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Nest building as an indicator of illness in laboratory mice

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ABSTRACT

Laboratory mice housed at typical temperatures and provided with crinkled paper nesting material build fully enclosed nests, increasing welfare, and reducing cold stress, but complicating daily animal observations by care staff. Anecdotal reports by animal care staff indicate that ill mice are not found within the nest and do not nest build. We hypothesized that both nest shape and whether or not ill mice were found outside the nest could be used as tools to identify ill mice. Forty two female C57BL/6NCrl mice were provided 10 g of nesting material and assigned to a social treatment of either solitary or group housing. Lipopolysaccharide (LPS) injected intraperitoneally at 1 mg/kg was used to induce malaise in 0, 1, 2, or 3 mice/cage; all others received saline. Prior to the study, mice were habituated to handling and injections with positive reinforcement. In order to blind the nest scorer to treatment novel, but experienced, handlers administered the experimental injections. Nest score, number of mice in the nest, and anhedonia measured by sugared cereal consumption were recorded at the following time points: baseline, cage change, saline injection, injection, and injection + cage change and data were analyzed using GLMs with post-hoc contrasts. The number of mice observed outside the nest was not affected by any treatment. Nest score was not significantly altered in group housed mice but LPS-injected solitary mice had significantly lower nest scores than saline-injected solitary mice at the injection + cage change time point. Saline-injected mice also had a significant reduction in nest score from baseline at injection + cage change. It is likely that receiving the injection from novel handlers were likely the cause for this alteration, yielding the unexpected result that nest building in mice is affected by a novel handler. LPS-injected mice, regardless of social treatment, ate ≈2 g less sugared cereal per mouse at both injection and injection + cage change time points compared to their baseline cereal consumption and saline-injected mice at the same time points. Group housing appears to mask changes in nest score if other cage residents are healthy and acutely ill individuals were not observed to have a location bias, in or out of the nest, after LPS injection. However, a reduction in nest score has the potential to be a useful tool to identify acute illness after cage change in solitary mice. Changes in nest complexity may be useful to identify illness earlier for general husbandry and welfare purposes and may be a more robust tool in chronic, rather than acute, disease models.

1. Introduction

In typical laboratory temperatures (20–26 °C), mice are exposed to temperatures resulting in chronic cold stress (Gordon, 2004). However, nesting material can be provided to aid in both thermoregulation (Gaskill et al., 2013a) and provide a biologically relevant enrichment. In the wild, nests aid mice in thermoregulation, provide shelter from predators, and are positively correlated with survival (Brown, 1953). The nests of wild mice are complex structures made from multiple materials, and replenished and manipulated daily (Brown, 1953; Latham and Mason, 2004). Laboratory mice are highly motivated to obtain nesting material (Gaskill et al., 2012; Gross et al., 2011; Van de Weerd et al., 1998) and will construct nests similar to those of wild mice if given the opportunity, but nest quality depends on the material provided (Gaskill et al., 2013b; Hess et al., 2008), and the strain and sex of the mice (Gaskill et al., 2012). Nesting material has been shown to reduce radiative heat loss (Gaskill et al., 2013a), increase feed conversion

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(Gaskill et al., 2013a; Olsson and Dahlborn, 2002) and decrease pup mortality (Gaskill et al., 2013c) in several laboratory strains studied.

Nesting material has one drawback when it is used to enrich laboratory mouse housing. Instructions for routine animal husbandry and care provided in the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011) stipulate that animals need to be checked for health and well-being daily. For larger laboratory animals, where few are typically housed or used, this can be a relatively simple process. For other animals, where there are often thousands, if not tens of thousands in a small space, this becomes a more complex problem. The solution has been to implement clear caging, which is not preferred by animals (Olsson et al., 2003; Porter et al., 1963; Sherwin and Glen, 2003), and to visually scan each cage for the presence of ill animals. Nesting material can complicate this scanning process, and ill animals may be overlooked. Anecdotally, most animal husbandry technicians and some research technicians report that once they are familiar with how mice behave with nesting material, nests do not impede this process, as mice will be in the nest when the lights are on, but sick animals are found outside the nest. Sick animals might be found outside the nest because they are isolating themselves from conspecifics (Yee and Prendergast, 2012), or because the conspecifics drive them away. The purpose of this study is not to determine the reasons why ill animals are found outside of the nest but instead to test if animals rendered ill would be found outside of the nest, since this would be simple and practical way for animal care staff to identify sick individuals. A general malaise can be induced by administration of a lipopolysaccharide (LPS) which activates the innate immune system through the release of cytokines, causing fever, body aches, and other signs of illness (Kelley et al., 2003) and this is the system we chose to use.

