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Burrowing and nest building behavior as indicators of well-being in mice

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HIGHLIGHTS

- Nest building and burrowing are spontaneous home cage behaviors of mice.
- Both behaviors reflect easy-to-quantify activities of daily living.
- Reduction of performance correlates with a variety of detrimental states in mice.
- Nest building and burrowing may be sensitive tools to assess animal well-being.

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ABSTRACT

The assessment of pain, distress and suffering, as well as evaluation of the efficacy of stress-reduction strategies, is crucial in animal experimentation but can be challenging in laboratory mice. Nest building and burrowing performance, observed in the home cage, have proved to be valuable and easy-to-use tools to assess brain damage or malfunction as well as neurodegenerative diseases. Both behaviors are used as parameters in models of psychiatric disorders or to monitor sickness behavior following infection. Their use has been proposed in more realistic and clinically relevant preclinical models of disease, and reduction of these behaviors seems to be especially useful as an early sign of dysfunction and to monitor disease progression. Finally, both behaviors are reduced by pain and stress. Therefore, in combination with specific disease markers, changes in nest building and burrowing performance may help provide a global picture of a mouse's state, and thus aid monitoring to ensure well-being in animal experimentation.

1. Introduction

Most countries have regulations for the breeding, housing and use of animals for scientific experimentation that aim to ensure laboratory animal well-being. These regulations emphasize the importance of reducing pain, distress and suffering by choosing refined breeding, housing and experimental procedures, and the importance of anesthetic and analgesic protocols for animals possibly experiencing pain, distress or suffering. In particular, they highlight the significance of the assessment and quantification of pain, distress and suffering, as well as evaluation of the efficacy of pain-, distress- and suffering-reduction strategies (see, for example, Directive 2010/63/EU). In addition, in many countries, including the countries of the European Union and Switzerland, it is mandatory to grade, prospectively and retrospectively, the level of discomfort and harm inflicted by experiment (Bundesamt für Veterinärwesen, 1994, 1995; The European Parliament and the Council of the European Union, 2010). The essential prerequisite of these practices is the reliable assessment of well-being or its deterioration in laboratory animals.

However, factors that determine well-being in mice – the most widely used laboratory species (Baumanns, 2004) – remain poorly understood (Clark et al., 1997) and hints of reduced well-being in these animals may be subtle (Peterson, 2004; Stasiak et al., 2003; van Sluyters and Obernier, 2004). Obvious clinical signs of reduced well-being in mice, such as sunken flanks, neglected grooming or piloerection, are evidence of a severely impaired, often moribund, health status in mice (FELASA, 1994). Diseases or interventions with a lesser impact seem not to evoke such clearly recognizable changes (Dawkins, 1980; Jirkof et al., 2010; Stasiak et al., 2003).

Behaviors that can be observed easily in a non-invasive manner might provide more sensitive cues as to the internal state of an animal compared to classical clinical monitoring tools. Observations in the home cage are especially advantageous as they impose minimal stress on the animal and reduce unwanted effects such as novelty stress, stress-induced analgesia or other changes in physiology and behavior that may be caused by the unfamiliar environment of a test apparatus. Recent studies have demonstrated the potential and

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promising use of complex behavioral indicators in the assessment of pain, distress and suffering in the laboratory mouse in veterinary research (Arras et al., 2007; Jirkof et al., 2010; Langford et al., 2010; Roughan et al., 2009) as well as in preclinical research (Deacon, 2006b, 2006c), but there remains a need to monitor species-typical behaviors in order to fully explore the underlying principles of murine disease and pain models, and to demonstrate the therapeutic effects of treatments (Blackburn-Munro, 2004; Mogil, 2009; Sano et al., 2009).

The assessment of pain- or distress-evoked aberrant behaviors or facial expressions (Langford et al., 2010; Roughan et al., 2009; Wright-Williams et al., 2007) has proved a sensitive approach toward a more clinically relevant estimation of well-being in mice.

As well as observing aberrant behaviors and signs of reduced well-being, indicators of positive well-being can also be assessed (Arras et al., 2007; Boissy et al., 2007; Jirkof et al., 2010). The display of behavioral diversity and so-called "luxury" behaviors or other highly motivated but, at least in the laboratory, non-essential behaviors, indicates that important needs of the animal are being met, and can serve as a sign of well-being. These kinds of behaviors are normally the first to be reduced in challenging situations (Boissy et al., 2007) and their absence might therefore indicate decreased well-being. These natural, spontaneous and often complex home cage behaviors may mirror activities of daily living (ADL) in humans that are affected by many clinical conditions, including chronic pain – a factor known to have an essential impact on quality of life in human patients (Lau et al., 2013; Torres-Lista and Gimenez-Llort, 2013; Urban et al., 2011).

