Significant Improvement in Survival of Tabby Jimpy Mutant Mice by Providing Folded-paper Nest Boxes

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Summary

There is little scientific evidence that environmental enrichment meets the physiological needs for laboratory animals. The purpose of this study was to evaluate whether the provision of nest boxes improved the lifespan of Tabby jimpy (Ta-jp) mutant mice carrying a lethal gene. Nest boxes tested in this study were small folded-paper trapezoid-shaped houses. The Nest box group was reared in cages with the nest boxes and the Control group was raised in only standard cages. Weaning results and survival rates in Ta-jp mice were compared between both groups. By the weaning stage, all of the Ta-jp mice survived in the Nest box group. There was a significant difference in weaning rates between both groups. The body weight of weanlings demonstrated a significant difference between both groups. The survival analysis indicated that the Nest box group (29.9 days) significantly had a longer lifespan than the Control group (25.7 days). The Nest box group developed few abnormal jumping behaviours. The nest boxes served as shelters from environmental stimuli. In conclusion, we confirmed that folded-paper nest boxes had a beneficial effect on weaning rates of Ta-jp mice. Our results revealed that there was significant improvement in survival of Ta-jp mice using the nest boxes.

Introduction

Environmental enrichment is currently considered a popular means for improving animal well-being, and various enrichment designs have been recommended on the basis of preference tests (*Sherwin, 1997; Marques & Olsson, 2005; van Loo et al., 2005*). Experimental support for beneficial effects of environmental enrichment includes reduced stereotypies, increased locomotory and exploratory behaviours, and prevention of fighting (*Würbel et al., 1998; Kaliste, 2006; Olsson & Sherwin, 2006*). However, the efficacy of environmental enrichment is prone to be judged subjectively from an anthropomorphic perspective. There are actually few preference and operant studies that show these environmental enrichment items (nest boxes, nesting materials, cardboard tubes, running-wheels and gnawing blocks) meet the physiological needs (natural behaviour, exercise, hiding and nesting) for laboratory mice (*Baumans, 2005; Huchinson et al., 2005*). In terms of food consumption and body weight loss, there is only a little definitive scientific evidence that mice with nesting materials are heavier and consume less food, and these studies indicated that mice with nesting materials need less energy for thermoregulation than those housed without nesting materials (*Olsson & Dahlborn, 2002*).

The dysmyelinating mutant jimpy (jp) arises from a point mutation in the mouse gene encoding proteolipid protein and is characterized by severe dysmyelination attributable to oligodendrocyte death (*Sidman et al., 1964*). Jp is an X-linked recessive mutation in the proteolipid protein gene. This mutant is considered an important model for the human leukodystrophy Pelizaeus-Merzbacher disease

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(*Gow et al., 1998*). Tabby (Ta) is the semidominant allele of the locus on the X chromosome, which affects a variety of tissues of ectodermal origin (*Sundberg, 1994*). In hemizygous and homozygous states, Ta produces characteristic findings such as narrowed palpebral fissures, reduced number of facial vibrissae, reduction in size and number of teeth, abnormal texture of the coat and impaired exocrine glands. Ta and jp were maintained by sib mating of the Ta-jp/++ genotype crossed to a Ta/Y or +/Y male. Although both Ta and jp are linked and located on the X chromosome, the jp allele causes premature death of homozygous male Ta-jp mice by 30 days commonly after generalized seizures, tonic convulsions and respiratory paralysis.

Jp mice have been used to investigate the regulation of oligodendrocyte progenitor proliferation in the postnatal spinal cord (*Knapp et al., 1986; Duncan, 1990; Griffiths et al., 1995; Lunn et al., 1995; Gow et al., 1998; Knapp et al., 1999; Baracskay et al., 2002; Duncan, 2005*). Although research on the pathophysiology and possible therapy of Pelizaeus-Merzbacher disease has been greatly aided by the availability of jp mice, this model includes the most severely affected myelin mutants. Therefore, in these studies, Ta-jp mice are used over time periods when the symptoms are only mild and transient, and the animals are euthanized before serious welfare problems occur.

To our knowledge, there has been no scientific evidence of possible life-prolonging effects of environmental enrichment. The purpose of this study was to evaluate improvement in survival of mutant Ta-jp mice provided with folded-paper nest boxes.

