lower distributed quartile for CV (≤ 33.9) (Figure 2b: encircled data points; photographs submitted as supplementary material). Further comparisons between the average total (Figure 2c) and daily (Figure 2d) nesting scores of animals expressing LNB and NNB respectively, revealed significant differences in pre-treatment nesting behaviour (Figure 2c: 28.95 ± 2.6 vs. 12.35 ± 1.9 , $t(22) = 4.76$, **** $p < 0.0001$; Figure 2d: 3.8 ± 0.2 vs. 1.75 ± 0.17 , $t(147) = 5.90$, **** $p < 0.0001$).

Nesting behaviour following escitalopram treatment

Chronic treatment with high-dose oral escitalopram significantly decreased the expression of LNB with respect to the total (Figure 3a) and daily (Figure 3b) nesting scores with significant interactions being displayed with respect to total nesting score (Figure 3a: $F(1,44) = 4.830$, $p = 0.03$) and daily nesting score (Figure 3b: $F(1,294) = 4.575$, $p = 0.03$). Furthermore, post-hoc analysis demonstrates significantly decreased total (***) $p = 0.0008$) and daily (***) $p = 0.0003$) nesting scores. In fact, without altering locomotor activity (Figure 3ci), the average total (Figure 3a: 15.45 ± 2.85) and daily (Figure 3b: 2.19 ± 0.4) post-treatment nesting scores of animals expressing LNB were found to be indistinct from the respective pre-treatment nesting scores of the group (Figure 3a: 12.35 ± 1.9 ; Figure 3b: 1.8 ± 0.17). While LNB still demonstrated significant post-treatment differences as compared to group NNB with respect to average total and daily nesting scores, these differences are less pronounced (Figure 3a: **** $p < 0.0001$ vs * $p = 0.03$; Figure 3b: **** $p < 0.0001$ vs. ** $p = 0.004$). Furthermore, a concomitant decrease in the locomotor activity of animals expressing NNB (Figure 3ci: $F(1, 42) = 0.87$; 131.8 ± 6.1 vs. 158.6 ± 9.7 , $p = 0.03$) was noted (Figure 3ci: 1.16 ± 0.12 vs. 1.75 ± 0.17 , * $p = 0.01$). The decrease in locomotor activity is only observed in the behaviour of N animals included in this group (Figure 3cii: $F(1, 28) = 1.21$; 136.6 ± 10.6 vs. 173.9 ± 14.9 , $p = 0.03$).

Discussion

The current investigation has demonstrated that 1) 30% of deer mice express a unique phenotype of LNB, and 2) LNB, and not NNB, is sensitive to chronic high-dose oral escitalopram treatment. Noteworthy is that stereotypy and nest-building are independent behaviours, which has significant implications.

OCD is a heterogeneous condition characterized by inter-patient differences in behavioural phenotype, response to treatment, and symptom severity (American Psychiatric Association, 2013; Stein, 2002). Similarly, animal models of OCD propose various behavioural manifestations to represent OC-like behaviour, i.e. increased MB behaviour (Egashira et al., 2008), pattern chewing (Chou-Green et al., 2003), lever pressing (Joel and Avisar, 2001), and cage stereotypy (Szechtman et al., 1998; Wolmarans et al., 2013), yet few have been tested for heterogeneity within the same model. Furthermore, within-species differences in NB behaviour have been suggested to be representative of normal vs. compulsive-like (viz. LNB) behaviour (Greene-Schloesser et al., 2011; Hoffman and Rueda Morales, 2009; Li et al., 2006),

even though between-species differences in nest size and structure are normal (Smithers, 1983). However, LNB as demonstrated in inbred house mice (*Mus musculus*) may more closely resemble compulsivity as it correlates positively with high MB behaviour (a putative measure of OC-behaviour (Thomas et al., 2009)) and demonstrates sensitivity to high dose SSRI treatment (Greene-Schloesser et al., 2011).

The deer mouse presents with time-consuming stereotypy that is attenuated following chronic, but not sub-chronic treatment with high dose escitalopram (Wolmarans et al., 2013). However, the possibility that deer mouse behaviour may represent more than one OC-like phenotype has not yet been established. Therefore, the current investigation assessed NB behaviour in deer mice, bearing in mind that LNB may be representative of an underlying OC phenotype.

Our data revealed that nest building behaviour in deer mice is an independent compulsive-like behaviour, being expressed in both N and H animals, while a unique pattern of nest building behaviour within either cohort could not be demonstrated (Figure 2a). However, a strong negative correlation between total nest size and variance in daily nesting behaviour (Figure 2b) revealed the presence of a unique nest building pattern in 30% of animals over both cohorts, viz. LNB (Figure 2b – d; 4H, 3N). Furthermore, while the NNB expressed by the rest of the group remained mostly unchanged, LNB (in both N and H animals) demonstrated significant attenuation following chronic high-dose escitalopram treatment, with adjustment to the pre-treatment NNB scores of the group (Figure 3a, b). The significant post-treatment decrease in daily nesting scores observed in the behaviour of animals expressing NNB (Figure 3b) was related to a parallel decrease in locomotor activity (Figure 3c i) and as such cannot be suggested as a behavioural response to drug treatment.

The above findings are in line with that observed in OCD, i.e. despite it being a clinically heterogeneous condition, the human disorder responds favourably to chronic SSRI treatment. Since 40% of deer mice are already genetically predisposed to develop OC-like behaviour, LNB may be one of a number of other possible behavioural similarities with OCD that have not yet been identified. It must be emphasized here that LNB also occurs naturally and without any external manipulation. In fact, the persistent and severe nature of LNB and its response to escitalopram treatment, as well as that it serves no apparent unique occupational or practical purpose compared to NNB, establishes the putative face and predictive validity for LNB as resembling a different, but also naturally developed OC-phenotype in addition to

spontaneous stereotypy already well-described in these animals. Furthermore, LNB expression could possibly be related to an altered coping response to environmental challenges (Sluyter et al., 1995) that may manifest as hoarding compulsions, security concerns and “just-right” perceptions that are well-described OC-related behaviours evident in OCD (Leckman et al., 1994).

In conclusion, the current investigation describes symptom heterogeneity in deer mice and has established LNB as a unique OC-phenotype within the behaviour of these animals. Although such behaviours occur in a smaller population of deer mice than does motor stereotypy, it is just as persistent while also responding to chronic SSRI treatment. Furthermore, apart from already being a robust and useful animal model of OCD, within-species manifestations of different OC-like behaviours in the deer mouse may constitute a valuable and useful naturalistic model to investigate the neurobiology, behavioural appearance and pharmacological response of various OC-related phenotypes.

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Conflicts of interest

The authors declare that over the past three years, Dan Stein has received research grants and/or consultancy honoraria from AMBRF®, Biocodex®, Cipla®, Lundbeck®, National Responsible Gambling Foundation, Novartis®, Servier®, and Sun®. In the past three years, Brian Harvey has participated in advisory boards and received honoraria from Servier®, and has

received research funding from Servier® and Lundbeck®. The authors declare that, except for income from the primary employer and research funding to BHH from the South African Medical Research Council, and the abovementioned exceptions, no financial support or compensation has been received from any individual or corporate entity over the past three years for research or professional services, and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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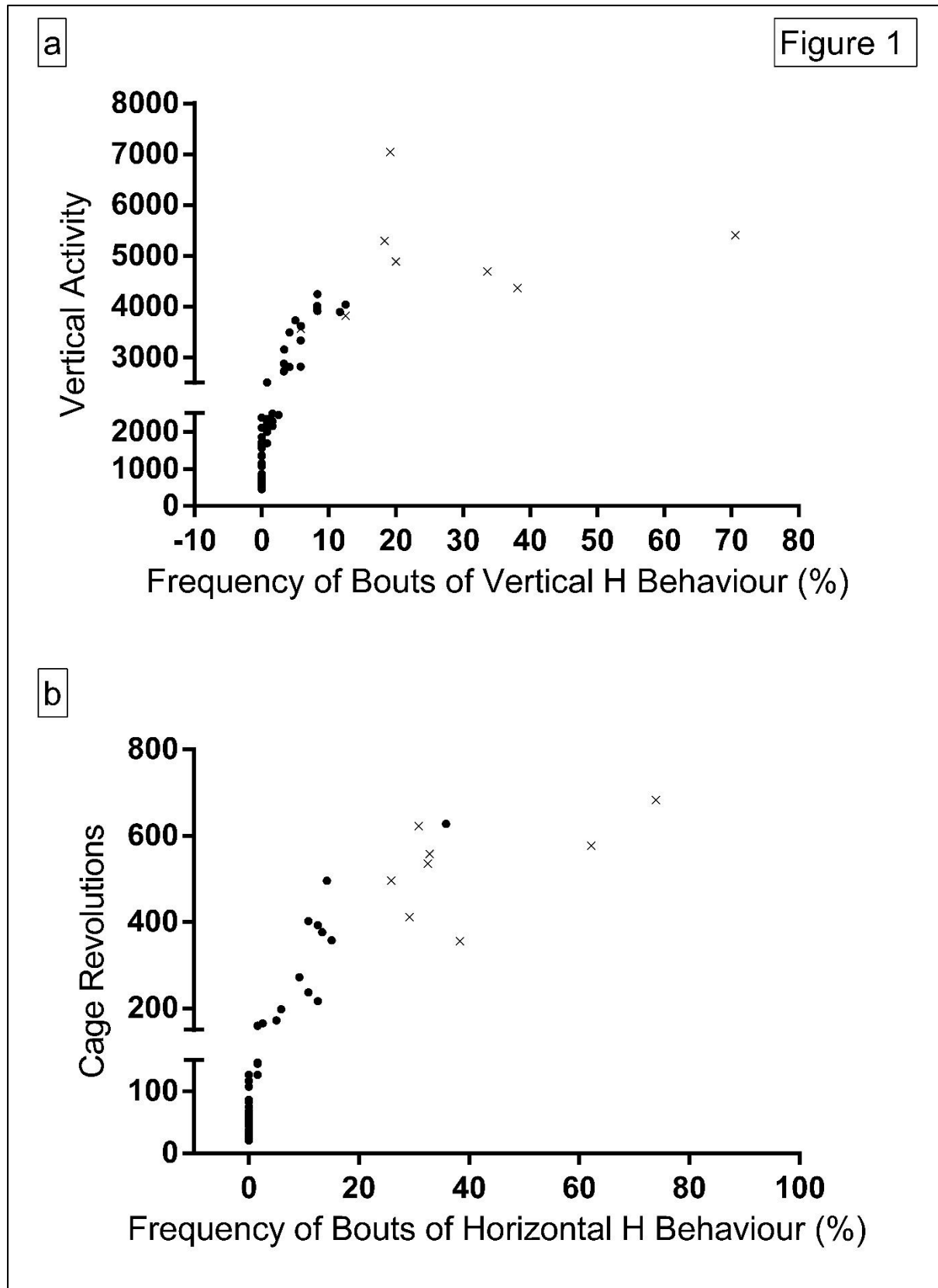


Figure 2

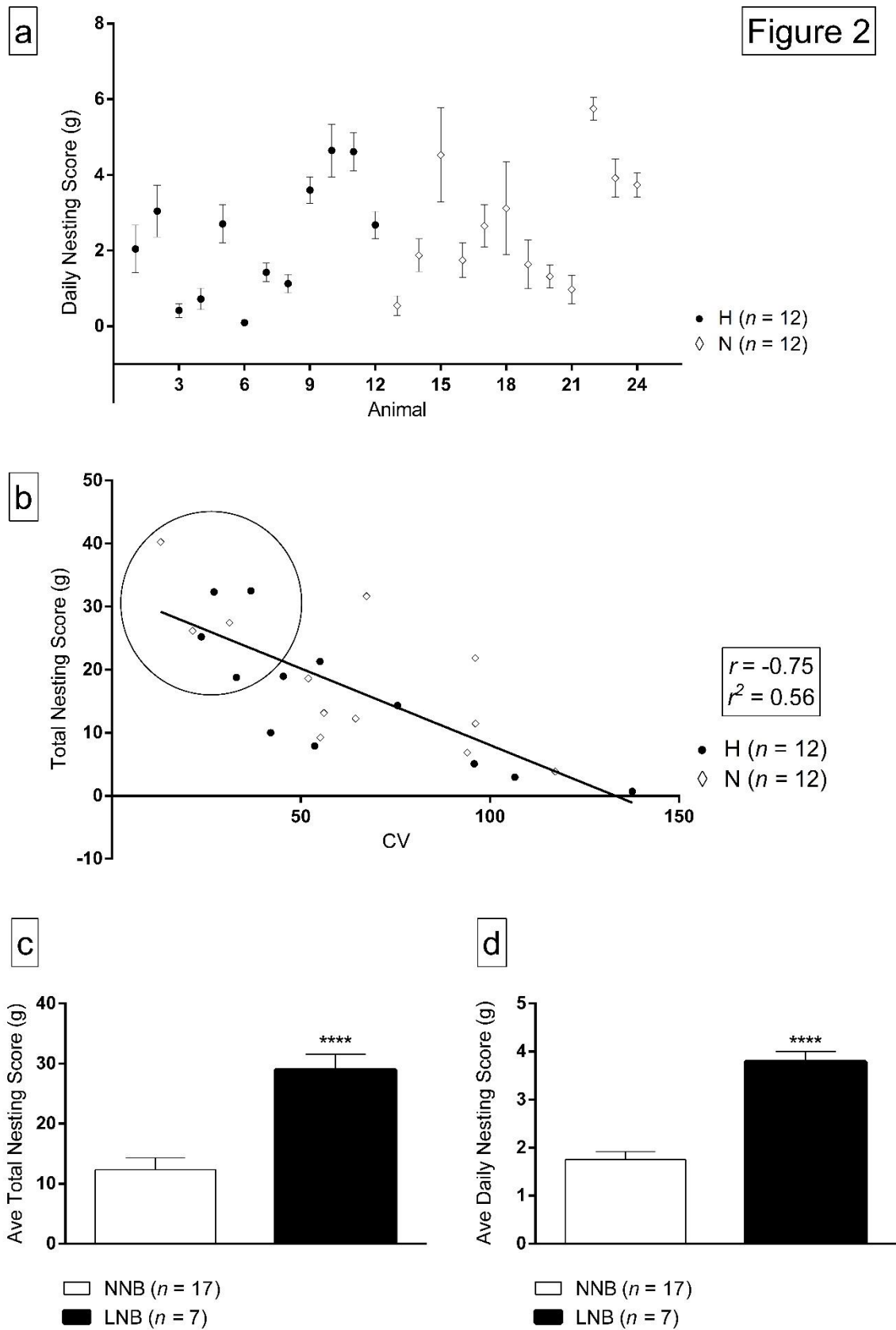


Figure 3

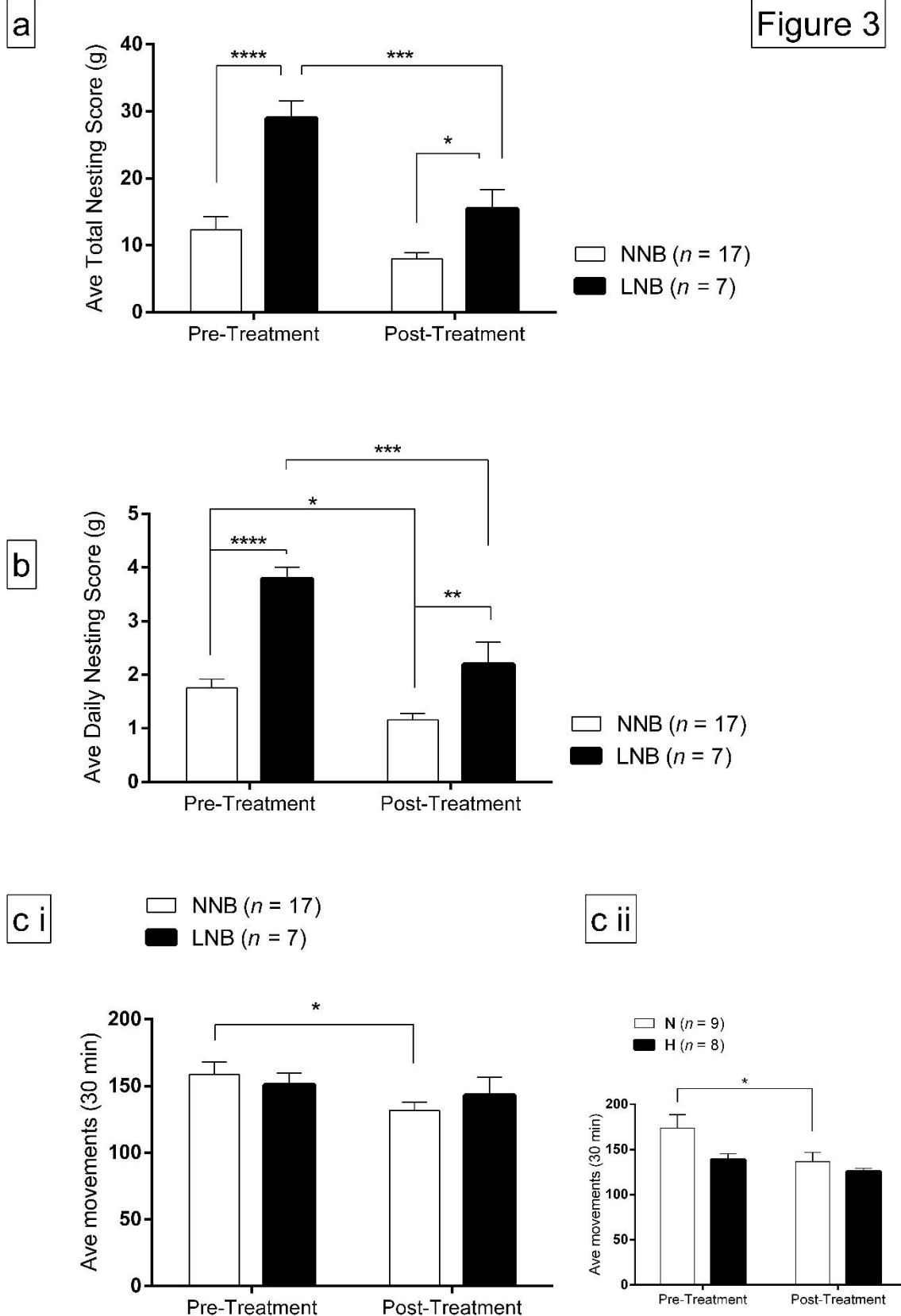


FIGURE CAPTIONS

Figure 1 – Stereotypy intensity vs. frequency of high stereotypical bouts

(a): Mean of highest individual daily vertical activity scores over the first five baseline behavioural screens vs. frequency of bouts of H activity.

(b): Mean of highest individual daily cage revolution scores over the first five baseline behavioural screens vs. frequency of bouts of H activity.

Crosses indicate H animals.

Figure 2 – Expression of pre-treatment nest building behaviour

(a): Average daily nesting scores expressed by animals of the H and N cohorts.

(b): Pearson's correlation between total weekly nesting scores and coefficients of variance (CV) with respect to daily nesting scores.

(c): Comparison of average total weekly nesting scores of animals expressing large nest building (LNB) and normal nest building (NNB) behaviour.

(d): Comparison of average daily nesting scores of animals expressing LNB and NNB.

Data represented as mean $\pm$ SEM

Figure 3 – Response of locomotor activity and nesting behaviour to chronic escitalopram treatment

(a): Average total weekly nesting scores in animals expressing LNB and NNB respectively. **** $p < 0.0001$, *** $p = 0.0008$, * $p = 0.03$.

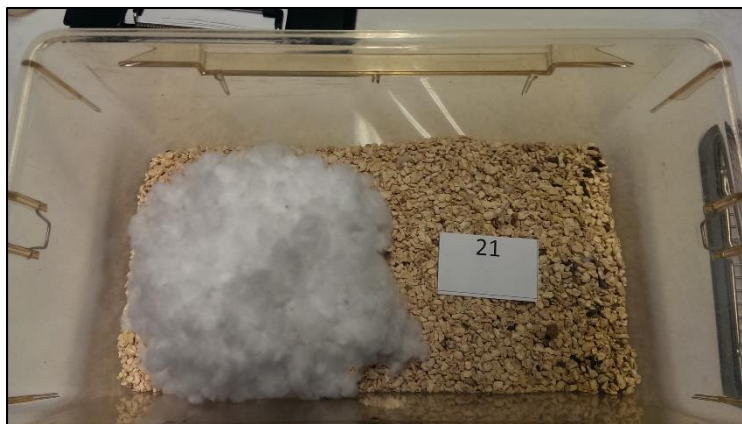
(b): Average individual daily nesting scores in animals expressing LNB and NNB respectively. **** $p < 0.0001$, *** $p = 0.0003$, ** $p = 0.004$, * $p = 0.01$.

(c i): Locomotor activity of animals expressing large nest building (LNB) and normal nest building behaviour (NNB) respectively. * $p = 0.04$.

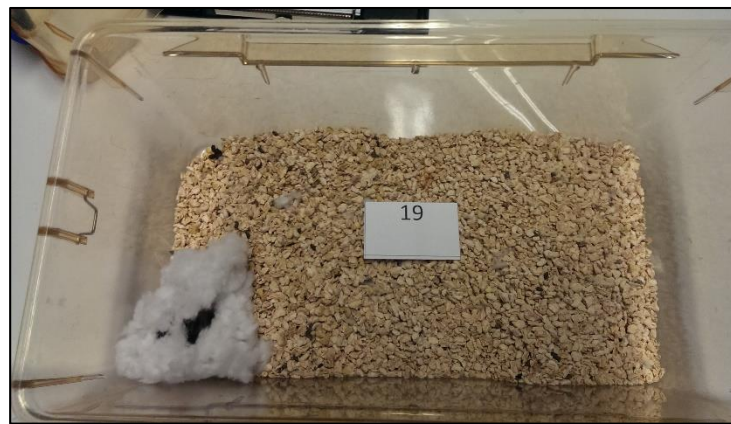
(c ii): Locomotor activity of H and N animals comprising the group of animals expressing normal nesting behaviour (a i). * $p = 0.03$. H: high-stereotypical, N: non-stereotypical.

Data represented as mean $\pm$ SEM

SUPPLEMENTARY DATA



Large nest-building example 1



Normal nest-building example 1



Large nest-building example 2



Normal nest-building example 2

6 Conclusion

The current investigation has strengthened the face and predictive validities of deer mouse stereotypy as a model of OCD by demonstrating significant differences in the social behavior of treatment-naïve H and N animals towards one another as well as towards animals from a different cohort and showing that said differences are responsive to chronic escitalopram treatment. A valuable contribution towards understanding MB as a model of neophobia and compulsivity has been made by confirming that MB is strain specific and manifests as normal, inherent behavior in the deer mouse. Furthermore, MB as expressed by deer mice is an inappropriate measure of neophobia and compulsivity within the current animal model of OCD. Importantly, the face and predictive validities of deer mouse behavior has been broadened by the demonstration that 30% of deer mice present with unique large nest building behavior. This behavior is 1) unrelated to the expression of stereotypy and 2) sensitive to chronic escitalopram treatment and is representative of a different OC phenotype present in deer mice.

* * *

Current findings relating to the social behavior of deer mice (Manuscript A) were realized following the development of a novel social interaction challenge (SIC) designed to observe *group* interactions between H and N animals both within and between behavioral cohorts. Moreover, this is the first study to explore the manifestation of group social interactions between rodents of the same and different behavioral cohorts and how this behavior responds to a typical drug treatment regimen employed in human OCD. The study has demonstrated that distinct differences exist between treatment-naïve behavior of N and H animals within and between cohorts, that N animals tend to interact with one another and not with an H animal following drug treatment, and that treatment increases the sociability of H animals towards one another as compared to an N animal. This is congruent with clinical evidence relating to the social behavior of OCD patients and their social experiences in the presence of healthy peers (Kim, Reynolds, & Alfano, 2012).

The novel methodology developed to observe the manifestation of social interactions between more than two animals makes a valuable contribution to current literature with respect to sociability in rodent models of psychiatric illness. In contrast to the majority of previous investigations between two animals of the same background and cohort (Möller, Du Preez, Emsley, & Harvey, 2011), it was demonstrated that unique patterns of social interaction are

recognized that would otherwise not have been realized. Furthermore, it is evident that as far as animal studies are concerned that focus on aspects of social behavior involving peer relationships and victimization, it is paramount that more complex social interaction paradigms be followed.

* * *

Based on findings from previous investigations into MB as a putative model of anxiety-like (*viz.* neophobia) and OC behavior (Egashira *et al.*, 2008; Nicolas, Kolb, & Prinssen, 2006) and given the heterogeneous nature of OCD, we quantified MB behavior in deer mice to assess 1) if H stereotypy is related to anxiety-like behavior or 2) whether H stereotypy can be associated with a different OC phenotype. In line with literature demonstrating MB to be a highly strain-specific behavioral phenomenon (Nicolas *et al.*, 2006), it was found that MB is inherent to the behavior of all deer mice across both cohorts and manifests as *normal* and *spontaneous* behavior without an apparent underlying neuropsychological construct (*viz.* inherent burying behavior, IBB). Furthermore, chronic escitalopram treatment failed to influence IBB supporting the argument that MB expressed by deer mice is a normal behavioral manifestation within the species with an investigative purpose. As such we propose that MB cannot be applied as a measure of either anxiety or compulsivity in the deer mouse model of OCD (Manuscript B). Furthermore, we recommend that its application as a measure of OCD-like behavior in a translational animal model be questioned.

However, the current investigation identified a unique, but severe and persistent burying phenotype expressed by 11% of animals of both stereotypical cohorts (*viz.* high burying behavior, HBB). Interestingly, HBB also did not respond to chronic escitalopram treatment. It is hypothesized that HBB may either be representative of a disorder related to persistent behavior, *i.e.* hoarding, or resemble a unique phenotype of treatment resistant OC behavior. Future behavioral and pharmacological studies in this regard are needed to elucidate the neuropsychological construct underlying HBB.

* * *

Considering that NB is a normal rodent activity (Jirkof, 2014) but that that *aberrant* NB behavior has been proposed as a putative animal model of OC behavior (Angoa-Pérez, Kane, Briggs, Francescutti, & Kuhn, 2013), we investigated NB behavior in deer mice. We identified a cohort of animals (30% of the experimental sample) that presented with unique treatment-

naive large nest building behavior (LNB), not related to the degree of stereotypy expressed (Manuscript C). Furthermore, this behavior was more persistent and exaggerated compared to the behavior of the larger group as demonstrated by 1) lower coefficients of variance with respect to the daily quantity of cotton wool utilized and 2) a significant increase in the cumulative amount of cotton wool utilized after seven days of assessment. Moreover, LNB demonstrated significant sensitivity to chronic escitalopram treatment, decreasing to levels equal to that of the pre-treatment nesting scores of animals expressing normal nesting behavior. Consequently, we hypothesize that LNB is representative of a unique OC behavioral phenotype, not related to stereotypy, present in deer mouse behavior and propose that deer mouse behavior in general, may be a useful animal model to investigate the bio-behavioral constructs of various OC phenotypes within a single model.

* * *

In conclusion, the present investigation highlighted the relevance, usefulness and validity of the deer mouse model of OCD (Table 6-1). The face and predictive validities of the model was strengthened by the demonstration that H deer mice present with altered, treatment-sensitive, social performance compared to N controls, while aberrant NB behavior as observed in 30% of animals, is proposed as a unique OC phenotype within the behavior of deer mice that responds to chronic treatment with high dose oral escitalopram. Findings that 11% of deer mice express unique aberrant MB behavior that does not demonstrate attenuation following chronic escitalopram treatment, necessitates further investigation with respect to its proposed resemblance of treatment-resistant OCD or another OC spectrum disorder.

