

Large nesting expression in deer mice remains stable under conditions of visual deprivation despite heightened limbic involvement: Perspectives on compulsive-like behavior

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Abstract

Visual stimuli and limbic activation varyingly influence obsessive-compulsive symptom expression and so impact treatment outcomes. Some symptom phenotypes, for example, covert repugnant thoughts, are likely less sensitive to sensory stimuli compared to symptoms with an extrinsic focus, that is, symptoms related to contamination, safety, and “just-right-perceptions.” Toward 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) and large (LNB) nesting deer mice, respectively (as far as possible, equally distributed between sexes). Briefly, after behavioral 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 light conditions or conditions of complete visual deprivation (VD). Nesting outcomes were assessed in terms of size and neatness. After nesting assessment completion, mice were euthanized, 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 behavioral 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 nesting behavior. 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 behaviors.

KEYWORDS

anxiety, deer mouse, nest building, obsessive-compulsive disorder, visual feedback

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1 | INTRODUCTION

Rigid and repetitive behavioral phenotypes are characteristic of obsessive-compulsive disorder (OCD), of which the main symptoms are obsessions (intrusive thoughts or ideas), compulsions (rigid, inflexible, and repetitive covert or overt behaviors), or both (APA, 2013). Compulsions are mostly expressed in response to an underlying, often anxiety-provoking obsession about specific themes, for example, safety or potential harm to oneself or others (Abramovitch & Cooperman, 2015). Also, obsessions related to perfectionistic ideals may contribute to among others, symmetry and ordering compulsions (Hood & Antony, 2016), and cognitive rigidity (Egan et al., 2007). While obsessions are born from dysfunctional implicit thought processes, some symptom phenotypes, for example, covert thoughts with repugnant themes, are likely less sensitive to sensory stimuli, compared to symptoms with an extrinsic focus, that is, 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), a psychotherapeutic intervention involving exposure of patients to aspects of their obsessive thoughts while they are prevented from engaging in compulsive neutralizing rituals (Hezel & Simpson, 2019). An important premise for the successful application of ERP is the ability of patients to process sensory feedback; thus, higher rates of treatment resistance are observed with covert mental routines that can, in many instances, not be triggered by external cues (Bednarek et al., 2022; Mataix-Cols et al., 2002). However, the relationship between external cues and compulsive symptom expression remains unclear, while poor insight and high anxiety sensitivity may also 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 orbito-frontal cortex (OFC), the anterior cingulate cortex (ACC), the ventro-lateral (vlPFC), ventro-medial (vmPFC), and dorso-lateral (dlPFC) 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, that is, the ventral affective, ventral cognitive, dorsal cognitive, fronto-limbic, and the sensorimotor circuit (Haber, 2016). For example, dysfunctional ventral affective processes, of which OFC hyperactivation is a core feature (Shephard et al., 2021), contributes to behavioral inflexibility (Hikosaka & Watanabe, 2004; Menzies et al., 2008). Considering the anxiogenic nature of obsessive intrusions, OCD is also associated with a high degree of limbic involvement (Wood & Ahmari, 2015). Hyperactivation of the limbic system, which includes the ACC and vmPFC, hippocampus, and amygdala, may contribute to intolerance of uncertainty and inflated safety-seeking behavior, both of which are seen in patients suffering from safety/checking OCD (Lind & Boschen, 2009). In turn,

Significance

The underlying motivational triggers of compulsive-like large nesting behavior (LNB) in deer mice remain unknown. The working hypothesis of the present work was that such behavior can be driven by either “just-right” feedback or inflated anxiety (as reflected by noradrenergic limbic activation), both of which could be dependent on visual feedback. We show here that neither of these notions were correct and that LNB in deer mice, though such behavior associating with increased hippocampal noradrenaline, is an inflexible habit-like phenotype. This finding is vital for understanding how rigid habit-like behaviors may promulgate obsessive-compulsive symptoms in some patients.

sensorimotor impairment can contribute to compulsive behaviors that are aimed at perfectionistic outcomes and it also underpins excessive habit formation, a neurocognitive endophenotype of OCD (Gillan et al., 2011).

