

A behavioural exploration of litter effect in normal and compulsive-like large nesting behaviour

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“I will say to the Lord, He is my refuge and my fortress; my God, in Him I will trust.”

Psalm 91:2

* * *

*“And we know that all things work together for good to those who love God, to those who are
the called according to His purpose.”*

Romans 8:28

* * *

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* * *

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Stoppel H., Harvey B., Wolmarans D. “Breeding success and offspring survival rates of normal- and compulsive-like large nesting deer mice”, July 2023, Indaba Hotel, Johannesburg.

Abstract

Obsessive compulsive disorder (OCD)¹ is a debilitating neuropsychiatric disorder which is diagnosed if the obsessions, compulsions, or both, meet specific diagnostic criteria. The disorder is characteristically different from general anxiety disorder, although anxiety is regularly present in OCD. Although selective serotonin reuptake inhibitors (SSRIs)² are currently considered to be the mainstay of its treatment, more than 40 % of patients remain non-responsive. The disorder is thus conceptualised as a condition of hyposerotonergic signalling. While its exact aetiology and pathophysiology are not yet fully understood genetic heritability is proposed to play a role. What remains entirely unclear, is if and to what extent genetic factors contribute to perturbations in one or more of the neurocognitive domains associated with OCD. Therefore, it remains difficult to advance our current understanding of the condition since the impact of specific gene-biology-behaviour interactions in the disorder, is not yet elucidated. Since longitudinal studies in clinical cohorts are difficult considering the lifespan of humans, research in preclinical models, like the present, is vital.

* * *

When housed under standard laboratory conditions, approximately 30 % of deer mice (*Peromyscus maniculatus bairdii*) spontaneously express excessive, compulsive-like large nesting behaviour (LNB)³. LNB is regarded as an excessive goal-directed behavioural routine. LNB can be born from genetic or other biological factors or processes related to experiential learning, especially since the evolutionary and naturalistic purpose of nesting is to ensure breeding and nursing success. With respect to LNB in deer mice, it is still unclear if LNB, as opposed to normal nesting behaviour (NNB)⁴, may represent a superior form of goal-directed behaviour that serves to improve breeding outcomes or whether we are correct to conclude that such behaviour is compulsive-like, and thus devoid of an actual, realistic function. It is further unknown if LNB is related to familial factors, which may point to a genetic construct underlying its presentation. The focus of the present investigation was to answer this question.

* * *

To fully address the aims and objectives of this work, fifty-seven deer mice from both sexes (28 female; 29 male) were bred and reared from eleven randomly selected breeding pairs at the vivarium of the

¹ obsessive compulsive disorder

² selective serotonin reuptake inhibitors

³ large nest building

⁴ normal nest building

Abstract

North-West University (NWU)¹, Potchefstroom, South Africa. All mice underwent nesting screening at the age of 12 – 20 weeks. From these, 14 LNB² expressing mice and 14 NNB³ expressing mice (equally distributed between sexes) were selected and paired, considering that littermates were not paired together. These seven NNB and LNB breeding pairs were left to breed two generations of offspring (for **Chapter 3**) and a third in LNB breeding pairs (to add to the adult offspring screened in **Chapter 4** of this work). Nesting expression was then assessed in all adult offspring of the seven NNB and LNB breeding pairs, and the data represented in **Chapter 4**.

* * *

The first main finding showed that despite being as fertile as NNB mice, offspring born from most LNB expressing pairs are notably less viable. Considering that nesting serves several evolutionary conserved functions, this finding was striking in showing that naturalistic LNB in deer mice is goalless and likely born from dysfunctional psychobiological processes.

Such a conclusion was supported by our second main finding, which showed that offspring from LNB expressing pairs built significantly larger nests than offspring from NNB pairs and that offspring from specific breeding pairs built similar sized nests, irrespective of the litter they came from. Since mice could not have expressed the present behaviour as an outcome of experiential learning, we can conclude with a relative degree of confidence that NNB and LNB are two distinct behavioural phenotypes founded upon heritable mechanisms. While the deer mouse model is a valuable model system in which to explore the psychobiological triggers that give rise to spontaneous compulsive-like behavioural persistence, future investigations should explore the nature of the familial influences shown here.

* * *

Keywords: deer mouse; compulsive; large nest building; breeding; offspring survival, heritability

¹ North-West University

² large nest building

³ normal nest building

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1 Introduction

1.1 Dissertation approach and layout

This dissertation was prepared in article format as specified by the North-West University (NWU)¹. The main experimental investigation is presented in the form of two journal articles (**Chapters 3 & 4**), one of which has been submitted to '*Behavioural Processes*' for editorial review (**Chapter 3**). Given the nature of the content, **Chapter 4** will also be submitted to '*Behavioural Brain Research*' for consideration.

The dissertation consists of five chapters. **Chapter 1** provides a broad overview of the problem statement, working hypothesis, aims and objectives of the present study, experimental (or study) layout, and ethical considerations. **Chapter 2** comprises a brief literature review that founds the present work on a theoretical background. As stated, **Chapters 3 & 4** represent the main experimental body of work, while **Chapter 5** concludes the dissertation with a summary of the overarching main findings of the present work and by providing some ideas for future consideration. One addendum is also included (**Addendum A**) in which additional experimental detail regarding the daily routine pertaining to animal husbandry and the experimental procedures are provided.

The entire dissertation has been prepared and referenced according to the seventh edition of the APA guidelines. However, Chapter 3 was prepared according to the referencing style prescribed by '*Behavioural Processes*' [[Guide for authors - Behavioural Processes - ISSN 0376-6357 \(elsevier.com\)](#)]. The dissertation was written in UK English.

¹ North-West University

1.2 Problem statement

Obsessive compulsive disorder (OCD)¹ is a neuropsychiatric disorder characterised by obsessions and compulsions (which can be described as either excessive goal-directed or habitual engagement) (APA, 2013). However, while compulsive behaviour is aimed at achieving specific behavioural outcomes, it should be said that seeking these outcomes, e.g., continuous door locking routines, are irrational. In this sense, compulsions are from a realistic point of view, functionally purposeless (Abramowitz & Jacoby, 2015). Although selective serotonin reuptake inhibitors (SSRIs)² are currently considered to be the mainstay of its treatment, more than 40 % of patients remain non-responsive (Gershkovich et al., 2017). Said response of OCD to SSRIs, though suboptimal, gave rise to the disorder being conceptualised as a condition of hyposerotonergic signalling in the cortico-striatal-thalamo-cortical (CSTC)³ circuits of the forebrain (Bernstein et al., 2016; Parmar & Sarkar, 2016). Various CSTC circuits are involved in complex executive functions, i.e., goal-directed action-outcome control, behavioural task switching, motor control, and reward-directed behavioural engagement (Graybiel & Rauch, 2000; Saxena et al., 1998; van den Heuvel et al., 2016). Dysfunction across all of these functional domains is variably involved in OCD.

While its exact aetiology and pathophysiology are not yet fully understood (which contributes to poor treatment outcomes), genetic heritability is proposed to play a role (Mataix-Cols et al., 2013). What remains entirely unclear, is if and to what extent genetic factors contribute to perturbations in one or more of the neurocognitive domains associated with OCD. Therefore, it remains difficult to advance our current understanding of the condition or improve its treatment outcomes in lieu of the fact that the impact of specific gene-biology-behaviour interactions on the aetiology and promulgation of obsessive-compulsive symptoms is not yet elucidated. As such, continued research with this question at its focus is needed. However, since longitudinal studies in clinical cohorts are difficult considering the lifespan of humans, research in preclinical models could be useful for this purpose. To this end, a thorough understanding and conceptual validation of naturalistic and seemingly compulsive-like behaviours is needed to ensure the appropriate application of such model systems to advance translational research. Working towards this ideal is the focus of the present work.

* * *

¹ obsessive compulsive disorder

² selective serotonin reuptake inhibitors

³ cortico-striatal-thalamo-cortical

When housed under standard laboratory conditions, approximately 30 % of deer mice (*Peromyscus maniculatus bairdii*) spontaneously express excessive large nesting behaviour (LNB)¹, which has been described against the background of compulsive-like behavioural persistence (Wolmarans et al., 2016). Such behaviour is entirely non-induced, is shown by mice of both sexes, and responds to chronic, high-dose escitalopram exposure (Wolmarans et al., 2022), as does clinical OCD². We regard LNB to be an excessive goal-directed behavioural routine for reasons described earlier (de Brouwer et al., 2020; Prinsloo, 2021). However, from an animal modelling perspective, drawing conclusions of translational relevance can be difficult, especially when modelling disorders with cognitive perturbation at its core. In terms of LNB, such behaviour can, for example, either be born from genetic or other biological factors (Greene-Schloesser et al., 2011), or from processes related to experiential learning (Roper, 1976). Also, the evolutionary and naturalistic purpose of nesting is to ensure breeding and nursing success and provide protection against predatory and environmental influences (Deacon, 2006). With respect to LNB in deer mice, it is still unclear if LNB, as opposed to normal nesting behaviour (NNB)³, may represent a superior form of goal-directed behaviour that serves to improve breeding outcomes, or alternatively, whether we are correct to conclude that such behaviour is compulsive-like, and thus devoid of an actual, realistic function. It is further unknown if LNB is related to familial factors, which may point to a genetic construct underlying its presentation. Answers to these two questions will contribute significantly to the present conceptualisation of LNB and its value for studying different aetiological and pathophysiological theories that may be of relevance for obsessive-compulsive symptom presentation.

1.3 Research hypothesis

Our research hypothesis is that LNB, though goal-directed in its execution (Hoffman & Rueda Morales, 2009; Roper, 1976), is an abnormal behavioural phenotype that is not related to or aimed at increased breeding success, and thus resembles compulsive-like, rather than functionally driven, behavioural execution. We further hypothesise that if NNB and LNB are founded solely upon familial mechanisms, mice from the same litter, irrespective of sex, will express similar nesting behaviours in adulthood. If so, the deer mouse model can further be explored in terms of its representation of a highly specific, functionally purposeless, excessive, and persistent behavioural phenotype which has a clear genetic or other biological origin.

¹ large nest building

² obsessive compulsive disorder

³ normal nest building

1.4 Research questions

- i. Is the expression of NNB and LNB differentially associated with breeding and nursing success as measured against litter survival through postnatal day (PND)¹ 21?
- ii. Do the respective offspring of breeding pairs that were prior selected for NNB² or LNB³ expression also express NNB and LNB behaviour in adulthood?
- iii. To what extent is the nesting expression of littermates in adulthood, similar?

1.5 Research aims and objectives

1.5.1 Aim

The main aim of the present investigation is to explore the functional and genetic basis of NNB and LNB in deer mice by:

- i. determining whether NNB and LNB are differentially associated with breeding success; and
- ii. determining whether the respective offspring of NNB and LNB breeding pairs also express NNB and LNB behaviour in adulthood.

1.5.2 Objectives

To achieve the abovementioned aims, the following objectives were met:

- i. Assessing the breeding performance of and collective litter survival of two subsequent litters of offspring from NNB and LNB breeding pairs as followed through PND 21 ($n = 7$ pairs per behavioural cohort); and
- ii. Assessing the nesting expression of all adult offspring (2 – 3 litters) from the NNB and LNB breeding pairs referred to in (i).

¹ postnatal day

² normal nest building

³ large nest building

1.6 Study layout

Figure 1-1 presents a schematic layout of the main investigation of this dissertation.

