Physiology & Behavior 139 (2015) 397–402

Contents lists available at ScienceDirect

Physiology & Behavior

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

Impaired gait pattern as a sensitive tool to assess hypoxic brain damage in a novel mouse model of atherosclerotic plaque rupture

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HIGHLIGHTS

- Spatial learning and motor coordination of ApoE$^{-/-}$ Fbn1$^{C1039G+/-}$ mice were studied.
- An increase in track width (gait analysis) revealed a disturbed balance.
- Pyknotic neurons in the parietal cortex confirmed hypoxic brain damage.
- Percentage of pyknosis and track width were correlated.
- Gait analysis is a sensitive tool to assess cerebral hypoxia in this model.

ARTICLE INFO

Article history:
Received 17 April 2014
Received in revised form 13 November 2014
Accepted 14 November 2014
Available online 20 November 2014

Keywords:
Atherosclerosis
ApoE$^{-/-}$ Fbn1$^{C1039G+/-}$ mouse model
Motor coordination
Gait
Pyknosis
Hypoxia

ABSTRACT

Apolipoprotein E deficient (ApoE$^{-/-}$) mice with a heterozygous mutation in the fibrillin-1 gene (Fbn1$^{C1039G+/-}$) show spontaneous atherosclerotic plaque ruptures, disturbances in cerebral flow and sudden death when fed a Western-type diet (WD). The present study focused on motor coordination and spatial learning of ApoE$^{-/-}$ Fbn1$^{C1039G+/-}$ mice on WD for 20 weeks ($n = 21$). ApoE$^{-/-}$ mice on WD ($n = 24$) and ApoE$^{-/-}$ Fbn1$^{C1039G+/-}$ mice on normal diet (ND, $n = 21$) served as controls. Starting from 10 weeks of diet, coordination was assessed every two weeks by the following tests: gait analysis, stationary beam, wire suspension and accelerating rotarod. The Morris water maze test was performed after 13 weeks of diet to study spatial learning. At the end of the experiment (20 weeks of WD), the mice were sacrificed and the brachiocephalic artery and brain were isolated. From 12 weeks onward, gait analysis of ApoE$^{-/-}$ Fbn1$^{C1039G+/-}$ mice on WD revealed a progressive increase in track width as compared to ApoE$^{-/-}$ mice on WD and ApoE$^{-/-}$ Fbn1$^{C1039G+/-}$ mice on ND (at 20 weeks: 29.8 ± 0.6 mm vs. 25.8 ± 0.4 mm and 26.0 ± 0.5 mm). Moreover, the stationary beam test showed a decrease in motor coordination of ApoE$^{-/-}$ Fbn1$^{C1039G+/-}$ mice on WD at 18 and 20 weeks. The wire suspension test and accelerating rotarod could not detect signs of motor impairment. Spatial learning was also not affected. Histological analysis of the brachiocephalic artery showed larger and more stenotic plaques in ApoE$^{-/-}$ Fbn1$^{C1039G+/-}$ mice on WD. Furthermore, the parietal cortex of ApoE$^{-/-}$ Fbn1$^{C1039G+/-}$ mice on WD showed pyknotic nuclei as a sign of hypoxia and the percentage of pyknosis correlated with track width. In conclusion, gait analysis may be an efficient method for analyzing hypoxic brain damage in the ApoE$^{-/-}$ Fbn1$^{C1039G+/-}$ mouse model. This test could be of value to assess the effect of potential anti-atherosclerotic therapies in mice.