TABLE 6-1

Study questions and final outcomes

SUMMARIZED STUDY QUESTION	FINAL OUTCOMES
1) Do H animals present with altered sociability compared to N controls?	H animals present with altered pre-treatment sociability compared to N controls. Furthermore, chronic treatment with oral escitalopram significantly improved social interactivity of H animals both within and between cohorts, while the social interactions of N animals remained unchanged.
2) Is H behavior associated with neophobia?	As we failed to demonstrate an anxiety-like construct underlying MB, data from the current investigation can neither confirm, nor deny an association between H stereotypy and anxiety.
3) Can H behavior be associated with a different phenotype of compulsive behavior or is deer mouse behavior in general representative of additional OC phenotypes?	Although no association between MB and an OC construct could be demonstrated, 30% of deer mice from both cohorts express unique treatment-sensitive LNB behavior. As such, putative face and predictive evidence is provided that deer mouse behavior may be representative of OC symptom heterogeneity.
4) Are any observable differences, as laid out in study questions 1 – 4, sensitive to chronic high dose escitalopram treatment?	Chronic escitalopram improved the sociability in H, but not N animals, while the expression of LNB attenuated to levels akin to the inherent nest-building scores of the larger group. These findings strengthen the face and predictive validities of the deer mouse model of OCD. The finding that aberrant MB expressed by 11% of deer mice does not respond to treatment, necessitates further investigation.

6.1 Shortcomings and future studies:

The current investigation has broadened the validity of deer mouse behavior as a robust and useful animal model of OCD. However, a number of shortcomings and questions have arisen from the current data that could be addressed in future investigations. In short, they can be summarized as follows:

Although our findings failed to associate H stereotypy with novelty-induced anxiety, we demonstrate that MB is an inappropriate measure of neophobia (Manuscript B). Given the proposed role of anxiety in the initiation of compulsive-like behavior in OCD (Bartz & Hollander, 2006; Robbins, Gillan, Smith, de Wit, & Ersche, 2012), future studies employing different behavioral screening tests for anxiety are needed to investigate a possible association between H behavior and anxiety. Results from these studies could prove useful to further distinguish deer mouse stereotypy from repetitive motor routines without a cognitive basis. Furthermore, we identified a unique burying phenotype (HBB) in 11% of animals that definitely requires further investigation. This behavioral anomaly in a sub-group of animals may represent another OC-spectrum disorder (i.e. hoarding) or a treatment-resistant manifestation of OCD. Moreover, the face validity of the model could be strengthened further by determining whether H animals present with altered reward-related behavior as demonstrated by tasks that evoke compulsive-like repetition, such as excessive lever pressing (Joel & Avisar, 2001), or studying its association with memory deficits.

* * *

The demonstration of a selective response of H deer mouse sociability and LNB to chronic escitalopram treatment has significantly bolstered the predictive validity of the model. As only 70% of patients respond favourably to chronic SSRI treatment and given the demonstration that HBB is not sensitive to chronic escitalopram treatment, it would be valuable to assess whether this behavior demonstrates attenuation following antipsychotic augmentation (Fineberg & Craig, 2007). Recent evidence has associated OCD with oxidative stress (Chakraborty *et al.*, 2009), while deer mouse stereotypy too is linked to cortico-striatal oxidative stress (Güldenpfennig, Wolmarans, du Preez, Stein, & Harvey, 2011). Since antioxidants such as N-acetyl cysteine (NAC) have recently been shown to be beneficial in the treatment of OCD (Lafleur *et al.*, 2006), undertaking NAC augmentation studies in deer mice would extend the predictive and construct validity of the model, and provide a valuable platform for further exploratory studies into the neurobiology of OCD (eg. altered immune function).

Conclusion

* * *

Although the current investigation mainly focused on the face and predictive validities of the deer mouse model, the construct validity of the model could be addressed by assessing whether altered 5HT_{1B/D} and 5HT_{2C} receptor expression can be demonstrated in animals. This could prove valuable to our understanding of the mechanisms underlying serotonin-directed control of repetitive behavior. Furthermore, employing accurate and appropriate neurochemical techniques, i.e. microdialysis and HTRF receptor binding, may shed light on the dopaminergic and serotonergic responses elicited when H deer mice are confronted with reward-related tasks.

* * *

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guidelines on statistical issues. These guidelines focus on the analysis and reporting of quantitative data. Many of the issues described below pertain to vulnerabilities in null hypothesis significance testing (NHST), in which the central question is whether or not experimental measures differ from what would be expected due to chance. Below we emphasize some steps that researchers using NHST can take to avoid exacerbating those vulnerabilities. Many of the guidelines are long-standing norms about how to conduct experimental research in psychology. Nevertheless, researchers may benefit from being reminded of some of the ways that poor experimental procedure and analysis can compromise research conclusions. Authors are asked to consider the following issues for each manuscript submitted for publication in a Psychonomic Society journal. Some of these issues are specific to NHST, but many of them apply to other approaches as well. We welcome feedback regarding these guidelines via email to info@psychonomics.org with the Subject heading “Statistical Guidelines.”

1. It is important to address the issue of statistical power. Statistical power refers to the probability that a test will reject a false null hypothesis. Studies with low statistical power produce inherently ambiguous results because they often fail to replicate. Thus it is highly desirable to have ample statistical power and to report an estimate of a priori power (not post hoc power) for tests of your main hypotheses. Best practice when feasible is to draw on the literature and/or theory to make a plausible estimate of effect size and then to test a sufficient number of participants to attain adequate power to detect an effect of that size. There is no hard-and-fast rule specifying “adequate” power, and Editors may judge that other considerations (e.g., novelty, difficulty) partially offset low power. If a priori power cannot be calculated because there is no estimate of effect size, then perhaps the analysis should focus on estimation of the effect size rather than on a hypothesis test. In any case, the Method section should make clear what criteria were used to determine the sample size. The main points here are to (a) do what you reasonably can to attain adequate power and (b) explain how the number of participants was determined.

2. Multiple NHST tests inflate null-hypothesis rejection rates. Tests of statistical significance (e.g., t-tests, analyses of variance) should not be used repeatedly on different subsets of the same data set (e.g., on varying numbers of participants in a study) without statistical correction, because the Type I error rate increases across multiple tests.

One concern is the practice of testing a small sample of participants and then analyzing the data and deciding what to do next depending on whether the predicted effect (a) is statistically

significant (stop and publish!), (b) clearly is not being obtained (stop, tweak, and start a new experiment), or (c) looks like it might become significant if more participants are added to the sample (test more participants, then reanalyze; repeat as needed). If this “optional stopping rule” has been followed without appropriate corrections, then report that fact and acknowledge that the Type I error rate is inflated by the multiple tests. Depending on the views of the Editor and reviewers, having used this stopping rule may not preclude publication, but unless appropriate corrections to the Type I error rate are made it will lessen confidence in the reported results. Note that Bayesian data analysis methods are less sensitive to problems related to optional stopping than NHST methods.

It is problematic to analyze data and then drop some participants or some observations, re-run the analyses, and then report only the last set of analyses. If participants or observations were eliminated, then explicitly indicate why, when, and how this was done and either (a) report or synopsise the results of analyses that include all of the observations or (b) explain why such analyses would not be appropriate.

Covariate analyses should either be planned in advance or be described as exploratory. It is inappropriate to analyze data without a covariate, then re-analyze those same data with a covariate and report only the latter analysis as confirmation of an idea. It may be appropriate to conduct multiple analyses in exploratory research, but it is important to report those analyses as exploratory and to acknowledge possible inflations of the Type I error rate.

If multiple dependent variables (DVs) are individually analyzed with NHST, the probability that at least one of them will be “significant” by chance alone grows with the number of DVs. Therefore it is important to inform readers of all of the DVs collected that are relevant to the study. For example, if accuracy, latency, and confidence were measured, but the paper focuses on the accuracy data, then report the existence of the other measures and (if possible) adjust the analyses as appropriate. Similarly, if several different measures were used to tap a construct, then it is important to report the existence of all of those indices, not just the ones that yielded significant effects (although it may be reasonable to present a rationale for why discounting or not reporting detailed results for some of the measures is justified). There is no need to report measures that were available to you (e.g., via a participant pool data base) but that are irrelevant to the study.

3. Rich descriptions of the data help reviewers, the Editor, and other readers understand your findings. Thus it is important to report appropriate measures of variability around means and

around effects (e.g., confidence intervals around means and/or around standardized effect sizes).

4. Cherry picking experiments, conditions, DVs, or observations can be misleading. Give readers the information they need to gain an accurate impression of the reliability and size of the effect in question.

Conducting multiple experiments with the same basic procedure and then reporting only the subset of those studies that yielded significant results (and putting the other experiments in an unpublished “file drawer”) can give a misleading impression of the size and replicability of an effect. If several experiments testing the same hypothesis with the same or very similar methods have been conducted and have varied in the pattern of significant and null effects obtained (as would be expected, if only due to chance), then you should report both the significant and the non-significant findings. Reporting the non-significant findings can actually strengthen evidence for the existence of an effect when meta-analytical techniques pool effect sizes across experiments. It is not generally necessary to report results from exploratory pilot experiments, such as when pilot experiments were used to estimate effect size, provided the final experiment has high power. In contrast, it is not appropriate to run multiple low-powered pilot experiments on a given topic and then report only the experiments that reject the null hypothesis.

Deciding whether or not to report data from experimental conditions post hoc, contingent on the outcome of NHST, inflates the Type I error rate. Therefore, please inform readers of all of the conditions tested in the study. If, for example, 2nd, 4th, and 6th graders were tested in a study of memory development then it is appropriate to report on all three of those groups, even if one of them yielded discrepant data. This holds even if there are reasons to believe that some data should be discounted (e.g., due to a confound, a ceiling or floor effect in one condition, etc.). Here again, anomalous results do not necessarily preclude publication (after all, even ideal procedures yield anomalous results sometimes by chance). Failing to report the existence of a condition that did not yield the expected data can be misleading.

Deciding to drop participants or observations post hoc contingent on the outcome of NHST inflates the Type I error rate. Best practice is to set inclusion/exclusion criteria in advance and stick to them, but if that is not done then whatever procedure was followed should be reported.

Be careful about using null results to infer “boundary conditions” for an effect. A single experiment that does not reject the null hypothesis provides only weak evidence for the absence of an effect. Too much faith in the outcome of a single experiment can lead to hypothesizing after the results are known (HARKing), which can lead to theoretical ideas being defined by noise in experimental results. Unless the experimental evidence for a boundary condition is strong, it may be more appropriate to consider a non-significant experimental finding as a Type II error. Such errors occur at a rate that reflects experimental power (e.g., if power is .80, then 20% of exact replications would be expected to fail to reject the null).

6. Authors should use statistical methods that best describe and convey the properties of their data. The Psychonomic Society does not require authors to use any particular data analysis method. The following sections highlight some important considerations.

Statistically significant findings are not a prerequisite for publication in Psychonomic Society journals. Indeed, too many significant findings relative to experimental power can indicate bias. Sometimes strong evidence for null effects can be deeply informative for theorizing and for identifying boundary conditions of an effect.

In many scientific investigations the goal of an experiment is to measure the magnitude of an effect with some degree of precision. In such a situation a hypothesis test may be inappropriate as it only indicates whether data appear to differ from some specific theoretical value. Sometimes stronger scientific arguments can be made with confidence intervals (of parameter values or of standardized effect sizes). Moreover, some of the bias issues described above can be avoided by designing experiments to measure effects to a desired degree of precision (range of confidence interval).

The Psychonomic Society encourages the use of data analysis methods other than NHST when appropriate. For example, Bayesian data analysis methods avoid some of the problems described above. They can be used instead of traditional NHST methods for both hypothesis testing and estimation.

Last Word

Ultimately, journal Editors work with reviewers and authors to promote good scientific practice in publications in Psychonomic Society journals. A publication decision on any specific manuscript depends on much more than the above guidelines, and individual Editors and re-

viewers may stress some points more than others. Nonetheless, all else being equal submissions that comply with these guidelines will be better science and be more likely to be published than submissions that deviate from them.

Resources

There are many excellent sources for information on statistical issues. Listed below are some that the 2012 Publications Committee and Editors recommend.

Confidence Intervals:

Cumming, G. (2012). *Understanding the new statistics: Effect sizes, confidence intervals, and meta-analysis*. New York, NY US: Routledge/Taylor & Francis Group. (see www.latrobe.edu.au/psy/research/projects/esci).

Masson, M. J., & Loftus, G. R. (2003). Using confidence intervals for graphically based data interpretation. *Canadian Journal of Experimental Psychology/Revue Canadienne de Psychologie Expérimentale*, 57, 203-220. doi:10.1037/h0087426

Effect Size Estimates:

Ellis, P. D. (2010). *The essential guide to effect sizes: Statistical power, meta-analysis and the interpretation of research results*. Cambridge University Press. ISBN 978-0-521-14246-5.

Fritz, C. O., Morris, P. E., & Richler, J. J. (2011). Effect size estimates: Current use, calculations and interpretation. *Journal of Experimental Psychology: General*, 141, 2-18.

Grissom, R. J., & Kim, J. J. (2012). *Effect sizes for research: Univariate and multivariate applications* (2nd ed.). New York, NY US: Routledge/Taylor & Francis Group.

Meta-analysis:

Cumming, G. (2012). *Understanding the new statistics: Effect sizes, confidence intervals, and meta-analysis*. New York, NY US: Routledge/Taylor & Francis Group. (see www.latrobe.edu.au/psy/research/projects/esci).

Littell, J. H., Corcoran, J., & Pillai, V. (2008). *Systematic reviews and meta-analysis*. New York: Oxford University Press.

Bayesian Data Analysis:

Kruschke, J. K. (2011). *Doing Bayesian data analysis: A tutorial with R and BUGS*. San Diego, CA US: Elsevier Academic Press. (See [www.indiana.edu/~kruschke/DoingBayesian DataAnalysis/](http://www.indiana.edu/~kruschke/DoingBayesianDataAnalysis/))

Kruschke, J. K. (in press). Bayesian estimation supersedes the t test. *Journal of Experimental Psychology: General*. For a preprint see <http://www.indiana.edu/~kruschke/BEST/BEST.pdf>.

Power Analysis

Faul, F., Erdfelder, E., Lang, A., & Buchner, A. (2007). G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods*, 39(2), 175-191. (See <http://www.pscho.uni-duesseldorf.de/abteilungen/aap/gpower3/>)

Manuscript style

Manuscripts are to adhere to the conventions described in the Publication Manual of the American Psychological Association (6th ed.). See www.apastyle.org/ for information on APA style, or type “APA style” into a search engine to find numerous online sources of information about APA style. Here we highlight only the most fundamental aspects of that style.

Layout: All manuscripts are to be double spaced and have 1” margins with page numbers in the upper right corner of each page.

Title Page: The title page must include the authors’ names and affiliations and the corresponding author’s address, telephone number, and e-mail address.

Abstract: There must be an abstract of no more than 250 words.

Sections: Manuscript should be divided into sections (and perhaps subsections) appropriate for their content (e.g., introduction/background, Method, Results, etc.), as per APA style.

Acknowledgments: The Author Note should include sources of financial support and any possible conflicts of interest. If desirable, contributions of different authors may be briefly described here. Reviewers and the Editor should not be thanked in the Author Note.

Figures and Tables: Figures and tables are to be designed as per APA style.

Location of Figures, Tables, and Footnotes: In submitted manuscripts, figures and tables can be embedded in the body of the text and footnotes can be placed at the bottom of the page on which the footnoted material is referenced. Note that this is a departure from APA style; if you prefer you can submit the manuscript with the figures, tables, and footnotes at the end,

but it is slightly easier for reviewers if these elements appear near the text that refers to them. When a paper is accepted, in the final version that the author submits for production each figure and table must be on a separate page near the end of the manuscript and all footnotes must be listed on a footnote page, as per the APA Publication Manual.

Citations and References: These should conform to APA style.

Acknowledgments and Funding Information

Acknowledgments of people, grants, funds, etc. should be placed in a separate section before the reference list. The names of funding organizations should be written in full. In addition, please provide the funding information in a separate step of the submission process in the peer review system. Funder names should preferably be selected from the standardized list you will see during submission. If the funding institution you need is not listed, it can be entered as free text. Funding information will be published as searchable metadata for the accepted article, whereas acknowledgements are published within the paper.

Supplemental material

Authors are encouraged to attach, in a separate file or files, supplemental material (e.g., data sets such as stimulus norms or raw data, demonstrations or pictorial, auditory, or video stimuli, additional information regarding methods, additional tables or figures, relevant program source code [excluding executable code] for modeling or stimulus generation, or supplementary analyses that are not central to the main thrust of an article). The supplemental material will be reviewed along with the submitted article, or may be added at the time of acceptance in consultation with the Editor. Supplemental material will be published online, linked to the accepted article. The Editor makes decisions regarding supplemental material.

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to CABN are ones that need to make use of color figures in order to most clearly present the data. As with most journals, we must charge for the publication of color pictures in the print version of articles. However, if authors wish, they may opt to publish a black and white version of pictures/tables in the print version (as long as they are understandable to readers) and publish color versions in the on-line versions of articles.

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* * *

Journal of Psychopharmacology – INSTRUCTIONS TO AUTHORS

<https://us.sagepub.com/en-us/nam/journal/journal-psychopharmacology#submission-guidelines>

I. Article types

Journal of Psychopharmacology is a peer-reviewed journal which welcomes the following article types for publication:

- Original Papers: Research Reports, describing new experimental findings; both full papers and short reports requiring rapid dissemination.
- Review Articles: The Editors wish to encourage the following types of review, but request that authors contact them (j.psychopharm@imperial.ac.uk) in advance:
- General reviews: providing a synthesis of an area of psychopharmacology;
- Perspectives: brief overviews, which are 4-6 printed pages in length including references, that address important new areas of general interest
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- Letters to the Editors: Readers' letters should address issues raised by published articles or should report significant new findings that merit rapid dissemination. The decision to publish is made by the Editors, in order to ensure a timely appearance in print.
- Case Reports will only be considered if they make a major impact on the field and generally need to reflect findings from more than a single case.

The journal no longer accepts Book Reviews. The British Association for Psychopharmacology (BAP) publishes book reviews in their newsletter. Please contact Prof Brian E. Leonard, Emeritus Professor of Pharmacology, National University of Ireland, Galway (email: belucg@iol.ie).

The journal is more flexible in terms of the length of the article. Therefore there are no word limits for any type of article.

2. Editorial policies

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Drafted the article or revised it critically for important intellectual content,

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Authors should meet the conditions of all of the points above. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content.

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Please ensure that a 'Declaration of Conflicting Interests' statement is included at the end of your manuscript, after any Acknowledgements and prior to the references. If no conflict exists, please state that "The Author(s) declare(s) that there is no conflict of interest."

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For research articles, authors are also required to state in the methods section whether participants provided informed consent and whether the consent was written or verbal.

Information on informed consent to report individual cases or case series should be included in the manuscript text. A statement is required regarding whether written informed consent for patient information and images to be published was provided by the patient(s) or a legally authorized representative.

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The relevant EQUATOR Network reporting guidelines should be followed depending on the type of study. For example, all randomized controlled trials submitted for publication should

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Other resources can be found at NLM's Research Reporting Guidelines and Initiatives

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See also Section 4.3. on supplementary material.

3. Publishing policies

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This journal is able to host additional materials online (e.g. datasets, podcasts, videos, images etc.) alongside the full-text of the article. These will be subjected to peer-review alongside the article. For more information please refer to our guidelines on submitting supplementary files, which can be found within our Manuscript Submission Guidelines.

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Dear examiner

19 October 2015

**PHD THESIS - D. WOLMARANS
PERMISSION TO INCLUDE MANUSCRIPTS FOR EXAMINATION PURPOSES**

As study leader and senior corresponding author on three manuscripts first authored by Mr De Wet Wolmarans, I hereby approve that the manuscripts listed below be included as part of the requirements for fulfillment of the PhD degree, and that these manuscripts be submitted for examination of Mr Wolmarans' thesis.

The three articles are as follows:

Manuscript A

Social behavior in deer mice as a novel interactive paradigm of relevance for obsessive-compulsive disorder (OCD)

In submission to *Social Neuroscience*

Manuscript B

Of mice and marbles – novel perspectives on burying behavior as a screening test for psychiatric illness

In submission to *Cognitive, Affective and Behavioral Neuroscience*

Manuscript C

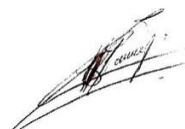
Excessive nest building is a unique behavioural phenotype in the deer mouse model of obsessive-compulsive disorder

In submission to *Journal of Psychopharmacology*

A letter of confirmation of submission from the respective journal editorial offices for each of the above manuscripts is included in the thesis, and appear at the start of each of the article chapters.

I trust that you will find this in order.

Sincerely,



Brian H Harvey, PhD

Study leader



Western Cape
Government
Health

Department of Psychiatry and Mental Health

Professor **Dan J Stein, FRCP, PhD, DPhil**

Head: Department of Psychiatry and Mental Health, UCT & GSH

Email: dan.stein@uct.ac.za

UCT Dept of Psychiatry and Mental Health, Groote Schuur Hospital (J-2), Anzio Rd,

Observatory 7925, Cape Town, South Africa

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22 October 2015

NWU Academic Board

Dear Sir/Madam

LETTER OF PERMISSION THAT THE ARTICLES ON DEER MOUSE BEHAVIOR MAY BE SUBJECT FOR MARKING

I hereby give permission for the three manuscripts to be subjected for marking with the intention of De wet Wolmarans attaining his degree.

Manuscript A

Social behavior in deer mice as a novel interactive paradigm of relevance for obsessive-compulsive disorder (OCD)

In submission to Social Neuroscience (Taylor and Francis)

Manuscript B

Of mice and marbles: Novel perspectives on burying behavior as a screening test for psychiatric illness

In submission to Cognitive, Affective and Behavioral Neuroscience (Springer)

Manuscript C

Excessive nest building is a unique behavioural endophenotype in the deer mouse model of obsessive-compulsive disorder

In submission to Journal of Psychopharmacology (Sage)

Yours sincerely

Prof Dan Stein
Co-author

"OUR MISSION is to be an outstanding teaching and research university,
educating for life and addressing the challenges facing our society."

Addendum C – Confirmations of Article Submissions

Manuscript A – Social Neuroscience

This document has been formatted without content change in the style of the current thesis. Original correspondence available on request.

23-Sep-2015

Dear Professor Harvey,

Thank you for submitting your manuscript entitled "Social behavior in deer mice as a novel interactive paradigm of relevance for obsessive-compulsive disorder (OCD)" to Social Neuroscience. It has been successfully submitted online and is with the editorial assistant awaiting further processing.

Your manuscript reference ID is SNS-RP 113.15.

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I will be in contact to inform you if your paper is sent to an action editor for reviewing, and I will keep you updated on the progress of your paper through the reviewing process though should you have any questions or concerns, at any stage of the reviewing process, please don't hesitate to contact me.

Thank you again for submitting your manuscript to Social Neuroscience.

Sincerely,

Lou Joseph

Lou Joseph

Editorial Assistant

Social Neuroscience

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Manuscript B – Cognitive, Affective and Behavioral Neuroscience

This document has been formatted without content change in the style of the current thesis. Original correspondence available on request.

13-Oct-2015

Dear Prof. Harvey:

Your manuscript entitled "Of mice and marbles – novel perspectives on burying behavior as a screening test for psychiatric illness" has been successfully submitted online and is presently being given full consideration for publication in the Cognitive, Affective, and Behavioral Neuroscience.

Your manuscript ID is CABN-RA-15-216.

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Thank you for submitting your manuscript to the Cognitive, Affective, and Behavioral Neuroscience.

Sincerely,

Cognitive, Affective, and Behavioral Neuroscience Editorial Office

Manuscript C – Journal of Psychopharmacology

This document has been formatted without content change in the style of the current thesis. Original correspondence available on request.

29-Oct-2015

Dear Mr. Wolmarans:

A manuscript titled Excessive nest building is a unique behavioural phenotype in the deer mouse model of obsessive-compulsive disorder (**JOP-2015-2687**) has been submitted by Mr. Dewet Wolmarans to Journal of Psychopharmacology.

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Addendum D –
Previous Work Published on the Deer
Mouse Model of OCD



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Research report

Reappraisal of spontaneous stereotypy in the deer mouse as an animal model of obsessive-compulsive disorder (OCD): Response to escitalopram treatment and basal serotonin transporter (SERT) density

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HIGHLIGHTS

- Stereotypy is time-consuming and effective for assessing severity and drug response.
- Chronic, but not sub-chronic treatment with escitalopram attenuates stereotypy.
- HS deer mice present with significantly lower striatal SERT density vs. controls.

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ABSTRACT

Obsessive-compulsive disorder (OCD) is characterized by recurrent thoughts and repetitive motor actions. Hyposerotonergic signalling in the cortico-striatal circuitry is believed to be central to the pathology of OCD, while many patients only respond to chronic treatment with high dose selective serotonin (5HT) reuptake inhibitors (SSRIs). Confined deer mice spontaneously develop two forms of stereotypy, namely vertical jumping and pattern running. The purpose of this investigation was to reappraise these behaviours and strengthen the validity of deer mouse stereotypy as an animal model of OCD within a framework of three study questions: (1) can the time spent executing stereotypical behaviours be employed as a measure of extent of stereotypy, (2) does deer mouse stereotypy only respond to chronic, but not sub-chronic treatment with a high-dose SSRI, and (3) is deer mouse stereotypy associated with altered cortico-striatal 5HT transporter (SERT) binding? The current study demonstrates that treatment naïve high stereotypical (HS) deer mice spend significantly more time executing stereotypical behaviours while significantly less time is spent indulging in stereotypy following chronic, but not sub-chronic, treatment with escitalopram. Furthermore, HS deer mice present with a significant decrease in striatal SERT density compared to non-stereotypical (NS) controls. Building on previous validation studies, we conclude that deer mouse stereotypy is a valid naturalistic animal model of OCD with robust face, construct and predictive validity.

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1. Introduction

Animal models of human psychiatric conditions may help elucidate the neurobiological mechanisms underlying the corresponding human disorder as well as provide a suitable framework for the development and pre-clinical evaluation of new treatment strategies. The current study follows on previous work describing the validation of spontaneous stereotypy in the deer mouse (*Peromyscus maniculatus bairdii*) as a putative animal model of obsessive-compulsive disorder (OCD) [1–3].

OCD is characterized by recurrent and intrusive thoughts (obsessions) and rigid repetition of certain motor actions

Abbreviations: OCD, obsessive-compulsive disorder; OC, obsessive-compulsive; SSRI, selective serotonin reuptake inhibitor; SERT, serotonin transporter; 5HT, serotonin; DA, dopamine; HS, high-stereotypical; [NS/LS], non- and low-stereotypical; VBI, vertical beam interruptions (indication of jumping activity); CR, cage revolutions (indication of pattern running activity).

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(compulsions) [4]. Although OCD has been classified as an anxiety disorder, much controversy remains over whether this is the best approach [5,6]. If indeed it is an anxiety disorder, it is noteworthy that traditional anxiolytics such as the benzodiazepines are ineffective in the clinical management of OCD [7,8]. OCD also demonstrates high comorbidity with a group of conditions collectively known as the obsessive-compulsive (OC) spectrum disorders [6,9], which includes trichotillomania, compulsive gambling, anorexia and body dysmorphic disorder, none of which responds to traditional anxiolytics.

