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Characterization of NFH-LacZ transgenic mice with the SHIRPA primary screening battery and tests of motor coordination, exploratory activity, and spatial learning

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Abstract

NFH-LacZ transgenic mice express a fusion protein between a truncated form of the endogenous neurofilament of heavy molecular weight and the complete *E. coli* β-galactosidase. NFH-LacZ transgenic mice could be distinguished from controls in the SHIRPA neurological battery by the appearance of action tremor and hindlimb clasping and a lower body weight. Despite normal exploratory activity and spatial learning, NFH-LacZ transgenic mice were deficient in stationary beam, coat-hanger, and rotorod tests of motor coordination. These results are concordant with neuropathological findings in spinal motoneurons and the cerebellum and indicate that despite the absence of paralysis, these transgenic mice may serve as an experimental model of the early stage of amyotrophic lateral sclerosis.

Keywords: Neurofilaments; Motor coordination; Cerebellum; Motor activity; Anxiety; Spatial learning; Spontaneous alternation

1. Introduction

The growing number of transgenic and knockout mice has increased our understanding on the behavioral effects of specific proteins (Anagnostopoulos et al., 2001; Surjo and Arndt, 2001). The use of a wide array of measurements should be encouraged, as genetic modifications often result in test-specific deficits. On the other hand, test standardization ensures a higher degree of interlaboratory replicability (Moldin et al., 2001). It is for these reasons that the SHIRPA (SmithKline/Harwell/Imperial College/Royal Hospital/Phenotype Assessment) test battery was presented (Rogers et al., 1997) and developed through large-scale analysis of mutations induced by *N*-ethyl-*N*-nitrosourea (ENU). The primary screen consists of quantitative and semi-quantitative evaluation of reflexes and basic sensorimotor functions as well as measurements of body weight and size. Secondary and tertiary screens encompass more detailed analyses of motor coordination, spatial orientation, and pain sensitivity. The SHIRPA protocol has been used

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in the description of several mouse strains (Rogers et al., 1999), *ApoE* and coloboma (*cm*) knockout mice (Hatcher et al., 2001), and the legs at odd angles (*Loa*) ENU-induced mutation (Rogers et al., 2001).

We have reported on the sensorimotor functions of transgenic mice with a fusion protein between the endogenous neurofilament protein of heavy molecular weight (NFH) and *E. coli* β-galactosidase (Lalonde et al., 1999; Dubois et al., 2002). The genetic modification causes the accumulation of neurofilaments in the cell body as opposed to their usual localization in axons, reduces axonal diameters in the peripheral and central nervous system, and reduces Purkinje cell number (Eyer and Peterson, 1994; Tu et al., 1997). This neuropathology resembles amyotrophic lateral sclerosis (ALS) (Pioro and Mitsumoto, 1996; Julien, 1997; Munoz et al., 1988). NFH-LacZ mice were impaired in several sensorimotor tests by comparison to normal mice of the B6C3 hybrid strain (Lalonde et al., 1999) as well as C3H and FVB strains (Dubois et al., 2002). The purpose of the present investigation was to determine whether the SHIRPA protocol is sensitive to the neural abnormalities observed in NFH-LacZ mice and whether the same behavioral phenotype can be reproduced on the DBA/2 background during testing in an open-field, an activity chamber, and three motor coordination tasks (stationary beam, coat-hanger, and rotorod).

Contrary to frontotemporal dementia with motor neuron disease causing hypometabolism in cortical association areas (Garraux et al., 1999), ALS patients are not demented. Nevertheless, impaired problem-solving (Strong et al., 1999) and working memory (Abrahams et al., 2000) abilities, as well as emotional lability (Newsom-Davis et al., 1999), giving rise to pathological laughing or crying when associated with pseudobulbar symptoms (McCullagh et al., 1999; Strong et al., 1999), have been described in ALS. It is, therefore, of interest to examine cognitive and emotional aspects of ALS-like murine models.

We determined whether NFH-LacZ mice differ from DBA/2 controls in three tests of exploration as well as spatial learning. Exploration was first evaluated with the spontaneous alternation test in a T-maze. After being forced to enter a maze arm, mice or rats usually choose the opposite arm, thereby exploring novel stimuli (Dember and Fowler, 1958). This test is sensitive to lesions of multiple brain regions, including the cerebellum, as well as induced mutations (Lalonde, 2002). Exploration tensencies were further evaluated in the elevated plus-maze test of anxiety (File, 2001; Pellow et al., 1985), as mice tend to explore safer (enclosed) arms as opposed to more anxiogenic (open) arms, taking into account the normal rodent response of avoiding open spaces (agoraphobia). The elevated plus-maze has been pharmacologically validated in DBA/2J mice for the GABAergic synapse, as diazepam decreased anxiety whereas picrotoxin increased it (Dalvi and Rodgers, 1996). This test is sensitive to several induced mouse mutations (Clément et al., 2002; Holmes, 2001). In the final method of investigating exploration, the emergence test was used (Holson, 1986; Holmes, 2001). This test evaluates the tendency of mice to remain inside a small safe compartment as opposed to a larger more anxiogenic one and is sensitive to induced mutations (Holmes, 2001). Lesions of the medial frontal cortex increased the time taken before rats emerged as well as elevating anxiety levels in several other tests, underlining the convergent validity of this measure (Holson, 1986). Spatial learning was assessed in the Morris maze, in which mice were trained to escape from a pool of water by reaching a platform (Morris et al., 1982). This test is sensitive to several mutations, including NFH-LacZ mice when compared to B6C3 controls (Lalonde et al., 1999).