Given the impact of anxiety on clinical OCD symptomology, interest into the potential contributions of the noradrenergic system also increased in recent years (Hatayama & Aruga, 2023). However, since antidepressants with a noradrenergic mechanism of action are mostly ineffective in the treatment of OCD (Kellner, 2022), some background is needed. Under resting state conditions in healthy subjects, moderate concentrations of frontal-cortical noradrenaline, which is primarily released from noradrenergic projections originating in the locus coeruleus (LC), inhibit amygdalar reactivity, and thus also anxiety- and fear-related processing in the limbic system (Bouras, 2023; Coley et al., 2021). This allows for normal executive functions to take precedent. However, once specific sensory or emotional stimuli activate the limbic system, a transient increase in limbic noradrenaline release 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 limbic hyperactivation (Boehme et al., 2014), which may point to a lower anxiety sensitivity threshold or alternatively, a loss in top-down frontal-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 OCD, 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 ERP (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 behaviors (Lustberg, Iannitelli, et al., 2020; Lustberg, Tillage, 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 behavior (LNB). Within a controlled environment, such large nests serve no unique purpose. In fact, in the wild, rodents nest for thermoregulatory, safety, breeding, and nursing purposes as well as to defend and maintain a social hierarchy (Wolmarans et al., 2016; Jirkof, 2014; Lewarch & Hoekstra, 2018; Smithers, 1983). None of these functions are arguably 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 behavioral persistence, since it is a highly goal-directed, though excessive, behavioral phenotype (de Brouwer, Fick, et al., 2020) shown by mice of both sexes. It further persists over the course of several assessment sessions (Wolmarans et al., 2022) and despite self-harming consequences (de Brouwer, Harvey, & Wolmarans, 2020), while the expression thereof prevents flexible behavioral switching to or engagement in other rodent behaviors (Wolmarans et al., 2022). While a detailed review of biobehavioral differences between NNB and LNB mice falls beyond the scope of the present work (see Wolmarans et al. (2022), Saaïman et al. (2023), Wolmarans et al. (2018), and Scheepers et al. (2019) for an overview), 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 behavior in tests of generalized anxiety, that is, a standard open field test (Saaïman et al., 2023), certain traces of evidence point to differences in anxiety sensitivity that must be highlighted. Firstly, and as alluded to above, LNB, compared to NNB mice, are overly resistant to negative and aversive feedback and will voluntarily recruit nesting material despite being demotivated to do so through self-inflicted punishment, that is, electric foot shock (de Brouwer, Harvey, & Wolmarans, 2020). Secondly, LNB mice show more risk-engaging behavior than NNB mice and will access a 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 potentially harming and detrimental consequences.

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 behavior. Indeed, mice have

been shown to rely on visual cues to guide specific aspects of their behavior, for example, learning and executing behavioral sequences (as is true for the motor routines recruited during nesting) (Gavornik & Bear, 2014). It would thus be important to establish if LNB in deer mice is related to “just-right” visual feedback, as may be true for some other nesting animal species, for example, rabbits (Hoffman & Rueda Morales, 2009). Last, the question remains whether LNB is associated with a distinct degree of limbic involvement that is either extrinsically, that is, visually, 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 complete visual deprivation (VD). We further measured frontal-cortical and hippocampal serotonin and noradrenaline concentrations as markers of anxiety-related limbic involvement.

2 | MATERIALS AND METHODS

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 1 week. From these, 24 mice were selected for inclusion in NNB and LNB 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), that is, VD. Following the last night of the P2 assessment, mice were euthanized to harvest frontal-cortical and hippocampal tissue (Figure 1).

2.2 | Mice

Since only 20%–30% of laboratory housed deer mice express LNB (Wolmarans et al., 2013, 2016), an initial pool of 100 mice (*Peromyscus maniculatus bairdii*) of both sexes were included for initial nesting assessment (ethical approval number: NWU-00769-22-A5; AnimCare Research Ethics Committee, NWU). 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 (SAVC registration number: FR15/13458; AAALAC accreditation file: 1717). All behavioral experiments were performed in the same facility and complied with the South African National Standard (SANS) 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) cages [35 cm (l) × 20 cm (w) × 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-h light/dark cycle (lights on at 6h00), except if stated otherwise. Food

