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Burrowing and Nest Building Behavior as Indicators of Well-being in Mice

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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.

Keywords

Burrowing

Nest building

Animal well-being

Mouse

Behavior

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 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 towards 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;Jirkof et al., 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

2.2. 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 (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).

2.2.1 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 towards 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. (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), (Figure 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). Therefore, nest quality or other parameters are frequently assessed after a complete dark phase (Deacon, 2006b) but shorter intervals are also used (Jirkof et al., 2013b;Paumier et al., 2013) and assessments may be conducted repeatedly (Arras et al., 2007;Moretti et al., 2005).

Nest building behaviour, as a thermoregulatory adaptation, may be increased due to cold ambient temperature (Barnett, 1965;Bult and Lynch, 1997) and complex nests could indicate cold stress. Therefore changes in nest building activity must be assessed under constant ambient temperature and interpreted with reference to appropriate control values.

2.2.1 Using nest building performance to monitor dysfunction and impairment

Non-maternal nest building performance has been shown to be sensitive to hippocampus damage and the progression of neurodegenerative diseases, and is used as a parameter in murine models of psychiatric disorders. In particular, post-surgical alterations in nest building support the use of this behavioral parameter in routine assessment of mouse well-being.

The important role of hippocampus regions in the display of nest building behavior is supported by several studies. Hippocampal damage, unlike medial prefrontal cortex lesions, (Deacon et al., 2002;Deacon et al., 2003) and malfunctions or small size of the hippocampus (Chen et al., 2005;Jedynak et al., 2012;Kondratiuk et al., 2013) reduce nest building in mice. This might be explained by the essential role of hippocampus cells in spatial memory and

orientation as well as in recognition of nest-like structures in mice (Deacon et al., 2002;Lin et al., 2007).

As expected, nest building is affected by hippocampal scrapie infection in mice, and deterioration of nest quality has been proposed as a sensitive and early sign of disease progression in this model (Cunningham et al., 2003;Cunningham et al., 2005;Deacon et al., 2005).

Alzheimer's disease is characterized by neuropathological changes found, amongst others, in hippocampus regions (Filali et al., 2009). Consequentially, in most, but not all (Filali et al., 2012), transgenic mouse models of Alzheimer's disease, impairment in nest quality or a prolongation of latency to initiate nest building has been observed (Deacon et al., 2008;Filali et al., 2009;Orta-Salazar et al., 2013;Torres-Lista and Gimenez-Llort, 2013;Wesson and Wilson, 2011). A combination of sensorimotor and memory impairment (Wesson and Wilson, 2011), deficits in behavioral planning and organization (Filali et al., 2011) as well as apathy or a depression-like state (Filali et al., 2009) is thought to underlie deterioration of this behavior, rather than any single gross general physical impairment.

Changes in the availability of neurotransmitters or hormones and their receptors play an important role in neurodegenerative and psychiatric disorders and may also influence nest building behavior. In different murine Parkinson`s disease models, for example after MTPT systemic injection or inactivation of the tyrosine hydroxylase gene, dopaminergic dysfunction or dopamine deficiency occur and seem to lead to nest building impairment (Paumier et al., 2013;Sager et al., 2010;Szczypka et al., 2001). In such models, nest building is used as a fine and sensorimotor, goal-directed and probably motivation-dependent task, and is thought comparable to the premotor symptoms human patients suffer (Paumier et al., 2013).

Genetically induced NMDA receptor hypofunction has been proposed as a model for schizophrenia and is correlated with reduced nest building (Ballard et al., 2002;Barkus et al., 2012;Belforte et al., 2010;Halene et al., 2009). Nevertheless, as NMDA receptors are distributed widely in the brain, their reduction may have several effects, and reduction of nest building might be indicative more of global impairment rather than a specific disease (Barkus et al., 2012).

The noradrenergic and dopaminergic systems play a major role in mouse models of Rett syndrome—a neurodevelopment autistic spectrum disorder—and seem to impact nest building, as malfunctions in these systems reduce home cage activities like nest building without resulting in gross motor deficits (Lang et al., 2013;Moretti et al., 2005).

Ablation of vitamin D receptors (VDR), which play an important role in the regulation of behaviors, affects the prolactin system in mice, and studies have shown that altered serum prolactin levels in VDR mutants may underlie impaired nest building and increased anxiety (Kalueff et al., 2006;Keisala et al., 2007).