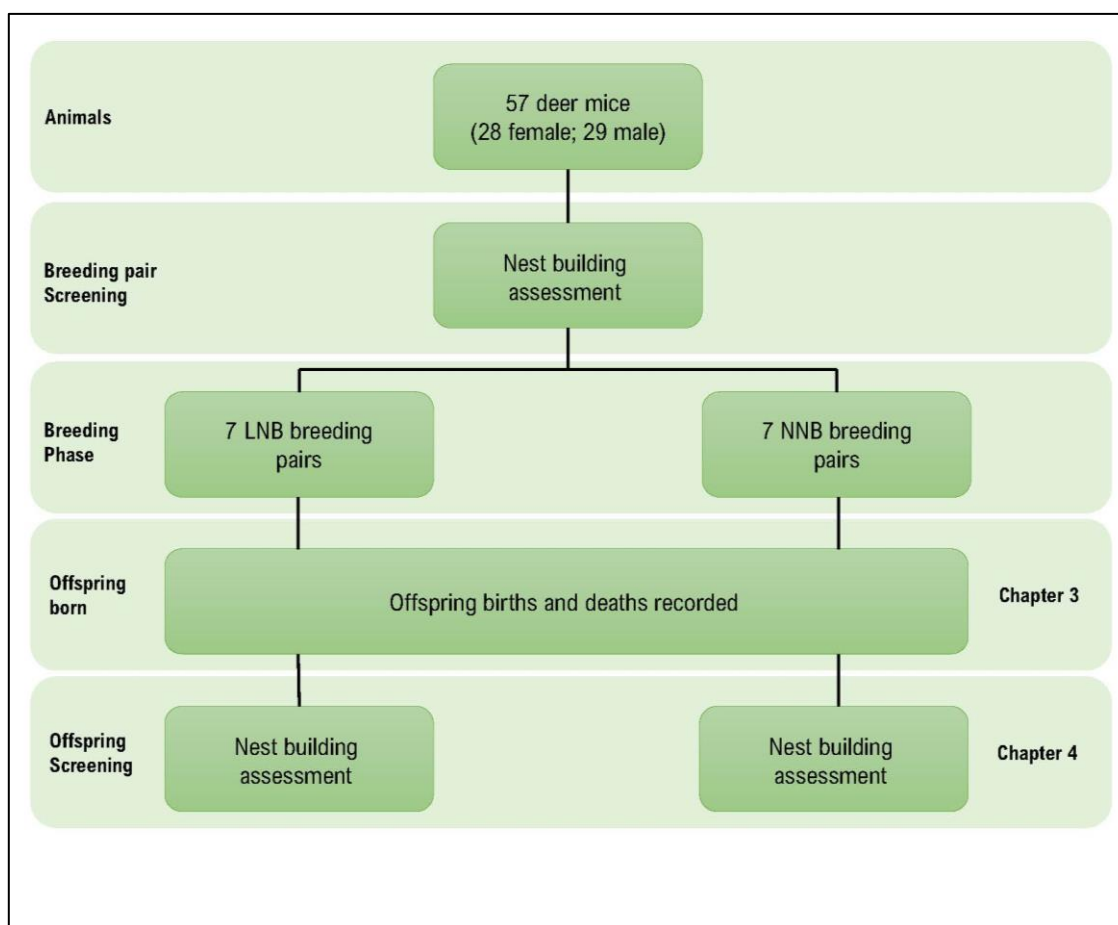


Figure 1-1 – Schematic representation of study layout
NNB: normal nest building | LNB: large nest building

1.7 Ethical approval

The present investigation was approved by the ‘Translational Neuroscience and Neurotherapeutics’ scientific committee and the NWU AnimCare Research Ethics Committee (ethics approval number: **NWU-00770-22-A5**). During the present study, the 4Rs (replacement, refinement, reduction, and responsibility), as it pertains to preclinical research in laboratory-housed animals, have been adhered to.

1.8 References

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2 Literature Review

2.1 A clinical overview of OCD

Obsessive-compulsive disorder (OCD)¹ which is prevalent in 0.9 – 1.8 % of the global population (Fawcett et al., 2020), mainly presents with intrusive thoughts (obsessions) or repetitive and persistent motor or cognitive routines (compulsions). Since obsessions are usually associated with a significant degree of implicit, i.e., self-generated concern or anxiety (APA, 2013; Stein et al., 2010) about specific themes, e.g., contamination, 'neutralising' compulsions, e.g., excessive handwashing (Ahmari, 2016; APA, 2013; Burguiere et al., 2015), are usually expressed in an attempt to reduce the level of anxiety that is born from these irrational obsessions (APA, 2013). However, since obsessive thoughts are implicitly generated, compulsive engagement instils relief for brief periods only. Over time, which can vary from minutes to hours, obsessive thoughts once again intrude, resulting in renewed compulsive engagement (Ahmari, 2016). The condition is thus described as 'self-reinforcing' (McNally, 2016). Importantly, patients suffering from OCD mostly show adequate insight, i.e., they know that the content of their obsessive-compulsive symptoms is irrational (APA, 2013). Although being fundamentally different from delusions, a condition characterised by poor insight, it is theorised that OCD patients present with inferential confusions in that a possible or imaginary situation is confused with a real-life situation, thus contributing to the psychobiological picture of OCD (Aardema et al., 2005; Julien et al., 2016).

The condition is clinically heterogeneous, meaning that different symptom dimensions, all equally intrusive, repetitive, and persistent, are diagnosed (APA, 2013; Storch et al., 2008). Currently, four main dimensions are recognised (APA, 2013) i.e.,

- contamination obsessions associated with washing or cleaning compulsions;
- obsessions about harm to oneself or others, which are associated with safety or checking compulsions;
- symmetry obsessions associated with ordering compulsions; and
- covert intrusive obsessions relating to repugnant themes, i.e., violence or sex transgressions, associated with covert mental compulsions.

The condition has a bimodal age of onset (Anholt et al., 2014; Delorme et al., 2005) with early-onset OCD diagnosed during childhood or early adolescence (before the age of 19 years), and late-onset OCD

¹ obsessive compulsive disorder

from the age of 20 years (Anholt et al., 2014). Men tend to present with early-onset OCD¹ more often than women (APA, 2013; Lensi et al., 1996). Importantly, early-onset OCD is linked to greater symptom severity (Janowitz et al., 2009).

Although OCD was historically viewed as an anxiety disorder (APA, 1994; van Ameringen et al., 2014), the condition is now regarded as the principal disorder in a class of related disorders, as per the most recent version of the Diagnostic and Statistical Manual of Mental Disorders (DSM)² (APA, 2013). Anxiety is often present in OCD, but there are distinguishing characteristics between the obsessional thoughts of OCD and the excessive worrying of generalised anxiety disorder (APA, 2013). In generalised anxiety disorder, the extreme worry about future situations or occurrences is abnormal, and mostly about actual, real-world concerns (APA, 2013). Obsessions in OCD present as inappropriate ideas in the form of images, urges and thoughts that are intrusive and unwanted and generally related to irrational fears (APA, 2013). Interestingly, traditional anxiolytics, i.e., benzodiazepines, are not effective for the treatment of OCD (Fineberg & Craig, 2007).

2.2 The neurobiology of OCD

2.2.1 Neuroanatomy

Although the exact neurobiological constructs of OCD have not yet been fully elucidated (Sinopoli et al., 2017), literature is congruent in pointing to cortico-striatal-thalamo-cortical (CSTC)³ involvement (Bernstein et al., 2016; Parmar & Sarkar, 2016; Pittenger et al., 2011). The CSTC circuitry develops from early childhood to young adulthood (van den Heuvel et al., 2016) and consists of several parallel loops that connect distinct, functionally diverse areas of the brain (Haber, 2003; Haber & Knutson, 2010; Parkes et al., 2019). It is proposed that OCD develops due to dysfunction of the neurocognitive processes underlying goal- and reward-directed function (Gillan & Robbins, 2014; Vaghi et al., 2017), a domain of human functioning that is controlled by the CSTC circuitry (Gillan et al., 2014; Gillan et al., 2011; Parkes et al., 2019).

Viewed collectively, CSTC circuits comprise direct and indirect cortico-striatal pathways (Saxena et al., 1998). Direct pathways are traditionally viewed to be responsible for cognitive and behavioural activation by promulgating signals via the internal aspect of the globus pallidus/substantia nigra, which subsequently activates the thalamus (van den Heuvel et al., 2016). The thalamus serves as an integration and relay station in the central nervous system that bridges striatal and cortical processing

¹ obsessive compulsive disorder

² diagnostic and statistical manual of mental disorders

³ cortico-striatal-thalamo-cortical

(Friedlander & Desrocher, 2006; Huey et al., 2008; Kumar et al., 2017; Sherman & Guillery, 2006). Within the present context, one of its major functions is to act as a gateway that regulates the movement of instructional information from the striatum back to the cortex for final execution (Sherman, 2006).

In contrast, indirect pathway activation inhibits thalamo-cortical signalling via a negative feedback loop that prevents cortical activation. Of note, upon cortical planning of cognitive and behavioural output, both pathways are simultaneously stimulated, resulting in executive conflict. Such conflict is resolved by dopamine, as will be explained later (Balasubramani et al., 2015). Suffice it to state at this point that an imbalance between direct and indirect pathway activity, could contribute to the psychobiological picture of OCD (Graybiel & Rauch, 2000; van den Heuvel et al., 2016).

2.2.2 Neurotransmission

2.2.2.1 Dopamine

Dopamine is primarily synthesised and released by the neurons of the midbrain (Bjorklund, 1984; Björklund & Dunnett, 2007; Ott & Nieder, 2019). From here, dopaminergic neurons project to various areas of the brain, including components of the CSTC¹ circuits and the limbic system, via nigro-striatal, and tubero-infundibular nerve bundles (Ott & Nieder, 2019). Within the context of OCD², we have previously referred to the fact that executive, i.e., ‘go-no-go’, conflict is generated upon the cortical planning of specific instructions (Nambu, 2008). Simultaneous dopamine release, which is triggered by the expectation of a beneficial or positive outcome of a planned routine (Fischer & Ullsperger, 2017), is crucial to resolving this conflict. To explain, the neurons of the direct pathway express dopamine D₁ receptors, which if activated, promulgates signalling (Graybiel, 1990; Graybiel & Rauch, 2000; Saxena et al., 1998). In contrast, when D₂ receptors which are expressed on the neurons of the indirect pathway are stimulated, activation of the indirect pathway is prevented, thus blunting signalling in this normally behaviourally inactivating pathway. As such, the executive balance switches to execution (Alexander & Crutcher, 1990; Graybiel, 1990; Graybiel & Rauch, 2000).

This function of dopamine also has a direct bearing on obsessive-compulsive symptoms. For example, increased dopaminergic neurotransmission has been shown in patients with OCD (Denys et al., 2013), explaining the use of dopaminergic antagonists as augmentative interventions to treat individuals that are resistant to selective serotonin reuptake inhibitors (SSRIs)³ (Zhou et al., 2019).

¹ cortico-striatal-thalamo-cortical

² obsessive compulsive disorder

³ selective serotonin reuptake inhibitors

2.2.2.2 Serotonin

In contrast with the mostly circumscribed distribution and functions of dopamine, serotonin, which is also a major — no, fundamental — neurobiological role player in OCD¹, is globally distributed throughout the brain (Sinopoli et al., 2017). It plays a major role in emotional, cognitive and behavioural functions, and thus, it is the therapeutic target of major drug classes used to treat a range of both affective and non-affective neuropsychiatric conditions, including OCD (Celada et al., 2013; Pizarro et al., 2014). Tryptophan is the precursor of among others, serotonin in the body (Comai et al., 2020). Tryptophan is an amino acid utilised in protein synthesis and is otherwise metabolised along two different pathways, i.e., the kynurenine pathway and the serotonin pathway (Comai et al., 2020), both of which produce neuroactive compounds (Davis & Liu, 2015). The kynurenine pathway accounts for approximately 90 % of tryptophan breakdown (Badawy, 2017) while the serotonin pathway metabolises a further 1 – 2 % (Bender, 1983; Keszthelyi et al., 2009).

Against the background of obsessions and compulsions, serotonin can be regarded as the behavioural opponent of dopamine (Daw et al., 2002). Serotonin therefore is proposed to inhibit executive routines (Daw et al., 2002). Given the excessive behavioural engagement typical of OCD, the disorder is often proposed to be related to hyposerotonergic signalling (Lissemore et al., 2018). Further, genetic polymorphisms in genes encoding different components involved in serotonergic signalling, e.g., the serotonin transporter (SERT)² (Atmaca et al., 2011; Cengiz et al., 2015; Maron et al., 2009), have been shown.

2.2.2.3 Glutamate

Glutamate and gamma-aminobutyric acid (GABA)³ are respectively the primary excitatory and inhibitory neurotransmitters in the mammalian brain (Karthik et al., 2020; Pittenger et al., 2011). Both are found in high concentrations in the cerebrospinal fluid (CSF)⁴ and central nervous systems of humans and other mammals (Danbolt, 2001; Pittenger et al., 2011; Snyder & Ferris, 2000). Further, both GABA and glutamate are hypothesised to play a role in the CSTC circuit development and the maintenance of a balance between the direct and indirect pathways (Karthik et al., 2020). Glutamatergic neurons form part of most of the subcortical as well as cortical networks (Karthik et al., 2020; Rubio-Casillas & Fernández-Guasti, 2016), with most of the axon terminals found in the striatum being glutamatergic (Wu et al., 2012). Glutamate transporters are key components in ending excitatory glutamatergic neurotransmission (Pittenger et al., 2011). Polymorphism in the solute carrier family 1 member 1

¹ obsessive compulsive disorder

² serotonin transporter

³ gamma-aminobutyric acid

⁴ cerebrospinal fluid

(SLC1A1)¹ gene — that encodes the neural glutamate transporter EAAT3 (Nieoullon et al., 2006; Pittenger et al., 2011) — has been associated with OCD² (Arnold et al., 2006; Dickel et al., 2006; Pittenger et al., 2011). In fact, abnormalities in genes implicated in glutamatergic functioning may cause defective CSTC³ circuit development (Karthik et al., 2020) and contribute to the promulgation of obsessive-compulsive symptoms (Carlsson, 2000; Pittenger et al., 2011; Pittenger et al., 2006).

