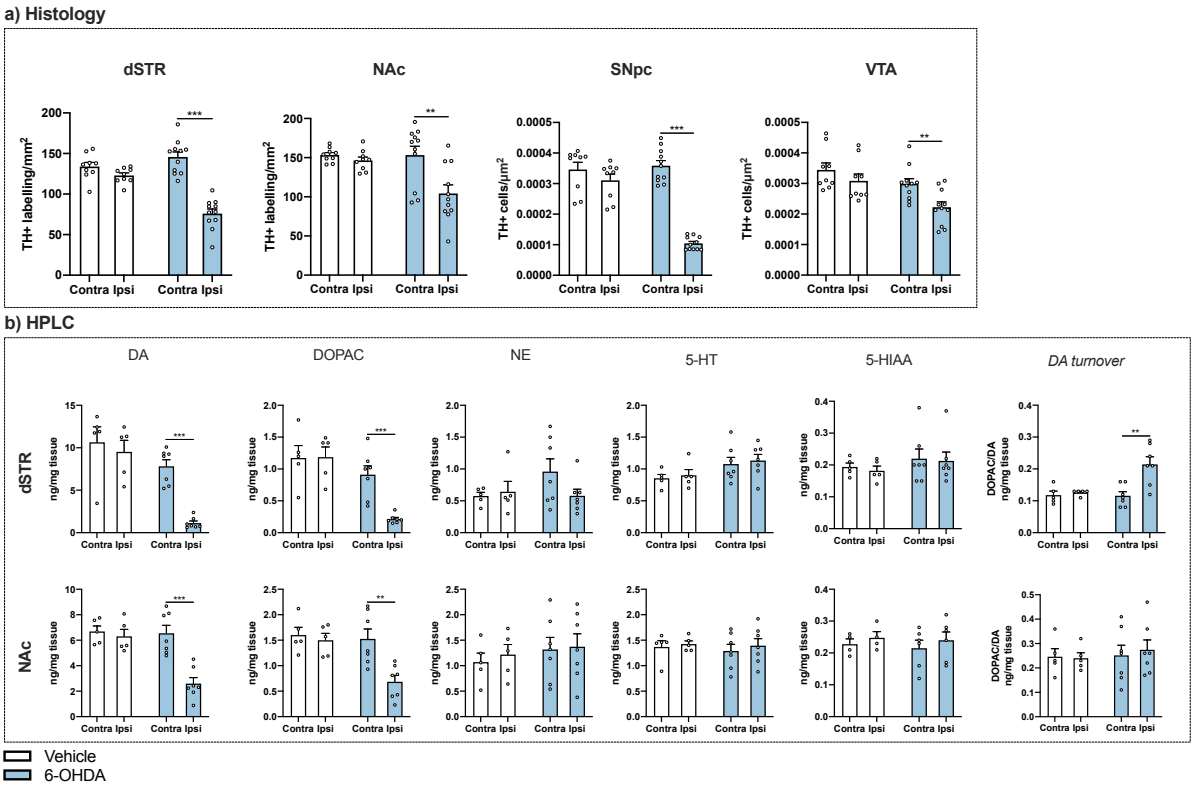
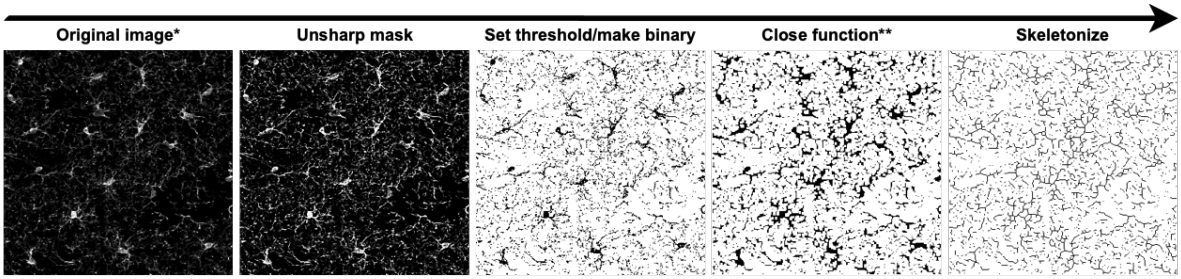



