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Analysis of respiratory movements in a mouse model of late Parkinson's disease submitted to stress

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ABSTRACT

Parkinson's disease (PD) is known to cause tremor and rigidity, but other symptoms such as respiratory and autonomic dysfunctions are a major cause of disability and mortality in patients. In this study, we examined respiratory movements by using cineradiography on a murine model of late/advanced PD. Under surgical anesthesia, C57BL/6J mice received an injection of either 6-OHDA or vehicle solution to the right striatum. Two weeks after surgery, the animals had their respiratory movements recorded by video X-ray without any restraint. During recordings the animals were submitted to a mild acute-stress challenge. Behavioral tests were performed to assess the severity of the 6-OHDA lesion. As a result, behavioral tests confirmed severe motor impairments in 6-OHDA mice as compared to controls. 6-OHDA mice showed a predominantly thoracic respiratory pattern with reduced diaphragmatic excursion, and reduced respiratory frequency after stress. These results suggest that advanced nigrostrial degeneration may cause respiratory alterations with the features of obstructive-type respiratory disorders.

1. Introduction

Parkinson's disease (PD) often displays respiratory disorders, particularly at its later stages. Such disorders as aspiration pneumonia account for a greater mortality among PD patients than PD itself (Hoehn and Yahr, 1967; Mehanna and Jankovic, 2010). The progression of the disease can be divided in stages according to specific symptoms and severity of such symptoms (Braak et al., 2003). The most prominent features of PD, the so-called motor symptoms, are attributed to the degeneration of dopaminergic circuits in the mid-brain and basal nuclei (Kalia and Lang, 2015). At earlier stages, non-motor symptoms of PD are attributed to disrupted activity in the medulla, enteric nervous system, and olfactory bulb, whereas at later stages, involvement of the cortex may cause cognitive and limbic symptoms (Tolosa et al., 2006). In addition to specific areas of the brain, the duration of the disease can be associated with the degree of degeneration of the dopaminergic nigrostriatal pathway (Kordower et al., 2013). It is estimated that at the time when motor symptoms present themselves and a diagnosis of PD can be made, most patients will have about a 60% reduction in the population of dopamine neurons in the nigrostriatal pathway (Jankovic and Sherer, 2014). It is possible that respiratory disorders associated with PD can be staged in a similar manner; in other words, a certain pattern of respiratory symptoms may emerge in relation to the progression of the disease. We have previously reported respiratory changes in a mouse model mimicking the initial stages of PD (de Campos et al., 2015). In this study we looked for respiratory changes in a more severe model of PD. In addition, we report the results of a stress challenge on the severe PD model. A stress challenge is the process of inducing a physiological response by exposing an animal to an aversive (i.e. stressful) stimulus.

2. Material and methods

The methods described here were reviewed and approved by the institutional animal welfare committee (Approval no. 0140089C). All animal experiments complied with the ARRIVE guidelines and were carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines.

2.1. Animals

Wild-type male C57BL/6J mice (Nihon Clea, Tokyo, Japan) were

Abbreviations: 6-OHDA, 6-hydroxydopamine; PD, Parkinson's disease; CPA, costophrenic angle

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used. The animals weighed 22–25 g (aged 8 weeks) at the beginning of experiments. The animals were housed individually in 20 cm × 20 cm × 30 cm acrylic cages lined with absorbing bedding material, with freely available food (standard chow) and water. The animals were kept in a temperature-controlled room (23 ± 1°C) and in a 12-h dark/light cycle (lights on at 8:00). A total of 36 mice were randomly and equally divided into experimental (6-OHDA) and control groups. After surgery 9 animals in the 6-OHDA group were excluded because of poor health conditions. The total number of animals in each group was 6-OHDA n = 9, control n = 18. All animals were humanely killed after the experiments by aesthetic overdose.

2.2. Surgical procedures

Animals were anesthetized with a mixture of ketamine (90–120 mg/kg) and xylazine (10 mg/kg), and supplemented as needed. Thoughout the surgery, appropriate surgical level of anesthesia was verified by the lack of withdrawal reflexes from a pinch stimulus applied to the tail. The animals were then placed on a stereotaxic apparatus (Narishige, Tokyo, Japan) and received two microinjections of either 6-OHDA or saline solutions into the striatum on the left side. The coordinates were 1) AP = +1.0, L = −2.1, DV = −2.9; and 2) AP = +0.3, L = −2.3, DV = −2.9; AP: +0.5; L: −2.0 and DV: −3.0 mm (relative to bregma, sagittal suture, and dural surface) (Paxinos and Franklin, 2008). All solutions were prepared on the day of the surgery and kept in light-protected vials and stored in an ice box until use. 6-OHDA solution consisted of 12 µg 6-OHDA hydrochloride (Sigma-Aldrich, Tokyo, Japan) dissolved in 0.9% NaCl with 0.02% ascorbic acid in sterile water. Saline solution consisted of 0.02% ascorbic acid and 0.9% NaCl in sterile water. The total injected volume of either solution was 4 µL (2 × 2-µL) with the flow rate of 0.5 µL/min. Injections were made through a 32G needle attached to a 10 µL Hamilton syringe on an injection pump. The injection needle was left in place for 2 min before and 2 min after the injection. The animals were monitored until recovered from anesthesia, then returned to their home cages.

