Behavioural Processes 216 (2024) 105004

Contents lists available at ScienceDirect

Behavioural Processes

journal homepage: www.elsevier.com/locate/behavproc

Short Communication

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

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ARTICLE INFO

Keywords:
Deer mouse
Rodent wellbeing
Offspring survival
Nest
Obsessive-compulsive

ABSTRACT

Nesting is a normal, evolutionary conserved rodent behavioural phenotype that is expressed for purposes of breeding, safety, and thermal regulation. Further, nesting is commonly assessed as marker of overall rodent health and wellbeing, with poorer nesting performance generally proposed to resemble a worse state of health. Deer mice can be bidirectionally separated with 30 % of mice presenting with excessively large nesting behaviour (LNB). All laboratory-housed deer mice are exposed to identical environmental conditions. Thus, the functional purpose of LNB remains unknown. Considering the evolutionary functions of nesting, we hypothesized that LNB will be related to an inflated drive to breed and nurse offspring. After breeding two generations of offspring from six 'normal' nesting (NNB) and seven LNB expressing pairs, our data showed that while as fertile as NNB expressing pairs, offspring survival of LNB mice were notably worse (67.9 % vs. 98.3 %). In conclusion, variance in nesting behaviour should be considered when animal health and wellbeing is considered, since it may point to underlying biobehavioural perturbations.

1. Introduction

Deer mice *(Peromyscus maniculatus bairdii)* are studied for their varying expression of rigid behavioural phenotypes, e.g., high stereotypy and large nesting behaviour (LNB).(Scheepers et al., 2018) Nesting is an evolutionarily conserved rodent behaviour that is expressed for purposes of breeding, safety, and thermal regulation.(Smithers, 1983) However, bidirectional separation of deer mice based on nesting performance revealed 30 % of mice of both sexes to show LNB, which is since studied for its resemblance of compulsive-like behavioural persistence.(Wolmarans et al., 2022) Against this background, LNB is highly stable and seemingly purposeless.(Wolmarans et al., 2016) It is characterised by dysfunctional cortico-striatal serotonergic (Wolmarans et al., 2022) and adenosinergic (Saaiman et al., 2023) signalling, while LNB, but not 'normal' nesting (NNB) expressing mice are overly resilient to the impact of negative sensory feedback (i.e. electrical shock (de Brouwer et al., 2020b) and anxiogenic open spaces (Wolmarans et al., 2022)), i.e., presenting with less anxiety in these instances, compared to their NNB counterparts. Since all laboratory-housed mice are exposed to identical environments, the question arises as to what the functional purpose of LNB is? Although nesting performance in laboratory-housed rodents is applied as a measure of overall health and wellbeing (poor nesting performance being indicative of a worse state of health; Jirkof, 2014) the functional purpose of LNB remains entirely unknown. Considering the evolutionary functions of nesting, we hypothesized that the behavioural output of LNB expressing mice will be driven by an inflated motivational drive to breed and that the breeding performance and offspring survival of LNB breeding pairs would therefore be higher, compared to that of NNB expressing pairs.

2. Methods and materials

2.1. Mice

Fifty-seven adult deer mice of both sexes (that were later used to constitute the NNB and LNB breeding pairs used in this investigation) were bred from eleven progenitor breeding pairs (12 – 18 months old) maintained at the vivarium (Preclinical Drug Development Platform,

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https://doi.org/10.1016/j.beproc.2024.105004
Received 11 July 2023; Received in revised form 8 February 2024; Accepted 11 February 2024
Available online 13 February 2024

Table 1
Descriptive data pertaining to breeding and offspring survival. Breeding pairs 1–7 are LNB. Breeding pairs 8–14 are NNB.