**Figure S1.** Raw data relative to main Figure 3. Histological characterization **(a)** of intrastriatal 6-OHDA lesions. TH+ labelling was measured in the dorsal striatum and NAc, and TH+ cell counts were performed in the SNpc and VTA. For all regions, significant differences were found between contra- and ipsilateral sides for the 6-OHDA group. Neurochemical analysis **(b)** using HPLC quantification combined with electrochemical detection, showing the dorsal striatum and NAc monoamine profile. Results showed a significant decrease of DA and its metabolite DOPAC for both regions, and an enhancement of DA turnover in the dorsal striatum, when comparing the contra- and ipsilateral sides of the 6-OHDA group. No differences were found for the other monoamines and its derivates (NE, 5-HT, and 5-HIAA) (Student's t test between contra and ipsilateral sides for each group; statistical summary in Table S2; data are presented as mean ± SEM). For histological analysis vehicle n = 9, 6-OHDA n = 11; for HPLC vehicle = 4-5, 6-OHDA n = 6-7. ** $p < 0.01$, *** $p < 0.001$. Abbreviations: dSTR, dorsal striatum; DA, Dopamine; DOPAC, 3,4-Dihydroxyphenylacetic acid; NAc, nucleus accumbens; NE, Norepinephrine; SNpc, substantia nigra pars compacta; TH, tyrosine hydroxylase; VTA, ventral tegmental area; 5-HIAA, 5-Hydroxyindoleacetic acid; 5-HT, serotonin; 6-OHDA, 6-hydroxydopamine.




*Representative section (supposed to be applied to the whole image)

**Joins pixels less than 2 pixels apart

**Analyze skeleton function**

| # Branches | # Junctions | # End-point voxels | # Junction voxels | # Slab voxels | Average Branch Length | # Triple points | # Quadruple points | Maximum Branch Length |
|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 2 | 0 | 2 | 3.045 | 0 | 0 | 3.045 |
| 0 | 0 | 1 | 0 | 0 | 0.000 | 0 | 0 | 0.000 |
| 1 | 0 | 2 | 0 | 12 | 12.318 | 0 | 0 | 12.318 |
| 0 | 0 | 1 | 0 | 0 | 0.000 | 0 | 0 | 0.000 |
| 0 | 0 | 1 | 0 | 0 | 0.000 | 0 | 0 | 0.000 |
| 0 | 0 | 1 | 0 | 0 | 0.000 | 0 | 0 | 0.000 |
| 3 | 1 | 3 | 3 | 2 | 2.076 | 1 | 0 | 2.716 |
| 0 | 0 | 1 | 0 | 0 | 0.000 | 0 | 0 | 0.000 |
| 1 | 0 | 2 | 0 | 0 | 1.125 | 0 | 0 | 1.125 |
| 0 | 0 | 1 | 0 | 0 | 0.000 | 0 | 0 | 0.000 |
| 1 | 0 | 2 | 0 | 8 | 8.477 | 0 | 0 | 8.477 |
| 0 | 0 | 1 | 0 | 0 | 0.000 | 0 | 0 | 0.000 |
| 1 | 0 | 2 | 0 | 0 | 0.795 | 0 | 0 | 0.795 |
| 1 | 0 | 2 | 0 | 5 | 5.102 | 0 | 0 | 5.102 |
| 1 | 0 | 2 | 0 | 0 | 0.795 | 0 | 0 | 0.795 |
| 1 | 0 | 2 | 0 | 0 | 0.795 | 0 | 0 | 0.795 |
| 1 | 0 | 2 | 0 | 5 | 5.432 | 0 | 0 | 5.432 |
| 0 | 0 | 1 | 0 | 0 | 0.000 | 0 | 0 | 0.000 |
| 1 | 0 | 2 | 0 | 0 | 0.795 | 0 | 0 | 0.795 |
| 3 | 1 | 3 | 4 | 5 | 3.466 | 1 | 0 | 3.841 |
| 4 | 1 | 4 | 5 | 15 | 5.151 | 0 | 1 | 14.046 |
| 0 | 0 | 1 | 0 | 0 | 0.000 | 0 | 0 | 0.000 |
| 0 | 0 | 1 | 0 | 0 | 0.000 | 0 | 0 | 0.000 |
| 1 | 0 | 2 | 0 | 0 | 0.795 | 0 | 0 | 0.795 |
| 0 | 0 | 1 | 0 | 0 | 0.000 | 0 | 0 | 0.000 |

**Figure S2.** Microglia morphology quantification workflow. Photomicrographs (20x magnification) were analyzed using a semi-automated workflow on FIJI. Microglial branching was assessed with the skeletonize tool after the application of an unsharp mask and the close function. Average branch length ≤ 2 were removed from the analysis (considered as noise). Total branch length (Average branch length x Branches) was normalized by the number of microglial cells (IBA1+ cells) within each image.