2. Material and methods

2.1. Animals

Six- to eight-month-old NFH-LacZ transgenic mice ($n = 10$, 6 females and 4 males) and littermate controls ($n = 12$, 6 females, 6 males) on the DBA/2 background were bred at Angers and then shipped to Rouen. The mice were kept in group cages with woodchip bedding in a room with a light-dark cycle of 12/12 h (lights on at 7:00 h). This research protocol adhered to the guidelines of the European Council Directive (86/609/EEC).

2.2. Apparatus and procedure

After a 5-day adaptation period to the new surroundings and to handling by the experimenters, the

evaluation began with the SHIRPA primary screen. Over a 10-day period, spontaneous alternation was evaluated and then followed by tests of motor activity (open-field and activity chamber), anxiety (plus-maze and emergence from a toy object), and motor coordination on the stationary beam and the coat-hanger. The battery was completed by rotorod and Morris water maze tests on Days 11–19.

The SHIRPA primary screen began with observations in a perspex jar, followed by a 30 s motor activity test and other measures in an open-field, as well as functional evaluations during tail-lifting, supine body restraint, and placement in a cylinder or a vertical grid (Rogers et al., 1997; Hatcher et al., 2001). This battery is described in detail at the ENU Mutagenesis Programme web site (www.mgu.har.mrc.ac.uk) with standardized material.

Spontaneous alternation was tested in a T-maze made of plywood, containing a central stem and two side arms, each measuring 30 cm in length and 10 cm in width, with walls 20 cm in height. On the initial trial, the mice were placed in the stem with the right arm blocked by a plastic barrier (forced choice). After entering the available arm (four-paw criterion), the mice were kept in it for 60 s by closing the barrier behind them. The mice were then retrieved and, after removing the barrier, were immediately placed back in the stem for a free-choice trial. On the following 9 days, the same procedure was followed, except that the blocked arm on the initial trial was changed alternatively from right to left. The number of alternations and the latencies before responding were measured, with a cut-off period of 60 s per trial. After this time, the mice were briefly prodded from behind, usually not more than once, and only when the mice were far from the choice-point, so that a response could be measured on all trials. As in all tests of exploration, the floor was wiped clean with a damp cloth and dried with a towel.

Motor activity was measured in an open-field (57 cm × 33 cm) made of opaque white plastic and surrounded by walls 26 cm in height. The floor was divided by masking tape markers into 18 rectangles (six rows of three), 14 at the periphery and 4 at the center. The mice were placed in one corner and the number of segments crossed at the periphery and at the center (four-paw criterion) were recorded during 5 min per session for 3 days. The activity chamber (Letica model LE 8811, Bioseb, France) measured 45 cm × 45 cm, surrounded by walls of transparent plastic 36 cm in height. The mice were placed in the center of the apparatus during 5 min per session for 3 days. The number of fast (>10 cm/s) or slow (<10 cm/s) ambulatory, stereotyped, and rearing movements were recorded by infrared photocell detectors (intercell distance: 2.5 cm; height of first and second sensors: 4 and 8 cm, respectively, from floor level).

Anxiety was measured in elevated plus-maze and emergence tests. The elevated plus-maze was made of opaque beige plastic, consisting of four arms (length: 28 cm, width: 5 cm, height from floor: 38 cm) in a cross-shaped form and a central region (5 cm × 5 cm). Two of the arms were enclosed on three sides by walls (height: 16 cm), whereas the other two were not. The two enclosed arms, as the two open arms, faced each other. The mice were first placed in the center region and their behavior evaluated for 5 min per session for 2 days. The number of entries (four-paw criterion) and the time spent in either the enclosed or the open arms were measured, together with the open/total number of entries and duration ratios. The open arm duration began once the mice first entered either open arm from the central region and was accumulated until entry into either enclosed arm. Conversely, enclosed arm duration was accumulated until entry into either open arm. In the emergence test, the mice were placed inside a small toy object (orange-colored plastic shoe; length: 13 cm, width: 6 cm, height: 7.5 cm) perforated with three holes (diameter: 3 cm) and situated in the middle of a larger enclosure (41 cm × 27 cm, height of walls: 18 cm) made of opaque white plastic. The mice had not been previously exposed to either the object or the larger enclosure. The latencies before emerging with either two or four paws were determined for 2 days, with a cut-off period of 5 min per session. The mice were allowed to explore the enclosure for 10 s after leaving the object.

Motor coordination was measured in the stationary beam test. The beam (diameter: 2 cm, length: 110 cm) was made of wood and covered by a layer of white masking tape in order to provide a firm grip. The beam was separated into 11 segments by line drawings and placed at a height of 38 cm from the cushioned floor. A piece of cardboard was inserted at each end in order to prevent the mice from escaping sideways. The mice

were placed in the middle part of the beam and the number of segments crossed (four-paw criterion), the latencies before falling, and the number of falls were measured during four trials in a single session, with a cut-off period of 1 min per trial and an intertrial interval of 15 min.