Pair no.	Male Total score (g)/% CV	Male age	Female Total score (g)/%CV	Female age	Litter 1 date of birth	Number of pups in litter	Mortalities (Litter 1)	Litter 2 date of birth	Number of pups in litter	Mortalities (Litter 2)
								Offspring from LNB expressing pairs		
1	22.9/23.5	149	29.4/13.1	151	20 Jan 23	3	**0**	16 Feb 23	4	**4**
2	21.0/20.0	90	30.5/39.1	151	21 Jan 23	3	**3**	15 Feb 23	2	**2**
3	24.8/15.1	117	29.2/43.4	132	24 Jan 23	3	**0**	24 Feb 23	3	**0**
4	23.5/31.5	132	23.9/32.8	151	22 Jan 23	5	**5**	15 Feb 23	6	**0**
5	23.9/36.3	141	23.8/28.7	151	20 Jan 23	3	**1**	18 Feb 23	5	**0**
6	22.5/34.2	149	20.3/26.9	123	22 Jan 23	4	**1**	21 Feb 23	4	**1**
7	20.2/23.4	132	20.0/27.0	151	21 Jan 23	2	**0**	17 Feb 23	5	**0**
AVE	**22.7/26.3**	**130**	**25.3/30.2**	**144**		**3.2**			**4.1**	
								Offspring from NNB expressing pairs		
8	4.5/21.0	149	7.0/26.3	106	-		-	-		-
9	9.8/35.0	132	8.9/73.6	141	19 Jan 23	5	**0**	17 Feb 23	7	**0**
10	6.0/63.3	106	6.6/81.4	112	20 Jan 23	5	**0**	14 Feb 23	5	**0**
11	8.3/76.7	117	9.6/92.5	123	02 Jan 23	1	**1**	26 Feb 23	6	**0**
12	5.9/32.8	132	3.7/72.9	112	30 Jan 23	5	**0**	27 Feb 23	6	**0**
13	6.1/71.0	141	4.4/74.9	132	20 Jan 23	4	**0**	24 Feb 23	5	**0**
14	7.6/34.8	149	12.9/39.0	132	19 Jan 23	5	**0**	07 Feb 23	5	**0**
AVE	**6.8/48.8**	**132**	**7.6/65.8**	**122**		**3.8**			**5.3**	

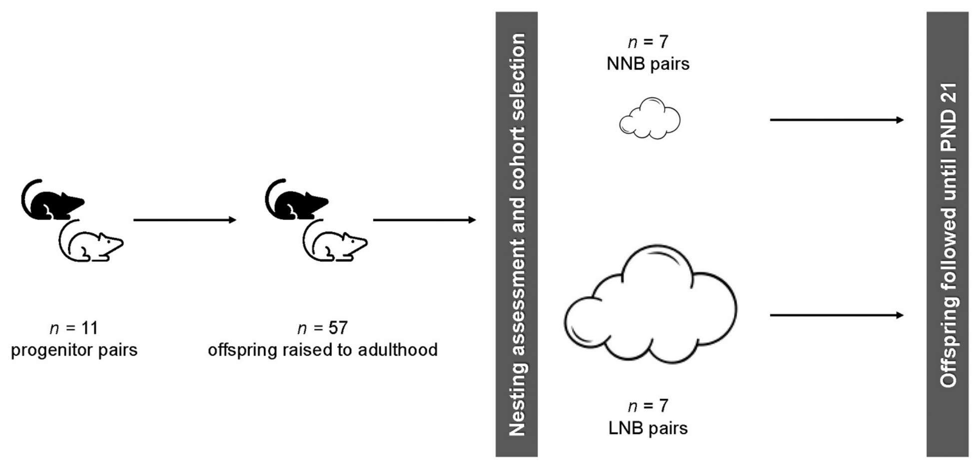

Fig. 1. Schematic layout of study design.

North-West University; SAVC reg: FR15/13458; AAALAC accreditation file: 1717). These pairs were randomly allocated, i.e. without litter or nesting bias (ethics approval no.: **NWU-00770-22-A5**). All mice were housed and bred in individually ventilated cages (35 cm (l) x 20 cm (w) x 13 cm (h); Techniplast® S.P.A., Varese, Italy) kept ambient at 23 °C on a 12-h light/dark cycle (06:00/18:00). Food and water were provided ad lib through study termination. Cages were cleaned and new bedding (corncob) added, weekly. A piece of white polyvinyl chloride pipe (10 cm (l) x 4 cm (Ø)) and paper towel were provided as a form of environmental enrichment and nesting material (except during periods of nesting assessment; see below), respectively. Since not all 57 offspring of the 11 progenitor breeding pairs were born within the same period, breeding continued until 57 pups were born. Pups were weaned on postnatal day (PND) 21 and raised until adulthood (PND 103-151; Table 1) when their nesting behaviour was assessed. This paper reports data pertaining to the breeding performance of and the offspring survival rates of pups born from the respective NNB and LNB expressing breeding pairs that were selected from these 57 mice (Fig. 1; see below); these pups were followed until PND 21, the typical time at which deer mice are weaned.

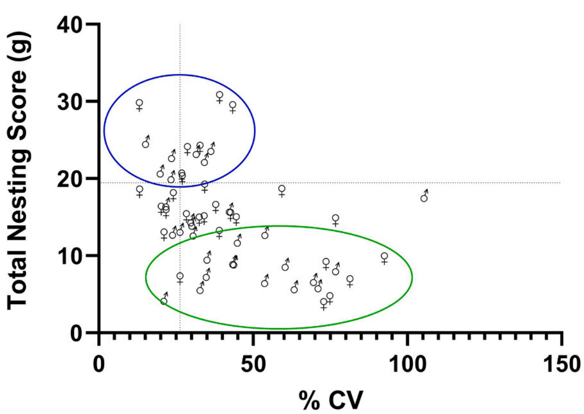

Fig. 2. Selection of NNB- (examples enclosed in green circle) and LNB- (enclosed in blue circle) expressing deer mice. % CV: percentage coefficient of variance with respect to the daily nesting scores generated by each mouse over seven consecutive days.

