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Research report

Motor function deficits in the 12 month-old female 5xFAD mouse model of Alzheimer's disease

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ABSTRACT

Motor problems occur early in some patients with Alzheimer's disease (AD) and as the disease progresses many patients develop motor dysfunction. Motor dysfunction has been reported in some mouse models of AD, including the 5xFAD mouse, thus this model may be particularly useful for studying motor dysfunction in AD. In order to determine the extent of motor dysfunction in these mice, we tested 11–13 month old female 5xFAD and wildtype (WT) control mice in a battery of motor behaviour tasks. The 5xFAD mice showed hind limb clasping, weighed less and had slower righting reflexes than WT mice. In the open field, the 5xFAD mice travelled a shorter distance than the WT mice, spent less time moving and had a slower movement speed. The 5xFAD mice fell faster than the WT mice from the balance beam, wire suspension, grid suspension and rotarod tasks, indicating dysfunctions in balance, grip strength, motor co-ordination and motor learning. The 5xFAD mice had a short, shuffling gait with a shorter stride length than WT mice and had a slower swim speed. The 5xFAD mice also failed to show an acoustic startle response, likely due to motor dysfunction and previously reported hearing impairment. The 5xFAD mice did not show deficits in the ability of peripheral motor nerves to drive muscle output, suggesting that motor impairments are not due to dysfunction in peripheral motor nerves. These results indicate that the aged 5xFAD mice are deficient in numerous motor behaviours, and suggest that these mice may prove to be a good model for studying the mechanisms of motor dysfunction in AD, and motor behaviour might prove useful for assessing the efficacy of AD therapeutics. Motor dysfunction in 5xFAD mice must also be considered in behavioural tests of sensory and cognitive function so that performance is not confounded by impaired locomotor or swimming behaviour.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by age-related development of Aβ-plaques, neurofibrillary tangles and cognitive dysfunction [1–4]. Although relatively less studied, sensory-motor dysfunctions frequently develop in AD [5], and motor behaviour deficits (e.g. abnormal gait) often occur in the early stages of AD [6,7]. Aberrant motor behaviours occur in 25–38% of AD patients [8] and interfere with the performance of activities of daily living [6]. Gait disturbances are of a particular concern as reduced mobility decreases quality of life and increases in falls are related to increased morbidity and mortality [9]. Sensory-motor dysfunction in AD has often been neglected in the treatment of AD, but reduction of sensory-motor deficits may enhance patient function as AD progresses [5]. Patients with mild cognitive impairment (MCI) plus motor dysfunction are more likely to develop AD than MCI patients without motor impairment [10], and motor dysfunction in AD is correlated with severity of cognitive dysfunction [11]. Once the ability to walk is lost in the later stages of AD, institutionalization is often required [12,13].

Transgenic mouse models of AD successfully recapitulate many of the neuropathological hallmarks and cognitive dysfunction in AD, and are used in the pre-clinical assessment of therapeutic interventions [14–17]. Some of these mouse models of AD develop motor as well as cognitive deficits [18–20]. However, very little is known about the extent of motor dysfunction in these AD model mice, nor has the usefulness of motor phenotypes been considered in the assessment of therapeutic efficacy. What research has been completed shows conflicting results, with some mouse models of AD having better motor function than wildtype (WT) controls (3xTg; [21]), some showing no difference in motor function (APPswe/PS1dE9; [22]), and others showing impaired motor ability (Tg2576; [23]). The double transgenic 5xFAD mouse model of AD carries the mutant amyloid precursor

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protein (APP) and presenilin-1 (PS1) transgenes, each expressed via the thymus antigen-1 (Thy-1) promoter [24]. These mice are particularly suited for the study of motor dysfunction in AD, given that they rapidly develop AD-related pathology and cognitive dysfunction and also show motor dysfunction beginning at nine months of age and progress to a profound dysfunction across multiple motor domains by 12 months of age [25]. These motor impairments coincide with the development of AD-related pathology within the motor regions of the CNS including (1) extra-cellular plaques in the spinal cord, (2) the development of swellings in the axonal processes of motor neurons which contain β-amyloid and may impede axonal transport, and (3) loss of neurons within layer 5 of the cerebral cortex, presumably including the motor cortex [25].

Since the initial characterization of motor dysfunction in 5xFAD mice, motor impairment has been used in only a few pre-clinical studies of therapeutics [26–29]. The infrequent assessment of motor phenotype may be due to an inability to replicate impairments across studies and/or across different aspects of motor function. There are a number of different tests for assessing motor function in mice [30,31] and in this study, we used a battery of such tests to provide an in-depth characterization of the motor function of 5xFAD mice at 11–13 months of age. This provides a method for characterizing a number of different parameters of motor function in order to determine the specific nature of the motor disorders shown by the 5xFAD mice. Because motor dysfunction can confound performance on tests of cognitive function, we also tested the swimming ability of 5xFAD mice in the Morris water maze. Measurements of the hind-limb clasping response, and behaviour on the balance beam, wire-suspension test and open field were done to replicate the results of Jawhar et al. [25]. The acoustic startle response was reported by O'Leary et al. [32]. We have added behavioural tests of motor coordination and motor learning on the rotarod, grip strength on the grid suspension test, footprint gait analysis, and swim speed in the Morris water maze to complete our motor behaviour test battery. Lastly, while previous examination of the mechanism for motor dysfunction has focused on the development of AD-related pathology within the pyramidal and extra-pyramidal motor systems of the brain and spinal cord of the 5xFAD mice, there has been no examination of the ability of the motor nerves in the peripheral nervous system to drive motor output. Therefore, we also examined the functional ability of the motor nerves in 5xFAD mice for generating output via neuro-muscular junctions, using ex-vivo isometric tension recordings from soleus muscle [33].