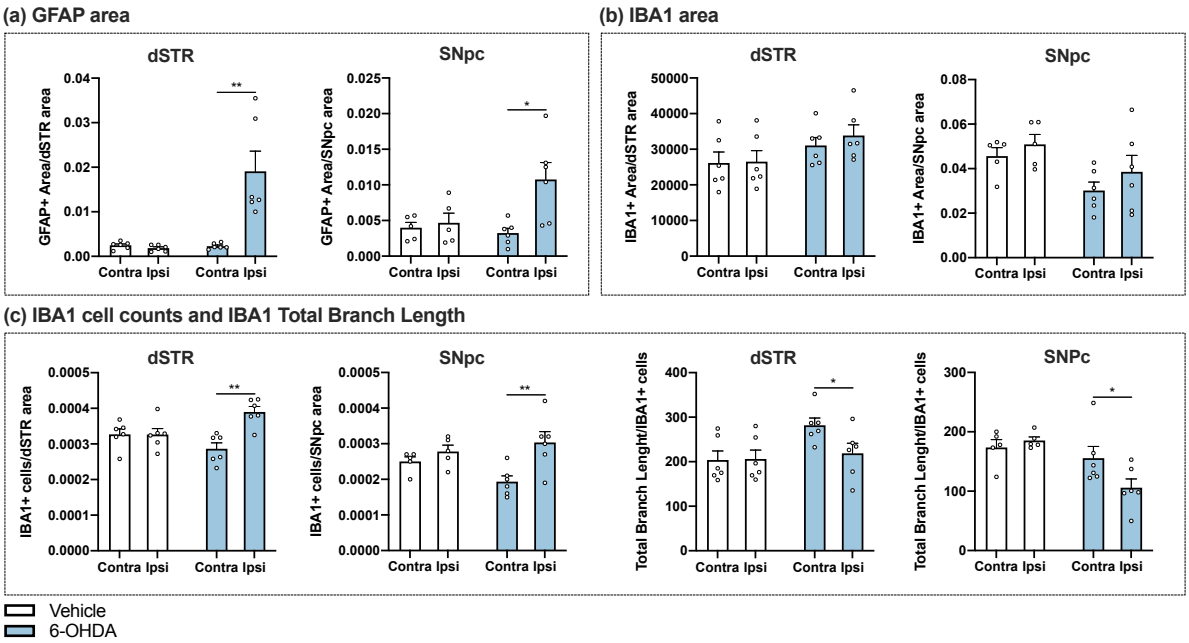



**Figure S3.** Raw data relative to main Figure 4 and Figure 5. Quantification of area coverage by GFAP+ staining **(a)** showed a significant astrocytic reaction between the contra- and ipsilateral sides for the 6-OHDA group. No differences were found between the contra- and ipsilateral sides of both groups after quantification of area coverage by IBA1+ staining in the dorsal striatum and SNpc **(b)**. A significant increase of IBA1+ cells, as wells as, a significant decrease of IBA1+ total branch length **(c)** in the ipsilateral areas of the 6-OHDA group was observed, indicating the presence of proliferative and activated microglia (Student's t-test; statistical summary in Table S2; data are presented as mean ± SEM). Vehicle n = 4-5, 6-OHDA n = 6-7 (4 slices/animal). * $p < 0.05$, ** $p < 0.01$. Abbreviations: dSTR, dorsal striatum; GFAP, glial fibrillary acidic protein; SNpc, substantia nigra pars compacta; 6-OHDA, 6-hydroxydopamine.