40–60% of OCD cases respond preferentially to chronic high dose treatment with selective serotonin reuptake inhibitors (SSRIs) [7,10], possibly implicating perturbations in serotonin functioning in brain areas such as the prefrontal cortex (most notably the orbitofrontal and anterior cingulate cortices), the basal ganglia and the thalamus [11,12]. In fact, a decrease in serotonin transporter (SERT) density has been demonstrated in OCD [13,14], with decreased availability of SERT associated with increased symptom severity [15–17]. These clinical findings suggest a major role for serotonin in the neuropathology and treatment of OCD.

Suitable animal models of OCD are necessary to understand the complex neurobiological mechanisms underlying OC behaviour. Obsessions may play a prominent role in the compulsive-like repetition evident in OCD and, by virtue of their association with cognitive function, are difficult to demonstrate in animals. However, a diagnosis of OCD does not necessarily require the presence of obsessions [4]. By associating compulsive-like repetition of certain motor actions in animals with the fundamental constructs of OCD, certain conclusions of relevance to the human disorder can be made. Thus, by associating unique stereotypies with altered serotonergic signalling, demonstrating involvement of the prefrontal-cortex and basal ganglia, as well as a favourable and selective response to chronic, *but not sub-chronic* high dose SSRIs, it may be possible to distinguish certain repetitive behaviours in an animal without a confounding cognitive association [18,19].

Ideal animal models of human psychiatric conditions are measured against an array of different criteria and while it is difficult to accurately mimic psychiatric conditions in animals, most established models are founded on either robust face, predictive or construct validity [20], or a combination thereof. Although authors differ as to the exact definitions of these criteria [20–23], face and predictive validity respectively refers to the accuracy with which an animal model mimics the clinical presentation of the human condition, and how it reacts to the different treatment modalities used in the human disorder [23,24]. Construct validity was initially described by Geyer and Markou [20] as the accuracy with which a certain test measures what it is intended to measure. However, since it is difficult to accurately measure mood and cognition in animals, this definition has evolved to reflect common neurochemical perturbations and underlying etiological mechanisms with the human condition [23,25].

With this in mind, spontaneous stereotypy in the deer mouse is a useful and highly regarded naturalistic animal model of OCD [25–27]. Deer mouse stereotypy can be categorized into repetitive vertical jumping and pattern running [2,28], which occur as a natural within species behavioural variation that mimics the rigid repetitive motor actions of OCD and contribute to the face validity of the model. Chronic treatment with an SSRI, but not a noradrenergic reuptake inhibitor, significantly decreases the expression of these behaviours [2], thus supportive of the predictive validity of the model. Furthermore, stereotypical but not non-stereotypical animals present with altered cortico-striatal cyclic adenosine monophosphate (cAMP)/phosphodiesterase-4 (PDE₄) signalling [3], a second messenger signalling system known to undergo adaptive changes via actions of an SSRI on 5HT_{1A/B/D}

receptors [3,29]. This observation has important implications for construct validity of the model [25,26,30].

The current study aims to extend and strengthen the validity of the deer mouse model for OCD, firstly by assessing deer mouse behaviour throughout the dark cycle and demonstrating the varying and time-consuming nature of deer mouse stereotypy, contributing to the face validity of the model. Indeed, the level of distress and functional impairment suffered by the patient with OCD [31] is routinely assessed with rating scales that equate severity with the time-consuming nature of compulsions and the degree of resistance to, or control over these behaviours, e.g. the Yale-Brown obsessive-compulsive scale (Y-BOCS; [32]), the Florida obsessive-compulsive inventory (FOCI; [33]), the dimensional obsessive-compulsive scale (DOCS; [34]), and the Clark-Beck obsessive-compulsive inventory (CBOCI; [35]). Secondly, we will demonstrate that the response of deer mouse stereotypy to an SSRI is dependent on treatment duration, presenting with differences in response between sub-chronic (7 days) and chronic (28 days) treatment. Thirdly, we will demonstrate an association between deer mouse stereotypy and altered cortico-striatal SERT availability, as is evident in human OCD [13–17].

2. Methods

2.1. Subjects

Two groups of animals were used during the course of the investigation. The first group ($n=39$) was employed to assess the time-consuming nature of stereotypy and its response to treatment. A second group of animals was used to determine the differences in SERT density between treatment naïve high stereotypic (HS) and non to low-stereotypic (LS/LS) mice. Animals were obtained from the deer mouse colony maintained and housed at the vivarium of North-West University (NWU), Potchefstroom, South Africa (Ethical Approval Number – NWU-00066-10-S5). As stereotypical behaviour expressed by deer mice is established by the age of 8 weeks [2], only mice between the ages of 10 and 12 weeks were chosen as experimental subjects at the onset of behavioural assessments (day 0). As earlier studies have noted that severity of stereotypy in deer mice is independent of sex [2,28], mice were randomly chosen from different litters, without sex or weight bias. One day before the start of the behavioural assessments (day –1), each animal was allocated individually to an automatic climate-controlled laboratory cage (35 cm (l) × 20 cm (w) × 13 cm (h); Techniplast® S.P.A., Varese, Italy) and maintained at 23 °C on a 12-h light/dark cycle (lights on at 06h00 and off at 18h00). Food and water were provided ad lib. for the duration of the study. Cages were cleaned and new bedding material added weekly.

2.2. Behavioural assessments

2.2.1. Generating the behavioural data

All animals underwent 12-h behavioural screening once a week for eight weeks during the dark cycle. On any specific assessment day, animals were moved from their housing environment to the behavioural screening room. These areas are located on the same floor of the vivarium and are environmentally controlled with respect to temperature (23 °C), relative humidity (55%), and light cycle (same as for home cages). Subsequently each animal was introduced to a test cage (21 cm (w) × 21 cm (l) × 35 cm (h); Accuscan® Inc., Columbus, OH, USA) constructed from clear, translucent Plexiglas®. Bedding material was provided in quantities enough to cover the floor of the test cages, but also ensuring that it did not interrupt the scoring of behavioural data. Food was also provided ad lib on the floor of the cage in the form of broken-up rodent

Table 1
Cut-off values for each cohort as a function of the behavioural topography expressed.

Cohort	Vertical beam interruptions/30 min	Cage revolutions/30 min
HS	>2000	>200
LS	500–2000	150–200
NS	<500	<150

chow pellets. Depending on the week during which behavioural screening was performed, either bottled water or escitalopram solutions (see Section 2.3) were provided through a tight-fit hole in the wall of each test cage. Cages were covered with lids that allow uninterrupted airflow. The animals were introduced to these environments by 16h00 and habituated for at least 2 h before the behavioural assessments started at the onset of the dark cycle. Test cages were cleaned after each screen using a laboratory disinfectant (Virkon®, DuPont de Nemours®, Sandton, South Africa).

Behavioural assessment was performed using the Fusion® Animal Activity Monitoring System (Accuscan® Inc., Columbus, OH, USA), and analyzed using Fusion® software. Each test cage presents with a grid of infra-red light beams that crosses the cage 2 cm above the cage floor along the X- and Y-axes (constituting a grid), while another set of beams (Z-axis) crosses the cage 10 cm above the cage floor, but parallel to the bottom Y-axis. Mice need to jump in order to break the beams in the Z-axis, as these are placed high enough not to be interrupted by normal rearing activity [2,36]. All parallel beams are spaced 2.5 cm apart and record activity every time they are interrupted by movement. Thus, the software can track the movements of the animals and calculate the distance moved, while also recording different forms of behaviour, such as vertical jumping and locomotor activity. Importantly, the number of vertical beam interruptions (VBI) was used as a relative and robust measure of vertical jumping activity. As animals break more than one beam in the Z-axis during the execution of a jump, one beam break does not imply one jump. The number of clockwise and anti-clockwise cage revolutions (CR) was used to assess the expression of pattern running.

During the recording session, the Fusion® software generates data continuously, allowing for experimental playback and the export of behavioural reports the following day.

2.2.2. Analysing the behavioural data

2.2.2.1. Determining the time-consuming nature of deer mouse stereotypy and response to treatment. Each recording session comprised the whole 12-h dark cycle. Data was extracted and divided into 30-min increments for analysis. First, data generated during the light cycle (habituation period) were excluded. In order to assess the general locomotor activity of the animals, the horizontal movements (defined as a beam interruption in the horizontal grid at least one second apart) was recorded. To classify animals as either HS or [NS/LS] following the first four weeks of pre-treatment behavioural screening, the average number of VBI and CR for each individual animal was calculated from the *three highest respective values obtained as incremented in 30 min intervals* during each weekly 12-h dark cycle. Following the first four weeks of behavioural (baseline) assessments, the median value of the four weekly averages was used to categorize the animals as either [NS/LS] or HS.

Animals were classified according to the criteria set out in Table 1. These criteria have been refined and differ in two aspects from that previously published [2,3,28] and can shortly be explained as follows. Firstly, the time increments for behavioural analysis were reduced from 1 h to 30 min. Normally an animal generating a vertical score of 2000 counts/h would be classified as HS. However, it is possible that some animals generate the total count/h during the first 10 min of the hour, while others execute

jumps at a slower rate but still generate the required 2000 vertical beam interruptions within the hour. If the duration of the individual increments of behavioural analysis is reduced to 30 min, the animal that breaks the vertical beams 2000 times within the first 10 min of the hour will still generate a stereotypy score of 2000 counts/30 min, while the animal expressing the behaviour at a slower rate will generate a lower score and will therefore not be classified as HS, resulting in a more robust distinction between [NS/LS] and HS cohorts. Secondly, we applied different criteria to categorize the behaviour of pattern runners and vertical jumpers. After performing a pilot study (data not shown), it became apparent that more often than not pattern runners are not included in the HS cohort as they only complete a few hundred cage revolutions in the same time period during which jumpers generate a far higher vertical stereotypy score. Thus while HS pattern runners run in circles continuously, the absolute stereotypy counts for these animals are lower than that achieved by jumpers due to the longer period of time taken to complete a cage revolution and given the fact that vertical jumpers break more than one beam in the vertical grid during the execution of one jump (see the statistical differences between the number of VBI and CR generated by [NS/LS] and HS jumpers and pattern runners respectively – Tables 2 and 3). Therefore, in order to classify a “runner” as compulsive, we needed to categorize their movements differently by applying different classification criteria to their behaviour.

To be included in the [NS/LS] cohort, animals *must* have demonstrated [NS/LS] behaviour with respect to both the vertical and horizontal topographies. Animals classified as being HS could have demonstrated HS behaviour in either one or both of the behavioural topographies. Furthermore, for inclusion in the combined [NS/LS] cohort, animals needed to present with statistically significant differences in counts/30 min in the above parameters vs. animals in the HS cohort (VBI, Table 2; CR, Table 3).

Following classification of animals as either HS or [NS/LS], data sets were reanalyzed to assess the time-consuming and varying nature of stereotypy throughout a whole dark cycle. Instead of applying only the three 30 min intervals during which an animal expressed the highest amounts of stereotypy (as explained above), all twenty-four 30-min intervals over the course of a 12-h dark cycle assessment period were analyzed. Two forms of analyses, namely *intervals of no stereotypic activity* and *intervals of HS activity*, were defined and applied to these data sets. The current study aimed to clearly distinguish between the behaviour of HS animals and that of [NS/LS] animals. As deer mice express stereotypic behaviour within a wide margin of variance, we excluded the grey margin of animals by defining an *interval of no stereotypic activity* as a 30 min interval during which a stereotypy score of 10 or lower has been generated. Furthermore, applying a cut-off criterion of less than 10 stereotypic movements per 30-min provides for a robust measurement of treatment outcome, as it is the exact opposite of an interval of HS activity. *Intervals of HS activity* are defined when an animal expresses HS behaviour (see Table 1 for criteria), irrespective of the behavioural topography executed.

Importantly, the layout of the investigation performed in these studies comprised four weeks of baseline screening, during which the animals only received food and drinking water. This four-week pre-treatment period (day 1–28) represents the placebo control and was followed immediately thereafter by a four-week (day 29–56) drug treatment period. As animals were screened on a weekly basis, day 28 was considered a *pre-treatment (baseline) control* value. Values obtained on day 35 (one week after onset of treatment) were considered to be the result of *sub-chronic* treatment, with values obtained on day 56 (four weeks after the onset of treatment) the result of *chronic* treatment.

Table 2

Weekly differences between the mean number of VBI generated by 12 animals classified as [NS/LS] and 9 animals classified HS vertical jumpers, respectively. Animals representative of these data were used in the determination of baseline SERT density.

Day	[NS/LS] (mean VBI $\pm$ SEM) $n = 12$	HS (mean VBI $\pm$ SEM) $n = 9$	p Values (unpaired t -test)
7	711 $\pm$ 114	3177 $\pm$ 298	<0.0001
14	965 $\pm$ 183	3071 $\pm$ 308	<0.0001
21	814 $\pm$ 126	3416 $\pm$ 427	<0.0001
28	1216 $\pm$ 283	3219 $\pm$ 403	0.0007
35	1068 $\pm$ 303	3377 $\pm$ 422	0.0003
42	828 $\pm$ 152	2774 $\pm$ 375	0.0001
49	817 $\pm$ 205	2749 $\pm$ 349	0.0002
56	971 $\pm$ 265	2726 $\pm$ 327	0.0006
Average	923	3063	

2.2.2.2. Behavioural classification of animals for the determination of SERT density. A second group of animals (treatment-naïve) was behaviourally screened for eight weeks (as explained in Section 2.2.1) and classified according to the criteria set out in Table 1. The same methodology was applied as that described in Section 2.2.2.1 with respect to the initial classification of animals after the first four weeks of behavioural screening.

2.3. Drugs and drug treatment

Escitalopram oxalate (H. Lundbeck, Copenhagen) was prepared for oral administration by dissolving it in the drinking water (see description of dose calculation below). Physical handling of the mice was kept to a minimum to prevent any possible anxiogenic effects. For the same reason, oral gavage was not considered as an appropriate dosage route.

Escitalopram was administered orally in the drinking water at a dose of 50 mg/kg/day. This dose was based on fluoxetine dosing described in an earlier study using a putative mouse model of OCD [24]. Although both fluoxetine and escitalopram are SERT inhibitors, escitalopram is a more potent and selective inhibitor of this transporter than fluoxetine [37]. The final concentration of the drinking solution was constituted at 20 mg/100 ml ensuring that each animal would receive 50 mg/kg/day (within close limits) of escitalopram. The dose calculation was based on a prior pilot study where the average water intake per mouse was determined as 0.25 ml/g/day (data not shown), and confirmed by others [38]. The average water intake of the animals did not change as a function of the concentration of escitalopram administered (data not shown). As all mice first underwent four weeks of pre-treatment screening, oral administration of escitalopram began on day 29. New solutions of escitalopram were prepared every day of which 20 ml was transferred to a water bottle in each cage. The replacement of escitalopram solutions continued throughout the four weeks of drug administration. Depending on the week of analysis, animals that were behaviourally screened during the dark cycle received either water (first four weeks) or the same escitalopram solution during behavioural assessment.

2.4. Determination of frontal-cortical and striatal SERT density

2.4.1. Pooling of tissue

Due to the small amount of brain tissue obtained from one mouse, pooling of brain tissue was required for the individual assays of regional brain SERT density. For SERT analysis by saturation binding assay, striatal and frontal-cortical tissues (identified and dissected according to [1]) from at least 3 animals (or 90 mg of tissue) classified in the same behavioural cohort were pooled for one binding assay. A minimum of three assays were needed to guarantee statistical credibility. Consequently 9 animals per behavioural cohort were used to determine the average regional SERT density. Of the 31 animals used, the striatal and frontal-cortical tissue of the 9 animals presenting with the lowest ([NS/LS]) and the highest (HS) average weekly stereotypy scores were used in the saturation binding studies. This ensured the exclusion of a small number of animals that constitute a 'grey' margin of individuals that generated stereotypy scores near to the cut-off values for the separation of cohorts (Table 1), a rationale also followed during previous investigations [39,40].

2.4.2. Saturation binding assay

The determination of SERT density was adapted from a previously described method [41]. At least three assays were performed per brain area. Briefly, brain tissue from the identified animals of a given cohort were weighed and pooled and kept on ice. 50 mM Tris-HCl buffer (containing 120 mM NaCl and 5 mM KCl; pH adjusted to 7.4) was added to constitute a 100 volumes dilution of each pool. Subsequently, the pooled tissue was homogenized with a Brinkman Polytron® PT10 homogenizer (setting 6, 20 s) and centrifuged at 50,000 $\times$ g for 10 min and the supernatant discarded.

This pelleted membrane fraction was re-suspended using a Teflon® drill bit and centrifuged twice, where after it was reconstituted in 100 volumes of the buffer by using a Polytron®. All of the above steps took place on ice. Aliquots of 450 μ l of the membrane preparation were incubated with ten concentrations (0.1–2.0 nM) of the radioactive SERT ligand paroxetine-[phenyl-6- ^{3} H] (22.9 Ci/mmol) and buffer or drug to a final volume of 500 μ l

Table 3

Weekly differences between the mean number of HR executed by 12 animals classified as [NS/LS] and 3 animals classified as HS pattern runners, respectively. Animals representative of these data were used in the determination of baseline SERT density.

Day	[NS/LS] (Mean HR $\pm$ SEM) $n = 12$	HS (Mean HR $\pm$ SEM) $n = 3$	p Values (unpaired t -test)
7	46 $\pm$ 11	211 $\pm$ 46	0.0001
14	71 $\pm$ 19	294 $\pm$ 46	0.0003
21	60 $\pm$ 16	319 $\pm$ 33	<0.0001
28	44 $\pm$ 11	333 $\pm$ 19	<0.0001
35	57 $\pm$ 11	276 $\pm$ 54	<0.0001
42	75 $\pm$ 31	351 $\pm$ 39	0.0013
49	49 $\pm$ 16	288 $\pm$ 30	<0.0001
56	41 $\pm$ 10	297 $\pm$ 66	<0.0001
Average	55	296	

for 60 min at room temperature. Non-specific binding was defined as that binding inhibited by 10 μ M fluoxetine.

Following the 1 h incubation period, the assay was terminated by rapid vacuum filtration through Whatman® GF/B glass microfiber filters pre-soaked in ice-cold buffer and washed twice rapidly with 4 ml of ice-cold buffer. Radioactivity trapped on the filters was determined by liquid scintillation counting (Packard® scintillation counter). Protein was determined by Bradford assay. The binding data were analyzed using GraphPad Prism® 5 software (GraphPad® Software, Inc., La Jolla, CA, USA) to obtain values for the maximal number of binding sites (B_{max}) and the binding affinity (K_D).

2.5. Statistical analysis

To compare the pre- and post-treatment expression of stereotypy, two-way repeated measures (RM) ANOVA was performed. Where significant interactions were displayed, one-way RM ANOVA was applied to the data, followed by Bonferroni post-tests. Behavioural cohorts were set as between group factor and time as within factor. To compare the baseline SERT densities of [NS/LS] and HS animals, two-way RM ANOVA was performed. Where significant interactions were displayed, post hoc *t*-tests were applied to validate the findings. Behavioural cohorts were set as between groups factor and brain region as within factor. Significance was set at $p < 0.05$.

3. Results

3.1. Deer mouse stereotypy and response to treatment (objectives 1 and 2)

Of the 39 animals used to address these study objectives, 11 animals were classified as HS vertical jumpers, 3 animals as HS pattern runners, and 2 animals as both HS vertical jumpers and pattern runners.

One of the primary aims of the current study was to assess the behaviour of deer mice holistically during the whole dark cycle (objective 1). Integral to this objective, we also assessed whether treatment with high dose oral escitalopram would lower the three highest scores of stereotypy generated by deer mice during a dark cycle (objective 2). Interestingly, no significant differences could be demonstrated after 7 (sub-chronic) or 28 (chronic) days of oral escitalopram treatment (50 mg/kg/day) in [NS/LS] and HS animals vs. pre-treatment control with respect to vertical jumping (VBI; Fig. 1A). The same lack of effect was evident in the [NS/LS] and HS cohorts of pattern runners respectively (CR; Fig. 1B), indicating that HS animals continued to express HS behaviour during at least some of the 30 min dark cycle intervals (a full discussion of this data follows later). Two-way RM ANOVA also revealed no significant interactions with respect to VBI [$F(2,38) = 0.26$, $p = 0.7724$] and CR [$F(2,30) = 0.3446$, $p = 0.7113$].

3.1.1. The time-consuming nature of stereotypy: effect of escitalopram treatment on the number of intervals of no stereotypic activity

Although neither sub-chronic, nor chronic treatment with oral escitalopram (50 mg/kg/day) significantly changed the number of intervals of no stereotypic activity observed in the behaviour of [NS/LS] animals between days 28 and 35 (sub-chronic), and between days 28 and 56 (chronic Fig. 2A, left), a statistically significant increase in the number of these intervals was demonstrated in HS animals after chronic, but not sub-chronic treatment with escitalopram (* $p < 0.05$, Fig. 2A, right). Two-way RM ANOVA also revealed no significant interactions [$F(2,44) = 0.2697$, $p = 0.7649$].

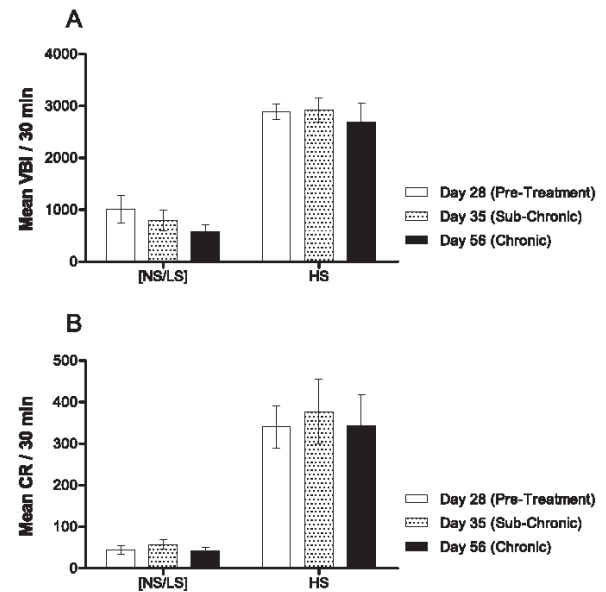


Fig. 1. Mean VBI (A) and CR (B) generated by animals of the [NS/LS] and HS cohorts prior to treatment (day 28), and following sub-chronic (1 week; day 35) or chronic (4 weeks; day 56) escitalopram treatment. These values represent the mean of the three 30-min intervals during which animals generated the highest stereotypy scores.

3.1.2. The time consuming nature of stereotypy: effect of escitalopram treatment on the number of intervals of HS activity

Two-way RM ANOVA revealed a significant between groups factor interaction [$F(2,42) = 7.088$, $p = 0.0022$]. Subsequently, a post hoc one-way RM ANOVA was performed to validate the findings. Neither sub-chronic, nor chronic treatment with oral escitalopram

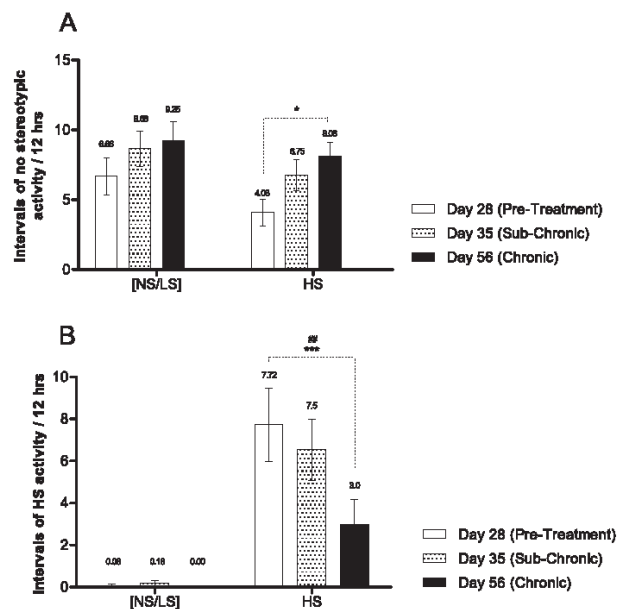


Fig. 2. Intervals of no stereotypic activity (A) and intervals of HS activity (B) observed in the behaviour of [NS/LS] and HS cohorts prior to treatment (day 28), and response to 1 (day 35) or 4 (day 56) weeks of escitalopram treatment. [2A] HS: two-way RM ANOVA * $p < 0.05$. [2B] HS: two-way RM ANOVA *** $p < 0.001$, one-way RM ANOVA, ## $p = 0.0054$.

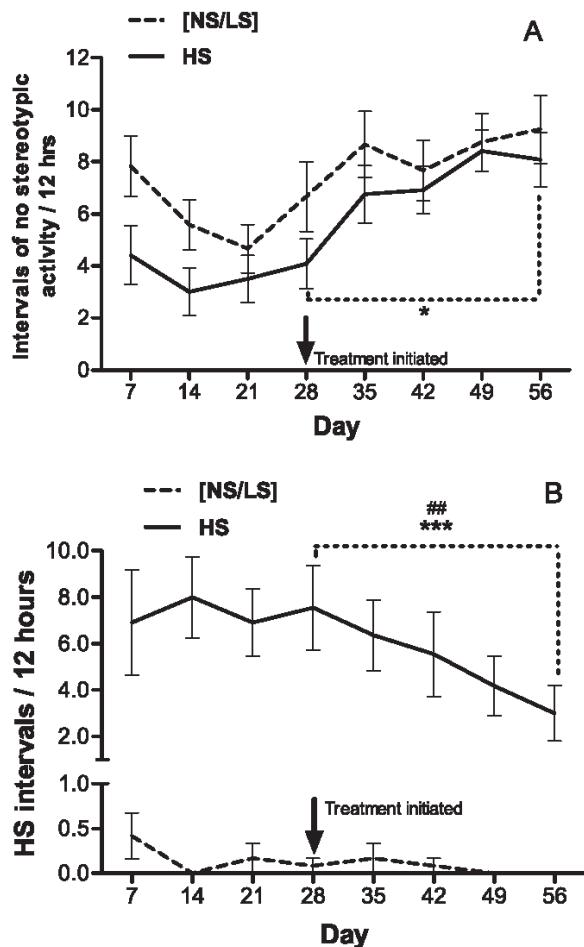


Fig. 3. Average weekly occurrence of intervals of no stereotypic activity (A) and intervals of HS activity (B) in [NS/LS] and HS cohorts prior to (day 28), and response to 1 (day 35) or 4 (day 56) weeks of escitalopram treatment. [3A] HS: two-way RM ANOVA $^{*}p < 0.05$; [3B] HS: two-way RM ANOVA $^{***}p < 0.001$, one-way RM ANOVA $^{**}p = 0.0054$.

(50 mg/kg/day) significantly altered the number of HS-intervals generated by [NS/LS] animals between days 28 and 35 (sub-chronic), and between days 28 and 56 (chronic) (Fig. 2B, left). However, chronic, but not sub-chronic treatment significantly decreased the number of HS-intervals in the behaviour of HS animals (two-way RM ANOVA $^{***}p < 0.001$; one-way RM ANOVA $^{**}p = 0.0054$; Fig. 2B, right).

A week-by-week comparison between the number of intervals of no stereotypic activity (Fig. 3A) and intervals of HS activity (Fig. 3B) expressed prior to (day 28) and after the onset of escitalopram treatment (from day 29) indicates that the number of intervals of no stereotypic activity and intervals of HS activity expressed by HS animals not only varies week by week, but begins to change after seven days, reaching statistical significance after 28 days of treatment. Furthermore, no effect of treatment on behaviour was observed in [NS/LS] animals.

