



The impact of trait anxiety and visual perception on nesting expression in deer mice

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This dissertation is dedicated to our heavenly Father, who bestowed His grace and knowledge upon us
to decipher the riddles of His great creation.

* * *

Rather be the light than the darkness that encloses it.

* * *

Oupa Harry Marx (10 April 1942 – 31 Augustus 2022)

Ouma Nita Marx (14 August 1943 – 30 April 2023)

Liefste Oupa en Ouma,

Vir die oneindigende liefde, omgee en geduld.

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Die Marx-Stooges

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Congress proceedings

The results from this investigation will be presented at the annual meeting of the Society for Neuroscience which will be hosted in Washington D.C., USA, 11 – 15 November 2023. The full reference for the poster is:

Marx H., Krahe T., Wolmarans D. *“Large nesting behavior in deer mice is an inflexible habit that persists despite absolute visual deprivation: perspectives on compulsivity”*, November 2023, Walter E. Washington Convention Centre, Washington D.C.

Abstract

Rigid and repetitive behavioural phenotypes are characteristic of obsessive-compulsive disorder (OCD)¹, which is characterized by obsessions, compulsions, or both. Compulsions are mostly expressed *in response to* an underlying, often anxiety-provoking obsession about specific themes, e.g., safety, or potential harm. Specific symptom themes, e.g., safety-seeking and symmetry and ordering compulsions, are more sensitive to visual environmental feedback that potentially promotes limbic anxiety-related responses.

The neurobiological foundation of OCD shows perturbation across several domains of cortico-striatal function, i.e., limbic processing, sensorimotor control, and habit formation. Furthermore, OCD is commonly associated with serotonergic and dopaminergic dysfunction, but here a third, lesser investigated neurotransmitter in OCD literature, i.e., noradrenaline will be afforded attention. Noradrenaline is a crucial modulator of fronto-limbic fear and anxiety processing. While OCD is not explicitly regarded as a condition of fear, patients experience noteworthy anxiety about their obsessive-compulsive symptoms. Further, increased anxiety sensitivity and perpetual limbic system activation could drive dysfunctional executive task engagement, behavioural inflexibility, and a default to automatic and habitual behaviours. Even though limbic involvement in OCD is irrefutable, the aetiological direction of such involvement is unclear and therefore, an improved understanding of how limbic and executive processes interact to promulgate OCD is necessary.

Approximately 30 % of deer mice (*Peromyscus maniculatus bairdii*) spontaneously display excessively large nesting behaviour (LNB)², which has previously been validated in terms of its representation of naturalistic compulsive-like behavioural expression. LNB serves no unique purpose within a controlled laboratory environment. In this sense, it is without function, realistic goal, and futile. While the evolutionary functions of nesting are well described, the executive triggers and modulators of nesting behaviour, notably so in deer mice, remain uncertain. In other words, it is unknown if LNB resembles a mere habit, a form of behavioural inflexibility, or if it is born from anxiety-related perturbation. Thus, and considering the diverse range of psychobiological underpinnings that may drive obsessive-compulsive symptoms, this investigation aimed to divulge the neurocognitive underpinnings of LNB as it is compared with a normal (NNB)³ behavioural control.

* * *

Briefly, we screened an initial pool of 100 deer mice of both sexes for nesting expression over one week (ethics approval number: **NWU-00769-22-A5**). This constituted the first phase (P1) nesting assessment.

¹ obsessive-compulsive disorder

² large nest building

³ normal nest building

Abstract

From these mice, 24 NNB¹- and 24 LNB²-expressing mice were identified and used in further study. Mice of each cohort were again divided into either a standard 12-h light-dark (SL)³ or visually deprived (visual deprivation; VD)⁴ group and reassessed for nesting expression during a second phase (P2). Specifically, approximately half of the mice in each cohort were maintained under a SL cycle, with the other half under conditions of utter VD applied during the dark cycle. After completion of P2, all mice were euthanised, the frontal-cortical and hippocampal tissues removed, and analysed for serotonin and noradrenaline concentration.

From these experiments, our main findings were that 1) in contrast to NNB, LNB represents an inflexible and excessive behavioural phenotype that is not dependent on visually guided action-outcome processing, and 2) despite fronto-limbic noradrenaline concentrations not being influenced by VD, LNB associates with increased frontal-cortical and hippocampal noradrenaline, a neurobiological signature which may contribute to its expression.

* * *

The first main finding of this investigation points to separation between the neurocognitive underpinnings of NNB and LNB, whereby NNB, but not LNB, inflates over time. Such a response cannot be related to overt limbic activation (see below) and thus, we propose NNB behaviour to be founded on evolutionary conserved mechanisms that enable nesting, but that is in the same sense, subject to improvement based on prior experience. In contrast, LNB is excessive, rigid, and representative of maximal habit-like behavioural activation that is not subject to temporal or experiential influences. Further, while mice of both cohorts were sensitive to VD so that nesting sizes varied less between different days, the consistency with which nest construction was executed relied less on visual feedback in LNB, than in NNB mice. These results are in line with theories pointing to a role of behavioural rigidity and habitual engagement in compulsive symptom expression, both of which are linked with cortico-striatal hyperactivation.

The second main finding of this study was particularly striking and potentially indicates a novel noradrenergic mechanism which may underlie compulsive-like behavioural expression. To explain, LNB expressing mice showed increased fronto-limbic noradrenaline concentrations that, although not traditionally associated with OCD⁵, may play a profound role in promoting limbic activation and compromising frontal-cortical regulation of executive goal-directed decision making. These findings contribute in a meaningful way to our understanding of LNB and how this naturalistic phenotype could

¹ normal nest building

² large nest building

³ standard 12-h light-dark

⁴ visual deprivation

⁵ obsessive-compulsive disorder

Abstract

be positioned to elaborate our understanding of how fronto-limbic mechanisms may underlie compulsive symptom promulgation.

Keywords: deer mouse; obsessive-compulsive; nest building; visual deprivation; limbic activation; noradrenaline

Solemn declaration: I, Harry Marx , herewith declare that this dissertation is my own work and that no part thereof has been copied from any other sources.

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1 Introduction

1.1 Dissertation approach and layout

This dissertation has been prepared in article format, as specified by the North-West University (NWU)¹, South Africa. The main experimental body of research will be presented in the form of a journal article, i.e., **Chapter 3**. As such, **Chapter 3** will be submitted for publication in an internationally accredited, peer-reviewed journal, i.e., '*Behavioural Brain Research*'.

The complete dissertation will comprise four chapters. **Chapter 1** provides an overall summary of the investigation, describing the problem, working hypothesis, study aims and objectives, experimental layout, and ethical considerations. In **Chapter 2**, a detailed but focused literature review lays the theoretical foundations for the body of research presented here. Last, **Chapter 4** contains a broad summary of the complete dissertation against the background of the translational problem investigated, the potential study shortcomings that were identified, and how these may be addressed in future.

One addendum (**Addendum A**) is included that contains supplementary background to the methods followed in this investigation **Chapter 3**. These details were regarded as excessive for inclusion in **Chapter 3**.

The dissertation is present in UK English, while the referencing style used (as per Endnote®, Clarivate™), is Author-Date.

¹ North-West University

1.2 Problem statement

Rigid and repetitive behavioural phenotypes are characteristic of obsessive-compulsive disorder (OCD)¹, which presents with obsessions (intrusive thoughts or ideas), overt or covert compulsions (inflexible, and repetitive behaviours), or both (APA 2013). An important point of departure that also contextualises the current research is that compulsions are mostly expressed *in response to* an underlying, often anxiety-provoking obsession about specific themes, e.g., safety, potential harm to oneself or others, or fears of eternal damnation (Abramovitch and Cooperman 2015). However, the anxiety relief is usually short-lived which promotes continuous engagement in compulsive routines; the condition is thus regarded as self-reinforcing (Abramovitch et al. 2019). It follows that some symptom themes, e.g., safety-seeking, symmetry and ordering compulsions (which may involve perfectionistic ideals), and contamination/washing, may potentially be more sensitive to visual environmental feedback that promotes limbic anxiety-related responses (Egan et al. 2011, Abramovitch and Cooperman 2015, Hood and Antony 2016), compared to others, e.g., implicitly generated repugnant thoughts. Such differential association between visual cues and obsessive-compulsive symptom promulgation is clearly observed in psychotherapeutic treatment outcomes. To explain, patients subjected to exposure and response prevention (ERP)², are—mostly visually—exposed to the thematic content of their obsessions, e.g., contaminants, while being prevented from engaging in compulsive neutralising rituals (Hezel and Simpson 2019). In this way, they come to realise over time that their prior ‘reactive’ compulsive responses to such cues, are futile and meaningless. Therefore, in instances where exposure is more problematic due to the obsessive theme being implicitly generated, ERP have less optimal outcomes (Starcevic and Brakoulias 2008, Schneider et al. 2020). An incomplete understanding of how intrinsic psychobiological processes and extrinsic factors interact to contribute to OCD, is one of the key obstacles in the path of improved treatment outcomes.

The obsessive-compulsive brain shows perturbation across several domains of cortico-striatal function (Calzà et al. 2019, Robbins et al. 2019), i.e., limbic processing (Wood and Ahmari 2015, Sha et al. 2020, Brooks and Stein 2022), sensorimotor control (Xu et al. 2021, Park et al. 2022), habit formation (Hadjas et al. 2019, Simmler and Ozawa 2019), and reward-feedback valuation (Wood and Ahmari 2015, Calzà et al. 2019). Further, these constructs of dysfunction are not similar between patients, with different obsessive-compulsive phenotypes showing unique psychobiological fingerprints (Shephard et al. 2021). As such, patients showing increased habit-proneness, are not necessarily showing inflated anxiety, while those with a lower anxiety-threshold, may not be as prone to develop habits (Fineberg et al. 2018). To use this example, compulsions may be born from habit formation (i.e., striatal dysfunction) in the former,

¹ obsessive-compulsive disorder

² exposure and response prevention

while irrational anxiety related to certain themes (which is modulated by the limbic system), may be the major symptom driver in the latter.

On a neurobiological level, OCD¹ is commonly associated with serotonergic (Robbins et al. 2019) and dopaminergic (Wong et al. 2015) perturbation. Extensive earlier study divulged the role of these two neurotransmitters in OCD and other related conditions associated with deficits in behavioural control (see Sgambato-Faure and Tremblay (2018) for a review) and as such will not be further focussed on here. Another lesser investigated neurotransmitter within the context of OCD is noradrenaline, which—although not directly implicated in the pathogenesis of full-blown OCD—is a pivotal role player in fear- and anxiety processing (Charney 2003, Katayama et al. 2010, Nikolaus et al. 2010, Sakolsky et al. 2012, Terbeck 2016, Mäki-Marttunen et al. 2020, Fineberg and Robbins 2021, Lu et al. 2021, Bierwirth and Stockhorst 2022). Under states of anxiety or when experiencing fear, noradrenergic signalling in the limbic regions exerts control over and inhibits prefrontal-cortical executive processing; this facilitates a state of hypervigilance and fight-or-flight readiness, while silencing irrelevant neurocognitive programs aimed at goal-directed task execution (Szabadi 2013, McBurney-Lin et al. 2019). However, in patients showing increased anxiety sensitivity, perpetual activation of the limbic system may contribute to dysfunctional executive task engagement, behavioural inflexibility, and defaulting to automatic and habitual behaviours (Mäki-Marttunen et al. 2019, McBurney-Lin et al. 2019, Mäki-Marttunen et al. 2020). In this sense, while direct noradrenergic manipulation might not be successful on its own to treat OCD, it may assist in restoring proper control over executive functions in the cortico-striatal circuitry. It would be important to highlight an obvious conundrum here. Even though limbic involvement in OCD is irrefutable, the direction of such involvement is unclear. In other words, while increased anxiety sensitivity can contribute to behavioural inflexibility, the converse may also be true, where patients suffering from compulsions, reciprocally generate feelings of anxiety, which in turn will further contribute compulsive behavioural inflexibility. Since these psychobiological uncertainties may influence both treatment choice and therapeutic outcomes, an improved understanding of how limbic and executive processes may interact in the promulgation of OCD, is necessary. Towards this end, investigations in naturalistic animal models can be useful.

When bred and reared under standard laboratory conditions, approximately 30 % of deer mice (*Peromyscus maniculatus bairdii*) spontaneously display excessively large nest building behaviour (LNB)². Since laboratory-housed deer mice are housed under identical circumstances in climate controlled cages, such behaviour is perplexing, since it serves no functional purpose compared to

¹ obsessive-compulsive disorder

² large nest building

smaller ('normal'; NNB)¹ nests (Smithers 1983, Jirkof 2014, Wolmarans et al. 2016, Lewarch and Hoekstra 2018). Given it's the highly persistent and repetitive nature of this clearly goal-directed (though not aimed at achieving a *functional* goal) behaviour, we have previously described LNB² as a compulsive-like behavioural phenotype (Wolmarans et al. 2022). Shown by mice of both sexes, LNB shows moderate response to the same pharmacotherapeutic interventions used in clinical OCD³, i.e., high-dose treatment with the selective serotonin reuptake inhibitor (SSRI)⁴, escitalopram (Wolmarans et al. 2022), in this sense aligning the model with current hyposerotonergic theories of the condition (Lissemore et al. 2018, Sinopoli et al. 2020). Compared to NNB, LNB is also associated with cortico-striatal perturbation, specifically increased striatal activation (Saaiman et al. 2023). With reference to the focus of the present investigation, LNB mice do not show overly anxious behaviours in standard open-field assessments of rodent anxiety (Wolmarans et al. 2022, Saaiman et al. 2023). That said, considering the highly thematic nature of anxiety in OCD, it is possible that any potential involvement of heightened limbic activation might be overseen by standard anxiety tests.

Nevertheless, despite the body of research framing LNB against a background of compulsivity, the motivational triggers that drive LNB are yet unknown. Specifically, it is unclear to what end deer mice depend on visual feedback to recruit the necessary executive starting and stopping mechanisms that regulate this behaviour (i.e., is LNB related to visually perceived external triggers of compulsive-like behavioural engagement?). Also, considering the diverse range of psychobiological underpinnings that may drive obsessive-compulsive symptoms, it is unknown if LNB is related to 'just-right' feedback, as is the case for some rabbit species (Hoffman and Rueda Morales 2009), or whether it is merely a persistent habit-like behaviour which remains inflexible irrespective of visual feedback. Alternatively, LNB may be associated with a distinct degree of limbic involvement which is either extrinsically or implicitly modulated. As such, to expand on our understanding of the model system and so contribute to its application as a framework in which to study the aetiology and pathophysiology of compulsive-like behaviour, these research themes will form the core focus of the present work.

1.3 Research hypothesis

Briefly, we hypothesise that LNB, an abnormal phenotype shown by some mice in the laboratory-housed deer mouse population, will be representative of neurocognitive perturbations that give rise to excessive and inflexible, though 'goal-directed', nesting behaviour, inflated limbic processing, or a combination of both. Specifically, we posit that LNB will be dependent on visual feedback, either in respect of its

¹ normal nest building

² large nest building

³ obsessive-compulsive disorder

⁴ selective serotonin reuptake inhibitor

dependence on visual cues for optimal task execution (thus distinguishing LNB¹ as a goal-directed response from habit-like behavioural engagement), or in terms of LNB expressing mice being more sensitive to visually perceived and potentially anxiogenic environmental cues. Further to the latter, we hypothesise that LNB expressing mice, assessed under standard dark conditions, will show a heightened degree of limbic activation as reflected by increased hippocampal and frontal-cortical noradrenaline concentrations, compared to LNB mice assessed under conditions of complete visual deprivation (VD)². In contrast to the biobehavioural profile expected in LNB mice, we hypothesise that NNB³, as a normal goal-directed behaviour, will be dependent on visual feedback only insofar as this is needed to guide motor actions towards nesting construction, but that neither NNB at large, nor its assessment under conditions of VD, will be associated with heightened limbic involvement.

1.4 Research questions

- i. Will VD differentially impact nesting outcomes in NNB and LNB expressing mice?
- ii. Will changes in nesting outcomes in NNB and LNB mice under conditions of VD point to LNB being a behavioural phenotype related to increased anxiety sensitivity towards visually perceived environmental cues or alternatively, seeking 'just-right' outcomes?
- iii. Will LNB mice, compared to NNB mice, show heightened limbic system activation as resembled by increased hippocampal and frontal-cortical noradrenaline concentrations?
- iv. Depending on the answers gained in terms of (i) – (iii) above, what will the translational conclusion in terms of the psychobiological fingerprint of LNB as a model of compulsive-like behavioural persistence be?

¹ large nest building

² visual deprivation

³ normal nest building

1.5 Study aims and objectives

1.5.1 Aims

The current research aimed to:

- i. Investigate the extent to which the expression of NNB¹ and LNB² may be dependent on visual feedback as reflected by changes in quantitative and qualitative nesting outcomes under conditions of VD³, compared to SL⁴; and
- ii. Determine whether NNB and LNB is differentially associated with limbic involvement under both standard and visually deprived dark conditions, as measured by hippocampal and frontal-cortical noradrenaline concentrations.

1.5.2 Objectives

To realise the aims of this work, the following objectives were addressed:

- i. Identifying 24 NNB and 24 LNB-expressing deer mice, as far as possible equally distributed between sexes, from an initial pool of 100 mice;
- ii. Subdividing each of the two behavioural cohorts referred to in (i), into the following experimental groups ($n = 10 - 14$ per group; refer to Chapter 3 for a full description of methods), that were reassessed for nesting expression as explained:
 - NNB expressing mice reassessed under standard dark conditions ($n = 14$);
 - NNB expressing mice reassessed under conditions of VD, i.e., utter darkness ($n = 10$);
 - LNB expressing mice reassessed under standard dark conditions ($n = 12$);
 - LNB expressing mice reassessed under conditions of VD ($n = 12$);
- iii. Comparing both the quantitative (nesting size) and qualitative (nesting attribute) nesting scores of NNB and LNB expressing mice assessed under standard dark conditions and conditions of VD; and
- iv. Quantifying the hippocampal and frontal-cortical serotonin and noradrenaline concentrations of mice in each of the groups referred to in (ii).

¹ normal nest building

² large nest building

³ visual deprivation

⁴ standard light

1.6 Study layout

Following from the above, the present investigation comprised two individual nesting assessment phases, i.e., 1) a baseline assessment which served to categorise mice into either the NNB¹ or LNB² cohorts, and 2) following a further separation of mice into the two lighting conditions, a second assessment which were either again conducted under standard dark conditions, or under conditions of VD³ (**Figure 1-1**). Following the conclusion of phase 2, all mice were euthanised, the relevant brain tissues sampled, and serotonin and noradrenaline quantified.