2.3 The treatment of obsessive-compulsive disorder

2.3.1 Pharmacotherapy

Initial indications that OCD—which was at the time overly difficult to treat—may indeed be responsive to pharmacotherapy, came from findings made more than 50 years ago that showed the tricyclic antidepressant (TCA)⁴, clomipramine, to be effective in some cases (Decloedt & Stein, 2010; Fernández & Lopez-Ibor, 1967; Fineberg & Gale, 2005). A noteworthy finding at the time was that another tricyclic antidepressant, nortriptyline was not as effective as clomipramine (Kellner, 2022; Thorén et al., 1980). The major difference between the two drugs is that clomipramine targets serotonergic and noradrenergic reuptake (McTavish & Benfield, 1990), while nortriptyline mainly bolsters noradrenergic signalling (Dell’Osso et al., 2011). As such, this finding provided the first proof-of-concept that OCD might be more related to serotonergic than noradrenergic dysfunction, distinguishing the disorder on a neurobiological level from major depressive disorder and anxiety (Goodman et al., 1990). Over time, the more selective serotonin reuptake inhibitors (SSRIs)⁵ became the preferred choice of therapy, considering the side-effect profile of the TCAs, which includes anticholinergic effects, e.g., cardiotoxicity (Pittenger & Bloch, 2014).

To be clinically effective for the treatment of OCD, SSRIs should be administered chronically in high doses (Kellner, 2022) and without interruption. Also, the onset of action of SSRIs in the treatment of OCD is longer than what is true for major depression, i.e., 8 – 12 weeks, a factor that contributes to poor treatment adherence (Pittenger & Bloch, 2014). Although the development and use of SSRIs signified a significant step forward for patients suffering from OCD, 40 – 60 % of patients still show only a partial response or even no improvement (Gershkovich et al., 2017). In such cases, several strategies can be followed. For example, even higher doses of currently used SSRIs can be used, or patients can be switched to a different drug in the same class (Bandelow et al., 2023). Mostly though, prescribers

¹ solute carrier family 1 member 1

² obsessive compulsive disorder

³ cortico-striatal-thalamo-cortical

⁴ tricyclic antidepressant

⁵ selective serotonin reuptake inhibitors

favour augmentation regimens where low doses of D₂ receptor antagonists, e.g., risperidone, are added to an existing SSRI¹ treatment regimen (Erzegovesi et al., 2005). Nevertheless, all these strategies are varyingly met with therapeutic resistance, which necessitated the field of neuropsychiatry to explore different avenues of investigation. Given the role of glutamatergic signalling in the neurobiology of OCD² alluded to earlier in this chapter, anti-glutamatergic agents are of special interest (Kellner, 2022). These drugs, which include riluzole, a benzothiazole (Maraone et al., 2021; Zarate & Manji, 2008), and memantine, a non-competitive N-methyl-D-aspartate (NMDA)³-receptor antagonist (Maraone et al., 2021; Parsons et al., 1999), used either as monotherapy or adjunctive therapy to SSRI regimens, have shown mixed efficacy in clinical trials (Maraone et al., 2021).

2.3.2 Psychotherapy

Another treatment option is non-pharmacological interventions, i.e., behavioural therapy, which includes exposure and response prevention (ERP)⁴ (Kellner, 2022). ERP is based on the exposure of patients to stressful situations or objects that are thematically related to the content of their obsessions. However, they are then prevented from engaging in neutralising compulsive behaviours (Foa, 2022). This approach aims to coach patients to face and endure a stimulus that usually bolsters obsessions and compulsions without acting on it (Reid et al., 2021). Of the different symptom dimensions, individuals presenting with contamination, harm, or symmetry obsessions have shown better responses to ERP than those suffering from covert obsessions and compulsions, i.e., sexual or violence related symptoms (Mataix-Cols et al., 2002; Rufer et al., 2006). Some individuals are however reluctant to adhere to ERP because of the anxiogenic nature of the treatment (Maiti et al., 2023; Storch & Merlo, 2006).

2.4 Towards clinical progress: novel targets to expand the current understanding of OCD

From the above, our understanding of OCD is not sufficient to improve current therapeutic outcomes. In recent years, the field of neuropsychiatry broadened to focus on complex gene-environment interactions studied within an integrated systems approach to address issues of therapeutic non-response (Keers & Uher, 2012; Klengel & Binder, 2013; Tsuang et al., 2004). This is not different for OCD (Alemany-Navarro et al., 2019). In this section, we will afford attention to some of these fields of interest since the present work sought to explore for one, familial influences in compulsive-like large

¹ selective serotonin reuptake inhibitors

² obsessive compulsive disorder

³ N-methyl-D-aspartate

⁴ exposure and response prevention

nesting behaviour (LNB)¹ in a naturalistic model of compulsive-like behavioural persistence, i.e., the deer mouse (*Peromyscus maniculatus bairdii*).

2.4.1 Genes and our environment: a confluent burden carried over between generations

Evidence for a genetic component in the aetiology and pathophysiology of OCD² initially came from family and twin studies (Hanna et al., 2005; Rotge et al., 2010). Specifically, the incidence of OCD is notably higher in monozygotic (identical) twins compared to dizygotic (non-identical) twins (Hettema et al., 2001; Inouye, 1965; Smoller et al., 2009). Apart from family studies, subsequent genome-wide association studies sought to investigate specific genetic constructs that might contribute to the aetiology and promulgation of OCD. A candidate gene approach is frequently followed which entails investigating various genes that, based on the disorder's characteristics, could potentially be connected to the disease phenotype (Schindler et al., 2000). These studies yielded interesting findings and implicated dysfunction in several neurotransmission systems at large.

Of all the genes studied, those involved in the functioning of the serotonin system are the most studied (Sinopoli et al., 2017). As alluded to earlier, polymorphisms in the SLC6A4 gene, which encodes the SERT³ protein (McDougle et al., 1998; Sinopoli et al., 2017), are commonly shown in patients with OCD (Cengiz et al., 2015). The functional relevance of these findings largely concurs with protein expression patterns, with OCD cohorts presenting with lower SERT expression compared to healthy controls (Hesse et al., 2005; Zitterl et al., 2007), supporting a theoretical description of OCD based on hyposerotonergic signalling.

In terms of dopamine, findings are less clear with both decreased (Hesse et al., 2005) and increased dopamine transporter (DAT)⁴ expression reported in OCD (Kim et al., 2003; Van Der Wee et al., 2004). We have also referred to other candidate genes investigated in OCD, i.e., the neuronal and epithelial glutamate transporter gene (SLC1A1)⁵ (Mattheisen et al., 2015; Pittenger et al., 2011; Wu et al., 2012), which strikingly, is regarded as the most dominant candidate gene of relevance for OCD (Escobar et al., 2019; Goodman et al., 2021). Polymorphisms in SLC1A1, specifically in men, are believed to predispose an OCD diagnosis (Arnold et al., 2006).

Still, it remains unclear which genes play the most prominent roles in an increased familial risk for OCD (Sinopoli et al., 2017; Taylor, 2013). Further, it is unlikely that specific gene polymorphisms on their

¹ large nest building

² obsessive compulsive disorder

³ serotonin transporter

⁴ dopamine transporter

⁵ solute carrier family 1 member 1

own, could solely be responsible for triggering and causing OCD¹. Rather, observational studies point to a need to investigate gene-environment interactions as important determinants of neuropsychiatric outcomes (Hettema et al., 2001; Mahjani et al., 2021). For example, first-degree relatives of individuals with paediatric-onset OCD have a higher risk of developing OCD compared to the first-degree relatives of individuals with adult-onset OCD (Browne et al., 2014; Nestadt et al., 2000; Pauls et al., 1995), and so points to a higher genetic predisposition in the former, compared to the latter (Browne et al., 2014). Interestingly, since the risk for OCD decreases from first to third-degree relatives of OCD probands (Mataix-Cols et al., 2013), it seems as if temporal and lifetime-specific environmental influences indeed play a crucial role in determining the outcomes of genetic polymorphism. Specifically with respect to OCD, said influences are mostly related to early-life stress or distressing life events (Gothelf et al., 2004; McGregor et al., 2016). Further, genetic polymorphisms that are not directly related to the known neurobiological systems involved in OCD, can influence interactions between brain function and other systems that may be involved as aetiological triggers in OCD. One such example pertains to brain-immune crosstalk. Specifically, an association between the mannose-binding lectin 2 (MBL2)² gene, which plays a role in the activation of the complement immune system, and paediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS)³ (Çelik et al., 2019), an immune-triggered subtype of OCD with a sudden onset, was shown.

Collectively viewed, although these findings point to genetic factors underlying OCD, it is likely that interactions between genetic polymorphism and environmental influences may differentially contribute to OCD in different populations. If and how these interactions may contribute to the various obsessive-compulsive phenotypes in men and women, remains to be established.

2.4.2 Gut-brain interactions and its implications for anti-compulsive therapy

Heritable influences do not only have bearing on genetic factors. It also transcends to the gut microbiome. The human gut is host to trillions of microorganisms (Cryan & Dinan, 2012; Cryan & O'Mahony, 2011; Cusotto et al., 2018), most of which are bacteria (Belkaid & Naik, 2013; Petra et al., 2015), but also viruses, archaea, parasites, and fungi (Iannone et al., 2019). Although it is uncertain whether colonisation of the gut mainly transpires before or after birth (Margolis et al., 2021; Perez-Muñoz et al., 2017), the microbiome is a crucial mechanism of crosstalk between the brain and the periphery and an important determinant of normal brain development (Collins et al., 2012; Cryan et al., 2019; Margolis et al., 2021; Rhee et al., 2009). Irrespective, at the age of three years, the gut-microbiota

¹ obsessive compulsive disorder

² mannose-binding lectin 2

³ paediatric autoimmune neuropsychiatric disorders associated with streptococcal infections

composition of most children becomes stable, resembling that of healthy adults (Claesson et al., 2011; Cresci & Bawden, 2015).

Gut microbiota dysbiosis, loosely defined as a disruption of the microbial composition in the gastrointestinal tract (Settanni et al., 2021), contributes to neuropsychiatric outcomes, including OCD¹ (Bendriss et al., 2023). Although it is not entirely certain how gut microbiota dysbiosis contribute to neuropsychiatric illness, the gut microbiota influences monoaminergic signalling and immune system functioning (Sandhu et al., 2017), both of which are central neurobiological constructs of central nervous system disorders. It stands to reason that gut microbiota dysbiosis can thus contribute to dysfunctional brain processes, which may give rise to neuropsychiatric illness. With respect to OCD, this is exemplified in children diagnosed with paediatric acute-onset neuropsychiatric syndrome (PANS)² related OCD, in whom differences between the bacterial diversity of OCD individuals and control groups have been observed (Quagliariello et al., 2018). Differences in the microbial composition of adult OCD patients and healthy controls have also been shown, with a reduction in alpha diversity being shown in individuals with OCD (Domènech et al., 2022).

* * *

In the present investigation, we will explore the extent to which LNB³ has an actual purpose within the evolutionary context of nesting behaviour or whether indeed, it is an artefact of dysfunctional psychobiological architecture. We will further investigate whether the expression of LNB is heritable to inform the basis of future studies into compulsive-like nesting behaviour. This point-of-departure will become clearer in the following section.

2.5 The deer mouse model for OCD

Considering that our understanding of the aetiology and pathophysiology of OCD is lacking, preclinical research in animal models that resemble specific traits of human conditions, is valuable (Fernando & Robbins, 2011). However, caution is advised, since animal models can never fully mimic the complete psychobiological scope of a human neuropsychiatric disorders, and thus, translational conclusion should be drawn with care (Szechtman et al., 2017). Irrespective of the condition modelled, animal models are validated according to a set of criteria, the most common of which speak to face, construct, and predictive validity (Albelda & Joel, 2012). Briefly, face validity refers to similarities between behavioural and cognitive outputs observed in both humans and the animal model applied, whereas predictive validity

¹ obsessive compulsive disorder

² paediatric acute-onset neuropsychiatric syndrome

³ large nest building

is based on similarities in treatment response (or non-response). Last, construct validity is based on shared underlying biological mechanisms (Monteiro & Feng, 2016). As is true for other psychiatric conditions, suitable animal models are also needed to understand the mechanisms central to obsessive-compulsive behaviour (Wolmarans et al., 2013). Be it as it may that obsessions are difficult to identify in animal models, compulsions (or compulsive-like behaviour) can be observed in animal models, as will be explained here (Wolmarans et al., 2013).

Notwithstanding their value, the relevance of translational animal models as tools that can be used to expand our understanding of human disorders is increasingly being doubted, mostly due to the fact that not many major and novel advances in the field of neuropsychiatry have recently resulted from animal research (Cope et al., 2016; Malfait & Little, 2015). Our laboratory has recently argued that said slow progress may partly be related to poor between-laboratory reproducibility, inadequate theoretical and conceptual reasoning prior to study onset, and irrelevant conclusions being drawn from research data (de Brouwer et al., 2019; de Brouwer & Wolmarans, 2018). Thus, we described a fourth criterion against which to validate animal models, i.e., methodological validity, which speaks to the manner in which translational methods are followed to obtain specific, meaningful, and relevant results (de Brouwer, 2020). To this end, the application of the methodology followed in this work, i.e., assessing animals for nest-building activity, follows such an approach. This, we will allude to in a moment.