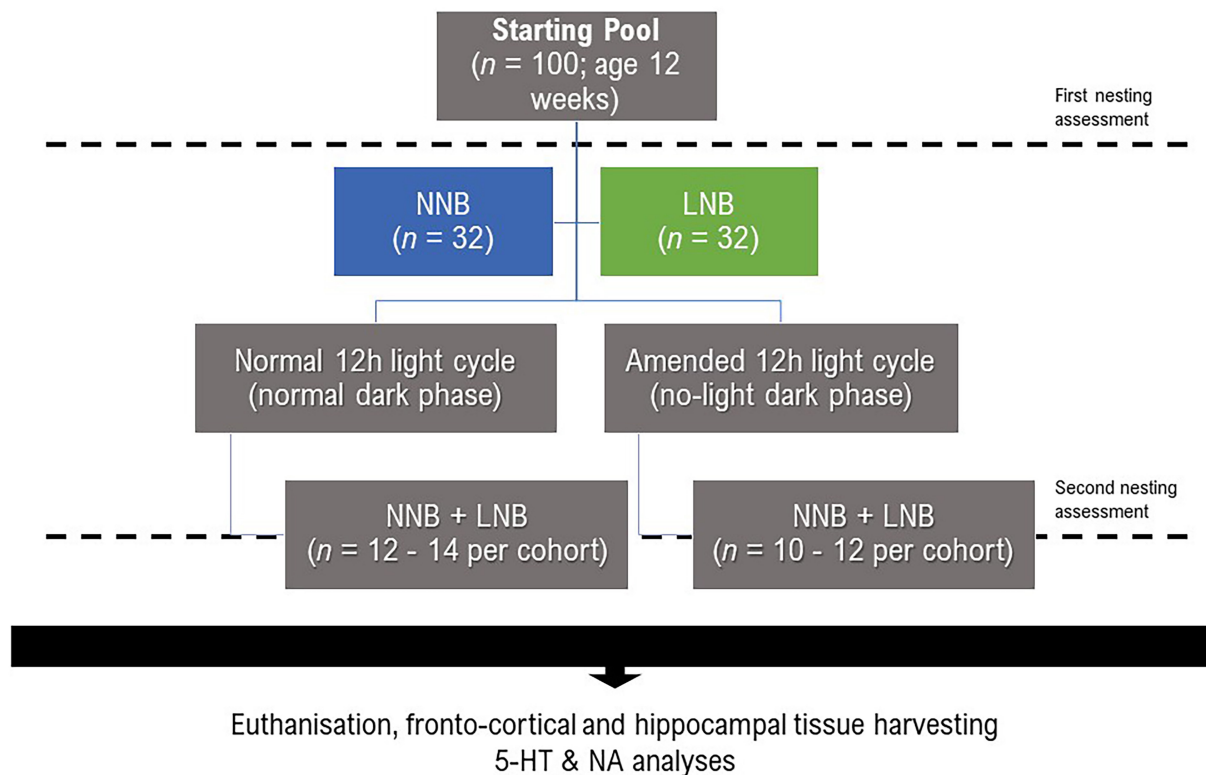


FIGURE 1 Schematic representation of study layout. 5-HT, serotonin; LNB, large nest building; NA, noradrenaline; NNB, normal nest building; SL, standard light; VD, visual deprivation.

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.

2.3 | Nesting assessment

2.3.1 | Conventional nesting assessment and behavioral categorization

During P1 (days 1–7), nesting assessment was aimed at measuring the baseline nesting expression of deer mice and selecting 24 NNB and LNB expressing mice for further study from the 100 initially included mice (de Brouwer, Fick, et al., 2020; Wolmarans et al., 2016). For 1 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, that is, wool not utilized for nesting purposes, was weighed, and the built nests discarded. Thus, mice had access to nesting material for at least 14h of every 24-h cycle, which included the complete dark cycle, that is, with normal ambient night-time lighting able to penetrate cages. Mice mostly engage in nesting behavior during the dark cycle, especially in the period prior to dawn (or lights on) (Wolmarans et al., 2016; Jirkof, 2014). 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 LNB is primarily based on (i) the total nesting-score generated over 7 days (i.e., the cumulative weight of all the cotton wool used to construct nests over 1 week), and (ii) the behavioral variance observed with respect to the between-day nesting performance over this period, reflected as a percentage coefficient of variance (% CV) 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 the 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 2). In contrast, NNB is identified as total nesting scores that consistently clustered below the 50th percentile of the total score distribution. After selection of the 24 NNB and LNB expressing mice, the remaining 52 mice were euthanized.

2.3.2 | Assessment of nesting behavior 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 VD, that is, without any ambient

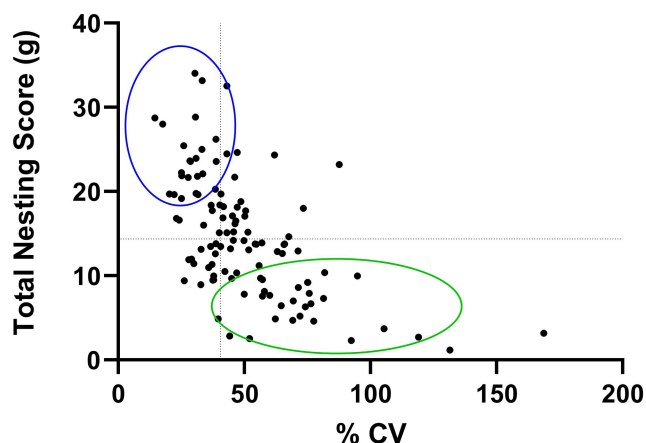


FIGURE 2 Selection of NNB and LNB mice following Spearman's rank order correlation of the individual total nesting scores (in grams) and the respective coefficients of variance pertaining to the inter-day nesting scores. LNB mice are enclosed in blue (upper left), NNB mice are enclosed in green (lower right). LNB, large nest building behavior; NNB, normal nest building behavior.

visible, ultraviolet (UV), or infrared (IR) light able to penetrate cages (see later). This was necessary, since mice—which mostly build nests during the dark cycle—may depend on both visible and UV light to guide some behaviors under nocturnal conditions (Leinonen & Tanila, 2018). For the NNB group, 14 mice (5 female) were allocated to the standard light (SL) group, 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, IVCs were equipped with pre-weighed cotton wool and wrapped in light-tight, black polyvinyl chloride (PVC) covers (550g/m², Neucoat®, Johannesburg, South Africa, 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) environment from 06:00 to 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 behavioral output parameters observed in P2, and that of P1, that is, $\left(\frac{\text{BehP2} - \text{BehP1}}{\text{BehP1}}\right) * 100$, where “Beh” represents the measured output (total nesting score or % CV) for both nesting assessment phases.

2.3.3 | Qualitative scoring of nesting behavior

To assess the impact 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 of each cage were taken 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, f1.8, 12 MP, Sony® Corporation, Tokyo, Japan). 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).