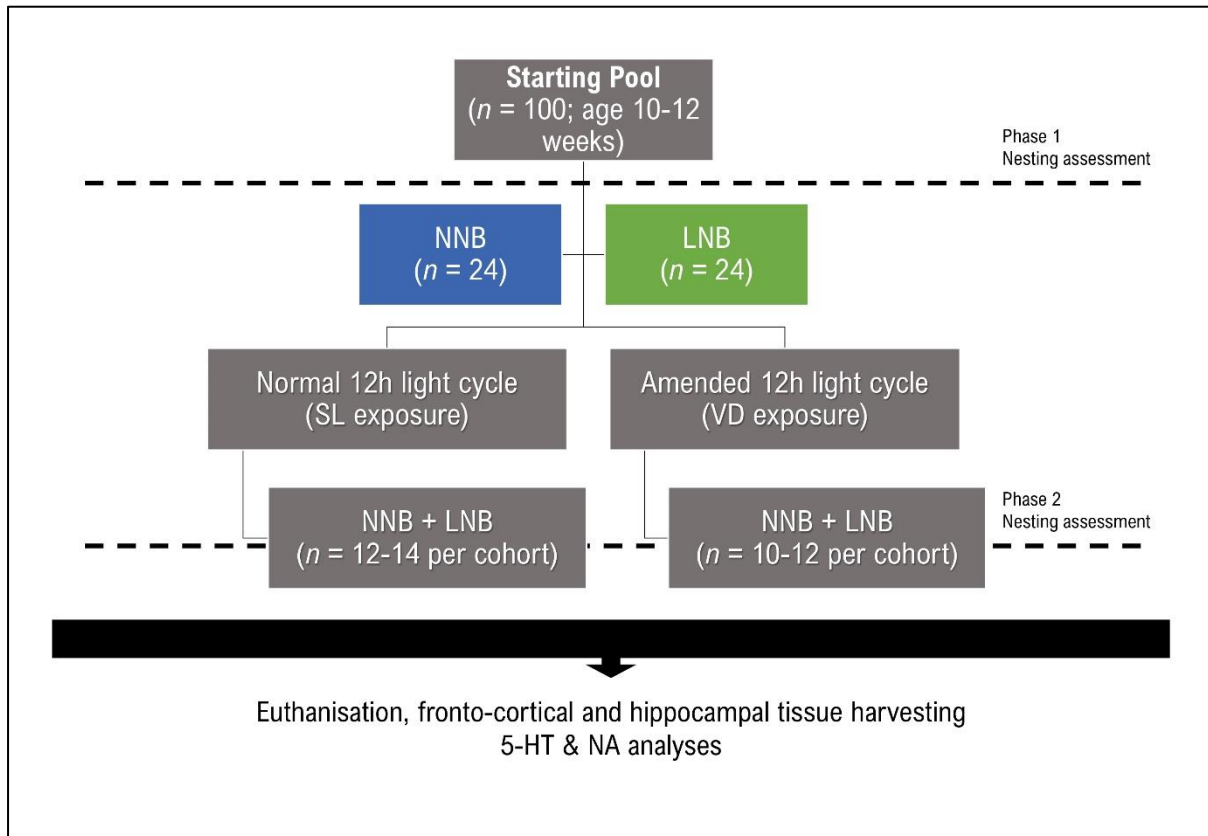


FIGURE 1-1: Schematic representation of study layout

NNB: normal nest building; LNB: large nest building; SL: standard 12-h light-dark; VD: visual deprivation; 5-HT: serotonin; NA: noradrenaline.

1.7 Ethical approval

Prior to the onset of experimentation, this investigation was approved by both the Translational Neuroscience and Neurotherapeutics scientific committee of PharmaCen (Faculty of Health Sciences, North-West University; NWU⁴) and by the NWU AnimCare Research Ethics Committee (approval number:

¹ normal nest building

² large nest building

³ visual deprivation

⁴ North-West University

NWU-00769-22-A5). Throughout the investigation, the four R's (4R) as they pertain to experimentation with laboratory housed animals, were considered (Hooijmans et al. 2010, Arora et al. 2011, Ferdowsian and Beck 2011, Doke and Dhawale 2015). All procedures were performed in accordance with the code of ethics stipulated in the relevant national legislation (South African National Standard for the Care and Use of Animals for Scientific Purposes, i.e., SANS¹ 10386:2008).

¹ South African National Standard

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2 Literature overview

2.1 Epidemiology and clinical features

Obsessive-compulsive disorder (OCD)¹ has an estimated lifetime prevalence of 2 – 3 % in the general population (Chamberlain et al. 2005, Schruers et al. 2005, Grabill et al. 2008, Walitza et al. 2020). According to the World Health Organization (WHO)², OCD is one of the ten leading causes of illness-related disability globally (Koran 2000, Ahmari and Dougherty 2015, Ahmari 2016). Clinically, the condition is recognised by two main symptom clusters, i.e., obsessions and compulsions (APA 2013). Obsessions are recurrent, intrusive, and troubling thoughts about specific themes that are mostly a product of one's own mind and that cause noteworthy anxiety in the sufferer. Compulsions, which are mostly expressed in response to the underlying obsessions, are repetitive, persistent and seemingly purposeless behaviours or mental rituals that are expressed in an attempt to neutralise the anxiety caused by obsessive intrusions (Pittenger et al. 2005, Abramowitz 2006, APA 2013, Abramowitz and Jacoby 2014, Ahmari and Dougherty 2015). However, since obsessions are implicitly generated, the relief gained from engaging in compulsive neutralising rituals is usually short-lived. Thus, the condition is self-reinforcing in the sense that patients must constantly engage in compulsive rituals to attenuate the obsession-induced anxiety.

The symptoms of OCD are clinically heterogeneous, meaning that different phenotypes or 'symptom clusters' are seen in different patients (Mataix-Cols et al. 2005, Anholt et al. 2013, Ahmari and Dougherty 2015). Specifically, four main symptom clusters are currently recognised, i.e., (1) contamination obsessions accompanied by washing and cleaning compulsions, (2) safety-related obsessions and checking compulsions, (3) symmetry and exactness obsessions which are paired with rituals that include counting, arranging, and ordering, and (4) intrusive obsessions of a repugnant nature and covert mental compulsions, e.g., excessive praying (Mataix-Cols et al. 2005, Hoffman 2011). Hoarding, which was previously included as an obsessive-compulsive phenotype, is now classified as its own condition in the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-V)³ (APA 2013).

In terms of its development, OCD is commonly regarded as an "adulthood disorder" (Fineberg and Robbins 2021); this is misguided. In reality, OCD is no less common in children and teenagers (Zohar 1999, Fineberg and Robbins 2021), with the median age of onset being 19.5 years (Ruscio et al. 2010). Further, over half of patients diagnosed with OCD report that they experienced symptom onset after a

¹ obsessive-compulsive disorder

² World Health Organisation

³ Diagnostic and Statistical Manual of Mental Disorders, fifth edition

significant, life-altering event, e.g., major financial problems, dramatic childhood experiences, or even the unexpected death of a loved one (Lensi et al. 1996, Clark 2019). That said, OCD¹ also seems to have a bimodal age of onset with the first peak in diagnoses being noted at the age of 11 years (early-onset OCD), and another peak seen in early adulthood between the ages of 19 and 22 years (so-called late-onset OCD) (Swedo et al. 1989, Fineberg and Gale 2005, Walitza et al. 2020, Fineberg and Robbins 2021). Importantly, research increasingly points to early- and late-onset OCD to be different conditions, with early-onset OCD associating more with genetic predisposition, compared to late-onset OCD (Anholt et al. 2013). Also, men are more likely to present with early-onset OCD, while their symptoms are also often more severe (Stewart et al. 2004, Fineberg and Robbins 2021). While men commonly present with symmetry and perfectionism obsessions and intrusive thoughts about repugnant, e.g., aggressive, sexual, or religious themes, women mostly present with contamination/washing OCD (Ahmari and Dougherty 2015, Clark 2019).

2.2 Diagnosis

Because the symptom content of OCD overlaps with every-day concerns, its diagnosis can be problematic and thus it should be made only by appropriately qualified clinicians (Mathes et al. 2019). Briefly, a diagnosis of OCD is based on a combination of patient self-reports and clinician-administered assessments during a clinical interview (Goodman et al. 1989, Hirschtritt et al. 2017). Based on these, OCD is diagnosed according to specific criteria, as stipulated by the DSM-V² (APA 2013), summarised as follows:

- Obsessions and/or compulsions (*note that only one is needed for a diagnosis*) must be present for at least 30 days and take up more than one hour per day;
- Symptoms cannot be linked with, or be ascribed to substance or medication use;
- Symptoms must not be better described by another mental disorder (e.g., generalised anxiety disorder, hoarding disorder, body dysmorphic disorder, or trichotillomania).

Further, reference to the level of insight (i.e., poor, moderate, or good) is also made at the time of diagnosis, since OCD is distinguished from conditions where patients show no insight into the irrationality of their symptoms, e.g., delusions (Phillips et al. 2012, de Avila et al. 2019). Indeed, obsessions are ‘ego-dystonic’ and not ‘ego-syntonic’, meaning that, as opposed to delusional patients, individuals suffering from OCD realise that their symptoms are irrational and futile (Schwartz 1999, Abramowitz and Jacoby 2014). Interestingly, symptoms can be triggered by environmental and circumstantial stimuli, e.g., seeing other individuals at risk of injury or coming into contact with

¹ obsessive-compulsive disorder

² Diagnostic and Statistical Manual of Mental Disorders, fifth edition

contaminated surfaces (Abramowitz and Jacoby 2014). The association between such sensory feedback and obsessive-compulsive symptom expression is the basis of anti-compulsive psychotherapeutic interventions like exposure-response prevention (ERP)¹ (Reid et al. 2021) and will form a core focus of investigation in the current work. Nevertheless, in terms of *actual* functional outcome, compulsive behaviours are not realistically connected to a functional, goal-directed outcome, since the triggers for their expression, are in fact, irrational (Pittenger et al. 2005, Abramowitz 2006, APA 2013).

2.3 The treatment of OCD

The primary outcome of treating any psychiatric illness is not only to instil a significant degree of symptom control, but also to restore quality of life. Patients suffering from OCD² experience a drastic decline in quality of life, which hampers their ability to function normally within society (Subramaniam et al. 2013, Hirschtritt et al. 2017). So as to attain the best therapeutic outcomes across different types and severities of OCD, treatment strategies are divided into three main groups, i.e., psychotherapy, pharmacotherapy, and neurosurgical interventions (Schruers et al. 2005, Gragnani et al. 2022).

Psychotherapy, i.e., ERP and cognitive behavioural therapy (CBT)³, is regarded as the first-line intervention for OCD due to its robust efficacy and long-lasting therapeutic benefit post-treatment (Reid et al. 2021). Briefly, ERP involves the systematic, gradual exposure of patients to obsession- or compulsion-provoking scenarios. While being exposed, patients are guided by the therapist to abstain from engaging in compulsive behaviours (Abramowitz 2006, Stein et al. 2019). Thus, over time, patients learn to inhibit their compulsive responding and come to realise that this was futile, unnecessary, and that it served no actual purpose. In other words, they gradually adapt to the idea that most every-day circumstances involving a low to moderate degree of risk, for example, are actually harmless (Reid et al. 2021). A very important premise for the successful administration of ERP is that patients should be able to perceive, and in turn, attempt to respond to their environment. As alluded to above, this contingency between sensory feedback and compulsive engagement forms a pivotal focus of the present work and will be revisited later in this chapter.

In terms of its mechanism of action, ERP involves three main constructs, i.e., a behavioural mechanism, a cognitive mechanism, and ultimately improved self-regulation. The behavioural mechanism involves learned response inhibition achieved over successive uninterrupted sessions, thereby decreasing conditioned ‘fear’ responses to a perceived, but irrational ‘threat’ (Abramowitz 2006). On a cognitive level, ERP thus corrects dysfunctional beliefs that may have been maintained with respect to a specific

¹ exposure-response prevention

² obsessive-compulsive disorder

³ cognitive behavioural therapy

theme. Extending the behavioural mechanism, the cognitive construct of ERP¹ ensures that patients relearn the difference between self-perceived and inflated fears and apply this new knowledge in future scenarios (Clark 2019). Ultimately, this change, i.e., realising that previously feared consequences are not likely to occur and that preventative compulsive rituals are therefore not needed, enables the third mechanistic construct, i.e., improved self-regulation by the patient self (Abramowitz 2006).

With respect to CBT², the psychological process of psychological reprogramming is akin to that employed in ERP, with the exception that patients are not explicitly brought into contact with compulsion-provoking scenarios (Reid et al. 2021). Rather, patients are guided through discussions with their therapists to reprogram their 'dysfunctional' belief system. While CBT is associated with similar therapeutic outcomes compared to ERP, results may be slower to manifest. Still, many clinicians prefer CBT over ERP, given the less invasive and stressogenic nature of its administration (Gragnani et al. 2022). Patient adherence to ERP remains a significant obstacle, with at least 25 to 30 % of patients refusing ERP therapy (Emmelkamp 1987, Foa 2010, Gragnani et al. 2022), while another estimated 30 % of those that commenced with therapy, drop out prematurely (Abramowitz 2006, Foa 2010).

Psychotherapeutic strategies can be followed as singular interventions or in combination with pharmacotherapy (Goodman et al. 2021). In this regard, the mainstay of pharmacological interventions for OCD³ remains to this day, serotonin-based, with the serotonin reuptake inhibitors (SRIs)⁴ and selective serotonin reuptake inhibitors (SSRIs)⁵ being the preferred agents (Fineberg and Robbins 2021). The mechanism of action of serotonergic agents in the treatment of OCD is based on the hyposerotonergic theory of OCD (Preskorn 1997, Feighner 1999, Xue et al. 2016), which will be explained later in this chapter. Briefly, serotonin is believed to counterbalance the behaviourally activating functions of dopamine within the cortico-striatal-thalamo-cortical (CSTC)⁶ circuits of the brain, which collectively govern voluntary motor output (Shephard et al. 2021). Thus, by increasing serotonin concentrations in the synaptic cleft, a pro-serotonergic tone is being maintained, thus contributing to behavioural inhibition. Whereas both SRIs and SSRIs show equal anti-compulsive efficacy, preference is based on among others, side-effect profiles (which are often more deleterious with SRIs, e.g., clomipramine) and the individual response of patients to therapy (Pittenger and Bloch 2014). Irrespective, both SRIs and SSRIs have a delayed onset of action, i.e., 8 – 12 weeks, and should be used chronically, without interruption (Koran et al. 2007, Pittenger and Bloch 2014). Alternative treatment, in the case of treatment non-response, should also only be considered after said time has elapsed. In

¹ exposure-response prevention

² cognitive behavioural therapy

³ obsessive-compulsive disorder

⁴ serotonin reuptake inhibitor

⁵ selective serotonin reuptake inhibitor

⁶ cortico-striatal-thalamo-cortical

cases where the first agent of choice proved ineffective, switching to a higher dose of the same agent, or to another drug entirely is advised (Fineberg and Robbins 2021). Alternatively, as is the case with 30 – 60 % of patients initiated on SRIs¹/SSRIs², augmentation therapy, i.e., SRI/SSRI combined with low-dose anti-dopaminergic drugs, is advised. However, even with such approaches, therapeutic outcomes are suboptimal, necessitating urgent efforts to identify novel treatment targets. Towards this end, an improved understanding of how obsessions and compulsions develop is needed.

Last, the neurosurgical interventions for OCD³, which are reserved for the most severe and treatment refractory cases, include deep-brain stimulation (DBS)⁴, electroconvulsive therapy (ECT)⁵, and transcranial magnetic stimulation (TMS)⁶ (Robbins et al. 2019). Considering their restricted use in a small minority of patients and taking the focus of the present work into consideration, a review of neurosurgical interventions is beyond the scope of the present literature overview. However, suffice to say that all three techniques are aimed at restoring executive balance in the CSTC⁷ circuits, as is the goal of pro-serotonergic therapy. For a detailed review of these therapeutic alternatives, please see Rasmussen and Goodman (2022).

2.4 The neurobiology of OCD

As briefly alluded to earlier in this chapter, OCD is mostly, but to varying degrees, associated with serotonergic and dopaminergic perturbations in the frontal-cortical, striatal, and thalamic areas of the brain, three structures which collectively govern goal-directed output (Shephard et al. 2022). However, since OCD is also fundamentally associated with anxiety, the limbic system is also involved its psychobiology (Haber et al. 2021). In this section, a summary of these neurobiological constructs will be provided against the background of OCD.

2.4.1 Neuroanatomy

The CSTC circuits comprise a series of discrete functional loops that originate in the frontal cortex, connect through the basal ganglia to the thalamus, and ultimately terminate in distinct areas of the frontal cortex from where it originated (Gao et al. 2019, Robbins et al. 2019). In humans, any one or more of five discrete cortico-striatal loops, i.e., the ventral affective circuit, the ventral cognitive circuit, the dorsal cognitive circuit, the fronto-limbic circuit, and the sensorimotor circuit, are active, depending on the

¹ serotonin reuptake inhibitor

² selective serotonin reuptake inhibitor

³ obsessive-compulsive disorder

⁴ deep-brain stimulation

⁵ electroconvulsive therapy

⁶ transcranial magnetic stimulation

⁷ cortico-striatal-thalamo-cortical

nature of the executive function that dominates at any specific time (Haber 2022). Importantly, considering the array of psychobiological perturbations that are associated with OCD¹, including those within the motor and cognitive domains, dysfunction in any of these circuits can contribute uniquely to obsessive-compulsive symptom presentation.

2.4.1.1 *The frontal cortex*

The brain enables humans and animals to coordinate their behaviour. The mechanisms that underlie goal-directed behaviour, is intrinsically associated with cognitive control, a higher function of the frontal cortex (Frith and Dolan 1996, Miller and Cohen 2001, Carlén 2017). Although cognition is a construct that has been investigated extensively (Carlén 2017), the precise architecture of its origins still remains one of the greatest mysteries in animal and human psychobiology (Szczepanski and Knight 2014). Although cognitive control is born from the concerted effort of most brain areas, the frontal cortex is regarded as the principal orchestrator of this ability (Miller and Cohen 2001). Importantly, the frontal cortex does not play a key role in automatic, habitual behaviours (Miller and Cohen 2001), but rather guides or adapts our behavioural output based on the internal, i.e., implicit, state of an individual or specific expected outcomes; this ability is critical for our survival. This form of cognitive control is otherwise also known as *top-down* control, as opposed to the *bottom-up* mechanisms, rooted in the basal ganglia (see below), that facilitate automated responses and habits (Miller and Cohen 2001, Carlén 2017). In line with its functions of enabling control over cognitive processing, the frontal cortex is responsible for emotional processes, the planning and selection of behaviour and thus behavioural flexibility, task-switching, and reward and punishment processing (Frith and Dolan 1996, Miller and Cohen 2001, Carlén 2017).

The frontal cortex is broadly divided into five distinct neuroanatomical regions, i.e., 1) the orbitofrontal cortex (OFC)², 2) the ventrolateral prefrontal cortex (vlPFC)³, 3) the dorsolateral prefrontal cortex (dlPFC)⁴, 4) the ventromedial prefrontal cortex (vmPFC)⁵, and 5) the anterior cingulate cortex (ACC)⁶. Other areas of the cortex that are variably linked with the clinical symptomology of OCD are the presupplementary and supplementary motor areas and the motor cortex (Stein et al. 2019, Shephard et al. 2021). Linking extensively with most other areas of the brain, including the sensory and sub-cortical motor areas and the limbic system (Miller and Cohen 2001, Crouson et al. 2005, Szczepanski and Knight 2014), fronto-cortical dysfunction is associated with marked impairments across most cognitive

¹ obsessive-compulsive disorder

² orbitofrontal cortex

³ ventrolateral prefrontal cortex

⁴ dorsolateral prefrontal cortex

⁵ ventromedial prefrontal cortex

⁶ anterior cingulate cortex

domains, including memory formation, emotional responding and sensory feedback processing (Barbey et al. 2013, Szczepanski and Knight 2014). Such impairment often results in dysregulated behaviour that could manifest as impulsive, repetitive, and disorganised behaviour (Miller and Cohen 2001), phenotypes that are often typical of OCD¹. As briefly alluded to above, the prefrontal cortex (PFC)² can be viewed as both a global trigger, and terminator of behavioural and cognitive output.