The present work focuses on LNB, as opposed to normal nest building (NNB)¹ behaviour in deer mice (*Peromyscus maniculatus bairdii*), as a form of compulsive-like behavioural persistence that is spontaneously expressed by a subpopulation of deer mice housed in the laboratory (Scheepers et al., 2020; Wolmarans et al., 2016a). To provide a suitable background for the present work, we will also afford some attention to the history of the model and explain why various behaviours expressed by animals of this species are argued to be reminiscent of compulsive-like behavioural routines.

The *Peromyscus* genus is commonly used in behavioural studies (Davis et al., 2023), including those related to OCD². Deer mice are native to North America (Vrana et al., 2014) and stereotypic behaviour, which includes somersaulting, repetitive jumping, and pattern running, develops spontaneously in some mice, notably so when housed in confinement (Güldenpfennig et al., 2011). Stereotypy imitates the recurrent and persistent motor actions that are seen in individuals with OCD (Wolmarans et al., 2013), since they are highly repetitive, occur in a waxing and waning nature, and are entirely purposeless. Stereotypy further presents in only 45 % of deer mice, irrespective of sex (de Brouwer et al., 2021; Korff

¹ normal nest building

² obsessive compulsive disorder

et al., 2008; Wolmarans et al., 2013). Further, chronic treatment with SSRIs¹, but not noradrenalin reuptake inhibitors, notably reduces the expression of stereotypy, a finding that provided the initial foundations for the model's predictive validity (Korff et al., 2008; Wolmarans et al., 2013). The construct validity with respect to stereotypical expression as a form of compulsive-like behaviour is based on reduced striatal SERT² density (Wolmarans et al., 2013). We have subsequently shown that high stereotypical (HS)³ and non-stereotypical (NS)⁴ deer mice differ with respect to immune functioning, with HS male mice showing an increased neutrophil-lymphocyte ratio (NLR)⁵, compared to female high stereotypical mice and normal mice, irrespective of sex (Meiring, 2021). We have also shown HS to be distinct from NS on the level of cognitive involvement, with HS mice presenting with social deficits, but only in the presence of NS mice, and not among themselves (Wolmarans et al., 2017). Further, while HS is not associated with cognitive inflexibility per se, mice of this cohort respond uniquely to the novel cognitive enhancer, levetiracetam, compared to NS mice (de Ridder et al., 2022). Specifically, spontaneous alternation behaviour in HS, but not NS mice, improves following chronic levetiracetam intervention (de Ridder et al., 2022), pointing to some degree of cognitive impairment in these mice that are not observed in NS mice.

In recent years, we expanded the model in terms of exploring deer mouse behaviour with respect to its possible resemblance to obsessive-compulsive symptom heterogeneity (Wolmarans et al., 2016a). As such, two additional behavioural phenotypes were described, i.e., high marble burying (expressed by 11 % of the population) (Wolmarans et al., 2016b) and LNB. Given the focus of the present work, we will afford attention to the latter.

Nest building is considered an innate mouse behaviour which is characterised by several finely coordinated steps, including swift and recurrent motions to pull cotton (or other forms of nesting material) through the metal bars at the top of the cage (Greene-Schloesser et al., 2011) and the construction of nests by means of forepaw movement. Rodents build nests for survival, for protection against predators, to regulate temperature and to breed and nurse offspring (Deacon, 2006). In the laboratory, none of these functions, except maybe for providing a superior environment for breeding and nursing, are realistically applicable. It would thus be expected that all same-sex mice of a specific species that are housed under the same laboratory conditions will build similar nests (Deacon, 2012; Wolmarans et al., 2022). This is not the case, with 30 % of deer mice from both sexes presenting with

¹ selective serotonin reuptake inhibitors

² serotonin transporter

³ high stereotypical

⁴ non-stereotypical

⁵ neutrophil-lymphocyte ratio

spontaneous and voluntary repetitive LNB¹ (Wolmarans et al., 2022; Wolmarans et al., 2016a). Like stereotypy, LNB is persistent and excessive and since these mice are housed under controlled thermoregulated laboratory conditions that are suitable for breeding and devoid of predators or other life-threatening risks, LNB is not connected to a realistic 'goal' that should be achieved (Wolmarans et al., 2016a). In this way, it is similar to clinical compulsions (APA, 2013).

During recent years, LNB has been studied in several investigations which collectively, yielded significant insights. Specifically, LNB responds to chronic SSRI² exposure (Wolmarans et al., 2022), with or without the addition of low-dose antidopaminergic agents, e.g., flupentixol (de Brouwer et al., 2020). Additionally, LNB expressing mice present with lower striatal and frontal-cortical tryptophan and serotonin concentrations (Karsten, 2022), highlighting that hyposerotonergia might be to blame for its presentation. Further, LNB and NNB³ mice also differ with respect to their naturalistic gut microbiota composition (Scheepers et al., 2020), with LNB mice showing an abundance of potentially inflammatory taxa, compared to NNB mice (Scheepers et al., 2020). Recent studies further highlighted that LNB expressing mice also show dysfunctional goal-directed behavioural control (Prinsloo, 2021) and increased cortico-striatal activation as evinced by a lower degree of adenosine A_{2A} receptor expression, which thus promulgates dopaminergic activation of striatal circuits (Saaiman et al., 2023).

Despite the abovementioned research and findings pertaining to LNB in deer mice, we still do not know if and to what extent this behaviour may be expressed as a superior phenotype that will contribute to greater breeding success. We also do not know if LNB and NNB are inherited behavioural phenotypes. In the absence of answers to these questions, we are unable to properly investigate the aetiological origins of LNB and appropriately contextualise the model as a research framework in which to study compulsivity. This will therefore be the focus of the present work.

¹ large nest building

² selective serotonin reuptake inhibitors

³ normal nest building

2.6 References

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3 Journal Article A

Higher offspring mortality in deer mice (*Peromyscus maniculatus bairdii*) that spontaneously present with large nest building behaviour

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Abstract

Nesting is a normal, evolutionary conserved rodent behavioural phenotype that is expressed for purposes of breeding, safety, and thermal regulation. Further, nesting is commonly assessed as marker of overall rodent health and wellbeing, with poorer nesting performance generally proposed to resemble a worse state of health. Deer mice can be bidirectionally separated with 30 % of mice presenting with excessively large nesting behaviour (LNB)¹. All laboratory-housed deer mice are exposed to identical environmental conditions. Thus, the functional purpose of LNB remains unknown. Our research hypothesis is that LNB, though goal-directed in its execution, is an abnormal behavioural phenotype that is not related to or aimed at increased breeding success, and thus resembles compulsive-like, rather than functionally driven, behavioural execution. After breeding two generations of offspring from six 'normal' nesting (NNB)² and seven LNB expressing pairs, our data showed that while as fertile as NNB expressing pairs, offspring survival of LNB mice was notably worse (67.9 % vs. 98.3 %). In conclusion, variance in nesting behaviour should be considered when animal health and wellbeing are considered, since it may point to underlying biobehavioural perturbations.

Keywords

deer mouse; rodent wellbeing; offspring survival; nest; obsessive-compulsive

¹ large nest building

² normal nest building

3.1 Introduction

Deer mice (*Peromyscus maniculatus bairdii*) are studied for their varying expression of rigid behavioural phenotypes, e.g., high stereotypy and large nesting behaviour (LNB)¹ (Scheepers, Stein, and Harvey 2018). Nesting is an evolutionary conserved rodent behaviour that is expressed for purposes of breeding, safety, and thermal regulation. (Smithers 1983). However, bidirectional separation of deer mice based on nesting performance revealed 30 % of mice of both sexes to show LNB, which has since been studied for its resemblance to compulsive-like behavioural persistence. (Wolmarans et al. 2022). Against this background, LNB is highly stable and seemingly purposeless. (Wolmarans et al. 2016). It is characterised by dysfunctional cortico-striatal serotonergic (Wolmarans et al. 2022) and adenosinergic (Saaiman et al. 2023) signalling, while LNB, but not 'normal' nesting (NNB)² expressing mice are overly resistant to negative sensory feedback (i.e., electrical shock (de Brouwer, Harvey, and Wolmarans 2020) and anxiogenic open spaces (Wolmarans et al. 2022)). Since all laboratory-housed mice are exposed to identical environments, the question arises as to what the functional purpose of LNB is? Although nesting performance in laboratory-housed rodents is applied as a measure of overall health and wellbeing (poor nesting performance being indicative of a worse state of health; (Jirkof, 2014)) the functional purpose of LNB remains entirely unknown. Considering the evolutionary functions of nesting, and that such objectives can be achieved by means of normal-sized nests, we hypothesised that LNB is an abnormal behavioural phenotype that is not related to or aimed at increased breeding success, and thus resembles compulsive-like, rather than functionally driven, behavioural execution.

3.2 Methods and materials

3.2.1 Mice

Fifty-seven adult deer mice of both sexes (that were later used to constitute the NNB and LNB breeding pairs used in this investigation) were bred from eleven progenitor breeding pairs (12 – 20 weeks old) maintained at the vivarium (Preclinical Drug Development Platform, North-West University; SAVC reg: FR15/13458; AAALAC accreditation file: 1717). These pairs were randomly allocated, i.e., without litter or nesting bias (ethics approval no.: **NWU-00770-22-A5**). All mice were housed and bred in individually ventilated cages (35 cm (l) x 20 cm (w) x 13 cm (h); Techniplast® S.P.A., Varese, Italy) kept ambient at 23 °C on a 12-h light/dark cycle (06:00/18:00). Food and water were provided *ad lib* through study termination. Cages were cleaned and new bedding (corncob) was added, weekly. A piece of white polyvinyl chloride pipe (10 cm (l) x 4 cm (Ø)) and a paper towel were provided as a form of

¹ large nest building

² normal nest building

environmental enrichment and nesting material (except during periods of nesting assessment; see below), respectively. Since not all 57 offspring of the 11 progenitor breeding pairs were born within the same period, breeding continued until 57 pups were born. Pups were weaned on postnatal day (PND)¹ 21 and raised until adulthood (PND 84 – 140) when their nesting behaviour was assessed, and animals allocated to either NNB² or LNB³ breeding pairs. This paper reports data pertaining to the breeding performance and the offspring survival rates of pups born from these NNB and LNB expressing breeding pairs (**Table 3-1**; see below).

Table 3-1 - Litter breakdown and date of birth

Pair no.	Male age	Female age	Date of Birth (n)	Mortalities (Litter 1)	Date of Birth (n)	Mortalities (Litter 2)
Offspring from LNB expressing pairs						
1	149	151	20 Jan 23 (3)	0	16 Feb 23 (4)	4
2	90	151	21 Jan 23 (3)	3	15 Feb 23 (2)	2
3	117	132	24 Jan 23 (3)	0	24 March 23 (3)	0
4	132	151	22 Jan 23 (5)	5	15 Feb 23 (6)	0
5	141	151	20 Jan 23 (3)	1	18 Feb 23 (5)	0
6	149	123	22 Jan 23 (4)	1	21 Feb 23 (4)	1
7	132	151	21 Jan 23 (2)	0	17 Feb 23 (5)	0
Offspring from NNB expressing pairs						
8	149	106	-	-	-	-
9	132	141	19 Jan 23 (5)	0	17 Feb 23 (7)	0
10	106	112	20 Jan 23 (5)	0	14 Feb 23 (5)	0
11	117	123	02 Feb 23 (1)	1	26 Feb 23 (6)	0
12	132	112	30 Jan 23 (5)	0	27 Feb 23 (6)	0
13	141	132	26 Jan 23 (4)	0	24 Feb 23 (5)	0
14	149	132	19 Jan 23 (5)	0	07 March 23 (5)	0