In contrast to the aforementioned neurotransmitters and hormones, serotonin does not seem to play a role in nest building behavior, as disruption of the serotonin transporter increased anxiety but did not elicit changes in nest building (Kalueff et al., 2007).

Also, in two possible models of psychiatric disorders (Nlgn4 null mutant mice suffering from synapse malfunction—a model for autism spectrum disorders—and Dvl1 deficient mice, which may have altered development processes), nest building is decreased. In both models, nest building is correlated with impaired social behavior (El-Kordi et al., 2013;Lijam et al., 1997).

Even though systemic inflammation caused by low dose LPS injections results in anhedonia and reduced motivation to engage in non-essential activities like burrowing behavior, nest building was not affected in a study by Teeling et al. (Teeling et al., 2007). This might be due to the low dose of LPS used, as other authors observed a decrease in maternal nest building of lactating mice following injection of higher doses of LPS (Aubert et al., 1997). However, deficiency of Schnurri-2, discussed as a model for schizophrenia, induces mild chronic inflammation in the brain and led to a reduction in nest building behavior that can be restored with anti-inflammatory drugs (Takao et al., 2013).

Nest building performance has been shown to decrease after minor laparatomy as well as after telemetry transponder implantation in mice. The assessment of additional behavioral and clinical signs and telemetric recordings support the conclusion that nest building was reduced by post-surgical pain in these experiments (Arras et al., 2007;Jirkof et al., 2012;Jirkof et al., 2013b;Van Loo et al., 2007). Nevertheless, the fact that nest quality could not be alleviated with analgesia close to control group values in at least one study (Jirkof et al., 2013b) may hint at a more complex correlation between nest building and post-surgical impairment in mice.

Interestingly, recovery of nest building and other parameters was better in female mice housed socially after surgery, while mice separated with a grid did not recover better than single housed females (Van Loo et al., 2007). Housing healthy male mice with an unfamiliar male separated by a grid resulted in long-term social stress and decreased nest building performance distinctly (Rettich et al., 2006).

2.3 Burrowing in laboratory mice

The burrowing test—a simple experimental setup with which to assess changes in spontaneous burrowing behavior—was first described by Deacon and co-authors (Deacon et al., 2001). The test is based on the species-typical behavior of mice to spontaneously displace items (normally with a type of push digging) from tubes within their home cage (Figure 2). The tube probably represents semi-natural circumstances imitating the natural environment of burrow digging animals. Burrowing very likely represents tunnel construction and maintenance, like burrow cleaning behavior (Deacon et al., 2001;Schmid-Holmes et al., 2001). While gerbils, rats and hamsters burrow in the burrowing test, Egypt spiny mice do not as they do not build burrows in the wild (Deacon et al., 2009). Burrowing should be discriminated from several similar behaviors like hoarding, where edible material is carried from the tube to be stored somewhere else (Deacon, 2006a), or digging, which is normally conducted with bedding (Deacon, 2006b). Burrowing may be used in the marble burying test, the defensive burrowing paradigm and the escape digging test, which can be used to assess anxiety or withdrawal symptoms, respectively (Deacon, 2006b;el-Kadi and Sharif, 1995). The ability to display burrowing behavior persists under laboratory conditions (Adams and Boice, 1981) and most commonly used mice strains burrow (see e.g., (Deacon, 2006c); nevertheless, some strains are poor burrowers, e.g., CBA, 129 substrains (Deacon, 2006c); author's personal observations). As burrowing behavior appears to be highly motivated (Sherwin et al., 2004), but with no obvious essential need or reinforcing consequences under laboratory conditions, a self-rewarding component in burrowing is suspected (Teeling et al., 2007).

2.3.1 Assessment of burrowing performance

Protocols to quantify burrowing performance are more consistent than those in nest building assessment. Measuring the amount of burrowed material, either short term (e.g., 2h) or overnight (Deacon, 2006c) is the approach most widely used, but burrowing duration (Jirkof et al., 2013a;Jirkof et al., 2012) and the latency to burrow can also be measured (Huang et al., 2013;Jirkof et al., 2010). Size and material of tubes may vary but plastic should be favored over other materials such as metal. Additionally, there might be a possible effect of tube diameter as normal mouse burrows have a 2- to 3-cm diameter entrance and tubes with markedly different openings might be ignored (Deacon, 2012;Schmid-Holmes et al., 2001). Food pellets of the usual diet work best in mice but sand, gravel, etc., can also be an option

(Deacon, 2006c). An additional shelter tube is optional as it seems not to decrease or increase burrowing performance ((Deacon et al., 2001); author's personal observations). Burrowing is known to increase slightly with practice (Deacon et al., 2001) and can be socially facilitated (McLinden et al., 2012). After a baseline is established, the test can be used repeatedly in the same individual (Deacon et al., 2001;Jirkof et al., 2013c), although a break between trials is recommended (Deacon, 2012).