The OFC³ forms part of the affective orbitofrontal-striatal circuit, otherwise known as the ventral affective circuit within the CSTC⁴-circuitry (Shephard et al. 2021). The OFC receives input from both the sensory regions, e.g., via visual, olfactory and gustatory stimuli, as well as from the dorsal raphe nuclei and limbic regions (Padoa-Schioppa and Conen 2017). Once activated, the OFC projects through the basal ganglia via the globus pallidus interna (GPi)⁵ to the mediodorsal thalamus, before the signal terminates in the OFC again (Menzies et al. 2008). Activation of this loop facilitates mental planning and executive routines (Petrides and Pandya 2006, Padoa-Schioppa and Conen 2017) and is thus a pivotal role player in reward-based and reversal learning, social aptitude, and outcome-directed decision-making (Clark et al. 2004, Elliott and Deakin 2005, Menzies et al. 2008). With respect to OCD, neuroimaging studies point to orbito-frontal dysfunction, i.e., mostly hyperactivation, on the levels of affect, psychobiological inhibition, decision-making, and reward-related learning and reverse learning (Eslinger and Damasio 1985, Bechara et al. 1994, Rauch et al. 1994, Menzies et al. 2008, Wood and Ahmari 2015, Goodman et al. 2021). Congruent with the clinical symptomology typical of OCD, impairment across these and other related cognitive domains prevents patients from adapting their behavioural output once specific tasks, i.e., locking a door or turning off an oven, have been completed (Hikosaka and Watanabe 2004, Menzies et al. 2008).

The ventrolateral and dorsolateral prefrontal cortices are two of the main structures within the ventral and dorsal cognitive CSTC circuits (Shephard et al. 2021). The vIPFC⁶, like the OFC, receives neurological input from sensory areas e.g., the dorsal and ventral visual pathways, as well as from the OFC itself and subcortical structures (Sakagami and Pan 2007, van den Heuvel et al. 2016). However, as opposed to the mainly executive functions of the OFC, the vIPFC interprets such feedback and facilitates behavioural adaptation in response to changing circumstances (Sakagami and Pan 2007). The sensory information needed to accomplish this is interpreted by the vIPFC and the context thereof relayed to the dIPFC⁷ and pre-motor cortex, which are responsible for the subsequent cognitive and behavioural output (Sakagami

¹ obsessive-compulsive disorder

² prefrontal cortex

³ orbitofrontal cortex

⁴ cortico-striatal-thalamo-cortical

⁵ globus pallidus interna

⁶ ventrolateral prefrontal cortex

⁷ dorsolateral prefrontal cortex

and Pan 2007). It is postulated that patients suffering from OCD¹ show deficits in context-related response inhibition, i.e., not being able to apply the necessary stop signal once a specific context, e.g., and unsafe environment, is no longer present. Such dysfunction is linked to vIPFC² dysfunction (Shephard et al. 2021) and likely contributes to behavioural persistence.

The vmPFC³ and ACC⁴ are integral components of the limbic system which enables the CSTC⁵ circuitry to respond to and act on either innately or extrinsically generated emotional and affective stimuli and emotional memory (Clark et al. 2004, Rudebeck et al. 2008, Roy et al. 2012, Shephard et al. 2021). These brain areas are therefore highly innervated by projections to and from structures in the limbic system, e.g., the amygdala, ventral striatum, and the hippocampus (Rudebeck et al. 2008, Hiser and Koenigs 2018). To integrate contextual information with limbic processing, the vmPFC also serves as an integration point for sensory input, before projecting to the ventral striatum and the amygdala where the information is subjected to emotional interpretation (Roy et al. 2012, Hiser and Koenigs 2018). In addition, the vmPFC is responsible for the regulation of negative emotions and fear memory consolidation via its top-down control over the amygdala and hippocampus (Hiser and Koenigs 2018). Working in concert with the vmPFC, the ACC facilitates automatic, emotionally triggered behaviours and visceral responses and mediates complex emotional and social behaviour (Rudebeck et al. 2008). It has been shown that individuals suffering from obsessive-compulsive symptoms that are linked to ‘fearful’ content, e.g., contamination- or safety-related fears, often present with underlying hyperactivity of the limbic circuits (van den Heuvel et al. 2016). This data point to two possible dysfunctional mechanisms in the limbic circuitry (Shephard et al. 2021), i.e., a dysregulated fear response or an intolerance of uncertainty. Dysregulated fear responses are seen with intense, mostly irrational reactions to fearful and anxiogenic circumstances, and can mostly be ascribed to dysfunctional safety-signalling mechanisms within the vmPFC. Intolerance of uncertainty refers to the inability to adapt to and function in situations with unfamiliar or ambiguous outcomes (Tolin et al. 2003, Sarawgi et al. 2013). This is often seen in OCD cohorts, which display inflated emotional reactions in the form of anxiety, which then drives ‘anxiolytic’ compulsive behaviours. This work will primarily focus on the potential involvement of the limbic system in compulsive-like large nesting behaviour (LNB)⁶ expression in deer mice by investigating whether moderation of potential anxiogenic stimuli by means of visual deprivation, could abrogate the expression of LNB.

¹ obsessive-compulsive disorder

² ventrolateral prefrontal cortex

³ ventromedial prefrontal cortex

⁴ anterior cingulate cortex

⁵ cortico-striatal-thalamo-cortical

⁶ large nest building

The sensorimotor circuit is comprised of various sub-cortical regions, including the basal ganglia, as well as the somatosensory cortex, the primary motor cortex, and the supplementary motor complex, which consists of supplementary motor area (SMA)¹, the pre-SMA, and the supplementary eye field (Nachev et al. 2008, van den Heuvel et al. 2016, Shephard et al. 2021). As a collective, the sensorimotor circuit integrates sensory information and generates appropriate motor output (Shephard et al. 2021), which are mostly automatic responses to sensory cues gradually born from repetitive exposure to specific circumstances (Hoshi and Tanji 2004). In this sense, sensorimotor impairment may contribute to compulsions related to ‘not-just-right’ perceptions or feelings of ‘incompleteness’ by triggering uncomfortable feelings or sensations via heightened visual, auditory, or tactile stimulation; this in turn is believed to drive repetitive, ‘restorative’ behaviours (Hoffman and Morales 2009, Shephard et al. 2021). Additionally, the sensorimotor system can also contribute to habit formation, another neurocognitive process that drives certain forms of compulsive behaviour, i.e., checking, counting, and ordering (Graybiel and Rauch 2000, van den Heuvel et al. 2016, Shephard et al. 2021). Such ‘compulsions’ are hypothesised to be dissociated from obsessive content, meaning that they arise from extensive experience with a previously goal-directed action-outcome contingency, which gradually becomes a habitual response that is triggered by specific environmental, i.e., sensory, cues (Gillan et al. 2011, Simmler and Ozawa 2019). This concept is of utmost importance within the context of the present research question.

2.4.1.2 *The basal ganglia*

The basal ganglia consist of interconnected sub-cortical nuclei that are deeply embedded within the brain (Albin et al. 1989, Lanciego et al. 2012). Acting as the main ‘yes-no’ bridge between cortical-striatal output and thalamo-cortical innervation, it plays an essential role in reinforcement learning, the control of movement and emotion, as well as executive function, e.g., goal-directed behaviour, reward-based learning and action gating (i.e., switching between go- and no-go signals) (Alexander et al. 1986, Chakravarthy et al. 2010, Lanciego et al. 2012, Steiner and Tseng 2016). To explain, the basal ganglia, in close concert with the cerebellum (Seger and Miller 2010, Koziol et al. 2011), relay executive instructions from the different areas of the frontal cortex referred to earlier, to the thalamus via two main pathway clusters, i.e., direct and indirect (Steiner and Tseng 2016). Although an in-depth review of the neuroanatomical structures of the basal ganglia falls beyond the scope of this review, it suffices to summarise that the direct pathway is commonly viewed as being behaviourally activating, while activation of the indirect pathway results in behavioural suppression (Lanciego et al. 2012, Calabresi et al. 2014). Since both pathways are activated simultaneously via cortical projections, executive conflict is generated, which is solved by dopamine. This will be explained later in this chapter.

¹ supplementary motor area

2.4.1.3 *The thalamus*

The thalamus, acting as the main integrator of cortico-striatal signalling reciprocally connects with various neuroanatomical regions in the brain, i.e., the basal ganglia (Lanciego et al. 2012), amygdala (Simon et al. 2014), hypothalamus (Buijs and Van Eden 2000), hippocampus (Li et al. 2019), and the cerebellum, which enables habitual behaviours and the acquisition of behavioural sequences by new sensory information (Imamizu et al. 2003, Glickstein et al. 2011, Koziol et al. 2011). The thalamus is therefore a critical integration point for inputs from most areas responsible for sensory, motor, and cognitive processes (Ward 2013). In this sense, the thalamus can be viewed as the main executive trigger which signals cortico-striatal instructions via thalamo-cortical efferents, thereby closing the respective CSTC¹ loops (Posner et al. 2014, Keeley et al. 2022). Given the diverse range of the psychobiological perturbations that are characteristic of OCD², three thalamic subregions have been found to contribute to the symptomology, i.e., the left sensory, right posterior parietal, and right occipital regions (Li et al. 2019). Abnormalities noted within these areas include changes in thalamic volumes (Menzies et al. 2008), dysfunctional connectivity between sub-thalamic regions and the sensory cortex, as well as decreased connectivity between the PFC³ and the thalamus (Ping et al. 2013, Li et al. 2019). Nevertheless, current pharmacological and surgical treatment approaches are generally not aimed at restoring thalamic function, highlighting the complexity of its involvement in the condition.

2.4.2 The neurotransmission of OCD

For the purposes of this review, attention will be afforded to three neurotransmitter systems which are involved in the pathophysiology of OCD, i.e., the dopaminergic, serotonergic, and noradrenergic systems. Although the gamma-aminobutyric acid (GABA)⁴-ergic and glutamatergic systems, as well as some other neurotransmitters, e.g., adenosine, are also involved in cortico-striatal signalling, an overview of these fall beyond the scope of the current research framework. As such, they will not be summarised here.

2.4.2.1 *Dopamine*

In the mammalian brain, the dopaminergic system mainly stems from the midbrain, from where it projects via three main pathways to the basal ganglia (via the nigrostriatal pathway), the limbic system (via the mesolimbic-cortical pathway), and the hypothalamus (via the tuberoinfundibular pathway), the

¹ cortico-striatal-thalamo-cortical

² obsessive-compulsive disorder

³ prefrontal cortex

⁴ gamma-aminobutyric acid

first two of which are especially important for an understanding of OCD¹ (Kapur and Remington 1996, Di Giovanni et al. 2008, Di Giovanni et al. 2008).

Earlier in this chapter, reference was made to executive conflict which is generated in the basal ganglia upon the simultaneous activation of both the direct and indirect cortico-striatal pathways (Lanciego et al. 2012, Cui et al. 2013, Calabresi et al. 2014). Since the direct pathway is known to promote behavioural engagement, with the opposite being true for indirect pathway activation, such conflict is to be resolved lest behavioural output is not activated; for this, dopamine is paramount. To explain, the neurons of the direct pathway express dopamine D₁ receptors, whereas those of the indirect pathway mainly express D₂ receptors. When goal-directed actions are planned and anticipated, dopamine is released in the nigrostriatal pathways and so stimulates both the D₁ and D₂ receptors of the respective direct and indirect neuronal clusters (Nambu 2008). This results in the direct pathway promulgating executive signals to the thalamus, while activity in the indirect pathway (that normally prevents behavioural output), is inhibited. Thus, simultaneous cortico-striatal signalling and nigrostriatal dopamine release is needed for the successful promulgation of cognitive and behavioural action (Hauser et al. 2017). In this sense, dopamine can be regarded as a behaviourally activating neurotransmitter. These functions of dopamine also explain the use of anti-dopaminergic therapy in the treatment of OCD symptoms that is non-responsive to serotonergic interventions. This will be explained in more detail later in this chapter.

It stands to reason that patients with OCD will present with a hyperdopaminergic state or heightened direct pathway activity. While research is not overly conclusive as to the extent of dopaminergic dysfunction in the disorder, functional neuroimaging studies have implicated at least some degree of imbalance between direct and indirect pathway activity (Calzà et al. 2019), as well as dopamine receptor changes in patients suffering from the illness (Denys et al. 2004, Perani et al. 2008, Keeler et al. 2014). For example, Denys et al. (2004) reported increased striatal dopamine transporter density in conjunction with decreased D₂ receptor expression in OCD patients compared with healthy controls. Further, OCD cohorts have also been shown to present with lower catechol-O-methyl transferase (COMT)² activity, an enzyme responsible for the breakdown of dopamine (Karayiorgou et al. 1997, Karayiorgou et al. 1999). While said perturbations all have bearing on the nigrostriatal system and its regulation of cognitive and behavioural output, dopamine is also responsible for heightened amygdalar and hippocampal activity via its modulation of the vmPFC³ through mesolimbic projections (Rosenkranz and Grace 2002). This may have clinical implications for obsessive-compulsive phenotypes that may be more associated with

¹ obsessive-compulsive disorder

² catechol-O-methyl transferase

³ ventromedial prefrontal cortex

inflated fear responses, i.e., safety-checking or contamination-washing OCD¹ (Figeet al. 2011, Grupe and Nitschke 2013, Eagle et al. 2014, Fineberg et al. 2018, Fick 2019, Robbins et al. 2019, Shephard et al. 2021). Blunted dopaminergic signalling, as elicited by antidopaminergic drug intervention, may thus also contribute to improved clinical outcomes in these patients.

2.4.2.2 Serotonin

Serotonergic neurons, which mainly originate in the raphe nuclei, are distributed throughout the brain and are involved in the regulation of executive processes, mood and emotion, and homeostasis (Baumgarten and Grozdanovic 1998). Within the present context, serotonin can also be regarded as the functional opponent of dopamine, with serotonergic stimulation in the CSTC²-circuitry, generally suppressing behavioural engagement (Soubrié 1986, Baumgarten and Grozdanovic 1998, Crockett et al. 2009, Eagle et al. 2009). Further, serotonin (5-HT)³ also modulates the expression of anxiety, with increased serotonergic tone in the limbic system often being associated with inflated anxiety-related symptoms (Lowry et al. 2005, Sargin et al. 2019). That said, long-term hyper-serotonergic transmission, as which prevails during the treatment of phobia with very high doses of SSRIs⁴, is associated with serotonin receptor downregulation and thus anxiety and phobia relief over longer periods of time (Boyer 2000, Nutt 2004, Akimova et al. 2009, Goodman et al. 2021). It follows that OCD can be regarded as a condition of hyposerotonergia, since the use of pro-serotonergic agents is associated with anti-compulsive outcomes (Shephard et al. 2021), whether by suppressing the pro-executive effects of cortico-striatal dopamine release (Tranulis et al. 2005, Goddard et al. 2008, Koo et al. 2010, Karpinski et al. 2017), or by modulating implicit anxiety-related processes (Fernandez and Gaspar 2012, Bauer 2015, Pourhamzeh et al. 2022). Serotonin exerts its effects on seven main receptor subtypes, i.e., 5-HT₁₋₇. Of relevance for executive output, and thus also for OCD are the 5-HT₁ and 5-HT₂ subtypes.

5-HT₁ receptors, which include the 5-HT_{1A/C}, 5-HT_{1D}, 5-HT_{1E}, and the 5-HT_{1F} receptors, are mainly found within the substantia nigra and other mesolimbic-cortical areas e.g., the amygdala and the frontal cortex (Pazos et al. 1985, Pazos and Palacios 1985, Kapur and Remington 1996, Di Giovanni et al. 2008, Di Giovanni et al. 2008). Here they are responsible for regulating serotonergic output to the rest of the brain. In line with the theoretical understanding of OCD, activation of 5-HT_{1A/B/D} autoreceptors can significantly exacerbate compulsive-like repetitive behaviour, impair working memory performance, and decrease cognitive flexibility (Pahua et al. 2019, Oliver et al. 2022).

¹ obsessive-compulsive disorder

² cortico-striatal-thalamo-cortical

³ serotonin

⁴ selective serotonin reuptake inhibitor

5-HT₂ receptors include the 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} subtypes. They are mainly expressed in the basal ganglia, amygdala, olfactory tubercle, frontal cortex and the entorhinal cortex (which is part of the medial temporal lobe and the limbic system) (Pazos et al. 1985, Pazos and Palacios 1985, Di Giovanni et al. 2008, Di Giovanni et al. 2008). In OCD¹, it is mostly the 5-HT_{2A} and 5-HT_{2C} receptors that implicated (Flaisher-Grinberg et al. 2008, Papakosta et al. 2013, Alvarez et al. 2022). Although an extensive body of work has been done to explore the exact nature of 5-HT_{2A/C} receptor modulation on the expression of obsessive-compulsive behaviour, their exact pathophysiological role remains elusive (Papakosta et al. 2013); this lack of a clear understanding is reflected in current treatment approaches that are mostly not receptor specific, but rather target hyposerotonergia in general.

2.4.2.3 *Noradrenaline*

Early research on the release of adrenalin and noradrenaline within the sympathetic nervous system has highlighted its importance for our ability to function under stressful or threatening circumstances (Terbeck 2016). Indeed, activation of the central noradrenergic system is imperative for maintaining vigilance, attention, and arousal (Bouras 2023). While it is not traditionally regarded as being an important role player in OCD (mostly due to agents with a noradrenergic mechanism of action not being effective for the treatment of OCD; Fineberg and Robbins (2021)), an increasing body of research points to its potential role in mediating the underlying fear responses, and hence the psychobiological triggers, of some phenotypes of the disorder (Charney 2003, Katayama et al. 2010, Sakolsky et al. 2012, Terbeck 2016, Lustberg et al. 2020, Bierwirth and Stockhorst 2022).

The major source of noradrenaline in the mammalian brain is the locus coeruleus (LC)² (Szabadi 2013, Terbeck 2016, Bouras 2023), which in concert with both cortical and subcortical structures, plays an important role in the modulation and facilitation of higher order functions and the inhibition of irrelevant behaviours when confronted with a stressful or threatening environment (Szabadi 2013, McBurney-Lin et al. 2019). In fact, under such circumstances, the brain defaults to pre-programmed fight- or flight and other relevant habitual behaviours, which is made possible by the inhibitory effects of noradrenaline on normal cognitive and goal-directed executive routines (McBurney-Lin et al. 2019). Further, heightened noradrenaline release also blunts emotional processing which further enables the enactment of automatic responses.

Although direct traces of evidence are still overly lacking, it stands to reason that since OCD is a condition implicitly linked to inflated anxiety, noradrenergic modulation may change the long-term outcomes of pathophysiological trajectories that ultimately give rise to obsessive-compulsive symptoms (Tanaka et al. 2000). In fact, where specific fears, whether rational or not, e.g., failure to be control of one's safety,

¹ obsessive-compulsive disorder

² locus coeruleus

give rise to neutralising obsessive-compulsive symptoms, noradrenergic moderation could be of benefit. This theory has been tested in both pre-clinical (Lustberg et al. 2020) and clinical studies (Bandelow, Sher et al. 2012, Locher et al. 2017, Tilaki, Hasanzadeh et al. 2023) with success. In this work, we will also afford attention to the potential interactions between so-called visually perceived ‘danger-signals’ and limbic noradrenaline concentrations in the manifestation of compulsive-like LNB¹, as will be explained in **Chapter 3** of this dissertation.

2.5 Neurocognitive obsessive-compulsive theory of relevance for the present work

In the previous sections, it became clear that OCD² is highly heterogeneous and that different symptom phenotypes may arise because of perturbations across a range of neurocognitive domains. In this investigation, closer attention will be afforded to the impact of visual feedback, and thus so-called potential danger-signalling triggers or perceptions of ‘imperfection’, on the expression of naturalistic compulsive-like behaviour. Indeed, given the extensive crosstalk between the sensory systems and the neurobiological targets of relevance for OCD, an improved understanding of how visual cues may impact the expression of compulsivity, could be useful to advance our current approaches to psychological and neuropharmacological interventions. To this end, a summary of current neurocognitive theories, and how these might be explained by in OCD is needed.