3.1.3. General locomotor activity and response to treatment

To affirm that the effects of escitalopram treatment on the number of intervals of no stereotypic activity and the number of HS intervals were not attributable to changes in general activity, we also performed locomotor analyses. Chronic, but not sub-chronic

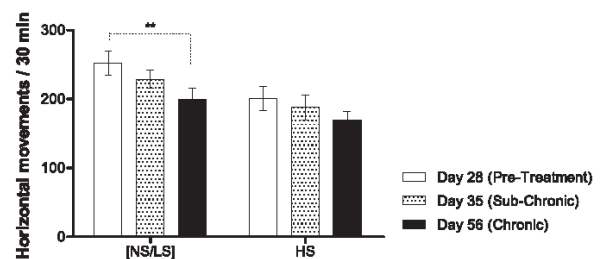


Fig. 4. Average number of horizontal movements generated in [NS/LS] and HS cohorts following sub-chronic or chronic escitalopram treatment. [NS/LS]: Two-way RM ANOVA, $^{**}p < 0.01$.

treatment with escitalopram was associated with a significant decrease in the average locomotor activity (horizontal movements) in [NS/LS] animals (two-way RM ANOVA $^{**}p < 0.01$; Fig. 4, left). However, neither sub-chronic nor chronic treatment significantly influenced the average general locomotor activity of HS deer mice ($p > 0.05$; Fig. 4, right). Furthermore, no interactions were demonstrated [$F(2,44) = 0.494$, $p = 0.6135$].

3.2. Association of HS behaviour with cortico-striatal SERT density (objective 3)

Two-way RM ANOVA of the SERT data revealed significant within and between group factor interactions [$F(1,8) = 17.28$, $p = 0.0032$] that necessitated post hoc t -tests to validate the findings. Both two-way RM ANOVA (Fig. 5; $^{**}p < 0.001$) and an unpaired t -test ($^{***}p = 0.0009$) revealed a significantly lower striatal SERT density in treatment naïve HS animals (2.31 ± 0.09 fmol/mg protein) compared to [NS/LS] animals (12.96 ± 1.42 fmol/mg protein). Although narrowly missing statistical significance ($p < 0.1$), the frontal-cortical SERT density in treatment-naïve HS animals (4.15 ± 0.25 fmol/mg protein) also tended to be lower than that in [NS/LS] animals (5.81 ± 1.61 fmol/mg protein). Due to

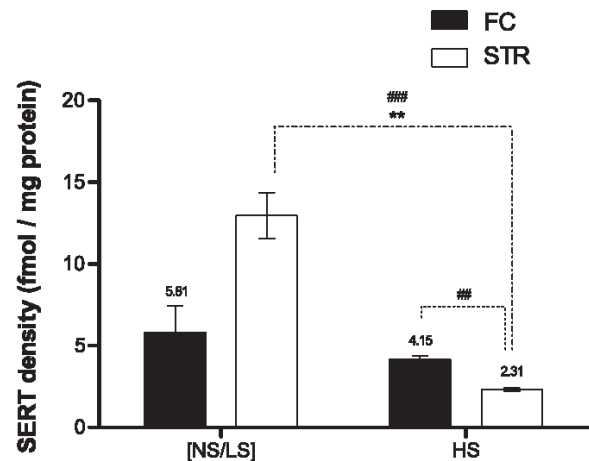


Fig. 5. Comparative striatal (STR) (two-way RM ANOVA $^{**}p < 0.001$; unpaired t -test $^{***}p = 0.0009$; B_{max} ([NS/LS]) = 12.96 ± 1.42 fmol/mg/protein; B_{max} (HS) = 2.31 ± 0.09 fmol/mg protein) and frontal cortical (FC) ($p > 0.05$; B_{max} ([NS/LS]) = 5.81 ± 1.61 fmol/mg/protein; B_{max} (HS) = 4.15 ± 0.25 fmol/mg protein) SERT densities in [NS/LS] vs. HS animals, as well as a comparison between frontal-cortical and striatal SERT densities in treatment naïve [NS/LS] ($p > 0.05$; B_{max} (frontal cortex) = 5.81 ± 1.61 fmol/mg protein; B_{max} (striatum) = 12.96 ± 1.42 fmol/mg protein) and HS (two-way RM ANOVA $^{**}p = 0.0087$; B_{max} (frontal cortex) = 4.15 ± 0.25 fmol/mg/protein; B_{max} (striatum) = 2.31 ± 0.09 fmol/mg protein) animals. In each figure, the data represent the mean $\pm$ SEM of three assays per brain region.

the significant within factor interaction revealed by two-way RM ANOVA, paired *t*-tests were applied to analyze the within-cohort differences (i.e. brain region) in the expression of SERT. This demonstrated that HS animals present with significantly lower striatal (2.3 ± 0.09 fmol/mg protein) than frontal-cortical (4.15 ± 0.25 fmol/mg protein) SERT density (Fig. 1; $^{***}p=0.0087$). Finally, a definite trend towards lowered SERT density was noted in the frontal cortex vs. striatum in [NS/LS] animals ($p<0.1$).

4. Discussion

Considering current trends in validity criteria for animal models (see Section 1), the present findings strengthen deer mouse stereotypy as a valid animal model of OCD. The most important observations are (1) that we have demonstrated the time consuming nature of stereotypy in HS animals and that the time spent executing stereotypical behaviour is an appropriate and effective criterion for assessing drug treatment response (face validity), (2) that treatment naïve HS deer mice spend significantly less time indulging in stereotypy following chronic, *but not sub-chronic*, treatment with the SSRI, escitalopram (predictive validity) and (3) that HS deer mice present with a significant decrease in striatal SERT density compared to their [NS/LS] controls (construct validity).

Regarding the behavioural expression of stereotypy in deer mice, we have established that deer mouse behaviour not only varies during the course of a 12-h dark cycle, but that the expression of stereotypy by HS animals is also *time-consuming*. This has allowed us to re-appraise the behavioural assessment of stereotypy in deer mice and to revise the scoring of behavioural topographies presented in these animals. This was undertaken by screening the behaviour of animals throughout the course of a whole dark cycle and in so doing improve behavioural assessment during and following drug treatment. Since time spent executing OC behaviour during the day is an important diagnostic criterion of OCD according to the Y-BOCS [32], FOCI [33], DOCS [34], and CBOCI [35], behavioural assessment of the animals throughout the whole dark cycle is not only sensible, but also improves phenotypic selection of the animals and bolsters sensitivity. Deer mice express stereotypic behaviour within a wide margin of variance (viz. non-, low, and high numbers of stereotypy counts) [2,36] making the measurement of both *intervals of no stereotypic activity* and *intervals of HS activity* particularly valuable for the acquisition of reliable behavioural data. For example, we now demonstrate that treatment naïve HS mice not only experience more bouts of HS activity, but also fewer bouts of *no* stereotypic activity compared to the [NS/LS] controls. Together these findings imply that the normal wake cycle of HS animals is characterized by more time spent indulging in high stereotypical behaviour, yet interweaved with episodes of *normal* rodent behaviour, not unlike that evident in many human OCD phenotypes [31]. The above-mentioned assessment scales also determine the degree of resistance to, or control over, the expression of OC behaviour as a measure of symptom severity. We hypothesize that animals executing repetitive behaviour at a more rapid pace (as per the redefined cut-off criteria, Table 1) exert less resistance to the expression of stereotypy, and have less control over the 'need' to express stereotypical behaviour. This brings the new cut-off criteria more in line with the clinical scenario and strengthens the face validity of the model. Furthermore, as far as we are aware this is the first behavioural analysis of a putative model of OCD to be conducted throughout the entire active period, adding a valuable dimension to the current methodologies employed to assess compulsive or repetitive animal behaviour.

Deer mouse stereotypy responds selectively to chronic treatment with fluoxetine, but not a noradrenergic reuptake inhibitor

[2], typical of the pharmacological response observed in OCD [7,42]. Apart from the greater serotonergic vs. noradrenergic selectivity required in the pharmacological management of OCD, treatment response also takes far longer than that for depression (as long as 8–12 weeks) [43,44]. That chronic *but not sub-chronic* treatment with high dose oral escitalopram significantly increased intervals of no stereotypical activity (Fig. 2A) and decreased high stereotypical bouts (Fig. 2B) in HS (but not [NS/LS]) animals is particularly noteworthy. Although changes in these parameters ensue after one week of treatment (Fig. 3), significance is only attained after four weeks, not unlike the response observed in the clinical scenario [43,44]. Interesting is that contrary to expected, HS animals continued to experience bouts of high stereotypic behaviour up to four weeks after the onset of oral escitalopram treatment (Fig. 1). To clarify these findings, it must be noted again that a 12 h dark cycle is incremented in no less than twenty-four 30 min intervals while animals do not express the same levels of stereotypy continuously during the dark cycle, including HS animals. Rather they experience variable *sporadic bouts* of HS activity during this period, which may explain these unexpected findings. Indeed, on average, treatment naïve HS animals only expressed HS behaviour during 7.7 of the 24 evaluation periods (data on day 28; Fig. 2B). It can be argued that as treatment naïve HS animals spend on average 30% of the dark cycle (or 7.7 out of the 24 intervals) expressing stereotypical behaviour, a positive treatment response is implicated by reducing this figure to 12% (or 3 out of the 24 intervals). This argument is supported by the fact that chronic, *but not sub-chronic* treatment increased the average number of bouts of no stereotypical (*normal*) behaviour in HS animals (from 4 to 8 out of 24 bouts or 17% to 33% of the dark cycle), indicating that chronic treatment with high dose escitalopram not only dampens the expression of stereotypy, but that it increases the total time of *normal* functioning in the dark cycles of HS deer mice. Furthermore, that HS animals experience more intervals of no stereotypic activity following chronic escitalopram treatment could indicate that the drug engenders improved control over the impulse to express HS behaviour. These findings are supportive of the face and predictive validity of the model.

A well-accepted construct underlying OCD is its preferential response to SSRIs, thereby suggesting a causal role for altered 5-HT function in the underlying neurobiology of the illness. A vast number of studies have been performed and various angles of approach have been followed in an effort to delineate the role of serotonin in the pathology of OCD. It is commonly accepted that OCD involves a hyperactive direct dopaminergic pathway in the cortico-striatal-thalamic-cortical (CSTC) circuitry [12,45]. Given the evidence that serotonin opposes the functional role of dopamine [46] and therefore suppresses complex motor behaviour [46–48], and that increasing synaptic serotonin levels with SSRIs seems to be effective in many cases of OCD [7,8], it can be argued that hyposerotonergic functioning in the CSTC circuitry underlies the pathology of OCD. In an effort to further elucidate the role of serotonin in the expression of OCD-like behaviour, a number of studies have employed positron emission tomography (PET) and single-photon emission computed tomography (SPECT) to investigate the availability of SERT in the brain areas implicated in the normal functioning of the CSTC circuitry. Evidently, OCD presents with significant decreases in SERT availability within the striatum [15], thalamus, midbrain and brain stem [16,17]. Furthermore investigators have noted a correlation between increased symptom severity and a decrease in midbrain SERT binding in patients with OCD and moreover that reduced pre-treatment SERT availability is associated with poor treatment response [17]. It is therefore plausible that a decrease in striatal, thalamic and midbrain SERT density in OCD, and in the current animal model, is related to hyposerotonergic signalling.

Reduced striatal SERT density in HS animals described here is congruent with the above findings from human studies [15] as well as data from another naturalistic animal model of OCD [49], albeit the latter demonstrating perturbations in the hypothalamus. HS animals also present with significantly lower striatal vs. frontal-cortical SERT density, with the opposite relationship evident in the [NS/LS] cohort, albeit narrowly missing statistical significance (Fig. 5). However, two-way RM ANOVA showed significant within and between group factor interactions, indicating that the regional pattern of SERT expression does indeed differ between the cohorts. This finding suggests that a perturbation of serotonergic functioning in the striatum relative to the frontal cortex may play a role in the elevated stereotypy present in HS deer mice. Earlier studies in the deer mouse have indeed highlighted cortico-striatal differences between HS and [NS/LS] animals but with respect to other biological parameters, although favouring a greater disturbance in the frontal-cortex [1,3]. Interestingly, in a different mouse model of OCD that also focused on rigid locomotor behaviour [50], stimulation of orbito-frontal 5HT_{1B/D}-receptors was associated with the expression of rigid hyperlocomotion and perseveration along a certain set of locomotor paths, leading the authors to conclude that activation of orbitofrontal, but not striatal, 5HT_{1B/D} receptors is necessary for the expression of OCD-like behaviour. Although SERT binding described here confirms a perturbation in striatal serotonin functioning, it is important to note that the two models differ with respect to the etiological mechanisms underlying compulsive-like behaviour. Since our data suggest a predominant striatal dysfunction in HS deer mice, studies comparing frontal-cortical and striatal 5HT_{1B/D} receptor densities in treatment-naïve HS and [NS/LS] deer mice can be valuable. Considering the strong interaction between serotonin and dopamine in the CSTC circuit, and that deer mouse stereotypy involves disturbances in both serotonin and dopamine [2,39,40], our results suggest that deficits in serotonin-mediated control over dopamine (or hyposerotonergic signalling), may underlie increased stereotypy in these animals, thus supportive of the construct validity of the model. Congruent with this hypothesis, studies in animal models, including the deer mouse, have demonstrated that direct acting serotonin receptor agonists attenuate compulsive or stereotypic behaviour [2,51–53].

5. Conclusion

This study has confirmed and extended the validity of the deer mouse model as a naturalistic animal model of OCD by demonstrating (1) that time spent executing stereotypical behaviour is an appropriate and effective criterion for assessing symptom severity and drug treatment response (face validity), (2) attenuation of HS behaviour following chronic, but not sub-chronic, high dose escitalopram treatment (predictive validity), and (3) that HS behaviour in deer mice is associated with decreased striatal SERT availability (construct validity).

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Conflicts of interest

The authors declare that Dan Stein has received research grants and/or consultancy honoraria from Abbott®, Astra Zeneca®,

Biocodex®, Eli-Lilly®, GlaxoSmithKline®, Jazz Pharmaceuticals®, Johnson & Johnson®, Lundbeck®, Orion®, Pfizer®, Pharmacia®, Roche®, Servier®, Solvay®, Sumitomo®, Takeda®, Tikvah®, and Wyeth®. In the past three years, Brian Harvey has participated in advisory boards and received honoraria from Servier®, and has received research funding from Lundbeck®. The authors declare that, except for income from the primary employer and research funding to BHH from the South African Medical Research Council, and the above-mentioned exceptions, no financial support or compensation has been received from any individual or corporate entity over the past three years for research or professional services, and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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Addendum E –
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(Chapter 3)

Social behavior in deer mice as a novel interactive paradigm of relevance for obsessive-compulsive disorder (OCD)

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ABSTRACT

Greater obsessive-compulsive (OC) symptom severity may be associated with poor social adjustment. Rather than possessing deficits in social skill per se, OCD patients may be more socially isolative in the presence of normal controls. We aimed to apply a novel social interaction challenge (SIC) to an established animal model of OCD, viz., the deer mouse, to assess complex social behavior in animals by investigating group sociability and its response to chronic escitalopram treatment (50 mg/kg/day × 28 days), both within and between non (N)- (viz., normal) and high (H)- (viz., OCD-like) stereotypical cohorts. Using automated screening, we scored approach behavior, episodes of proximity, duration of proximity, and relative net weighted movement. H animals socialized more with one another within cohort in all of the above parameters compared to the within-cohort behavior of N animals. Furthermore, the social behavior of H animals toward one another, both within and between cohort demonstrated significant improvements following chronic escitalopram treatment. However, the study also demonstrates that the social interaction between H and N animals remain poor even after chronic escitalopram treatment. To conclude, findings from the current investigation support clinical data demonstrating altered sociability in patients with OCD.

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Obsessive-compulsive disorder (OCD); deer mouse; animal model; social interaction; escitalopram

Introduction

Obsessive-compulsive disorder (OCD) has a lifetime prevalence of 1–3% (Stein, 2002) and is characterized by recurrent and intrusive thoughts (obsessions) and rigid repetition of certain motor actions (compulsions) (American Psychiatric Association, 2013). According to DSM-V, OCD is classified under the OC spectrum (American Psychiatric Association, 2013).

OCD is often comorbid with social anxiety disorder (SAD) (Kim, Reynolds, & Alfano, 2012) or poor social adjustment (Rosa et al., 2012). Adults with OCD report higher rates of unemployment, marital discord, and financial instability (Kim et al., 2012), while children display an impaired ability for making and keeping friends (Kim et al., 2012; Piacentini, Bergman, Keller, & McCracken, 2003). Both social phobia and OCD follow a course of varying symptom intensity, are characterized by severe occupational infringement, and respond preferentially to selective serotonin reuptake inhibitors (SSRIs) (Baldwin, Brandish, & Meron, 2008; Lochner et al., 2003; Niederauer, Braga, De Souza,

Meyer, & Volpato Cordioli, 2007), while comorbid OCD and social impairment demonstrate greater OC symptom severity and treatment resistance (Alarcon, Libb, & Spitler, 1993; Khanna, Rajendra, & Channabasavanna, 1988) with a poor treatment outcome (Stewart et al., 2010). Finally, increased OC symptom severity may worsen social impairment and vice versa (Rosa et al., 2012). Although youth with OCD have fewer friendships and struggle to maintain these relationships, they do not perceive themselves as different from normal controls (Piacentini et al., 2003). Instead of being unable to socialize per se, they may only be more socially isolative in scenarios where normal peers may observe their behavior (Piacentini et al., 2003).

Spontaneous stereotypy in the deer mouse (*Peromyscus maniculatus bairdii*) is a naturalistic animal model of OCD that is founded on robust face, construct and predictive validity (Albelda & Joel, 2012; Joel, 2006; Joel, Stein, & Schreiber, 2008; Wang, Simpson, & Dulawa, 2009). Deer mouse stereotypy can generally be categorized into repetitive jumping and pattern

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running (Korff, Stein, & Harvey, 2008; Powell, Newman, Pendergast, & Lewis, 1999), behaviors that correspond with the repetitive and compulsive-like motor actions of OCD. As is true for stereotypy, compulsivity is an inappropriate and persistent behavior with no apparent goal (Bartz & Hollander, 2006). Furthermore, the deer mouse demonstrates varying symptom intensity as is the case in OCD (Korff et al., 2008; Wolmarans, Brand, Stein, & Harvey, 2013). As with human OCD (El Mansari & Blier, 2006; Fineberg, 2004), chronic but not subchronic SSRI treatment decreases stereotypic behaviors while it also replaces high-stereotypical bouts with periods of normal rodent activity (Wolmarans et al., 2013). High stereotypic deer mice also present with decreased striatal serotonin transporter (SERT) density (Wolmarans et al., 2013), the latter also described in OCD (Atmaca et al., 2011; Reimold et al., 2007; Zitterl et al., 2008).

Given that the complex nature of social relationships between OCD patients and normal peers is relatively understudied, and that it is uncertain how well these social impairments resolve following treatment, the *primary aim* of the current investigation was to apply the deer mouse model of OCD to assess the social behavior of N- (*viz.*, healthy controls) and H- (*viz.*, OCD-like) stereotypical deer mice. The deer mouse presents with unique species-specific challenges relating to behavioral analysis that complicates experimental design, for example, their high stereotypical nature complicates behavioral assessment of anxiety in the elevated plus maze (EPM) and open field test (OFT). Similarly, the standard social interaction challenge (SIC) for rodents (Duxon, Kennett, Lightowler, Blackburn, & Fone, 1997; Jenkins, Harte, McKibben, Elliott, & Reynolds, 2008; Kask, Nguyen, Pabst, & Von Hörsten, 2001; Möller, Du Preez, Emsley, & Harvey, 2011) is likewise complex. While the SIC is most often performed with two sex and weight-matched animals from identical behavioral and treatment backgrounds (Duxon et al., 1997; Jenkins et al., 2008; Kask et al., 2001; Möller et al., 2011; Scattoni, Martire, Cartocci, Ferrante, & Ricceri, 2013; Willey, Varlinskaya, & Spear, 2009; Wongwitdecha & Marsden, 1996), a new SIC paradigm will be developed to closely simulate the social interaction between OCD patients and normal peers.

Drawing from clinical literature relating to social behavior in patients with OCD and taking the high stereotypical nature of these animals into account, we hypothesized that (1) the behavior of animals from both the N and H cohorts will differ depending on the cohort structure of the group, (2) chronic escitalopram treatment will significantly influence the behavior of H animals toward one another and toward N controls, and (3) different criteria than those published previously for evaluating the social behavior of rodents need to be

applied to attain the aforementioned objectives. The *secondary aim* was to develop a novel SIC that would enable investigation of *group* social behavior of N and H animals both within and between cohorts, and how such behavior responds to the typical pharmacotherapy regime used in OCD, *viz.*, chronic SSRI treatment. To this end, performing a SIC between three, and not two animals in different combinations of cohorts will be applied to study the social dynamics between animals of different behavioral cohorts and how the presence of an animal from either an H or N cohort will affect the behavior of an animal from a different stereotypical cohort. Employing a pharmacotherapy arm (escitalopram 50 mg/kg/day $\times$ 28 days) will reveal how treatment alters social competence of H deer mice as well as the behavioral cohort structure of the group. As far as we are aware, this is the first study in a translational animal model of OCD that has assessed the complicated nature of group social interaction between animals of different behavioral classifications.

Materials and methods

Animals

Seventy deer mice were obtained from the deer mouse colony maintained and housed at the vivarium of North-West University (NWU), Potchefstroom, South Africa (Ethical Approval Number—NWU-00066-10-S5). The original colony was established using breeding pairs obtained from the *Peromyscus* Genetic Stock Centre at the University of South Carolina. As reported previously (Korff et al., 2008), stereotypical behavior in the deer mouse is established at the age of 8 weeks and only mice between the ages of 10 and 12 weeks were chosen as experimental subjects at the onset of behavioral assessments (day 0). Mice were randomly chosen from different litters, without sex or weight bias and housed in groups of 6 same-sex animals per cage. Earlier studies have already noted that the severity of stereotypy in deer mice is independent of sex (Hadley, Hadley, Ephraim, Yang, & Lewis, 2006; Powell et al., 1999; Presti, Watson, Kennedy, Yang, & Lewis, 2004; Wolmarans et al., 2013). One week before the start of the behavioral assessments, each animal was allocated individually to an automatic climate-controlled laboratory cage (35 cm (l) $\times$ 20 cm (w) $\times$ 13 cm (h); Techniplast® S.P.A., Varese, Italy) and maintained at 23°C on a 12-hour light/dark cycle (lights on at 06h00 and off at 18h00). Food and water were provided *ad lib* for the duration of the study. Cages were cleaned and new bedding material added weekly.

Initial classification of N and H animals

Before performing the SIC, animals were categorized as N or H according to a previously published protocol (Wolmarans et al., 2013). In short, all animals underwent 12-hour behavioral screening once a week for five consecutive screens. On any specific assessment day, animals were moved from their housing environment to the behavioral screening room. These areas are located on the same floor of the vivarium and are environmentally controlled with respect to temperature (23°C), relative humidity (55%), and light cycle (same as for home cages). Subsequently, each animal was introduced to a behavioral test cage (21 cm (w) × 21 cm (l) × 35 cm (h); Accuscan® Inc., Columbus, Ohio, USA) constructed from clear, translucent Plexiglas®. Bedding material was provided in quantities enough to cover the floor of the test cages, but also ensuring that it did not interrupt the scoring of behavioral data. Food was provided ad lib on the floor of the cage in the form of broken-up rodent chow pellets. Bottled water was provided through a tight-fit hole in the wall of each test cage. Cages were covered with lids that allowed uninterrupted airflow. The animals were introduced to these environments by 16h00 and habituated for at least 2 hours before the 12-hour behavioral assessments started at the onset of the dark cycle. Test cages were cleaned after each screen.

Behavioral assessment was performed using the Fusion® Animal Activity Monitoring System (Accuscan® Inc., Columbus, Ohio, USA), and analyzed using Fusion® software. Briefly, each testing cage presents with a grid of infrared light beams that cross the cage roughly 2 cm above the cage floor along the X- and Y-axes (constituting a grid), while another set of beams (Z-axis) crosses the cage 10 cm above the cage floor, but only parallel to the lower Y-axis. Parallel beams are spaced 2.5 cm apart and record activity every time it is interrupted by movement. The number of vertical beam interruptions (VBI) was used as a broad measure of vertical jumping activity, while the number of clockwise and anti-clockwise cage revolutions (CR) was used to evaluate the expression of pattern running (Wolmarans et al., 2013). During the recording session, the Fusion® software generates data continuously, allowing for experimental playback and the export of behavioral reports the following day.

Following the first five behavioral screens, animals were divided into N and H groups according to our previously published protocol (Wolmarans et al., 2013). As findings from previous studies (Wolmarans et al., 2013) demonstrated the time-consuming nature of stereotypy, we amended the protocol to include both

the intensity of stereotypical bouts (average of the highest individual 30-minute stereotypy counts generated during each of the five respective trials) and the time spent executing stereotypy (number of 30-minute H-stereotypical bouts generated over all five trials) as indicators of behavioral severity. The published criteria for defining vertical and horizontal H intervals are represented in Table 1. This data was plotted for both vertical (Figure 1A) and horizontal activity (cage revolutions; Figure 1B) and, as no distinction was made between the different forms of stereotypy, H animals could demonstrate stereotypical behavior in either one

Table 1. Criteria for defining N and H intervals of stereotypical activity with respect to vertical activity and cage revolutions.

Stereotypy intensity	Vertical beam interruptions/ 30 min	Cage revolutions/ 30 min
N	<500	<150
H	>2000	>200

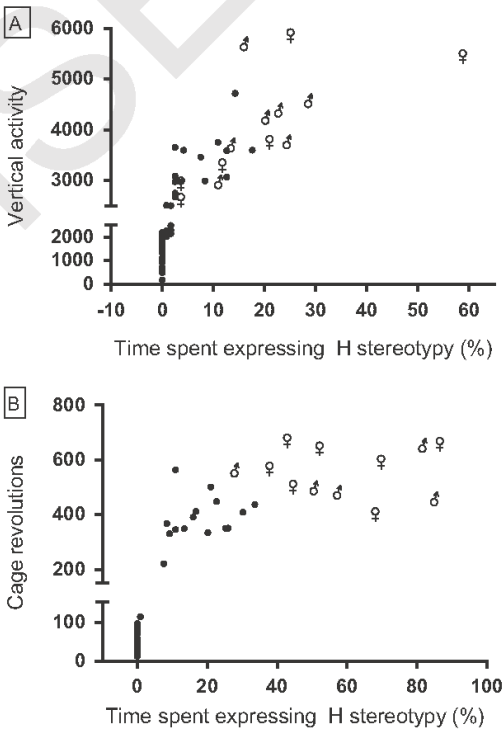


Figure 1. Stereotypy intensity versus time spent expressing H stereotypy. 1A: Mean of highest individual daily vertical activity scores over the first five baseline behavioral screens versus time spent expressing H stereotypy. 1B: mean of highest individual daily cage revolution scores over the first five baseline behavioral screens versus time spent expressing H stereotypy. Sex symbols indicate high stereotypical males and females, respectively.

or both of the behavioral topographies. We subsequently included 18 animals in the H group (Figures 1A and 1B combined; subjects identified with appropriate sex symbols) that expressed both the highest intensity of stereotypy for the longest cumulative duration of time, irrespective of whether an individual did so in only one or both of the topographies (the sum total of sex symbols from Figures 1A and 1B, i.e., 25, exceeds 18 as more than one animal displayed H behavior in both topographies). The group of N animals was simply constituted from the 18 animals that consistently generated the lowest stereotypy counts (Figures 1A and 1B combined; black dots plotted at $X = 0$).

As only 18 animals were included in each cohort following the first five behavioral screens, a gray margin of 34 animals was excluded from the experimental design. The rationale for the exclusion of a gray margin of animals is to improve sensitivity, as described elsewhere (Wolmarans et al., 2013).

