Published in final edited form as:

Behav Brain Res. 2008 November 21; 193(2): 315–319. doi:10.1016/j.bbr.2008.06.016.

Early deficits in motor coordination and cognitive dysfunction in a mouse model of the neurodegenerative lysosomal storage disorder, Sandhoff disease

Maria Gulinello^a, Fengying Chen^b, and Kostantin Dobrenis^{b,*}

^a Behavioral Core Facility, Dominick S. Purpura Department of Neuroscience, Albert Einstein College of Medicine of Yeshiva University, Bronx, NY, USA

^b Dominick S. Purpura Department of Neuroscience, Albert Einstein College of Medicine of Yeshiva University, Bronx, NY 10461, USA

Abstract

Mouse models of lysosomal storage diseases, including Sandhoff disease, are frequently employed to test therapies directed at the central nervous system. We backbred such mice and conducted a behavioral test battery which included sensorimotor and cognitive assessments. This is the first report of short-term memory deficits in a murine model of Sandhoff disease. We also document early onset of motor deficits using the balance beam test.

Keywords

Sandhoff disease; Mouse model; Novel object recognition test; Rotarod; Motor coordination; Balance beam; Open field; Memory

Lysosomal storage disorders (LSDs) are a family of inherited diseases with multi-organ involvement frequently including the nervous system. The G_{M2} gangliosidoses, one class of human LSDs that include Tay-Sachs and Sandhoff diseases, are marked by the lysosomal accumulation of G_{M2} ganglioside (GM2) in neurons throughout the nervous system. Sandhoff disease arises from defects in the gene encoding the β -subunit (*hexb*) of β -N-acetyl-d-hexosaminide N-acetylhexosaminohydrolase (EC 3.2.1.52) normally involved in the sequential catabolism of gangliosides and other glycoconjugates [11]. Sandhoff patients have widespread brain pathology which results in clinical symptoms including progressive mental and motor deterioration [10,11,21].

Two transgenic murine models of Sandhoff disease exist [23,25] that, like the human equivalent, exhibit premature death and display widespread neuronal storage of ganglioside, in addition to gliotic and inflammatory reactions and neuronal cell death [11,16,23,25,27]. The Sandhoff models have engendered much research directed at overcoming the challenges

that the CNS presents to therapy [1,2,4,11,14,15,18,20]. The knockout model [25] presented here displays ataxia, tremor, muscle wasting, and deficits in motor reflex, coordination and balance [1,4,7,8,13,14,16,18,20,22,25,27,28]. However, systematic studies examining cognitive or affective outcomes are lacking [25]. Clearly, these latter studies would be valuable to evaluate CNS-specific improvements, and would be most meaningful if assays pertinent to human neurologic assessments were employed to facilitate transition to clinical trials. Furthermore, the Sandhoff mice rarely show overt clinical signs before 3 months old and then rapidly decline, typically becoming moribund between 4 and 5 months of age [1,13–15,18,20,22,25,27,28]. Therefore, behavioral assays should ideally be able to identify cognitive deficits in subjects that have multiple behavioral outcomes. In addition, increased assay sensitivity could permit behavioral assessment at earlier ages thereby also providing better evaluation of therapeutic strategies which seek earlier intervention.

We have now backbred the Sandhoff mouse model, originally on a mixed background [25], onto the normal C57BL/6J strain and phenotyped these mice. Firstly, we used an assay of memory, the novel object recognition test [9]. This does not require food or water deprivation, which is a potential confound in metabolic disorders, does not require good motor coordination or stamina, is suitable for longitudinal and crossover designs, and is similar to tests conducted in humans [17]. Controls for visual acuity and exploratory deficits are intrinsic to the design of the task. We also performed a battery of tests, including assessments of motor coordination, such as the balance beam [26] and rotarod assays [3], a functional observation battery (FOB) [12,24] and open field tasks [5]. Furthermore, we tested whether deficits could be detected in relatively young mice prior to manifestation of overt symptoms.

Heterozygous breeders of the B6; 129S4-*Hexβ^{tm1Rlp}* strain [25] (Jackson Labs, Maine) were backbred through 10 generations by mating with C57BL/6J mice. Then the backbred heterozygotes were mated together and progeny genotyped using a standardized PCR protocol (provided by Jackson Labs) on tail-tip DNA extracts. Studies were performed using homozygous wild-type (WT; *hexβ*^{+/+}) and Sandhoff mutant (*hexβ*^{-/-}) littermates or age-matched progeny. Mice were group housed in filter cages under standard light/dark cycle and provided standard feed and water *ad libitum*. All procedures were conducted according to NIH, IUACAAC and internal guidelines for animal welfare, and every precaution taken to minimize distress and ensure the health and welfare of the mice.