Materials and Methods

Animals

Heterozygous female mice (Ta Plp^{jp}/++) carrying the Ta-jp mutation and wt males (B6CBACa-$A^{W\text{-}J}$/A) were used as breeding pairs. Mice were housed in polycarbonate cages (W 17.0 × D 35.0 × H 14.0 cm) with bedding made from pure-pulp (Care-feeaz, Hamri Co., LTD., Ibaraki, Japan) in our specific-pathogen-free (SPF) barrier facilities. The animal room was maintained at constant temperature (23 ± 1 °C) and relative humidity (55 ± 10 %). The room air was ventilated 10 to 15 times per hour automatically and 12 hr/12 hr light-dark cycle (lighting 06:00-18:00) was imposed. The animals received commercial radiation sterilized diets (CE-2, Clea Japan Inc., Tokyo, Japan; CMF Spraut (specified diets for transgenic mice which are difficult to rear), Oriental Yeast Co. Ltd., Tokyo, Japan) and water *ad libitum*. Ta-jp pups were put on the specified diets to ensure their eating and water requirements. These diets were previously sterilized by γ-irradiation at a dose of 30 kGy. The health status of animals in our SPF facilities was checked four times a year for the pathogens listed by the Japanese Association of Laboratory Animal Facilities of National University Corporations, and animals were free from all these pathogens.

Late-pregnant stage mice were assigned randomly to the following 2 groups: the Nest box group (dams which were kept in standard cages with the nest boxes) and the Control group (dams which were kept in cages with only bedding materials). After the birth, intact litters (Ta-jp and wt pups) were kept together with their dams in each group. After weaning, which occurred at 20 days of age, the Ta-jp pups were separated from their dams. Ta-jp pups were distinguished from wt pups on the basis of coat colour. Throughout the duration of the survival study, they were kept in the same housing conditions as in the nursing period.

Nest boxes

Figures 1 shows an appearance of the nest box. Nest boxes tested in this study were small folded-paper trapezoid-shaped houses (W 93.0 × D 14.3 × H 4.7 cm, 16.6 g) with a hole in each side. The surface was slightly rough. These boxes were prepared from uncoated white paper by Watanabe IS Co. Ltd. (Tokyo, Japan). Before the beginning of this study, these nest boxes were sterilized by the autoclave.

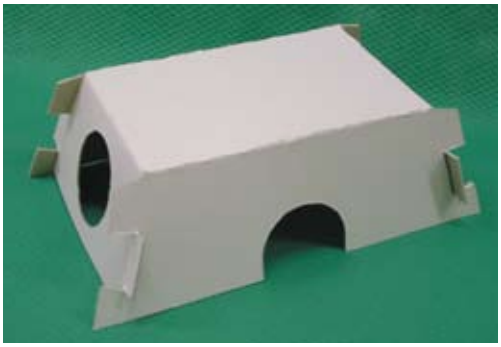

Figure 1. The folded-paper nest box.

Experimental procedures

In the present study, humane endpoints were defined as the early indicators of impending death. We used moribund condition as a humane experimental endpoint. Humane endpoint markers of the moribund condition comprised hind-limb paralysis, the inability to rise or ambulate, and the impossibility of food intake. We believe that euthanasia of moribund animals can terminate and prevent further pain and distress. The health and welfare of the animals was checked by daily observations. Litter size, the number of Ta-jp newborns, the number of Ta-jp weanlings, survival rate of Ta-jp mice and weaning weight of offspring were determined during this study. The behaviour of animals was also followed during the care routines. In view of humane endpoints, the mice were euthanized before serious welfare problems occurred.

All procedures involving animals were approved by the Animal Use Committee of National Institutes of Natural Sciences (NINS) and followed the guidelines of animal care and experiments of the NINS.

Statistical evaluation

Measurements were expressed as the mean ± standard deviation (SD) for each group of Ta-jp mice. In survival rates of Ta-jp weanlings, statistical analysis was performed using Fisher's exact probability test. In litter size and body weight of Ta-jp weanlings, statistical evaluation was done by the use of Welch's *t*-test. Survival curves were prepared by the Kaplan-Meier method. The log-rank test was performed for the comparison of survival curves.

Results

Observations

Many sheets of light nest boxes could be sterilized in piles by the autoclave. After autoclaving the paper-folding nest boxes, there were no changes in their shape or quality.

During pregnancy, birth and rearing, the dams spent the majority of a day in the nest boxes. The dams nibbled an extra hole in each side or shredded a part of the nest box. Some mice built their own nests by combining the bedding litter and shredded pieces of the paper nest boxes. This type of nest box allowed the Ta-jp offspring to climb and make better use of the three-dimensional space of the cages. Throughout this study, we found no avoidance behaviours toward the nest boxes. The dams used the nest boxes as nesting sites and/or sleeping places.