2.5.1 Inflated safety-seeking responses and an intolerance of uncertainty

As alluded to earlier, the expression of obsessions and compulsions is closely related to underlying anxiety (Stein et al. 2010, Gillan et al. 2016). It would be important to stress that patients with OCD are not overly anxious in general. In other words, except in cases where anxiety and OCD are comorbid, OCD patients do not suffer from generalised anxiety disorders per se (Abramovitch and Cooperman 2015). Thus, some authors propose compulsions to result from highly specific and implicitly generated fear or anxiety about specific themes only, e.g., personal safety, contamination, or uncertain outcomes (Salkovskis 1985). Though implicitly generated, such anxiety and fears are often found to be triggered by sensory feedback. In humans, this is especially true for visual feedback, since specific perceptions of one’s environment guide our immediate responses towards specific scenarios (Woody and Szechtman 2011), with some individuals ascribing a greater degree of importance to specific safety-related cues, than others. Closely related to this theme is the concept of ‘uncertainty intolerance’, i.e.,

¹ large nest building

² obsessive-compulsive disorder

the inability to cope with uncertain outcomes; this in turn gives rise to the expression of behaviours aimed at instilling a self-perceived level of control over one's circumstances (Sarawgi et al. 2013).

In this sense, safety-seeking compulsions, e.g., excessive door-locking, can be viewed as inflated coping responses, whereas excessive counting or hoarding compulsions are seen as mitigators of uncertain futures. For both these explanations of OCD¹, visual feedback is paramount, since the perception of one's environment triggers these and other similar behaviours which are maintained until said perception is altered (Reid et al. 2021). Within this framework of understanding, hyperactivation of the vmPFC² and amygdala (Mataix-Cols et al. 2003, Lammertsma and van Dyck 2005) contributes to safety-seeking compulsions and uncertainty intolerance.

2.5.2 Perfectionism

Perfectionism is a highly prevalent trait observed in patients diagnosed with OCD (Pinto et al. 2006, Pinto et al. 2017), especially in those presenting with hoarding or ordering compulsions (Pinto et al. 2017) and in those diagnosed with obsessive-compulsive personality disorder. Perfectionism was first defined as the tendency to set and pursue unrealistically high standards within specific domains of one's daily life, as well as the overly critical evaluation of one's actions once a task is completed (Frost et al. 1990). It was only later when Shafran, Cooper and Fairburn (2002) proposed a second definition of perfectionism, which further defined the cognitive behavioural view of perfectionism as *“the overdependence of self-evaluation on the determined pursuit of personally determined, self-imposed standards despite adverse/unwanted consequences”*. Thus, in cases where perfectionism is a pivotal driver of compulsions, excessive engagement in behaviours aimed at 'just-right' outcomes is common (Grützmann et al. 2022). In extension, striving towards perfection also implies that the outcome of one's actions should be monitored. As such, hyperactive and excessive performance monitoring has also been shown in these cohorts (Endrass and Ullsperger 2014, Riesel et al. 2019), leading to the repetition of specific 'tried and tested' behavioural sequences that are self-reinforcing, i.e., being repetitively engaged in to perfect the outcome (in itself, an unrealistic goal) (Coles and Ravid 2016). Importantly, perfectionism within the context of OCD may be related to perceptions of one's own standards or that of society (Hewitt and Flett 1991, Pinto et al. 2017). In both instances, compulsions aimed at 'perfect' outcomes may born from exaggerated concerns of making mistakes, setting unrealistic standards for oneself, mitigating and responding to criticism, and self-doubt (Frost et al. 1993, Pinto et al. 2017).

A critical observation to make at this stage is that behaviours aimed 'perfect' outcomes are highly goal-directed and thus are founded within the sensory, motor, and cognitive domains of our functioning

¹ obsessive-compulsive disorder

² ventromedial prefrontal cortex

(Pinto et al. 2017), including those processes involved in error monitoring. As such OCD¹ patients presenting with perfectionism-related symptoms show increased activation of among others the prefrontal-cortico-striatal circuitry (Schlösser et al. 2010, Sadaghiani and D'Esposito 2014, Grützmann et al. 2022). Further, as is the case for safety- and uncertainty-related symptoms, the expression of perfection-seeking symptoms also heavily depends on visual feedback, which provides the basis for both error and action-outcome monitoring.

2.5.3 Habit proneness and inadequate response inhibition

Another neurocognitive theory put forward to explain compulsions is related to habits and the fact that it develops more easily in some individuals than others (Gillan 2021). Habits are formed by performing a specific sequence of behaviours repetitively to attain the same outcome within a specific context (Gillan et al. 2016). Thus, while most habits are born from goal-directed origins requiring a high degree of cognitive involvement, they gradually become habitual in the sense that a lesser degree of cognitive processing is needed to reach the same outcome (e.g., automatically depressing a brake pedal when a traffic light turns red). Several important characteristics of habits need to be mentioned. Habits are highly automated behaviours that are triggered by specific environmental (i.e., sensory) stimuli. It can also be associated or dissociated from a relevant functional outcome (Gruner and Pittenger 2017). The balance between goal-directed behaviour and mere habits is important for our normal functioning (Gillan et al. 2014, Gruner and Pittenger 2017, Gillan 2021). Whereas planned behaviours enable humans to complete complicated tasks, habits ensure that everyday functions can be performed without excessive cognitive input. However, when the functionality of this balance becomes disturbed, i.e., when habits become rigid and unresponsive to changes in context, psychopathologies, including OCD and addiction disorder, may arise.

Against this background, habits are associated with deficits in response inhibition, whereby individuals engage in specific behaviours without prior consideration of its outcomes. Inadequate response inhibition is a neurocognitive trait observed in among others OCD, gambling disorder, impulsivity, and substance use disorder (Verbruggen and Logan 2008). Thus, compulsions are construed to be rigid habits which are unsensitive to changes in realistic or sought-after goals. In fact, from neuropsychological study it is evident that OCD patients also tend to over-rely on habit and are unable to disengage from a specific behavioural sequence once it is reinforced (Gillan et al. 2014). On a neurobiological level, the mechanisms involved in habit formation are also closely intertwined with those underlying compulsive symptom presentation (irrespective of whether such compulsions are associated

¹ obsessive-compulsive disorder

with habit). It includes striatal, amygdalar, and cerebellar hyperactivation (Gillan et al. 2015, Liljeholm et al. 2015, Amaya and Smith 2018).

2.6 Visual perception as an integral component of action regulation

Vision enables humans and animals to perceive and interact with their immediate environments which further facilitates the acquisition of much-needed skills and behavioural aptitude. In fact, up to 85 % of all processes related to perception, learning, and cognition are dependent on sight (Thomas Politzer 2000). Given the manner of our interactions with the environment around us, this is not surprising (Renaud and Bédard 2013, Khorrami-Nejad et al. 2016). Since the present work will explore the extent to which the underlying mechanisms driving compulsive-like goal-directed behaviour in an animal model depend on visual perception (and in so doing explore the relationships between said behaviour and visually perceived danger- or imperfection-related triggers), a brief overview of how the two most prominent visual pathways, i.e., the dorsal and ventral streams, innervate the various neuroanatomical targets of relevance for OCD¹, will be provided here.

The dorsal visual pathway, otherwise known as the ‘vision-to-action’ pathway, enables humans and animals to plan and execute complex coordinated behaviours. It also underlies the consolidation and recall of spatial working memory and so enables visually guided actions and spatial navigation (Kravitz et al. 2011, Hebart and Hesselmann 2012, Kravitz et al. 2013, Rauschecker 2018).

After being captured by the various photoreceptors of the retina, optical impulses are primarily relayed to the visual cortex in the occipital cortex. The dorsal pathway, which originates here, then project to the parietal cortex via various pathways before ending in the frontal cortex (Kravitz et al. 2011, Kravitz et al. 2013, Rauschecker 2018). These different pathways all have distinct functions. For example, the parieto-prefrontal pathway innervates the prefrontal cortical areas necessary for spatial navigation, while the parieto-premotor pathway is responsible for the visual guidance of planned behaviour (Kravitz et al. 2013). Both functions are vital to plan and execute goal-directed behaviour (Curtis 2006, Funahashi 2006). Indeed, over-consolidation of spatial and contextual memory, which is recalled upon processing and acting upon specific environmental cues (Milner and Goodale 1993, Gallese et al. 1996, Kravitz et al. 2011), could be to blame for some forms of compulsivity, especially considering the intricate relationship between visual cues and compulsive engagement that is exploited during psychotherapy.

In the same manner, the parieto-medial temporal pathway, which is one of the most complex visual systems of the mammalian brain (Kravitz et al. 2011), can contribute to the expression of compulsive-like (and normal everyday) behaviour since it enables humans and animals to interact with and navigate

¹ obsessive-compulsive disorder

in their environments based on specific landmarks (Kravitz et al. 2011). To explain, this pathway is less involved in the execution of different types of fine coordinated movements, but rather provides contextual and circumstantial information regarding one's immediate surroundings (Kravitz et al. 2011). This pathway could thus be involved in conveying danger-related signals to among others, structures of the limbic system, e.g., the hippocampus (Kravitz et al. 2011), thereby modulating the expression of fear-based compulsions.

Compared to the dorsal pathway, the ventral pathway, that also originates in the primary visual cortex, projects to the temporal cortex, before it branches to various main sub-cortical and cortical areas (Kravitz et al. 2013). Projections to the neostriatum affects motor output as well action-outcome programming and selection. Over time, interactions between the temporal cortex and the neostriatum facilitate the acquisition of short- and long-term working memory, and so enable the formation of, among others, habitual behaviour (Fernandez-Ruiz et al. 2001, Kravitz et al. 2013). Tempero-visual projections to the ventral striatum underlie reward-directed and pro-social behaviour and provide the necessary feedback mechanisms needed for emotional regulation and the evaluation of positive and negative stimuli via limbic processing (Báez-Mendoza and Schultz 2013, Kravitz et al. 2013). In extension, the ventral pathway also innervates the amygdala, which enables the processing of visual stimuli in terms of its danger- and fear-related properties (Mirolli et al. 2010, Kravitz et al. 2013). Without the visual input from these connections, emotional regulation (Pessoa 2010, Kravitz et al. 2013), fear-induced avoidance responses, and fear memory consolidation and recall will not be possible (Murray and Wise 2010).

Visual projections from the temporal cortex to the OFC¹ are, in concert with those branching to the ventral striatum, responsible for informing positive and negative feedback (Kravitz et al. 2013). These projections are also vitally important to update pre-encoded associations between specific actions and their outcomes and may thus be compromised in patients with OCD² (Clark et al. 2004, Elliott and Deakin 2005, Menzies et al. 2008). In turn, ventral efferents innervating the vIPFC³ is an integral component for the executive (i.e., 'what-to-do-with') processing of all sensory and its associated non-sensory (i.e., emotional) information (Kravitz et al. 2013).

In summary, visual perception impacts our ability to interact with and respond to our environments. Further, for most phenotypes of OCD, perception of the environment impacts symptom presentation significantly. Whether by triggering safety-seeking behaviours or by signalling 'not-just-right' feelings, the question begs whether some phenotypic symptoms of OCD would be expressed as rigidly and persistently, would patients not be able to visually interact with their surroundings. This question opens

¹ orbitofrontal cortex

² obsessive-compulsive disorder

³ ventrolateral prefrontal cortex

another potential door for investigation. It will, for logical reasons, never be feasible to impair visual perception as a form of therapeutic intervention in OCD¹. However, it might be possible to modulate the valence and importance that is ascribed to *specific visual cues and its related contextual memories* to prevent the emotional and behavioural responses to such information. This is an especially intriguing possibility in modern medicine, considering the newfound ability of science to manipulate neuronal functioning on a neuron-by-neuron basis (Liu et al. 2014, Smiley et al. 2021). Further, understanding to what degree visual feedback is important for the aetiology, pathophysiology and promotion of obsessions and compulsions, could assist the field of neuropsychiatry to answer questions of causality, an aspect of the disorder that remains elusive until this day (Szechtman et al. 2017). This will be the focus of the present investigation.

2.7 Animal models as preclinical tools for the exploration of obsessive-compulsive mechanisms

During the past few decades, several animal model systems of compulsive-like behavioural persistence have been described, each presenting with unique advantages (Alonso et al. 2015). Animal models are useful instruments with which questions of aetiology, pathophysiology and cognition can be explored (Alonso et al. 2015). Models can further be exploited to identify novel targets for future drug and surgical therapies (Fineberg et al. 2011, Alonso et al. 2015, Wolmarans et al. 2018). Although some major aspects of OCD, i.e., obsessions, are difficult to replicate in animal models, it is not entirely impossible, with novel approaches to these dilemmas taken in the recent past that delivered some very interesting results (de Brouwer et al. 2020, Wolmarans et al. 2022). For animal models to be translationally relevant, they need to meet specific validation criteria. First, models must be founded on good face validity, which means that there should be phenotypical similarities between the behavioural output of humans suffering from the modelled condition and the animal. Secondly, there should be neurobiological and cognitive overlap between the human condition and the animal model system, which speaks to construct validity, while predictive validity refers to similarities in the therapeutic response (or non-response, for that matter) (Hoffman 2011, Alonso et al. 2015). Importantly, no animal model can truly reflect the full scope of a human condition, especially in the field of psychiatry. It should also be noted that most model systems are in a perpetual state of development, while translational application is often coincidental (Stanford 2020).

The present body of research build on extensive prior work conducted in our laboratory over the past 15 years and will focus on the potential impact of visual feedback on the expression of compulsive-like

¹ obsessive-compulsive disorder

LNB¹ in deer mice. To fully appreciate the present point of departure, a brief overview of this model system will be provided in this section.

2.7.1 The deer mouse model of compulsive-like behavioural persistence: general background

Deer mice (*Peromyscus maniculatus bairdii*) is native to North America and is found across most parts of the United States and Mexico. The genetic stock centre, which maintains deer mouse breeding pairs of all subspecies and strains, is housed at the University of South Carolina at Columbia in the United States. When bred and housed in captivity, deer mice, like many other animal species, varyingly begin to present with repetitive behavioural phenotypes. However, as opposed to all other rodent species, which develop comparable repetitive behaviours, e.g., excessive grooming and tail-chasing, deer mice present with somewhat unique behaviours that are only found in this species (Scheepers et al. 2018). The nature of these repetitive behaviours makes the model especially well-suited to study the naturalistic development of excessive and functionally purposeless behaviours.

Briefly, adult deer mice of both sexes naturalistically and spontaneously present with three forms of repetitive behaviours, i.e., high motor stereotypy (seen in 45 % of mice), high marble burying (shown by 11 – 15 % of mice) and LNB, which is expressed by 30 % of mice (Scheepers et al. 2018). Further, mice mostly show only one of these phenotypes, with roughly 25 % of mice that show no form of behavioural repetition at all. Each of the three forms of behaviour resembles OCD² at face value in that they are purposeless (but nonetheless ‘goal-directed’) within a laboratory-housed environment and persist over several successive testing sessions. Further, the behavioural presentation waxes and wanes much like the symptoms of OCD do, and animals engaging in these behaviours seem to disregard the importance of non-related stimuli (Scheepers et al. 2018, Wolmarans et al. 2022). Animals presenting with stereotypical motor expression also show markedly altered social behaviours (Wolmarans et al. 2017) and are able to ‘unlearn’ the need to engage in said behaviour (Burke et al. 2022). This distinguishes stereotypy in deer mice from the mere motor disturbances observed in other models (Martino and Hedderly 2019), which are reminiscent of tics or involuntary muscle contractions often seen in conditions like Parkinson’s disease and autism spectrum disorder.

The model is also founded on robust predictive and construct validity, with both stereotypical and LNB-expressing mice showing sensitivity to pro-serotonergic intervention (Scheepers et al. 2018). Also, stereotypical mice present with decreased striatal serotonergic signalling compared to normal behavioural controls (Wolmarans et al. 2013), while evidence for an imbalance between the direct and

¹ large nest building

² obsessive-compulsive disorder

indirect cortico-striatal pathways, as is the case in clinical OCD¹, has also been shown (Saaiman et al. 2023).

2.7.2 Large nestbuilding in the deer mouse model of OCD

Since the present work will solely focus on improving our understanding of LNB² in the model, a brief conceptual overview of this behavioural phenotype will be provided against the background of OCD.

In the wild, nesting behaviour plays a crucial role in the normal everyday existence of rodents. Nesting is expressed for various reasons which include thermoregulation and insulation, to protect offspring, to maintain social structures and to breed (Jirkof 2014, Lewarch and Hoekstra 2018). Nesting is also highly goal-directed and irrespective of whether the act of nesting is a carefully selected and evolutionary conserved behavioural pattern executed with a high degree of automation (Gaskill et al. 2013), it remains functional. To nest, rodents collect vegetation and other useful materials from their surroundings, which they use to construct intricate nests. Given the aforementioned functions of built nests, it could be argued that mice of a specific species and strain would exhibit similar nesting behaviours, especially in the laboratory. However, this is not the case, with 30 % of deer mice expressing excessive, persistent, and repetitive LNB that serves no unique function in a laboratory setting other than that already served by smaller (or 'normal') nests.

On a neurobiological level, LNB is associated with dysfunctional cortico-striatal signalling, where adenosinergic modulation of the indirect pathway fails to modulate the expression of LNB. In contrast, the same intervention exacerbates the expression of normal nesting behaviour (NNB)³, clearly indicating that the act of nesting itself, is fundamentally subject to cortico-striatal action-outcome control (Saaiman et al. 2023). These data point to striatal hyperactivation in LNB, but not NNB mice, which remains insensitive to moderating interventions. Also, LNB, but not NNB is responsive to chronic intervention with high doses of the SSRI⁴, escitalopram, administered alone or in combination with low doses of the non-selective dopamine D_{1/2} receptor antagonist, flupentixol (de Brouwer et al. 2020); this finding links LNB with a similar neurobiological construct as that which prevails in the clinical condition. Recently, we have also shown that the gut microbiota of LNB and NNB expressing mice differ significantly, with the microbiome of LNB mice also responding differently to escitalopram, compared to that of NNB-expressing mice (Karsten 2021). While data pertaining to gut microbiota dysbiosis in humans with OCD is scant (Turna et al. 2016), research is conclusive in showing that patients with neuropsychiatric illness

¹ obsessive-compulsive disorder

² large nest building

³ normal nest building

⁴ selective serotonin reuptake inhibitor

often present with perturbations in the gut microbiome (Bastiaanssen and Cryan 2021). Thus, these findings support the behavioural separation of deer mice on a biological level as well.

At a behavioural level, LNB¹ expressing mice fixate on the act of nesting, despite having to overcome both actual (de Brouwer et al. 2020) and perceived punishing and negative outcomes (Wolmarans et al. 2022). While mice showing LNB are able to function normally under circumstances where the opportunity to engage in nesting does not exist, they compulsively engage in the act of nesting when the context so provides. Such a response is not unlike that which is seen in clinical OCD², when patients respond to specific environmental cues in a compulsive manner (Abramovitch et al. 2015).

It would thus be important to conclude at this stage that although the identification and selection of LNB-expressing mice is based on subjective observation (as will be explained in **Chapter 3**), LNB expressing mice do indeed differ on both a cognitive and neurobiological level from their NNB³ expressing counterparts, a finding that provides the foundation for this work.

Nevertheless, despite the goal-directed nature of nesting behaviour itself, it is still unclear whether LNB in deer mice is dependent on visual feedback which may be informing and guiding processes of action-outcome monitoring, and thus the expression of nesting. Specifically, it is unknown whether LNB is related to processes regulating 'just-right' (and thus 'perfectionism'-like) perceptions as was shown for other rodent species before (Hoffman and Morales 2009). Similarly, it is unknown if mice presenting with LNB, compared to mice showing NNB, show a lower threshold for anxiety-sensitivity in response to visual cues that they may perceive in the environment. Alternatively, the behaviour might be entirely driven by evolutionary conserved habitual processes that are inadequately governed by the normal stop-and-go signals that govern behavioural output. In this work, we will attempt to explore these questions to strengthen the model for future application in translational work seeking to improve the current therapeutic approaches to clinical OCD.