2. Methods

2.1. Subjects

This study used 13 wildtype and 13 5xFAD female mice between 11 and 13 months of age. Mice were the F2 progeny of male hemizygous C57BL/6J x SJL/J FN 5xFAD (B6SJL-Tg (APPSwFILon, PSEN1*M146L*L286V) 6799Vas/Mmjax) and female wild-type C57BL/6J x SJL/J F1 mice obtained from Jackson Laboratories (Bar Harbour, Maine) and bred in our laboratory. Mice were housed in same sex groups of 2–4 in polyethylene cages with wood-chip bedding, a small polyethylene tube for enrichment, and cages were covered by micro-isolator filters. Food (Purina rodent chow #5001) and tap water were available *ad-libitum*. Some 5xFAD mice showed difficulty rearing to reach the food hoppers, and therefore food pellets and dishes that contained powered food mixed with water were placed on the floor of the cages. Female mice were used because of the high aggression levels and mortality of 5xFAD males as they age, which reduced the number of transgenic males available for testing [34]. Mice were housed in a colony room under a 12:12 reversed light-dark cycle, with lights off from 9:30 am to 9:30 pm. Testing was completed during the dark phase of the light cycle and the Dalhousie University Committee on Animal Care approved all experimental procedures. Mice were genotyped for the APP and PS1 transgenes by Dr. Chris Sinal (Pharmacology Department, Dalhousie University) from tissue samples obtained from ear punches. Mice were tested in a battery of motor behaviour tests in the order described below. A different group of 12-month-old female mice (N = 10 per genotype) bred and housed under the same conditions, was used to analyze swimming ability in the Morris water maze.

2.2. Body-weight and basic reflexes

On the first day of the test battery, body-weight was recorded and a brief assessment of motor reflexes completed using a sub-set of measures from the SHIRPA test battery for neurobehavioural screening of mutant mice [35,36]. When suspended by the tail mice will normally splay the hind limbs outward (Fig. 1), whereas aged 5xFAD mice retract the hind limbs against the body, where they are then clasped together [25]. To determine if the limb-clasping response was present, mice were suspended by the tail for 20 s, and the total time that the hind-limbs were clasped was recorded. Cranial reflexes were then tested (scored as either present or absent) by lightly brushing the ear with a cotton swab to observe if pinna deflection occurred. The cotton swab was also brought close to the eye to observe if the corneal blinking reflex occurred. Finally the righting reflex was measured by placing mice in a transparent plastic tube (4 cm diameter, 11 cm length), which was rotated vertically 180°. The mean time taken for the mouse to return to an upright position over two consecutive trials was recorded

2.3. Locomotion and anxiety in the open field

Mice were tested in the open field (OF) to assess locomotor activity and anxiety-related behaviours [37]. The OF consisted of an open box (72 × 72 cm) made of plywood, with the floor and walls (36 cm height) painted white. A 4 × 4 grid and a central square (18 × 18 cm) were drawn on the floor to demark the center of the maze from the periphery, and the floor was covered with transparent Plexiglas. One of the walls was replaced with transparent Plexiglas so that mice were visible while in the apparatus. The test room (1.8 × 4.6 m) was lit by a 60 W light placed above the OF. The Ethovision (Noldus) video-tracking system was used to record the activity of the mice, and an additional camera was positioned beside the maze to record species typical behaviours. For each trial, a single mouse was placed into one of the corners of the OF using a plastic container (450 ml) and allowed to explore for 10-min. Locomotor activity was measured by distance travelled, percentage time spent mobile (velocity > 4 cm/sec), the average velocity while mobile and the number of rears. To measure anxiety-like behaviour, entries into the center of the OF, the number of defecations, and time spent grooming were recorded. The floor was cleaned with 70% ethanol and dried after each trial.

2.4. Motor coordination and motor learning on the rotarod

Mice were tested on the AccuRotor rotarod (Accuscan Instruments Inc. Columbus Ohio) to assess motor coordination and motor learning [21]. The rotarod consisted of an acrylic rod (3 cm diameter), separated into four 11 cm sections by circular Plexiglas dividers (15 cm high), so that up to four mice could be tested simultaneously. For each trial, mice were placed onto the rod that accelerated from 0 to 48 rotations per minute over 360 s. Mice completed 3 days of training with 6 trials per day. The latency to fall was recorded on each trial by sensors in the holding chambers located 39 cm beneath the rod. After each trial the mice remained in the holding chambers for a minimum of one minute before the next trial. The rotarod was cleaned with soap and water after groups of 3–4 mice completed a daily test session. The rotarod was located in a 1.2 × 2.60 m room, lit by a single 60 W red light.

2.5. Balance beam

Mice were tested on the balance beam to assess motor coordination