

Characterization of Parkinson Model: Cellular Loss Following Unilateral Lesion of the Substantia Nigra with 6-OHDA

Introduction

Unilateral brain lesioning with 6-Hydroxydopamine (6-OHDA) has been extensively used in rodents to model Parkinson's disease (PD) because of its ability to reproduce selective dopaminergic lesions. Charles River provides several rat models of Parkinson's disease, two of which are medial forebrain bundle lesion and unilateral intrastriatal lesion with 6-OHDA.

The objective of this project was to characterize the degree of cell loss in the substantia nigra following unilateral lesion with 6-OHDA. This work was conducted in collaboration with University of Pennsylvania*: Charles River provided rats with 6-OHDA lesions and the University of Pennsylvania characterized the cellular loss.

Methods

Surgical Approach: Three adult male 250-275 g CD® IGS rats (CrI:CD(SD)) were anesthetized and placed in stereotaxic apparatus. The Parkinson's model was created by injecting 6-OHDA into the substantia nigra using 12 μ g free base of 6-OHDA in 4 μ L vehicle (PBS). The solution was administered at a rate of 0.67 μ L/min for 6 minutes and delivered to the following coordinates relative to bregma (left side of brain): Anterior Posterior (mm) -1.5; Medial Lateral (mm) \pm 1.8; Dorsal Ventral (mm) -7.5. The incisor bar was positioned at +2.5 mm. The animals were given an apomorphine challenge (0.2 mg/kg, SC) 5-7 days post-

surgery and the threshold for success was set at 5 rotations per minute, and all three animals met this criteria. The animals were then shipped to the University of Pennsylvania for evaluation of cell loss at 14 days post-surgery.

Tissue Preparation: Upon receipt, the animals were transcardially perfused with heparinized saline and 10% formalin and the brains removed. The brains were divided coronally at the infundibulum and the anterior and posterior regions processed for histological analysis.

Histological Analysis: The anterior and posterior portions of the brain were placed in 30% sucrose *in toto* until saturated. The posterior portion was sectioned at 20 μ m on the cryostat and mounted on slides. The sections containing the substantia nigra (-4.8 to -5.2 bregma; 3 sections separated by at least 100 μ m) were stained with an antibody to tyrosine hydroxylase (Abcam, AB112) and visualized using the Vector Labs Universal ABC kit and DAB (Vector Labs, PK6200 & SK4100, respectively).

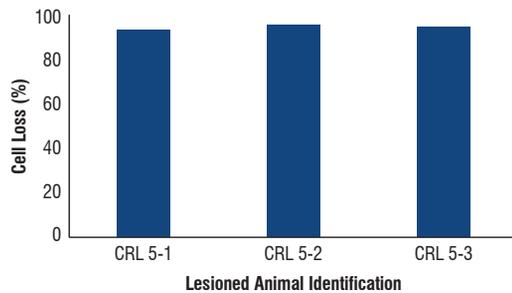
Cell Loss: Tyrosine hydroxylase-positive (TH+) cells of the substantia nigra pars compacta (SNpc) were counted manually by a trained observer. Only cells with a visible nucleus were counted. Counts were tabulated for each of the ipsilateral and contralateral sides of the brain. Percent loss was determined by comparing the number of ipsilateral cells to the number of contralateral cells.

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Results

As shown in Figure 1, direct unilateral lesion of the substantia nigra with 6-OHDA resulted in a dramatic loss of TH+ cells ($94\% \pm 1.4\%$; mean \pm standard deviation; Fig. 1). Figure 2 shows representative immunostaining of non-lesioned (A,B,E,F) and lesioned (C,D,G,H) animals. Figures 2I and 2J demonstrate cell loss in the SNpc as confirmed by cresyl violet (Nissl) staining.

Figure 1: Quantitative analysis of TH+ cells. Unilateral lesion with 6-OHDA resulted in 94% loss of cells.



Conclusions

The results of this evaluation indicate that direct injection of 6-OHDA into the rat substantia nigra is an effective and reproducible model provided by Charles River Laboratories. The approach results in a dramatic reduction in TH+ cells (94%) in the ipsilateral SNpc at 14 days post-injection that is associated with Parkinson's-like behavior issues as measured by an apomorphine challenge. While the amount of 6-OHDA injected is on par with published literature, it may be possible to adjust the dose in order to modify the resultant loss in TH+ cells.

Figure 2: Representative photomicrographs of the substantia nigra following lesioning with 6-OHDA. In control animals, tyrosine hydroxylase staining showed bilateral staining in the SNpc at -4.8 Bregma (A,B) and -5.2 Bregma (E,F). In comparison, lesioned animals showed a loss of nigral cells at the same levels (C,D,G,H; respectively). Scale bars=1 mm. Cresyl violet staining corroborated the immunohistochemistry findings (I,J).