Behavioral tests were conducted in the following order: FOB, open field, novel object recognition test, balance beam and rotarod. All animals were tested between 9:00 am and 5:00 pm in matched blocks to ensure that time of day did not confound interpretation. To do this, each block included animals of each condition and age. The FOB is a standard method for assessment of general arousal, reflexes, autonomic, motor and neurological deficits, and was performed as described previously [12,24]. This battery was used as a primary screen in order to verify the absence of gross deficits that would prevent or confound the principal behavioral tests. Methodology of the many tests would be too long to include here, thus only the most relevant methods are described. Grip strength was assessed by latency to fall from a horizontal wire (s). Visual placing was determined by lowering the mice toward a grid and recording the distance at which the mouse reached for the grid.

In the open field test, mice were placed in a 16 × 16 in. square opaque testing arena after acclimation to the testing room for 30 min. Behaviors were assessed for 9 min with an automated video-tracking system (Viewer, Biobserve, Germany) to measure total tracklength (general locomotor activity) and center track-length. Thigmotaxis was defined as the percent center exploration [(center tracklength/outside tracklength) × 100]. As this is effectively the proportion of exploration in the center zone, this measure is not readily confounded by alterations in total locomotor activity, and has been previously validated to reflect anxiety-like behavior [5]. The number of entries into the center zone was also analyzed as an adjunct measure of anxiety-like behavior.

The novel object recognition test of memory is based on the robust tendency of rodents to preferentially explore novel objects and was performed essentially as described [9] in an opaque perspex arena (16 × 16 in.). In Trial 1, mice were given 3 min to freely explore two identical, non-toxic objects (glass salt shakers). Mice were then removed to the home cage for a 60 min retention interval, and returned to the arena for 3 min in which one object was replaced with a novel object, a glass candlestick holder (Trial 2). Data from Trial 2 were expressed as a preference score—(time exploring novel object/total time exploring) × 100. A preference score of 50% indicates chance performance. Exploration of the objects was defined as whisking, sniffing, rearing on or touching the object, and approach and obvious orientation to the object within 3 cm. Novelty-induced exploration of the objects in Trial 1 was also assessed and expressed as the total time spent exploring both the objects. Any animal having <2 s exploration in either trial was excluded from preference score analysis, but was included in the analysis and representation of novel object exploration.

In the balance beam test, latency to cross a round beam and number of slips were recorded [19,26]. To minimize latency variation and acclimatize animals to the task, mice were first exposed to walking over a flat wooden plank with a hide (containing a palatable food—honey-nut cheerios) at one end to encourage crossing. Animals were pre-exposed to the food for 2–4 days prior to reduce neophobia. After this pre-training, two 28 in. long round beams of 1.5 in. (easy) and 1.2 in. (hard) diameter were used to assess motor coordination. Both latency (time to cross the beam from end to end) and number of slips (each time a paw fell under the beam midline) were assessed as measures of motor coordination. In the rotarod test, latency to fall from a rotating rod (acceleration increased by 0.5 cm/s every 5 s) was scored automatically with infrared sensors in a Rotamex 5 rotarod (Columbus Inst, Ohio) with four trials per day over 2 days.

Behavioral tests were primarily conducted on mice of 79(±2) days old, an age that in most previous assays showed no deficits, but were also performed on some at 66(± 2) and 97(± 2) days when had been typically reported as normal or displaying deficits, respectively [1,4,7,8,13,14,16,18,20,22,25,27,28]. All data are shown as mean ± S.E.M. and were statistically tested (JMP and Statview: SAS, Cary, NC, USA) using a two-way ANOVA (age × genotype), except in the rotarod where a two-way repeated measures ANOVA (age × genotype × trial no.) was used. *Post hoc* tests (Bonferroni–Dunnnett) were conducted when significant ($p < 0.05$) main effects and/or interactions were found in the primary ANOVA.

