Neuroscience 148 (2007) 825–832

QUANTITATIVE MEASUREMENT OF POSTURAL SWAY IN MOUSE MODELS OF HUMAN NEURODEGENERATIVE DISEASE

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Abstract—Detection of motor dysfunction in genetic mouse models of neurodegenerative disease requires reproducible, standardized and sensitive behavioral assays. We have utilized a center of pressure (CoP) assay in mice to quantify postural sway produced by genetic mutations that affect motor control centers of the brain. As a positive control for postural instability, wild type mice were injected with harmaline, a tremorigenic agent, and the average areas of the 95% confidence ellipse, which measures 95% of the CoP trajectory values recorded in a single trial, were measured. Ellipse area significantly increased in mice treated with increasing doses of harmaline and returned to control values after recovery. We also examined postural sway in mice expressing mutations that mimic frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17) (T-279, P301L or P301L–nitric oxide synthase 2 (NOS2)$^{-/-}$ mice) and that demonstrate motor symptoms. These mice were then compared with a mouse model of Alzheimer's disease (APPSwDI mice) that demonstrates cognitive, but not motor deficits. T-279 and P301L-NOS2$^{-/-}$ mice demonstrated a significant increase in CoP ellipse area compared with appropriate wild type control mice or to mice expressing the P301L mutation alone. In contrast, postural instability was significantly reduced in APPSwDI mice that have cognitive deficits but do not have associated motor deficits. The CoP assay provides a simple, sensitive and quantitative tool to detect motor deficits resulting from postural abnormalities in mice and may be useful in understanding the underlying mechanisms of disease.

Key words: tremor, models, center of pressure assay, postural sway, behavior, neurodegeneration.

The development of multiple mouse models of human disease has led to the requirement for reliable, reproducible and sensitive assays of mouse behaviors (van der Staay and Steckler, 2002; Meredith and Kang, 2006). Behavioral phenotyping is particularly important as an outcome measure because of the potential relevance for direct comparison to human disease. Our laboratory has focused on mouse models of chronic neurodegenerative diseases such as frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17), Parkinson's disease (PD) and Huntington's disease (HD). Each of these diseases is associated with pathological changes in those regions of the brain that control motor behavior. For example in Huntington's disease, abnormal gait and changes in muscle activity are typically observed and can be correlated with damage to the corticostriatal input to the striatal neuron pool as well as the striatum itself (Schilling et al., 1999; Sieradzan and Mann, 2001). Loss of dopaminergic neurons in the substantia nigra in PD is well known to be associated with progressive motor dysfunctions that include bradykinesis and rigidity (Braak et al., 2004; Bhidayasiri, 2005; von Bohlen Und Halbach, 2005; Meredith and Kang, 2006). In addition, neurodegenerative diseases such as Huntington's and PD demonstrate tremor (Hefter et al., 1987; Mangiarini et al., 1996; Bhidayasiri, 2005). In PD the involuntary tremor resulting from loss of dopaminergic neurons in the basal ganglia is typically a mixture of postural and resting tremor and can be lessened with voluntary movements (Bhidayasiri, 2005). The other major type of tremor in the human population is essential tremor which is the most common form of action tremor and is produced by voluntary muscle contraction (Bhidayasiri, 2005).

Motor symptoms that are reminiscent of neurodegenerative diseases in humans can be at least partially reproduced in both pharmacological and genetic mouse models. For example, harmaline is a well-described tremorigenic compound that acts through modulation of dopamine release in the basal ganglia and the inferior olive nucleus (Miwa et al., 2000; Fowler et al., 2002). Harmaline treatment is associated with a dose-dependent increase in action tremor with a frequency of 10–12 Hz (Wang and Fowler, 2001; Martin et al., 2005). Treatment with 1-methy-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) produces motor symptoms in mice that mimic PD, including akinesia and tremor (Haobam et al., 2005; von Bohlen Und Halbach, 2005). These behavioral changes correlate with loss of tyrosine hydroxylase immunopositive neurons in the striatum and confirm that dopaminergic neuronal function is compromised. In mouse models of FTDP-17 that express mutated human tau, overt disease onset is commonly manifest by motor impairment and dystonia progressing to limb paralysis (Nasreddine et al., 1999; Lewis et al., 2000; Arendash et al., 2004; Ramsden et al., 2005). Changes in movement, thus, can be used as a reliable indicator of disease pathology and progression in rodent models of human disease.

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Abbreviations: APP, amyloid precursor protein; CoP, center of pressure; FTDP-17, frontotemporal dementia with Parkinsonism linked to chromosome 17; NOS2, nitric oxide synthase 2; PD, Parkinson's disease.

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doi:10.1016/j.neuroscience.2007.07.025

A variety of behavioral tests is commonly used to study movement disorders in rodents, including the wire hang test for general muscle strength, the balance beam or pole test for general motor function, and the rotorod assay for motor coordination (Sango et al., 1996; Hamm, 2001; Arendash et al., 2004; Meredith and Kang, 2006). Measurement of postural instability and tremor in rodents has primarily relied on subjective measures such as counting of head shakes. More recently, however, new techniques using load sensors or force plate actometers have been developed for detection and quantification of mouse activities such as rotation, rearing activity and gait (Fowler et al., 2001; Martin et al., 2005). The force platform is particularly useful in detecting loss of balance (postural sway) by measuring differences in the center of pressure (CoP). This technique has recently been used to measure postural sway in a healthy elderly population and in patients with essential tremor (Masui et al., 2005; Bove et al., 2006).