¹ postnatal day

² normal nest building

³ large nest building

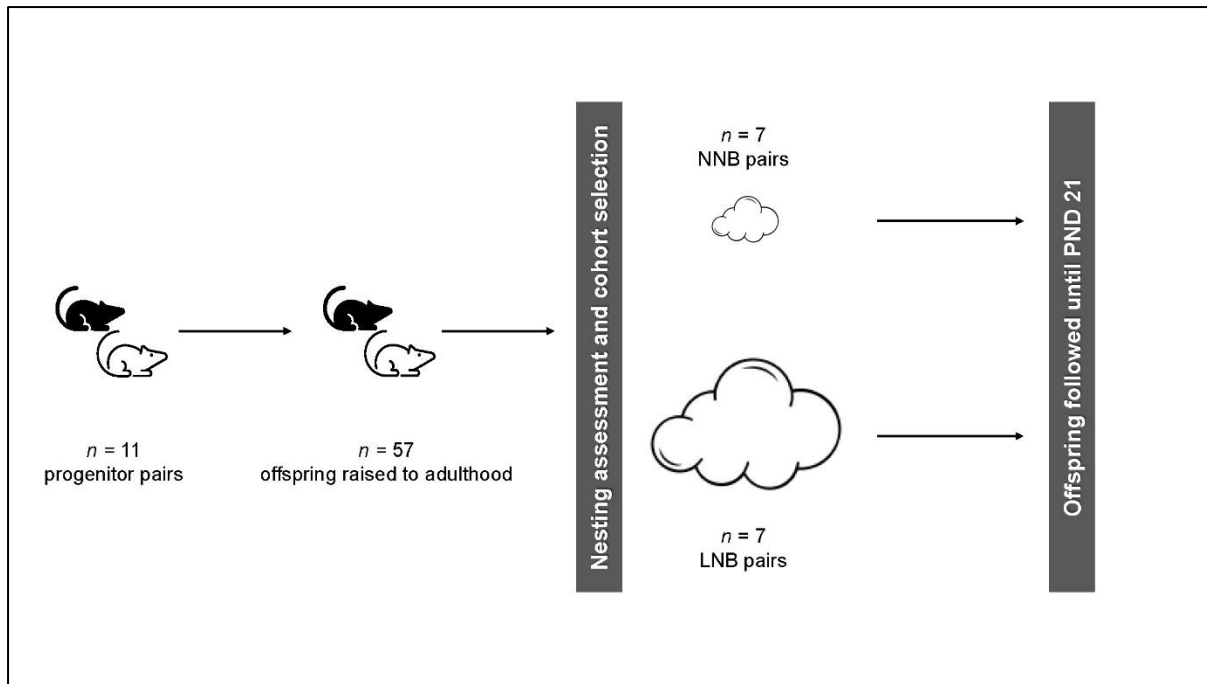


Figure 3-1 – Schematic representation of study layout

3.2.2 Nesting assessment and behavioural selection

Nesting performance was assessed in each of the 57 first-generation offspring over seven consecutive days. Briefly, to classify mice into the NNB¹- or LNB²-expressing cohorts, we applied the protocol of (Saaiman et al. 2023) without modification. Each mouse was screened for nesting behaviour over the course of seven consecutive days. On each day between 12:00 and 14:00, an excess of pre-weighed, unscented cosmetic cotton wool was introduced into the roof of each home cage. Every subsequent day, also between 12:00 and 14:00, the remaining cotton wool, i.e., not used for nesting, was weighed, and the built nests removed and discarded. This process was repeated over all seven days of testing. At the end of the assessment period, a grand total nesting score (the sum of the seven daily scores, expressed in grams), was calculated for each mouse. Considering that compulsivity is both excessive and persistent, the classification of LNB behaviour was based on the extreme end of the total nesting score distribution of all assessed mice (Wolmarans et al. 2016). Specifically, LNB behaviour was broadly defined as those scores that clustered near to or above the upper 75th percentile of the total nesting score distribution, but that were also associated with coefficients of variance pertaining to the inter-day nesting scores that clustered within the lower 25th percentile of distribution (**Fig. 3-2**). Conversely, NNB behaviour was characterised by total nesting scores that clustered below the 50th percentile of the distribution. From this data, 14 NNB and 14 LNB-expressing mice (7 female; 7 male per cohort), were

¹ normal nest building

² large nest building

selected for further study (**Fig. 3-2**) and seven breeding pairs per behavioural cohort were randomly constituted. All breeding pairs were left to breed two generations of offspring.

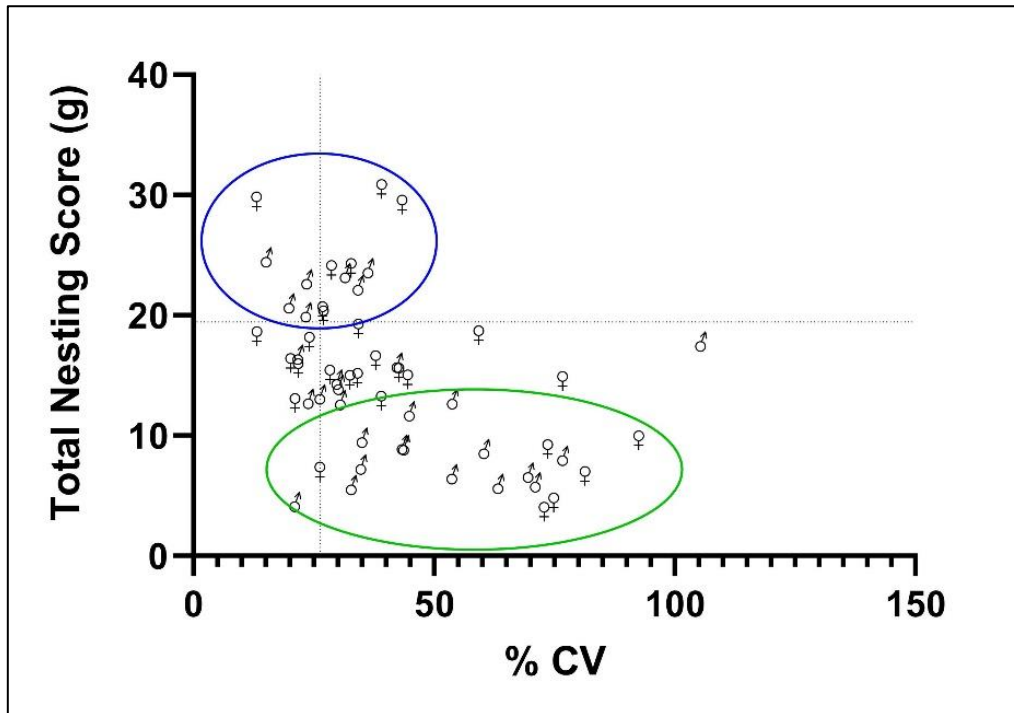


Figure 3-2 – Selection of NNB (green oval) and LNB (blue oval) expressing mice

3.3 Results

The descriptive data pertaining to NNB¹ and LNB² expressing breeding pairs, births and survival rates are shown in **Table 3-1**. Six of the seven NNB dams gave birth to a total of 59 pups (average between-litter interval: 30.33 days), of which 58 pups survived until PND³ 21 (survival rate: 98.3 %). LNB dams gave birth to 53 pups (average between-litter interval: 31.57 days). Of these, 36 pups survived through PND 21 (survival rate: 67.9 %).

3.4 Discussion

The present data show 1) that NNB and LNB expressing pairs present with similar breeding performance, but 2) that mortalities were higher in offspring of most LNB, compared to NNB expressing pairs.

Our results remarkably show that despite being as fertile as NNB mice, offspring born from most LNB expressing pairs are notably less viable. This is important since nesting is commonly assessed in laboratory-housed rodents as a measure of overall health and wellbeing. Because nesting serves several

¹ normal nest building

² large nest building

³ postnatal day

evolutionary conserved functions, (Saaiman et al. 2023; Hoffman and Rueda Morales 2009) we believed that LNB¹, compared to NNB², would represent an abnormal behavioural phenotype which is dissociated from improved breeding outcomes. This theory held true. Our findings support theories that naturalistic LNB in deer mice is an overly goalless behavioural outcome of dysfunctional psychobiological processes (Saaiman et al. 2023; Wolmarans et al. 2022; de Brouwer, Harvey, and Wolmarans 2020; de Brouwer et al. 2020). Since parental care was not investigated *per se*, continued investigation should determine whether the high mortality rate in LNB-bred offspring is related to genetic and biological impairment, or rather the outcome of poor parental care. Although the latter may be possible due to the focused and rigid engagement of LNB expressing mice in nesting behaviour shown before (Wolmarans et al. 2022), several traces of evidence point to LNB being founded upon dysfunctional neurobiological (Saaiman et al. 2023; Wolmarans et al. 2022), cognitive (de Brouwer, Harvey, and Wolmarans 2020) and inflammatory processes (Scheepers et al. 2019), all of which could have contributed to the present findings. In conclusion, variance in nesting behaviour should be considered when animal health and wellbeing are considered, since it may point to underlying biobehavioural perturbations.

Data availability statement

The datasets generated during the current study are available from the corresponding author.

¹ large nest building

² normal nest building

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4 Journal Article B

Adult offspring of deer mouse breeding pairs selected for normal and compulsive-like large nesting expression invariably show the same behaviour without prior in-breeding

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Keywords: deer mouse, nest building, compulsive, heritability

Abstract

Obsessive-compulsive disorder (OCD)¹ is a debilitating and potentially heritable neuropsychiatric condition. Indeed, twin studies show higher concordance rates for OCD in monozygotic, compared to dizygotic twins. Against this background, laboratory-housed deer mice of both sexes varyingly present with excessive and persistent large nesting behaviour (LNB)², which has been validated for its resemblance to clinical compulsivity. While LNB differs from normal nesting behaviour (NNB)³ on both a biological and cognitive level, it is unknown to what extent the expression of LNB and NNB is related to familial background. Here, we randomly selected 14 NNB and 14 LNB expressing mice (equally distributed between sexes) to constitute seven breeding pairs of each phenotype. Pairs were each allowed to breed two to three generations of offspring, which were raised until adulthood (12 weeks) and assessed for nesting expression. Remarkably, our findings show that offspring from LNB expressing pairs build significantly larger nests compared to offspring from NNB expressing pairs and that the nesting expression of the offspring of each breeding pair, irrespective of parental phenotype or litter (i.e., NNB and LNB), is family specific. Collectively, the results of this investigation indicate that LNB can be explored for its potential to shed light on heritable neurocognitive mechanisms that may underlie the expression of specific persistent behavioural phenotypes.

¹ obsessive compulsive disorder

² large nest building

³ normal nest building

4.1 Introduction

Obsessive compulsive disorder (OCD)¹ is a neuropsychiatric condition that shows a noteworthy degree of heritability (Mataix-Cols et al., 2013; Nestadt et al., 2000). With an overall lifetime prevalence of more than 1 % (Fawcett et al., 2020), it is estimated from twin studies that about 47 % of cases are predominantly related to a familial history due to genetic factors (Mataix-Cols et al., 2013). Also, higher concordance for OCD has been shown in monozygotic vs. dizygotic twins (Browne et al., 2014). The disorder is characterised by intrusive, disquieting, and often anxiogenic thoughts, i.e., obsessions, and repetitive acts or behaviours, i.e., compulsions, which are expressed as neutralising behaviours in response to obsessions (APA, 2013). Different thematic symptom phenotypes are described, e.g., obsessions about contamination, which are associated with excessive cleaning compulsions or safety obsessions associated with compulsive checking behaviour (Ahmari, 2016; Burguiere et al., 2015). Although research is congruent in pointing to cortico-striatal-thalamo-cortical (CSTC)² involvement (Bernstein et al., 2016; Saxena et al., 1998), conclusions of causality remain inconclusive (Sinopoli et al., 2017). For example, while patients present with hyperactivation of the CSTC circuitry (Beucke et al., 2013; Kang et al., 2013; Sakai et al., 2011), inhibiting these processes via serotonergic and anti-dopaminergic pharmacotherapy is only varyingly effective (Decloedt & Stein, 2010; Gershkovich et al., 2017; Kaplan & Hollander, 2003). Also, diverse psychobiological mechanisms underlie different cases of OCD. Indeed, neurocognitive evidence shows that compulsions can be born from among others, processes of excessive habit formation (Gillan et al., 2014), inadequate response inhibition (Mar et al., 2022), deficits in reward sensitivity (Huey et al., 2008), or a higher degree of uncertainty intolerance in some symptom dimensions (Tolin et al., 2003). It is further unknown how genetic polymorphisms known to be associated with OCD, may contribute to these unique psychobiological underpinnings. For this purpose, animal model systems, especially those with a naturalistic aetiology, are valuable experimental tools.

Deer mice (*Peromyscus maniculatus bairdii*) of both sexes that are bred and housed in confinement, varyingly present with persistent and repetitive high motor stereotypy (HS³; seen in 40 – 45 % of the population) (de Brouwer et al., 2021; Korff et al., 2008; Wolmarans et al., 2013), high marble burying (shown by 11 % of mice) (Wolmarans et al., 2016b) and large nest building (LNB)⁴ which is expressed by 30 % of deer mice (Wolmarans et al., 2016a). While a complete review of these phenotypes and their relevance for studies of compulsivity falls beyond the scope of the present work, it suffices to

¹ obsessive compulsive disorder

² cortico-striatal-thalamo-cortical

³ high stereotypy

⁴ large nest building

mention that except for high marble burying, both high stereotypy and LNB¹ are associated with CSTC² hyperactivation (Presti & Lewis, 2005; Saaiman et al., 2023), and varyingly respond to high-dose chronic exposure to the selective serotonin reuptake inhibitor, escitalopram (Wolmarans et al., 2013; Wolmarans et al., 2016a). Such a response is congruent with clinical findings (Kellner, 2022). Further, these behaviours are not mere motor disturbances, but rather related to dysfunctional neurocognitive processes. For example, high stereotypical (HS)³ mice show a unique response to pharmacological interventions targeting both cognitive and motor routines, compared to normal controls (de Brouwer et al., 2021; de Ridder et al., 2022). Since the present work focuses on LNB, it is important to highlight also that LNB, compared to normal mice, shows deficits in reward- and punishment processing (de Brouwer et al., 2020) and engages in high-risk behaviour to satisfy an underlying motivational drive to nest (Wolmarans et al., 2022). Nesting is a normal rodent behaviour aimed at protection against predators, reproduction, and thermoregulation (Deacon, 2006). However, since mice are housed and reared under identical laboratory conditions, it would be reasonable to expect that same-sex laboratory-reared and housed mice of the same species would build similar nests (Deacon, 2012).