Hexb^{-/-} mice exhibited cognitive deficits compared to *hexb*^{+/+} mice as assessed in Trial 2 of the novel object recognition task (Fig. 1A). Two-way ANOVA (age × genotype) indicated a main effect of genotype ($F = 9.4, p < 0.005$). There was no main effect of age and no interaction between age and genotype. When assessed as the numbers of mice that have a preference, 100% of the control mice of both ages successfully prefer the novel object. In contrast, 50% of *hexb*^{-/-} at 66 days and the majority (67%) of the 79-day-old *hexb*^{-/-} mice perform at chance levels (Chi square, $p < 0.02$ for *hexb*^{-/-} mice of each age compared to age-matched controls). The absence of differences between ages for the *hexb*^{+/+} mice reflects the robustness of this response in normal animals and its suitability for longitudinal studies.

In contrast to differences in memory evident in Trial 2 of the novel object recognition test, initial levels of novel object exploration (Trial 1; Fig. 1B) were not statistically different between *hexb*^{-/-} and age-matched *hexb*^{+/+} mice, demonstrating that the motivation and ability to explore was intact in all mice at 66 and 79 days. Furthermore, all mice (within the age range tested) had normal pupil dilation and visual placing in the FOB (all control mice and all but one of the mutant mice reach for the grid within 2–6 cm) [12,24]. By 97 days, most *hexb*^{-/-} mice showed little object exploration which resulted in a sample size too small to analyze statistically.

Hexb^{-/-} mice showed deficits in motor coordination in the balance beam test. On the easier (i.e. thicker) balance beam, *hexb*^{-/-} mice had significantly more slips (main effect of genotype and age: $F_{\text{genotype}} = 10.5, p < 0.002$; $F_{\text{age}} = 4.3, p < 0.02$; Fig. 2A) which was evident only at 79 days (Bonferroni–Dunnett, $p < 0.001$). However using the harder (i.e. thinner) beam (Fig. 2B), the number of slips was significantly higher for *hexb*^{-/-} than *hexb*^{+/+} mice at all ages (main effect of genotype and age: $F_{\text{genotype}} = 20.8, p < 0.001$; $F_{\text{age}} = 4.2$; Fig. 2A). Thus, employing a more difficult task revealed motor coordination deficits at a very early stage of the disease (Bonferroni–Dunnett, $p < 0.05$ for each age when *hexb*^{-/-} was compared to age-matched *hexb*^{+/+}). The latency to cross—either beam was also significantly higher in mutants than in controls (main effect of genotype: $F_{\text{easy}} = 15.2, p < 0.001$; $F_{\text{hard}} = 15.3, p < 0.001$, main effect of age $F_{\text{hard}} = 3.66, p < 0.04$; Fig. 2C and D).

Assessment of the mice in the rotarod test (Fig. 3) also indicated deficits in motor coordination. The latency to fall on the first trial on day 1 (D1T1) primarily reflects motor coordination, while the combination of coordination and motor learning is evident by an increased latency to fall over subsequent trials [3]. *Hexb*^{-/-} mice had significantly shorter latencies to fall in the first trial compared to *hexb*^{+/+} mice as indicated by a significant main effect of genotype in a two-way ANOVA ($F_{\text{genotype}} = 7.8, p < 0.01$). This pattern of poorer performance in *hexb*^{-/-} mice was also evident across trials (repeated measures ANOVA—age × genotype × trial no.: $F_{\text{genotype}} = 7.7, p < 0.01$; $F_{\text{trial no.}} = 3.9, p < 0.001$).

Total locomotor activity assessed in the open field test was lower in *hexb*^{-/-} than *hexb*^{+/+} mice. Two-way ANOVA indicated a main effect of genotype ($F = 5.7, p < 0.03$) with no significant effect of age (Fig. 4A). *Hexb*^{-/-} mice also had fewer entries into the center of the open field compared to *hexb*^{+/+} mice ($F_{\text{genotype}} = 20.5, p < 0.001$; Fig. 4B) and a decreased proportion of ambulation in the center ($F_{\text{genotype}} = 12.9, p < 0.008$; $F_{\text{age}} = 3.0, p < 0.057$).

(Fig. 4C) indicative of increased “anxiety-like” behavior in the *hexb*^{-/-} mice. We have employed a behavioral test battery to assess a mouse model of Sandhoff disease that we backbred onto the C57BL/6J background.

We demonstrated deficits in motor activity, and coordination consistent with those previously reported [1,7,8,13,14,16,18,20,22,25,27,28] suggesting no significant effect of genetic background. However, we were also able to detect deficits as early as 66 days using the balance beam test. Importantly, we have now extended the range of behavioral assays to include tests of cognitive function which demonstrate memory deficits and increased “anxiety-like” behavior.