The social interaction challenge (SIC)

Before the SIC (performed on day 0), all animals were habituated individually to the test environment three times for 10 minutes during the dark cycle from day -3 to day -1. Briefly, the SIC was performed during the dark cycle under dim white light (40 lux) in black Plexiglas® cages (50 cm × 50 cm) between 19:00 and 24:00. On the night of testing, three same-sex animals were color marked (color stickers attached on the back

of the animal) and simultaneously introduced to the testing cages to prevent possible resident-intruder interactions. Animals were combined in groups consisting of HHH, NNN, HHN, and NNH animals, respectively (Table 2). All animals were naive with respect to social contact and each animal was only used once (thus 18 N and H animals, respectively). The SIC was performed three times before the onset of treatment (pretreatment control) and again for three times following four weeks of escitalopram treatment with the same animals. However, individuals introduced to the cages for the posttreatment SIC were combined differently than during the pretreatment SIC to exclude the possible influence of familiarization. Therefore, each animal had contact with another animal only once over the course of both the pre- and posttreatment challenges.

Animals were left to socialize for 5 minutes and the number of approaches (interaction distance set as ≤ 15 cm), episodes of proximity (set as ≤ 5 cm), cumulative duration of proximity and net weighted movement with respect to one another was calculated using Ethovision XT® 11 software (Noldus Information Technology B.V., Wageningen, Netherlands; Table 3). Net weighted movement is an objective measure of the intensity of approach and avoidance behavior (Spruijt, Hol, & Rousseau, 1992), allowing the movement of subjects toward (*viz.*, more positive values; approach behavior) or away (*viz.*, more negative values; avoidance behavior) from each other to be calculated, regardless of the direction of movement. Video files

Table 2. Description of group structures in the social interaction paradigm and the nature of interactions measured.

Group name Each performed three times before and after treatment	Description of group structure and interactions measured (<i>n</i>) values as obtained during one assessment
HHH/NNN	• Three H or N animals grouped together • Number of approaches measured in both directions between respective animals (<i>viz.</i>, a-b, a-c; b-a, b-c; c-a, c-b; <i>n</i> = 6) • Number of episodes of proximity between all subjects measured (<i>viz.</i>, a-b, a-c, b-c; <i>n</i> = 3) • Cumulative duration of proximity (in seconds) measured between all subjects (<i>viz.</i>, a-b, a-c, b-c; <i>n</i> = 3) • Net weighted movement calculated between respective animals in both directions (<i>viz.</i>, a-b, a-c; b-a, b-c; c-a, c-b; <i>n</i> = 6)
HHN	• Two H animals and one N animal grouped together • HH(N): Social behavior measured between two H animals only but with an N animal in the cage. Number of approaches in both directions (<i>n</i> = 2), number of episodes of proximity (<i>n</i> = 1), duration (sec) of proximity (<i>n</i> = 1) and net weighted movement in both directions (<i>n</i> = 2). • [HH]N: Social behavior of two H animals toward the N animal. Number of approaches of each H animal toward the N animal (<i>n</i> = 2), number of episodes of proximity (<i>n</i> = 2), duration of proximity (<i>n</i> = 2), and net weighted movement of each H animal toward/away from the N animal (<i>n</i> = 2)
NNH	• Two N animals and one H animal grouped together • NN(H): Social behavior measured between two N animals only but with an H animal in the cage. Number of approaches in both directions (<i>n</i> = 2), number of episodes of proximity (<i>n</i> = 1), duration of proximity (<i>n</i> = 1), and net weighted movement in both directions (<i>n</i> = 2). • [NN]H: Social behavior of two N animals toward the H animal. Number of approaches of each N animal toward the H animal (<i>n</i> = 2), number of episodes of proximity (<i>n</i> = 2), duration of proximity (<i>n</i> = 2), and net weighted movement of each N animal toward/away from the H animal (<i>n</i> = 2)

Table 3. Definitions and descriptions of the social interaction parameters measured.

Behavior measured by Ethovision®	Description/Definition
Number of approaches (viz, moving to)	The state is "moving to" when the focal subject (actor) is moving toward another subject (receiver). Interaction distance set as ≤ 15 cm
Number of episodes and cumulative duration of proximity (s)	A discrete variable with two possible states, "in proximity" and "not in proximity". • The state is "in proximity" when the distance between the selected body points of the focal subject (actor) and the body points of another subject (receiver) is lower than a user-defined "in proximity" threshold (set as ≤ 5 cm) • The state is "not in proximity" when the distance between the selected body points of the actor and the body points of another subject receiver is greater than 5 cm
Net weighted movement	The signed, distance-weighted change in distance between two subjects from one sample to the next. Net weighted movement is weighted by the distance between two subjects. Changes in positions of subjects which are at a large distance from each other have a lower weight, so they can be distinguished from movements at close distance, which have a different biological meaning. Unlike "relative movement", this is a continuous variable (in distance units). The "net weighted movement" is positive if the subject (actor) is getting closer to another subject (receiver), negative in the other case

Adapted from the Ethovision XT® Reference Manual. See also (Spruijt et al., 1992).

(not shown) were scrutinized and cross-analyzed with the behavioral data to verify the presence of social and not aggressive behavior. Although controversy remains as to the exact nature, pathology and clinical relevance of rodent aggression (Blanchard, Wall, & Blanchard, 2003), we excluded the presence of aggressive behavior (biting, scratching, boxing, and wrestling (Kalinine et al., 2014; Lewis, Gariépy, Gendreau, Nichols, & Mailman, 1994)), through careful scrutiny of the video files.

For more information regarding Ethovision XT® 11 software, please visit <http://www.noldus.com>.

Drug and administration

As published previously (Wolmarans et al., 2013), escitalopram oxalate was prepared for oral administration by dissolving it in the drinking water. Physical handling of the mice was kept to a minimum to prevent any possible anxiogenic effects. For the same reason, oral gavage was not considered as an appropriate dosage route.

Following the first five screens during which all animals only received tap water, escitalopram was administered orally for 4 weeks by dissolving it in the drinking water. The final concentration of the drinking solution was constituted at 20 mg/100 ml ensuring that each animal would receive 50 mg/kg/day (within close limits) of escitalopram. The dose calculation was based on a prior pilot study where the average water intake per mouse was determined as 0.25 ml/g/day (data not shown), and confirmed by others.

Statistics

Due to social behavior of animals demonstrating notable variance, data are not distributed normally (Shapiro–Wilk tests run for each data set, significance was $p < 0.05$ for all sets). As such, Mann–Whitney

analysis was applied to comparisons of the between-group treatment-naïve social behavior of H and N deer mice (Figure 2) and the within-group responses to treatment (Figures 3i and 4i). To compare the treatment-naïve social behavior of N and H animals in mixed paradigms with the behavior of N and H animals in the NNN and HHH control groups, respectively (Figure 2), Kruskal–Wallis ANOVA was applied. Furthermore, Kruskal–Wallis ANOVA was also applied to compare the statistical significance of end point differences in the average response to treatment (Figures 3i and 4i) and the percentage change from baseline (Figures 3ii and 4ii) in the behavior of N and H animals in mixed paradigms, with the NNN and HHH groups as respective controls. Statistical significance was set at $p < 0.05$. All statistical analysis was followed by Cohen's d calculations to establish the practical significance of effect magnitude. Cohen's d is an effect size used to indicate the standardized difference between two means. Effect sizes are considered large at values between 0.8 and 1.29 and very large at values higher than 1.3. Statistical analysis was performed with IBM® SPSS® Statistics 22.0 and GraphPad Prism® 6.01 software under the guidance of the statistical consultation service of North-West University, Potchefstroom.

Results

Considering that no form of aggressive behavior was observed during scrutiny of video files (not shown), the results of the SIC will be discussed under three headings viz., Figure 2, Figure 3, and Figure 4. For a detailed description of the different groups and the interactions measured, see Table 2. Table 3 provides a description of the various parameters measured. Figure 2 presents findings regarding the social behavior of treatment-naïve N and H animals while Figures

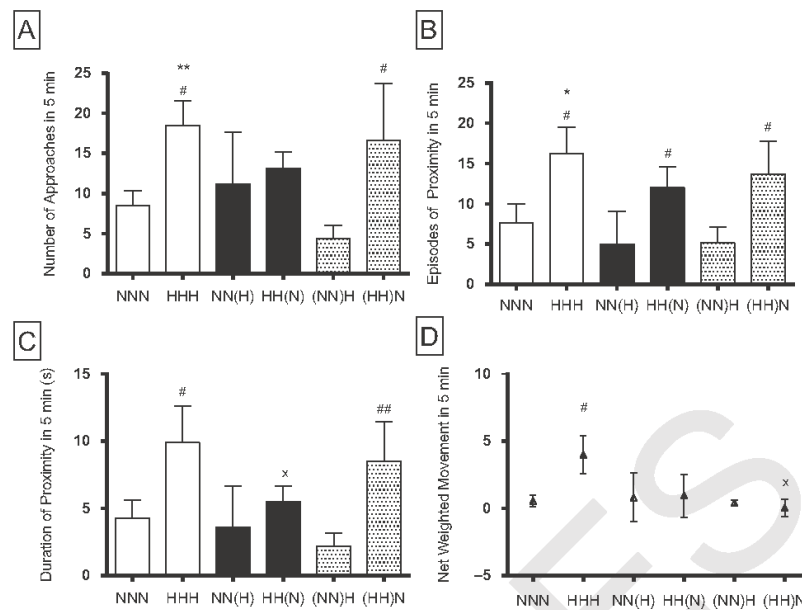


Figure 2. Baseline social behavior of N and H animals for four separate social interactive behaviors (A–D). For a description of the different group structures, see Table 2. Descriptive statistics are provided in Tables 4 and 5. Data is presented as mean $\pm$ SEM. (*Pairwise Mann–Whitney *U* test; #Cohen's *d* effect size of pairwise comparisons; xCohen's *d* effect size between between social behavior of N and H animals in mixed paradigms with respective NNN and HHH controls).

3i and 3ii presents comparisons between the pre- and posttreatment manifestation of social behavior of N animals. Figures 4i and 4ii presents the same comparisons as Figure 3, but with respect to social behavior of H animals. Descriptive statistics for all analyses are provided in Tables 4–6.

Figure 2 (Tables 4 and 5)—baseline social behavior of N and H animals

Animals in the HHH group demonstrated significantly higher treatment-naïve approaches (2A, 18.5 ± 3.1 , $**p = 0.009$) as well as episodes of proximity between one another (2B, 16.2 ± 3.3 , $*p = 0.04$), both characterized by large effect sizes ($^{\#}d = 0.95$ and $^{\#}d = 1.0$, respectively), when compared to the behavior of animals in the NNN group (2A, 8.5 ± 1.8 and 2B, 7.7 ± 2.4 , respectively). These data are validated by the trends toward increased duration of proximity (2C, 9.9 ± 2.7 , $^{\#}d = 0.92$) and net weighted movement (2D, 4.0 ± 1.4 , $^{\#}d = 1.03$) observed between animals in the HHH group compared to that of the NNN group (2C, 4.3 ± 1.3 and 2D, 0.5 ± 0.4 , respectively), indicating that H subjects approached, rather than avoided one another throughout the 5-minute challenge.

Although H animals in the HHN group demonstrated a trend toward an increased number of episodes spent together in the presence of an N animal (2B, HH[N]; 12.0 ± 2.6 , $^{\#}d = 1.2$) compared to the same behavior of N animals in the presence of an H animal (2B, NN[H]; 5.0 ± 4.0), this was not demonstrated in any of the other parameters (2A, C and D; HH[N] vs. NN[H]). However, in contrast to the behavior of N animals toward an H animal (Figure 2A–D, [NN]H), large (2A and B) and very large (2C) effect sizes were observed with respect to the behavior of H animals toward an N animal ([HH]N) vs. [NN]H (2A: 16.7 ± 7.0 vs. 4.3 ± 1.7 , $^{\#}d = 1.15$; 2B: 13.7 ± 4.1 vs. 5.2 ± 2.0 , $^{\#}d = 1.13$; 2C: 8.5 ± 2.9 vs. 2.2 ± 1.0 , $^{\#}d = 1.32$).

Considering comparisons of the behavior of N and H animals in mixed groups (*viz.*, NNH and HHN) with their behavior toward one another in the respective NNN and HHH control groups (Table 5), N animals demonstrated indistinguishable social behavior toward one another (NN[H]) and toward an H animal ([NN]H) (Figure 2A–D). However, although no statistical significance could be established, large negative effect sizes were observed in the behavior of H animals in the mixed group toward one another with respect to duration of proximity (2C, HH[N], 5.5 ± 1.2 , $^{\times}d = -0.86$) and toward an N animal with respect to net weighted

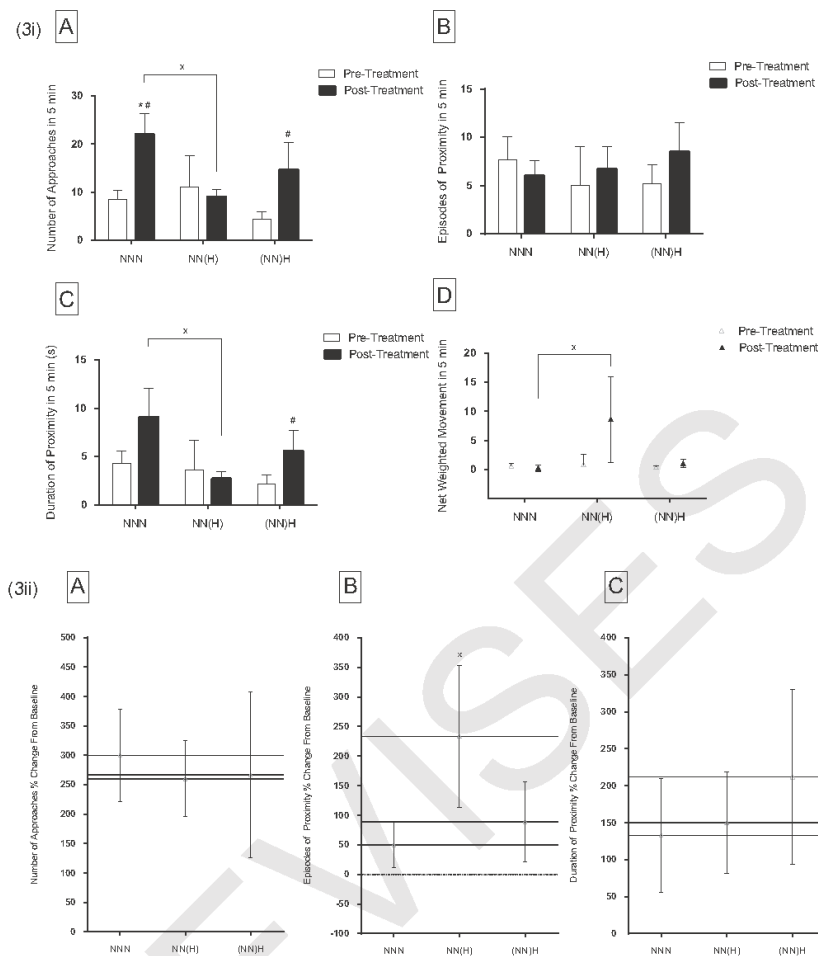


Figure 3. Comparisons of pre- and post treatment social behavior of N animals. *3i*: Within- and between group comparisons of pre- and post-treatment social behavior for 4 separate social interactive behaviors (A-D). For a description of the different group structures, see Table 2. Descriptive statistics are provided in Table 6. Data is presented as mean $\pm$ SEM. (*Pairwise Mann-Whitney *U* test; #Cohen's *d* effect size of within-group comparisons; $\times$ Cohen's *d* effect size of end point differences in the posttreatment social behavior between N animals within the NNH paradigm and those in the NNN control group). *3ii*: Posttreatment percentage change from average baseline social behavior with respect to approach behavior, episodes of proximity, and duration of proximity. ($\times$ Cohen's *d* effect size of end point differences in the posttreatment social behavior between N animals within the NNH paradigm and those in the NNN control group).

movement (2D, [HH]N, 0.035 ± 1.61 , $\times d = -1.03$) compared to the behavior of H animals in the HHH group (2C, 9.9 ± 2.7 and 2D, 3.4 ± 1.4 respectively).

Figure 3 (Table 6)—pre- and posttreatment social behavior of N animals

Chronic treatment with escitalopram significantly increased the number of approaches with a large effect size between animals in the NNN grouping (3i A, 22.0 ± 4.3 vs. 8.5 ± 1.8 , $\#d = 1.03$) but failed to influence

either episodes and duration of proximity or net weighted movement within the same group (3i B-D). Furthermore, although the behavior of N animals toward one another in a mixed group remained unchanged, (Figure 3i A-D, NN[H]), N animals demonstrated trends with large effect sizes toward increased approach behavior and duration of proximity with respect to social interaction with H animals (3i A, [NN] H, 14.7 ± 5.7 vs. 4.3 ± 1.7 , $\#d = 1.15$ and 3i C, [NN]H, 5.7 ± 2.1 vs. 2.1 ± 0.9 , $\#d = 0.9$) following treatment. Interestingly, Cohen's *d* calculations revealed

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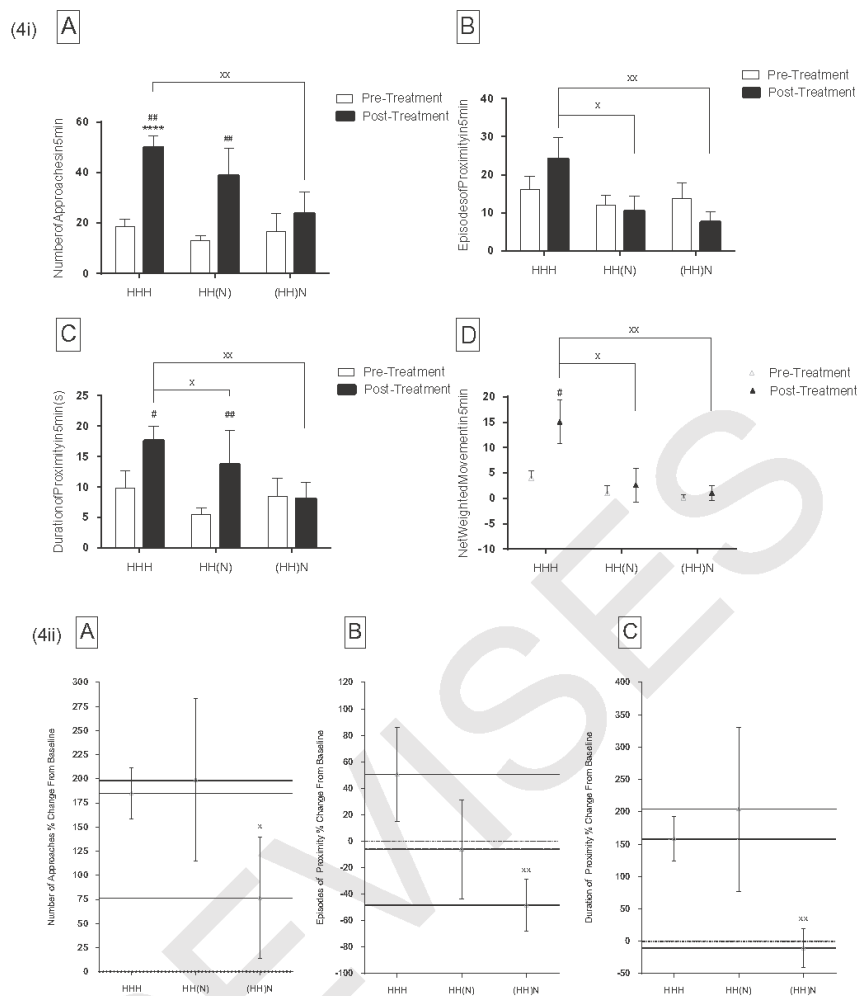


Figure 4. Comparison of pre- and posttreatment social behavior of H animals. *4i*: Within- and between-group comparisons of pre- and posttreatment social behavior for four separate social interactive behaviors (A–D). For a description of the different group structures, see Table 2. Descriptive statistics are provided in Table 6. Data is presented as mean $\pm$ SEM. (*Pairwise Mann–Whitney U test; #Cohen’s d effect size of within-group comparisons; xCohen’s d effect size of end point differences in the posttreatment social behavior between H animals within the HHN paradigm and those in the HHH control group). *4ii*: Posttreatment percentage change from average baseline social behavior with respect to approach behavior, episodes of proximity, and duration of proximity. (xCohen’s d effect size of end point differences in the posttreatment social behavior between N animals within the NNH paradigm and those in the NNN control group).

posttreatment differences with large negative effect sizes with respect to the number of approaches toward one another (**3i A**, NN[H], 9.0 ± 1.6 , $^x d = -1.16$) and duration of proximity between one another (**3i C**, NN[H], 2.7 ± 0.7 , $^x d = -1.23$) in the behavior of N animals in the NNH group, compared to animals in the NNN group (**3i A**, 22 ± 4.3 and **3i C**, 9.0 ± 3.0 , respectively). However, taking the net weighted movement into account, a trend toward a more positive posttreatment value was observed in the behavior of N animals

toward one another in the NNH group, compared to the NNN controls (**3i D**, NN[H], 8.6 ± 7.4 vs. 0.2 ± 0.5 , $^x d = 0.82$).

Considering the percentage change from average baseline with respect to the number of approaches, episodes of proximity and duration of proximity, chronic escitalopram treatment had minimal effect on magnitudes of change, with the exception being a noteworthy increase in the episodes of proximity between N animals in the NNH group compared to

Table 4. Descriptive statistics of pairwise between-group social behavior of treatment naive N and H animals.

			Pretreatment comparisons between N and H behavior (Figure 2, Mann–Whitney U test)		
			<i>p</i>	Mann–Whitney <i>U</i>	Cohen's <i>d</i>
Number of approaches	NNN vs. HHH	<i>n</i> = 18	**0.009	80.5	# 0.95
	NN(H) vs. HH(N)	<i>n</i> = 6	0.37	12.0	0.19
	(NN)H vs. (HH)N	<i>n</i> = 9	0.14	8.5	# 1.15
Episodes of proximity	NNN vs. HHH	<i>n</i> = 9	*0.04	17.0	# 1.00
	NN(H) vs. HH(N)	<i>n</i> = 3	0.4	2.0	# 1.20
	(NN)H vs. (HH)N	<i>n</i> = 6	0.17	9.0	# 1.13
Duration of proximity	NNN vs. HHH	<i>n</i> = 9	0.11	22.0	# 0.92
	NN(H) vs. HH(N)	<i>n</i> = 3	0.7	3.0	0.51
	(NN)H vs. (HH)N	<i>n</i> = 6	0.13	8.0	## 1.32
Net weighted movement	NNN vs. HHH	<i>n</i> = 18	0.18	119.0	# 1.03
	NN(H) vs. HH(N)	<i>n</i> = 6	0.9	17.0	0.03
	(NN)H vs. (HH)N	<i>n</i> = 6	0.47	13.0	0.38

Descriptive statistics of between-group social behavior of N and H animals (Figure 2). Statistical significance and large effect sizes as determined by Cohen's *d* calculations are provided in bold script. (*n*-values are provided as a total of three repetitions).

Table 5. Descriptive statistics of comparisons between the behavior of N and H animals in mixed paradigms (NNH and HHN) with the behavior of animals within the respective NNN and HHH control groups.

			Pretreatment comparisons of N and H behavior in mixed paradigms with NNN and HHH controls, respectively (Figure 2, Kruskal–Wallis ANOVA)	
			<i>p</i>	Cohen's <i>d</i>
Number of approaches (Control <i>n</i> = 18)	NN(H)	<i>n</i> = 6	>0.99	0.22
	HH(N)		0.93	(-) 0.60
	(NN)H	<i>n</i> = 6	0.58	(-) 0.71
Episodes of proximity (Control <i>n</i> = 9)	(HH)N		0.95	(-) 0.12
	NN(H)	<i>n</i> = 3	0.66	(-) 0.37
	HH(N)		>0.99	(-) 0.58
Duration of proximity (Control <i>n</i> = 9)	(NN)H	<i>n</i> = 6	>0.99	(-) 0.42
	(HH)N		>0.99	(-) 0.26
	NN(H)	<i>n</i> = 3	0.85	(-) 0.15
Net weighted movement (Control <i>n</i> = 18)	HH(N)		>0.99	× (-) 0.86
	(NN)H	<i>n</i> = 6	0.55	(-) 0.67
	(HH)N		>0.99	(-) 0.18
	NN(H)	<i>n</i> = 6	>0.99	(-) 0.08
	HH(N)		0.63	(-) 0.61
	(NN)H	<i>n</i> = 6	> 0.99	(-) 0.10
	(HH)N		0.32	× (-) 1.03

Statistical significance and large effect sizes as determined by Cohen's *d* calculations, are provided in bold script. (*n*-values are provided as a total of three repetitions).

the behavior of animals in the NNN control group (Figure 3ii B, NN[H], 233 ± 120.2 vs. 50 ± 38.6 , $\times d = 1.13$).

450 **Figure 4 (Table 6)—pre- and posttreatment social behavior of H animals**

Chronic escitalopram induced meaningful changes in the behavior of H animals in the HHH grouping, with a significant increase demonstrating a very large effect size in the number of approaches observed (4i A, 49.9 ± 4.6 vs. 18.50 ± 3.1 , $**** p < 0.0001$, $## d = 1.92$). Furthermore, although narrowly missing statistical significance, H animals within the HHH group also demonstrated trends with large effect sizes toward increased duration of proximity (4i C, 17.6 ± 2.4 vs. 9.9 ± 2.7 , $p = 0.06$, $# d = 1.0$) and net weighted movement (4i D, 15.0 ± 4.3 vs. 4.0 ± 1.4 ; $p = 0.18$, $# d = 0.91$) following

treatment. Moreover, escitalopram treatment tended to increase the number of approaches and duration of proximity between H animals in the presence of an N animal (HH[N]; 4i A, 38.8 ± 10.9 vs. 13.2 ± 1.2 , $## d = 1.62$ and 4i C, 13.7 ± 5.7 vs. 5.5 ± 1.2 , $## d = 1.38$). However, comparisons between the posttreatment behavior of H animals toward one another in the HHH and HHN groups, respectively, revealed noteworthy differences displaying large negative effect sizes with respect to the number of episodes of proximity, and net weighted movement (HH[N]; 4i B, 10.3 ± 4.1 vs. 24.1 ± 5.7 , $\times d = -1.14$ and 4i D, 2.6 ± 3.3 vs. 15.0 ± 4.3 , $\times d = -0.94$).

In contrast to the increased posttreatment socializing between H animals, the social interaction of H animals toward an N animal ([HH]N; 4i A–D) remained poor, even after 4 weeks of chronic treatment with escitalopram. As such, H animals approached N animals less often (4i A, 23.8 ± 8.5 vs. 49.9 ± 4.6 , $p = 0.06$,

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Table 6. Descriptive statistics of within and between-group comparisons of the pre- and posttreatment social behavior of N and H mice.