Nest building (also described as nesting) and burrowing are spontaneous behaviors that have been proposed to represent such ADL in mice (Deacon, 2012), and good performance in these home cage behaviors might be indicative of normal behavioral function or well-being in mice and rats (Arras et al., 2007; Deacon, 2012; Huang et al., 2013; Jirkof et al., 2010, 2013b; Van Loo et al., 2007). This article reviews data on nest building and burrowing behavior from basic research and applied animal welfare research that may give hints as to the feasibility of using these behaviors for monitoring well-being in laboratory mice.

2. Species-typical behaviors to monitor well-being in mice

2.1. Nest building in laboratory mice

The construction of nests is common in rodent species. Wild house mice build nests to provide heat conservation, shelter from elements, predators, and competitors and to allow successful reproduction (Deacon, 2006b; Hess et al., 2008; Latham and Mason, 2004). Nest building increases lifetime reproductive success and is an essential thermoregulatory adaption (Berry, 1970; Bult and Lynch, 1997).

The motivation and ability to perform the behavioral sequence culminating in a finished nest persists also in domesticated mice and in laboratory animal facilities (Estep et al., 1975). Aside from "brood" or maternal nests, built specifically for reproduction, laboratory mice of both sexes provided with suitable nest building materials build "sleeping" or non-maternal nests of comparable size (Lisk et al., 1969; Sherwin, 1997). The literature discussing maternal nest building behavior in rodents is extensive but will not be reviewed here. In the laboratory setting, non-maternal nests might allow the mouse to shield itself from conspecifics, as well as from humans and external stimuli such as direct light (Clough, 1982). Also, as most standard animal facilities have ambient temperatures beneath their thermoneutral temperature, laboratory mice build nests for thermoregulatory reasons (Gaskill et al., 2012) as nest material reduces heat loss and associated food consumption

Fig. 1. Example of a nest built by a healthy female C57BL/6J mouse using a commercially available nestlet (Indulab).

(Gaskill et al., 2013). The motivation for nest building is high, and nest building material is highly valued by laboratory mice (Roper, 1973; Van De Weerd et al., 1998) see, for example Olsson and Dahlborn (2002) for a review. Additionally, it could be shown that providing nest material can result for example in the reduction of corticosterone production (Gurfein et al., 2012).

Nest building in mice is, to some extent, genetically determined and therefore strain differences in performance may occur (Bult and Lynch, 1997; Gaskill et al., 2012, 2013; Lynch, 1980; Van Oortmerssen, 1970). Nevertheless, nest building is present among the most widely used inbred and outbred laboratory strains; see Sherwin (1997) for literature examples. It is a complex, goal-directed behavior consisting of different aligned actions like pulling, carrying, fraying, push digging, digging, sorting and fluffing of nest material and bedding (Gaskill et al., 2012).

2.1.1. Assessment of nest building performance

Since maternal and non-maternal nest building performance has been used for decades as a monitoring tool in several scientific fields, a wealth of different protocols to assess nest building is available. Parameters to quantify focus either on the final goal toward which this behavior is directed, i.e., the completed nest, or on the display of the behavior per se. Nest quality is often quantified with complexity scores of 4–6 grades (Deacon et al., 2003; Paumier et al., 2013), ranging from no nests to complex nests with walls surrounding the mice; the height of the nest (Lijam et al., 1997; Moretti et al., 2005); or the amount of used or not used nest material (Deacon, 2006b). Sager et al. (2010) also recorded the numbers of entries into a plastic igloo blocked with nest building material to estimate the quality of a nest within a shelter. Nest quality is of course dependent on the material provided (Hess et al., 2008): paper cloth (Chen et al., 2005) and nestlets (Deacon, 2006b) (Fig. 1), are by far the most used materials in systematic assessment of nest building performance, and both enable mice to build at least moderately complex nests. Nest quality scoring has to be performed with special caution as schemes dealing with complexity scores may be especially prone to inter- as well as intra-rater variability. When provided with fresh nest material, the majority of healthy, naïve mice of both sexes start to manipulate it within a few minutes to less than an hour (Jirkof et al., 2013b; Sherwin, 1997). Therefore, the latency to use nest material (Jirkof et al., 2013b; Torres-Lista and Gimenez-Llort, 2013) or the time to build a "proper nest" (Lijam et al., 1997), as well as the duration of nest building (Jirkof et al., 2012), have been used to quantify nest building behavior. Mice generally build and repair their nests just before dawn but may show one or two additional nest building bouts during the dark phase (Jirkof et al., 2013b; Roper, 1973; Van Oortmerssen, 1970).