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Comparison of Age-Related Peripheral Nerve Changes in Mice Housed in Either Plastic Cages with Sawdust-Covered Solid Flooring or Wire-Mesh-Floor Cages

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Abstract: A comparative histologic survey was conducted on the dorsal root, sciatic, tibial and medial plantar nerves of 90- and 110-week-old B6C3F1 female mice reared in either solid-floor cages covered in sawdust or wire-mesh-floor cages. Age-related peripheral nerve lesions, characterized by axonal degeneration and remyelination, were present in all nerves surveyed, and were especially prominent in the sciatic and medial plantar nerves at 110 weeks of age but, there were no differences associated with the type of cage floor in clinical signs, grasping power of the fore- and hind-limbs, motor nerve conduction velocity or histopathologic findings at these ages.

Key words: cage floor, mice, peripheral nerves, pressure neuropathy

Neuropathy is a spontaneous complication which often arises during aging. Localized peripheral nerve lesions, including demyelination, Wallerian degeneration and Renaut bodies, are reported to arise in the hindfeet of rats as a result of living in cages with wire-grate flooring [6, 12]. Mizisin *et al.* [6] demonstrated that wire grates rapidly induce hindpaw tactile hyperesthesia and plantar neuropathy in rats, which could complicate or invalidate studies which assess hindlimb function and structure. This effect is not limited to rats; pressure neuropathy also arises in the plantar nerves of guinea pigs kept in cages with wire mesh floors [1, 10]. Although spontaneous, age-related, peripheral neuropathy has been studied extensively in rats [2–4, 7, 11], there are few reports concerning this phenomenon

in mice [5, 8]. There is marked variation between CD-1 and B6C3F1 strains in the incidence of the spontaneous peripheral neuropathy, suggesting increased susceptibility to B6C3F1 strain [5]. In this study, a histologic survey was conducted on the dorsal root, sciatic, tibial and plantar nerves of 90- and 110-week-old B6C3F1 female mice housed in either plastic cages or wire-mesh-floor cages to attempt to delineate the clinical and functional disorders which compose this peripheral neuropathy.

Eighty 6-week-old female B6C3F1 mice were purchased from Charles River Japan (Kanagawa, Japan) and housed inside a barriered rodent facility. Groups of five were first maintained in plastic cages (22^L × 38^W × 20^H cm) with solid floors covered by a deep

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layer of sawdust (Beta-chip, Charles River Japan, Kanagawa, Japan) to a depth of about 4 cm from the bottom. The room was maintained at a temperature of $23 \pm 2^\circ\text{C}$, and a relative humidity of 40–70%, and with a 13 hr fluorescent lighting period (0800–2100). The mice were fed pelleted food (CRF-1, Oriental Industries, Japan) and tap water *ad libitum*. After 52 weeks, mice that showed no sign of abnormality in general health, ophthalmologic or auricular reflex tests were randomly divided into 2 groups of 30 animals each, based on their body weight which ranged from 29.0–40.3 g. None had any clinical or functional disorders at this age (Table 1). To confirm this observation, 10 mice were soon sacrificed. No histopathologic abnormalities in hindlimb nerves were found in any mouse at this age. At 52 weeks, one group were transferred to suspended wire cages. The flooring was a grid of 2 mm intersecting round stainless steel wires, with each open space having dimensions of 8 mm by 8 mm.

The mice were observed daily for clinical signs. Body weight and food consumption were measured

weekly. Blood was collected at weeks 52, 70, 90 and 110 for blood chemistry tests, including glycemic parameters; blood pH and hematocrit, glucose, HbA_{1c}, 1, 5-anhydroglucitol, fructosamine, triglycerides, cholesterol, phospholipids and total protein. To assess the animals' general health, sole and tail skin temperatures were taken monthly. At 52, 90 and 110 weeks, rectal temperature, grip strength, blood pressure, heart rate and tail flick latency were determined. On the sacrifice day, motor nerve conduction velocity (MNCV) was measured by the method described by Sharma *et al.* [9]. A neural circuit consisting of the right sciatic and tibial nerves was stimulated at the sciatic notch (proximal end) and the posterior tibial nerve at the ankle (distal end), by means of a monopolar needle electrode from an MS92 electromyograph (Medelec, London, UK). Muscle action potentials were recorded from the interdigital plantar muscle of the right hindlimb with a bipolar needle electrode. The MNCV was calculated from the latency of the M-response and the distance between two stimulation points.

