Behavioural Brain Research 312 (2016) 265–271

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Behavioral deficits and cholinergic pathway abnormalities in male Sanfilippo B mice

Shih-hsin Kan[a], Steven Q. Le[a], Quang D. Bui[a], Braeden Benedict[a], Jesse Cushman[b], Mark S. Sands[c], Patricia I. Dickson[a,*]

[a] Department of Pediatrics, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA, USA
[b] Department of Psychology, Behavioral Testing Core, University of California, Los Angeles, Los Angeles, CA, USA
[c] Department of Medicine, Washington University School of Medicine, Saint Louis, MO, USA

HIGHLIGHTS

- We studied behavior and cholinergic pathways in male Sanfilippo B mice.
- We found reduced fearfulness and abnormal social interaction in Sanfilippo B mice.
- These occurred early in the disease, before hearing and vision loss are expected.
- We found reduced acetylcholinesterase activity in the brain of Sanfilippo B mice.
- The findings further link Sanfilippo B syndrome with adult-onset dementias.

ARTICLE INFO

Article history:
Received 8 March 2016
Received in revised form 12 May 2016
Accepted 13 June 2016
Available online 23 June 2016

Keywords:
Mucopolysaccharidosis
Lysosomal
Neurodegeneration
Acetylcholinesterase
Glycosaminoglycan
Inborn error of metabolism

ABSTRACT

Sanfilippo B syndrome is a progressive neurological disorder caused by inability to catabolize heparan sulfate glycosaminoglycans. We studied neurobehavior in male Sanfilippo B mice and heterozygous littermate controls from 16 to 20 weeks of age. Affected mice showed reduced anxiety, with a decrease in the number of stretch-attend postures during the elevated plus maze ($p = 0.001$) and an increased tendency to linger in the center of an open field ($p = 0.032$). Water maze testing showed impaired spatial learning, with reduced preference for the target quadrant ($p = 0.01$). In radial arm maze testing, affected mice failed to achieve above-chance performance in a win-shift working memory task (*t*-test relative to 50% chance: $p = 0.289$), relative to controls ($p = 0.037$). We found a 12.4% reduction in mean acetylcholinesterase activity ($p < 0.001$) and no difference in choline acetyltransferase activity or acetylcholine in whole brain of affected male animals compared to controls. Cholinergic pathways are affected in adult-onset dementias, including Alzheimer disease. Our results suggest that male Sanfilippo B mice display neurobehavioral deficits at a relatively early age, and that as in adult dementias, they may display deficits in cholinergic pathways.

1. Introduction

Sanfilippo B syndrome is a devastating neurological disorder of childhood [1,2]. The biochemical defect is deficiency of the lysosomal enzyme alpha-*N*-acetylglucosaminidase (Naglu), leading to accumulation of heparan sulfate throughout the body and brain [3]. Affected children experience progressive neurological deterioration and early death. Work by others showed that behavioral abnormalities in Sanfilippo children with the phenotypically-similar type A include a Klüver-Bucy like syndrome with reduced fearfulness [4,5]. This reduced fear response was found to correlate with volume loss in the amygdala [4]. The mouse model of Sanfilippo B was similarly found to demonstrate reduced fear, evidenced by increased time spent in the center of an open field [6].

While there are no available, effective treatments for Sanfilippo B, several promising therapeutic options have been explored in animal models [7–10]. Better characterization of the murine phenotype is needed in order to evaluate the effectiveness of potential therapies for neurological disease due to Sanfilippo B prior to trans-

Abbreviations: AChE, acetylcholinesterase; ChAT, choline acetyltransferase; Naglu, alpha-*N*-acetylglucosaminidase.

* Corresponding author at: 1124W. Carson St, HH1, Torrance, CA 90502-2006, USA.
E-mail address: pdickson@labiomed.org (P.I. Dickson).

http://dx.doi.org/10.1016/j.bbr.2016.06.023
0166-4328/

lation to patients. The ideal phenotypic read-outs for preclinical therapeutic development would be detectable relatively early in life, in order to avoid age-associated physical disease that can confound neurobehavioral testing. These read-outs would also ideally not be overly dependent upon hearing and vision, both of which are abnormal in Sanfilippo B mice [11].

2. Materials & methods

2.1. Animals

Mutant male Sanfilippo B mice (B6.129S6-Naglu$^{tm1Efn/J}$ backbred onto C57BL6/J; [6]; *Naglu*$^{-/-}$) were mated with heterozygous females (*Naglu*$^{+/-}$) to obtain homozygous affected mice and heterozygous controls. Genotype was performed with primers: NAG 5′: TGGACCTGTTTGCTGAAAGC; NAG 3′: CAGGCCATCAAATCTGGTAC; Neo 5′: TGGGATCGGCCATTGAACAA; Neo 3′: CCTTGAGCCTGGCGAACAGT. The design was cross-sectional. Male Sanfilippo B mice and controls were housed in groups of 4 mice/cage. Behavioral core personnel were blinded as to genotype of each cage. Data were entered on a coded spreadsheet, so that core laboratory staff remained blinded as to the group assignment of individual mice but could see group means and trends. The experiment began with $n = 14$ Sanfilippo B and $n = 12$ control mice. During neurobehavioral testing, two Sanfilippo B mice were excluded due to trauma from fighting, and one control mouse was excluded due to malocclusion and poor weight gain. For the radial arm maze, any mice that did not eat all of the pellets in phase 1 did not move on to phase 2 and were excluded from further analysis. The exact numbers of animals used in each experiment are indicated in the figures. Mice received *ad libitum* food and water (except when fasted for radial arm maze testing) and a 12-h light/dark cycle. All study procedures were reviewed and approved by the Institutional Animal Care and Use Committees at the Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center and UCLA.