In the Control group, the weaning pups simultaneously jumped up out of the cages in response to slight external stimuli. In contrast, the mice in the Nest box group developed few of the abnormal jumping behaviours found in the Control group. The mice hid themselves from disturbances, and then they kept still in their nest boxes. Although these jumping behaviours were not scored, the nest boxes served as shelters from environmental stimuli.

Survival effects

In litter size (Ta-jp and wt mice), there were no apparent differences between the Nest box group (8.6 ± 1.7) and the Control group (7.8 ± 2.9). In each group, the number of newborns was 4-13/cage. There were some females, which did not produce Ta-jp pups (Nest box group: 3 females, Control group: 7 females). The mean numbers of Ta-jp pups were 1.8 ± 1.2 in the Nest box group and 1.4 ± 1.5 in the Control group. The other pups were wt mice.

The weaning results in Ta-jp mice are shown in Table 1. After weaning, the number of Ta-jp pups was 1-6/cage. The mice delivered pups in the nest

Table 1. The weaning results in Tb-jp mice

Group	The number of dams	The number of Tb-jp newborns	The number of Tb-jp weanlings	Weaning rates (%)	Body weight (g) of Tb-jp weanlings
Nest box	19	35	35	100 *	6.1 ± 1.0 *
Control	21	28	24	86	5.4 ± 1.6

*: $p < 0.05$ (Significantly different between the Nest box and the Control group).

boxes and the dams subsequently used these boxes during maternal care (Figure 2). By weaning at 20 days of age, all of the Ta-jp mice survived in the Nest box group. In contrast, the housing condition without the nest box reduced murine survival rates to as much as 86 % of their initial number. There was a significant difference in weaning rates between the Nest box and the Control group ($p < 0.05$). In addition, the body weight of Ta-jp weanlings demonstrated a significant difference between the Nest box (6.1 ± 1.0 g) and the Control group (5.4 ± 1.6 g) ($p < 0.05$).

Figure 2. Ta-jp mice and nest box.

Figure 3 shows survival analysis of Ta-jp mice in both groups. In both the groups, Ta-jp mice older than 10 days were identified by tremors of the hindquarters when attempting movement. After 3 weeks of age, they began to develop some convulsions. The Kaplan-Meier analysis indicated that the Nest box group had a longer lifespan than the Control group throughout the observation period. In the Control group, almost all the Ta-jp mice showed their lifespan ranging from 20 days to 30 days after the birth. In contrast, in the Nest box group, nearly half of Ta-jp mice survived at 30 days after the beginning of this study. The average lifespan was 29.9 days for the Nest box group and 25.7 days for the Control group. Survival of animals of the Nest box group was significantly greater than that of the Control group ($p < 0.05$). Maximum survival duration of mice was 40 days for the Nest box group and 34 days for the Control group.

Discussion

The folded-paper nest boxes were autoclavable and these items disturbed no daily behaviours of Ta-jp mice bearing a lethal gene. As shown by the Control group, mice are generally housed in small 'shoe box' cages, with no further structuring of the cage other than a layer of sawdust litter. The standard cages are quite different from natural rodent natural habitats and this artificial environment restricts their ability for social interaction. This housing condition provides few beneficial stimuli and gives limited opportunities for mice to perform natural behaviour patterns such as exploring, hiding, foraging, gnawing and nesting. Mice prefer a more complex cage to the standard cage, and are willing to collect

