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POSTURAL DYSFUNCTION IN A TRANSGENIC MOUSE MODEL OF SPINOCEREBELLAR ATAXIA TYPE 3

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Abstract—During voluntary limb movements, humans exert anticipatory postural adjustments (APAs) to prevent any upcoming equilibrium disturbance that might be provoked by limb movements. Dysfunction in generation or control of APAs is associated with postural deficits in some human patients with cerebellar damage. To examine the role of the cerebellum in APAs, we investigated a conditional transgenic mouse of spinocerebellar ataxia type 3 (SCA3Tg) that has defective cerebellar Purkinje cells. Kinematic analyses and monitoring of electromyographic activities during quadrupedal standing showed that SCA3Tg mice exhibited greater hindlimb instability than wild-type (WT) mice. This instability increased during a reaching task that required postural adjustments associated with voluntary neck movements. Normally, the activities of the hindlimb muscles are synchronized with those in the neck that are the agonists for movement of the head in this reaching task; however, in SCA3Tg mice, activities in the hindlimbs were markedly delayed compared to the neck. These observations cannot simply be explained as a secondary outcome of the muscle atrophy that occurs in SCA3Tg mice. In WT mice with muscle atrophy induced by immobilization of the hindlimbs, we did not find impairment of APAs. These findings suggest that the deficits in APAs during the reaching task in SCA3Tg mice were not due to muscle atrophy in the hindlimbs, but were mainly caused by cerebellar degeneration. Therefore, we conclude that the cerebellum is critically involved in APAs.

Key words: spinocerebellar ataxia type 3, anticipatory postural adjustments, cerebellum, reaching movement, Purkinje cell.

INTRODUCTION

The cerebellar vermis plays a crucial role in adaptive movements in posture and during locomotion (Ito, 1984; Yanagihara and Kondo, 1996; Morton and Bastian, 2004). This role is exemplified by the severe postural abnormalities adopted by patients with cerebellar damage when attempting to stand upright (Diener and Dichgans, 1992; van de Warrenburg et al., 2005; Ilg et al., 2009) and by their abnormal responses to perturbation of their stance (Nashner, 1976; Horak and Diener, 1994; Bakker et al., 2006). Cerebellar disorders are divided into those that occur sporadically and those with an inherited basis (Manto and Marmolino, 2009a; Seidel et al., 2012). As a result of advances in identification of the causal genetic changes in cerebellar hereditary ataxias (Schöls et al., 2004; Seidel et al., 2012), it has been possible to genetically engineer mouse models of these diseases (Torashima et al., 2008; Yamada et al., 2008; Manto and Marmolino, 2009b; Colomer Gould, 2012). These models have been used to investigate the mechanisms of neuronal degeneration and to establish therapeutic strategies. Although mice with cerebellar degeneration exhibit severe movement disorders, conventional tests, such as footprint and rotarod analyses, have been used to assess their ataxia. To date, however, very little data are available from detailed kinematic analyses or from monitoring electromyographic activities in hindlimbs during standing in model systems.

Goal-directed limb movements are accompanied by postural adjustments during standing; these latter movements are termed anticipatory postural adjustments (APAs). Analysis of the APAs that accompany voluntary arm movements in humans has shown that activities of the leg muscles required for postural adjustment precede those of the arm muscles used for the intended primary movements (Bouisset and Zattara, 1981; Gahéry and Massion, 1981; Cordo and Nashner, 1982). During voluntary limb movements, the central nervous system has to predict any forthcoming internal perturbation that might result; thus, APAs are crucial to minimizing potential equilibrium disturbance. A feedforward postural signal may be important for the generation of APAs (Gahéry and Massion, 1981; Massion, 1992). In addition,

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Abbreviations: 1C2, expanded polyglutamine; ANOVA, analysis of variance; APAs, anticipatory postural adjustments; BF, biceps femoris; EMG, electromyographic; GA, gastrocnemius; HA, hemagglutinin; P, postnatal day; RMS, root mean square; SCA3, spinocerebellar ataxia type 3; SCA6, spinocerebellar ataxia type 6; SCA3Tg, transgenic mouse of spinocerebellar ataxia type 3; Sol, soleus; TA, tibialis anterior; VL, vastus lateralis; WT, wild-type.

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projections from the cortical motor areas to the cerebellar vermis have been hypothesized to be involved in the neural mechanisms for APAs (Diedrichsen et al., 2005; Coffman et al., 2011; Galgiani et al., 2011). Although the various studies listed here have provided some insights into the role of the cerebellum in APAs associated with voluntary movements, much of the details of this process remain to be elucidated.

In this study, we used quantitative kinematic and electromyographic analyses during static and dynamic postural tasks to investigate the postural deficits associated with voluntary movements in a conditional transgenic mouse with spinocerebellar ataxia type 3 (SCA3), which has defective cerebellar Purkinje cells induced by the use of the Purkinje cell-specific L7 promoter (transgenic mouse of spinocerebellar ataxia type 3 (SCA3Tg) mouse). Hence, the SCA3Tg mice will be a valuable resource to directly test the influences of cerebellar disorders on postural control. Postural deficits in other SCA3Tg mice have been determined through negative geotaxis tests (Cemal et al., 2002) and righting reflex tests (Goti et al., 2004). However, to date, APAs in mice with cerebellar neurodegenerative disorders have not been investigated in detail. Previous studies have reported that patients with SCA3 frequently display characteristic muscle atrophy (Watanabe et al., 1996; Maruyama et al., 1997; Schmitz-Hübsch et al., 2008). A similar observation has been made in rats in which muscle atrophy of the forelimbs or hindlimbs adversely affects postural stability and locomotion (Canu et al., 2001). Since the SCA3Tg mice used in the present study are likely to show atrophy of the limb muscles, we decided to compare the effects of hindlimb muscle atrophy in these mice with those in a specially prepared group of wild-type (WT) mice. Here, we demonstrate that postural deficits in SCA3Tg mice can be ascribed to cerebellar dysfunction rather than muscle atrophy and that the cerebellum plays an important role in APAs associated with voluntary movements.