2.2. Neurobehavior

Mice were transferred to the UCLA Behavioral Testing Core at age of 16 weeks. After an acclimatization period of approximately 1 week, mice underwent behavioral testing from age 17–20 weeks in the following order: SHIRPA primary screen, elevated plus maze, rotarod, socialization approach test, open field test, novel object recognition test, Morris water maze (visible, then hidden), radial arm maze. Behavior was tracked *via* an overhead camera using Topscan automated behavioral analysis software (Clever Sys Inc., Reston, VA).

2.2.1. SHIRPA primary screen

A SHIRPA primary screen was performed to assess general health, gross hearing, vision and coordination, modified from [12]. The following behaviors were assessed over a period of approximately ten minutes for each mouse: body position, spontaneous activity, respiration rate, tremor, urination, defecation transfer arousal, locomotor activity, palpebral closure, piloerection, startle response, gait, pelvic elevation, tail elevation, touch escape, positional passivity, trunk curl, limb grasping, visual placing, whisker brush, whisker placement, grip strength, body tone, pinna reflex, corneal reflex, toe pinch, wire maneuver, body length, skin color, limb tone, abdominal tone, lacrimation, salivation, provoked biting, penlight vision, righting reflex, contact righting reflex, negative geotaxis, fear (present/absent), irritability, aggression, and vocalization.

2.2.2. Elevated plus maze

Mice were placed in a standard elevated plus maze apparatus for 5 min. The percent time in the open and closed arms and the number of stretch attend postures were tracked with automated behavioral tracking software.

2.2.3. Rotarod

To test balance (vestibular and cerebellar function), mice were studied on a Rotamex-5 rotarod (Columbus Instruments International, Columbus, OH). Rod speed was accelerated from an initial speed of 5 rpm to a maximum of 60 rpm over period of 300 s. The latency to fall and the final rpm were recorded.

2.2.4. Socialization approach test

We assessed the interaction of Sanfilippo B mice with same-sex, non-littermate, non-cagemate mice. This task utilized a three-chamber apparatus and procedures as described [13]. Briefly, mice were habituated to the three chamber apparatus for 10 min. Wire cups were then placed in the side chamber with a novel interaction mouse placed in one cup and no mouse placed in the other cup. Time spent in the two side chambers over a 10 min test period was then tracked to determine the relative amount of time spent interacting with the interaction mouse vs. the empty cup. A preference ratio was calculated as: (time in mouse side − time in empty cup side)/(time in mouse side + time in empty cup side)*100.

2.2.5. Open field test

Activity in an open field was assessed over 10 min in a square plexiglass enclosure (27.5 cm × 27.5 cm) during light and dark as previously described [14]. Lights in the experimental room were on during the first 5 min (25 lx) and off during the second 5 min. Overall distance moved and time spent in the center was analyzed by automated behavioral analysis software.

2.2.6. Novel object recognition test

Mice also underwent a novel object recognition task utilizing the same open field apparatus described above. Dim lighting conditions (5 lx) were used across the three days of this test. Mice were habituated to the open field for 10 min on Day 1. On Day 2 two identical objects were placed in the open field in opposite corners 6 cm equidistant from the nearest two walls. Distance moved and object interaction was tracked over 10 min. The novel and familiar objects were 50 mL Erlenmeyer flasks that were either empty or contained green tissue paper (for half of the animals the filled flask was familiar and the empty flask was novel, for the other half of the animals this was reversed). On Day 3 the familiar object was switched with a novel object (counterbalanced across animals for which object was novel vs. familiar and which side of the arena the novel object was placed in). Distance moved and object interaction was tracked over 10 min.

2.2.7. Morris water maze

Mice were trained (4 training trials per day) in a custom water maze pool (122 cm diameter) at 24 °C with an overhead camera. An escape platform in the center of one quadrant (target quadrant) was submerged under 1 cm of water that was made opaque using non-toxic tempura paint. The two trials within each block were separated by five minutes and the two blocks were separated by 2 h. The mice were placed in the pool at one of three start locations at the center of each non-target quadrant, with the order of placement randomized each day. The latency to find the platform was tracked each day. Approximately 2 h after the end of training on the 5th day the platform was removed and the mice were given a 60 s probe trial where percent quadrant occupancy was determined. Mice were then tested in the visible version where the escape plat-