Although we cannot rule out peripheral factors that could affect motor performance [13,22,25], we suggest this is not primary cause of the poor coordination in the balance beam and rotarod that we demonstrate here. The mice did not have abnormal grip (*hexb*^{-/-} -8.6 ± 1.4 s and *hexb*^{+/+} 13.3 ± 2.5 s) and exhibited voluntary activity and exploration within normal ranges at the time points tested. Furthermore, the fact that all mice improve with successive trials in the rotarod and that the motor incoordination was worse in the harder balance beam task would tend to argue against solely peripheral causes, such as muscle wasting and hind limb rigidity. Finally, the deficits in motor coordination and/or learning are in accord with the neuronal loss and cellular pathology reported in the cerebellum, basal ganglia and striatum [16,18,22, 25,28].

The age of onset of motor deficits previously reported has widely varied, even when the same test was employed, such as the rotarod [18,20,22,25,27,28] or horizontal beam crossing test [1,4,13–15], in which deficits were first detected as late as 104 days [13]. Varied findings for the same test may be attributable to different genetic backgrounds (the original mice were not purebred and colony breeding schemes have differed between reports) and/or to different experimental parameters. Evidence for onset of motor incoordination as early as 60–70 days in LSDs, as we have detected using the balance beam test, is uncommon [15,27,28]. The use of this test, not previously employed on Sandhoff mice, should be particularly valuable to evaluation of efficacy in therapeutic strategies involving early intervention.

Most importantly, this is the first time an assay of short-term memory has been employed in Sandhoff mice. Despite the relevance to a CNS pathology, well-controlled assessments of cognitive deficits have not been conducted on this model save for brief mention of a passive-avoidance learning task in the initial description of the model. That test was performed only at 9 weeks of age and found no deficit [25]. Our assays demonstrate that cognitive deficits in the novel object recognition task are evident by 11–12-weeks in *hexb*^{-/-} mice. This type of cognitive deficit is consistent with the neurohistopathology reported in the mouse model [1,6,13,16,18,22], notably in the hippocampus [6]. Additional behavioral tasks would be useful in future studies to further evaluate the extent of cognitive dysfunction and affective outcomes present in this disease model. The relevance of thigmotaxis as a genuine symptom of anxiety and its relationship to psychiatric manifestations [11] in human patients merits further investigation.

We considered the possibility that visual system pathology may have influenced the performance in the test battery conducted here. Previous electrophysiological studies of visual function in Sandhoff mice have been reported in older mice (3.5–4 months). Electroretinograms were normal even at this late stage while visual evoked potentials were generally present [7]. These data, in combination with the normal levels of novel object exploration in Trial 1, normal pupil dilation and normal visual placing in the FOB suggest that deficits in visual function are not sufficient to explain the poor performance in the memory tests.

Inherited neurodegenerative LSDs are debilitating and frequently fatal. Much of the preclinical research is currently focused on molecular aspects and gross neurological symptoms. However, these human disorders can present with a complex behavioral phenotype including psychosis, mood disorders and cognitive impairment [10,11,21] and such aspects have rarely been addressed in murine models. In the present study with a Sandhoff disease model we found for the first time evidence of cognitive deficits in a memory task, and increased thigmotaxis in an open field test. We also found early onset of motor coordination deficits in the balance beam test. These assays should prove useful both in studies aimed at better delineating the pathophysiologic mechanisms and at evaluating efficacy of therapeutic strategies for the GM2 gangliosidosis.

Acknowledgments

This work was supported by a grant from the Lysosomal Storage Disease Research Consortium.