The coat-hanger consisted of a horizontal steel bar (diameter: 2 mm, length: 41 cm) and two side-bars (length: 19 cm in length) oriented at an angle of 35° from the horizontal axis. The bar was placed at a height of 50 cm from a cushioned table. The mice were placed upside-down in the middle part of the horizontal wire and were released only when all four paws gripped it. The test was performed in a single session of four trials with a cut-off period of 1 min and an intertrial interval of 15 min. Seven types of movement time (MT) were compiled, namely, latencies before reaching (snout criterion) the first 10 cm segment (MT-1) or the extremity (MT-2) of the horizontal wire, latencies before reaching either side-bar with two, three or four paws, and latencies before reaching (snout criterion) either the midway or the top of the side-bar. The latencies before falling and the number of falls were also recorded. Whenever a mouse reached the top of the apparatus, it was immediately retrieved and a maximal score of 60 s given for latencies before falling. A trial ended whenever the mice fell from any part of the apparatus.

The accelerating rotorod (Letica Rota-Rod/RS, Bioseb, France) consisted of a beam (diameter: 3 cm, width: 5 cm) made of knurled white plastic and covered with a layer of masking tape. The cylinder was flanked by two round plates in order to prevent any escape from the side and suspended at a height of 17 cm above a plastic plate. The mice were placed on top of the already revolving beam (4 rpm) and facing away from the experimenter's view in the orientation opposite to that of beam movement, so that forward locomotion was necessary for avoiding a fall. The rotorod accelerated gradually from 4 to 40 rpm over the 2-min cut-off period. Latencies before falling were measured during four trials per session for 3 days, with an intertrial interval of 15 min. Since passive rotation on the beam was rarely observed, the time spent walking reflects almost exactly the latencies.

Spatial orientation was evaluated in the Morris water maze, consisting of a basin (diameter: 86 cm, height of the wall: 30 cm) made of opaque white plastic and filled with water (22 °C) at a height of 21 cm. Yellow plastic beads were evenly spread over the water surface in order to camouflage the escape platform (diameter: 8 cm) made of white plastic and covered with a wiremesh grid to ensure a firm grip. The pool was placed in a room with abundant extramaze visual cues, such as light fixtures, a dangling wire, a pole, and a wall poster. The mice were placed next to and facing the wall successively in north (N), east (E), south (S), and west (W) positions, with the escape platform hidden 1 cm beneath water level in the middle of the NW quadrant. The experimenter was hidden from the view of the animal, but was able to follow swimming trajectories on a videomonitor, on which the pool was separated into four equally spaced quadrants. The number of quadrant entries (four-paw criterion) indicating swim path length and the escape latencies were measured for four trials per session for 5 days, with an intertrial interval of 15 min. After their swim, the mice were kept dry in a plastic holding cage filled with paper towels. Whenever the mice failed to reach the escape platform within the maximally allowed time of 60 s, it was manually placed on the platform for 5 s. The day after the acquisition phase, a probe test was conducted by removing the platform and placing the mouse next to and facing the N side. The time spent in the previously correct quadrant was measured during a single 60 s trial. Two hours later, the visible platform version was performed, when the escape platform was lifted at a height of 1 cm above water level and shifted to the SE quadrant. A pole (height: 7 cm) was inserted on top of the platform as an aid for viewing. In an identical manner to the place learning task, the number of quadrant entries and the escape latencies were measured for four trials per session, the animals stayed on the platform for 5 s, and a cut-off period of 60 s per trial with an intertrial interval of 15 min was used, except that the test was conducted in a single day.

3. Results

3.1. SHIRPA protocol

As presented in Table 1, NFH-LacZ transgenic mice could be distinguished from controls in the SHIRPA primary screen by the appearance of action tremor in 10/10 mice ($U_{10,10} = 0$, $P < 0.001$), hindlimb

Table 1
Mean±S.E.M. values of the SHIRPA primary screen in NFH-LacZ transgenic mice ($n = 10$) and controls ($n = 12$)