Collectively, the findings related to LNB reviewed here, point to a useful degree of overlap between LNB and clinical compulsivity that could be exploited to expand our current understanding of the aetiological and pathophysiological processes contributing to the promulgation of compulsive-like behavioural persistence. However, it has not yet been established if LNB is solely founded upon an inherited or other litter-specific biological mechanism. In the absence of a clear answer to this question, continued investigations aimed at identifying and targeting specific underlying aetiological triggers, and in so doing, informing clinical research, remains difficult. Therefore, the present work aimed to explore this theme, by investigating whether normal nesting behaviour (NNB)⁴ and LNB are indeed related to familial background.

4.2 Methods

4.2.1 Study layout

Since only 30 % of deer mice present with LNB, 57 randomly selected adult mice (28 females and 29 males, aged at least 12 weeks at the onset of experimentation), which were bred from 11 progenitor pairs (also randomly paired and of which the behavioural background was entirely unknown), were initially assessed for nesting expression. Of these, 14 NNB and 14 LNB expressing mice (equally

¹ large nest building

² cortico-striatal-thalamo-cortical

³ high stereotypical

⁴ normal nesting behaviour

distributed between sexes) were selected for within-phenotype pairing after accounting for litter (i.e., mice from the same familial background were not paired together). NNB and LNB pairs were allowed to breed a total of two to three generations of offspring of which the collective data are reported in this work. After weaning, all offspring were raised in same-litter, same-sex cages until postnatal day (PND)¹ 84, at which time they were also screened for nesting expression (**Fig. 4-1**). Importantly, none of the offspring assessed at adulthood had any prior experience with the nesting material used for nesting analyses, nor did breeding pairs have access to such material while pups were nursed.

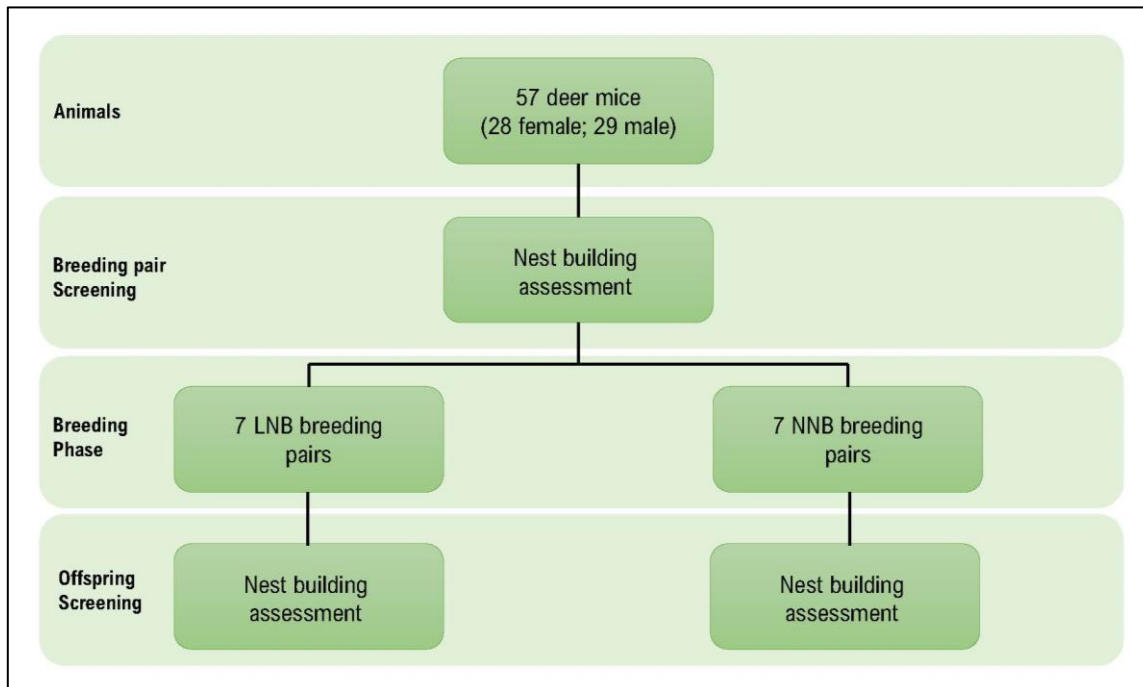


Figure 4-1 - Schematic representation of study layout
NNB: normal nest building | LNB: large nest building

4.2.2 Mice

All mice used in this investigation were bred, housed, and maintained in the Vivarium of the North-West University (South African Veterinary Council registration number: FR15/13158; AAALAC accreditation file:1717; AnimCare Research Ethics Committee approval number: **NWU-00770-22-A5**). Except if stated otherwise, mice were group-housed in standard laboratory cages [35 cm (l) x 20 cm (w) x 13 cm (h); Tecniplast® S.P.A., Varese, Italy] that were individually ventilated and maintained at a relative humidity of 60 % and a temperature of 23 °C. A standard 12-h light-dark cycle was always applied (lights on at 6:00 AM). Food and water were supplied *ad lib* throughout the investigation and mice were monitored daily for health status and to record births (where applicable). Except during periods of nesting

¹ postnatal day

assessment, cages were supplied with a piece of paper towel as a form of nesting material and a section of white polyvinyl chloride pipe [10 cm (l) x 4 cm (Ø)] for purposes of environmental enrichment. Cages were cleaned, and new corncob bedding and nesting material were supplied, weekly.

4.2.3 Nesting assessment

During the nesting assessment, irrespective of the phase of study, mice were single housed with no access to other forms of nesting material, except that which was provided for the purpose of the experiment. Nesting assessment was performed over seven consecutive days for each mouse. At noon of each assessment day, cages were supplied with an excess of pre-weighed, white, sterile cotton wool in the roof of the cage. At noon on the next day, built nests were removed from the cage and the remaining cotton wool, i.e., not used for nesting, was weighed. Thus, mice had access to nesting material for 23 to 24 h of each assessment day. Daily nesting scores were recorded, and a total nesting score calculated at the end of the seven-day period. Mice expressing LNB¹ were identified based on the method of (Wolmarans et al., 2016a), which accounts for both the magnitude of nesting scores and the daily variance in nesting behaviour. Thus, only mice that consistently built larger nests (scores clustering above the 75th percentile of the distribution), and that showed the least between-day variance as reflected by the coefficient of variance with respect to daily nesting scores, were included in the LNB cohort (**Fig. 4-2**; nesting data of the 57 adult deer mice initially screened to select the 14 breeding pairs; refer ‘**Study layout**’). Conversely, mice showing nesting scores that averaged below the 50th percentile, were chosen for inclusion in the NNB² group.

¹ large nest building

² normal nest building

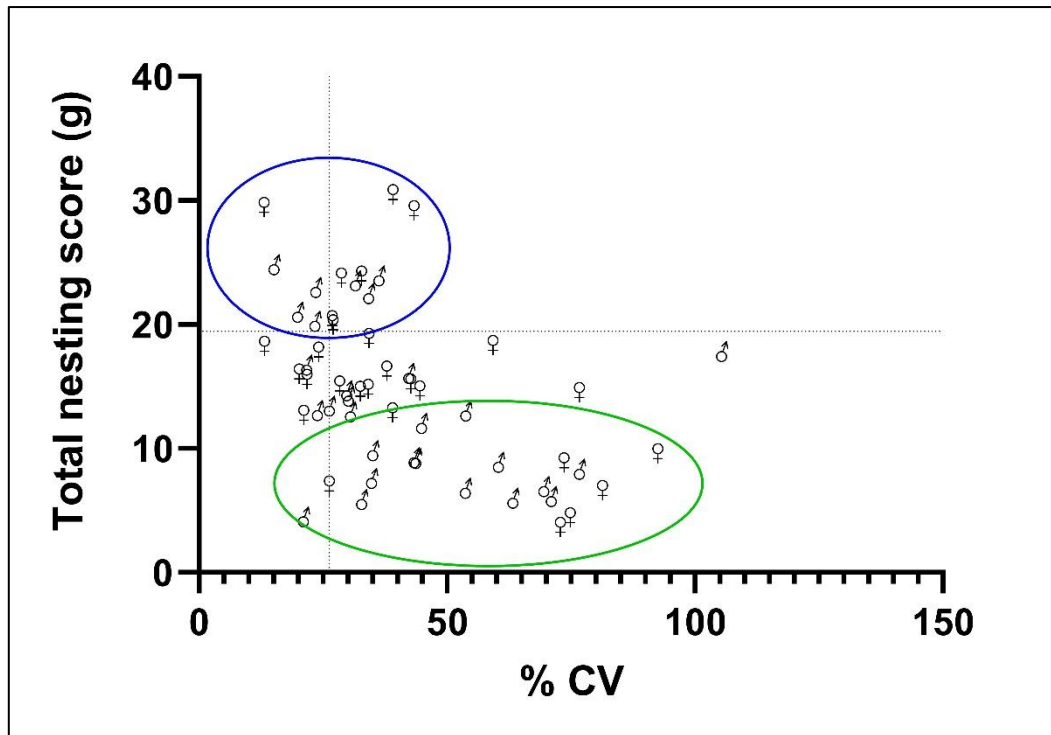


Figure 4-2 - Total nesting scores vs. % CV of each of the 57 deer mice initially screened for NNB and LNB cohort selection

4.2.4 Statistical analysis

All statistical analyses were performed, and graphs drawn using GraphPad® Prism® version 10. The relationship between total nesting scores and the coefficient of variance in terms of daily nesting scores was assessed with a Spearman's rank-order correlation. Per-litter nesting scores of the adult offspring of the 7 NNB¹ and LNB² breeding pairs were assessed for normality with Shapiro-Wilk tests. Since some data sets violated the assumption of normal distribution, a Welch's t-test was used to determine the overall difference in nesting scores of NNB and LNB offspring, while Kruskal-Wallis H tests were used to compare the nesting scores of the respective adult litters of the different breeding pairs. Statistical significance was set at $p < 0.05$ for all analyses.

4.3 Results

4.3.1 Selection of NNB and LNB expressing breeding pairs

A significant negative correlation was shown between the total nesting scores of the 57 mice and the respective coefficients of variance calculated with respect to the daily nesting scores generated by each mouse ($r_s = -0.46$ [-0.65, -0.22], $p = 0.0003$; **Fig. 4-2**). Thus, descriptive statistics (quartiles of data

¹ normal nest building

² large nest building

distribution for both the X- and Y-datasets) were used to broadly cluster NNB¹ and LNB² expressing mice together as explained earlier.

4.3.2 Nesting data of offspring

4.3.2.1 Litter data

A total of 93 deer mice offspring were assessed for nest building expression in this investigation. These included 52 offspring from NNB and 41 offspring from LNB breeding pairs. From the six NNB breeding pairs (one pair failed to breed), a total of 26 males and 26 females were assessed, whereas 26 males and 15 females born from the seven LNB pairs were assessed.

4.3.2.2 Correlations between nesting scores and daily variance

For both the offspring of NNB and LNB breeding pairs, significant negative correlations were shown between total nesting scores and respective coefficients of variance calculated for each mouse (**NNB offspring: 52; LNB offspring: 41; Fig. 4-3**). Subsequent analysis of the data distribution revealed the 75th percentile (for nesting scores) to be higher and the 25th percentile (for the coefficient of variance) to be lower for offspring from LNB, compared to NNB breeding pairs (**LNB**, 75th nesting score: 23.22 g, 25th CV: 34 %; **NNB**, 75th nesting score: 15.69 g, 25th CV: 44 %).

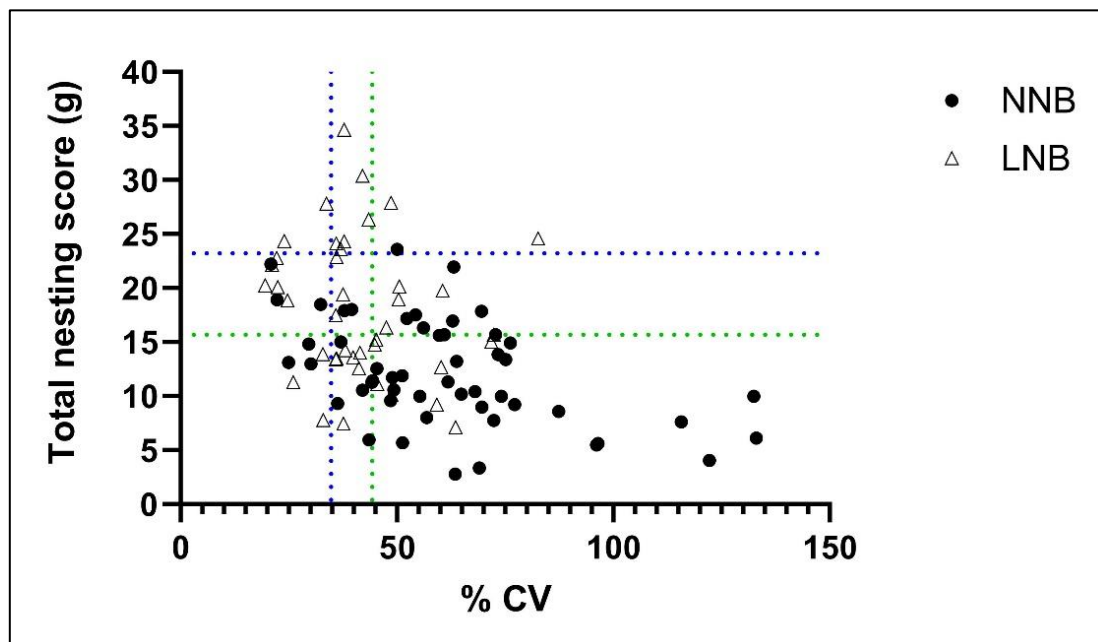


Figure 4-3 - Total nesting scores vs. % CV of each of all offspring

¹ normal nest building

² large nest building

4.3.2.3 Comparisons of the total nesting scores of all offspring of NNB and LNB expressing breeding pairs

For this comparison, the total nesting scores of all offspring from the NNB¹ ($n = 52$) and LNB² ($n = 41$) expressing breeding pairs were compared with a Welch's t -test (**Figure 4-4**). Offspring from NNB expressing pairs generated a mean total nesting score of 12.2 ± 4.9 g, and offspring from LNB expressing pairs a mean total score of 18.0 ± 6.6 g over one week of assessment. This difference was both significant ($t[72.1] = 4.7$, $p < 0.0001$ [3.36, 8.29]) and of a great magnitude ($d = 1.0$ [0.58, 1.45]).