2.4 | Quantification of frontal-cortical and hippocampal monoamines

2.4.1 | Reagents and chemicals

Serotonin, noradrenaline, and 5-hydroxymethyl tolterodine (5-HMT; internal standard) were all acquired from Sigma-Aldrich Chemicals Pty (Ltd) (Johannesburg, South Africa). Chemicals that were used for the mobile phase included high-performance liquid chromatography (HPLC) grade deionized water (Milli-Q® water), HPLC grade acetonitrile (ACN), sodium formate, 1-heptanesulphonic acid sodium salt, ethylenediaminetetraacetic acid (EDTA) 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), which were also obtained from Merck Pty (Ltd) (Johannesburg South, Africa).

2.4.2 | Procedure

All mice were euthanized by means of cervical dislocation (Underwood & Anthony, 2020) on the morning following the last night of P2. The whole brain of each mouse was removed on ice and the complete right and left components of the frontal cortices and hippocampi 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 .1 µL/mL. Tissues were then homogenized via sonication (twice for 12 s 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 (14,000 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 .1 M HClO₄-in-water solution was added.

Tubes were vortexed for 15s and again centrifuged for 20min as described above. The resultant supernatants were then analyzed for serotonin and noradrenaline according to the method of Viljoen et al. (2018).

Briefly, standards and samples were analyzed on an UltiMate® 3000 ultra high-performance liquid chromatography (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 μ m column (4.6 $\times$ 250mm; Bonna-Angela Technologies®, Torrance, CA, USA). 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 200ng/mL. The mobile phase consisted of a .1M sodium formate buffer containing 5mM sodium 1-heptanesulfonate, .17mM EDTA and 5% v/v acetonitrile. The pH of the mobile phase was set at 4 with orthophosphoric acid. The system injected 25 μ L of each sample onto the column, with the flow rate set at 1.0mL/min. Data were analyzed 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.

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 % CV calculated for each mouse (Figure 2). Changes in total nesting scores and % CV from P1 to P2 (expressed as a percentage change from P1 to P2), as well as the qualitative nesting attributes scored during P2, were compared by means of ordinary two-way analysis of variance (2-way ANOVA). 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 condition, or main effects of either or both independent factors were observed, ANOVA analyses were followed by Bonferroni's post-hoc pairwise comparisons. Statistical significance was set at $p < .05$ for all analyses. 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). Only large effects sizes ($d \geq .8$) 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 | RESULTS

3.1 | Nesting behavior

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 2). A statistically significant and strong negative linear relationship was found ($r_s(112) = -.595$ [$-.71, -.46$], $p < .0001$). The distribution of data points was subsequently applied to select NNB and LNB mice, as explained earlier.

3.1.2 | Quantitative changes in nesting behavior 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 NNB VD group. Two-way ANOVA did not reveal a significant phenotype-condition interaction [$F(1, 43) = .03$, $p = .874$; Figure 3a]. However, a significant main effect of phenotype [$F(1, 43) = 14.60$, $p = .0004$], but not of condition [$F(1, 43) = 1.55$, $p = .220$] was shown. Specifically, compared to the total nesting scores of LNB mice, the scores of NNB mice inflated during P2, irrespective of light condition (SL: $1.5\% \pm 31.6\%$ vs. $41.8\% \pm 40.4\%$, $p = .017$, $d = 1.1$ [.26, 1.92]; VD: $13.5\% \pm 22.9\%$ vs. $57.2\% \pm 52.0\%$, $p = .021$, $d = 1.1$ [.20, 2.08]).

In terms of changes in nesting consistency as reflected by the % CV values of P2 versus 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 [$F(1, 42) = .00$, $p = .983$; Figure 3b]. A main effect of condition [$F(1, 42) = 9.88$, $p = .003$], but not of phenotype [$F(1, 42) = 1.24$, $p = .272$], was however observed. Subsequent pairwise comparisons showed that mice of both phenotypes trended toward expressing nesting activity with less variance under conditions of VD, compared to SL (NNB: $-29.2\% \pm 21.7\%$ vs. $14.4\% \pm 44.3\%$, $p = .070$, $d = 1.1$ [.25, 2.07]; LNB: $-14.0\% \pm 29.1\%$ vs. $30.2\% \pm 70.2\%$, $p = .058$, $d = -.8$ [-1.65, .05]).

3.1.3 | Qualitative nest attributes scored for P2

Nesting roundness

Nesting roundness (expressed as an index from 0 to 1, with "1" being the most round) was scored as a measure of neatness. No statistically significant interaction between phenotype and condition was shown [$F(1, 44) = .12$, $p = .735$; Figure 4a]. Phenotype [$F(1, 44) = 9.73$, $p = .003$], but not condition [$F(1, 44) = .17$, $p = .679$], significantly impacted nesting roundness, with LNB mice in the VD group,