Table 1. Body weight, body temperature, heart rate, blood pressure, grip strength, tail flick latency, MNCV and blood biochemistry parameters in mice housed in plastic cages with sawdust-covered solid flooring or wire-mesh-floor cages

	52 weeks old Sawdust-plastic	90 weeks old Sawdust-plastic	90 weeks old Wire-mesh flooring	110 weeks old Sawdust-plastic	110 weeks old Wire-mesh flooring
Survival Rate (%)	100	97	93	68	72
Body Weight (g)	37.3 ± 2.3	41.5 ± 2.2	$36.7 \pm 2.0^{**}$	41.6 ± 2.2	$35.0 \pm 2.0^{**}$
Body Temperature ($^\circ\text{C}$)					
Rectal	37.7 ± 0.4	37.2 ± 0.4	36.5 ± 0.2	37.0 ± 0.4	36.7 ± 0.2
Sole Skin	29.7 ± 0.6	29.2 ± 0.6	28.5 ± 0.5	28.9 ± 0.5	28.6 ± 0.5
Tail Skin	29.7 ± 0.5	29.2 ± 0.7	29.0 ± 0.7	28.9 ± 0.7	28.5 ± 0.6
Heart Rate (bpm)	745 ± 24	715 ± 19	702 ± 20	737 ± 22	722 ± 21
Blood Pressure (mmHg)	85 ± 5	83 ± 7	83 ± 8	83 ± 7	82 ± 6
Grip Strength (g)	510 ± 18	515 ± 15	542 ± 20	500 ± 15	505 ± 20
Tail Flick Latency (sec)	5.7 ± 0.4	5.8 ± 0.3	5.9 ± 0.4	6.2 ± 0.3	6.9 ± 0.4
MNCV (m/sec)	52.4 ± 2.5	41.4 ± 2.5	43.5 ± 2.0	39.4 ± 2.7	38.5 ± 2.9
<i>Blood chemistry</i>					
pH	7.33 ± 0.4	7.28 ± 0.4	7.32 ± 0.3	7.28 ± 0.4	7.32 ± 0.3
Hematocrit(%)	42 ± 4	41 ± 2	42 ± 3	40 ± 2	40 ± 3
Glucose (mg/dl)	144 ± 8	149 ± 8	137 ± 7	143 ± 10	138 ± 4
HbA _{1c} (%)	3.2 ± 0.1	3.0 ± 0.1	2.9 ± 0.1	2.8 ± 0.1	2.6 ± 0.1
Fructosamine ($\mu\text{mol/l}$)	44 ± 4	46 ± 2	53 ± 3	57 ± 2	58 ± 3
1,5-Anhydroglucitol ($\mu\text{g/ml}$)	9.2 ± 0.2	9.0 ± 0.2	9.4 ± 0.3	8.3 ± 0.2	8.7 ± 0.3
Cholesterol (mg/dl)	171 ± 6	164 ± 6	$128 \pm 10^{**}$	177 ± 6	$119 \pm 10^{**}$
Triglycerides (mg/dl)	169 ± 9	163 ± 10	$118 \pm 10^{**}$	188 ± 11	$130 \pm 10^{**}$
Phospholipids (mg/dl)	308 ± 9	298 ± 9	$227 \pm 10^{**}$	319 ± 11	$242 \pm 12^{**}$
Total protein (g/dl)	5.4 ± 0.1	5.4 ± 0.1	5.3 ± 0.1	5.3 ± 0.1	5.1 ± 0.1

Significant difference of sawdust-plastic flooring cage. $^{**}p < 0.01$ MEAN $\pm$ SE (n=10)

Ten mice from each group were anesthetized with ether at 90 and 110 weeks, respectively. Intracardiac infusion was performed with 4% phosphate-buffered paraformaldehyde after an infusion of phosphate-buffered saline. The medial plantar nerve, tibial nerve, sciatic nerve and L4–L6 lumbar dorsal and ventral roots were then dissected out from the same site in each animal and postfixed with 4% phosphate-buffered paraformaldehyde. After fixation, these nerves were cut into 0.5 mm lengths, embedded in paraffin or plastic (Historesin plus, Jung, Germany), and sectioned longitudinally. The sections were then stained with either hematoxylin and eosin or hematoxylin, eosin and Luxol fast blue for light microscopy. In addition, portions of the medial plantar and tibial nerves were dissected out and fixed in 2.5% phosphate buffered glutaraldehyde, post-fixed in osmium tetroxide, and dehydrated in serial alcohol solutions and propylene oxide prior to embedding in epon. The sections (1 μ m thick) were stained with toluidine-blue. One hundred myelinated fibers in cross sections were examined per animal, and the number of nerve fibers exhibiting axonal degeneration, myelin body formation and remyelination were noted.

All results are expressed as mean values with standard errors. The values were tested for significant differences against the age-matched sawdust group by Student's *t*-test.

