American Pain Society
RESEARCH
EDUCATION
TREATMENT
ADVOCACY
PUBLISHED BY ELSEVIER

The Journal of Pain, Vol 17, No 1 (January), 2016: pp 50-64
Available online at www.jpain.org and www.sciencedirect.com

Bedding Material Affects Mechanical Thresholds, Heat Thresholds, and Texture Preference

Francie Moehring, Crystal L. O'Hara, and Cheryl L. Stucky

Department of Cell Biology, Neurobiology, and Anatomy, Medical College of Wisconsin, Milwaukee, WI.

Abstract: **It has long been known that the bedding type on which animals are housed can affect breeding behavior and cage environment, yet little is known about its effects on evoked behavior responses or nonreflexive behaviors. C57BL/6 mice were housed for 2 weeks on 1 of 5 bedding types: aspen Sani-Chips (standard bedding for our institute), ALPHA-Dri, Cellu-Dri, Pure-o'Cel, or TEK-Fresh. Mice housed on aspen exhibited the lowest (most sensitive) mechanical thresholds and those on TEK-Fresh exhibited 3-fold higher thresholds. Although bedding type had no effect on responses to punctate or dynamic light touch stimuli, TEK-Fresh-housed animals exhibited greater responsiveness in a noxious needle assay than did those housed on the other bedding types. Heat sensitivity was also affected by bedding because animals housed on aspen exhibited the shortest (most sensitive) latencies to withdrawal, whereas those housed on TEK-Fresh had the longest (least sensitive) latencies to response. Slight differences between bedding types were also seen in a moderate cold temperature preference assay. A modified tactile conditioned place preference chamber assay revealed that animals preferred TEK-Fresh to aspen bedding. Bedding type had no effect in a nonreflexive wheel running assay. In both acute (2 day) and chronic (5 week) inflammation induced by injection of complete Freund's adjuvant in the hindpaw, mechanical thresholds were reduced in all groups regardless of bedding type, but TEK-Fresh and Pure-o'Cel groups exhibited a greater dynamic range between controls and inflamed cohorts than aspen-housed mice.**

Perspective: ***These findings indicate that the bedding type routinely used to house animals can markedly affect the dynamic range of mechanical and heat behavior assays under normal and tissue injury conditions. Among beddings tested, TEK-Fresh bedding resulted in the least sensitive baseline thresholds for mechanical and thermal stimuli and the greatest dynamic range after tissue injury. Therefore, selection of routine cage bedding material should be carefully considered for animals that will be tested in behavioral somatosensory assays.***

Key words: *Mechanical behavior, mechanotransduction, bedding, cold behavior, chronic inflammation, acute inflammation, tactile place preference, wheel running.*

In recent years, we noticed that the median von Frey thresholds obtained in our laboratory from noninjured wild-type mice are typically lower than those of many other groups.[7,18-20,23,37,38,67] It has been shown that many factors can affect behavioral response properties, such as density of mice in the cage, time allowed for acclimation before tests, stress levels of the animals, stress levels of the experimenters, noise in the animal facility, odors from compounds worn by experimenters or animal caretakers, or the gender of the operator.[25,56] In addition, pain behavior is influenced by the time of day when the animals are tested; mice show a greater pain sensitivity during the light period than during the dark period, with the greatest pain sensitivity in the late afternoon,[33,46] suggesting that circadian rhythms influence behavioral responses. It has also been shown that environmental enrichment attenuates hypersensitivity to mechanical and cold stimuli in mice after nerve injury.[58] We have attempted to control these environmental factors as much as possible over the past 5 years, but nonetheless, we have found that the von Frey thresholds that we obtain are lower (more sensitive)[18-20,37] compared with values published by other groups.[7,38,67] Therefore, we

Received March 26, 2015; Revised August 31, 2015; Accepted August 31, 2015.

The research reported in this manuscript was supported by NIH grants NS040538 and NS070711 to C.L.S. Partial support for this work was provided by the Research and Education Component of the Advancing a Healthier Wisconsin Endowment at the Medical College of Wisconsin. The authors have no conflicts of interest to report.

Address reprint requests to Cheryl L. Stucky, PhD, 8701 Watertown Plank Road, BSB 428, Milwaukee, WI 53226. E-mail: cstucky@mcw.edu

1526-5900/$36.00

http://dx.doi.org/10.1016/j.jpain.2015.08.014

asked whether the type of bedding on which our animals are housed sensitizes their behavioral responses to evoked environmental stimuli or in nonreflexive assays for spontaneous painlike behaviors. Other laboratories[1,6,29,30,51] have shown that animals show preferences for some bedding materials over others, based on color and texture. We reasoned that the type of bedding that animals are constantly stepping on might affect normal baseline mechanical sensitivity if the bedding components have properties such as sharp edges, splinters, grooves, or other surface attributes in their physical properties. In behavioral assays, we routinely test the plantar surface of the hindpaw, which is consistently exposed to the bedding surface. Furthermore, bedding that contains dyes or chemicals might affect the animals' sensitivity to mechanical, thermal, or chemical stimuli. Therefore, we set out to quantitatively determine, in an otherwise controlled (to the greatest extent possible) environment, the effects of different bedding types on behavioral responses in mice under normal conditions and after acute and chronic peripheral hindpaw inflammation. We tested evoked behavior to mechanical, heat, and cold stimuli as well as spontaneous (nonreflexive) behavior assays for mice housed on different bedding types that are typically used in many animal care facilities.