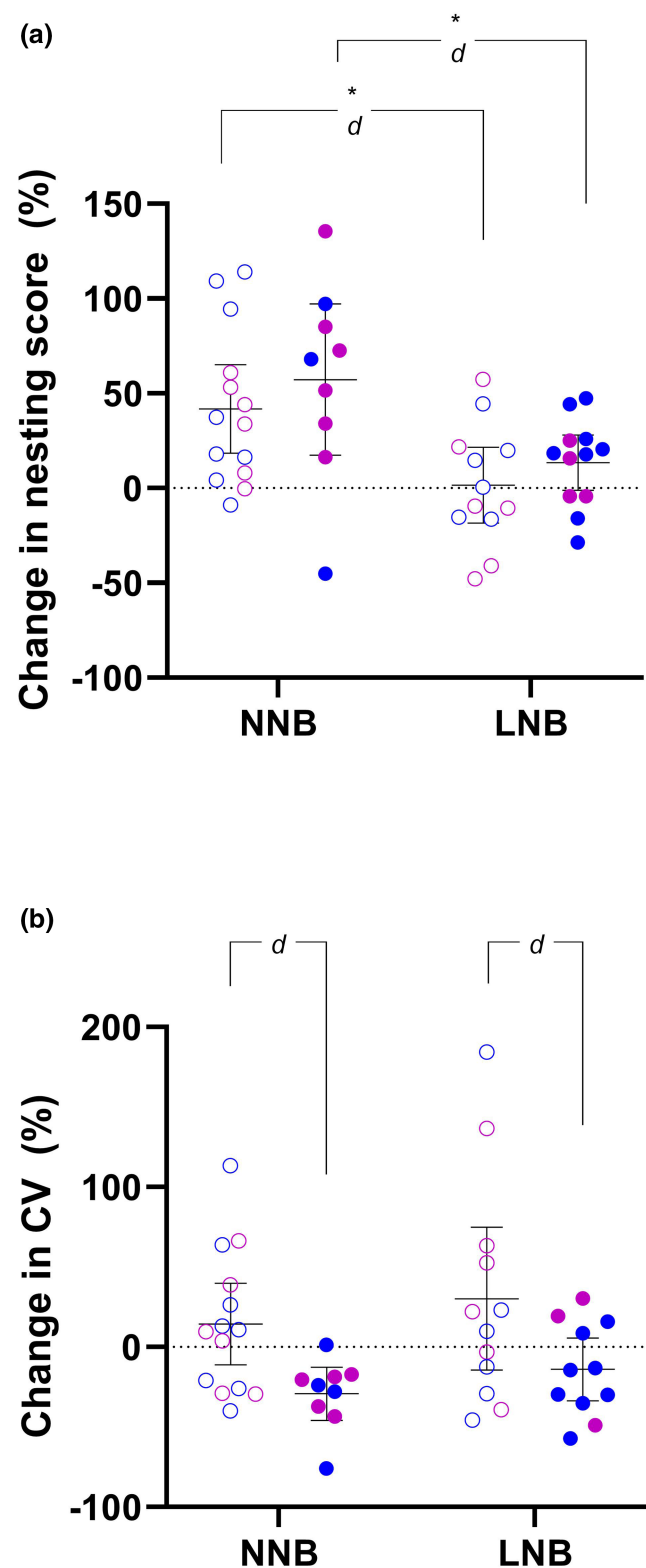


FIGURE 3 Percentage change in the quantity of cotton wool utilized by NNB and LNB expressing mice when subjected to standard lighting and conditions of visual deprivation (a). Percentage change in the coefficients of variance of NNB and LNB behavior when subjected to standard lighting and no-light conditions (b). Error bars represent mean with 95% confidence intervals. LNB, large nest building; NNB, normal nest building; blue dots: male mice; purple dots: female mice.

constructing nests with a higher roundness index, than VD NNB mice ($.8 \pm .03$ vs. $.7 \pm .1$, $p = .047$, $d = 1.0$ [.14, 1.94]). This difference was not significant for mice assessed in the SL condition ($.8 \pm .05$ vs. $.7 \pm .1$, $p = .092$, $d = .8$ [−.03, 1.58]).

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 to 1 (with “1” being the most “solid,” i.e., with the least shadow variance). Again, no significant interaction was evident between phenotype and condition [$F(1, 44) = .52$, $p = .475$; Figure 4b]. A main effect of phenotype [$F(1, 44) = 8.79$, $p = .005$], but not for condition [$F(1, 44) = .003$, $p = .960$] was shown. Specifically, VD LNB mice built nests with a higher solidity index compared to VD NNB mice ($.9 \pm .01$ vs. $.8 \pm .1$, $p = .032$, $d = .9$ [.06; 1.84]), a difference that was not seen between LNB and NNB mice housed under SL conditions ($.9 \pm .03$ vs. $.8 \pm .1$, $p = .209$, $d = .7$ [−.07, 1.53]).

Nesting area

Nesting area (expressed as pixels) was analyzed as a third measure of nesting neatness and integrity. A significant main effect of phenotype [$F(1, 44) = 24.22$, $p < .0001$], but not of light condition [$F(1, 44) = .42$, $p = .522$] was again shown in the absence of a significant two-way interaction [$F(1, 44) = .03$, $p = .864$; Figure 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: $193878 \pm 47,480$ vs. $118,397 \pm 51,784$ pixels, $p = .002$, $d = 1.5$ [.62, 2.38]; VD: $206874 \pm 60,782$ vs. $125,906 \pm 58,895$ pixels; $p = .002$, $d = 1.3$ [.40; 2.27]).

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 1.