Tests	Controls	NFH-LacZ
Viewing jar		
Body position	3.0 ± 0	3.0 ± 0
Activity	1.4 ± 0.1	1.2 ± 0.1
Respiration rate	2.0 ± 0	2.0 ± 0
Tremor	0 ± 0	2.0 ± 0**
Fecal boli	3.6 ± 0.7	1.8 ± 0.7
Open-field		
Arousal	2.8 ± 0.2	2.3 ± 0.2
Activity	12.6 ± 4.5	9.8 ± 1.9
Eye closure	0 ± 0	0 ± 0
Coat status	0 ± 0	0 ± 0
Startle response	0 ± 0	0 ± 0
Gait	0 ± 0	0 ± 0
Pelvic elevation	1.9 ± 0.1	1.6 ± 0.2
Tail elevation	1.7 ± 0.1	1.5 ± 0.2
Touch escape	1.8 ± 0.2	2.1 ± 0.3
Position passivity	1.1 ± 0.2	0.8 ± 0.2
Tail-lifting		
Trunk curl	1.0 ± 0	1.0 ± 0
Limb grasp	1.0 ± 0	1.0 ± 0
Visual placing	3.0 ± 0	3.0 ± 0
Grip strength	2.0 ± 0	1.9 ± 0.1
Body tone	1.0 ± 0	1.0 ± 0
Horizontal grid		
Pinna reflex	1.0 ± 0	1.0 ± 0
Corneal reflex	1.0 ± 0	1.0 ± 0
Toe pinch	2.9 ± 0.1	2.5 ± 0.3
Horizontal wire		
Wire maneouvre	0.6 ± 0.3	1.4 ± 0.4
Supine restraint		
Skin color	1.0 ± 0	1.0 ± 0
Heart rate	1.0 ± 0	1.0 ± 0
Limb tone	1.0 ± 0	1.0 ± 0
Abdomen tone	1.0 ± 0	1.0 ± 0
Lacrimation	0 ± 0	0 ± 0
Salivation	0 ± 0	0 ± 0
Biting	0.4 ± 0.1	0.4 ± 0.2
Drop righting	0 ± 0	0 ± 0
Tube		
Contact righting	1.0 ± 0	1.0 ± 0
Vertical grid		
Geotaxis	0.7 ± 0.2	0.6 ± 0.2
Handling		
Fear	1.0 ± 0	1.0 ± 0
Irritability	0.9 ± 0.1	1.0 ± 0
Aggression	0 ± 0	0 ± 0
Vocalization	0.4 ± 0.1	0.4 ± 0.2
Body measures		
Weight (g)	23.7 ± 1.0	19.3 ± 0.7**
Size (cm)	8.8 ± 0.1	8.4 ± 0.2
Additional signs		
Paw clasping	0 ± 0	0.1 ± 0.1

** $P < 0.01$ vs. controls (Mann–Whitney U-test).

clasping in 1/10 mice, and reduced body weight (female: $t_{10} = 2.46$, $P < 0.05$; male: $t_8 = 5.05$, $P < 0.01$; total: $t_{20} = 3.3$, $P < 0.01$). In contrast, the gait of NFH-LacZ transgenic mice was normal, as were their scores in tests of ambulation, grip strength, limb tone, sensitivity to toe pinch, the ability to lift their hindlimbs on a horizontal wire and to turn on a vertical grid, and the appearance of various reflexes. Some mice in either group displayed vocalization during handling, but neither group responded to a 90 db auditory stimulus meant to startle.

3.2. Spontaneous alternation

The spontaneous alternation rate of the control group over the 10-day period was 56% ± 6, not above chance ($U_{12,12} = 54$, $P > 0.05$). Paradoxically, the 65% ± 3 alternation rate of NFH-LacZ mice reached significance ($U_{10,10} = 22.5$, $P < 0.01$). The groups did not differ in response latencies ($t_{20} = 1.17$, $P > 0.05$; controls: 15 s ± 4 per trial; NFH-LacZ: 11 s ± 2 per trial).

3.3. Motor activity

One fewer mouse of each group could be tested in the open-field (Fig. 1), because of the onset of a manic-type behavior and a huge number of segment crossings that could not be recorded even by a trained observer. However, these mice appeared normal in the other tests. Two-way ANOVAs with repeated measures revealed that the motor activity of NFH-LacZ transgenic mice was identical to that of controls ($P > 0.05$); only the day effect was significant for peripheral ($F_{2,36} = 5.52$, $P < 0.01$) and central ($F_{2,36} = 9.61$, $P < 0.001$) crossings, as both groups had lower values across days. As seen in Table 2, the only significant difference in the activity chamber was the lower