2.3.2 Using burrowing performance to monitor dysfunction and impairment

Like nest-building, burrowing performance has been shown to be sensitive to hippocampus damage and the progression of neurodegenerative diseases, and is used as a parameter in murine models of psychiatric disorders and to monitor sickness behavior after treatments that mimic viral or bacterial infections. Post-surgical alterations in burrowing suggest the use of this behavioral parameter in routine assessment of mouse well-being—a view supported by recent studies in rats aimed at establishing the burrowing test for pain assessment.

Hippocampal cytotoxic lesions as well as medial prefrontal cortex lesions and malfunctions of the hippocampus in mice reduce burrowing (Chen et al., 2005;Deacon et al., 2002;Deacon et al., 2003;Sano et al., 2009). The authors of these latter studies suggest a reduced motivation to approach the tube and initiate burrowing behavior as well as impaired spatial memory, while other behavioral tests do not show impairment of gross motor or learning function under the conditions described (Deacon et al., 2002;Deacon et al., 2003;Sano et al., 2009).

Hippocampal scrapie infection also impairs burrowing performance distinctly while effects on motor function occur significantly later, and decreased burrowing behavior is proposed as an early sign of disease progression (Cunningham et al., 2003;Cunningham et al., 2005;Deacon et al., 2001;Deacon et al., 2005;Felton et al., 2005;Guenther et al., 2001). Burrowing performance seems to be a more sensitive indicator than nest quality to monitor prion disease progression as changes in burrowing behavior occur long before nest quality decreases in scrapie-infected mice (Guenther et al., 2001).

In at least one Alzheimer`s disease model, neuropathological changes found in, amongst others, hippocampus regions, could be correlated with a significant reduction in burrowing performance (Deacon et al., 2008;Deacon et al., 2009).

In murine models of schizophrenia and anxiety, burrowing was used successfully as a parameter of dysfunction. As observed in nest building, NMDA receptor hypofunction in a

model of schizophrenia reduces burrowing. As NMDA receptors are distributed widely in the brain, general impairment rather than specific symptoms are measured in the affected mice (Barkus et al., 2012). 5-HT transporter knockout mice, which serve as a model of anxiety disorders, fail to burrow. Whether increased anxiety or changes in hippocampal regions inhibit the approach towards the tube remains unclear (Line et al., 2011).

A sickness behavior response following immune system activation is normally characterized by changes in motivation to engage in certain activities as an animal's priorities are altered by the immune challenge (Aubert, 1999). Additionally, areas of the hippocampus are affected by the neuroinflammatory response involved (Cunningham et al., 2007;Konat et al., 2009). Burrowing and nest building therefore appear as promising tools with which to assess sickness behavior. However, while there is only scant evidence that nest building assessment fulfils this promise, several studies show a decrease in burrowing performance during inflammation. Inflammation following even low doses of LPS, which mimics bacterial infection in mice, reduces burrowing for 24–48h. This decrease is accompanied by typical symptoms of illness such as fever response, decrease in locomotion and reduced reward-seeking behavior (Cunningham et al., 2009;Puntener et al., 2012;Tarr et al., 2012;Teeling et al., 2010;Teeling et al., 2007); piloerection and hunched posture were also observed after repeated doses of LPS (Puntener et al., 2012). Burrowing behavior can be restored by treatment with non-steroidal anti-inflammatory drugs (Teeling et al., 2010;Teeling et al., 2007). Similarly, systemic viral challenge induced by nasally instilled Piry virus or mimicked with double-stranded RNA injection, decreases burrowing for up to 2 days (Cunningham et al., 2007;de Sousa et al., 2011;Konat et al., 2009). Both bacterial and viral infections seem to have more deleterious effects on burrowing performance in aged mice compared to younger mice (Hart et al., 2012;McLinden et al., 2012).