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 [$F(1, 37) = 2.79$, $p = .103$; Figure 5a], nor any significant main effect of phenotype [$F(1, 37) = .1902$, $p = .665$] or condition [$F(1, 37) = 1.52$, $p = .225$] influenced the result. With regards to hippocampal serotonin concentrations of NNB and LNB mice, four samples were below the limit of detection. However, while there was also no interaction observed with respect to hippocampal serotonin [$F(1, 38) = .14$, $p = .714$; Figure 5b], condition significantly impacted the data [$F(1, 38) = 5.35$,

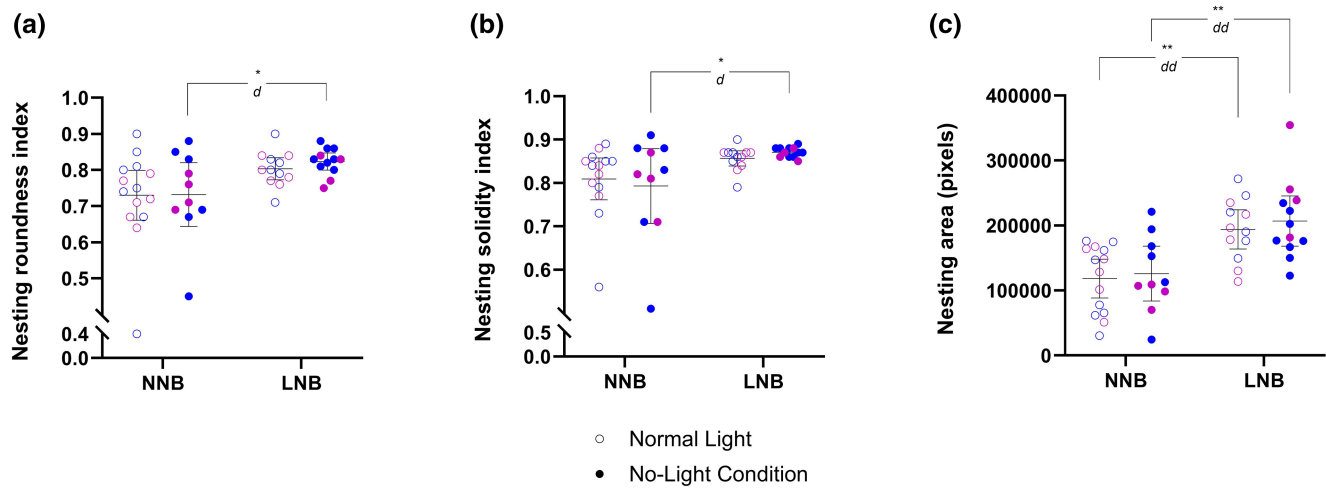


FIGURE 4 Roundness index (a), solidity index (b), and area (in pixels) (c) of nests built by NNB and LNB expressing mice when subjected to standard lighting and conditions of visual deprivation. Error bars represent mean with 95% confidence intervals. LNB, large nest building; NNB, normal nest building; blue dots: male mice; purple dots: female mice.

TABLE 1 Group allocation for frontal-cortical and hippocampal monoamine analysis.

	Frontal cortex (n)	Hippocampus (n)
NNB SL	14	14
Group n=14	–	(5HT: 2 bld)
LNB SL	10	12
Group n=12	(NA: 1 bld; 5HT: 1 bld)	–
NNB VD	9	9
Group n=10	–	(5HT: 1 bld)
LNB VD	11	11
Group n=12	(NA: 2 bld; 5HT: 2 bld)	(5HT: 1 bld)

Abbreviations: 5HT, serotonin; bld, below detection limit; LNB, large nesting; NA, noradrenaline; NNB, normal nesting; SL, standard light conditions; VD, visual deprivation.

$p=.026$). The same was not true for phenotype [$F(1,38)=1.52$, $p=.225$]. Nevertheless, Bonferroni post-hoc tests failed to reveal significant between-condition pairwise comparisons.

3.2.2 | Noradrenaline

Three frontal-cortical samples were below the limit of detection. A significant phenotype-condition interaction was shown [$F(1, 37)=4.59$, $p=.0388$; Figure 5c]. Bonferroni post-hoc revealed VD LNB mice to present with significantly higher frontal-cortical noradrenaline concentrations, compared to VD NNB mice (363.4 ± 61.1 vs. 290.8 ± 76.8 , $p=.027$, $d=1.0$ [.04, 2.02]).

Further, while no significant interaction was shown for the measured hippocampal concentrations [$F(1, 42)=.48$, $p=.4912$; Figure 5d], phenotype [$F(1, 42)=16.62$, $p=.0002$], but not condition [$F(1, 42)=1.77$, $p=.191$] significantly impacted the result. This phenotypic difference was most prominent in the SL condition, with LNB mice presenting with higher noradrenaline concentrations compared to NNB mice (307.1 ± 60.4 vs. 168.3 ± 104.8 ng/g, $p=.002$, $d=1.5$ [.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, $p=.060$, $d=.9$ [-.04, 1.82]).