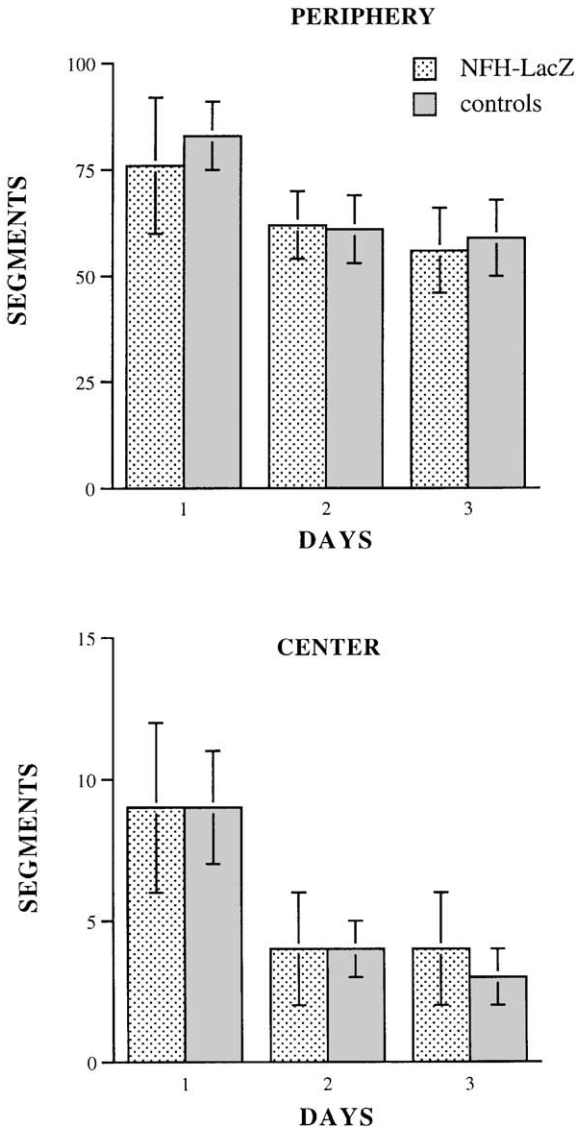

Fig. 1. Motor activity (means±S.E.M.) by NFH-LacZ mice ($n = 9$) and controls ($n = 11$) in the peripheral and in the central part of the open-field during 5 min sessions.

number of slow stereotyped movements by NFH-LacZ mice ($F_{2,36} = 5.52$, $P < 0.01$).

3.4. Plus-maze

NFH-LacZ transgenic mice did not differ from controls in any aspect of the elevated plus-maze test. As presented in Table 3, the number of arm entries

Table 2
Number of movements by NFH-LacZ mice ($n = 10$) and controls ($n = 12$) during a 5-min exposure to the activity monitor (means± S.E.M.) over 3 days

Tests	Controls	NFH-LacZ
Fast ambulation		
Day 1	463 ± 218	281 ± 102
Day 2	254 ± 99	190 ± 123
Day 3	353 ± 119	222 ± 97
Slow ambulation		
Day 1	166 ± 15	141 ± 32
Day 2	130 ± 25	98 ± 23
Day 3	128 ± 20	132 ± 25
Fast stereotypy		
Day 1	262 ± 46	208 ± 50
Day 2	229 ± 36	271 ± 88
Day 3	236 ± 32	221 ± 48
Slow stereotypy		
Day 1	78 ± 7	51 ± 9*
Day 2	71 ± 7	49 ± 9*
Day 3	65 ± 7	54 ± 8*
Fast rears		
Day 1	3 ± 1	2 ± 1
Day 2	2 ± 1	3 ± 1
Day 3	4 ± 1	3 ± 1
Slow rears		
Day 1	13 ± 1	10 ± 3
Day 2	10 ± 2	7 ± 2
Day 3	11 ± 1	9 ± 2

* $P < 0.05$ for transgene effect on two-way ANOVA.

(enclosed: $t_{20} = 1.93$, $P > 0.05$; open: $t_{20} = 0.55$, $P > 0.05$) and the time spent (enclosed: $t_{20} = 0.36$, $P > 0.05$; open: $t_{20} = 0.36$, $P > 0.05$) in either maze arm as well as open/total arm entry ($t_{20} = 0.96$, $P > 0.05$) and duration ($t_{20} = 0.39$, $P > 0.05$) ratios by NFH-LacZ transgenic mice were similar to those of controls on Day 1. The same results were found on Day 2 ($P > 0.05$).

3.5. Emergence

As presented in Table 4, normal controls emerged from the toy with similar latencies on both test days. The latencies of NFH-LacZ mice differed on the 2 days, but the groups did not differ either on Day 1 when the intergroup variances were homogeneous (two-paw criterion: $t_{20} = 0.46$, $P > 0.05$; four-paw criterion: $t_{20} = 0.9$, $P > 0.05$) or on Day 2, when the

Table 3
Mean ± S.E.M. open and enclosed arm entries and duration (s) in the elevated plus-maze by NFH-LacZ mice ($n = 10$) and controls ($n = 12$) during 2 days of testing

Tests	Controls	NFH-LacZ
Day 1		
Open arms		
Entries	1.3 ± 0.4	1.0 ± 0.5
Duration	15.8 ± 5.2	19.6 ± 9.6
Enclosed arms		
Entries	7.3 ± 1.0	4.6 ± 0.9
Duration	284.2 ± 5.3	280.4 ± 9.6
Open/total ratio (%)		
Entries	13.7 ± 3.6	11.5 ± 5.1
Duration	5.1 ± 1.7	6.5 ± 3.2
Day 2		
Open arms		
Entries	0.2 ± 0.2	0.3 ± 0.2
Duration	0.7 ± 0.7	1.0 ± 0.7
Enclosed arms		
Entries	5.1 ± 1.2	3.1 ± 0.8
Duration	299.3 ± 0.6	299.0 ± 0.7
Open/total ratio (%)		
Entries	0.9 ± 0.9	3.5 ± 2.6
Duration	0.2 ± 0.3	0.3 ± 0.2

intergroup variances were heterogeneous and, therefore, analyzed by the Mann–Whitney U non-parametric test (two-paw criterion: $U_{10,12} = 50.5$, $P > 0.05$; four-paw criterion: $U_{10,12} = 51.5$, $P > 0.05$).