1. Introduction

Atherosclerosis is a progressive inflammatory disease of the large and medium-sized arteries and is characterized by the formation of plaques in the vessel wall. Because rupture of atherosclerotic plaques remains the main cause of acute cardiovascular syndromes and death, the need for plaque-stabilizing therapies is high [1]. We recently reported that Apolipoprotein E deficient (ApoE$^{-/-}$) mice with a heterozygous mutation in the fibrillin-1 gene (Fbn1$^{C1039G+/-}$) develop increased arterial stiffness due to fragmentation of the elastin fibers [2]. On a Western-type diet (WD), this mutation in ApoE$^{-/-}$ mice causes

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http://dx.doi.org/10.1016/j.physbeh.2014.11.047
0031-9384/

accelerated atherosclerosis, spontaneous plaque rupture, disturbed cerebral flow and sudden death [3]. This model allows evaluating potential plaque-stabilizing therapies on important end points of plaque rupture, such as myocardial infarction and brain hypoxia. In the present study, we focused on spatial learning and motor coordination of ApoE$^{-/-}$ Fbn1$^{C1039G+/-}$ mice (1) to characterize the neurological aspects of this mouse model and (2) to establish a method for the early detection of brain damage. This approach might contribute to the evaluation of potential anti-atherosclerotic drugs in the ApoE$^{-/-}$ Fbn1$^{C1039G+/-}$ mouse model.

2. Material and methods

2.1. Mice

Female ApoE$^{-/-}$ ($n = 24$, control group) and ApoE$^{-/-}$ Fbn1$^{C1039G+/-}$ mice ($n = 21$) were fed a WD (TD88137, Harlan Teklad) starting at an age of 6 weeks. Female ApoE$^{-/-}$ Fbn1$^{C1039G+/-}$ mice on a normal diet (ND, $n = 21$) were also included to control for the phenotype (e.g. scoliosis). At the end of the experiment (20 weeks of ND or WD) the mice were sacrificed. All experiments were approved by the ethics committee of the University of Antwerp. One ApoE$^{-/-}$ mouse was excluded from the study after 19 weeks of WD due to illness not related to the experiment.

2.2. 'Fast' Morris water maze test

Memory and spatial learning of all mice was analyzed after 13 weeks of diet by a 'fast' Morris water maze (MWM) test. Compared with the classic protocol [4,5], we performed a 'fast' version consisting of two acquisition trial blocks daily with an interval of 4 h on four consecutive days. Animals' trajectories were recorded using a computerized video-tracking system (Ethovision, Noldus, The Netherlands) logging path length, escape latency, and swim speed. Four days after the final acquisition trial block, a probe trial was performed. The platform was removed from the maze, and each mouse was allowed to swim freely for 100 s. Spatial accuracy was expressed as percentage of time spent in each quadrant of the MWM and the number of crossings through the target position, i.e., the specific location of the platform during the acquisition phase.

2.3. Motor coordination assessment

Motor coordination of the mice was analyzed at 10, 12, 14, 16, 18 and 20 weeks of diet.

2.3.1. Wire suspension test

To test grip strength and endurance, the front paws were positioned on a horizontal steel wire (0.6 mm thick) suspended at a height of 46 cm above tabletop. Test parameters were latency to the first fall and the number of falls during a 2-min assessment period.

2.3.2. Accelerating rotarod test

Motor abilities were evaluated on an accelerating rotarod apparatus (Bioseb, France). After two adaptation trials lasting up to 2 min each at a constant speed (4 rpm), the mice were placed on the rotating rod for four test trials during which the rotation speed was gradually increased from 4 to 40 rpm. The time that an animal was able to stay on the rod was measured up to a maximum of 5 min. An intertrial interval of 1 min was respected between each training and/or test trial.

2.3.3. Stationary beam test

The setting consisted of a wooden beam (diameter: 25 mm, length 110 cm) covered with a layer of masking tape to provide a firmer grip. The beam was divided into 11 segments and placed at a height of 38 cm above a cushioned floor. A piece of cardboard was inserted at each end to prevent escaping of the mice. Testing commenced by placing an animal in the middle of the beam. The number of segments crossed (four-paw criterion), the latencies before falling, and the number of falls were measured in four trials with a cut-off period of 1 min per trial and an intertrial interval of 10 min.