Beyond simply observing the animals, assessing sickness in mice is another problem, as their small size, unfamiliar body language, quick movements, nocturnal tendencies, prey stoicism, and sheer number usually limits detailed assessments unless part of a research protocol. Some clinical signs are universally recognized as mouse sickness behavior, such as hunched posture, reluctance to move, piloerection, lethargy, lack of grooming, unsteady gait, and reduced food consumption. Nest building in the context of illness has been studied, where gathering behavior is restored in sick lactating mice at cold temperatures (Aubert et al., 1997). Other studies have tested alteration of nest conformation when mice are in pain but the observations were not statistically evaluated (Arras et al., 2007) or results were not completely clear (Jirkof et al., 2013b).

Mice and other animals assess sickness and parasitism in their own species using behavioral and olfactory cues unavailable to humans. Sickness can lead to avoidance by conspecifics (Arakawa et al., 2010), changes in social interactions (Renault et al., 2008; Yee and Prendergast, 2010, 2012), disinterest from potential mates (Penn et al., 1998), and even aggression (Hart, 1990). The anecdotal reports from caretakers and researchers, the literature on sickness behavior, and our own experience led us to hypothesize that nest quality would be reduced with an increasing number of sick mice, making it easier for care staff to observe mice in a cage and that any sick animals in a group would be found outside the nest during routine health checks. We also predict that cages receiving LPS would be readily apparent after cage cleaning because animals experiencing general malaise would be less likely to gather material and build a nest.

2. Materials and methods

2.1. Animals and husbandry

All work was conducted at Charles River's AAALAC-accredited Wilmington, MA, facility and was approved by the IACUC. Animals were free of a list of common mouse pathogens; further details may be found at http://www.criver.com/files/pdfs/rms/hmsummary.aspx. For all tests, female C57BL/6NCrl (B6) were used. Mice were housed in disposable, transparent, individually ventilated cages (Innovive, San Diego, CA; LxWxH: 37.3 × 23.4 × 14.0 cm). The cages were bedded with irradiated aspen shavings (NEPCO, Warrensburg, NY), and mice were provided with 10 g of fresh nesting material at every cage change (Enviro-Dri, Shepherd Specialty Papers, Watertown, TN). Food (5L79, LabDiet, St. Louis, MO) and hyperchlorinated water via water bottle were provided *ad libitum*. The light cycle was 12:12 light:dark (on at 06:30, off at 18:30), temperature was maintained at 21 °C ± 1 °C, and humidity was maintained between 30 and 70%. Animals were observed daily for appearance and general health; if animals had appeared ill more than 48 h after experimental onset, they would have been euthanized and the cage replaced. All randomization of animals and cages was accomplished through the use of the random integer generator at random.org.

Table 1
Number of cages (n) assigned to each treatment.

Social treatment		LPS treatment			
		Saline	1LPS	2LPS	3LPS
	Group (3 mice)	3	3	3	3
	Solitary (1 mouse)	3	3		

Forty two 8 week old female mice were randomly assigned to a social treatment (single or group housing) using a Latin square experimental design (N = 3 per combination). The authors intended to conduct this study in both sexes, however due to a large amount of injurious aggression in the group housed males prior to the study start, too many data points were lost and we could not include males in the analysis. Handling rats at the time of LPS administration alters their hyperthermic response to LPS (Romanovsky et al., 1998). Therefore, before any tests were performed, all mice were habituated by an investigator (B. Gaskill) to handling and injections using a reward of several microliters of chocolate milk for three weeks, with this habituation consisting of 3–4 sessions per week lasting approximately 10–60 s per animal. Solitary cages of mice were randomly allocated to either saline or LPS treatments (1 mg/kg IP, from *E. coli* 0111:B4, Sigma-Aldrich, St. Louis, MO; see Table 1). Group housed cages were also randomly assigned an LPS treatment: all 3 mice injected with saline (Saline); 1 mouse injected with LPS and the other 2 mice injected with saline (1LPS); 2 mice injected with LPS and the other injected with saline (2LPS); or all 3 mice injected with LPS (3LPS). Different numbers of mice were injected in group housing to determine if nest scoring or observing mice outside the nest site would change as a function of the number of ill mice. All animals received intraperitoneal injections at approximately 17:00, one hour before the start of the dark cycle. Mice were injected at this time of day because it corresponds to the end of the daily inactivity period, meaning it would be more likely to disrupt overall activity as well as nest building peaks found toward the end of the dark and beginning of the light period (Jirkof et al., 2013a).

Measures collected from the cage were: individual mouse weights (averaged per cage), nest score, food consumption, and the number of mice in-or-out of the nest. Illness is often accompanied by a depressive state, which may be assessed by a decrease in enjoyment of pleasurable experiences (Aubert, 1999). In rodents, this is often evaluated by the consumption of sweet solutions. Sugar solution could not be used since cage design did not allow for a second water bottle. Therefore we added a test that determined the change in consumption of a sweetened cereal as a measure for anhedonia. In this test, 5 g of FrootLoops® (Kellogg's, Battle Creek, MI; a fruit-flavored cereal with approximately 12 g of sugar per 29 g of cereal) per mouse are placed in the cage at 17:00, at the start of the activity

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