3.3 | Biobehavioral correlations

To explore potential relationships between the different behavioral outputs, and frontal-cortical and hippocampal monoamine concentrations, we ran Spearman's rank order correlations. Of all the analyses run, the only significant correlation was shown between the total nesting score and hippocampal noradrenaline concentrations under conditions of standard lighting ($r_s=.51$, 95CI [.14, .75], $p=.008$; remainder of the statistical analyses not reported).

4 | DISCUSSION

The present work assessed the dependency of nesting expression in the deer mouse model of compulsive-like behavioral persistence on visual feedback. We further aimed to determine how NNB and LNB uniquely associates with limbic involvement. The main findings were

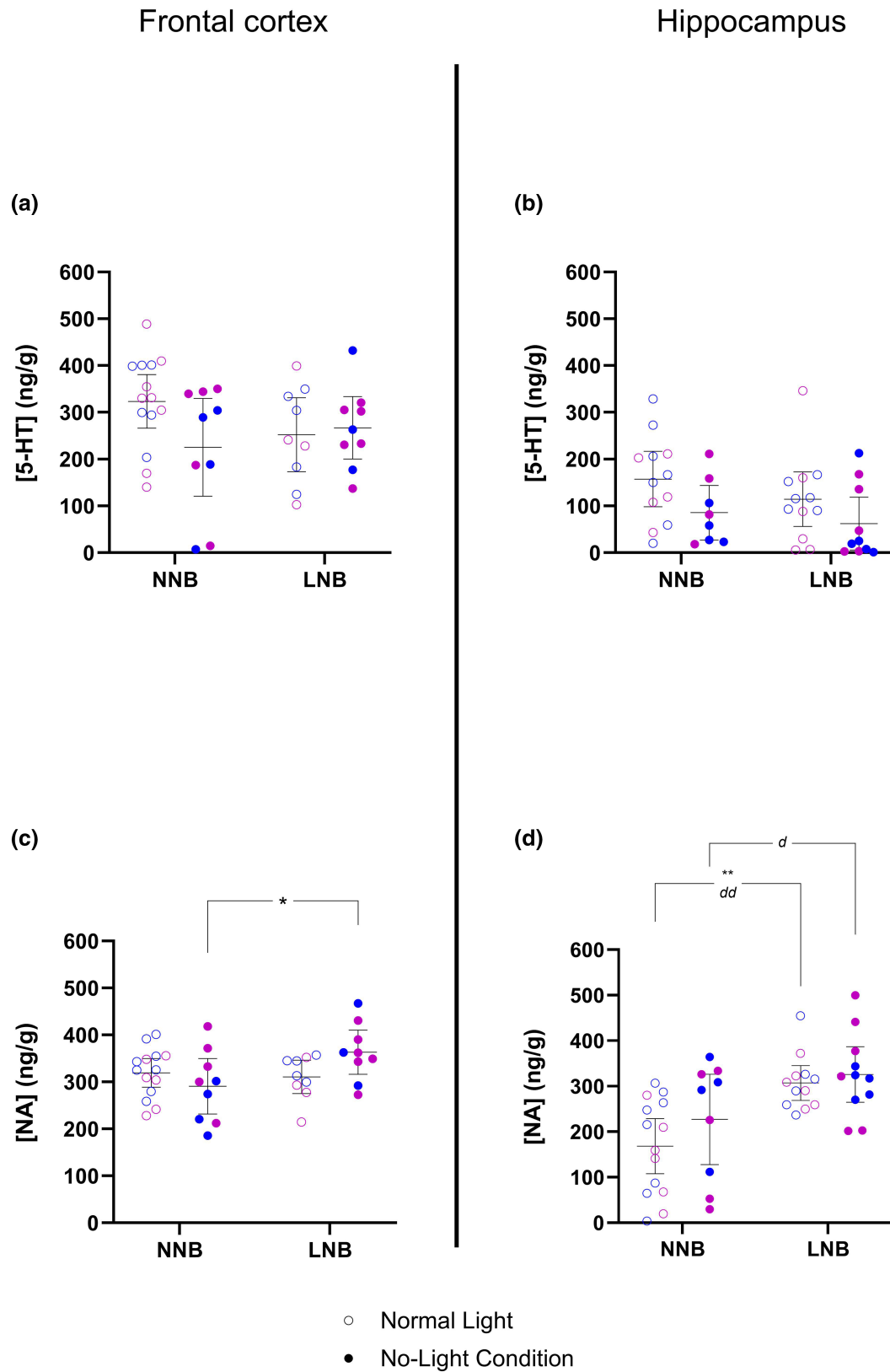


FIGURE 5 Serotonin concentrations in the prefrontal cortex (a) and hippocampi (b) and noradrenaline concentrations in the prefrontal cortex (c) and hippocampi (d) of NNB and LNB expressing mice when subjected to standard lighting and conditions of visual deprivation. Error bars represent mean with 95% confidence intervals. 5-HT, serotonin; LNB, large nest building; NA, noradrenaline; NNB, normal nest building; blue dots: male mice; purple dots: female mice.

as follows: (1) as opposed to NNB, LNB represents an inflexible and excessive behavioral 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 concentrations which may contribute to its expression.