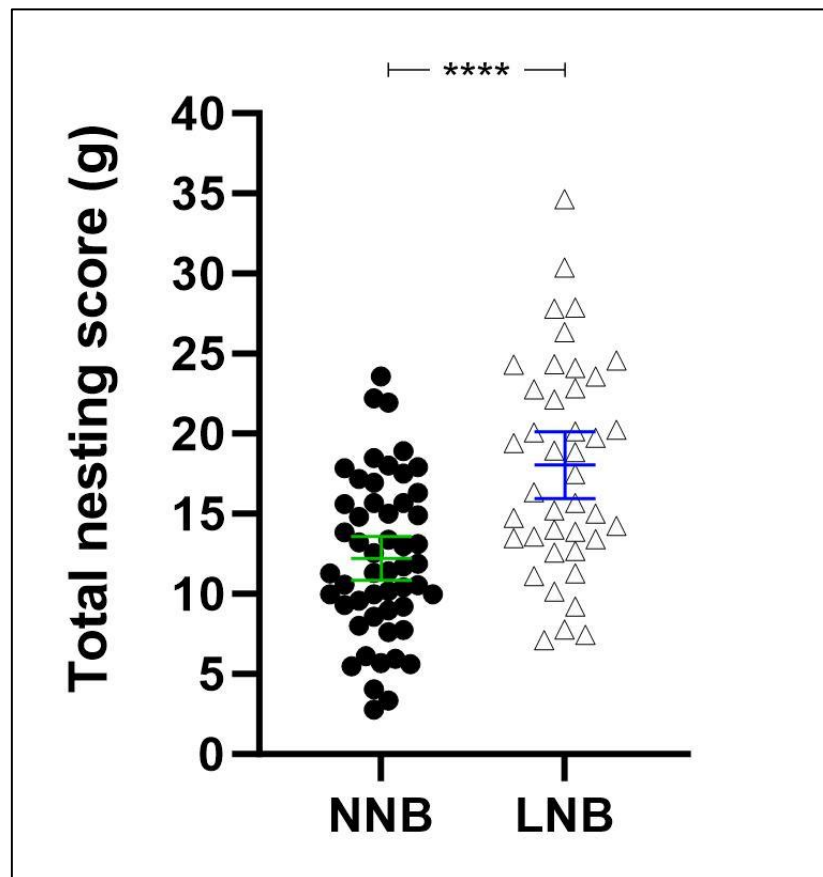


Figure 4-4 - Total nesting scores of all offspring from NNB and LNB breeding pairs

The collective nesting scores of all litters from the six NNB and seven LNB expressing pairs respectively, are shown in **Fig. 4-5A & B**. Descriptive data are provided in **Table 4-1**. Kruskal-Wallis H tests revealed the median nesting scores of offspring from the different NNB and LNB breeding pairs to vary significantly within each cohort (**NNB**: $H[5] = 11.7$, $p = 0.04$; **LNB**: $H[6] = 16.7$, $p = 0.01$). Further, only five offspring from NNB expressing breeding pairs generated total nesting scores higher than the median nesting score of offspring from LNB pairs (represented with a blue line in **Fig. 4-5A**), while only seven

¹ normal nest building

² large nest building

offspring from LNB¹ expressing pairs generated nesting scores that were lower than the median score generated by offspring from NNB² pairs (represented by the green line in **Fig. 4-5B**).

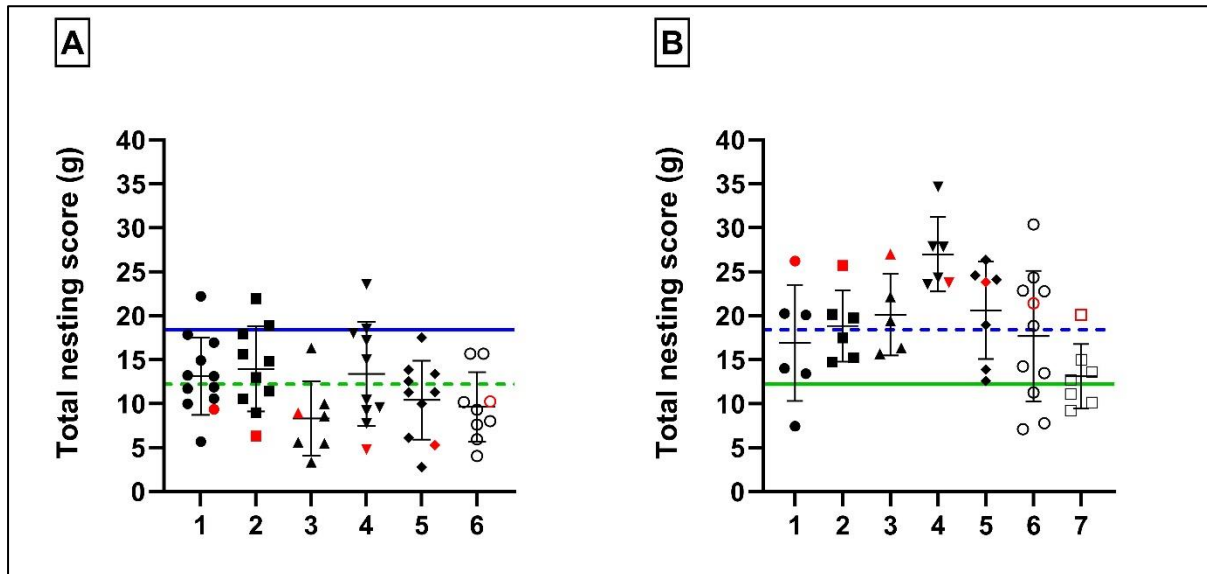


Figure 4-5 – Nesting scores of offspring from the respective breeding pairs, irrespective of litter

* * *

Table 4-1 – Descriptive statistics pertaining to offspring nesting scores

	NNB	LNB
<i>n</i>	52	41
Minimum (g)	2.780	7.100
25 th percentile (g)	9.043	13.45
Median (g)	11.56	17.49
75 th percentile (g)	15.69	23.22
Maximum (g)	23.57	34.65
Range (g)	20.79	27.55
Mean (g)	12.21	18.04
Std. Deviation (g)	4.932	6.590
Std. Error of mean	0.6840	1.029

4.4 Discussion

In this observational experiment, we explored the relationship between nesting expression and familial background in adult offspring from NNB and LNB expressing breeding pairs. The main findings of this work are that 1) offspring from LNB expressing pairs build significantly larger nests compared to

¹ large nest building

² normal nest building

offspring from NNB¹ expressing pairs and 2) the expression of nesting behaviour by the offspring of each breeding pair, irrespective of parental phenotype or litter (i.e., NNB and LNB²), is family specific.

In the introduction to this paper, we alluded to the fact that nesting is a typical, evolutionary conserved rodent behaviour aimed at specific outcomes, including but not limited to ensuring protection against predation and adverse environmental influences, and providing a suitable nursery in which to breed and care for offspring (Deacon, 2006). However, since laboratory-housed mice are maintained under similar circumstances, it remains unclear why nesting behaviour in a specific species and population bidirectionally separate into 'normal' and large nesting phenotypes. We have previously investigated and validated spontaneous LNB in deer mice as a preclinical model system of relevance for studying the aetiology and pathophysiology of compulsive-like behavioural expression (Wolmarans et al., 2016a). Given the high degree of heritability in OCD³, an important question that remains unanswered in this model system, is whether NNB and LNB are related to familial background. In other similar work, bidirectional separation of mice into 'small' and 'big' nesting groups is also performed, albeit in an inbred model (Mitra & Bult-Ito, 2021). To ensure that deer mice remain a naturalistic research framework suitable for studying the spontaneous promulgation of repetitive behaviours, mice are not inbred and as such, questions of heritability have not yet been answered.

The present data are striking in showing that the adult offspring from randomly selected NNB and LNB breeding pairs differ in their average expression of nesting behaviour, with offspring from NNB pairs utilising significantly less cotton wool than the offspring of LNB mice (**Fig. 4-4**). Importantly, while both cohorts of offspring can be separated into mice that build smaller and larger nests (**Fig. 4-3**), nesting scores clustering above the 75th percentile of the distribution, are substantially higher, compared to the same cluster of scores generated by NNB offspring. We considered that this difference could be related to experiential learning, as has been shown for mice before (Mainardi et al., 1986). However, neither the breeding pairs, nor the offspring had any exposure to cotton wool during any of the breeding, nursing, or rearing windows. As such, the skill of nesting could not be transferred from one mouse to another via experiential learning. Also, while parental behaviour can significantly impact the social, anxiety-like, and aggressive behaviour of offspring (Braun & Champagne, 2014), and considering that nesting behaviour can be modified by changes in these domains (Gaskill et al., 2013; Jirkof, 2014), such influences are normally associated with paternal deprivation (Braun & Champagne, 2014), which was not applied in the present work. Rather, we propose the present result to be directly related to distinct

¹ normal nest building

² large nest building

³ obsessive compulsive disorder

heritable psychobiological mechanisms that regulate the execution of goal-directed motor behaviour in NNB¹ and LNB² mice, with maladaptive processes prevailing in LNB offspring. Putative support for this suggestion is twofold. First, earlier work in our laboratory showed LNB expressing mice to present with a lower degree of striatal adenosine A_{2A} receptor activation, which is believed to contribute to excessive striato-cortical signalling (Saaiman et al., 2023) by promoting the actions of dopamine on the dopamine D₂ receptor (Fuxe et al., 2010). Given that heritable differences in the expression of adenosine receptor variants are well described in clinical cohorts (Alsene et al., 2003; Byrne et al., 2012), it is likely that said difference in A_{2A} receptor activity in LNB mice, may be related to a genetic influence.

The second trace of evidence to support a conclusion that LNB is heritable comes from our second main finding. We demonstrate here that, whether from the first or second litter, offspring from specific breeding pairs build remarkably similar sized nests (**Figure 4-5A & B**). This finding is paramount, because for the first time in a wildtype model, we show that behavioural phenotypes are transferred with striking concordance from one generation to the next. The translational meaning of this finding is further emphasised, considering that nesting assessment is performed over one week; thus, it would be reasonable to expect a greater degree of variation in the nesting scores of offspring. Interestingly, the average historic nesting score of each breeding pair (**Figure 4-5**; indicated in red colour) did not predict the magnitude of nesting in offspring, but rather the overall cohort to which they would belong. It is prudent to reiterate that none of the mice used in this investigation were inbred. It is therefore concluded that the nesting expression of offspring can be traced back to innate neurobiological differences between deer mice of the two cohorts that are sufficiently stable to influence behavioural outcomes in successive generations. This is valuable since the deer mouse model can now be applied as a truly naturalistic murine model to study the nature of genetic and other family-specific biological contributions to the spontaneous manifestation of compulsive-like behaviour.

While the present results are informative, some questions for future consideration remain. First, while the impact of familial factors in nesting expression is clearly highlighted in the present work, we did not explore genetic influences per se. This would be an important focus for future consideration, especially since heritability is not only related to genetic involvement. Indeed, litter-specific differences in gut microbial composition and immune functioning that are postnatally induced, could also influence behavioural expression (Robertson et al., 2019; Zhang et al., 2015). In this regard LNB deer mice

¹ normal nest building

² large nest building

present with a higher loading of potentially pro-inflammatory taxa (Scheepers et al., 2020), e.g., *Proteobacteria* (Rizzatti et al., 2017), compared to NNB¹ mice.

4.5 Conclusion

In the present work, we investigated whether familial background influences the expression of NNB and LNB² behaviour in deer mice. Our data revealed that offspring from LNB expressing pairs build significantly larger nests than offspring from NNB pairs. We also showed that offspring from specific breeding pairs build remarkably similar sized nests, irrespective of the litter they came from. Since mice could not have expressed the present behaviour as an outcome of experiential learning, we conclude that NNB and LNB are two distinct behavioural phenotypes founded within heritable mechanisms. While the deer mouse model is a valuable model system to explore the psychobiological triggers that give rise to spontaneous compulsive-like behavioural persistence, future investigations should explore the nature of the familial influences shown here.