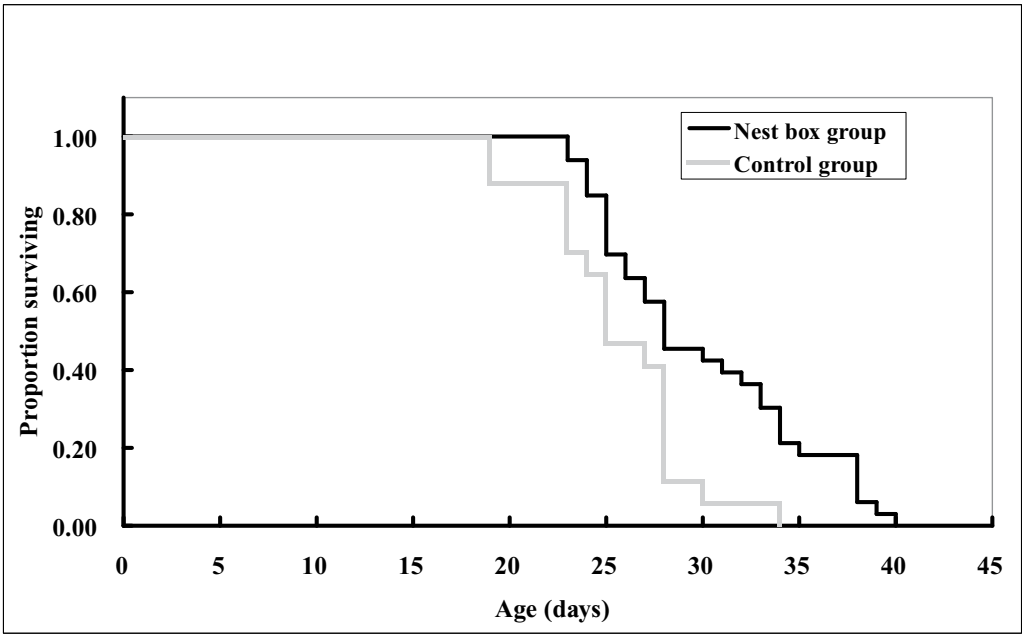

Figure 3. Survival analysis of Ta-jp mice. Survival of Ta-jp mice of the Nest box group was significantly greater than that of the Control group ($p < 0.05$) by the log rank test.

to nesting materials (*Olsson & Dahlborn, 2002; Smith & Corrow, 2005; Olsson & Sherwin, 2006; Kostomitsopoulos et al., 2007*). Our observations were in close agreement with the experience of other investigators.

Modifications of housing conditions for mice provide materials and structures which can serve as hiding and climbing places. With the use of insets for climbing and hiding, the enhancement of complexity of cage environment provided the dams and Ta-jp offspring with shelter and increased opportunities for exploration.

Laboratory mice build nests if they are provided with suitable materials (paper towel, toilet tissue, processed corn husk, wood-wool, gauze cotton batting and compressed virgin cotton fibers) (*Olsson & Dahlborn, 2002*). A number of preference experiments suggest that easily available materials such as tissues and paper towels should fulfil the nesting requirements of mice (*van de Weerd et al., 1997; Dahlborn et al., 1996; Olsson & Dahlborn, 2002*). The preference test for nest boxes shows that mice favour folded-paper nest boxes over resin-treated ones (*van Loo PL et al., 2005*). Our findings also revealed that this folded-paper nest box served as a suitable living space for Ta-jp mice. The functional role of the nest boxes was to alter the living environment in order to provide opportunities to express their natural behaviours. It was probable that mice preferred nest boxes with some kind of sheltering structures.

In our study, we could not quantify the difference in neuropathic state of weaning Ta-jp mice housed with or without nest boxes (i.e. the alleviation of convulsions and decrease of abnormal behaviours). However, it was probable that favourable weaning body weight of Ta-jp mice was associated with greater longevity in the Nest box group. When

mice persist in gnawing the bars of the cage lid or jumping up-and-down along the cage wall, their energy consumption reaches up to 50% of their total daily activities (*Würbel, 2001*). The decrease in the abnormal behaviours (jumping-up) allowed Ta-jp mice to keep in good physical strength. Several studies have demonstrated that the provision of nesting materials affected body weight and/or food intake (*Dahlborn et al., 1996; van de Weerd et al., 1997*).

The most important aim of environmental enrichment is to meet essential behavioural needs of animals in order to improve their well-being in captivity. From this standpoint, we recommend that the use of nest boxes should be incorporated into the standard housing of mice. The flat roof added an elevated horizontal area to the standard cage and the window (a hole in each side) allowed observers to see the condition of mice. Because folded-paper nest boxes with a flat roof had structures combining shelters with rearing sites, this environmental enrichment improved animal welfare by life-prolonging effects on mutant Ta-jp mice.

In conclusion, we confirmed that folded-paper nest boxes had a beneficial effect on weaning rates of Ta-jp mice. Our results revealed that there was significant improvement in survival of Ta-jp mice using the nest boxes.

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References

Baumans V: Environmental enrichment for laboratory rodents and rabbits: requirements of rodents, rabbits, and research. ILAR J 2005, *46*, 162-170.