Methods

Animals

Adult male C57BL/6 mice from Jackson Laboratories (Bar Harbor, ME) at least 6 weeks of age were used and housed for at least 2 weeks on the specific indicated bedding type at our facility before behavior tests were performed. Before our facility received the mice, they were housed on a 1-to-1 mixture of aspen Sani-Chips and aspen shavings (Northeastern Products Corp, Warrensburg, NY) at the Jackson Laboratories. Mice were housed in a 14/10 hour light/dark cycle and were provided with food and water ad libitum. All animals were maintained with experimental protocols approved by the Medical College of Wisconsin and performed in accordance with the Institutional Animal Care and Use Committee.

Bedding Types

To test differences in behavioral responses, we housed the animals on 5 different bedding types, with Enviro-dri[54] nesting material (Shepherd Specialty Papers, Watertown, TN). The animal facility at our institute typically uses an aspen wood chip bedding called Sani-Chips (Fig 1A; P.J. Murphy Forest Products, Montville, NJ), which when touched feels prickly with sharp edges, even although the wood pieces are small. The aspen chips are dried to about 8% moisture content and then screened to the National Institute of Health[28] specifications, which includes a size range from 8 to 20 mesh. The aspen Sani-Chips we use come from P.J. Murphy Forest Products (Montville NJ), and are virtually dust free, contain no chemical additives or paper sludge, and are not a food source for animals. TEK-Fresh bedding (Fig 1B; Envigo, Cambridgeshire, UK) is a very soft bedding made of 100% virgin wood pulp and has the consistency of soft egg cartons.[22] Importantly, TEK-Fresh is virtually dust free and does not irritate either human or animal respiratory systems. TEK-Fresh does not contain any silica, resins, or aromatic hydrocarbons that could irritate the animals' respiratory system.[22] The third bedding we tested was Pure-o'Cel bedding (Fig 1C; Andersons Lab Bedding, Maumee, OH), which is made out of white 19-mm (.75-in) paper chip squares and is hard with relatively sharp edges. The paper chips offer 74% more surface area than typical squares, which makes the Pure-o'Cel bedding more absorptive than other paper square bedding types.[57] Cellu-Dri Soft (Fig 1D; Shepherd Specialty Papers) is a very soft bedding made out of recycled cellulose fiber. The soft texture of the bedding enhances enrichment of the environment and encourages nest building, Cellu-Dri also helps eliminate dust

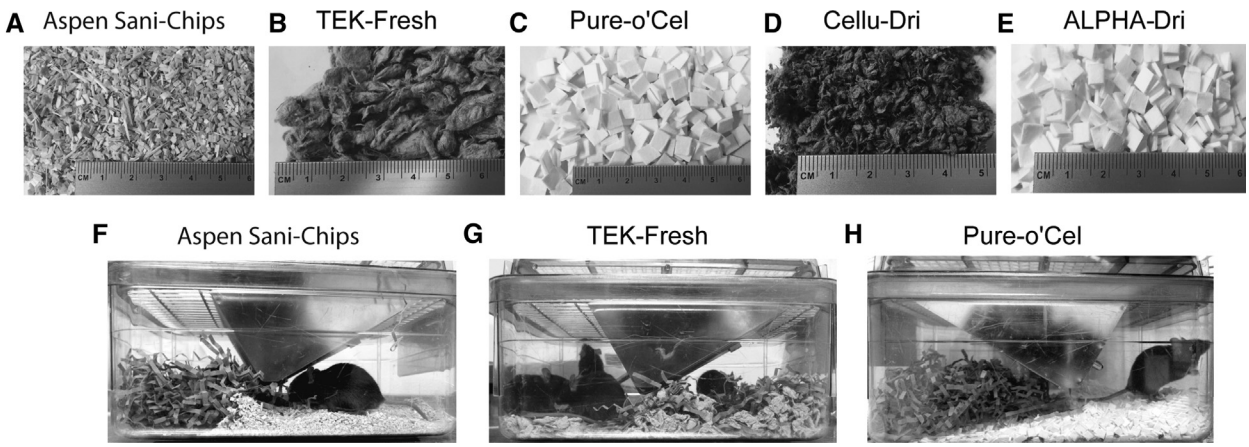

Figure 1. Bedding materials used for housing mice 2 weeks before behavioral testing. **(A)** aspen Sani-Chips. **(B)** TEK-Fresh. **(C)** Pure-o'Cel. **(D)** Cellu-Dri Soft. **(E)** ALPHA-Dri. **(F)** Mice housed on aspen Sani-Chips build small nests out of Enviro-dri. **(G)** Mice housed on TEK-Fresh bedding mix the Enviro-dri with their bedding to build larger nests. **(H)** Mice housed on Pure-o'Cel push bedding material away from Enviro-dri nest to the other side of the cage.