¹ large nest building

² obsessive-compulsive disorder

³ normal nest building

2.8 References

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Article title

Naturalistic large nest building in deer mice is associated with increased hippocampal noradrenergic signalling despite showing visuomotor stability: perspectives on compulsive-like behavioural persistence

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Author contributions

HM designed and executed the experimental work, performed all statistical analyses and interpreted the results of this study under the supervision of DWW. HM also drafted the first version of the manuscript. DWW and TEK were the supervisor and co-supervisor of the M.Sc. project, respectively. DWW conceptualised and designed the investigation with HM and performed some of the behavioural experimentation. All authors contributed equally to the revision of the first version of this manuscript and drafting the final version, included here.

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Important information

- As per the guidelines to authors of *Behavioural Brain Research*, figures are provided at the end of this manuscript following the reference list.

Naturalistic large nesting behaviour in deer mice is associated with increased hippocampal noradrenaline and shows visuomotor stability: perspectives on compulsive-like behavioural persistence

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Abstract

Visual stimuli and limbic activation can variably influence obsessive-compulsive symptom expression and so impact treatment outcomes. Indeed, some symptom phenotypes, e.g., covert thoughts with repugnant themes, are likely less sensitive to sensory stimuli compared to symptoms with an extrinsic focus, i.e., symptoms related to contamination, safety, and 'just-right-perceptions' and thus show a poor response to psychotherapies based on exposure-response prevention. Towards an improved understanding of the neurocognitive underpinnings of obsessive-compulsive psychobiology, work in naturalistic animal model systems is useful. Here, we explored the impact of visual feedback and limbic processes on 24 normal (NNB)¹ and large (LNB)² nesting deer mice, respectively (as far as possible, equally distributed between sexes). Briefly, after behavioural classification into either the NNB or LNB cohorts, mice of each cohort were separated into two groups each and assessed for nesting expression under either standard 12-h light-dark conditions (SL)³ or conditions of complete visual deprivation (VD)⁴ applied during the dark cycle. Nesting outcomes were assessed in terms of size and neatness. After nesting assessment completion, mice were euthanised, and samples of frontal-cortical and hippocampal tissues were collected to determine serotonin and noradrenaline concentrations. Our results show that LNB, as opposed to NNB, represents an inflexible and excessive behavioural phenotype that is not dependent on visually guided action-outcome processing, and that it associates with increased frontal-cortical and hippocampal noradrenaline concentrations, irrespective of lighting condition. Collectively, the current results are informing of the neurocognitive underpinnings of normal nesting behaviour. It also provides a valuable foundation for continued investigations into the noradrenergic mechanisms that may influence the development and promulgation of excessive, rigid, and inflexible behaviours.

Keywords

obsessive-compulsive disorder; anxiety; deer mouse; visual perception; nest building

¹ normal nest building

² large nest building

³ standard 12-h light-dark

⁴ visual deprivation

3.1 Introduction

Rigid and repetitive behavioural phenotypes are characteristic of several neuropsychiatric disorders, including obsessive-compulsive disorder (OCD)¹, of which the main symptoms are obsessions (intrusive thoughts or ideas), compulsions (rigid, inflexible, and repetitive covert or overt behaviours) or both (APA 2013). Compulsions are mostly expressed in response to an underlying, often anxiety-provoking obsession about specific themes, e.g., safety or potential harm to oneself or others (Abramovitch and Cooperman 2015). Also, obsessions related to perfectionistic ideals may contribute to among others, symmetry and ordering compulsions (Hood and Antony 2016) and cognitive rigidity (Egan et al. 2007). Although obsessions and compulsions are mostly ego-dystonic, that is, patients show insight into the irrationality of their symptoms, disengagement from compulsive symptom expression is problematic (Abramovitch et al. 2019). While all obsessions are born from implicit processes, some symptom phenotypes, e.g., covert thoughts with repugnant themes, are likely less sensitive to sensory stimuli, compared to symptoms with an extrinsic focus, i.e., symptoms related to contamination, safety, and 'just-right-perceptions'. This is reflected by the mostly positive response of the latter symptoms to exposure and response prevention (ERP)². This modality of psychotherapy exposes patients to aspects of their obsessive thoughts while they are prevented from engaging in compulsive neutralising rituals (Hezel and Simpson 2019). An important premise for the successful application of ERP is the ability of patients to process sensory feedback and thus higher rates of treatment resistance are observed with covert mental routines that can, in many instances, not be triggered by external cues (Mataix-Cols et al. 2002, Bednarek et al. 2022). Still, treatment resistance remains a clinical obstacle as ERP, which is highly anxiogenic, is associated with high patient drop-out rates (Middleton et al. 2019). Thus, the psychobiological underpinnings of different obsessive-compulsive symptom phenotypes must be better understood. In fact, the relationship between external cues and compulsive symptom expression remains unclear, while poor insight and high anxiety sensitivity may contribute to poor treatment outcomes (Abramovitch et al. 2015, Blakey et al. 2017, Ojalehto et al. 2020).

Research is congruent in pointing to dysfunctional serotonergic and dopaminergic regulation of cortico-striatal processes underlying the disorder (Calzà et al. 2019, Robbins et al. 2019). Specifically, heightened activation of cortico-striatal circuits, which comprise the orbitofrontal cortex (OFC)³, the anterior cingulate cortex (ACC)⁴, the ventro-lateral (vlPFC)⁵, ventro-medial (vmPFC)⁶, and dorso-lateral

¹ obsessive-compulsive disorder

² exposure and response prevention

³ orbitofrontal cortex

⁴ anterior cingulate cortex

⁵ ventro-lateral prefrontal cortex

⁶ ventro-medial prefrontal cortex

(dlPFC)¹ prefrontal cortices, as well as the basal ganglia, is a common finding (Calzà et al. 2019, Shephard et al. 2021). Further, different obsessive-compulsive phenotypes are likely related to perturbations in discrete neural circuits (Shephard et al. 2021). Five of these circuits are of special relevance, i.e. the ventral affective, ventral cognitive, dorsal cognitive, fronto-limbic, and the sensorimotor circuit (Haber 2022). For example, dysfunctional ventral affective processes, of which OFC² hyperactivation is a core feature (Shephard et al. 2021), contributes to behavioural inflexibility (Hikosaka and Watanabe 2004, Menzies et al. 2008). In turn, inadequate behavioural adaptation to action-outcome feedback and changes in context, is associated with vlPFC³ and dlPFC perturbations (Sakagami and Pan 2007), both integral components of the ventral and dorsal cognitive circuits (Sakagami and Pan 2007, Haber 2022). Considering the anxiogenic nature of obsessive intrusions, OCD⁴ is also associated with a high degree of limbic involvement (Wood and Ahmari 2015). Hyperactivation of the limbic system, which includes the ACC⁵ and vmPFC⁶, the hippocampus, and the amygdala, may contribute to intolerance of uncertainty and inflated safety-seeking behaviour, both of which are seen in patients suffering from safety/checking OCD (Lind and Boschen 2009). Last, sensorimotor impairment can contribute to compulsive behaviours that are aimed at perfectionistic outcomes and it also underpins excessive habit formation, a neurocognitive endophenotype of OCD (Gillan et al. 2011).

Recently, interest into the potential contributions of the noradrenergic system to OCD has also emerged (Hatayama and Aruga 2023). However, since antidepressants with a noradrenergic mechanism of action are mostly ineffective in the treatment of OCD (Kellner 2022), some explanation is needed. Under resting state conditions, moderate concentrations of frontal-cortical noradrenaline, which is primarily released from noradrenergic projections originating in the locus coeruleus (LC)⁷, inhibit amygdalar reactivity, and thus also anxiety- and fear-related processing in the limbic system (Coley et al. 2021, Bouras 2023). This allows for normal executive functions to take precedent. However, once specific sensory or emotional stimuli activate the limbic system, a transient increase in amygdalar noradrenaline exerts inhibitory control over prefrontal-cortical processing (McCall et al. 2015) and so facilitates an optimal degree of arousal, attention, and fight-or-flight readiness (Arnsten 2011). Importantly, patients suffering from anxiety disorders consistently show amygdalar hyperactivation (Boehme et al. 2014), which may point to a lower anxiety sensitivity threshold or alternatively, a loss in top-down frontal-

¹ dorso-lateral prefrontal cortex

² orbitofrontal cortex

³ ventro-lateral prefrontal cortex

⁴ obsessive-compulsive disorder

⁵ anterior cingulate cortex

⁶ ventro-medial prefrontal cortex

⁷ locus coeruleus

cortical control over limbic activation (Bouras 2023); this also explains why alpha (α) 1- and beta (β) adrenoceptor activation contributes to the manifestation of anxiety- and fear-related symptoms post-trauma (Gazarini et al. 2013).

Against this background and considering the known anxiogenic role of environmental cues on the phenotypic presentation of certain phenotypes of OCD¹, it is important to highlight specific findings and case reports that implicate a role for noradrenergic processes in the disorder. For example, patients with contamination-related OCD present with increased noradrenaline release when undergoing ERP² (Fluitman et al. 2010). Also, the prevention of fear memory consolidation by administering D-cycloserine prior to ERP, was associated with significantly decreased obsession-related distress, post-treatment (Kushner et al. 2007). Recent data from a preclinical murine model also supports a role for noradrenergic neurotransmission in the manifestation of stressor-induced repetitive behaviours (Lustberg et al. 2020, Lustberg et al. 2020). It is therefore plausible that noradrenaline, via its modulation of limbic processes, could contribute to the pathogenesis of certain obsessive-compulsive phenotypes that may be more contingent on anxiety- and fear-related stimuli, than others. Furthermore, noradrenaline is also a key neuromodulator of perceptual sensitivity and visual awareness (Gelbard-Sagiv et al. 2018), both fundamentally related to how environmental cues are perceived.

Under standard laboratory conditions, approximately 30 % of deer mice (*Peromyscus maniculatus bairdii*) spontaneously display excessively large nesting behaviour (LNB)³. Within a controlled environment, such large nests serve no unique purpose. In fact, in the wild, all rodents nest for thermoregulatory, safety, breeding, and nursing purposes as well as to defend and maintain a social hierarchy (Smithers 1983, Jirkof 2014, Wolmarans et al. 2016, Lewarch and Hoekstra 2018). None of these functions are of relevance (or needed) in a controlled laboratory environment, where mice are assessed for nesting expression in isolation and under the same circumstances. Therefore, LNB is reminiscent of compulsive-like behavioural persistence, since it is a highly goal-directed, though excessive, behavioural phenotype (de Brouwer et al. 2020) shown by mice of both sexes. It further persists over the course of several assessment sessions (Wolmarans et al. 2022), and the expression thereof prevents flexible behavioural switching or engagement in other rodent behaviours (Wolmarans et al. 2022). In terms of drug response, LNB, but not normal nesting behaviour (NNB)⁴ responds to chronic high-doses of the selective serotonin reuptake inhibitor (SSRI)⁵, escitalopram, administered either as monotherapy, or in combination with low doses of the non-selective anti-dopaminergic drug,

¹ obsessive-compulsive disorder

² exposure and response prevention

³ large nest building

⁴ normal nest building

⁵ selective serotonin reuptake inhibitor

flupentixol (de Brouwer et al. 2020). On a neurobiological level, the expression of nesting behaviour itself is rooted within and controlled by cortico-striatal processes, with NNB¹ being inflated by anti-adenosinergic activation of striatal signalling (Saaiman et al. 2023). In contrast, LNB² is insensitive to the same, hinting at a perpetual state of striatal hyperactivation in LNB, compared to NNB mice (Saaiman et al. 2023). Neurobiological dissimilarity also extends to the gut microbiome, with NNB and LNB mice presenting with distinct gut microbiota profiles. This highlights the involvement of gut-brain control mechanisms in uniquely regulating the behavioural output (Scheepers et al. 2019) of large, as opposed to normal nesting mice. NNB and LNB expressing mice have also been studied for potential differences in their anxiety-like profiles. While the two cohorts do not separate in terms of their behaviour in tests of generalised anxiety, i.e., a standard open field test (Saaiman et al. 2023), certain traces of evidence point to differences in anxiety sensitivity that must be highlighted. Firstly, LNB, compared to NNB mice, are overly resistant to negative and aversive feedback and will voluntarily recruit nesting material despite being demotivated through self-inflicted punishment, i.e., electric foot shock (de Brouwer et al. 2020). Secondly, LNB mice show more risk-engaging behaviour than NNB mice and will access potentially dangerous and anxiogenic environment in order to obtain nesting material (Wolmarans et al. 2022). In this sense, LNB expressing mice, in contrast to NNB mice, seem to fixate on the act of nesting itself, despite the potentially harming and detrimental consequences of their behaviour.

Nevertheless, the motivational triggers that drive LNB are unknown. Specifically, it is unclear to what end deer mice depend on visual feedback to recruit the necessary executive starting and stopping mechanisms that regulate this behaviour. In extension, it would be important to establish if LNB in deer mice is related to 'just-right' feedback, as is true for some other nesting animal species, e.g., rabbits (Hoffman and Rueda Morales 2009), or alternatively, whether it is merely a persistent habit-like behaviour which remains inflexible irrespective of visual feedback. Last, it would be valuable to ascertain if LNB is associated with a distinct degree of limbic involvement that is either extrinsically or implicitly modulated. The present work therefore sought to explore NNB and LNB under both standard and amended light conditions, where some mice were assessed for nesting expression under conditions of total visual deprivation (VD)³. We further measured frontal-cortical and hippocampal serotonin and noradrenaline concentrations as markers of anxiety-related limbic involvement.

¹ normal nest building

² large nest building

³ visual deprivation

3.2 Materials and methods

3.2.1 Study layout

The present work was divided into two nesting assessment phases (P1 and P2), as will be explained further down. In P1, an initial pool of 100 mice was screened for nesting expression over one week. From these, 24 mice were selected for inclusion in ‘normal’ (NNB)¹ and LNB² expressing cohorts, respectively ($n = 48$ in total). These 48 mice were again assessed in P2, with half of each cohort maintained under a standard 12-h light dark cycle, and the other half under conditions of total light deprivation during the dark cycle (see below). Following the last night of the P2 assessment, mice were euthanised to harvest frontal-cortical and hippocampal tissue (**Figure 3-1**).

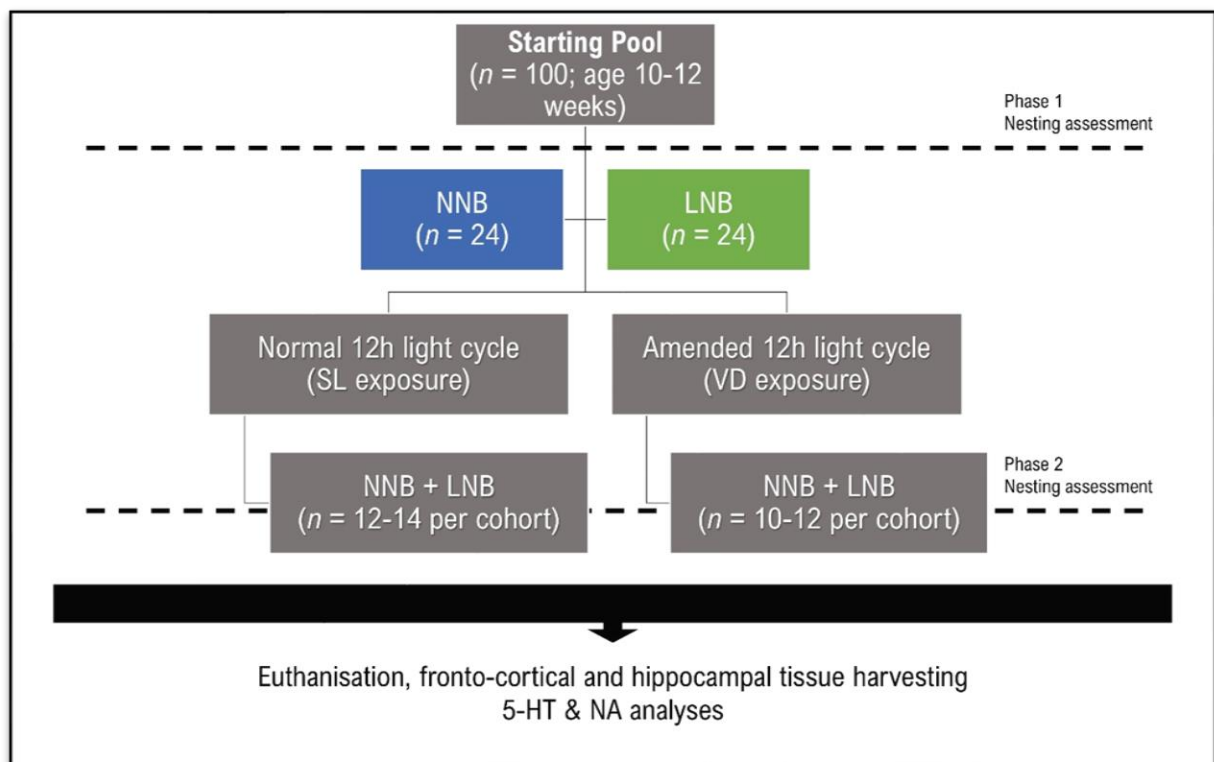


FIGURE 3-1: Schematic representation of study layout

NNB: normal nest building; LNB: large nest building; SL: standard 12-h light-dark; VD: visual deprivation; Serotonin: 5-HT; Noradrenaline (NA).

¹ normal nest building

² large nest building

3.2.2 Mice

Since only 20 – 30 % of deer mice express LNB¹ in the laboratory (Wolmarans et al. 2013, Wolmarans et al. 2016), an initial pool of 100 deer mice (*Peromyscus maniculatus bairdii*) of both sexes were included for initial nesting assessment (ethical approval number: **NWU-00769-22-A5**; AnimCare Research Ethics Committee, North-West University (NWU)². All mice were between the ages of 10 and 12 weeks at the onset of the experimentation. Animals were bred, reared, and housed in the North-West University Vivarium, Potchefstroom, South Africa (SAVC³ registration number: FR15/13458; AAALAC accreditation file: 1717). All behavioural experiments were performed in the same facility and complied with the South African National Standard (SANS)⁴ for the Care and Use of Animals for Scientific Purposes (SANS 10386:2008). Prior to nesting assessment, mice were group-housed (4 – 6 same-sex animals per cage) in individually ventilated and climate-controlled (IVC)⁵ cages [35 cm (l) x 20 cm (w) x 13 (h) cm; Techniplast® S.P.A., Varese, Italy], after which each mouse was allocated to its own home cage. Cages were kept ambient at 23 °C with a relative humidity of 55 % on a 12-hour light/dark cycle (lights on at 6h00), except if stated otherwise. Food and water were available *ad lib* during all phases of rearing and experimentation. Mice had access to paper towel as a form of nesting material until experimentation began. Henceforth, only cotton wool was supplied (see below). Cages were cleaned and fresh corncob bedding and paper towel (where applicable) added weekly.