2.3.4. Gait analysis

Gait characteristics (stride, track width and toe span) were analyzed by applying ink to the animals' hind paws and letting them walk on a

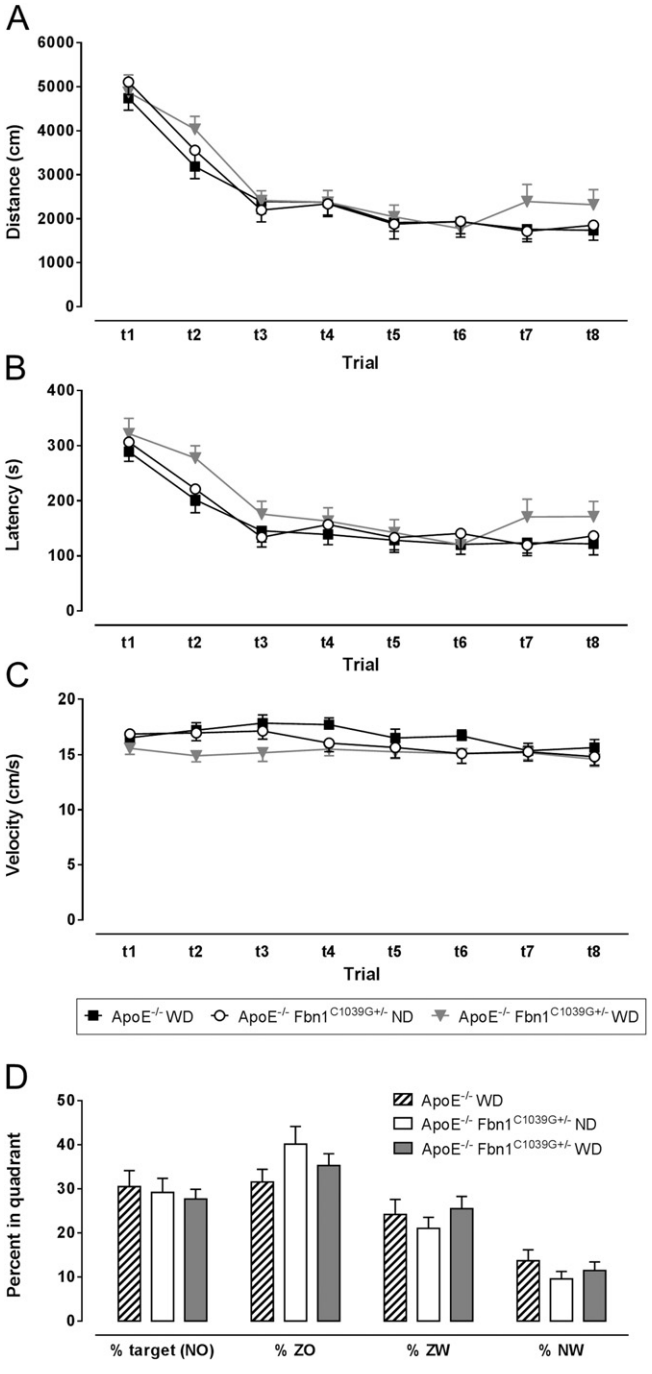

Fig. 1. Morris water maze test. A. The distance to reach the platform was not different between ApoE$^{-/-}$ Fbn1$^{C1039G+/-}$ mice on WD and the control groups. The gradual decrease over time proved that learning capacities were intact. B. Latency showed the same pattern as distance, with no differences between groups. C. Swim speed remained stable throughout the experiment. D. The probe trial showed no differences between ApoE$^{-/-}$ Fbn1$^{C1039G+/-}$ mice on WD, ApoE$^{-/-}$ Fbn1$^{C1039G+/-}$ mice on ND and ApoE$^{-/-}$ mice on WD for the percentage of time spent in the target and the three other quadrants of the pool. WD = Western-type diet, ND = normal diet. (t = trial block).