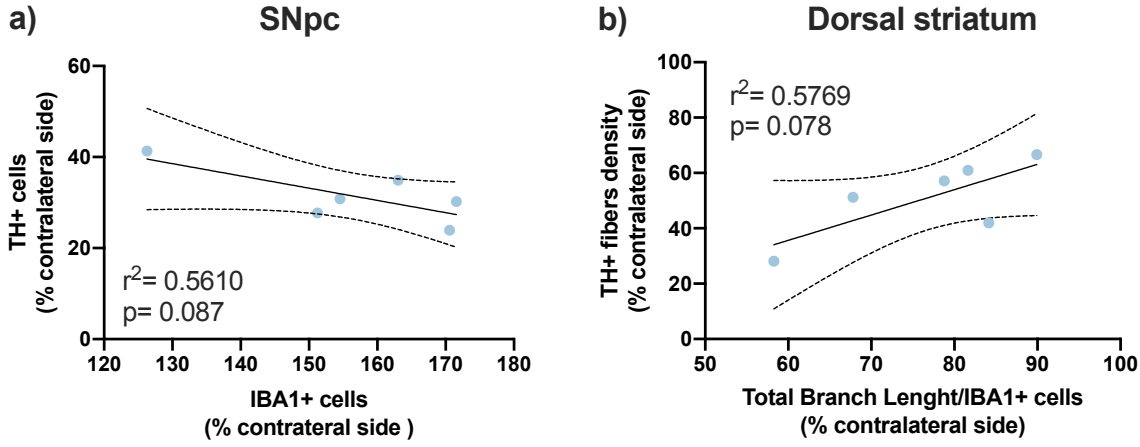



**Figure S4.** Correlation between dopaminergic loss and microglial cells. Scatter plots representing the correlation between TH+ cells and IBA1+ cells in SNpc **(a)** and TH+ fibers density with microglial branching (total branch length/IBA1+ cells) **(b)** for 6-OHDA-lesioned mice (Pearson correlation; statistical summary in Table S2). 6-OHDA n=6. Abbreviations: SNpc, substantia nigra pars compacta; TH, tyrosine hydroxylase; 6-OHDA, 6-hydroxydopamine.