		Pre- vs posttreatment within group comparisons (Figures 3i and 4i, Mann-Whitney U test)				End point comparisons of N and H behavior in mixed paradigms with NNN and HHH controls, respectively (Figures 3i and 4i, Kruskal-Wallis ANOVA)		Endpoint comparisons: % change from baseline in the behavior of N and H animals in mixed paradigms compared to NNN and HHH controls respectively (Figures 3ii and 4ii, Kruskal-Wallis ANOVA)	
		<i>n</i>	<i>p</i>	Mann-Whitney <i>U</i>	Cohen's <i>d</i>	<i>p</i>	Cohen's <i>d</i>	<i>p</i>	Cohen's <i>d</i>
Number of approaches	NNN	18	* 0.02	86.5	# 1.03		Control		Control
	NN(H)	6	0.42	12.5	(-) 0.20	0.28	× (-) 1.16	> 0.99	(-) 0.16
	(NN)H	6	0.36	12.0	# 1.15	> 0.99	(-) 0.45	> 0.99	(-) 0.09
Episodes of proximity	NNN	6	> 0.99	40.5	(-) 0.30		Control		Control
	NN(H)	3	0.80	3.5	0.30	> 0.99	0.15	0.50	×1.13
	(NN)H	6	0.44	13.0	0.54	0.89	0.41	> 0.99	0.27
Duration of proximity	NNN	9	0.16	24.0	0.74		Control		Control
	NN(H)	3	0.70	3.0	(-) 0.27	0.42	× (-) 1.23	> 0.99	0.09
	(NN)H	6	0.38	12.0	# 0.90	> 0.99	(-) 0.47	> 0.99	0.30
Net weighted movement	NNN	18	0.76	152.0	(-) 0.17		Control		Control
	NN(H)	6	0.45	13.0	0.70	0.61	× 0.82		
	(NN)H	6	0.81	16.0	0.52	> 0.99	0.40		
Number of approaches	HHH	18	***<0.0001	38.5	## 1.92		Control		Control
	HH(N)	6	0.37	12.0	## 1.62	0.86	(-) 0.47	> 0.99	0.09
	(HH)N	6	0.57	14.0	0.37	0.06	×× (-) 1.30	0.35	× (-) 0.81
Episodes of proximity	HHH	6	0.45	31.5	0.58		Control		Control
	HH(N)	3	0.90	4.0	(-) 0.39	0.56	× (-) 1.14	> 0.99	(-) 0.66
	(HH)N	6	0.33	11.5	(-) 0.72	0.09	×× (-) 1.39	0.17	×× (-) 1.30
Duration of proximity	HHH	9	0.06	19.0	# 1.00		Control		Control
	HH(N)	3	0.70	3.0	## 1.38	> 0.99	(-) 0.46	> 0.99	0.28
	(HH)N	6	0.67	15.0	(-) 0.06	0.08	×× (-) 1.40	0.09	×× (-) 1.91
Net weighted movement	HHH	18	0.18	119.0	# 0.91		Control		Control
	HH(N)	6	> 0.99	18.0	0.27	0.19	× (-) 0.94		
	(HH)N	6	0.47	13.0	0.39	0.31	×× (-) 1.30		

Statistical significance and large effect sizes as determined by Cohen's *d* calculations, are provided in bold script. (*n*-values are provided as a total of three repetitions).

××*d* = -1.3), spent fewer episodes of proximity near N animals (**4i B**, 7.5 ± 2.8 vs. 24.1 ± 5.7 , $p = 0.09$, ××*d* = -1.39), spent less time in the vicinity of N animals (**4i C**, 8.1 ± 2.6 vs. 17.6 ± 2.4 , $p = 0.08$, ××*d* = -1.4) and generated a more negative net weighted movement interacting with N animals (**4i D**, 1.0 ± 1.4 , vs. 15.0 ± 4.3 , $p = 0.31$, ××*d* = -1.3) compared to each other in the HHH control group. Furthermore, percentage change from average baseline behavior showed meaningful differences with large (**4ii A**) and very large (**4ii B, C**) negative effect sizes observed in the behavior of H animals toward N animals, compared to the behavior of H animals in the HHH control group ([HH]N; **4ii A**, 76.5 ± 63.0 vs. 185.1 ± 23.33 , ×*d* = -0.81; **4ii B**, (-) 48.3 ± 19.4 vs. 50.56 ± 35.5 , ××*d* = -1.3, and **4ii C**, (-) 10.5 ± 29.4 vs. 158.3 ± 34.7 , ××*d* = -1.91).

Discussion

The most important findings from this study are: (1) the demonstration that distinct differences between the treatment-naïve behavior are observed in N and H animals within and between cohorts (Figure 2), (2) a robust treatment-related increase in the interaction of H

animals with one another in both the HHH and HHN groups is observed, and (3) poor pre- and posttreatment social interaction of N animals toward H animals in the NNH group (Figure 3i).

OCD is a multifactorial condition that not only involves the experience of recurrent intrusive thoughts and/or expression of seemingly purposeless and repetitive behavior (Bartz & Hollander, 2006) but is also characterized by a number of cognitive (Belloch et al., 2011; Dietrich, Emrich, Becker, & Münte, 1996) and functional impairments (Jacoby, Leonard, Riemann, & Abramowitz, 2014; Nadeau et al., 2013; Storch et al., 2014). Furthermore, various clinical investigations have found distinct disturbances in the social behavior of OC patients compared to normal controls (Khanna et al., 1988; Rosa et al., 2012; Storch et al., 2006). Although many aspects of OCD-related changes in social behavior are still unknown, deficits manifest as altered social stability with respect to patient-parent, patient-sibling, and patient-peer relationships (Rosa et al., 2012), an increased incidence of comorbid conditions such as social phobia (Assunção et al., 2012) and poor social adjustment (Khanna et al., 1988; Rosa et al., 2012), and higher rates of occupational impairment such as

unemployment and financial instability (Kim et al., 2012). Furthermore, comorbid diagnosis of OCD and conditions of social impairment are characterized by increased OC symptom severity and poor treatment response (Rosa et al., 2012).

Given the complex nature of OC-related social behavior, the aim of the current investigation was to explore social behavior of animals in a validated animal model of OCD, to establish how these behaviors respond to a typical drug treatment regime applicable to OCD, and how these correlate with that noted in the human condition. In an attempt to do this, we set out to develop a novel SIC capable of assessing within- and between-cohort social interactions, which is of relevance in disorders such as OCD where the extent of social impairment is influenced by the proximity of peer groups. Since the study set out to investigate the group social behavior of same-cohort animals in the presence of an animal from another cohort, a typical two-animal SIC could not be followed. A novel SIC paradigm was therefore developed whereby the social behavior of three animals could be investigated simultaneously. Different group structures (*viz.*, NNN, HHH, NNH, and HHN) enabled us to investigate the social behavior of N (*viz.*, normal) and H (*viz.*, OCD-like) animals in all possible within- and between-cohort combinations (for a description of the various interactions measured, see Table 2).

In the light of H animals demonstrating increased treatment-naïve sociability within cohort compared to N controls (Figure 2), our hypothesis that H animals would interact with one another in a distinctly different manner may hold sway. Furthermore, treatment-naïve H animals in the HHN mixed group demonstrated trends of being more sociable toward one another (Figure 2B; HH[N]) and toward N animals (Figure 2A, B and C; [HH]N) compared to the interaction of N animals with each other or with an H counterpart (Figure 2A–C; NN[H] and [NN]H, respectively). However, given the social impairments often described in OCD patients, it would have been reasonable to anticipate that H animals would tend to act more isolative than sociable compared to N animals. Nevertheless, these findings support those described in earlier clinical investigations (Piacentini et al., 2003) which have concluded that OCD patients, rather than lacking social skill *per se*, are in fact capable of forming social bonds in terms of friendship. Furthermore, our findings with respect to the interactions of N animals with an H counterpart, showing that H animals tend to isolate themselves within a normal peer group, are congruent with clinical data that have reported that children and adolescents with OCD are more susceptible to peer victimization and

marginalization than nonpsychiatric controls (Storch et al., 2006).

Concerning the influence of chronic escitalopram treatment on the social interaction of N and H animals, we anticipated that the behavior of N animals (*viz.*, normal controls) would not be altered. Although N animals demonstrated increased approach behavior within cohort following chronic treatment (Figure 3i A; NNN), no significant differences or large magnitudes of effect were displayed in any of the other measured variables. As such, we interpret the change in approach behavior to be investigative rather than social in nature. Considering the behavior of N animals toward one another in the presence of an H animal, social cohesion remained poor even after chronic escitalopram treatment (Figure 3i A–C; NN[H]). However, as shown in the posttreatment difference between the net weighted movement scores of N animals interacting with one another in the NNN and NNH cohorts respectively (Figure 3i D), the presence of an H animal in the cage resulted in an increased tendency of N animals to group together. Furthermore, increased approach behavior and duration of proximity was observed with respect to the interaction of N animals with an H animal following drug treatment (Figure 3i A, C; [NN]H), suggesting that SSRI treatment tended to restore the abovementioned marginalization. Whether this finding results from an increase in investigative behavior noted in N animals, or from the possibility of H animals being more approachable following treatment (see below), is unclear, but it is nonetheless of interest how an SSRI may modify intergroup socialization between N and H animals. These data are of particular interest as for the first time we have support in an animal model that spontaneous marginalization of OCD patients by their normal peer group does indeed occur (Storch et al., 2006).

The baseline social behavior of H animals was significantly altered by 4-week escitalopram administration in that H animals demonstrated increased posttreatment sociability toward one another in both the HHH and HHN paradigms (Figure 4i A, C, and D). However, the magnitude of difference between the posttreatment sociability of H animals toward one another within these two groups demonstrates large effect sizes (Figure 4i B–D), suggesting that the presence of an N animal in the cage moderated the effects of treatment in H animals to some extent. Although clinical data regarding this phenomenon are limited, at least one case report documented that social phobia toward healthy peers experienced by a patient with OCD only responded to a combination of the SSRI paroxetine (high dose, 60 mg/day) and the atypical antipsychotic,

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risperidone (low dose, 1 mg/day) (Yamauchi, Ohta, Taniguchi, & Ohmori, 2004). This is consistent with our findings that show a more substantial increase in the social interactivity of H animals within cohort, compared to the interaction of H animals in the presence of an N animal. Nevertheless, that escitalopram treatment resulted in substantial trends toward increased social interactivity between H animals in the HHN paradigm, irrespective of the presence of an N animal (Figure 4i A, C), is in agreement with how SSRIs may improve OCD symptoms, *viz.*, by reducing social anxiety and improving social interaction with peers (Baldwin et al., 2008).

Chronic escitalopram treatment failed to influence the behavior of H animals toward N subjects. In fact, instead of improving the social relationships between H and N animals, escitalopram favorably altered the social interaction between animals of the H cohort only, as can be seen in Figures 4i A and C. This finding is particularly interesting in that it implies that the SSRI selectively modified social behavior in H animals in relation to themselves, but not toward normal peers. In fact, the social interaction of H animals with an N animal within the HHN group, and not between one another, seems to have deteriorated following treatment (Figure 4ii, A–C). It can be argued that as OCD patients respond preferentially to chronic high-dose SSRI treatment, an associated improvement in comorbid social deficits, including those involving interactions toward normal peers, could have ensued. However, our data agree with clinical evidence in that the social competence of H deer mice did indeed improve, albeit toward one another. We have demonstrated previously that although chronic escitalopram treatment decreases the number of high-stereotypical bouts, H animals persist in the expression of repetitive motor stereotypy (Wolmarans et al., 2013). OCD patients have been known to hide their symptoms from normal peers (Piacentini et al., 2003), irrespective of how often they occur. We therefore argue that, since H deer mice may still express OC bouts following drug treatment, a decrease in stereotypical bouts associated with an increase in social competence corresponds with clinical findings and that chronic escitalopram treatment dramatically improves the symptomology of H animals.

Limitations of the study include the complicated design of the SIC, although, at the same time, this approach has proven to be a valuable instrument with which to assess the complex nature of social structures. Nevertheless, the need for various comparisons to be made could have resulted in some comparisons narrowly missing statistical significance. Given the natural between-animal variance in social behavior, it is

possible that a larger sample pool could have resulted in more robust differences. Secondly, behavioral analysis with deer mice is problematic given the excessive species-specific expression of motor stereotypy. Social behavior, as is traditionally defined (Jenkins et al., 2008; Kask et al., 2001; Möller et al., 2011), is mostly absent in an SIC performed in deer mice and hence a combination of different behavioral measures and a novel means of investigation is required to distinguish social behavior from other forms of interaction (e.g., aggression). However, we believe that the combination of approach behavior, episodes of proximity, duration of proximity, and net weighted movement, co-analyzed with video data, provides a robust and reliable means of assessing social behavior in deer mice.

Conclusion

As far as we are aware, this is the first investigation in an animal model of OCD where the intergroup sociability of H (*viz.*, OCD-like) and N (*viz.*, normal) controls has been compared. This is the first study to explore the manifestation of *group* social interactions between rodents of the same and different behavioral cohorts and how this behavior can be manipulated by a typical drug treatment regimen employed in human OCD. Furthermore, it describes how social impairment may modify response to treatment, in particular how the presence of an N animal may abrogate SSRI response on social behavior in H animals. The study has demonstrated that distinct differences exist between treatment-naïve behavior of N and H animals within and between cohorts, that the sociability of H animals toward one another (both within and between cohorts) improves following chronic escitalopram treatment, while the interaction of H animals with N animals remains poor. Data from the current investigation are congruent with clinical evidence relating to the social behavior of OCD patients and their social experiences in the presence of healthy peers. However, our findings also suggest that greater focus on social behavior of OCD patients is needed; while they may experience increased social function after treatment with SSRIs, it is also possible that enduring social impairments require rigorous assessment and targeting. Not only do the results of the current investigation highlight the relevance, usefulness, and impact of the deer mouse model of OCD but also contribute to our understanding of the complex social behavior between animals of different behavioral cohorts. Finally, the model sheds new light on how more complex social interaction paradigms may be relevant to patients with OCD.

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(Chapter 4)

*Of mice and marbles: Novel perspectives
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Of mice and marbles: Novel perspectives on burying behavior as a screening test for psychiatric illness

De Wet Wolmarans¹ · Dan J. Stein² · Brian H. Harvey³

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Abstract Burying forms part of the normal behavioral routine of rodents, although its expression is species-specific. However, it has been suggested that aberrant burying behavior, of which marble-burying (MB) is an example, may represent neophobic and/or compulsive-like behavior. In the present investigation, we assessed MB in an established animal model of obsessive-compulsive disorder (OCD)—namely, spontaneous stereotypy in the deer mouse—to establish whether high (H) stereotypy is associated with neophobia and/or another compulsive endophenotype, i.e. MB, as compared to nonstereotypical (N) controls. A three-trial, one-zone MB test was performed over three consecutive evenings both before and after chronic treatment with high-dose (50 mg/kg/day) oral escitalopram. Neophobia was measured via the number of marbles buried during the first pre- and posttreatment MB trials, and compulsive-like behavior via the number of marbles buried over all pre- and posttreatment MB trials. The data from the present study support earlier findings that

burying is a normal behavioral routine (inherent burying behavior, IBB) that is expressed by all deer mice, irrespective of stereotypical cohort, and is not associated with either neophobia or compulsiveness. Indeed, chronic escitalopram treatment, which is similarly effective in treating clinical anxiety and OCD, as well as in attenuating H behavior, failed to influence IBB. Although 11 % of the animals presented with a unique burying endophenotype (high burying behavior), escitalopram also failed to attenuate said behavior, necessitating further investigation as to its relevance. In conclusion, MB cannot be regarded as a measure of anxiety-like or compulsive behavior in the deer mouse model of OCD.

Keywords Marble-burying · Obsessive-compulsive disorder · Neophobia · Deer mouse · Animal model · Escitalopram

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Burying, burrowing, and digging form part of the normal behavioral repertoire of rodents, in both the wild and the laboratory (Deacon, 2006; Smithers, 1983). These strain-specific behaviors are mostly aimed at searching for food, burying both noxious and harmless objects, and building adequate nurseries capable of protecting individuals against predators and providing suitable environments to breed (Smithers, 1983). Evidently, burying and digging are expressed in relatively nonanxiogenic scenarios (e.g., nesting, hoarding, foraging), as well as under anxiogenic circumstances (e.g., burying of noxious objects, confronting predators).

Defensive burying (Pinel & Treit, 1978) can be defined as the process of moving loose bedding material vertically upward and over potentially harmful stimuli or sources of aversive stimulation (e.g., a shock prod). Due to the stress-provoking nature of an electric shock, defensive burying has been used as a measure of avoidance-dependent anxiety in a

number of previous investigations (for a review, see De Boer & Koolhaas, 2003). Another anxiety-related form of burying (or neophobic burying) relates to novelty-induced anxiety (Diamant, Croiset, De Zwart, & De Wied, 1991; Torres-Lista, López-Pousa, & Giménez-Llort, 2015) following exposure to novel, but nonreactive and harmless, objects. In theory, whereas animals demonstrate nonhabituation toward defensive burying, neophobic burying should attenuate over time following repeated exposure to the same stimuli (Londei, Valentini, & Leone, 1998).

However, an abundance of literature (Egashira et al., 2008; Londei et al., 1998; Njung'e & Handley, 1991; Thomas et al., 2009) has demonstrated that rodents often persist in burying harmless forms of stimuli in the absence of anxiety—which, for the purpose of this article, will be referred to as *inherent burying*, of which marble-burying (MB) is a typical example. As such, it has been hypothesized that this represents nonfunctional repetitive behavior analogous to the behavioral symptoms of obsessive-compulsive disorder (OCD; Thomas et al., 2009; Umathe, Manna, & Jain, 2012). Although some studies have employed MB as a measure of avoidance-related anxiety (Nicolas, Kolb, & Prinssen, 2006), findings relating to nonhabituation to marbles in the absence of anxiety (Thomas et al., 2009) are more compatible with inherent than with defensive or neophobic burying. Indeed, MB is a screening test for potential OCD treatments (Egashira et al., 2008; Gyertyan, 1995; Millan, Girardon, Mullet, Brocco, & Dekeyne, 2002). However, the test lacks illness specificity and has been used to model treatment response in impulsivity (Schneider & Popik, 2007), autism (Angoa-Pérez, Kane, Briggs, Francescutti, & Kuhn, 2013), and dementia (Torres-Lista et al., 2015).

Defensive, neophobic, and inherent burying often respond to the same pharmacological agents (De Boer & Koolhaas, 2003; Nicolas et al., 2006). All three forms of burying have shown sensitivity to traditional anxiolytics—namely benzodiazepines (Nicolas et al., 2006; Njung'e & Handley, 1991)—and to various classes of noradrenergic and serotonergic antidepressants—e.g., desipramine, imipramine, citalopram, paroxetine, and fluvoxamine (Abe, Nakai, Tabata, Saito, & Egawa, 1998; Broekkamp, Rijk, Joly-Gelouin, & Lloyd, 1986; Millan et al., 2001). Haloperidol (Nicolas et al., 2006) and chlorpromazine (Bruins Slot, Bardin, Auclair, Depoortere, & Newman-Tancredi, 2008), which are traditional antipsychotics, also attenuate burying behavior. That said, the predictive validity of burying behavior as a screening test for anxiety and/or compulsive-like repetition is undermined by contradictory results—for instance, response to anxiogenic drugs (Nicolas et al., 2006) and sensitivity to a number of compounds not related to the clinical treatment of either anxiety or compulsivity (de Almeida, de Carvalho, Silva, de Sousa, & de Freitas, 2014; Honda, Kawaura, Soeda, Shirasaki, & Takahama, 2011; Krass, Rünkorg, Wegener, &

Volke, 2010; Uday, Pravinkumar, Manish, & Sudhir, 2007). Moreover, previous pharmacological studies have used mostly acute, single-dose treatments ranging from 30 to 90 min before recording burying behavior (Nicolas et al., 2006; Uday et al., 2007), despite antidepressants only being effective after chronic administration (Fineberg & Craig, 2007; Huh, Goebert, Takeshita, Lu, & Kang, 2011).

As opposed to learned or pharmacologically induced perseverative behavior, spontaneous stereotypy in the deer mouse (*Peromyscus maniculatus bairdii*) is a naturalistic animal model of OCD, founded on robust face, construct, and predictive validity (Güldenpfennig, Wolmarans, du Preez, Stein, & Harvey, 2011; Korff, Stein, & Harvey, 2008, 2009; Wolmarans, Brand, Stein, & Harvey, 2013). Deer mouse stereotypy can generally be categorized into repetitive jumping and pattern running (Korff et al., 2008; Powell, Newman, Pendergast, & Lewis, 1999), behaviors that correspond with the repetitive motor actions of OCD. Furthermore, deer mouse stereotypy demonstrates varying symptom intensities, as in OCD, and “time spent executing stereotypy” is employed as a marker of behavioral severity and treatment outcome (Wolmarans et al., 2013). Although deer mouse stereotypy has not yet been studied with respect to anxiety-like behavior, it demonstrates response to chronic, but not to subchronic, treatment with high doses of a selective serotonin reuptake inhibitor (SSRI) that is similarly effective in the treatment of anxiety and compulsive behavior in humans (Wolmarans et al., 2013).

Since MB may reflect both defensive/neophobic (anxiety-like) and inherent (compulsive-like) burying behavior, in the present investigation we attempt to contribute to an understanding of the phenomenology of OCD by challenging an established animal model—namely, spontaneous stereotypy in the deer mouse—with MB. The aims of the present study were (1) to investigate whether the compulsive-like expression of motor behavior by high-stereotypical (H) deer mice can be associated with unique MB behavior, relative to nonstereotypical (N) controls; (2) to assess whether deer mice habituate to burying behavior; and (3) to determine whether such behavior is attenuated by chronic (four-week), high-dose (50 mg/kg/day) oral escitalopram (Baldwin, Brandish, & Meron, 2008).

Materials and method

Animals

A total of 70 deer mice (males and females) were obtained from the deer mouse colony maintained and housed at the vivarium of North-West University (NWU), Potchefstroom, South Africa (Ethical Approval No. NWU-00066-10-S5). As has been reported previously (Powell et al., 1999),

stereotypical behavior in the deer mouse is established by the age of 8 weeks, and only mice between the ages of 10 and 12 weeks were chosen as experimental subjects at the onset of behavioral assessments (Day 0). Mice were randomly chosen from different litters, without sex or weight bias, and housed in groups of six same-sex animals per cage. Earlier studies have already shown that the severity of stereotypy in deer mice is independent of sex (Hadley, Hadley, Ephraim, Yang, & Lewis, 2006; Korff et al., 2008). One week before the onset of our behavioral assessments, each animal was allocated individually to an automatic climate-controlled laboratory cage [35 (l) × 20 (w) × 13 (h) cm; Techniplast S.P.A., Varese, Italy] and maintained at 23 °C on a 12-h light/dark cycle (lights on at 06 h00 and off at 18 h00). Food and water were provided ad lib for the duration of the study. The cages were cleaned and new bedding material was added weekly.

Generating behavioral data and the classification of N and H animals

In order to screen animals for baseline expression of stereotypy, the same protocol as published previously (Wolmarans et al., 2013) was followed. In short, all animals underwent a 12-h behavioral screening once a week for five consecutive screens. On any specific assessment day, animals were moved from their housing environment to the behavioral screening room. These areas were located on the same floor of the vivarium and were environmentally controlled with respect to temperature (23 °C), relative humidity (55 %), and light cycle (same as for the home cages). Subsequently, each animal was introduced to a behavioral test cage [21 (w) × 21 (l) × 35 (h) cm; Accuscan Inc., Columbus, Ohio, USA] constructed from clear, translucent Plexiglas. The home cage bedding material was provided in quantities enough to cover the floor of the test cages, while also ensuring that it did not interrupt the scoring of behavioral data. Food was provided ad lib on the floor of the cage in the form of broken-up rodent chow pellets. Bottled water was provided through a tight-fit hole in the wall of each test cage. The cages were covered with lids that allowed uninterrupted airflow. The animals were introduced to these environments by 16 h00 and habituated for at least 2 h before the 12-h behavioral assessments started at the onset of the dark cycle. The test cages were cleaned after each screen.

Behavioral assessment was performed using the Fusion Animal Activity Monitoring System (Accuscan Inc., Columbus, Ohio, USA), and analyzed using the Fusion software. Each testing cage was fitted with a grid of infrared light beams that crossed the cage roughly 2 cm above the cage floor along the *x*- and *y*-axes (constituting a grid), and another set of beams (*z*-axis) that crossed the cage 10 cm above the cage floor, but parallel to the lower *y*-axis. Parallel beams were spaced 2.5 cm apart and recorded activity every time they were interrupted by movement. The number of vertical beam

interruptions was used as a measure of vertical jumping activity, whereas the numbers of clockwise and counterclockwise cage revolutions were used to evaluate the expression of pattern running. During the recording session, the Fusion software generated data continuously, allowing for experimental playback and export of the behavioral reports the following day.

Following the first five behavioral screens, the animals were divided into N and H groups according to our previously published protocol (Wolmarans et al., 2013). Because the findings from previous studies had demonstrated the time-consuming nature of stereotypy, we amended the protocol to include both the *intensity* (number of stereotypical counts per 30 min) and *frequency* (number of 30-min H bouts per 12-h dark cycle or time spent executing stereotypy) as indicators of behavioral severity. The published criteria for defining vertical and horizontal H intervals are reproduced in Table 1. These data were plotted for both vertical activity (Fig. 1A) and horizontal activity (cage revolutions; Fig. 1B) and, since no distinction was made between the different forms of stereotypy, H animals could demonstrate stereotypical behavior in either one or both of the behavioral topographies. We subsequently included 18 animals in the H group (Fig. 1A and B combined; subjects are indicated with crosses) that expressed both the highest intensity of stereotypy and the highest frequency of H bouts, irrespective of whether an individual did so in only one or in both of the topographies (the sum total of crosses from Fig. 1A and B—i.e., 25—exceeds 18 as more than one animal displayed H behavior in both topographies). The group of N animals was simply constituted from the 18 animals that consistently generated the lowest stereotypy counts (Fig. 1A and B combined; black dots plotted at *x* = 0).

Because only 18 animals were included in each cohort following the first five behavioral screens, a gray margin of 34 animals were excluded from the experimental design. The rationale for the exclusion of a gray margin of animals is to improve sensitivity, as we have described elsewhere (Wolmarans et al., 2013).

Marble-burying test

In the present investigation, the MB test was applied as previously described (Broekkamp et al., 1986) with respect to the

Table 1 Criteria for defining nonstereotypical (N) and high-stereotypical (H) intervals of activity with respect to vertical activity and cage revolutions

Stereotypy Intensity	Vertical Beam Interruptions/ 30 min	Cage Revolutions/ 30 min
N	<500	<150
H	>2,000	>200

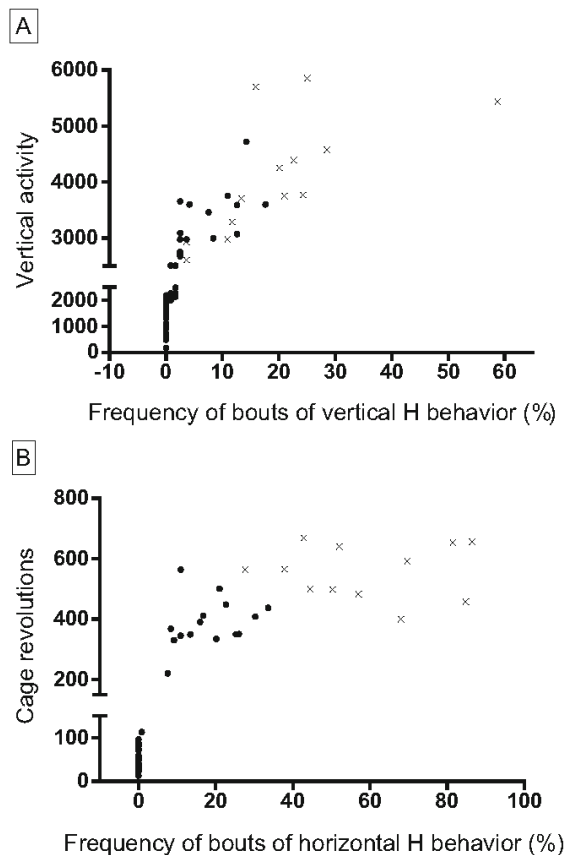


Fig. 1 Stereotypy intensity versus frequency of high-stereotypical (H) bouts. (A) Mean of the 15 highest individual daily vertical activity scores over the first five baseline behavioral screens versus frequency of bouts of H activity. (B) Mean of the 15 highest individual daily cage revolution scores over the first five baseline behavioral screens versus frequency of bouts of H activity. Crosses indicate the H animals

setup for a one-zone paradigm, with slight modifications. Following the last baseline behavioral screen, the bedding material of the 18 N and the 18 H animals was exchanged with sawdust. Animals were habituated in these cages in the absence of marbles for at least 24 h before the first MB test. All experiments were carried out under dim red light during the dark cycle. To assess MB but prevent avoidance behavior, 15 glass marbles (ϕ 1 cm) were evenly spaced on sawdust (obtained from South African Vaccine Producers, Sandringham, South Africa; the average flake size was 4 mm, in a layer 5 cm thick) in exact copies of the home cages. Each mouse was allocated to a marble-containing cage and allowed 30 min to explore. After returning the animals to their home cages, the marbles were counted. Scoring took place blindly by an observer unfamiliar with the classification status of the animals. A marble was considered buried when 2/3 or more of its size was covered with sawdust. MB was measured

in the same animals during three separate trials on three consecutive evenings before treatment (T1–T3), and again during three trials following four weeks of treatment (T4–T6; see the Drugs section below). Locomotor activity was assessed separately from MB, and as stereotypy and locomotor activity were simultaneously recorded by the Fusion software, the data from the last baseline screen (pretreatment) were compared to those obtained following four weeks of treatment. Screening was performed one night before the first pre- (T1) and post- (T4) treatment MB trials.