3.6. Motor coordination

NFH-LacZ transgenic mice were deficient in all three motor coordination tasks. The latencies before falling ($U_{10,12} = 13.0$, $P < 0.01$) and the number of segments crossed ($U_{10,12} = 8.0$, $P < 0.001$) on the stationary beam (Table 5) were lower in NFH-LacZ mice than controls, while their number of falls was higher ($U_{10,12} = 16.0$, $P < 0.01$). Lower latencies before falling ($t_{20} = 3.45$, $P < 0.01$) and a higher number of falls ($t_{20} = 4.26$, $P < 0.001$) were also observed by the transgenics on the coat-hanger (Table 5). Intergroup differences were also revealed for the more difficult MT measures. Due to intercage fighting, there was one fewer male control in the final two tests (rotorod and Morris). The transgene effect ($F_{1,19} = 4.76$, $P < 0.05$) and the trial ($F_{12,209} = 9.33$, $P < 0.001$) main factors were significant for latencies before falling from the rotorod. NFH-LacZ mice had lower latencies, but both groups improved with repeated trials (Fig. 2).

Table 4
Mean ± S.E.M. two- and four-paw emergence latencies (s) from a toy object by NFH-LacZ mice ($n = 10$) and controls ($n = 12$) during 2 days of testing

Tests	Controls	NFH-LacZ
Day 1		
Two-paw	28.9 ± 24.7	46.2 ± 29.0
Four-paw	29.2 ± 24.7	68.8 ± 38.6
Day 2		
Two-paw	28.1 ± 24.7	7.7 ± 3.2
Four-paw	29.1 ± 24.7	9.5 ± 4.2

Table 5
Motor coordination (mean ± S.E.M. totals over four trials) by NFH-LacZ mice ($n = 10$) and controls ($n = 12$) on stationary beam and coat-hanger tests

Tests	Controls	NFH-LacZ
Stationary beam		
Segments	66 ± 10	14 ± 3**
Latencies (s)	220 ± 7	127 ± 20**
Falls	0.6 ± 0.2	2.6 ± 0.5**
Coat-hanger		
MT-1 (s)	35 ± 7	36 ± 9
MT-2 (s)	83 ± 15	89 ± 21
Two-paw (s)	135 ± 17	203 ± 20*
Three-paw (s)	161 ± 16	227 ± 13**
Four-paw (s)	173 ± 15	228 ± 13**
Midway (s)	186 ± 15	235 ± 5**
Top (s)	207 ± 15	235 ± 5*
Fall latencies (s)	176 ± 17	98 ± 14**
Falls	1.8 ± 0.4	3.8 ± 0.2**

* $P < 0.05$ vs. controls (unpaired t-test or Mann–Whitney U-test).
** $P < 0.01$ vs. controls (unpaired t-test or Mann–Whitney U-test).

3.7. Spatial learning

Even during the initial trials of place learning in the Morris water maze, rarely did mice of either group bump against the platform and continue swimming. Instead, the mice tended to climb on the platform as soon as they found it.

Only the day effect was significant (quadrant entries: $F_{4,76} = 4.27$, $P < 0.01$; escape latencies:

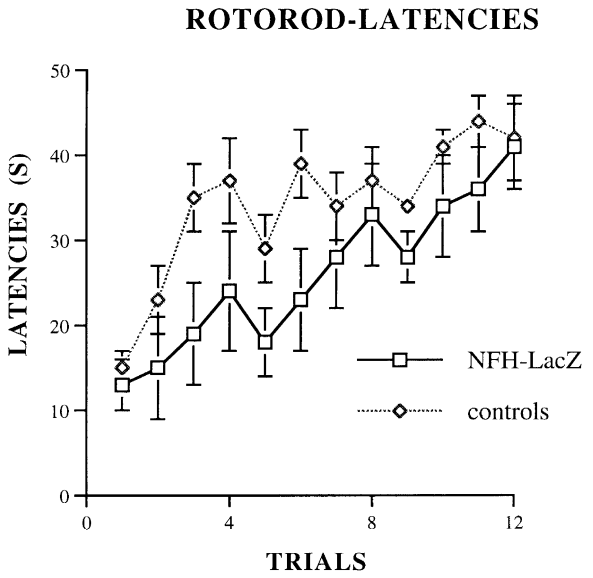

Fig. 2. Latencies before falling in s (means±S.E.M.) by NFH-LacZ mice ($n = 10$) and controls ($n = 11$) during acquisition of sensorimotor learning in the rotorod test.

$F_{4,76} = 6.15$, $P < 0.001$) for acquisition of the hidden platform version of the water maze, as both groups improved their scores with repeated trials (Fig. 3). The number of quadrant entries ($F_{1,19} = 0.12$, $P > 0.05$) and the escape latencies ($F_{1,19} = 0.47$, $P > 0.05$) were unchanged in NFH-LacZ mice relative to DBA/2 controls, and the gene × day interaction was not significant either (quadrant entries: $F_{4,76} = 1.59$, $P > 0.05$; escape latencies: $F_{4,76} = 0.86$, $P > 0.05$).

In the probe trial, NFH-LacZ mice did not differ from controls ($t_{19} = 1.44$, $P > 0.05$). Control mice only spent 33% of their time (20 s ± 2/60) in the target quadrant, not above 25% chance level ($U_{11,11} = 33$, $P > 0.05$), while NFH-LacZ mice spent 27% of their time (16 s ± 2/60) there, not above chance either ($U_{10,10} = 50$, $P > 0.05$).