We have applied a simplified "CoP" assay for use on mice in order to phenotype motor dysfunction in genetic mouse models of neurodegenerative diseases. In this study we used a well-established genetic model of human FTDP-17, that expresses the human P301L tau mutation and demonstrates hyperphosphorylated tau and neurofibrillary tangles in the brain stem, forebrain and spinal cord but does not exhibit dopaminergic neuronal loss (Lewis et al., 2000). P301L mice demonstrate motor deficits such as impaired rotorod and poor balance beam performance prior to paralysis in late disease stages (Arendash et al., 2004). A bigenic mouse created by crossing the P301L mouse to a nitric oxide synthase 2 $(NOS2)^{-/-}$ mouse was also examined since removal of NOS2 has recently been associated with enhanced tau pathology (Colton et al., 2006). We also tested a mouse model of amyloid deposition reminiscent of Alzheimer's disease (APPSwID) that displays cognitive but not motor deficits (Miao et al., 2005; Xu et al., 2007). Finally, we used the T-279 mouse that models FTDP-17 and demonstrates loss of dopaminergic neurons in the striatum (Dawson et al., in press). Overall, the CoP assay is simple to perform, is easily quantifiable and is highly sensitive allowing detection of significant differences in motor activity between closely related mouse models.

EXPERIMENTAL PROCEDURES

Animals

Transgenic mice (APPSwDI) containing the Swedish, Dutch (E22Q) and Iowa (D23N) amyloid precursor protein (APP) mutations, were generated as described (Davis et al., 2004) and were a generous gift from Dr. William Van Nostrand, Stony Brook University, Stony Brook, NY, USA. P301L mice expressing the P301L human tau mutation were generated as described by Lewis et al. (2000) and were a generous gift from Drs. Lewis and Hutton, Mayo Clinic at Jacksonville, Jacksonville, FL, USA. Bigenic mice were produced by crossing P301L mice with $NOS2^{-/-}$ mice (B6 129P2$NOS2^{tau1Lau}$/J) (Jackson Laboratories, Bar Harbor, ME, USA). T-279 mice expressing the N279K human FTDP-17 tau mutation under the control of the human TAU promoter were generated and characterized by Dr. Hana Dawson Duke University Medical Center, Durham, NC, USA. Control mice for the T-279 strain were generated from a backcrossed strain and were aged under the same conditions and for the same time period as the genetic strain. C57/Bl6 littermate mice bred in our facility served as control mice for all other strains. All mice were genotyped using standard procedures. Male and female mice were bred and housed under standard temperature and light conditions in an AAALAC/NIH-approved facility.

All experiments conformed to local and international guidelines on the ethical use of animals and were approved by the Duke University committee for Care and Use of Animals. Care was taken to minimize stress to each animal and to minimize the number of animals used.

CoP apparatus

The CoP analysis system is composed of the AMTI Biomechanics Force Platform (Model HE6x6; Advanced Mechanical Technology Inc., Watertown, MA, USA), automated data acquisition software (AMTI NetForce), analysis software (AMTI BioAnlaysis) and a digital video camera. The force platform was designed by AMTI to be sensitive enough to detect changes in force and moment initiated by tremor in 10–50 g mice. Zumwalt et al. (2006) have performed a detailed analysis of the force platform response linearity and accuracy. Reduction of spurious, externally generated vibrations was accomplished by placing the force platform on a large inertial mass (lead bricks), which was then placed on a tabletop vibration isolation platform (Model 66-500, Technical Manufacturing Corporation, Peabody, MA, USA). Finally, to prevent the mice from moving off of the platform, a Plexiglas box with a non-reflective inner surface was placed around the platform. The dimensions of the box were large enough to allow ¼ inch clearance between the box and platform. To provide a visual cue on the video at the time of measurement, a small, red LED was mounted inside the box near the top edge. This LED was activated by a small pushbutton switch connected to a battery. The placement of the box was checked prior to measurement of tremor to ensure that accidental interference of the force platform motion by the box did not occur. Fig. 1 shows the general apparatus configuration.

Measurements

Each mouse was weighed and then placed onto the platform within the box. The video camera and acquisition program were activated and the mouse was allowed to explore the platform for approximately 1–2 min. In order to avoid measuring voluntary movements such as walking or grooming, the mouse was watched by the operator and a video image recorded for each force measurement. When the mouse was observed to be resting still, that is, remaining in a prolonged stationary position on all four legs with no overt motion, the operator initiated signal acquisition. The software was set to record the forces experienced by the force platform for the 1 s prior to signal initiation, which corresponded to the time the operator observed that the animal was standing still. To further confirm that the mouse did not exhibit active movements of the limbs or head, the video was activated by a remote trigger and verbal descriptions of the animal's activity were recorded. In this manner, the video image of each force measurement could be used to confirm the "resting status" of each mouse. The measurements were repeated 8 to 10 times per mouse in each test period (approximately 30 min).

Pharmacological induction of tremor

Harmaline hydrochloride (Sigma Chemical Co., St. Louis, MO, USA) was dissolved into normal saline and injected s.c. at the nape of the neck in young adult mice. Doses given were 5 mg/4 ml/kg and 10 mg/4 ml/kg. Onset of tremor usually occurred at 15–20 min post-injection and was defined as the point where a