Drugs

Escitalopram oxalate was prepared for oral administration by dissolving it in the drinking water (Wolmarans et al., 2013). Physical handling of the mice was kept to a minimum to prevent any possible anxiogenic effects. For the same reason, oral gavage was not considered an appropriate dosage route. The final concentration of the drinking solution was constituted at 20 mg/100 ml, ensuring that each animal would receive 50 mg/kg/day (within close limits) of escitalopram. The dose calculation was based on a prior pilot study in which the average water intake per mouse was determined as 0.25 ml/g/day (data are not shown), which has been confirmed by others (Aschhoff, Schroff, Wildenauer, & Richter, 2000).

Statistics

Statistical analysis was performed using GraphPad Prism 6 and IBM SPSS Statistics 22 under the guidance of the Statistical Consultation Service of North-West University, Potchefstroom. Two-way analysis of variance followed by Bonferroni posttests was applied so as to compare the pre- and posttreatment expressions of MB behavior and locomotor activity both within and between N and H cohorts. Behavioral Cohort was set as a between-subjects (independent) factor, and Time and Treatment as within-subjects (dependent) factors. The same statistics were applied to compare high burying behavior (HBB) with inherent burying behavior (IBB). In this case, Burying Cohort was set as a between-subjects factor and Time and Treatment as within-subjects factors. HBB behavior was statistically separated from IBB on the basis of (1) the extent to which the average HBB scores deviated from the average IBB scores and (2) calculations of coefficients of variance in daily burying scores. Since the total average burying scores did not demonstrate a normal distribution, a Spearman's correlation between the total average number of marbles buried and the coefficients of variance was applied. As such, only animals that demonstrated burying behavior above the 75th percentile of the distribution, deviating from the mean by two times the standard deviation and that had a calculated coefficient of variance below the 25th percentile of the distribution, were included in the HBB group. To compare

the pre- and posttreatment locomotor activity of animals expressing HBB behavior, a two-tailed paired *t* test was applied. Statistical significance was set at $p < .05$ for all analyses.

Results

Marble-burying

Figure 2A and B describe the MB behavior in N and H deer mice that received escitalopram over three different trials. An initial evaluation of the data did not demonstrate significant differences in MB between the N and H cohorts with respect to the first trial (neophobic burying behavior), either before (Fig. 2A, T1) or after (Fig. 2A, T4) four weeks of treatment with escitalopram. Furthermore, a Pearson's correlation between the average stereotypy count and the pretreatment number of marbles buried failed to display a linear relationship [both sets of data were normally distributed, $r(34) = -.06$, $p = .74$; data not shown]. Moreover, chronic escitalopram failed to

influence the first-trial MB behavior within either of the cohorts. The average number of marbles buried throughout all pretreatment trials for N and H animals also did not differ, and treatment failed to influence this behavior either within or between behavioral cohorts (data not shown). Neither N nor H animals habituated toward burying behavior, irrespective of treatment, since no intertrial differences in MB could be observed either within or between behavioral cohorts (Fig. 2B, T1–T6).

Given the apparent lack of separation between H and N animals, the MB behavior of individual animals was analyzed independent of degree of stereotypy. A Spearman's correlation between the average numbers of marbles buried over all trials and the coefficients of variance calculated with respect to daily burying behavior revealed a strong negative association (Fig. 3A), $r(34) = -.64$, $p < .0001$. As such, four animals (one female, three male) were identified that consistently expressed increased MB behavior, as demonstrated by burying scores within the upper 75th percentile of the distribution, deviating by more than two standard deviations from the mean IBB score, and with little interday variance (i.e., coefficients of variance within the lower 25th percentile of the distribution). No interaction between burying behavior and treatment [$F(5, 204) = 0.97$, $p = .43$] was demonstrated, but a significant main effect of burying cohort [$F(1, 54) = 17.04$, $p < .0001$] was observed. This significant increase in HBB relative to the larger group was noted throughout all trials but one (Fig. 3B, $**p < .005$). Furthermore, taking the average pre- (T1–T3) and post- (T4–T6) treatment expressions of burying behavior together, no interaction between burying behavior and treatment [$F(1, 212) = 0.70$, $p = .4$] was found, although again the main effect of HBB was significant [$F(1, 21) = 58.16$, $p < .0001$], with post-hoc analysis demonstrating the significance to be even more profound (Fig. 3C, $****p < .0005$).

Importantly, it must be emphasized that apart from the four animals expressing HBB behavior, all of the deer mice in the present investigation displayed IBB (Table 2). Although this behavior varied in expression between individual animals, it also varied in intensity across different trials within the same animal (Fig. 3D; data sheet included as [supplementary material](#)). In fact, apart from the consistent burying behavior of the animals expressing HBB behavior, no apparent burying pattern could be recognized in the same animal, in the same behavioral cohort, or with respect to its response to treatment (Figs. 3A, B, D).

Locomotor activity

A significant two-way interaction was evident between cohort and treatment [$F(1, 34) = 6.319$, $p = .02$]. N deer mice displayed a significant increase in baseline locomotor activity as compared to H animals (Fig. 4A, $****p < .0005$), whereas

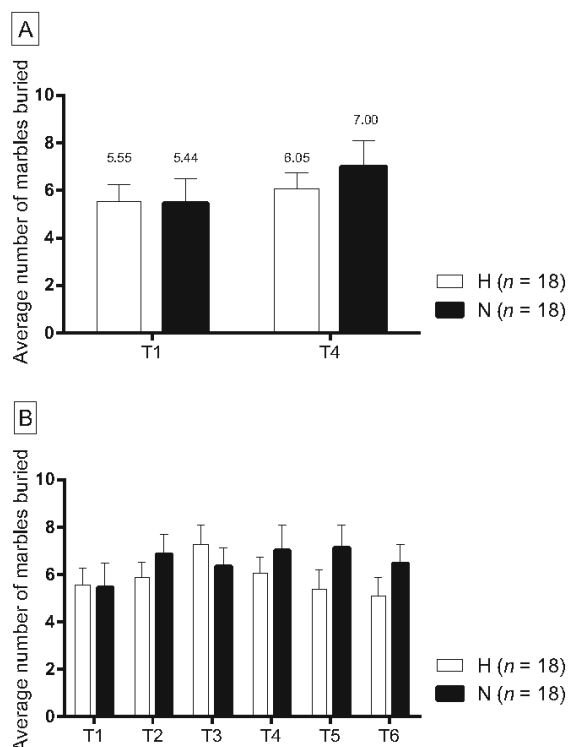


Fig. 2 Comparisons between the marble-burying behaviors of high-stereotypical (H) and nonstereotypical (N) deer mice. (A) Comparison of the average numbers of marbles buried during the first pretreatment (T1) and first posttreatment (T4) trials. (B) Intertrial comparisons of the average numbers of marbles buried (T1–T6). Data are represented as means $\pm$ SEMs

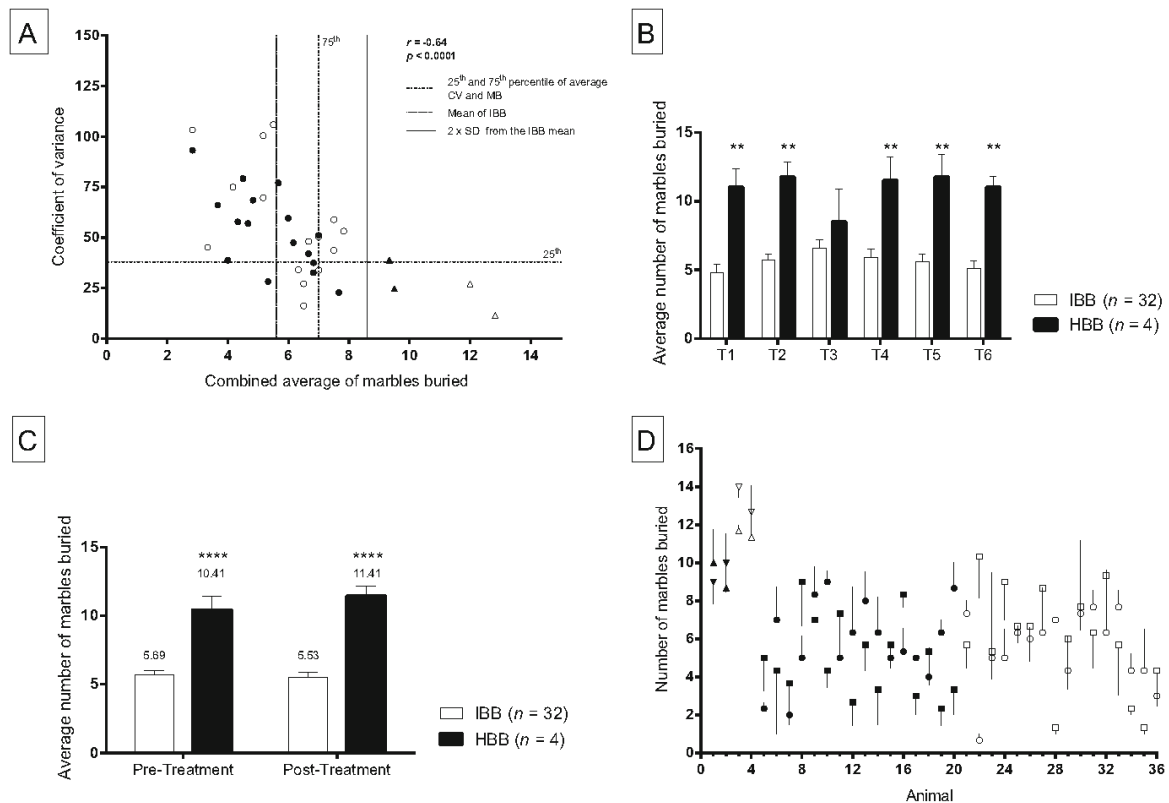


Fig. 3 Comparisons between the marble-burying (MB) behavior of animals expressing high or inherent burying behavior (HBB or IBB, respectively). (A) Coefficients of variance with respect to daily burying scores against the combined average numbers of marbles buried by each individual over all trials: closed triangles, high-stereotypical (H) animals expressing HBB; open triangles, nonstereotypical (N) animals expressing HBB; closed dots, H animals expressing IBB; open dots, N animals expressing IBB; CV, coefficient of variance; SD, standard deviation. (B) Intertrial comparisons of the average numbers of marbles buried

(T1–T6). (C) Comparison of the average numbers of marbles buried over the three pretreatment (T1–T3) and the three posttreatment (T4–T6) trials. (D) Average pre- and posttreatment numbers of marbles buried by individual animals: closed triangles, H animals expressing HBB; open triangles, N animals expressing HBB; upward triangles, pretreatment value; downward triangles, posttreatment value; closed squares and dots, IBB of H animals; open squares and dots, IBB of N animals; dots, pretreatment; squares, posttreatment. ** $p < .005$, **** $p < .0005$. Data are represented as means $\pm$ SEMs

chronic escitalopram treatment significantly decreased the locomotor activity of both N and H animals (Fig. 4A, **** $p < .0005$, * $p = .02$). Regarding the four animals expressing HBB, escitalopram significantly decreased locomotor activity in those animals [Fig. 4B; $t(3) = 3.99$, * $p = .03$].

Discussion

The major findings of the present study are (1) that H deer mice do not present with altered MB behavior relative to N animals; (2) that all deer mice exhibit a level of IBB that is not subject to habituation; (3) that a characteristically different form of burying behavior (HBB) has been identified within the species, but across different behavioral cohorts; and (4) that neither IBB nor HBB behavior

is sensitive to chronic (four-week) high-dose escitalopram treatment (50 mg/kg/day).

Although defensive and neophobic burying is a normal behavioral coping response in mice, and as such cannot be used to model a psychiatric illness (De Boer & Koolhaas, 2003), aberrant defensive, neophobic, and inherent burying behavior, of which MB has been suggested to be an example (Broekkamp et al., 1986; Njung'e & Handley, 1991; Thomas et al., 2009), might be useful for modeling illnesses analogous to compulsivity and anxiety. However, the diversity of the application and pharmacological sensitivity of this measure (Nicolas et al., 2006; Thomas et al., 2009) has contributed to uncertainty as to what behavioral phenotype(s) the test may in fact be measuring.

OCD responds preferentially to SSRIs, and invariably to a higher dose than the nominal antidepressant dose (El Mansari

Table 2 Average pretreatment, posttreatment, and combined numbers of marbles buried

Animal	Behavioral Cohort	Pretreatment Average Number of Marbles Buried	Posttreatment Average Number of Marbles Buried	Combined Average Number of Marbles Buried
1	H	10.0	9.0	9.5
2	H	8.7	10.0	9.3
3	N	11.7	14.0	12.8
4	N	11.3	12.7	12.0
Average		10.4	11.4	10.9
5	H	2.3	5.0	3.7
6	H	7.0	4.3	5.7
7	H	2.0	3.7	2.8
8	H	5.0	9.0	7.0
9	H	8.3	7.0	7.7
10	H	9.0	4.3	6.7
11	H	5.0	7.3	6.2
12	H	6.3	2.7	4.5
13	H	8.0	5.7	6.8
14	H	6.3	3.3	4.8
15	H	5.0	5.7	5.3
16	H	5.3	8.3	6.8
17	H	5.0	3.0	4.0
18	N	4.0	5.3	4.7
19	N	6.3	2.3	4.3
20	N	8.7	3.3	6.0
21	N	7.3	5.7	6.5
22	N	0.7	10.3	5.5
23	N	5.0	5.3	5.2
24	N	5.0	9.0	7.0
25	N	6.3	6.7	6.5
26	N	6.0	6.7	6.3
27	N	6.3	8.7	7.5
28	N	7.0	1.3	4.2
29	N	4.3	6.0	5.2
30	N	7.3	7.7	7.5
31	N	7.7	6.3	7.0
32	N	6.3	9.3	7.8
33	N	7.7	5.7	6.7
34	N	4.3	2.3	3.3
35	N	4.3	1.3	2.8
36	N	3.0	4.3	3.7
Average		5.7	5.5	5.6
Standard deviation		2.0	2.4	1.5

Animals 1–4 expressed HBB. Animals 5–36 expressed IBB. The average combined marble-burying values for Animals 1–4 fall within the upper 75th percentile of the normal distribution

& Blier, 2006). Although stereotypy is a perseverative behavior common to a number of neuropsychiatric disorders,

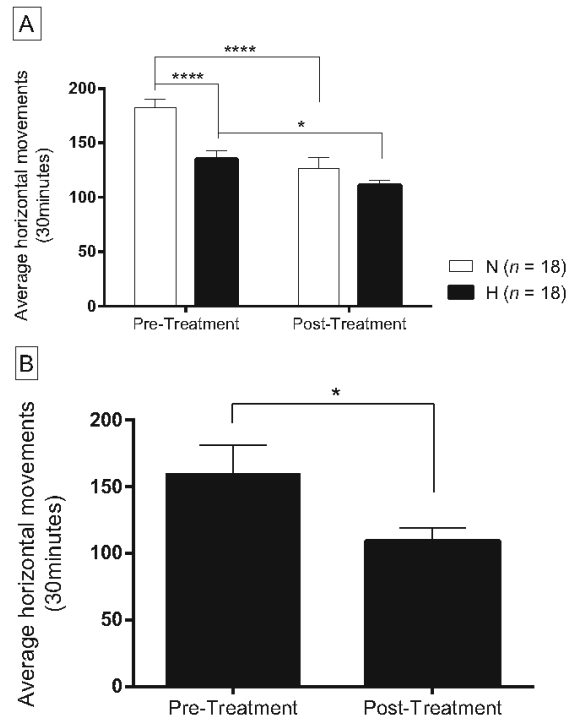


Fig. 4 Comparisons of pre- and posttreatment locomotor activity. (A) Pre- and posttreatment locomotor activity of nonstereotypical (N, open bars) and high-stereotypical (H, closed bars) animals. (B) Pre- and posttreatment locomotor activity of animals expressing high marble-burying behavior. * $p < .05$, **** $p < .0005$. Data are represented as means $\pm$ SEMs

including OCD (Powell et al., 1999), deer mouse stereotypy is reversed by serotonergic but not noradrenergic antidepressants (Korff et al., 2008), and chronic treatment is obligatory (Wolmarans et al., 2013). Furthermore, high stereotypy in deer mice correlates to reduced striatal serotonin transporter (SERT) density (Wolmarans et al., 2013), a recognized pathological feature of OCD (Zitterl et al., 2008), whereas SERT is also the preferential target for SSRIs (El Mansari & Blier, 2006). In this study, we challenged H animals with MB in order to screen for anxiety (neophobia) and compulsivity and to determine the response of said behavior to chronic escitalopram treatment, a known anxiety and OCD treatment modality.

The present investigation has delivered a number of fundamental observations that are relevant to MB behavior as a potential model for anxiety and compulsive-like behavior. Apart from MB failing to demonstrate significant differences between H and N animals (Fig. 2A and B), burying behavior in deer mice is not associated with novelty-induced anxiety (neophobia, Fig. 2A). Furthermore, the nonhabituation of deer mice toward marbles (Fig. 2B) is consistent with findings published previously (Thomas et al., 2009). This observation

and the fact that *all* deer mice exhibited burying behavior (Table 2) support the hypothesis that novel, but harmless and nonreactive, objects are devoid of anxiogenic properties, and that the MB test more appropriately resembles investigative, rather than anxious, behavior (Londei et al., 1998). That MB behavior varies between species (Nicolas et al., 2006), with IBB being displayed in both behavioral cohorts of deer mice (N and H), suggests that MB behavior is a normal, not a pathological, activity within the species. This finding is congruent with the lack of response to chronic escitalopram treatment (Fig. 3B, C, and D).

Interestingly, we found that a minority of deer mice exhibit a characteristically different phenotype of burying behavior that we refer to as *high burying behavior*. HBB could be statistically and behaviorally distinguished from IBB and was expressed in only four of the 36 animals (2 N, 2H; see Table 2 and Fig. 3C and D). This conclusion is based on the following data: First, a strong negative correlation was demonstrated between the average number of marbles buried over all trials and the coefficients of variance calculated from the daily burying scores (Fig. 3A). As such, HBB is statistically distinguishable from IBB in that the average pre- and posttreatment (as well as the combined) HBB values fall within the upper quartile of the burying distribution and deviate by more than twice the standard deviation from the mean IBB score (Fig. 3A, Table 2). Moreover, such behavior demonstrates very little intertrial variability (the respective coefficients of variance are contained within the lower quartile of the distribution; Fig. 3A). Second, animals expressing HBB *completely* buried the counted marbles underneath. As such, if face validity is considered, the phenotypical presentation of HBB behavior displayed by a small number of deer mice is characteristically different from the IBB expressed by the larger group, for which the “2/3-buried criterion” could be applied. Furthermore, as with IBB, HBB did not selectively track either the H or the N cohort, was not a result of neophobia, and did not respond to escitalopram treatment. Although we assumed HBB to be representative of compulsive behavior, given the strengths of the deer mouse model to emulate the biobehavioral and treatment response characteristics of OCD, as HBB did not selectively track H animals, these findings raise the question of what the behavioral and clinical significance of HBB might be. HBB might represent a unique within-species burying phenotype that resembles another behavioral manifestation related to perseverative behavior, such as hoarding. Indeed, this idea is supported by the delineation of hoarding disorder in DSM-5 (Morein-Zamir et al., 2014). Moreover, a recent study (Torres-Lista et al., 2015) demonstrated that only the number of completely buried, and not horizontally displaced or partly buried, marbles positively correlated

with increased anxiety in the open-field test, supporting our view that HBB may be related to a different neuropsychological construct than is IBB.

That N deer mice displayed increased baseline locomotor activity relative to H animals (Fig. 4A) may be attributable to the manner in which locomotor activity was scored. Since H deer mice expressed significantly more vertical activity than N animals, they spent less time in contact with the horizontal beams (responsible for recording locomotor behavior) than did the N animals. Whereas previous investigations in our laboratory have shown decreased locomotor activity following chronic escitalopram treatment in the N cohort only (not in H animals; Wolmarans et al., 2013), the present data demonstrate posttreatment decreases in locomotor activity in both the N and H cohorts. Methodological differences, such as the duration of the study (12 vs. 8 weeks) could be responsible, producing an age-related downward shift in locomotor activity. However, as no changes in the expression of either IBB or HBB following treatment could be demonstrated, it is unlikely that changes in locomotor activity could have contributed in any meaningful way.

In summary, the present work has broadened our understanding of the behavioral phenotypes that may be represented by MB behavior. Findings from the present investigation demonstrate that all deer mice express MB behavior, and that such behavior is not subject to habituation and does not respond to chronic high-dose escitalopram treatment. As such, we failed to demonstrate the relevance of MB behavior as a screening test for either neophobia or compulsive-like behavior in deer mice, and suggest that such behavior is primarily investigative in nature. Furthermore, we identified a different within-species burying phenotype (HBB), displayed by a small minority of deer mice, that will require further investigation. Burying behavior is a natural phenomenon inherent to most rodent species, and requires experimental and methodological manipulation, such as pretest restraint (Kedia & Chattarji, 2014) or correlations with other behavioral tests (Londei et al., 1998; Torres-Lista et al., 2015), to be a useful screening tool for either anxiety or compulsivity. Using a known OCD translational model has identified the inherent shortcomings of the MB test for obsessive-compulsive behavior, although this finding should be confirmed in other translational models (e.g., Joel & Avisar, 2001; Szechtman et al., 2001), as well as in the promising range of genetic models currently available (Berridge, Aldridge, Houchard, & Zhuang, 2005; Chou-Green, Holscher, Dallman, & Akana, 2003; Shanahan et al., 2009). Given the inherent tendency of rodents to bury, we strongly argue against drawing conclusions relating to anxiety and compulsive-like behavior from a test that is dependent on MB behavior alone. Our findings further support the conclusion of De Boer and

Koolhaas (2003) that, as far as face or predictive validity is concerned, MB behavior on its own is not a valid model for either anxiety or compulsive-like behavior.

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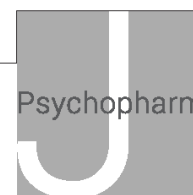
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Addendum G –
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(Chapter 5)

Excessive nest building is a unique behavioural phenotype in the deer mouse model of obsessive–compulsive disorder

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Abstract

Obsessive–compulsive disorder (OCD) is a phenotypically heterogeneous condition characterised by time-consuming intrusive thoughts and/or compulsions. Irrespective of the symptom type diagnosed, the severity of OCD is characterised by heterogeneity in symptom presentation that complicates diagnosis and treatment. Heterogeneity of symptoms would be invaluable in an animal model. Nest building behaviour forms part of the normal behavioural repertoire of rodents that demonstrate profound between-species differences. However, it has been proposed that within-species differences in nest building behaviour (i.e. aberrant vs. normal nest building) may resemble obsessive–compulsive-like symptoms. In an attempt to investigate whether other obsessive–compulsive-like behaviours are present in an animal model of OCD, or if aberrant nest building behaviour may represent a unique obsessive–compulsive phenotype in such a model, the current study assessed nest building behaviour in high (H, viz. obsessive–compulsive) and non (N, viz. normal) stereotypical deer mice. Subsequently, 12 N and H animals, respectively, were provided with an excess of cotton wool daily for one week prior to and following four weeks of high-dose oral escitalopram treatment (50 mg/kg/day). Data from the current investigation demonstrate daily nesting activity to be highly variable in deer mice, with stereotypy and nest building being independent behaviours. However, we identified unique aberrant large nest building behaviour in 30% of animals from both cohorts that was attenuated by escitalopram to pre-treatment nesting scores of the larger group. In summary, behavioural and drug-treatment evidence confirms that deer mouse behaviour does indeed resemble symptom heterogeneity related to OCD, and as such expands its face and predictive validity for the disorder.

Keywords

Deer mouse, symptom heterogeneity, perseverative behaviour, OCD animal model, SSRI response

Introduction

Obsessive-compulsive disorder (OCD) has a lifetime prevalence of 1–3% (Stein, 2002) and is characterised by recurrent intrusive thoughts (obsessions) and rigid repetition of certain behaviours (compulsions) (American Psychiatric Association, 2013). Furthermore, being clinically heterogeneous, OCD is characterised by a range of phenotypically different symptom types (Williams et al., 2013). Four major obsessive–compulsive symptom dimensions have been described, namely: (a) contamination obsessions and washing compulsions; (b) responsibility for harm obsessions and checking compulsions; (c) symmetry obsessions and ordering compulsions; and (d) unacceptable thoughts (e.g. sexual, religious, or aggressive in nature) and neutralising compulsions (e.g. thought suppression) (Abramovitch and Cooperman, 2015). Irrespective of the symptom dimensions identified, a diagnosis of OCD is based on the time-consuming nature of such symptoms and how their severity impacts the time spent on, degree of resistance to, and control over these behaviours (American Psychiatric Association, 2013).

Animal models of OCD have enjoyed a great deal of interest recently (Alonso et al., 2015; Hoffman, 2011). However, most if not all these models centre around one particular aspect of the behavioural phenomenology of OCD, such as non-goal-directed stereotypy, excessive grooming, or exaggerated nest building (NB). Considering the heterogeneity of OCD, an animal model that typifies a range of phenotypes that may separate it from other

known stereotypic disorders, that is, autism and tics would be invaluable (Cagnin et al., 2014; Edwards et al., 2004; Ghanizadeh, 2010). Spontaneous stereotypy in the deer mouse (*Peromyscus maniculatus bairdii*) is a validated naturalistic animal model of OCD (Alonso et al., 2015; Hoffman, 2011; Wolmarans et al., 2013). Deer mouse stereotypy can be categorised into repetitive jumping and pattern running (Powell et al., 1999) that correspond with the repetitive motor actions of OCD. Furthermore, as in human OCD the model demonstrates varying symptom intensity (i.e. high (H) vs. non-(N)-stereotypic), and employs ‘time spent executing stereotypy’ as a marker of behavioural severity and treatment outcome (Wolmarans et al., 2013). The predictive

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validity of the model is founded on its response to chronic but not subchronic treatment with high doses of a selective serotonin reuptake inhibitor (SSRI) (Wolmarans et al., 2013), and its lack of response to noradrenergic antidepressants (Korff et al., 2008), as are evident in OCD (Fineberg and Craig, 2007). Concerning construct validity, neurobiology studies have described the association of H behaviour with altered corticostriatal cyclic adenosine monophosphate (cAMP)/phosphodiesterase-4 (PDE4) signalling (Korff et al., 2009), purported to be linked to SSRI response (El Mansari and Blier, 2006; Goddard et al., 2008), and decreased striatal SERT density (Wolmarans et al., 2013), a finding described in OCD (Reimold et al., 2007; Zitterl et al., 2008).