A vast body of work explored nesting behaviors in laboratory housed nesting species, for example, bank voles (Kapusta et al., 2022; Schönecker, 2009), rats (Simpson & Kelly, 2011; Van Loo & Baumanns, 2004), mice (Balcombe, 2006; Jirkof et al., 2013) and rabbits (Lidfors et al., 2007; Verga et al., 2007). While nesting behavior is applied as an indicator of rodent welfare, substantial research into varying nesting expression in specific species and its relevance for studying compulsive-like behaviors has provided valuable insights. Within the context of the present investigation, earlier findings showed that in some species, for example, rabbits, nesting-expression is dependent on sensory feedback that regulates behaviors aimed at “just-right” outcomes (Hoffman & 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 behavior. In our laboratory, the same approach is followed to separate deer mice into NNB and LNB cohorts, with the exception that mice are not inbred for this purpose and thus the species resembles a wild-type model (Wolmarans et al., 2018). This is important, since the model lends itself well to studies of the naturalistic mechanisms that contribute to the etiology and promulgation of compulsive-like behavioral persistence. Nevertheless, despite previous work that provided valuable insights into the biobehavioral differences between NNB and LNB expressing mice, a deeper understanding of the underlying thought-action control of deer mouse nesting behavior, notably LNB, remains lacking. Therefore, in this work, we specifically aimed to establish if LNB is related to visually perceived environmental 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 SL and total VD.

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 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 & Hoekstra, 2018), prior experience may improve behavioral 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 behavioral 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 4a,b). While no significant differences in the neatness of nests within each cohort were shown between the two lighting conditions, VD influenced NNB and LNB distinctly, so that neatness became more varied in NNB, and less varied in LNB mice (Figure 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 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, for example, tactile or haptic feedback, to guide their nesting behavior, and that these mechanisms were not as effective as vision for this purpose. In contrast, since LNB resembles an excessive, rigid, and habit-like behavior, its expression is not as reliant on visual feedback. Within this conceptual framework, the influence of VD on fine coordinated behaviors 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 that prior learned goal-directed actions can be completed in the absence of simultaneous visual feedback, performance accuracy significantly deteriorated. 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 hypothesized 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 behavioral rigidity and habitual learning in compulsive symptom expression, both of which are linked with corticostriatal hyperactivation (Calabresi et al., 2014; Lanciego et al., 2012), 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 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 5c,d). Although not traditionally regarded as an essential role player in OCD, noradrenaline plays a critical role in regulating behavioral expression, notably under stressful or threatening circumstances (McBurney-Lin et al., 2019; Szabadi, 2013). The ability to adapt one's behavior based on immediate changes in the environment speaks to behavioral flexibility and is critical for survival. For this, the LC-noradrenaline system, is crucial (Aston-Jones

& Cohen, 2005; Mäki-Marttunen et al., 2020). Hyperactivation of this system is associated with heightened arousal and emotionality (Aston-Jones & Cohen, 2005), impaired cognitive processing (Mäki-Marttunen et al., 2020), and behavioral inflexibility (Borodovitsyna et al., 2017; Mäki-Marttunen et al., 2019, 2020). The present data pertaining to LNB mice are congruent with such an understanding. While visual feedback did not influence fronto-limbic noradrenaline concentrations in NNB mice, that is, 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 PFC inactivation and compromised behavioral flexibility and goal-directed decision making (Mäki-Marttunen et al., 2020). This will result in an overreliance on habitual and rigid behavioral programs, which lends further support to the first main finding as well. In extension, it should be underscored that while visual feedback did not directly influence nest size and neatness indices per se in LNB mice, putative evidence is presented that increasing nest sizes are correlated with increased hippocampal noradrenaline concentrations under conditions of standard lighting only. Thus, we cannot exclude the fact that visual feedback indeed contributed to an inflated sense of anxiety, as was hypothesized. The present findings are in line with work the work of Lustberg, Iannitelli, et al. (2020), who showed noradrenaline to be necessary for stress-induced compulsive-like behavioral 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 (Buot & Yelnik, 2012; Lu et al., 2021; Nikolaus et al., 2010), 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 behavioral expression in deer mice, a finding that could pave the way forward for investigations into the role of noradrenergic processing in clinical OCD, a topic that remains mostly understudied.

5 | CONCLUSION

The present work sought to build on previous study 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 than NNB. Further, a clear role for noradrenergic signaling in LNB is highlighted, which points to heightened innate limbic activation as a potentially novel neurobiological construct in the present model system. Collectively, the current results are not only informing of the neurocognitive underpinnings of normal nesting behavior, 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 behaviors.

AUTHOR CONTRIBUTIONS

HM: methodology, formal analysis, investigation, writing—original draft, writing—review and editing. TEK: conceptualization, methodology, formal analysis, investigation, writing—original draft, writing—review and editing; DWW: conceptualization, methodology, formal analysis, investigation, writing—original draft, writing—review and editing, supervision, funding.

DECLARATION OF TRANSPARENCY

The authors, reviewers and editors affirm that in accordance to the policies set by the *Journal of Neuroscience Research*, this manuscript presents an accurate and transparent account of the study being reported and that all critical details describing the methods and results are present.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

PEER REVIEW

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/jnr.25320>.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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