DSS-induced colitis is a murine model of chronic inflammatory bowel disease in humans and leads to a progressive reduction in burrowing performance (Jirkof et al., 2013c). The observed decrease in burrowing behavior in colitis might be correlated with inflammation and pain in this condition. Such a combination of pain, inflammation and probably fatigue and stress is also expected after surgical procedures. After minor laparotomy, burrowing behavior was impaired, as shown in a prolongation of latency to burrow (Jirkof et al., 2013a;Jirkof et al., 2012;Jirkof et al., 2010). The behavior could be restored, mainly with non-steroidal anti-inflammatory analgesics. Remaining differences from baseline performance hint at procedural stress that might also be measured sensitively by the burrowing test. These results in mice are supported by studies published recently in rats, which could show that neuropathic (Andrews

et al., 2011;Huang et al., 2013;Lau et al., 2013) as well as inflammatory (Andrews et al., 2011;Rutten et al., 2013b) pain reduced burrowing reliably and independently of the elicited tactile and thermal hypersensitivity (Andrews et al., 2011;Lau et al., 2013). Several analgesics, but not non-analgesic drugs that influence anxiety and locomotor activity, restored burrowing in most of these pain models (Andrews et al., 2011;Lau et al., 2013;Rutten et al., 2013a;Rutten et al., 2013b).

Studies analyzing the influence of housing conditions on the way mice react to stressful procedures were able to show that burrowing performance after surgery was improved by social housing and familiar environment (Jirkof et al., 2013a;Jirkof et al., 2012), and after Piry virus infection in mice housed in enriched cages (de Sousa et al., 2011). Grid floor housing seems to induce stress which affects burrowing performance (Bangsgaard Bendtsen et al., 2012).

3 Discussion

Nest building and burrowing performance have proved valuable tools with which to assess brain damage or malfunction as well as the progression of neurodegenerative diseases. These behaviors are also used in models of psychiatric disorders or to monitor sickness behavior, and have been proposed for use in more realistic and clinically relevant preclinical models of disease. They seem especially useful as early signs of beginning dysfunction and for the monitoring of disease progression, and therefore may be valuable in the testing of treatments (Deacon et al., 2005;Felton et al., 2005;Guenther et al., 2001). There is increasing evidence that both behaviors are reduced by pain and stress, suggesting their use as behavioral parameters to assess well-being in mice (Arras et al., 2007;Deacon, 2012;Jirkof et al., 2010). Both behaviors require optimal nervous system function, which might be compromised in various spontaneously occurring or experimentally induced detrimental conditions laboratory mice may experience. An intact hippocampus seems to be especially essential for burrowing and nest building behavior, both of which require a high degree of organization, planning and executive function (Deacon et al., 2002;Deacon et al., 2001;Filali et al., 2011;Guenther et al., 2001).

3.1 Complex home cage behaviors may mirror human activities of daily living

The ability to organise, plan and execute basic and complex behaviors is an essential prerequisite of the so-called activities of daily living (ADL). In humans, this term describes activities that define the degree of self-reliance that is still achieved by a patient and include basic self maintenance activities like eating without assistance and also so-called instrumental ADLs like housekeeping (Lawton and Brody, 1969). The deterioration of ADL is known to be an early sign of diseases like Alzheimer's in humans and represents a significant source of distress in these patients (Torres-Lista and Gimenez-Llort, 2013). The need to translate human ADL into animal ADL to achieve more clinically relevant preclinical models has been formulated, for example, in the field of geriatric medicine (Carter et al., 2001). Disruption of goal-directed home cage behaviors of mice is an obvious candidate with which to address this challenge. Burrowing and nest building probably resemble more complex instrumental ADL (Deacon, 2012), and the reviewed literature shows, with few exceptions, a decrease in murine ADL-like behaviors in conditions that would also affect human ADL negatively. This not only supports the appropriateness of the use of these behaviors in preclinical research, it also suggests that, like in humans, these behaviors may be used as parameters of well-being in mice.

3.2 Nest building and burrowing behavior as alternatives to simple reflexive pain measures

The impact of pain, especially chronic pain, on quality of life also results in changes to ADL and affective states in humans; frequent comorbidities of ongoing pain are anxiety, depression and cognitive impairment (Blackburn-Munro, 2004;Huang et al., 2013;Mogil, 2009;Urban et al., 2011). In contrast to nociception, the emotional component of pain and its comorbidities have been especially difficult to prove in non-human animals, and preclinical models have been criticized for rarely addressing this effect of pain. Therefore, a move beyond the simple reflex measures by which analgesic effectiveness is evaluated in preclinical trials towards more ethologically relevant measures that mirror the accompanying discomfort has been suggested (Blackburn-Munro, 2004;Huang et al., 2013;Mogil, 2009;Urban et al., 2011). Nest building and, especially, burrowing behavior might be alternatives to simple reflexive responses. Studies in mice and rats show that burrowing and nest building performance are reduced by pain and that effective analgesia can reinstate these spontaneous behaviors (Andrews et al., 2011;Arras et al., 2007;Jirkof et al., 2010;Rutten et al., 2013b). In rats, recent