In the wild, mice and other rodents build nests for protection against the elements and predators, and to provide a nursery to breed and raise young (Smithers, 1983). Under laboratory conditions, mice build nests to shelter from harsh lighting, low ambient temperatures and human handling (Jirkof, 2014). As such, motivation for nesting is high and NB behaviour is routinely assessed as a measure of general wellbeing of laboratory-housed mice (Jirkof, 2014). Although nesting forms part of the normal behavioural repertoire of mice, between-species differences in nest size, structure and locale are profound (Gaskill et al., 2013). However, nesting behaviour can also demonstrate within-species variance, and it has been suggested that large nest building (LNB) behaviour may represent compulsiveness (Greene-Schloesser et al., 2011; Li et al., 2006). Indeed, LNB has been associated with increased marble-burying behaviour (MB), a measure of compulsiveness, while chronic high-dose fluoxetine significantly attenuates LNB in mice (Greene-Schloesser et al., 2011).

Considering that nesting is a normal behavioural routine in rodents, but that aberrant LNB in animal behaviour may represent a different phenotype of OCD, the current investigation assessed NB behaviour in an established animal model of OCD. The aims were to establish: (a) if within-species differences in nesting behaviour can be demonstrated, irrespective of the stereotypical cohort (*viz.* can deer mouse behaviour in general be representative of different obsessive-compulsive phenotypes?), and (b) whether potential differences in nesting behaviour are sensitive to chronic treatment with high-dose oral escitalopram (50 mg/kg/day).

Materials and methods

Animals

Sixty deer mice were obtained from the deer mouse colony maintained and housed at the vivarium of North-West University (NWU), Potchefstroom, South Africa (ethical approval number NWU-00066-10-85). The original colony was established using breeding pairs obtained from the *Peromyscus* Genetic Stock Centre at the University of South Carolina. As reported previously (Powell et al., 1999) stereotypical behaviour in the deer mouse is established at the age of 8 weeks, and only mice between the ages of 10 and 12 weeks were chosen as experimental subjects at the onset of behavioural assessments (day 0). Mice were randomly chosen from different litters, without sex or weight bias and housed in groups of six same-sex animals per cage. Earlier studies have already noted that the severity of stereotypy in deer mice is independent of sex (Hadley et al., 2006; Powell et al., 1999; Presti and Lewis, 2005; Wolmarans et al., 2013). One

week before the start of the behavioural assessments, each animal was allocated individually to an automatic climate-controlled laboratory cage (35 cm (l) × 20 cm (w) × 13 cm (h); Tecniplast SPA, Varese, Italy) and maintained at 23°C on a 12-hour light/dark cycle (lights on at 06.00 and off at 18.00 hours). Food and water were freely provided for the duration of the study. Cages were cleaned and new bedding material added weekly.

Generating behavioural data and classification of N and H animals

Before assessing NB behaviour, animals were categorised as N or H according to a previously published protocol (Wolmarans et al., 2013). In short, all animals underwent 12-hour behavioural screening once a week for five consecutive screens. On any specific assessment day, animals were moved from their housing environment to the behavioural screening room. These areas are located on the same floor of the vivarium and are environmentally controlled with respect to temperature (23°C), relative humidity (55%), and light cycle (same as for home cages). Subsequently, each animal was introduced to a behavioural test cage (21 cm (w) × 21 cm (l) × 35 cm (h); Accuscan Inc., Columbus, OH, USA) constructed from clear, translucent Plexiglas. Bedding material was provided in quantities enough to cover the floor of the test cages, but also ensuring that it did not interrupt the scoring of behavioural data. Food was freely provided on the floor of the cage in the form of broken-up rodent chow pellets. Bottled water was provided through a tight-fit hole in the wall of each test cage. Cages were covered with lids that allowed uninterrupted airflow. The animals were introduced to these environments by 16.00 hours and habituated for at least two hours before the 12-hour behavioural assessments started at the onset of the dark cycle. Test cages were cleaned after each screen.

Behavioural assessment was performed using the Fusion animal activity monitoring system (Accuscan Inc., Columbus, OH, USA), and analysed using Fusion software. Briefly, each testing cage presents with a grid of infrared light beams that cross the cage roughly 2 cm above the cage floor along the X and Y axes (constituting a grid), while another set of beams (Z axis) crosses the cage 10 cm above the cage floor, but only parallel to the lower Y axis. Parallel beams are spaced 2.5 cm apart and record activity every time it is interrupted by movement. The number of vertical beam interruptions was used as a broad measure of vertical jumping activity, while the number of clockwise and anticlockwise cage revolutions was used to evaluate the expression of pattern running (Wolmarans et al., 2013). During the recording session, the Fusion software generates data continuously, allowing for experimental playback and the export of behavioural reports the following day.

Following the first five behavioural screens, animals were divided into N and H groups according to our previously published protocol (Wolmarans et al., 2013). As findings from previous studies (Wolmarans et al., 2013) demonstrated the time-consuming nature of stereotypy, we amended the protocol to include both the intensity (average of the highest individual 30-minute stereotypy counts generated during the five respective trials) and frequency (percentage of 30-minute H stereotypical bouts generated over all five trials, *viz.* time spent executing stereotypy) as indicators of behavioural severity. The criteria for defining vertical and horizontal H intervals are represented in Table 1.

Table 1. Criteria for defining N and H intervals of stereotypical activity with respect to vertical activity and cage revolutions.

Stereotypy intensity	Vertical beam interruptions/30 min	Cage revolutions/30 min
N	<500	<150
H	>2000	>200

N: non-stereotypical; H: high stereotypical.

These data were plotted for both vertical (Figure 1(a)) and horizontal activity (cage revolutions; Figure 1(b)) and, as no distinction was made between the different forms of stereotypy, H animals could demonstrate stereotypical behaviour in either one or both of the behavioural topographies. We subsequently included 12 animals in the H group (Figure 1(a) and (b) combined; subjects indicated with crosses) that expressed both the highest intensity of stereotypy and the highest frequency of H stereotypical bouts, irrespective of whether an individual did so in only one or both of the topographies (the sum total of crosses from Figure 1(a) and (b), i.e. 16, exceeds 12 as more than one animal displayed H behaviour in both topographies). The group of N animals was simply constituted from the 12 animals that consistently generated the lowest stereotypy counts (Figure 1(a) and (b) combined; black dots plotted at X=0). As only 12 animals were included in each cohort following the first five behavioural screens, a grey margin of 36 animals was excluded from the experimental design. The rationale for the exclusion of a grey margin of animals is to improve sensitivity, as described elsewhere (Presti and Lewis, 2005).

Assessing nest building behaviour

Following the last baseline behavioural screen, nesting behavior was assessed in the same animals for seven consecutive nights before and after chronic (four-week) treatment with oral escitalopram (50 mg/kg/day). An excess of preweighed standard cosmetic cotton wool (non-scented) was introduced in the roof of each home cage every day between 15.00 and 16.00 hours. As mice generally build their nests just before dawn (Jirko, 2014), the remaining cotton wool was removed and weighed between 13.00 and 14.00 hours on the following day. Daily nesting scores were expressed in grams of cotton wool utilised and added together for a total nesting score after one week (Greene-Schloesser et al., 2011). Animals did not have access to any other form of nesting material and food and water were supplied as normal.

Assessment of locomotor activity

As stereotypy and locomotor activity was simultaneously recorded by the Fusion software, data from the last baseline screen (pre-treatment) was compared to that obtained from another screen following four weeks of treatment, performed one night before assessment of post-treatment NB behaviour.

Drugs

As previously reported (Wolmarans et al., 2013), escitalopram oxalate was prepared for oral administration by dissolving it in the drinking water. Physical handling of the mice was kept to a

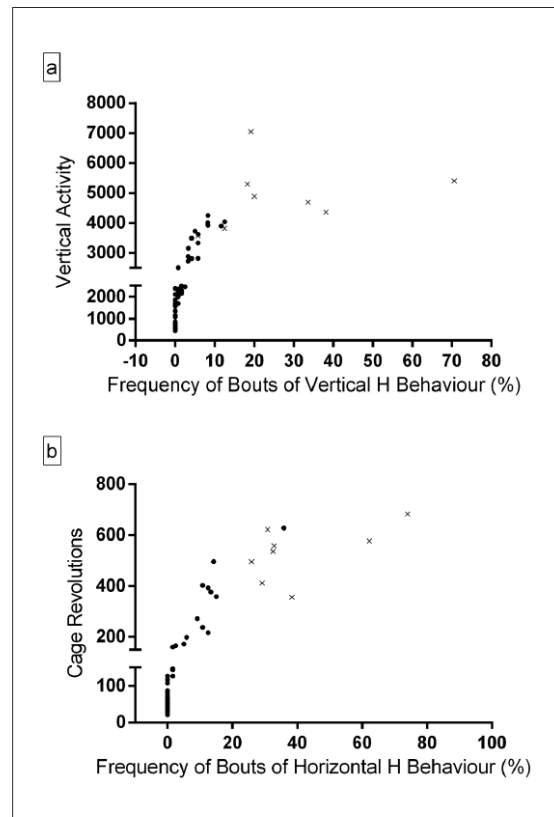


Figure 1. Stereotypy intensity versus frequency of high stereotypical bouts. (a) Mean of highest individual daily vertical activity scores over the first five baseline behavioural screens versus frequency of bouts of H activity. (b) Mean of highest individual daily cage revolution scores over the first five baseline behavioural screens versus frequency of bouts of H activity. Crosses indicate H animals.

minimum to prevent any possible anxiogenic effects. For the same reason, oral gavage was not considered an appropriate dosage route. The final concentration of the drinking solution was constituted at 20 mg/100 ml ensuring that each animal would receive 50 mg/kg/day (within close limits) of escitalopram. The dose calculation was based on a previous pilot study in which the average water intake per mouse was determined as 0.25 ml/g/day (data not shown), and confirmed by others (Aschhoff et al., 2000).

Statistics

Statistical analysis was performed using GraphPad Prism 6 under guidance of the statistical consultation service of North-West University, Potchefstroom. Two-way analysis of variance followed by post-hoc testing with Bonferroni corrections was applied to compare the pre and post-treatment expression of NB behaviour and locomotor activity both within and between N and H cohorts. The behavioural cohort was set as between subject (independent) factor and time and treatment as within (dependent) subject factors. The same statistics were applied to compare

the response of LNB and normal nesting (NNB) behaviour to treatment. In this case, the nesting cohort was set as between-subject factor and time and treatment as within-subject factor. Independent unpaired t-tests were applied to compare the baseline expression of NNB and LNB behaviour. Statistical significance was set at $P < 0.05$ for all analyses.

Results

Nesting behaviour in drug-naïve groups

Nest building behaviour in deer mice displays within and between-animal variance with respect to daily nesting scores, with no apparent differences in pre-treatment nesting behaviour noted between N and H animals (Figure 2(a)). Despite the lack of association between nesting and stereotypy (Figure 2(a)), Pearson's correlation revealed a strong negative relationship between the average total pre-treatment nesting scores and the coefficients of variance (CV) relating to the daily nesting scores within the N and H cohorts (Figure 2(b): $r(24) = -0.75$, $r^2 = 0.56$, $P < 0.0001$) demonstrating that animals expressing LNB, regardless of severity of stereotypy, displayed less between-day variance, suggesting a more consistent pattern of aberrant behaviour. Subsequently, seven animals (30%; 4H, 3N) were identified that consistently displayed LNB located in the upper quartile of the total nesting score distribution (≥ 25.9 g) and the lower distributed quartile for CV (≤ 33.9) (Figure 2(b): encircled data points; photographs submitted as supplementary material). Further comparisons between the average total (Figure 2(c)) and daily (Figure 2(d)) nesting scores of animals expressing LNB and NNB behaviour, respectively, revealed significant differences in pre-treatment nesting behaviour (Figure 2(c): 28.95 ± 2.6 vs. 12.35 ± 1.9 , $t(22) = 4.76$, $***P < 0.0001$; Figure 2(d): 3.8 ± 0.2 vs. 1.75 ± 0.17 , $t(147) = 5.90$, $***P < 0.0001$).

Nesting behaviour following escitalopram treatment

Chronic treatment with high-dose oral escitalopram significantly decreased the expression of LNB with respect to the total (Figure 3(a)) and daily (Figure 3(b)) nesting scores with significant interactions being displayed with respect to total nesting score (Figure 3(a): $F(1,44) = 4.830$, $P = 0.03$) and daily nesting score (Figure 3(b): $F(1,294) = 4.575$, $P = 0.03$). Furthermore, post-hoc analysis demonstrates significantly decreased total ($***P = 0.0008$) and daily ($***P = 0.0003$) nesting scores. In fact, without altering locomotor activity (Figure 3(c i)), the average total (Figure 3(a): 15.45 ± 2.85) and daily (Figure 3(b): 2.19 ± 0.4) post-treatment nesting scores of animals expressing LNB were found to be indistinct from the respective pre-treatment nesting scores of the group (Figure 3(a): 12.35 ± 1.9 ; Figure 3(b): 1.8 ± 0.17). While LNB still demonstrated significant post-treatment differences as compared to group NNB with respect to average total and daily nesting scores, these differences are less pronounced (Figure 3(a): $***P < 0.0001$ vs. $*P = 0.03$; Figure 3(b): $***P < 0.0001$ vs. $**P = 0.004$). Furthermore, a concomitant decrease in the locomotor activity of animals expressing NNB (Figure 3(c i): $F(1,42) = 0.87$; 131.8 ± 6.1 vs. 158.6 ± 9.7 , $P = 0.03$) was noted (Figure 3(c i): 1.16 ± 0.12 vs. 1.75 ± 0.17 , $*P = 0.01$). The decrease in locomotor activity is only observed in the behaviour of N

animals included in this group (Figure 3(c ii): $F(1,28) = 1.21$; 136.6 ± 10.6 vs. 173.9 ± 14.9 , $P = 0.03$).

Discussion

The current investigation has demonstrated that: (a) 30% of deer mice express a unique phenotype of LNB, and (b) LNB, and not NNB, is sensitive to chronic high-dose oral escitalopram treatment. Noteworthy is that stereotypy and NB are independent behaviours, which has significant implications.

OCD is a heterogeneous condition characterised by interpatient differences in behavioural phenotype, response to treatment and symptom severity (American Psychiatric Association, 2013; Stein, 2002). Similarly, animal models of OCD propose various behavioural manifestations to represent obsessive-compulsive-like behaviour, i.e. increased MB behaviour (Egashira et al., 2008), pattern chewing (Chou-Green et al., 2003), lever pressing (Joel and Avisar, 2001) and cage stereotypy (Szechtman et al., 1998; Wolmarans et al., 2013), yet few have been tested for heterogeneity within the same model. Furthermore, within-species differences in NB behaviour have been suggested to be representative of normal versus compulsive-like (viz. LNB) behaviour (Greene-Schloesser et al., 2011; Hoffman and Rueda Morales, 2009; Li et al., 2006), even though between-species differences in nest size and structure are normal (Smithers, 1983). However, LNB as demonstrated in inbred house mice (*Mus musculus*) may more closely resemble compulsivity as it correlates positively with high MB behaviour (a putative measure of obsessive-compulsive behaviour (Thomas et al., 2009)) and demonstrates sensitivity to high-dose SSRI treatment (Greene-Schloesser et al., 2011).

The deer mouse presents with time-consuming stereotypy that is attenuated following chronic, but not subchronic treatment with high-dose escitalopram (Wolmarans et al., 2013). However, the possibility that deer mouse behaviour may represent more than one obsessive-compulsive-like phenotype has not yet been established. Therefore, the current investigation assessed NB behaviour in deer mice, bearing in mind that LNB may be representative of an underlying obsessive-compulsive phenotype.

Our data revealed that NB behaviour in deer mice is an independent compulsive-like behaviour, being expressed in both N and H animals, while a unique pattern of NB behaviour within either cohort could not be demonstrated (Figure 2(a)). However, a strong negative correlation between total nest size and variance in daily nesting behaviour (Figure 2(b)) revealed the presence of a unique NB pattern in 30% of animals over both cohorts, namely LNB (Figure 2(b-d); 4H, 3N). Furthermore, while the NNB expressed by the rest of the group remained mostly unchanged, LNB (in both N and H animals) demonstrated significant attenuation following chronic high-dose escitalopram treatment, with adjustment to the pre-treatment NNB scores of the group (Figure 3(a) and (b)). However, the significant post-treatment decrease in daily nesting scores observed in the behaviour of animals expressing NNB (Figure 3(b)) was related to a parallel decrease in locomotor activity (Figure 3(c i)) and as such cannot be suggested as a behavioural response to drug treatment.

The above findings are in line with that observed in OCD, that is, despite it being a clinically heterogeneous condition, the human disorder responds favourably to chronic SSRI treatment. Since

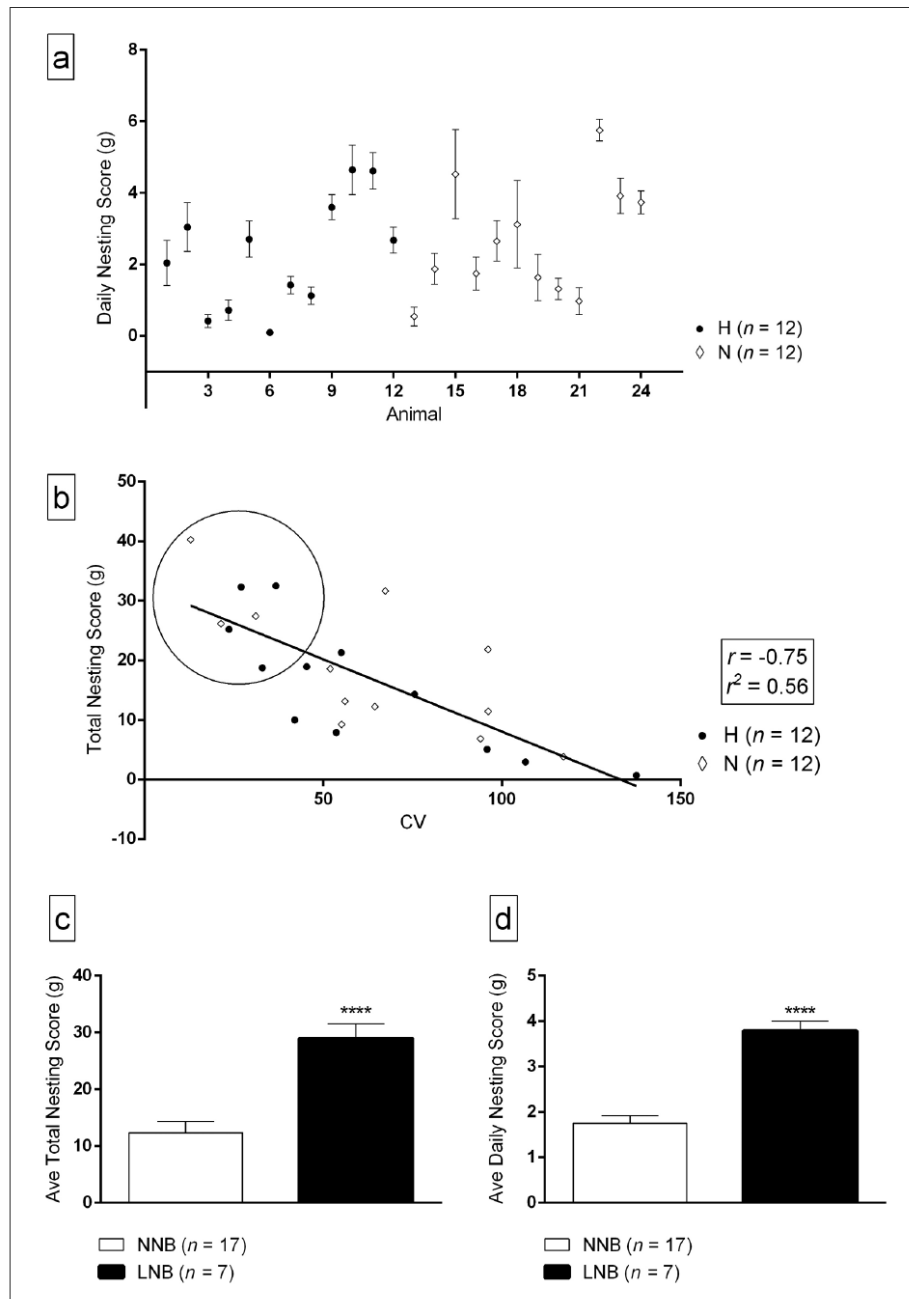


Figure 2. Expression of pre-treatment nest building behaviour. (a) Average daily nesting scores expressed by animals of the H and N cohorts. (b) Pearson's correlation between total weekly nesting scores and coefficients of variance (CV) with respect to daily nesting scores. (c) Comparison of average total weekly nesting scores of animals expressing large nest building (LNB) and normal nest building (NNB) behaviour. **** $P < 0.0001$. (d) Comparison of average daily nesting scores of animals expressing LNB and NNB behaviour. **** $P < 0.0001$. Data represented as mean $\pm$ SEM.

40% of deer mice are already genetically predisposed to develop obsessive-compulsive-like behaviour, LNB may be one of a number of other possible behavioural similarities with OCD that have

not yet been identified. It must be emphasised here that LNB also occurs naturally and without any external manipulation. In fact, the persistent and severe nature of LNB and its response to

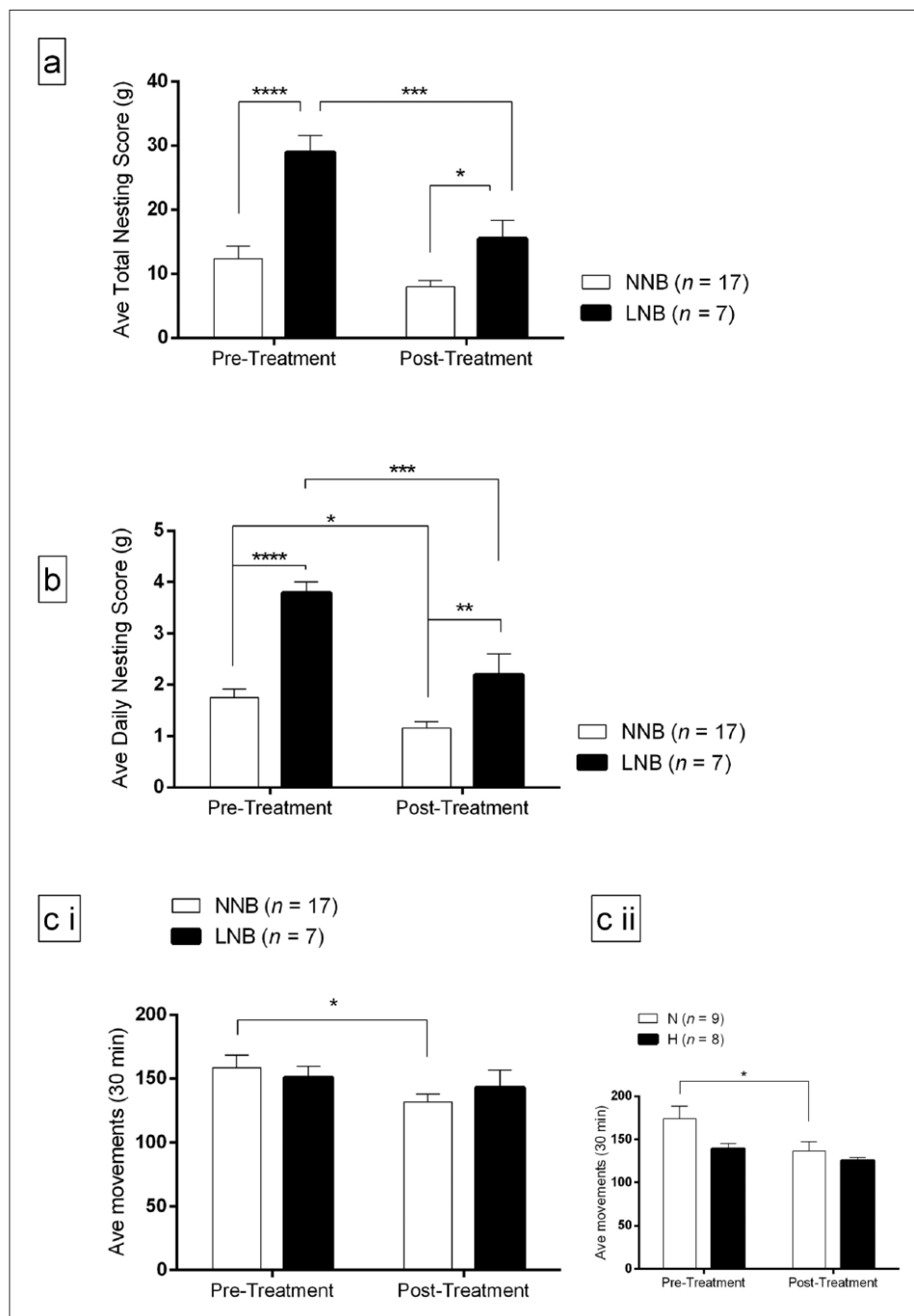


Figure 3. Response of locomotor activity and nesting behaviour to chronic escitalopram treatment. (a) Average total weekly nesting scores in animals expressing large nest building (LNB) and normal nest building (NNB) behaviour, respectively. **** $P < 0.0001$, *** $P = 0.0008$, * $P = 0.03$. (b) Average individual daily nesting scores in animals expressing LNB and NNB behaviour, respectively. **** $P < 0.0001$, *** $P = 0.0003$, ** $P = 0.004$, * $P = 0.01$. (c i) Locomotor activity of animals expressing LNB and NNB behaviour, respectively. * $P = 0.04$. (c ii) Locomotor activity of H and N animals comprising the group of animals expressing NNB behaviour (a). * $P = 0.03$. H: high stereotypical; N: non-stereotypical. Data represented as mean $\pm$ SEM.

escitalopram treatment, as well as that it serves no apparent unique occupational or practical purpose compared to NNB, establishes the putative face and predictive validity for LNB as resembling a different, but also naturally developed obsessive-compulsive phenotype in addition to spontaneous stereotypy already well described in these animals. Furthermore, LNB expression could possibly be related to an altered coping response to environmental challenges (Sluyter et al., 1995) that may manifest as hoarding compulsions, security concerns and 'just-right' perceptions that are well described obsessive-compulsive-related behaviours evident in OCD (Leckman et al., 1994). Given the attenuation of LNB with escitalopram, such coping may be mediated by the serotonergic system (Sluyter et al., 1995).

In conclusion, the current investigation describes symptom heterogeneity in deer mice and has established LNB as a unique obsessive-compulsive phenotype within the behaviour of these animals. Although such behaviours occur in a smaller population of deer mice than do motor stereotypy, it is just as persistent while also responding to chronic SSRI treatment. Furthermore, apart from already being a robust and useful animal model of OCD, within-species manifestations of different obsessive-compulsive-like behaviours in the deer mouse may constitute a valuable and useful naturalistic model to investigate the neurobiology, behavioural appearance and pharmacological response of various obsessive-compulsive-related phenotypes.

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