2.3. Behavioral tests

Behavioral tests were carried out 2 weeks after surgery. General motor function was assessed by the three tests described below. All behavioral tests were scored by an examiner who was blind to the animals group assignment.

Cylinder test: asymmetric paw preference in spontaneous exploratory behavior was examined by placing the mouse inside a glass beaker (8 cm diameter × 11 cm height) in front of vertical mirrors as to allow for a clear view of the mouse from all angles. The mice were filmed for 5 min while in the beaker. This test was performed only once, to avoid habituation, and just before lights-off time, when the animals are most active. The normal animal behavior is to explore the new environment by rearing inside the beaker, touching the glass walls with its forepaws. The number of weight-bearing wall contacts made by the right and left forepaws was counted.

Rotation test: this test is used to detect asymmetry in the use of the paws, cognitive deficits, or general welfare in mice. The animals were placed in individual compartments and filmed for 5 min. The number of turns that the mouse completed toward each side during the observation period were analyzed.

Apomorphine test: this test evaluates the severity of the injury and is used to detect the asymmetry in the use of the paws. In a manner similar to the above Rotation test, the animals were placed in individual compartments and filmed 20 min after an injection of apomorphine (0.5 mg/kg, i.p., Sigma-Aldrich, Tokyo, Japan). The drug has the effect of over-exciting the dopamine-deficient area of the neurotoxic lesion, causing the animal to move and turn in the direction contralateral to the injection of 6-OHDA. Except for the apomorphine test, all other behavioral tests were performed before the cineradiographic recordings of respiratory movements.

2.4. Cineradiographic recordings and stress challenge

Cineradiographic recordings were obtained 2 weeks after surgery. Before the recordings, the animals were habituated to the cineradiographic apparatus (Micro X-movie, NIC, Fujisawa, Japan) for 5 min during two consecutive days. On the following two consecutive days, 6-min movie recordings were made from each mouse on each day. The animals were transferred to a test cage inside the apparatus, one by one, without any restraint. Recordings consisted of one-minute of each: 1) habituation, 2) observation, 3) first rotation, 4) observation, 5) second rotation 6) observation. Measurements were made during the periods of observation. Stress challenge: during the recordings, the test cage inside the apparatus was rotated in order to provoke an aversive stimulus to the mice. Rotations consisted of 60 s of 1 Hz rotation around the animals center of mass. First and second rotations were applied toward opposing sides and counterbalanced. Images were obtained by passing an X-ray beam vertically onto the mouse by a micro-focus X-ray tube (Toshiba Electron Tubes and Devices Co. Ltd., Tokyo). Power settings on the X-ray tube were kept constant at 70 kV and 0.3 mA to obtain stable X-ray emission. The X-ray photons passing through mouse were converted into visible light by a beryllium fast-response image-intensifier (Toshiba Electron Tubes and Devices Co., Ltd., E5889BP-P1K). This image was then captured by a digital video camera positioned underneath the image intensifier (Hasegawa et al., 2014). Videos were recorded at 29 fps and 1920 × 1080 pixels.

2.5. Respiratory movements

We measured respiratory frequency, diaphragmatic excursion, amplitude of the costophrenic angle (CPA), and the distance beetween the CPAs during quiet respiration (Fig. 1), in a manner similar to that described before (de Campos et al., 2015). Each animal was recorded twice and each video was measured three times at each observation period (total 18 measurements/animal). Measurements were made by an examiner who was blind to whether the mice had received 6-OHDA or saline injection.

Measurement of respiratory frequency was derived from the number of frames between maximum inspiration and the next maximum expiration. Periods of apnea, defined as intervals ≥2 respiratory cycles

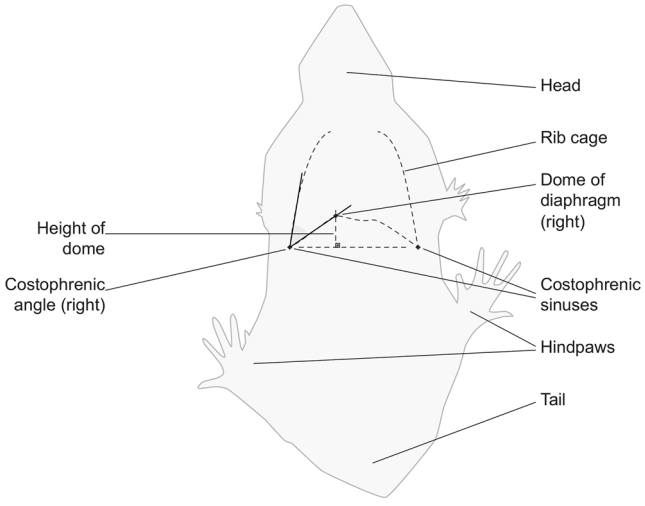

Fig. 1. Respiratory measurements. Measurements were made over the cineradiographic images of the mice in the horizontal plane. Video-still images were selected at the points of maximum inspiration and maximum expiration during quiet respiration. Measurements included linear (height of diaphragmatic dome and distance between costophrenic angles), angular (the costophrenic angles), and temporal (respiratory frequency) variables.