2.2. *Nesting assessment and behavioural selection*

Nesting performance was assessed in each of the 57 first-generation offspring over seven consecutive days. Briefly, to classify mice into the NNB- or LNB-expressing cohorts, we applied the protocol of Saaiman et al. (2023) without modification. Each mouse was screened for nesting behaviour over the course of seven consecutive days. On each day between 15:00 and 16:00, an excess of pre-weighed, unscented cosmetic cotton wool was introduced into the roof of each home cage. Every subsequent day, also between 15:00 and 16:00, the remaining cotton wool, i.e. not used for nesting, was weighed, and the built nests removed and discarded. This process was repeated over all seven days of testing. At the end of the assessment period, a grand total nesting score (the sum of the seven daily scores, expressed in grams), was calculated for each mouse. Considering that compulsivity is both excessive and persistent, classification of LNB behaviour was based on the extreme end of the total nesting score distribution of all assessed mice (Wolmarans et al., 2016). Specifically, LNB behaviour was broadly defined as those scores that clustered near to or above the upper 75th percentile of the total nesting score distribution, but that were also associated with coefficients of variance pertaining to the inter-day nesting scores that clustered within the lower 25th percentile of distribution (Fig. 2). Conversely, NNB behaviour was characterised by total nesting scores that clustered below the 50th percentile of the distribution. From this data, 14 NNB and 14 LNB-expressing mice were selected for further study (Fig. 2) and seven breeding pairs per behavioural cohort randomly constituted. All breeding pairs were left to breed two successive generations of offspring. Importantly, male and female counterparts of each pair remained co-housed throughout the course of this investigation. While males of this species show little interest in parental care (Khadraoui et al., 2022), co-housing in the model is preferred due to the unknown experiential impact of parental behaviour on the long-term behavioural outcomes in offspring.

3. Results

The descriptive data pertaining to NNB and LNB expressing breeding pairs, births, and survival rates are shown in Table 1. Six of the seven NNB dams gave birth to a total of 59 pups (average between-litter interval: 30.3 days), of which 58 pups survived until PND 21 (survival rate: 98.3 %). LNB dams gave birth to 53 pups (average between-litter interval: 26.6 days). Of these, 36 pups survived through PND 21 (survival rate: 67.9 %). Importantly, deaths occurred throughout the 21 days post-birth, with no specific timepoint being more significant in this respect.

4. Discussion

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

Our results remarkably show that despite being as fertile as NNB mice, offspring born from most LNB expressing pairs are notably less viable. This is important since nesting is commonly assessed in laboratory-housed rodents as a measure of overall health and wellbeing. Because nesting serves several evolutionary conserved functions,(Saaiman et al., 2023; Hoffman and Rueda Morales, 2009) we believed that LNB, compared to NNB, would be representative of an inflated motivational drive to breed and nurse offspring. This theory did not hold true. Rather, our findings support theories that naturalistic LNB in deer mice is an overly goalless behavioural outcome of dysfunctional psychobiological processes (Saaiman et al., 2023; Wolmarans et al., 2022; de Brouwer et al., 2020b, 2020a). Since parental care was not investigated per se and considering that we did not prospectively track the developmental outcomes of pups, continued investigation should determine whether the high mortality rate in LNB-bred offspring is related to genetic and biological impairment, or rather the outcome of poor parental care or malnourishment. Although the latter may be possible due to the focused and rigid engagement of LNB expressing mice in nesting behaviour shown before (Wolmarans et al., 2022), several traces of evidence point to LNB being founded upon dysfunctional neurobiological (Saaiman et al., 2023; Wolmarans et al., 2022), cognitive (de Brouwer et al., 2020b) and inflammatory processes (Scheepers et al., 2019), all of which could have contributed to the present findings. In conclusion, variance in nesting behaviour should be considered when animal health and wellbeing is considered, since it may point to underlying biobehavioural perturbations.

CRediT authorship contribution statement

De Wet Wolmarans: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Brian H Harvey:** Writing – original draft, Supervision, Resources, Project administration, Funding acquisition. **Heike Stoppel:** Writing – original draft, Methodology, Investigation, Data curation, Conceptualization.

Data availability

Data will be made available on request.

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