There was no indication of any adverse cage-related effect on the incidence or distribution of deaths. In

general, the body temperature and MNCV decreased with age, independent of the housing conditions. Although there were no cage-associated differences in clinical signs, body temperature, grasping power of fore- and hind-limbs, tail flick latency or MNCV, a significant decrease in body weight and the blood lipid level were observed in mice housed in wire cages (Table 1). A corresponding lower incidence of obesity was observed in mice housed in wire cages.

At 90 weeks of age, sporadic lesions corresponding to axonal degeneration, formation of myelin bodies and remyelination were present in the sciatic, tibial, plantar nerves and lumbar dorsal root of some of the mice. At 110 weeks of age, all the mice had prominent lesions in the sciatic and medial plantar nerves. There were sporadic lesions in other nerves. There were no histopathologic differences associated with either cage in the amount of tibial and plantar nerve damage at either 90- or 110 weeks of age (Table 2, Fig. 1). There were no histopathologic changes in dorsal root ganglia in mice housed either in plastic or wire-mesh cages at 90- or 110 weeks of age.

Grate-induced neuropathy in rats was characterized by edema, demyelination and some evidence of axonal degeneration only in the medial plantar nerve during the 9 weeks in the wire floor cage [6]. Similar grate-induced nerve injury was described in another study [12] where neuropathy developed earlier in heavier animals, indicating an etiology related to direct pressure or compression of plantar nerves. Nerve injuries docu-

Table 2. Incidence (%) of nerve lesions in mice housed in plastic cage with sawdust-covered solid flooring or wire-mesh-floor cages

	90 weeks old		110 weeks old	
	Sawdust-plastic	Wire-mesh flooring	Sawdust-plastic	Wire-mesh flooring
<i>Tibial nerve</i>				
Normal	95.2 $\pm$ 0.8	95.1 $\pm$ 1.8	92.4 $\pm$ 1.2	93.0 $\pm$ 1.1
Axonal degeneration	0.4 $\pm$ 0.2	0.2 $\pm$ 0.1	1.4 $\pm$ 0.3	1.3 $\pm$ 0.5
Myelin body formation	4.0 $\pm$ 0.6	4.1 $\pm$ 1.4	5.1 $\pm$ 0.7	5.0 $\pm$ 0.7
Remyelination	0.4 $\pm$ 0.2	0.6 $\pm$ 0.3	1.1 $\pm$ 0.3	0.7 $\pm$ 0.2
<i>Plantar nerve</i>				
Normal	90.1 $\pm$ 1.5	88.0 $\pm$ 1.9	82.3 $\pm$ 2.3	84.6 $\pm$ 3.0
Axonal degeneration	2.0 $\pm$ 0.7	2.4 $\pm$ 1.0	4.9 $\pm$ 1.4	5.0 $\pm$ 1.7
Myelin body formation	5.5 $\pm$ 0.6	7.1 $\pm$ 1.0	9.4 $\pm$ 1.0	8.1 $\pm$ 1.4
Remyelination	2.4 $\pm$ 0.6	2.5 $\pm$ 0.5	3.4 $\pm$ 0.4	2.3 $\pm$ 0.8

Histopathologic examination was conducted on traverse sections of epon-embedded tissues. One hundred myelinated nerve fibers were examined for each animal. MEAN $\pm$ SE (n=10)

Tibial nerve

Plantar nerve

Sawdust-plastic flooring

Wire-mesh flooring

Fig. 1. Semithin, toluidine blue-stained cross sections of nerve from 110-week-old mice. Scale bar=10 μ m. D: Axonal degeneration, O: Ovoid formation, R: Remyelination with Schwann cell proliferation.

mented elsewhere [1, 12] have features in common with animal and human entrapment neuropathies that develop as the result of repeated pressure or compression of nerve trunks. Although age-related peripheral neuropathy of the hind-limb and plantar pressure neuropathy were observed during the long-term rearing of mice in this study, there were no histopathologic differences associated with type of cage flooring. The formation of lesions in the medial plantar nerves of mice in both types of cages is probably caused by pressure on the back of the foot, in addition to age-dependent spontaneous neuropathy. The present age-related histopathologic findings are in close agreement with a previous report [5, 8] suggesting that neuropathy in senescent mice is not species specific, but is less severe, even in suscep-

tible B6C3F1 female mice, than neuropathies in rats or guinea pigs. A review of previously reported age-related changes and pressure-induced neuropathies in rats [3, 7] suggests a more severe nerve pathology than that seen in elderly mice [5, 8].

In conclusion, wire-grate cage flooring exerts few confounding effects on assessing hindlimb function and structure in mice, compared with that seen in the rat or guinea pig.

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