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Cineradiographic analysis of respiratory movements in a mouse model for early Parkinson's disease

P.S. de Campos[a], K. Hasegawa[b], Y. Kumei[c], J.L. Zeredo[a,c,*]

[a] Graduate Program in University of Brasilia, Health Sciences and Technologies, Brasilia, Brazil
[b] JAXA/Institute of Space and Astronautical Science, Sagamihara, Japan
[c] Department of Hard Tissue Engineering, Tokyo Medical and Dental University, Tokyo, Japan

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ABSTRACT

Parkinson's disease (PD) is a progressive degenerative disorder of the central nervous system known to cause a typical pattern of motor symptoms. In its later stages, PD is known to cause respiratory alterations including shortening of operational volumes and reduced velocity of respiratory-muscle contraction. It has been proposed that such changes are secondary to changes in posture and osteoarticular degeneration, leading to an alteration in the spinal axis that in turn could affect breathing mechanics. In this study, we examined respiratory movements by using cineradiography on a murine (C57BL/6J) model of mild hemi-PD. Under surgical anesthesia, PD mice received an injection of 6-OHDA solution to the right striatum, and were compared to control mice, which received an injection of saline solution. Two weeks after surgery, all mice had their respiratory movements recorded by video X-ray without any restraint. Behavioral tests were performed to assess the severity of the 6-OHDA lesion. As a result, behavioral tests confirmed mild motor impairments in PD mice as compared to controls. Parameters of respiratory function showed mild alterations in the PD group, suggestive of a restrictive-type respiratory disorder. These results suggest that respiratory alterations in PD may emerge simultaneously to other motor symptoms, and not as a consequence of the latter.

1. Introduction

Parkinson's disease (PD) is a currently incurable, highly prevalent neurodegenerative disorder. PD affects mostly, but not exclusively, the elder population, with age being its the most important risk factor (Abdullah et al., 2014). The estimated prevalence is 0.2–0.3% in the general population and as much as 3% or more in persons over 65 years old (Errea et al., 1999; Khatter et al., 1996; Moghal et al., 1994). The prevalence decreases in the population above 90 years old, possibly because of increased mortality associated with the disease (Errea et al., 1999).

PD is caused by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta, which results in well-known motor impairments that characterize the disease (Nicholson et al., 2002). The diagnosis is essentially based on clinical criteria: resting tremor, muscle rigidity, and slowness of movements (bradykinesia), in addition to therapeutic response to levodopa (Tolosa et al., 2006). Diagnosing PD is straightforward when all clinical features are clearly present, which is not the case in the initial stages of the disease. In early PD, the first detected symptoms are peripheral tremors, especially of the hands, and at rest (Hoehn and Yahr, 1967). In mild to severe PD, in addition to worsening of the tremors, motor impairments gradually evolve to bradykinesia, akinesia, rigidity, and loss of postural reflexes. Further, as the disease progresses, many patients also develop other neurological impairments such as deficits of cognition (Koster et al., 2014).

Respiratory symptoms are a common find in severe PD patients. However, it is less clear if such symptoms are present in the initial/mild stages of the disease. It is possible that respiratory symptoms are merely less evident because the other motor impairments reduce the demand on the respiratory system (Siderowf and Stern, 2008). Given that respiratory complications are the most common cause of death in PD patients (Nicholson et al., 2002), it is important to know the stage of the disease when these symptoms first emerge and if they are susceptible to early/preemptive treatments. Therefore, in this study we measured variables related to

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

* Corresponding author at: Graduate Program in Health Sciences and Technologies, University of Brasilia, Centro Metropolitano, Conjunto A, Lote 1, Brasília, DF 72220-900, Brazil. Tel.: +55 61 9975 9006.
E-mail address: jllzeredo@unb.br (J.L. Zeredo).

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the respiratory function in the early stages of an animal model of PD. For this purpose a novel micro-focus X-ray movie system was employed.

2. Material and methods

The methods used in this study followed the guidelines for animal welfare of the Tokyo Medical and Dental University and had institutional approval in advance of experimentation (Approval No. 0140089C).