In addition, NFH-LacZ mice performed normally while swimming to the visible platform (quadrant entries: $t_{19} = 0.41$, $P > 0.05$; escape latencies: $t_{19} = 0.47$, $P > 0.05$). The total number of quadrant entries for the four trials was 18 ± 4 for the control group and 20 ± 3 for the NFH-LacZ group. The escape latencies were 50 s ± 13 for the control group and 58 s ± 10 for the NFH-LacZ group.

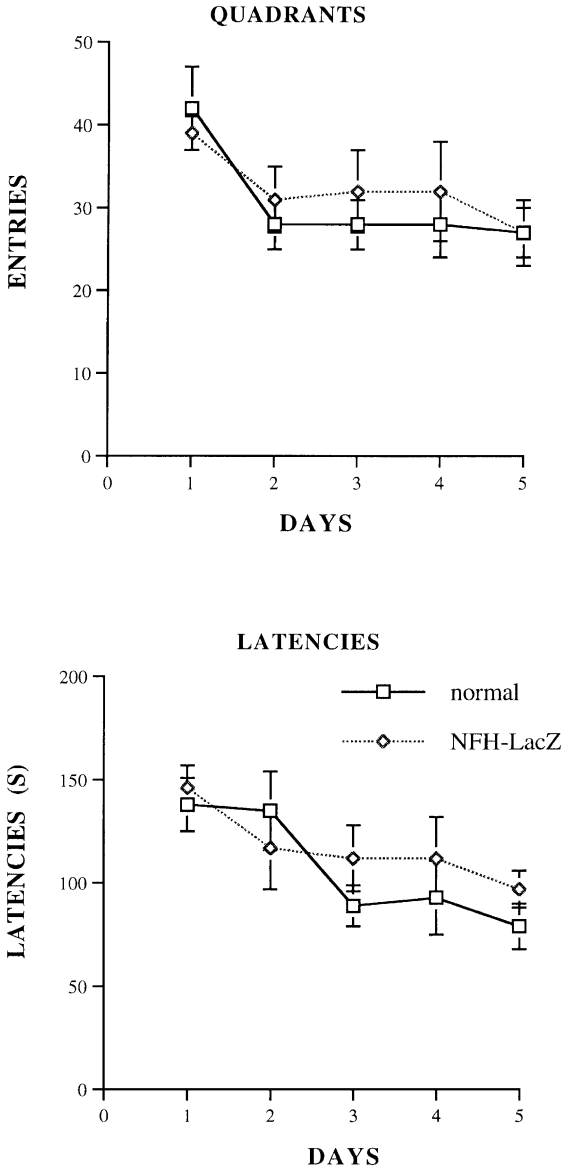

Fig. 3. Total number of quadrant entries and escape latencies in s (means ± S.E.M.) over the four trials of the day during acquisition of spatial learning in the Morris water maze by NFH-LacZ mice ($n = 10$) and controls ($n = 11$).

4. Discussion

4.1. SHIRPA protocol

Rogers et al. (1999) compared DBA/2 with five other strains in the SHIRPA primary screen as well as

tests of motor activity and coordination. Their results on 6-week-old DBA/2 mice resemble our own at 6 months of age. The data were identical in regard to body position in the viewing jar, visual placing, and grip strength and nearly the same for spontaneous activity in the viewing jar, transfer arousal, motor activity (means: 12.6 versus 14.7), touch escape in the open-field, and toe pinch. Slight differences in body measures (heavier weight and bigger size) may be explained by the aging factor. Their mice had homogeneous values for pelvic elevation, negative geotaxis, irritability, and vocalization. Although our mean values were similar, we found some variability in the individual scores. A particularity of this strain is the total absence of a startle reponse to a 90 db auditory stimulus, confirming previous results of age-dependent hearing deficits caused by cochlear nucleus abnormalities (Turner and Willott, 1998).

NFH-LacZ transgenic mice could be distinguished from controls in the SHIRPA primary screen based on action tremor and reduced body weight. The action tremor displayed by NFH-LacZ mice is age-related, being absent at 5 months (Lalonde et al., 1999; Dubois et al., 2002). The body weight of NFH-LacZ transgenic mice was also diminished relative to C3H and FVB controls, interpretable as a result of unequal access to milk during the weaning period because of competition (Dubois et al., 2002). An additional neurological sign in the transgenic mice was hindlimb clasping, but this was only seen in 10%. In contrast, muscular tone and a number of basic reflexes were normal. Cerebellar ataxia, observed in NFH-LacZ mice over 12 months of age (Lalonde et al., 1999) and explained by the loss of Purkinje cells (Tu et al., 1997), was not present in this age group. Thus, the action tremor appears before ataxia but is also probably caused by cerebellar dysfunction.

4.2. Exploratory activity

Despite neurofilament maldistribution in the central nervous system (Eyer and Peterson, 1994), the ambulatory activity of NFH-LacZ transgenic mice was unchanged in the open-field and in the automated chamber, confirming results on B6C6, C3H, and FVB backgrounds (Lalonde et al., 1999; Dubois et al., 2002). The only significant difference observed in the automated chamber was the lower number of slow stereotyped movements by NFH-LacZ mice. This measure reflects head and body swaying and grooming responses.