References

1. Andersson U, Smith D, Jeyakumar M, Butters TD, Cortina Borja M, Dwek RA, et al. Improved outcome of *N*-butyldeoxygalactonojirimycin-mediated substrate reduction therapy in a mouse model of Sandhoff disease. *Neurobiol Dis.* 2004; 16:506–15. [PubMed: 15262262]
2. Bourgoin C, Emiliani C, Kremer EJ, Gelot A, Tancini B, Gravel RA, et al. Widespread distribution of beta-hexosaminidase activity in the brain of a Sandhoff mouse model after coinjection of adenoviral vector and mannitol. *Gene Ther.* 2003; 10:1841–9. [PubMed: 12960974]
3. Buitrago MM, Schulz JB, Dichgans J, Luft AR. Short and long-term motor skill learning in an accelerated rotarod training paradigm. *Neurobiol Learn Mem.* 2004; 81:211–6. [PubMed: 15082022]
4. Cachon-Gonzalez MB, Wang SZ, Lynch A, Ziegler R, Cheng SH, Cox TM, et al. Effective gene therapy in an authentic model of Tay-Sachs-related diseases. *Proc Natl Acad Sci USA.* 2006; 103:10373–8. [PubMed: 16801539]
5. Choleris E, Thomas AW, Kavaliers M, Prato FS. A detailed ethological analysis of the mouse open field test: effects of diazepam, chlordiazepoxide and an extremely low frequency pulsed magnetic field. *Neurosci Biobehav Rev.* 2001; 25:235–60. [PubMed: 11378179]
6. Clark RE, Zola SM, Squire LR. Impaired recognition memory in rats after damage to the hippocampus. *J Neurosci.* 2000; 20:8853–60. [PubMed: 11102494]
7. Denny CA, Alroy J, Pawlyk BS, Sandberg MA, d'Azzo A, Seyfried TN. Neurochemical, morphological, and neurophysiological abnormalities in retina of Sandhoff and GM1 gangliosidosis mice. *J Neurochem.* 2007; 101:1294–302. [PubMed: 17442056]
8. Denny CA, Kasperzyk JL, Gorham KN, Bronson RT, Seyfried TN. Influence of caloric restriction on motor behavior, longevity, and brain lipid composition in Sandhoff disease mice. *J Neurosci Res.* 2006; 83:1028–38. [PubMed: 16521125]

9. Ennaceur A, Meliani K. A new one-trial test for neurobiological studies of memory in rats. III. Spatial vs. non-spatial working memory. *Behav Brain Res.* 1992; 51:83–92. [PubMed: 1482548]
10. Frey LC, Ringel SP, Filley CM. The natural history of cognitive dysfunction in late-onset GM2 gangliosidosis. *Arch Neurol.* 2005; 62:989–94. [PubMed: 15956171]
11. Gravel, RA.; Clarke, JTR.; Kaback, MM.; Mahuran, D.; Sandhoff, K.; Suzuki, K. The GM2 gangliosidosis. In: Scriver, CR.; Beaudet, AL.; Sly, WS.; Valle, D., editors. *The metabolic and molecular bases of inherited disease.* VII edition. McGraw-Hill; New York: 2001. p. 3827–76.
12. Irwin S. Comprehensive observational assessment. Ia. A systematic, quantitative procedure for assessing the behavioral and physiologic state of the mouse. *Psychopharmacologia.* 1968; 13:222–57. [PubMed: 5679627]
13. Jeyakumar M, Butters TD, Cortina Borja M, Hunnam V, Proia RL, Perry VH, et al. Delayed symptom onset and increased life expectancy in Sandhoff disease mice treated with *N*-butyldeoxynojirimycin. *Proc Natl Acad Sci USA.* 1999; 96:6388–93. [PubMed: 10339597]
14. Jeyakumar M, Norflus F, Tifft CJ, Corina-Borja M, Butters TD, Proia RL, et al. Enhanced survival in Sandhoff disease mice receiving a combination of substrate deprivation therapy and bone marrow transplantation. *Blood.* 2001; 97:327–9. [PubMed: 11133779]
15. Jeyakumar M, Smith DA, Williams IM, Cortina Borja M, Neville DCA, Butters TD, et al. NSAIDS increase survival in the Sandhoff disease mouse: synergy with *N*-butyldeoxynojirimycin. *Ann Neurol.* 2004; 56:642–9. [PubMed: 15505823]
16. Jeyakumar M, Thomas R, Elliot-Smith E, Smith DA, van der Spoel AC, d'Azzo A, et al. Central nervous system inflammation is a hallmark of pathogenesis in mouse models of GM1 and GM2 gangliosidosis. *Brain.* 2003; 126:974–87. [PubMed: 12615653]
17. Kurylo DD, Corkin S, Rizzo JF III, Growden JH. Greater relative impairment of object recognition than of visuospatial abilities in Alzheimer's disease. *Neuropsychology.* 1996; 10:74–81.
18. Kyrkanides S, Miller JH, Brouxhon SM, Olschowka JA, Federoff HJ. Beta-hexosaminidase lentiviral vectors: transfer into the CNS via systemic administration. *Brain Res Mol Brain Res.* 2005; 133:286–98. [PubMed: 15710246]
19. Lalonde R, Strazielle C. Motor coordination, exploration, and spatial learning in a natural mouse mutation (nervous) with Purkinje cell degeneration. *Behav Genet.* 2003; 33:59–66. [PubMed: 12645822]
20. Lee JP, Jeyakumar M, Gonzalez R, Takahashi H, Lee PJ, Baek RC, et al. Stem cells act through multiple mechanisms to benefit mice with neurodegenerative metabolic disease. *Nat Med.* 2007; 13:439–47. [PubMed: 17351625]
21. MacQueen GM, Rosebush PI, Mazurek MF. Neuropsychiatric aspects of the adult variant of Tay-Sachs disease. *J Neuropsychiatry Clin Neurosci.* 1998; 10:10–9. [PubMed: 9547461]
22. Norflus F, Tifft CJ, McDonald MP, Goldstein G, Crawley JN, Hoffmann A, et al. Bone marrow transplantation prolongs life span and ameliorates neurologic manifestations in Sandhoff disease mice. *J Clin Invest.* 1998; 101:1881–8. [PubMed: 9576752]
23. Phaneuf D, Wakamatsu N, Huang JQ, Borowski A, Peterson AC, Fortunato SR, et al. Dramatically different phenotypes in mouse models of human Tay-Sachs and Sandhoff diseases. *Hum Mol Genet.* 1996; 5:1–14. [PubMed: 8789434]
24. Rogers DC, Peters J, Martin JE, Ball S, Nicholson SJ, Witherden AS, et al. SHIRPA, a protocol for behavioral assessment: validation for longitudinal study of neurological dysfunction in mice. *Neurosci Lett.* 2001; 306:89–92. [PubMed: 11403965]
25. Sango K, Yamanaka S, Hoffmann A, Okuda Y, Grinberg A, Westphal H, et al. Mouse models of Tay-Sachs and Sandhoff diseases differ in neurologic phenotype and ganglioside metabolism. *Nat Genet.* 1995; 11:170–6. [PubMed: 7550345]
26. Stanley JL, Lincoln RJ, Brown TA, McDonald LM, Dawson GR, Reynolds DS. The mouse beam walking assay offers improved sensitivity over the mouse rotarod in determining motor coordination deficits induced by benzodiazepines. *J Psychopharmacol.* 2005; 19:221–7. [PubMed: 15888506]
27. Wu YP, Proia RL. Deletion of macrophage-inflammatory protein 1alpha retards neurodegeneration in Sandhoff disease mice. *Proc Natl Acad Sci USA.* 2004; 101:8425–30. [PubMed: 15155903]