3.2.3 Nesting assessment

3.2.3.1 Conventional nesting assessment and behavioural categorisation

During P1 (days 1 – 7), the assessment was aimed at measuring the baseline nesting expression of deer mice and to select 24 NNB⁶ and LNB expressing mice for further study from the 100 initially included mice (Wolmarans et al. 2016, De Brouwer et al. 2020). For one week, between 16:00 and 17:00, cages were supplied with an excess of pre-weighed, unscented hospital-grade, cosmetic cotton wool, which was placed in the roof of the cage. The following morning at 06:00, the remaining cotton wool, i.e. wool not utilised for nesting purposes, was weighed, and the built nests discarded. Thus, mice had access to nesting material for at least fourteen hours of every 24-h cycle, which included the complete dark cycle, i.e., with normal ambient night-time lighting able to penetrate cages. Mice mostly engage in nesting behaviour during the dark cycle, especially in the period prior to dawn (or lights on)

¹ large nest building

² North-West University

³ South African Veterinary Council

⁴ South African National Standard

⁵ individually ventilated and climate-controlled

⁶ normal nest building

(Jirkof 2014, Wolmarans et al. 2016). Importantly, prior to the onset of P1, mice had no experience with cotton wool as nesting material, nor with its placement in the roof of the home cage.

Classification of LNB¹ is primarily based on i) the total nesting-score generated over seven days (i.e., the cumulative weight of all the cotton wool used to construct nests over one week), and ii) the behavioural variance observed with respect to the between-day nesting performance over this period, reflected as a percentage coefficient of variance (% CV)² value (Wolmarans et al. 2016). For the purposes of this work, as well as all prior nesting studies done in our laboratory, LNB is broadly defined as nesting scores that group together within upper quartile (75th percentile) of the total nesting score distribution *and* the lower quartile (25th percentile) of the % CV distribution (Saaiman et al. 2023) (**Figure 3-2**). In contrast, NNB³ is identified as total nesting scores that consistently clustered between the 25th and the 50th percentile of the total score distribution. After selection of the 24 NNB and LNB expressing mice, the remaining 52 mice were euthanised.

3.2.3.2 *Assessment of nesting behaviour under conditions of visual deprivation*

In P2 (days 8 – 14), the NNB and LNB expressing mice selected in P1, were further divided into two groups each. One group of each cohort was again assessed as explained above (i.e., undergoing the same procedure for a second week). However, the other group was now assessed under conditions of total VD⁴, i.e., without any ambient visible, ultraviolet, or infrared light able to penetrate cages. For the NNB group, 14 mice (5 female) were allocated to a standard 12-h light-dark (SL)⁵ condition, while 10 mice (6 female) were included in the VD group. For the LNB group, 12 mice (6 female) were included in the SL group, while 12 mice (4 female) were assessed under conditions of VD. Although all effort was made to ensure the allocation of an equal number of mice to each of the groups, the use of mice of similar ages at the time of testing (which were not necessarily equally distributed between sexes), took precedence.

To assess mice under conditions of VD, IVCs⁶ were equipped with pre-weighed cotton wool and wrapped in light-tight, black polyvinyl chloride (PVC)⁷ covers (Chen et al. 2021) at 17:00 every day. At 06:00 every following day, covers were removed, nests weighed, and the remaining cotton wool discarded. The same timeline was followed for SL cages. Thus, all mice experienced a similar day (light)

¹ large nest building

² coefficient of variance

³ normal nest building

⁴ visual deprivation

⁵ standard 12-h light-dark

⁶ individually ventilated and climate-controlled

⁷ polyvinyl chloride

environment from 06:00 – 17:00 every day. Covers had tight-fit openings around air inlets and outlets to ensure uninterrupted flow of air.

The statistical analyses and graphical representations pertaining to these analyses, are expressed as values that reflect the difference (in percentage) between the behavioural output parameters observed in P2, and that of P1, i.e., $\left(\frac{BehP2 - BehP1}{BehP1}\right) * 100$, where “Beh” represents the measured output (total nesting score or % CV¹) for both nesting assessment phases.

3.2.3.3 Qualitative scoring of nesting behaviour

To assess the value of visual feedback on nesting performance, photographs of nests were scored for i) neatness (using a roundness index), ii) firmness (applying a shadow index), and iii) area (expressed in terms of image pixels). To ensure objective scoring, photographs were taken of each cage in the same location under the same lighting conditions, with the camera set at the same distance and magnification every day over all days of the P2 assessment (26 mm equivalent wide-angle, *f*1.8, 12 MP, Sony® Corporation, Tokyo, Japan; **supplementary images**). Image analyses were performed with ImageJ2® open-source software (National Institutes of Health and the Laboratory for Optical and Computational Instrumentation, University of Wisconsin, USA).

3.2.4 Quantification of frontal-cortical and hippocampal monoamines

3.2.4.1 Reagents and chemicals

Serotonin, noradrenaline, and 5-hydroxymethyl tolterodine (5-HMT²; internal standard) were all acquired from Sigma-Aldrich Chemicals Pty (Ltd) (Johannesburg, South Africa). Chemicals that were used for the mobile phase included HPLC grade deionised water (Milli-Q® water), HPLC grade acetonitrile (ACN)³, sodium formate, 1-heptanesulphonic acid sodium salt, ethylenediaminetetraacetic acid (EDTA)⁴ disodium salt, and orthophosphoric acid (H₃PO₄ 85 %), all of which were acquired from Merck Pty (Ltd) (Johannesburg South, Africa). Additionally, the chemicals used for the frontal-cortical and hippocampal sample preparation were HPLC grade methanol and perchloric acid (HClO₄)⁵, which were also obtained from Merck Pty (Ltd) (Johannesburg South, Africa).

All mice were euthanised by means of cervical dislocation (Underwood and Anthony 2020) on the morning following the last night of P2. The whole brain of each mouse was removed on ice and the

¹ coefficient of variance

² 5-hydroxymethyl tolterodine

³ acetonitrile

⁴ ethylenediaminetetraacetic acid

⁵ perchloric acid

frontal cortex and hippocampus collected. Tissues were immediately flash frozen in liquid nitrogen and stored at -80 °C until analysis.

On the day of analysis, tissues were allowed to thaw on ice, weighed and added to 1.5 mL Eppendorf Safelock[®] tubes containing 200 µL of the sample preparation solution (50 % HPLC grade methanol in Milli-Q[®] water) to which 5-HMT (internal standard) was added at a concentration of 0.1 µL/mL. Tissues were then homogenised via sonication (twice for 12 sec at an amplitude of 14 µ), which is the preferred method to achieve complete disruption of cell membranes in small samples (Keller et al. 1976). The tubes were subsequently allowed to rest on ice for 20 min to allow for protein precipitation and the extraction of monoamines. Samples were then vortexed and centrifuged at 20817 RCF (14000 rpm), while being maintained at 4 °C (Eppendorf 5430R centrifuge). The supernatant was collected and transferred to clean Safelock[®] tubes to which 25 µL of a 0.1 M HClO₄¹ in water solution was added. Tubes were vortexed for 15 sec and again centrifuged for 20 min as described above. The resultant supernatants were then analysed for serotonin and noradrenaline by means of ultra high-performance liquid chromatography with electrochemical detection (UHPLC-EC)² according to the method of Viljoen et al. (2018).

Briefly, standard curves for serotonin and noradrenaline were set up from readings obtained from six standards per analyte of which the concentrations ranged from 5 to 200 ng/mL. Samples were analysed on an UltiMate[®] 3000 UHPLC system (Thermo Fisher Scientific[®], Waltham, MA, USA) equipped with an ISO-3100SD isocratic pump, a WPS-3000TSL analytical autosampler and an electrochemical ECD-3000RS[®] detector (Thermo Fisher Scientific[®], Waltham, MA, USA). Chromatographical separation was achieved using a Venusil[®] ASB C8 5 µm column (4.6 × 250 mm; Bonna-Angela Technologies[®], Torrance, CA, USA). The mobile phase consisted of a 0.1 M sodium formate buffer containing 5 mM sodium 1-heptanesulfonate, 0.17 mM EDTA³ and 5 % v/v ACN⁴. The pH of the mobile phase was set at 4 with orthophosphoric acid. The system injected 25 µL of each sample onto the column, with the flow rate set at 1.0 mL/min. Data were analysed using Chromeleon[®] chromatography software (version 7.2; Thermo Fisher Scientific[®], Waltham, MA, USA). Results are expressed as ng/g of the wet weight of the brain tissue.

¹ perchloric acid

² ultra high-performance liquid chromatography with electrochemical detection

³ ethylenediaminetetraacetic acid

⁴ acetonitrile

3.2.5 Statistical analyses

All statistical analyses were performed with GraphPad® Prism® version 10 (GraphPad®, San Diego, California). A Spearman's rank-order correlation was used to assess the relationship between the individual total nesting scores generated by all mice assessed in P1 and the respective % CV¹ calculated for each mouse (**Figure 3-2**). Changes in total nesting scores and % CV from P1 to P2 (expressed as a percentage change from P1 to P2), as well as qualitative nesting attributes as scored during P2, were compared by means of ordinary two-way analysis of variance (2-way ANOVA²). The same was applied to compare the frontal-cortical and hippocampal serotonin and noradrenaline concentrations of each of the groups. The respective parameters that were measured were set as dependent factors, while nesting phenotype and light condition (hereafter 'condition') were set as independent variables. Where significant interactions between phenotype and light conditions, or a main effect of either independent factor, were observed, ANOVA analyses were followed by Bonferroni's post-hoc pairwise comparisons. Statistical significance was set at $p < 0.05$ for all analyses, and strong trends were identified where $0.05 > p > 0.07$. The magnitude of pairwise differences was further informed by means of Cohen's d effect size calculations, which were supported by the 95 % confidence interval of d (95CI)³. Only large effects sizes ($d \geq 0.8$) are shown. Prior to analysis, datasets were explored for outliers by means of Grubb's test. In cases where outliers have been removed, clear indication is given in the relevant results sections.

3.3 Results

3.3.1 Nesting behaviour

3.3.1.1 Baseline selection of LNB and NNB animals

A Spearman's rank-order correlation was run to explore the relationship between the total nesting scores generated by each of the 100 initially assessed mice and the respective % CV values calculated with respect to the daily nesting scores (**Figure 3-2**). A statistically significant and strong negative linear relationship was found ($r_s(112) = -0.595 [-0.71, -0.46]$, $p < 0.0001$). The distribution of data points was subsequently applied to select NNB and LNB mice, as explained earlier.

¹ coefficient of variance

² analysis of variance

³ 95 % confidence interval

3.3.1.2 Quantitative changes in nesting behaviour between P1 and P2

With respect to the change in the total nesting scores from P1 to P2, one outlier was identified and removed from the NNB¹ VD² group. Two-way ANOVA³ did not reveal a significant phenotype-condition interaction [$F(1, 43) = 0.03, p = 0.874$; **Figure 3-3A**]. However, a significant main effect of phenotype [$F(1, 43) = 14.60, p = 0.0004$], but not of condition [$F(1, 43) = 1.55, p = 0.220$] was shown. Specifically, compared to the total nesting scores of LNB⁴ mice, the scores of NNB mice inflated during P2, irrespective of light condition (**SL**⁵: 1.5 ± 31.6 vs. 41.8 ± 40.4 %, $p = 0.017, d = 1.1$ [0.26, 1.92]; **VD**: 13.5 ± 22.9 vs. 57.2 ± 52.0 %, $p = 0.021, d = 1.1$ [0.20, 2.08]).

In terms of changes in nesting consistency as reflected by the % CV⁶ values of P2 vs. P1, an outlier was removed from each of the VD LNB and VD NNB groups. Here again, phenotype and condition did not interact significantly to impact the result [$F(1, 42) = 0.00, p = 0.983$; **Figure 3-3B**]. A main effect of condition [$F(1, 42) = 9.88, p = 0.003$], but not of phenotype [$F(1, 42) = 1.24, p = 0.272$], was however observed. Subsequent pairwise comparisons showed that mice of both phenotypes trended towards expressing nesting activity with less variance under conditions of VD, compared to SL (**NNB**: -29.2 ± 21.7 vs. 14.4 ± 44.3 %, $p = 0.070, d = 1.1$ [0.25, 2.07]; **LNB**: -14.0 ± 29.1 vs. 30.2 ± 70.2 %, $p = 0.058, d = -0.8$ [-1.65, 0.05]).

3.3.1.3 Qualitative nest attributes scored for P2

3.3.1.3.1 Nesting roundness

Nesting roundness (expressed as an index from 0 – 1, with ‘1’ being the most round) was scored as a measure of neatness. No statistically significant interaction between phenotype and condition was shown [$F(1, 44) = 0.12, p = 0.735$; **Figure 3-4A**]. Phenotype [$F(1, 44) = 9.73, p = 0.003$], but not condition [$F(1, 44) = 0.17, p = 0.679$], significantly impacted nesting roundness, with LNB mice in the VD group, constructing nests with a higher roundness index, than VD NNB mice (0.8 ± 0.03 vs. $0.7 \pm 0.1, p = 0.047, d = 1.0$ [0.14, 1.94]). This difference was not significant for mice assessed in the SL condition (0.8 ± 0.05 vs. $0.7 \pm 0.1, p = 0.092, d = 0.8$ [-0.03, 1.58]).

3.3.1.3.2 Nesting solidity

The solidity of built nests was also scored as an index of neatness. For this, an analysis of shadow variance was performed with ImageJ2[®] and expressed as an index of solidity on a scale from 0 – 1 (with ‘1’ being the most ‘solid’, i.e., with the least shadow variance). Again, no significant interaction was

¹ normal nest building

² visual deprivation

³ analysis of variance

⁴ large nest building

⁵ standard 12-h light-dark

⁶ coefficient of variance

evident between phenotype and condition [$F(1, 44) = 0.52, p = 0.475$; **Figure 3-4B**]. A main effect of phenotype [$F(1, 44) = 8.79, p = 0.005$], but not for condition [$F(1, 44) = 0.003, p = 0.960$] was shown. Specifically, VD¹ LNB² mice built nests with a higher solidity index compared to VD NNB³ mice (0.9 ± 0.01 vs. $0.8 \pm 0.1, p = 0.032, d = 0.9 [0.06; 1.84]$), a difference that was not seen between LNB and NNB mice housed under SL⁴ conditions (0.9 ± 0.03 vs. $0.8 \pm 0.1, p = 0.209, d = 0.7 [-0.07, 1.53]$).

3.3.1.3.3 Nesting area

Nesting area (expressed as pixels) was analysed as a third measure of nesting neatness and integrity. A significant main effect of phenotype [$F(1, 44) = 24.22, p < 0.0001$], but not of light condition [$F(1, 44) = 0.42, p = 0.522$] was again shown in the absence of a significant two-way interaction [$F(1, 44) = 0.03, p = 0.864$; **Figure 3-4C**]. Bonferroni post-hoc testing revealed that the average nesting area generated by LNB mice was larger compared to that of NNB subjects, irrespective of light condition (**SL**: 193878 ± 47480 vs. 118397 ± 51784 pixels, $p = 0.002, d = 1.5 [0.62, 2.38]$; **VD**: 206874 ± 60782 vs. 125906 ± 58895 pixels; $p = 0.002, d = 1.3 [0.40; 2.27]$).

3.3.2 Frontal-cortical and hippocampal serotonin and noradrenaline

Due to unexpected sample loss on the day of analysis, the results reported in this section are based on the sample numbers as reflected in **Table 3-1**.

Table 1 - Group allocation for frontal-cortical and hippocampal monoamine analysis

	Frontal cortex (n)	Hippocampus (n)
NNB SL Group n = 14	14	14
LNB SL Group n = 12	10	12
NNB VD Group n = 10	9	9
LNB VD Group n = 12	11	11

3.3.2.1 Serotonin

With respect to the frontal-cortical serotonin concentrations of NNB and LNB mice, three samples were below the limit of detection. No significant phenotype-condition interaction [$F(1, 37) = 2.79, p = 0.103$; **Figure 3-5A**], nor any significant main effect of phenotype [$F(1, 37) = 0.1902, p = 0.665$] or condition [$F(1, 37) = 1.52, p = 0.225$] influenced the result. With regards to hippocampal serotonin concentrations

¹ visual deprivation

² large nest building

³ normal nest building

⁴ standard 12-h light-dark

of NNB¹ and LNB² mice, four samples were below the limit of detection. However, while there was also no interaction observed with respect to hippocampal serotonin [$F(1, 38) = 0.14, p = 0.714$; **Figure 3-5B**], condition significantly impacted the data [$F(1, 38) = 5.35, p = 0.026$]. The same was not true for phenotype [$F(1,38) = 1.52, p = 0.225$]. Nevertheless, Bonferroni post-hoc tests failed to reveal significant between-condition pairwise comparisons.

3.3.2.2 Noradrenaline

Three frontal-cortical samples were below the limit of detection. A significant phenotype-condition interaction was shown [$F(1, 37) = 4.59, p = 0.0388$; **Figure 3-5C**]. Bonferroni post-hoc revealed VD³ LNB mice to present with significantly higher frontal-cortical noradrenaline concentrations, compared to VD NNB mice (363.4 ± 61.1 vs. $290.8 \pm 76.8, p = 0.027, d = 1.0 [0.04, 2.02]$).

Further, while no significant interaction was shown for the measured hippocampal concentrations [$F(1, 42) = 0.48, p = 0.4912$; **Figure 3-5D**], phenotype [$F(1, 42) = 16.62, p = 0.0002$], but not condition [$F(1, 42) = 1.77, p = 0.191$] significantly impacted the result. This phenotypic difference was most prominent in the SL⁴ condition, with LNB mice presenting with higher noradrenaline concentrations compared to NNB mice (307.1 ± 60.4 vs. 168.3 ± 104.8 ng/g, $p = 0.002, d = 1.5 [0.686, 2.469]$). Although narrowly missing statistical significance, the same trend was observed in the VD group (325.6 ± 90.7 vs. 227.2 ± 129.2 ng/g, $p = 0.060, d = 0.9 [-0.04, 1.82]$).

3.4 Discussion

The present work assessed the dependency of nesting expression in the deer mouse model of compulsive-like behavioural persistence on visual feedback so as to explore its neurocognitive basis. We further aimed to determine how NNB and LNB uniquely associates with limbic involvement. The main findings were as follows: 1) as opposed to NNB, LNB represents an inflexible and excessive behavioural phenotype that is not dependent on visually guided action-outcome processing, and 2) despite fronto-limbic noradrenaline concentrations not being influenced by VD, LNB associates with increased frontal-cortical and hippocampal noradrenaline which may contribute to its expression.

A vast body of work explored nesting behaviours in laboratory housed nesting species, e.g., bank voles (Schönecker 2009, Kapusta et al. 2022), rats (Van Loo and Baumans 2004, Simpson and Kelly 2011), mice (Balcombe 2006, Jirkof et al. 2013) and rabbits (Lidfors et al. 2007, Verga et al. 2007). While nesting behaviour is applied as an indicator of rodent welfare, substantial research into varying nesting

¹ normal nest building

² large nest building

³ visual deprivation

⁴ standard 12-h light-dark

expression in specific species and its relevance for studying compulsive-like behaviours, has provided valuable insights. Within the context of the present investigation, earlier findings showed that in some species, e.g., rabbits, nesting-expression is dependent on sensory feedback that regulates behaviours aimed at ‘just-right’ outcomes (Hoffman and Rueda Morales 2009). House mice (*Mus musculus*) can also be bred for bidirectional separation into ‘small’ and ‘big’ nesting cohorts (Greene-Schloesser et al. 2011), with persistence and larger nesting size applied as criteria to identify compulsive-like behaviour. In our laboratory, the same approach is followed to separate deer mice into NNB¹ and LNB² cohorts, with the exception that mice are not inbred for this purpose and thus the species resembles a wild-type model (Scheepers et al. 2018). This is important, since it provides a valuable opportunity to investigate the naturalistic mechanisms that contribute to the etiology and promulgation of compulsive-like behavioural persistence. Nevertheless, despite previous work having provided valuable insights into the biobehavioural differences between NNB and LNB expressing mice (which has been summarised in the introduction), our understanding of the underlying thought-action control of deer mouse nesting behaviour, notably LNB, remains lacking. Therefore, in this work, we specifically aimed to establish if LNB is related to visually perceived environmental and action-outcome feedback and if LNB, compared to NNB, is uniquely associated with limbic involvement. We did so by assessing NNB and LNB under conditions of total VD³.