EXPERIMENTAL PROCEDURES

The SCA3Tg C57BL/6J mice used here express an NH_2-terminal-truncated Ataxin-3 with the Q69 mutation and an NH_2-terminal hemagglutinin (HA) epitope (Yoshizawa et al., 2000). Construction of the transgenic lines in which the transgene is driven specifically in cerebellar Purkinje cells by the Purkinje cell-specific L7 promoter has been described previously (Hirai et al., 2005). Since the L7 promoter becomes active after birth, the cerebellum of a SCA3Tg mouse is not affected at birth. However, dendritic differentiation of Purkinje cells becomes impaired in parallel with the nuclear accumulation of mutant Ataxin-3 after birth. Experiments were conducted using 21 SCA3Tg mice and 24 WT littermates. Approval for the experimental protocol was obtained from the Ethics Committee for Animal Experiments at the University of Tokyo, and the study was carried out in accordance with the Guidelines for Research with Experimental Animals of the University of Tokyo and the Guide for the Care and Use of Laboratory Animals (NIH Guide) revised in 1996.

To examine posture, the mice were placed in a custom-made acrylic box (130 mm wide × 60 mm deep × 150 mm high). The placement of all four paws was monitored by a mirror located beneath the box; the mirror was set at about 45° from the vertical. Mice were habituated to the box before recording. We analyzed three postural tasks: (1) standing on all four paws on a surface for 5 s (‘‘the stance task’’); (2) standing on all four paws while drinking water for 5 s (‘‘the drinking stance task’’); and (3) dorsiflexion of the neck while standing unrestrained in a quadrupedal manner and raising the mouth to the tube of a water flask. The tube was placed 30 mm above the surface and had a diameter of 7 mm. We called this third task ‘‘the reaching task.’’

Mice were briefly anesthetized with isoflurane (2%), and circular reflective markers (2 mm in diameter) were placed on the shaved skin of the right hindlimb at the iliac crest, the greater trochanter, the knee joint, the malleolus lateralis, the fifth metatarsophalangeal joint, and the toe. Tasks were captured at 100 frames/s using a high-speed digital image camera system (HAS-220, DITECT, Inc.), and images were stored for later analysis. Motion analysis was limited to the sagittal plane. Marker displacements, angular excursions (hip angle, knee angle, ankle angle) and mouth trajectory were analyzed using custom-designed motion analysis software (DIPP-Motion Pro 2D, DITECT, Inc.). Variability was defined as the root mean square (RMS) of displacement of the markers and angular excursions of the hip, knee, and ankle joints for each of the individual trials of each mouse.

Electromyographic (EMG) activities were recorded from the dorsal neck muscle (neck), the gastrocnemius muscle (GA), the tibialis anterior muscle (TA), the biceps femoris muscle (BF), and the vastus lateralis muscle (VL). After the mice were anesthetized with isoflurane (2%), a pair of thin insulated stainless steel wires with 1 mm of the tips exposed (76 μm in diameter, coated 140 μm, A-M systems, Inc.) was implanted. EMG signals were recorded and amplified (bandwidth 150 Hz–10 kHz), then digitized with a data acquisition system at 10 kHz using a personal computer. The high-speed digital image camera and EMG acquisition were synchronized. Digitized records were analyzed using custom-written Matlab software (MathWorks, Inc.). EMG signals were full-wave rectified and filtered with a 20-Hz low-pass second-order Butterworth filter. Onset of the EMG signal was defined as a deflection above six standard deviations from baseline. The baseline level was defined as the mean EMG signal in the resting state before the reaching movement.

For the hindlimb immobilization experiment, we used a slight modification of the previously described procedure on 7- to 19-week-old C57BL/6J mice (Booth and Kelso, 1973; Booth, 1977; Frimel et al., 2005; Caron et al., 2009). The mice were anesthetized with isoflurane (2%) and both hindlimbs were immobilized at their resting length. The hindlimbs were wrapped in sterile gauze, and then the hindlimbs were immobilized using papier-mâché. The mice were monitored on a daily basis for signs of chewing and breakage of the papier-mâché, and for problems with movement. The mice were free to move using their forelimbs, and to eat and drink *ad libitum*. After 2–3 weeks, the papier-mâché was removed and the mice were allowed to recover for 1–3 days in their cages before the behavioral experiments were performed. This group of mice is referred to as the ‘‘muscle atrophy group’’ in this paper. High-speed digital imaging of their movements were recorded and analyzed as described above.

Standard statistical tools (SPSS Japan, Inc.) were used. Muscle weights, body weights, joint angles, plantar distances, and movement times were compared using a one-way analysis of variance (ANOVA), and significant effects ($P < 0.05$) were confirmed by a post hoc comparison using the Bonferroni test. Parameters of the RMS, trajectory length ratios, ankle angle joints in the drinking stance task, and distance between the plantar of the forelimb in the drinking stance task were compared with the Kruskal–Wallis one-way ANOVA, and significant effects were confirmed using a post hoc Mann–Whitney *U*-test with Bonferroni adjustment (adjusted $P = 0.0167$). The differences in timing of the onset of neck