¹ normal nest building

² large nest building

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5 Conclusion

The present work aimed to elucidate our understanding of the large nesting behaviour (LNB)¹ in deer mice (*Peromyscus maniculatus bairdii*) by focusing on two ideas, i.e., i) that LNB does not have an evolutionary meaningful purpose (which is better breeding and offspring survivability), and ii) that such behaviour is founded upon biologically heritable mechanisms. We took this approach since LNB is contextualised in our laboratory as a pre-clinical model of compulsive-like behavioural persistence (Wolmarans et al., 2022), yet the functional significance and biological underpinnings of this behavioural phenotype are not yet known.

Taking one step back, obsessive-compulsive disorder (OCD)² is founded upon cortico-striatal perturbation with hyperactivation of these brain areas commonly shown in OCD (Bernstein et al., 2016, Parmar and Sarkar, 2016). Since cortico-striatal networks play a vital role in among others, goal-directed behaviour (Vaghi et al., 2017), OCD is associated with dysregulation in this cognitive domain (Vaghi et al., 2017, Gillan and Robbins, 2014). Further, while no specific gene is consistently implicated in the pathophysiology of OCD (Taylor, 2013), heritability has been investigated in probands of individuals with OCD. In this regard, research shows that the risk for OCD diagnoses decreases from first to third-degree relatives (Mataix-Cols et al., 2013), while twin studies are undoubtedly showing that the disorder is more prevalent in monozygotic twins compared to dizygotic twins (Inouye, 1965, Hettema et al., 2001, Smoller et al., 2009). In terms of deer mice, heritable influences have also been shown, albeit with respect to stereotypical behaviour, not LNB (Davis et al., 2023).

With respect to nesting behaviour, LNB (like clinical compulsivity) is excessive and persistent (Wolmarans et al., 2016). Also akin to the clinical treatment response of OCD, LNB attenuates following chronic, high-dose escitalopram exposure (Wolmarans et al., 2022). However, drawing conclusions of translational relevance for compulsivity can be difficult. Indeed, since the evolutionary purpose of nesting is to ensure breeding and nursing success (Deacon, 2006), it was unclear to us if LNB, as opposed to normal nesting behaviour (NNB)³, may represent a superior behaviour aimed at improving breeding outcomes.

* * *

¹ large nest building

² obsessive compulsive disorder

³ normal nest building

Conclusion

Fifty-seven deer mice from both sexes (28 female; 29 male) were bred and reared from eleven randomly selected breeding pairs at the vivarium of the North-West University (NWU)¹, Potchefstroom, South Africa. All mice underwent nesting screening at the age of 12 – 20 weeks. From these, 14 LNB² expressing mice and 14 NNB³ expressing mice (equally distributed between sexes) were selected and paired, considering that littermates were not paired together. These seven NNB and LNB breeding pairs were left to breed at least two generations of offspring (for **Chapter 3**), and a third in the case of LNB breeding pairs (to add to the adult offspring screened in **Chapter 4** of this work). Nesting expression was then assessed in all adult offspring of the seven NNB and LNB breeding pairs and the data represented in **Chapter 4**.

* * *

Our first main finding showed that despite being as fertile as NNB mice, offspring born from most LNB expressing pairs are notably less viable. Considering that nesting serves several evolutionary conserved functions (Saaiman et al., 2023, Hoffman and Rueda Morales, 2009), this finding was striking in showing that naturalistic LNB in deer mice is goalless and likely born from dysfunctional psychobiological processes.

Such a conclusion was supported by our second main finding which showed that offspring from LNB expressing pairs built significantly larger nests than offspring from NNB pairs and that offspring from specific breeding pairs built similar sized nests, irrespective of the litter they came from. Since mice could not have expressed the present behaviour as an outcome of experiential learning, we can conclude with a relative degree of confidence that NNB and LNB are two distinct behavioural phenotypes founded upon heritable mechanisms. While the deer mouse model is a valuable model system to explore the psychobiological triggers that give rise to spontaneous compulsive-like behavioural persistence, future investigations should explore the nature of the familial influences shown here.

¹ North-West University

² large nest building

³ normal nest building

5.1 Final summary of study outcomes

Table 5-1 – Summary of answers to the study questions asked

Study Question	Actual outcome
i. Is the expression of NNB and LNB differentially associated with breeding and nursing success as measured against litter survival through postnatal day (PND) 21?	 While the offspring survivability of NNB and LNB mice were indeed strikingly different, offspring of LNB expressing mice fared worse, compared to those of NNB expressing mice. As such, we can conclude that as far as the evolutionary function of nesting is concerned, LNB is not a superior phenotype, but rather a maladaptive behaviour founded upon perturbed psychobiological underpinnings.
ii. Do the respective offspring of breeding pairs that were prior selected for NNB or LNB expression, also express NNB and LNB behaviour in adulthood?	 We show here that offspring from each of the behavioural phenotypes, express nest building behaviour similarly to the respective maternal and paternal progenitors. In this sense, the nesting phenotype is carried over from one generation to the next, paving the way for continued investigation into the potential neurobiological underpinnings of NNB and LNB as frameworks to study normal and compulsive-like behavioural expression.
iii. To what extent is the nesting expression of littermates at adulthood, similar?	 Remarkably, such biological heritability, referred to in (ii) above, yielded highly consistent behaviours in the offspring of the respective phenotypes. The behavioural outcomes of such heritable mechanisms are therefore highly regulated and since mice could not learn from one another during their development and rearing, this finding is very informative for continued study. In fact, the expression of LNB may be entirely related to a biological mechanism which underlies the expression of excessive goal-directed motor output.

NNB: normal nest building | LNB: large nest building

5.2 Study shortcomings and future study directions

The present investigation was not without some shortcomings that should be noted. For example, in terms of **Chapter 3**, we did not monitor the parental nursing of offspring, while breeding pairs were also not supplied with cotton wool during nursing periods. As such, it remains difficult to confirm whether the decreased LNB¹ litter survival rate was due to poorer care per se. In future investigations, it will be valuable to provide cotton wool during stages of breeding and nursing to elucidate this answer. The shortcomings of the second investigation (**Chapter 4**) were that we did not have a group where NNB² and LNB offspring were housed together until adulthood. Although highly unlikely, the possibility of learning having an impact on nesting expression should be considered and investigated in future studies. Additionally, we also did not investigate specific genes in these mice. In future investigations, the role of serotonin transporter (SERT)³ gene polymorphisms, for example, may be a valuable starting point since SERT expression differences have previously been implicated in another compulsive-like behaviour shown by these mice, i.e., spontaneous stereotypy (Wolmarans et al., 2013).

END

¹ large nest building

² normal nest building

³ serotonin transporter

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Addendum A

In this addendum, additional information regarding animal husbandry is provided.

Supplementary information pertaining to deer mice husbandry, housing, and care

- Deer mice used in the present investigation were bred, reared, and housed at the vivarium of the North-West University, Potchefstroom, South Africa (SAVC registration number: FR15/13458; AAALAC accreditation file: 1717; SANAS GLP compliance number: G0019).
- Housing cages for mice were as follows: 35 cm (l) x 20 cm (w) x 13 cm (h); Tecniplast® S.P.A., Varese, Italy.
- During weekly cage changes, mice were placed in cleaned autoclaved cages with new corncob bedding and paper towels as nesting material.
- Food provided to mice (mouse pellets; Labchef; Nutritionhub®, Reg nr: 2012/141960/07) were autoclaved for 15 minutes at 90 °C.
- During daily checks, available water and food were checked and added where necessary.
- The temperature, humidity, and ventilation of cages were monitored by Tecniplast® smart flow.

Prior to study-onset (initial breeding of breeding pairs for the present study)

- New births were recorded every day.
- Mice were weaned on postnatal day (PND)¹ 21, unless too small, in which case weaning took place one to two days later. After weaning, mice were housed in groups of two to seven same-sex, mixed-litter cages until the onset of the present investigation. Mice in the same cage did not differ with more than eight days in age.
- Additionally, extra corncob was added to cages halfway through every week.

After study-onset

- All breeding pair and offspring cages had access to environmental enrichment, i.e., a piece of white polyvinyl chloride pipe [10 cm (l) x 4 cm (Ø)], except during the nest building assessment period.
- Births and deaths were monitored and recorded daily.
- Offspring from NNB and LNB breeding pairs were weaned at PND 21, except in large litters where offspring were too small, at which they were then weaned one to two days later, and offspring were housed in **same-litter** same-sex cages of two to five mice per cage.
- Mice were single housed two hours before the onset of nest building assessment without any paper towel in the cage as nesting material. During the nesting assessment, cotton wool was placed in the roof of the cage that mice had to pull through the grid to nest.

¹ postnatal day

Supplementary images of general husbandry, housing, and care during the investigation

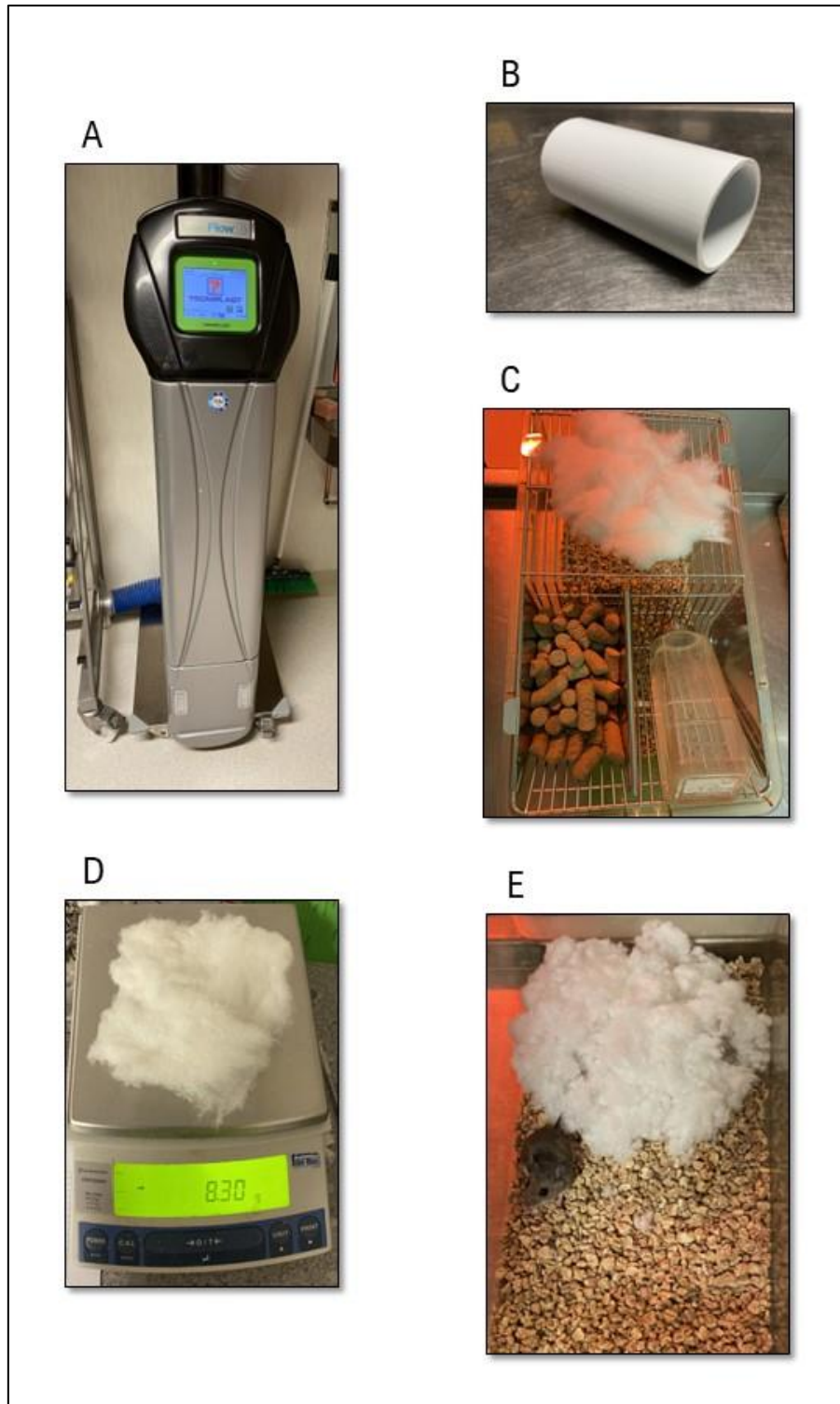


Figure A-1 – (A): Techniplast® smart flow for temperature, humidity, and ventilation regulation; (B): white polyvinylchloride pipe as environmental enrichment; (C): Nest building assessment cage set-up; (D): Pre-weighing of cotton wool to be placed in the roof of the cage for nesting assessment; (E): Typical example of a large nest builder nest.