In this regard, our first main finding is informing since it points to clear separation between the neurocognitive underpinnings of NNB and LNB. Specifically, we show that NNB, but not LNB, inflates over time (**Figure 3-3A**), irrespective of lighting condition. This finding is congruent with previous work in the model; however, whereas we previously argued this response of NNB mice to resemble an anxiety-like manifestation after longer term social isolation (Saaiman et al. 2023), the present data regarding hippocampal noradrenergic involvement (see below), are counterintuitive to such a conclusion. Rather, it is likely that while NNB is founded on evolutionary conserved mechanisms that enable mice to nest (Lewarch and Hoekstra 2018), prior experience may improve behavioural outcomes in this cohort. In this sense, NNB can be regarded as an innate skill that is sensitive to goal-directed experiential learning. Conversely, the expression of LNB is excessive, rigid, and representative of maximal habit-like behavioural activation that is not subject to temporal or experiential influences. We have argued the same before (Wolmarans et al. 2022, Saaiman et al. 2023). Here, we base our conclusion also on the response of NNB and LNB to VD, where significant differences between the neatness indices of nests built by NNB and LNB mice were only observed under conditions of VD (**Figure 3-4A & B**). While no significant differences in the neatness of nests within each cohort were shown between the two lighting

¹ normal nest building

² large nest building

³ visual deprivation

conditions, VD¹ influenced NNB² and LNB³ in an opposite manner, so that neatness became more varied in NNB, and less varied in LNB mice (**Figure 3-4A & B**). This finding must be viewed together with the overall effect of VD on the between-day variance in nesting size, represented in **Figure 3-3B**). While mice of both cohorts were sensitive to VD so that nesting sizes varied less between different days, the consistency with which nest construction was executed relied less on visual feedback in LNB, than in NNB mice.

A working hypothesis that arises from this data that should be explored in future work, is that VD necessitated NNB mice to rely on other sensory mechanisms, e.g., tactile or haptic feedback, to guide their nesting behaviour, and that these mechanisms were not as effective as vision for this purpose. In contrast, since LNB resembles an excessive, rigid, and habit-like behaviour, its expression is not as reliant on visual feedback. Within this conceptual framework, the influence of VD on fine coordinated behaviours has also been studied in human populations, where VD was shown to cause fallback to haptic perception in healthy individuals (Fujisaki 2012). However, while the authors showed it to be possible that prior learned goal-directed actions can be completed in the absence of simultaneous visual feedback, performance accuracy significantly deteriorates. This is similar to what the present data also show. Sadly, clinical research into the effects of VD on motor habits remains lacking. While we hypothesised that LNB might be related to processes aimed at achieving ‘just-right’ outcomes, the present results refute such a conclusion. In fact, the performance of LNB mice in this work is in line with theories pointing a role of behavioural rigidity and habitual learning in compulsive symptom expression, both of which are linked with cortico-striatal hyperactivation (Lanciego et al. 2012, Calabresi et al. 2014), as was also shown in LNB compared to NNB mice before (Saaiman et al. 2023).

Considering the known interactions between visual feedback, limbic activation and compulsive symptom expression, another core focus of the present work was to establish if LNB is uniquely associated with fronto-limbic involvement. We show here that while frontal-cortical and hippocampal serotonin concentrations are similar across both phenotype and light condition (**Figure 3-5A & B**), LNB mice present with significantly elevated noradrenaline concentrations in the frontal cortex under conditions of VD, and in the hippocampus under both light conditions (**Figure 3-5C & D**). Although not traditionally regarded as an essential role player in OCD⁴, noradrenaline plays a critical role in regulating behavioural expression, notably under stressful or threatening circumstances (Szabadi 2013, McBurney-Lin et al. 2019). The ability to adapt one’s behaviour based on immediate changes in the environment speaks to

¹ visual deprivation

² normal nest building

³ large nest building

⁴ obsessive-compulsive disorder

behavioural flexibility and is critical for survival. For this, the LC¹-noradrenaline system, is crucial (Aston-Jones and Cohen 2005, Mäki-Marttunen et al. 2020). Hyperactivation of this system is associated with heightened arousal and emotionality (Aston-Jones and Cohen 2005), impaired cognitive processing (Mäki-Marttunen et al. 2020), and behavioural inflexibility (Borodovitsyna et al. 2017, Mäki-Marttunen et al. 2019, Mäki-Marttunen et al. 2020). The present data pertaining to LNB² mice are congruent with such an understanding. While visual feedback did not influence fronto-limbic noradrenaline concentrations in NNB³ mice, i.e., the expression of NNB did not inflate over time due to increased levels of anxiety (see earlier), the increased noradrenaline concentrations shown in LNB mice may be related to hyperactivation of the limbic system (Mäki-Marttunen et al. 2020), leading to PFC⁴ inactivation which compromises behavioural flexibility and goal-directed decision making (Mäki-Marttunen et al. 2020). This will result in an overreliance on habitual and rigid behavioural programs, which lends further support to the first main finding as well. The present findings are also in line with work the work of Lustberg et al. (2020), who showed noradrenaline to be necessary for stress-induced compulsive-like behavioural expression in *Dbh* mice. While we have not directly explored the influence of stressors on nesting expression in deer mice, it is possible that innately inflated limbic activation might have been at play here. We should also reiterate that such limbic control over frontal-cortical processes as contemplated here, is likely mediated via striatal, instead of direct cortical modulation (Nikolaus et al. 2010, Buot and Yelnik 2012, Lu et al. 2021), especially considering that the frontal-cortical serotonin concentrations of NNB and LNB mice were similar. Overall, this result is striking, since it points for the first time to noradrenergic mechanisms that may underlie compulsive-like behavioural expression in deer mice, a finding that could pave the way forward for investigations into the role of noradrenergic processing in clinical OCD⁵, a topic that remains mostly understudied.

3.5 Conclusion

The present work sought to build on previous work in the same model system by investigating the influence of visual feedback and limbic processes in the expression of compulsive-like LNB in deer mice. Our data are mostly congruent with earlier results in showing that LNB is excessive, rigid, and habit-like. In contrast to NNB, LNB is not subject to experiential learning and significantly less reliant on visual feedback. Further, a clear role for noradrenergic signalling in LNB is highlighted, which points to heightened innate limbic activation as a potentially novel neurobiological construct in the present model

¹ locus coeruleus

² large nest building

³ normal nest building

⁴ prefrontal cortex

⁵ obsessive-compulsive disorder

system. Collectively, the current results are not only informing of the neurocognitive underpinnings of normal nesting behaviour, but it also provides a valuable foundation for continued investigations into the noradrenergic mechanisms that may influence the development and promulgation of excessive, rigid, and inflexible behaviours.

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Figures

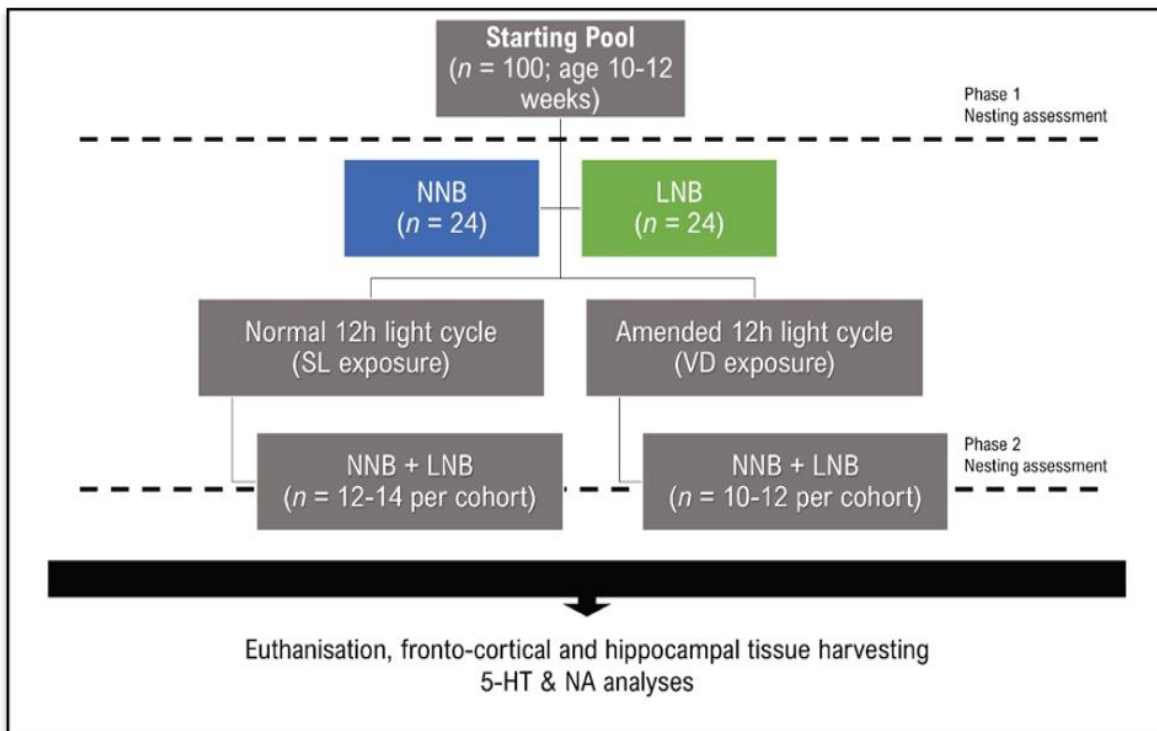


FIGURE 3-1: Schematic representation of study layout

NNB: normal nest building; LNB: large nest building; SL: standard 12-h light-dark; VD: visual deprivation; 5-HT: serotonin; NA: noradrenaline

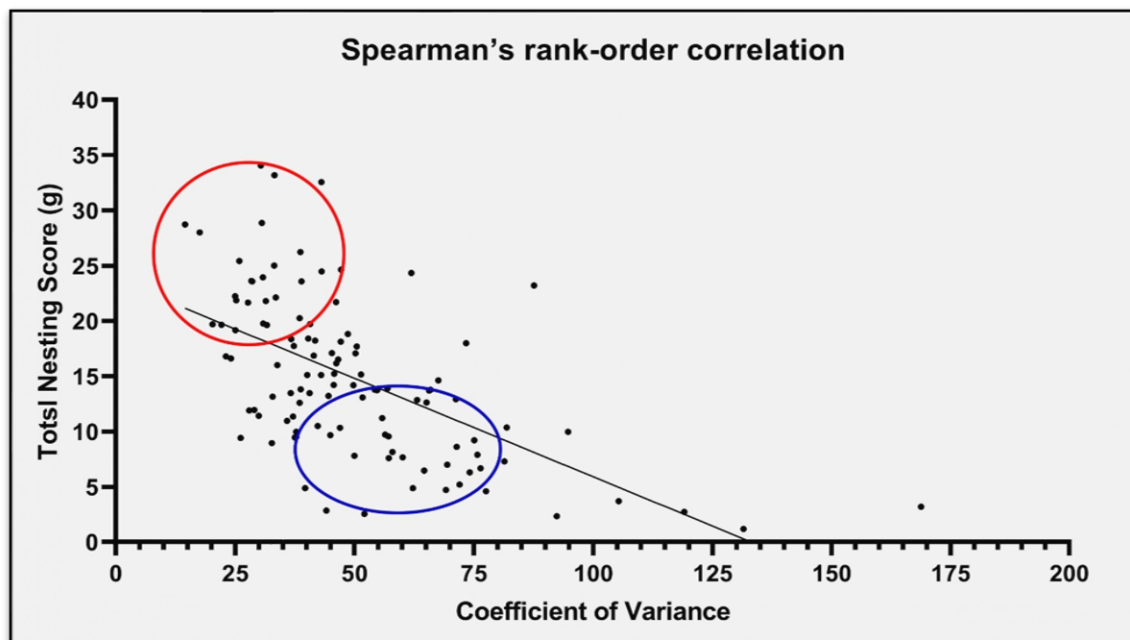


FIGURE 3-2 - Spearman's correlation of the individual total nesting scores (g) plotted against the respective coefficients of variance pertaining to the inter-day nesting scores. LNB animals are enclosed in the red circle, while the NNB animals can be found in the blue oval.

NNB: normal nest building behaviour; LNB: large nest building behaviour.

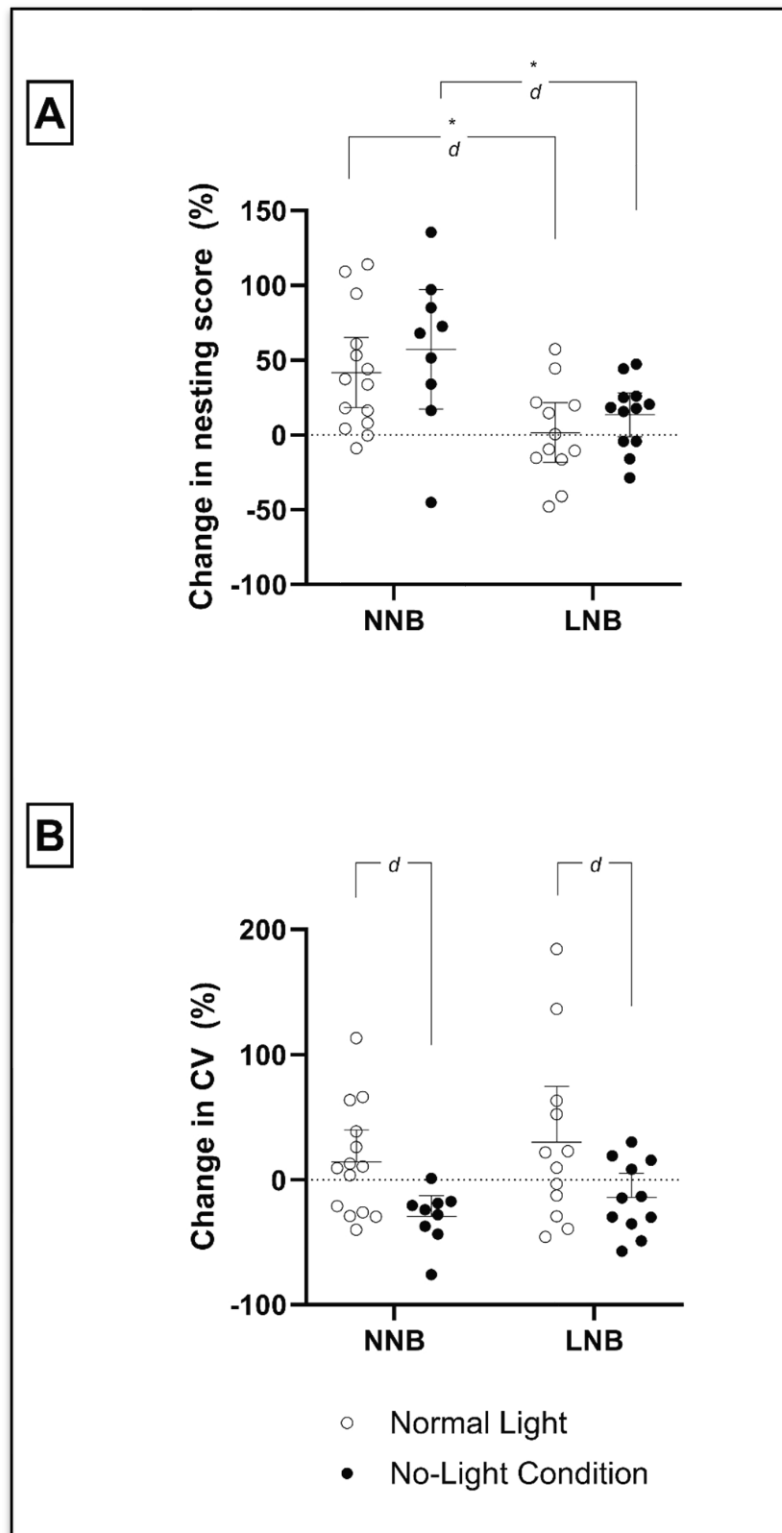


FIGURE 3-3 - Percentage change in amount of cotton wool utilised by NNB and LNB expressing mice when subjected to standard lighting and conditions of visual deprivation (A). Percentage change in coefficient of variance of NNB and LNB, when subjected to standard lighting and no-light conditions (B). Error bars represent means with 95% confidence intervals. Ordinary two-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparisons tests and Cohen's *d* effect size calculations.

NNB: normal nest building; LNB: large nest building; SL: standard 12-h light-dark; VD: visual deprivation.

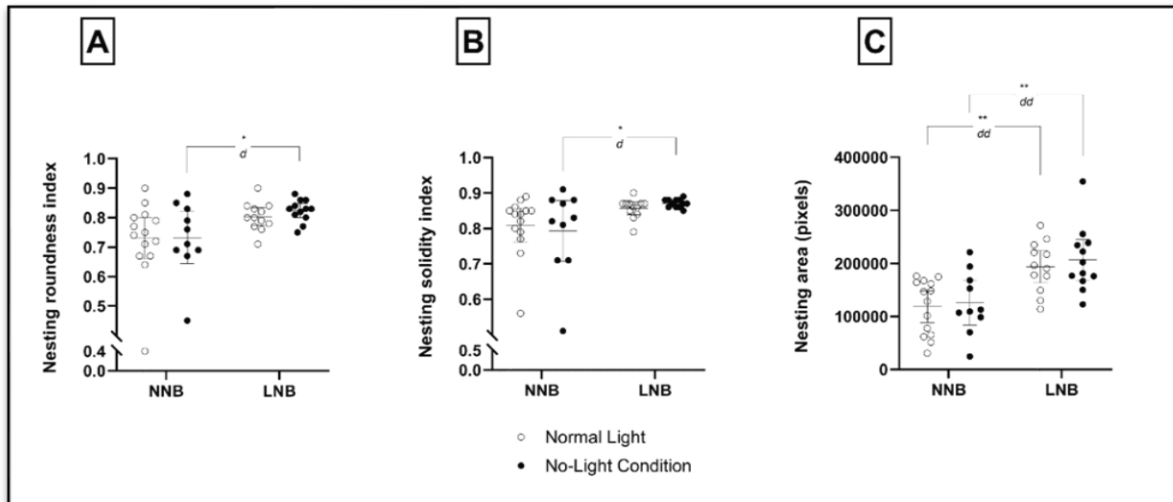


FIGURE 3-4 - Roundness index (A); Solidity index (B); Area size (pixels) (C) of nests built by NNB and LNB expressing mice when subjected to standard lighting and conditions of visual deprivation. Error bars represent means with 95% confidence intervals. Ordinary two-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparisons tests and Cohen's *d* effect size calculations.

NNB: normal nest building; LNB: large nest building; SL: standard 12-h light-dark; VD: visual deprivation.