2.1. Animals

We used wildtype male C57BL/6J mice acquired from a commercial breeder (Nihon Clea, Tokyo, Japan). The animals were 8 weeks old (body weight: 23–25 g) 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. A total of 22 mice were randomly divided in two groups, 6-OHDA ($n = 14$) and saline ($n = 8$). 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).

2.2. Surgical procedures

Mice were anesthetized with a mixture of ketamine (90–120 mg/kg) and xylazine (10 mg/kg). 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 microinjections of either 6-OHDA or saline solutions into the striatum on the right side. The coordinates were AP: +0.5; L: −2.0 and DV: −3.0 mm (relative to bregma, sagittal suture, and dural surface) (Paxinos and Franklin, 2008). The solutions were prepared on the day of the surgery and kept in light-protected vials in an ice box until use. 6-OHDA solution consisted of 3 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. For either solution the total injected volume was 2 μL with the flow rate of 0.5 μL/min. Injections were made through a 32 G 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. Behavior tests

Behavior tests were carried out 2 weeks after surgery. General motor function was assessed by the three tests described below. All behavior 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.

Pole test: bradykinesia and motor coordination were assessed by placing the mice on top of a pole (50 cm in length, 1 cm in diameter) facing upwards. The normal animal behavior is to turn and climb down the pole. The mice were trained on this task before surgery, on two consecutive days. The test session was recorded on video and analyzed for the time spent to turn facing downwards, and to climb down the pole into the home-cage below. Each mouse completed the task three times and the average time was calculated. The cutoff time was 60 s.

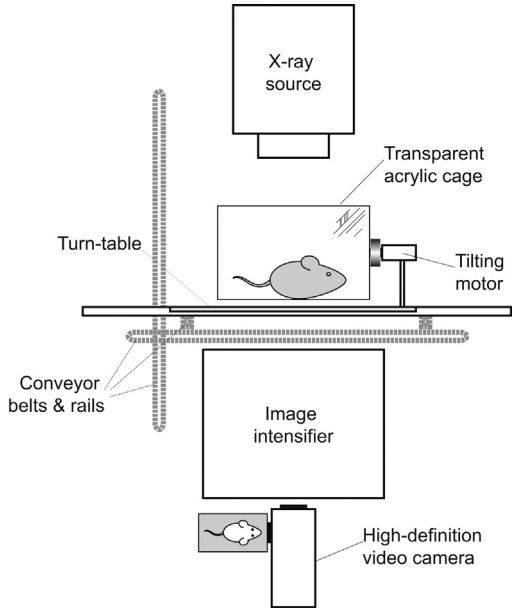

Fig. 1. Scheme of the cineradiography apparatus. A lateral view shows the test cage holding a mouse between the X-ray source and the image intensifier. The test cage can be gently moved by motors in the three dimensions, rotated, and tilted for optimal positioning of the mouse even as it moves in the cage. X-ray images were captured in real-time by a high-definition video camera.

Nest-building test: motor activity and sensory-motor function were tested by placing squares of pressed cotton on the cage's chow bin one hour before lights-off. Mice will use the cotton to build a nest overnight. The following morning, 12 h later, we collect and weight the amount of cotton material left unused in the bin, and calculated the amount that had been used out of an average initial weight of 2.67 g (±0.01 SD) of material.

2.4. Respiratory movements

Respiratory movements in each mouse were recorded 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, three 5-min recordings were made from each mouse. The animals were transferred one by one to a test cage inside the apparatus (Fig. 1). An X-ray beam was emitted vertically onto the unrestrained mouse by a micro-focus X-ray tube (Toshiba Electron Tubes and Devices Co. Ltd., Tokyo). Power on the X-ray tube was kept constant at 70 kV and 0.3 mA to obtain stable X-ray emission. The X-ray photons passing through mouse reached the 100 mm diameter entrance field of the high speed-response type beryllium image intensifier (Toshiba Electron Tubes and Devices Co., Ltd., E5889BP-P1K), which converted the X-ray photons into visible-light photons, creating an image that was captured by a digital video camera positioned underneath the image intensifier (Hasegawa et al., 2014). Video recordings were made at 29 fps and 1920 × 1080 pixels.

We measured respiratory frequency, diaphragmatic excursion, and amplitude of the costophrenic angle (CPA) (details below). Three measurements were made from each recording, during quiet respiration, at the positions of maximum inspiration and the next