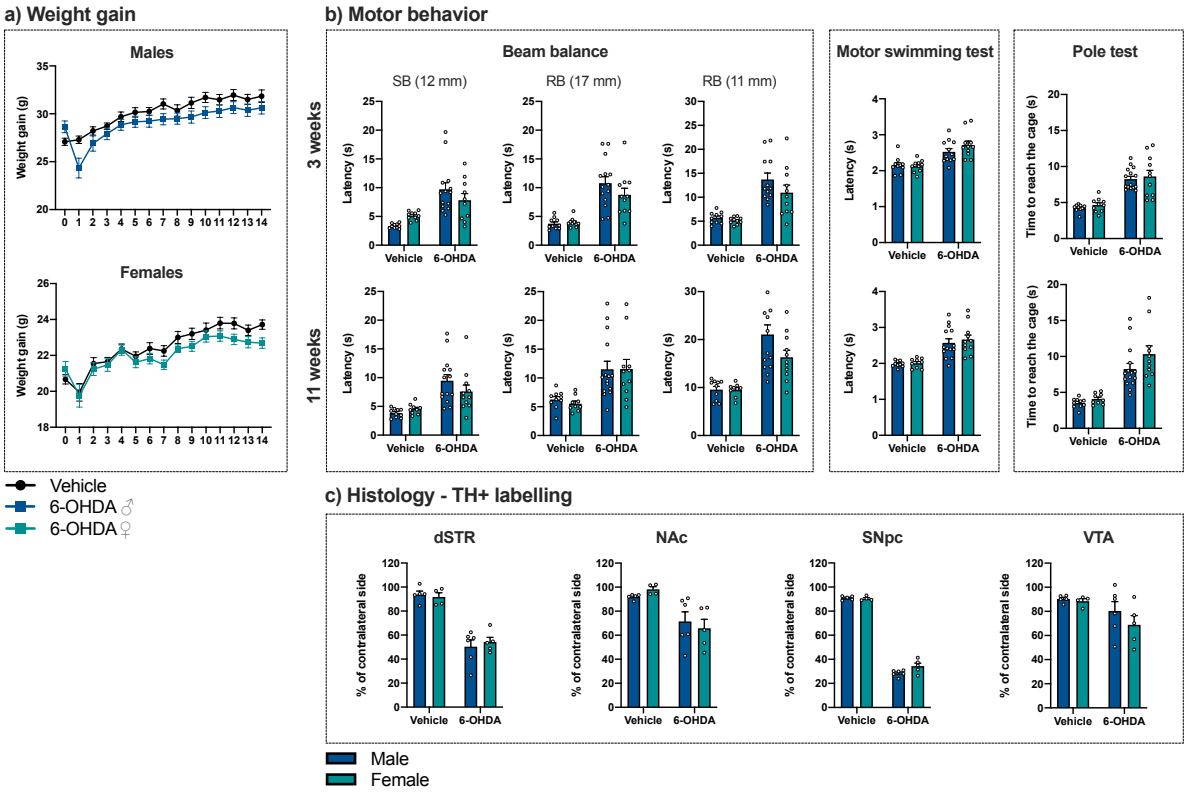



**Figure S5.** Impact of sex in behavioral and histological outcomes after 6-OHDA intrastriatal lesion. No differences were found in the weight gain for both males **(a)** and females **(b)** after 6-OHDA injections, when compared to the vehicle group, until de the end of the in vivo experiments (14 weeks) (Repeated measures ANOVA; statistical summary in Table S2; data are presented as mean ± SEM). No impact of sex or interaction between sex and group were significative in the motor behavior **(b)** and histological analysis **(c)** (Two-way ANOVA; statistical summary in Table S2; data are presented as mean ± SEM). The only source of variation was the group, i.e. the 6-OHDA lesion (these differences are not represented on the graphs, but are summarized in Table S2). For weight gain and motor behavior: $vehicle_{males}$ = 10, $6\text{-}OHDA_{males}$ = 15, $vehicle_{females}$ = 9-10, $6\text{-}OHDAfe_{males}$ = 11; for histological analysis $vehicle_{males}$ = 5, $6\text{-}OHDA_{males}$ = 6, $vehicle_{females}$ = 4, $6\text{-}OHDAfe_{males}$ = 5. Abbreviations: 6-OHDA, 6-hydroxydopamine.

**Table S1.** Statistical reports of all performed analysis in main Figures

| Measure | | Statistical report | |
|---|---|---|---|
| | | **3 weeks** | **11 weeks** |
| **Beam balance** (Figure 1a) *Latency (s)* | Square beam (12 mm) | $t_{(42)}$=5.03, p<0.001, d=1.58 | $t_{(42)}$=4.83, p<0.001, d=1.56 |
| | Round beam (17 mm) | $t_{(43)}$=6.48, p<0.001, d=2.04 | $t_{(42)}$=4.44, p<0.001, d=1.43 |
| | Round beam (11 mm) | $t_{(41)}$=6.10, p<0.001, d=1.93 | $t_{(42)}$=5.88, p<0.001, d=1.90 |
| **Motor swimming test** (Figure 1b) *Latency (s)* | | $t_{(41)}$=5.51, p<0.001, d=1.71 | $t_{(42)}$=6.31, p<0.001, d=2.04 |
| **Pole test** (Figure 1c) *Time to reach the cage (s)* | | $t_{(43)}$=7.92, p<0.001, d=2.54 | $t_{(43)}$=6.67, p<0.001, d=2.16 |
| **Cylinder test** (Figure 1d) *Left paw contacts as % of total* | | $t_{(17)}$=4.52, p<0.001, d=2.10 | $t_{(15)}$=5.33, p<0.001, d=2.54 |
| **Open arena** (Figure 1e) | Nr. of squares traveled | U=45, p<0.001 | U=73.50, p<0.001 |
| | Gait quality | U=120, p<0.001 | U=171, p<0.05 |
| **Rotameter test** (Figure 1f) *Net rotation* | | U=0, p<0.001 | |
| **Open field** (Figure 2a) *Time in center* | | $t_{(43)}$=0.89, p=0.3785 | |
| **Elevated-plus maze** (Figure 2b) *Time in opened arms* | | $t_{(43)}$=1.44, p=0.156 | |
| **Forced swim test** (Figure 2c) *Immobility time* | | $t_{(39)}$=2.30, p<0.05, d=0.75 | |
| **Splash-sucrose test** (Figure 2d) *Time grooming* | | $t_{(41)}$=2.46, p<0.05, d=0.78 | |
| **TH+ labelling** (Figure 3c/d) | **dSTR** | $t_{(18)}$=9.58, p<0.001, d=4.43 | |
| | **NAc** | $t_{(18)}$=4.25, p<0.001, d=2.00 | |
| **TH+ labelling** (Figure 3e/f) | **SNpc** | $t_{(18)}$=33.76, p<0.001, d=15.85 | |
| | **VTA** | $t_{(18)}$=2.33, p<0.05, d=1.10 | |
| **Neurochemical analysis** (Figure 3g/h) | | **dSTR** | **NAc** |
| | **DA** | $t_{(10)}$=6.08, p<0.001, d=3.22 | $t_{(10)}$=4.20, p<0.01, d=2.46 |
| | **DOPAC** | $t_{(10)}$=7.76, p<0.001, d=4.21 | $t_{(10)}$=3.25, p<0.01, d=2.00 |
| | **NE** | $t_{(10)}$=1.63, p=0.135 | $t_{(10)}$=0.48, p=0.635 |
| | **5-HT** | $t_{(10)}$=0.07, p=0.946 | $t_{(10)}$=0.19, p=0.856 |
| | **5-HIAA** | $t_{(10)}$=0.48, p=0.644 | $t_{(8)}$=0.53, p=0.609 |
| **DA turnover (Figure 3i) dSTR** | | $t_{(10)}$=2.40, p<0.05, d=1.51 | |
| **GFAP+ Area** (Figure 4c/d) | **dSTR** | $t_{(10)}$=3.36, p<0.01, d=1.94 | |
| | **SNpc** | $t_{(9)}$=3.07, p<0.05, d=1.95 | |
| **IBA1+ area** (Figure 5c/f) | **dSTR** | $t_{(10)}$=0.89, p=0.396 | |
| | **SNpc** | $t_{(9)}$=0.69, p=0.506 | |
| **IBA1+ cells** (Figure 5d/g) | **dSTR** | $t_{(10)}$=3.51, p<0.01, d=2.03 | |
| | **SNpc** | $t_{(9)}$=4.56, p<0.01, d=2.77 | |
| **IBA1 branch length** (Figure 5e/h) | **dSTR** | $t_{(10)}$=4.08, p<0.01, d=2.36 | |
| | **SNpc** | $t_{(9)}$=3.49, p<0.01, d=2.11 | |