392 systematic analyses of burrowing claimed to increase the face, predictive and construct
393 validity of preclinical studies assessing the efficacy of pain treatment (Andrews et al.,
394 2011;Huang et al., 2013;Lau et al., 2013;Rutten et al., 2013b).
395 One important advantage of nest building and burrowing as a tool in the assessment of
396 spontaneous pain, whether in basic pain research or to estimate the painful impact of
397 experimental procedures and to adjust analgesic regimens, is that they are reduced but not
398 evoked by pain. Thus, no false positive results occur due to sedative side effects of the
399 analgesic drug used that may have reduced activity in general (Andrews et al., 2011;Rutten et
400 al., 2013b). Therefore, complex behaviors reduced by pain may be a welcome addition to the
401 pain-evoked spontaneous behaviors already used in the routine assessment of pain in
402 laboratory mice and rats.
403

404 ## 3.3 Nest building and burrowing behavior to detect stressful housing
405 ## conditions

406 To date there are only few studies utilising nest building and burrowing performance to
407 evaluate housing conditions of laboratory mice. Nevertheless there are hints that stress evoked
408 by housing conditions (Bangsgaard Bendtsen et al., 2012;Rettich et al., 2006) can also be
409 reflected in a change in nest building and burrowing performance. Several studies have shown
410 that these behaviors can be useful in monitoring the possible recovery-supporting effect of
411 housing conditions after surgery and infection that may increase the ability of the individual
412 to cope with these stressors (de Sousa et al., 2011;Jirkof et al., 2013a;Jirkof et al., 2012;Van
413 Loo et al., 2007).
414

415 ## 3.4 Advantages and drawbacks of nest building and burrowing behavior
416 ## in monitoring mouse well-being

417 Most studies reviewed here did not reveal a correlation of gross motor deficits with reduced
418 performance in nest building or burrowing, although some fine motor or sensorimotor deficits
419 might have occurred (Ballard et al., 2002;Paumier et al., 2013;Wesson and Wilson, 2011).
420 Although overall activity might decrease during progression of a disease, mice show
421 unchanged or even higher activity in open field tests and in the home cage at the time at
422 which burrowing and nest building performance are already affected (Ballard et al.,
423 2002;Barkus et al., 2012;Cunningham et al., 2003;Cunningham et al., 2005;de Sousa et al.,
424 2011;Deacon et al., 2001;Guenther et al., 2001;Jirkof et al., 2013a;Jirkof et al., 2012). An
425 exception are mice showing sickness behavior (Cunningham et al., 2009;Cunningham et al.,

2007;Teeling et al., 2010;Teeling et al., 2007) as well as mice having undergone major impact surgery (Van Loo et al., 2007), which are found to have reduced overall activity and impaired motor coordination. In addition, in rats, hypersensitivity due to Complete Freund`s Adjuvant injection in paw or hind limb showed no correlation with decreased burrowing behavior (Andrews et al., 2011;Lau et al., 2013). Taken together, these results indicate that a reduction in nest building and burrowing behavior due only to the animal avoiding using an affected limb, other motor impairment or reduced general activity is unlikely in most conditions that affect nest building and burrowing. It can be assumed that mice in poor health, pain or stress indeed reduce their involvement in normal home cage behaviors, not due primarily to a disability in perform these behaviors but rather to a reduced motivation to engage in them. Nest building and burrowing behavior seem to be self-rewarding behaviors for mice and a reduction of these behaviors hints that normally attractive stimuli like nesting material and a filled tube appear less attractive to the mouse. This change in the priorities of the animal might hint at a global reduction in well-being.

As mentioned above, increased anxiety may also accompany detrimental conditions like chronic pain. Anxiety may of course reduce approach motivation towards the nest material or the tube and may therefore inhibit these behaviors. Nevertheless, while some studies found a correlation between reduced burrowing or nest building behavior and increased anxiety (Belforte et al., 2010;Kalueff et al., 2006;Keisala et al., 2007;Line et al., 2011) others did not (Barkus et al., 2012;Deacon et al., 2003;Halene et al., 2009).