Baracskay KL, CS Duchala, RH Miller, WB Macklin & BD Trapp: Oligodendrogenesis is differentially regulated in gray and white matter of jimpy mice. J Neurosci Res 2002, *70*, 645-654.

Dahlborn K, BAA van Gils, HA van de Weerd, JE van Dijk & V Baumans: Evaluation of long-term environmental enrichment in the mouse. Scandinavian J Lab Anim Sci 1996, *23*, 97-106.

Duncan ID: Dissection of the phenotype and genotype of the X-linked myelin mutants. Ann N Y Acad Sci 1990, *605*, 110-121.

Duncan ID: The PLP mutants from mouse to man. J Neurol Sci 2005, *228*, 204-205.

Gow A, CM Southwood & RA Lazzarini: Disrupted proteolipid protein trafficking results in oligodendrocyte apoptosis in an animal model of Pelizaeus-Merzbacher disease. J Cell Biol 1998, *140*, 925-934.

Griffiths IR, A Schneider, JM Anderson & KA Nave: Transgenic and natural mouse models of proteolipid protein (PLP)-related dysmyelination and demyelination. Brain Pathol 1995, *5*, 275-281.

Huchinson E, A Avery & S Vandewoude: Environmental enrichment for laboratory rodents. ILAR J 2005, *46*, 148-161.

Kaliste EK, SM Mering & HK Huuskonen: Environmental modification and agonistic behavior in NIH/S male mice: nesting material enhances fighting but shelters prevent it. Comp Med 2006, *56*, 202-208.

Knapp PE, RP Skoff & DW Redstone: Oligodendroglial cell death in jimpy mice: an explanation for the myelin deficit. J Neurosci 1986, *6*, 2813-2822.

Knapp PE, WP Bartlett, LA Williams, M Yamada, K Ikenaka & Skoff RP: Programmed cell death without DNA fragmentation in the jimpy mouse: secreted factors can enhance survival. Cell Death Differ 1999, *6*, 136-145.

Kostomitsopoulos NG, E Paronis, P Alexakos, E Balafas, P van Loo & V Baumans: The influence of the location of a nest box in an individually ventilated cage on the preference of mice to use it. J Appl Anim Welf Sci 2007, *10*, 111-121.

Lunn KF, ML Fanarraga & ID Duncan: Myelin

mutants: new models and new observations. Microsc Res Tech 1995, *32*, 183-203.

Marques JM & IA Olsson: The effect of preweaning and postweaning housing on the behaviour of the laboratory mouse (*Mus musculus*). Lab Anim 2005, *41*, 92-102.

Olsson IA & K Dahlborn: Improving housing conditions for laboratory mice: a review of "environmental enrichment". Lab Anim 2002, *36*, 243-270.

Olsson IA & CM Sherwin: Behaviour of laboratory mice in different housing conditions when allowed to self-administer an anxiolytic. Lab Anim 2006, *40*, 392-399.

Sherwin CM: Observations on the prevalence of nest-building in non-breeding TO strain mice and their use of two nesting materials. Lab Anim 1997, *31*, 125-132

Sidman RL, MM Dickie & SH Appel: Mutant mice (qurking and jimpy) with deficient myelination in the central nervous system. Science 1964, *144*, 209-211.

Smith AL & D Corrow: Modifications to husbandry and housing conditions of laboratory rodents for improved well-being. ILAR Journal 2005, *46*, 140-147.

Sundberg JP. The tabby (Ta Sundberg JP.), tabby-c (Ta^c), and tabby-j (Ta^j) mutations, chromosome X. In: *Handbook of mouse mutations with skin and hair abnormalities: animal models and biomedical tools* (*Sundberg JP*, eds). CRC Press, Boca Raton 1994, pp 455-462.

van Loo PL, HJ Blom, MK Meijer & V Baumans: Assessment of the use of two commercially available environmental enrichments by laboratory mice by preference testing. Lab Anim 2005, *39*, 58-67.

van de Weerd HA, PLP van Loo, LFM van Zutphen, JM Koolhaas & V Baumans: Nesting material as environmental enrichment has no adverse effects on behavior and physiology of laboratory mice. Physiol Behav 1997, *62*, 1019-1028.

Würbel H, R Freire & CJ Nicol: Prevention of stereotypic wire gnawing in laboratory mice: effects on behaviour and implications for stereotypy as a coping response. Behav Process 1998, *41*, 61-72.

Würbel H: Ideal homes? Housing effects on rodent brain and behaviour. Trends in Neurosci 2001, *24*, 207-211.