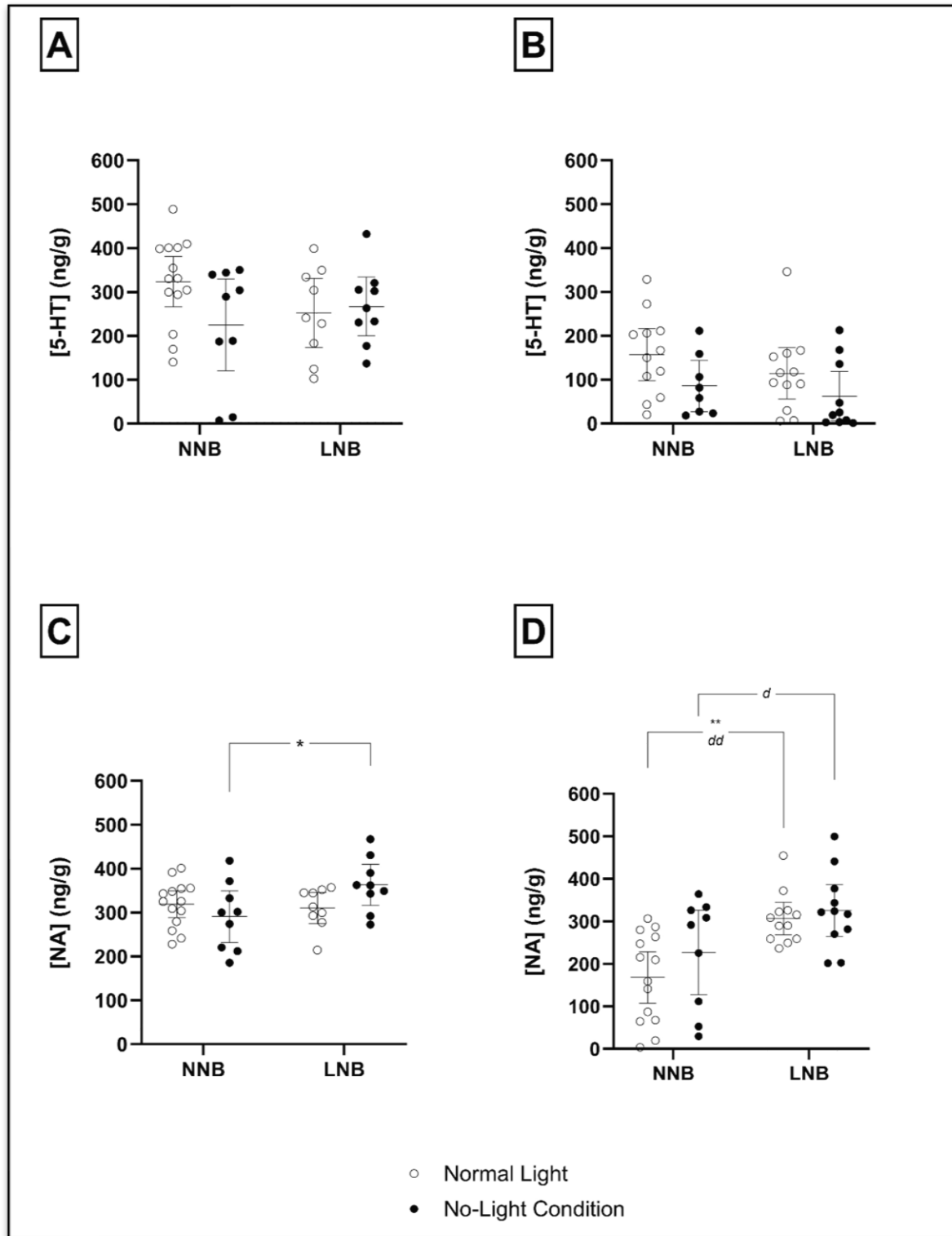


FIGURE 3-5 - The 5-HT levels in the prefrontal cortices (A); 5-HT levels in the hippocampi (B); NA levels in the prefrontal cortices (C); NA levels in the hippocampi (D) of NNB and LNB expressing mice when subjected to standard lighting and conditions of visual deprivation. Error bars represent means with 95% confidence intervals. Ordinary two-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparisons tests and Cohen's *d* effect size calculations.

LNB: large nest building behaviour; NNB: normal nest building; LNB: large nest building; SL: standard 12-h light-dark; VD: visual deprivation; 5-HT: serotonin; NA: Noradrenaline.

4 Conclusion and summary

The present investigation explored the neurocognitive underpinnings of large nesting behaviour (LNB)¹ in deer mice (*Peromyscus maniculatus bairdii*) within the context of its resemblance of compulsive-like behavioural persistence. We specifically aimed to establish how interactions between visual feedback and limbic processing may modulate the expression of LNB, compared to its ‘normal’ (NNB)² behavioural control. The present data indicates that 1) LNB represents an inflexible and excessive behavioural phenotype that is not dependent on visually guided action-outcome processing, and 2) despite fronto-limbic noradrenaline concentrations not being influenced by visual deprivation (VD)³, LNB associates with increased frontal-cortical and hippocampal noradrenaline and thus, heightened limbic involvement.

The neurocognitive mechanisms underlying obsessive-compulsive disorder (OCD)⁴ are diverse, spanning numerous domains of central nervous system functioning. While intrusiveness, persistence, and repetition are all core components of an OCD diagnosis, their promulgation from aetiology to symptomology take different routes between patients and between symptom phenotypes (Mataix-Cols et al. 2003, Van Den Heuvel et al. 2009, Grassi et al. 2022). This complicates our understanding of the condition and hampers treatment outcomes. Indeed, OCD only shows suboptimal response to currently available pharmacological interventions and psychotherapies, with an observed non-response rate of 40 % - 60 %, depending on study cohort (Pallanti et al. 2002, Stein et al. 2008, Abdolhosseinzadeh et al. 2019, Roth et al. 2020). The contributions of diverse neurocognitive mechanisms to treatment outcomes, are further illustrated by the varying response rates of different symptom phenotypes to specific treatment strategies. For example, contamination/washing and safety/checking OCD respond better to exposure-response prevention (ERP)⁵ than do hoarding compulsions or obsessions about repugnant themes (Starcevic and Brakoulias 2008, Muroff et al. 2011, Clark and Hilchey 2017, Puccinelli et al. 2023). ERP centres around the sensory perception by patients of environmental nuances that resemble the thematic content of their obsessions and thus, sensory—notably visual—feedback is an important modulator of OCD symptoms. With multiple exposures, patients come to realise the futility of their prior compulsive behavioural responses and that the obsessions about these themes were irrational to begin with (Hezel and Simpson 2019). Interestingly, the pathophysiological interaction between visual feedback and limbic processing and its effect on obsessive-compulsive symptom presentation is elegantly illustrated by the high percentage of drop-out rates seen with ERP (Ojalehto et al. 2020). Patients experience exposure therapy to be highly anxiogenic and thus they often fail to

¹ large nest building

² normal nest building

³ visual deprivation

⁴ obsessive-compulsive disorder

⁵ exposure-response prevention

complete therapeutic programmes. It thus stands to reason that heightened anxiety sensitivity and hyperactivity of the limbic system, may reciprocally interact with cortico-striatal processes that drive the expression of compulsions. However, since our understanding of how these and other neurocognitive mechanisms may contribute to the pathogenesis of OCD¹ across phenotypes remain lacking, improved treatment outcomes remain a future goal.

We therefore investigated this theme in a naturalistic animal model of compulsive-like behavioural repetition, i.e., the deer mouse, a species native to North America. When housed under standard laboratory conditions, a subpopulation of mice of both sexes show excessive and persistent LNB². While nesting behaviour is normally applied as an indicator of rodent welfare, excessive expression of nesting, as is true for LNB, has been studied for its resemblance of compulsive-like behaviour. While the neurocognitive triggers of and processes underlying LNB is not yet known, previous findings showed that in some species, e.g., rabbits, nesting-expression is dependent on sensory feedback that regulates behaviours aimed at 'just-right' outcomes (Hoffman and Rueda Morales 2009). House mice also bidirectionally separate into 'small' and 'big' nest builders after multiple rounds of inbreeding (Greene-Schloesser et al. 2011). In our laboratory, the same approach is followed, albeit applying deer mice as a wild-type model, instead of breeding mice to exhibit specific behavioural traits. This approach enables investigation of the naturalistic mechanisms that may contribute to the aetiology and promulgation of compulsive-like behavioural persistence. Nevertheless, our understanding of the underlying neurocognitive architecture of LNB could be expanded on. Specifically, it was not known whether LNB is expressed in response to potentially anxiogenic environmental cues (which for some mice might be more anxiogenic than for others), or if LNB associates with heightened limbic involvement, irrespective of visual influences. Alternatively, the possibility also existed that LNB could be a rigid, inflexible habit-like behaviour that is expressed without any sensory control or limbic involvement, i.e., a mere motor routine that is founded upon dysfunctional starting or stopping mechanisms. In lieu of this gap in the existing knowledge, translational application of the model system towards advancing therapeutic outcomes, is problematic. This investigation therefore sought to address this issue by exploring the neurocognitive basis of LNB, specifically asking whether LNB is related to visually perceived environmental and action-outcome feedback and if LNB, compared to NNB³, is uniquely associated with limbic involvement.

Our first main finding was striking since it indicated remarkable separation between the neurocognition underlying NNB and LNB. Specifically, we showed that NNB, but not LNB, inflated over time and that

¹ obsessive-compulsive disorder

² large nest building

³ normal nest building

this transpired irrespective of lighting condition. This response cannot be related to social isolation induced anxiety (as was our earlier view; de Brouwer et al. (2020)); rather, it seems that while NNB¹ may be founded on evolutionary conserved nesting mechanisms (Lewarch and Hoekstra 2018), it is adaptable according to prior experience, which may improve its behavioural presentation. Conversely, LNB² which was also not robustly influenced by VD³, can be regarded as excessive, rigid, and representative of maximal habit-like behavioural activation that is not further modifiable by either external or implicit processes. We base this conclusion on the response of NNB and LNB to VD, where significant differences between the neatness indices of nests built by NNB and LNB mice were only observed under conditions of VD. In fact, we believe that VD necessitated NNB mice to rely on other sensory mechanisms to guide their nesting behaviour, and that these mechanisms were not as effective as vision for this purpose. In contrast, since LNB resembles an excessive, rigid, and habit-like behaviour, its expression was not as reliant on visual feedback.

Another core focus of the present work was to establish whether LNB is uniquely associated with fronto-limbic involvement. Here, we showed that LNB expressing mice broadly present with significantly elevated frontal-cortical (under conditions of VD) and hippocampal noradrenaline concentrations, a highly informing finding. Given its role in regulating behaviour under stressful or threatening circumstances (Szabadi 2013, McBurney-Lin et al. 2019), an increased noradrenergic tone contributes to heightened arousal, impaired cognitive processing, and behavioural inflexibility (Borodovitsyna et al. 2017, Mäki-Marttunen et al. 2019, Mäki-Marttunen et al. 2020). At large, our data point to hyperactivation of the limbic system (Mäki-Marttunen et al. 2020), which potentially contributed to inflexible nesting outputs. This result points for the first time to noradrenergic mechanisms that may underlie compulsive-like behavioural expression in deer mice, a finding that now paves the way forward for investigations into the role of noradrenergic processing in clinical OCD⁴, a topic that remains mostly understudied.

* * *

Viewed in its entirety, the body of work presented in this dissertation contributed much to the present understanding of nesting behaviour in deer mice, but most importantly, to how its application to model compulsive-like, ‘abnormal’ behaviours, should be conceptualised. As alluded to throughout the text, OCD is born from diverse psychobiological perturbations which are poorly understood and that contribute to suboptimal treatment outcomes. This dilemma can, for ethical and practical reasons, not

¹ normal nest building

² large nest building

³ visual deprivation

⁴ obsessive-compulsive disorder

be addressed without robust and reliable animal models. While animal model systems will never truly mimic any human neuropsychiatric disorder in terms of all aspects of its presentation, we show here that deer mice may be useful to model at least some of the psychobiological perturbations shown in clinical OCD¹. The data from this investigation are highly informing for future work, since it positions LNB² within a framework of study that could focus on exploring the psychobiological processes that contribute to the development of habit-like behavioural rigidity, notably with an emphasis on how limbic processes exert control over frontal-cortical executive flexibility.

¹ obsessive-compulsive disorder

² large nest building

4.1 Final summary of study aims and outcomes

Expected outcome	Actual outcome
i) Will VD¹ differentially impact nesting outcomes in NNB² and LNB³ expressing mice?	  Although VD did not significantly alter nesting outcomes in NNB and LNB mice, respectively (compared to a standard 12-h light-dark (SL)⁴ conditions), VD resulted in a diverging behavioural response where the behaviour of NNB mice became less uniform, and that of LNB mice more rigid, so that significant differences between the qualitative nesting scores generated by NNB and LNB mice, were only shown under conditions of VD.
ii) Will changes in nesting outcomes in NNB and LNB mice under conditions of VD point to LNB being a behavioural phenotype related to increased anxiety sensitivity towards visually perceived environmental cues or alternatively, seeking 'just-right' outcomes?	 No, the lack in adaptation to VD seen in the nesting outcomes of LNB mice, indicated LNB to be highly rigid, inflexible, repetitive and, considered with the fact that LNB remained stable over time (as opposed NNB which inflated), representative of maximal habit-like behavioural execution.
iii) Will LNB mice, compared to NNB mice, show heightened limbic system activation as resembled by increased hippocampal and frontal-cortical noradrenaline concentrations?	 Yes. The neurobiological data from this investigation point to significantly increased hippocampal and frontal-cortical noradrenaline concentrations, irrespective of lighting condition. Thus, while prior study indicated LNB mice not to be overly anxious in standard rodent assessments of anxiety, the present data point to an underlying limbic mechanism that may drive behavioural inflexibility.

¹ visual deprivation

² normal nest building

³ large nest building

⁴ standard 12-h light-dark

iv) What will the translational conclusion in terms of the psychobiological fingerprint of LNB¹ as a model of compulsive-like behavioural persistence be?	 Here, we conclude that when applying the deer mouse model of OCD², the model system will lend itself well to investigations of fronto-limbic interactions that may drive rigid, repetitive and inflexible behavioural output. Since these mechanisms, especially with reference to noradrenergic involvement in OCD, are not well studied, the present findings are highly informative.
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4.2 Study shortcomings and future directions

Although the present findings shed critical light on our understanding of deer mouse nesting behaviour, especially in terms of LNB in its psychobiological resemblance of compulsive-like behavioural repetition, some noteworthy shortcomings deserve being mentioned.

Firstly, in terms of sensory perception, we solely focused on visual feedback (or the lack thereof) on the nesting ability of NNB³ and LNB expressing deer mice (*Peromyscus maniculatus bairdii*). As is often observed in humans (Weisser et al. 2005), mice begin to over-rely on other forms on sensory stimulation in circumstances where one of their key senses is damaged or disabled (Zhou et al. 2017). The present study design did not afford attention to the question of whether ‘just-right’ feedback, driven by other forms of sensory stimulation, e.g., tactile or olfactory perception in deer mice, guides the expression of LNB, as opposed to NNB. We can therefore not draw a definitive conclusion that our argumentative approach is correct in this respect. Future work, aimed at manipulating these and possibly other forms of feedback in NNB and LNB expressing deer mice, will be crucial to address this question in its entirety.

Secondly, it remains largely unknown whether the act of nesting itself is genetically conserved to such extent that no prior experience is needed for its expression. With respect to the behavioural output of NNB and LNB deer mice under conditions of VD⁴, we must explore the possibility that nesting can be learned and that prior learning experience, as introduced here in the phase 1 assessment, could have contributed to the nesting scores of mice generated under conditions of VD. This question can be solved by investigating nesting expression under conditions of VD, but in the absence of any prior exposure to nesting material before said assessment period. Clarity on this issue is vital, since it will enable us to understand if LNB expression is an *acquired* habit, (i.e., learned), or whether such behaviour is implicit and inherent to deer mice. Further to this, this knowledge will guide future research in the

¹ large nest building

² obsessive-compulsive disorder

³ normal nest building

⁴ visual deprivation

Conclusion and summary

model with respect to the study approach followed, i.e., to explore the model in terms of genetic targets of cognitive flexibility, or against a background of cognitive perturbation, whereby rigid behaviours are learnt.

Finally, we showed here that LNB¹ mice presented with elevated fronto-limbic noradrenaline concentrations, compared to NNB² mice. While the data is irrefutable in terms of pointing to heightened limbic activation, we cannot conclude with certainty that LNB mice do not show increased stressogenic limbic system activation, that may subsequently drive the expression of LNB. Deeper investigation into the stress axis reactivity of LNB, compared to NNB mice will be necessary. For this, investigations of stress hormones, e.g., corticotrophin releasing hormone (CRH)³ and corticosterone, will be vital. Insights into this issue is necessary, given the intricate relationship between anxiety and obsessive-compulsive symptom expression.

* * *

THE END

¹ large nest building

² normal nest building

³ corticotrophin releasing hormone

4.3 References

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Addendum A

This Addendum contains supplementary methodological detail that was not relevant for inclusion in the main body of work, i.e., **Chapter 3**. As such, the information contained in this Addendum should be read with **Chapter 3**.

Additional information regarding the husbandry, housing, and care of mice before the commencement of the study

- Prior to the onset of this investigation, deer mice were bred in the North-West University (NWU)¹ vivarium, Potchefstroom (SAVC² registration number: FR15/13458; AAALAC accreditation file: 1717).
- Mice were weaned at the age of 21 days, and group housed (4 - 6 same sex mice per cage; 35 cm (l) x 20 cm (w) x 13 cm (h); Tecniplast[®] S.P.A., Varese, Italy), until the age of 10 – 12 weeks.
- Welfare monitoring included checking and recording of 1) food and water availability, 2) temperature of the housing room (22 ± 1°C), and 3) the humidity of the housing room (40 – 55 %). These routine checks were done daily between the hours of 08:00 and 13:00. In cases where any abnormal behaviour or other causes for concern were noted in terms of animal welfare, the vivarium staff and other relevant stakeholders were informed, whereafter the cause of the specific problem was identified and resolved according to standard AnimCare-REC and vivarium protocol.
- At the beginning of each week, cages were cleaned, and fresh corncob bedding added. Cages were cleaned with F10[®] veterinary disinfectant cleaning solution (Reg No Act 29, GNR 529/29990/040/150; DALRRD³ Registration Number: G3073). Before the onset of the experimental period, paper towel and polyvinyl chloride pipe (100 mm x 40 mm (Ø)) was placed in each home cage as forms of nesting material and environmental enrichment. However, this was not provided during periods of experimental nesting assessment.
- From the onset of the baseline nesting assessment, all mice were allocated individually to new single housing cages. Housing conditions remained the same throughout the entire study, i.e., as alluded to above, until study endpoint.
- All food (mouse pellets; Labchef[®] Nutrition Hub, DALRRD Reg nr: 2012/141960/07) were autoclaved at 90°C for 15 min before use.

¹ North-West University

² South African Veterinary Council

³ Department of Agriculture, Land Reform and Rural Development

Behavioural experimentation

Information regarding conventional nest building assessment and nest building assessment under conditions of visual deprivation

- During this investigation, two nest building assessments were conducted as described in detail in **Chapter 3**. The first nesting assessment served as the baseline assessment of nest building output. For this purpose, an initial pool of 100 deer mice was used from which 24 large (LNB)¹, and 24 normal nest building (NNB)² mice were identified, respectively. These mice were further divided and assessed under either standard 12-h light-dark (SL)³ conditions or conditions of visual deprivation (VD)⁴.
- During periods of nest building analysis, food and water were available as normal, although animals were not provided with any additional form of nesting material or environmental enrichment.

Information regarding Nest integrity analysis

- Every day, all built nests were photographed and allocated a cage, date and mouse number.
- Prior to photographing, nests were not disrupted with mice being left in the cages until photographing each cage was concluded.
- After the second nesting assessment concluded, each image was analysed for the parameters described in **Chapter 3**.
- ImageJ2[®] software (Fiji[®]) was used to manually outline the border of nest, where after the software automatically processed the image.

¹ large nest building

² normal nest building

³ standard 12-h light-dark

⁴ visual deprivation

Addendum A

Supplementary images



FIGURE A-1: (A): Techniplast® smart flow for the monitoring of ventilation, humidity, and temperature in the cages; (B) daily monitoring sheet for recording ventilation, humidity and temperature; (C): deer mice housing system (cages: 35 cm (L) X 20 cm (W) X 13 cm (H); Techniplast® S.P.A., Varese, Italy). Each cage was marked according to the specific cohort and lighting condition; (D-K): Daily routine, which was followed during the second nesting assessment, i.e., weighting of unscented cotton wool to nesting image analysis.