Nest building and burrowing performance are simple to observe under controlled laboratory conditions in mice, and many studies report large effect size and individual consistency. Drawbacks of using nest building and burrowing performance as parameters may include non-parametric data, high inter-individual variability as well as possible ceiling effects (Cunningham et al., 2003;Deacon, 2012;Lau et al., 2013). High inter-individual variability can easily be buffered with sufficient sample numbers and non-parametric statistics but may hamper the use of these behaviours as parameters of well-being in individual mice. The fact that both nest building and burrowing are affected negatively by a variety of detrimental conditions makes these behaviours attractive tools for several scientific questions and especially for the assessment of global well-being in mice. Their lack of specificity, however, limits their use as sole parameters.

4 Conclusions

Nest building and burrowing behavior are likely to represent ADL in laboratory mice that are impaired by pain, stress, infection and in several psychiatric and neurodegenerative murine disease models. With few exceptions this impairment is not due to motor deficits or reduced overall activity per se. As all of these states are known to decrease quality of life in human patients, and are assumed to do so also in animals, both behaviors offer a sensitive and easy to use tool with which to assess well-being in laboratory mice.

In combination with pain or stress-induced aberrant behaviors, and specific markers of disease like certain clinical signs, nest building and burrowing may help provide a more global picture of a mouse's status than can be achieved in routine monitoring based solely on standard signs of impaired health. This may help estimate the global impact of phenotypes as well as scientific or husbandry procedures, and to fulfil ethical and legal obligations to reduce pain, distress and suffering by choosing the best breeding, housing and experimental procedures and allowing the application of effective analgesic protocols for mice possibly experiencing pain, suffering or distress.

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Legends

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

Figure 2: Example of a simple burrowing test setup. Cage contains two water bottles, one as burrowing test apparatus filled with food pellets, one as shelter, and additional nesting material (nestlet).

	Nest building	Burrowing	References
Mouse model			
Prefrontal lesions		x	Deacon et al. 2003
Hippocampal lesions or malfunctions	x	x	Deacon et al. 2002, Chen et al. 2005, Sano et al. 2009, Jedynak et al. 2012, Kondratiuk et al. 2013
Alzheimer's disease	x	x	Deacon et al. 2008, Deacon et al. 2009, Filali et al. 2009, Filali et al 2011, Wesseon and Wilson 2011, Orta-Salazar et al. 2013, Torres-Lista and Gimenez-Llort 2013
Parkinson`s disease	x		Szczypka et al. 2001, Sager et al. 2010, Paumier et al. 2013
Scrapie and prion disease	x	x	Deacon et al. 2001, Guenther et al. 2001, Cunningham et al. 2003, Cunningham et al. 2005, Deacon et al. 2005, Felton et al. 2005
Bacterial infection		x	Teeling et al.2007, Cunningham et al. 2009, Teeling et al. 2010, Hart et al. 2012, Puntener et al. 2012, Tarr et al. 2012
Viral infection		x	Cunningham et al. 2007, Konat et al. 2009, de Sousa et al. 2011, McLinden et al. 2012
Post-surgical pain	x	x	Arras et al. 2007, Van Loo et al. 2007, Jirkof et al. 2010, Jirkof et al. 2012, Jirkof et al. 2013a, Jirkof et al. 2013b
Inflammatory bowel disease		x	Jirkof et al. 2013c
Anxiety models		x	Line et al. 2011
Schizophrenia models	x	x	Ballard et al. 2002; Halene et al. 2009, Belforte et al. 2010, Barkus et al. 2012, Takao et al. 2013
Autism, autistic spectrum disorder, Rett syndrome	x		El Kordi et al. 2013, Lang et al. 2013, Moretti et al. 2005
Obsessive–compulsive disorder	x		Greene-Schloesser et al. 2011
Vitamin D receptor mutant	x		Kalueff et al. 2006, Keisala et al. 2007
Use in phenotyping battery			
	x		e.g.: Lijam et al. 1997, Gerdin et al. 2006
Housing conditions			
	x	x	Rettich et al. 2006, Van Loo et al. 2007, de Sousa et al. 2011, Bangsgaard Bendtsen et al. 2012, Jirkof et al. 2012, Jirkof et al. 2013a
Diet conditions			
		x	Lavin et al. 2011

Table 1: Summary table of publications using non-maternal nest building and/or burrowing performance in laboratory mice successfully as behavioral parameters in different mouse models and under different husbandry conditions. Find a discussion of the listed publications in the corresponding sections; with the exception of (Gerdin et al., 2006;Greene-Schloesser et al., 2011;Lavin et al., 2012). Literature search was conducted with PubMed.

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