28. Yamaguchi A, Katsuyama K, Nagahama K, Takai T, Aoki I, Yamanaka S. Possible role of autoantibodies in the pathophysiology of GM2 gangliosidosis. *J Clin Invest.* 2004; 113:200–8. [PubMed: 14722612]

Fig. 1.

Deficits in short-term memory (A) were evident in Sandhoff mutant mice (-/-) compared to WT (+/+) controls despite normal levels of object exploration (B) in the novel object recognition test. Numbers inside bars indicate sample size in this and the following graphs. (A) Chance performance is indicated by a preference score of 50% (gray line). *Hexb*^{-/-} had significantly lower preference scores than age-matched *hexb*^{+/+} mice ($p < 0.01$). (B) Initial object exploration in the training trial (exploration time in s) was normal in mutant mice.

Fig. 2.

Hexb^{-/-} have motor coordination deficits in the balance test beam compared to age-matched *hexb*^{+/+} mice. Motor coordination was assessed on easier (thicker; A and C) and harder (thinner; B and D) beams as number (#) of slips (A and B) and latency to cross (C and D; in s). Main effects of genotype were evident in all assays (see text). Asterisks indicate ages at which significant differences ($p < 0.05$) were found in *post hoc* tests between genotypes.

Fig. 3.

Hexb^{-/-} mice had motor coordination deficits in the rotarod. The latency to fall was determined over 2 days (D) of testing in four trials (T) per day. No significant differences were evident between 79- and 97-day-old *hexb*^{+/+} mice. However, *hexb*^{-/-} mice perform significantly worse than control mice in D1T1 ($p < 0.01$) and across trials ($p < 0.001$).

Fig. 4.

Hexb^{-/-} mice show hypoactivity and thigmotaxis. Reduced locomotor activity (A), and increased anxiety-like behavior as assessed by significantly fewer entries into the center zone of the open field (B) and by decreased proportion of exploration in the center zone (C), was evident in *hexb*^{-/-} compared to *hexb*^{+/+} mice.