Although NFH-LacZ transgenic mice exhibited action tremor and impaired motor coordination abilities in comparison to DBA/2 controls, the groups did not differ during exploration of the elevated plus-maze and emerged with similar latencies from a toy object to a larger enclosure. The interpretation of the spontaneous alternation test is complicated by the lack of alternation shown in the control strain. The absence of spontaneous alternation seen in our DBA/2 mice confirms the findings of Bertholet and Crusio (1991) and Gerlai (2001) and stands in contrast to the high alternation rates observed in other mouse strains. The normal response of alternating above chance in the T-maze was observed in C3H controls and their NFH-LacZ counterparts, but not in FVB controls and their NFH-LacZ counterparts (Dubois et al., 2002). On the normal-performing B6C3 background, NFH-LacZ mice had an age-related deficit in spontaneous alternation (Lalonde et al., 1999). Although several behavioral measures have been related to interstrain differences in hippocampal morphology, this was not the case for spontaneous alternation rates (Bertholet and Crusio, 1991). The reason why some strains fail to alternate is uncertain. An intriguing finding is that although NFH-LacZ mice did not differ from DBA/2 controls in reponse latencies, the transgenics nevertheless alternated above chance. It is possible that although deficits in balance and equilibrium are caused by neurofilament maldistribution, compensatory brain changes occurred which counteracted the abnormality seen in DBA/2 controls. Paradoxical improvements have been reported in other transgenic mice. For example, the introduced A246E mutation of the *PS1* gene causing the familial form of Alzheimer's disease on a murine knockout background resulted in a higher mean body weight than the wild-type human gene (Lalonde et al., 2003).

Altered anxiety levels in elevated plus-maze and in emergence tests have been reported for several transgenic mice as well as targeted null mutations (Holmes, 2001). For example, in the elevated plus-maze, anxiety was increased in two $GABA_A$ receptor null mutations as well as the knockout for the GABA synthesizing enzyme, glutamate decarboxylase (Holmes, 2001). In the emergence test, altered anxiety was

found in null mutations for dopamine, serotonin, and corticotropin-releasing hormone receptors (Holmes, 2001). In the present study, NFH-LacZ mice were not different from controls in either test. These results indicate that the transgene-induced motor deficits observed in stationary beam, coat-hanger, and rotorod tests (see below) were not sufficient for reducing ambulation and did not appear to affect those brain regions involved in anxiety. No transgene effect on regional brain metabolism was detected in the amygdala, crucially involved in anxiety (Strazielle et al., 2002).

4.3. Motor coordination

In contrast to their normal ambulation, NFH-LacZ mice were deficient in all three motor coordination tasks. The deficits on the stationary beam, coat-hanger, and rotorod tests once again reproduce results on B6C3, C3H and FVB backgrounds (Lalonde et al., 1999; Dubois et al., 2002). These deficits are age-related. For example, latencies before falling from the rotorod were lower in 12- but not in 3-month-old NFH-LacZ mice relative to B6C3 controls (Lalonde et al., 1999; Stone et al., 2001). NFH-LacZ mice were deficient in the coat-hanger test despite an intact ability for performing the wire manoeuvre described by Rogers et al. (1999).

4.4. Spatial learning

By contrast to the impaired hidden and visible platform subtests of the Morris water maze observed in NFH-LacZ mice on the B6C3 background (Lalonde et al., 1999), no such impairment occurred on the DBA/2 background. Although DBA/2 controls improved their scores during acquisition of the hidden platform version, they showed no tendency for remaining in the target quadrant during the probe trial. Thus, their improved scores may be explained by the development of appropriate strategies, such as abandoning exploration of the maze wall, as opposed to the ability of precisely locating the spatial coordinates of the platform. The superior performance of C57BL/6 over DBA/2 in annulus crossings during the probe trial has been ascribed to interstrain differences in morphology at the level of the hippocampus (Schöpke et al., 1991). On the other hand, Owen et al. (1997) found equally good probe trial performance in the two strains. In any case, the B6C3 strain showed good acquisition and probe trial performance, as opposed to DBA/2, C3H, and FVB strains (Dubois et al., 2002), and it is only on that hybrid background that NFH-LacZ mice were impaired relative to controls. These results illustrate the importance of using several genetic backgrounds for interpretation of transgene-mediated effects. Deficits are generally obvious only with the use of a genetic background performing at a high level. The impaired maze performance on the B6C3 background is concordant with poor problem-solving (Strong et al., 1999) and working memory (Abrahams et al., 2000) abilities described in ALS. On the other hand, we did not find in the present study any evidence of behavioral disinhibition described in ALS (McCullagh et al., 1999; Newsom-Davis et al., 1999; Strong et al., 1999).

5. Conclusions

The motor deficits of NFH-LacZ transgenic mice are concordant with neuropathological findings in motoneurons and the cerebellum (Eyer and Peterson, 1994; Tu et al., 1997) expected of an experimental model of ALS (Pioro and Mitsumoto, 1996; Julien, 1997; Munoz et al., 1988). Nevertheless, the motoneuron abnormalities do not result in paralysis, the hallmark of this disease. Therefore, the transgenic mice may serve as a model for its early form.

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