Validation of intrastriatal 6-OHDA infusion lesion model

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Supplemental 1

6-OHDA treatment: Male Sprague-Dawley rats (~300g) were anesthetized with 50 mg/kg sodium pentobarbital. Animals were placed in a stereotaxic frame (Stoelting, Wood Dale, IL, USA) with the incisor bar positioned 3.3 mm below the interaural line. 20µg 6-OHDA (Sigma, H4183, measured as free base) in 4 µl saline solution containing 0.02% ascorbic acid or vehicle (0.02% ascorbic acid in saline) was injected into the left striatum at the following coordinates relative to bregma and dura: AP 0.0mm, ML+3.00 mm, and DV-5.00 mm at 0.5 µL/min, using a motor-drive injector with a 26-gauge needle.

Figure 1 Dopamine levels

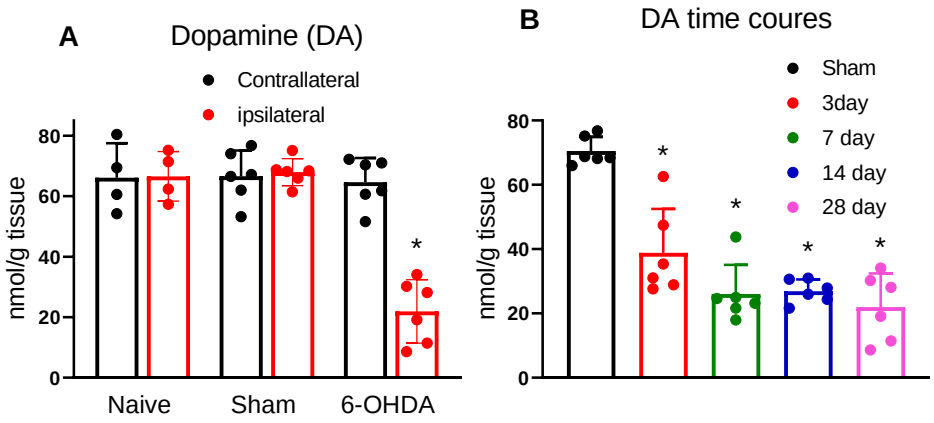

Figure 1 (A) Striatal DA levels in naïve (no infusion), sham (vehicle infusion) and 6-OHDA in rats 4 weeks post infusion. (B) The levels of striatal DA in rats 3, 7, 14 and 28 days post 6-OHDA infusion. Striatal DA levels were depleted 65-70 % 28 days post 6-OHDA infusion compared with vehicle intrastriatal infusion (sham). There was no striatal dopamine depletion in sham rats 4 weeks post infusion sham compared with naïve rats or the contralateral striatum 4 weeks post 6-OHDA infusion. This suggests that the data from the sham group can serve as an appropriate control group. Bars represent mean + S.D. * p<0.01 vs. sham, one-way ANOVA test. n=4-6 rats per group.

Figure 2 Rotation Behavioral Test

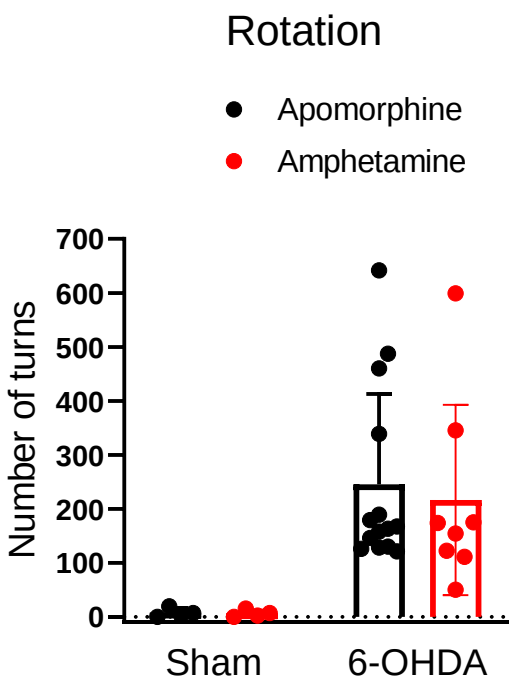

Figure 2 Rotation behavioral test in rats 4 weeks post 6-OHDA (20ug) or vehicle intrastriatal infusion was recorded for 60 min after apomorphine (0.5mg/kg, i.p) or amphetamine (5mg/kg, i.p.) challenge. Rotational behavior was measured by placing animals in a circular cage where they were connected with an automated rotometer. The number of complete turns performed by the animals was monitored by an automated recording system for 60 min. Bars represent mean + S.D. * p<0.01 vs. sham, one-way ANOVA test. n=4-12 rats per group. The numbers of turn in rats 4 weeks post 6-OHDA intrastriatal infusion with apomorphine or amphetamine were ~200 to 250 turns

in a total of 60 min. This indicates the lesions were significant. The rats that received vehicle infusions (sham) did not have any significant rotational behavior with apomorphine or amphetamine.

Figure 3 Immunohistochemical tyrosine hydroxylase (TH) staining

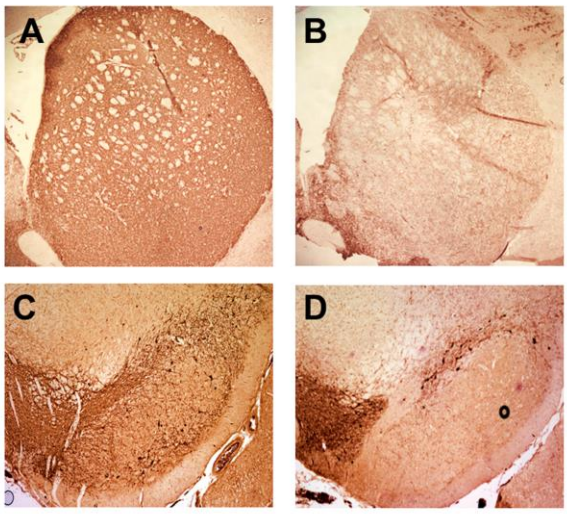

Figure 3 Representative immunohistochemical tyrosine hydroxylase (TH) staining images in the striatum (A, B) and in the substantial nigra (C, D) of the rats 4 weeks post vehicle (A and C) or 6-OHDA (B and D) infusion. TH immunostaining performed with a rabbit antibody to TH (Chemicon, Temecula, CA) using the ABC method (ABC Elite Kit, Vector Laboratories, Burlingame, CA). The dopamine terminal in the striatum (TH positive fibers) and dopaminergic neurons in the substantial nigra (TH positive neurons) were significantly decreased in the 6-OHDA lesioned rats compared with the vehicles (sham).