**Table S2.** Statistical reports of all performed analysis in supplementary Figures

| Measure | | | | Statistical report |
|---|---|---|---|---|
| **Correlation** (Figure S2a) *DA neurons with IBA1+ cells* | | | | r2=0.561, p=0.087 |
| **Correlation** (Figure S2b) *DA fibers with Branch Length* | | | | r2=0.577, p=0.078 |
| **Weight gain** (Figure S3a) | Males | | | Time: $F_{(3.7, 86.1)}$=36.41, p<0.001; Group: $F_{(1, 23)}$=2.13, p=0.158<br>Time x Group: $F_{(14, 322)}$=2.98, p<0.001 |
| | Females | | | Time: $F_{(2.7, 48.7)}$=37.52, p<0.001; Group: $F_{(1, 18)}$=1.85, p=0.191<br>Time x Group: $F_{(14, 252)}$=1.339, p=0.185 |
| **Time Point (**Figure S5b) | | | | **3 weeks** | **11 weeks** |
| **Beam balance** *Latency (s)* | Square beam (12 mm) | | | Group: $F_{(1, 40)}$=24.47, p<0.001<br>Sex: $F_{(1, 40)}$=0.004, p=0.952<br>Group x Sex: $F_{(1,40)}$=4.07, p=0.051 | Group: $F_{(1, 40)}$=22.11, p<0.001<br>Sex: $F_{(1, 40)}$=0.42, p=0.521<br>Group x Sex: $F_{(1,40)}$=1.93, p=0.521 |
| | Round beam (17 mm) | | | Group: $F_{(1, 41)}$=40.68, p<0.001<br>Sex: $F_{(1, 41)}$=0.96, p=0.333<br>Group x Sex: $F_{(1,41)}$=1.44, p=0.237 | Group: $F_{(1, 41)}$=18.81, p<0.001<br>Sex: $F_{(1, 41)}$=0.07, p=0.788<br>Group x Sex: $F_{(1,41)}$=0.09, p=0.772 |
| | Round beam (11 mm) | | | Group: $F_{(1, 39)}$=37.68, p<0.001<br>Sex: $F_{(1, 39)}$=2.16, p= 0.150<br>Group x Sex: $F_{(1,39)}$=0.92, p=0.343 | Group: $F_{(1, 40)}$=34.83, p<0.001<br>Sex: $F_{(1, 40)}$=2.41, p=0.128<br>Group x Sex: $F_{(1,40)}$=2.28, p=0.139 |
| **Motor swimming test** *Latency (s)* | | | | Group: $F_{(1, 39)}$=31.49, p<0.001<br>Sex: $F_{(1, 39)}$=0.94, p=0.340<br>Group x Sex: $F_{(1,39)}$=1.65, p=0.210 | Group: $F_{(1, 40)}$=38.71, p<0.001<br>Sex: $F_{(1, 40)}$=0.32, p=0.574<br>Group x Sex: $F_{(1,40)}$=0.12, p=0.734 |
| **Pole test** *Time to reach the cage (s)* | | | | Group: $F_{(1, 42)}$=62.07, p<0.001<br>Sex: $F_{(1, 42)}$=0.57, p=0.456<br>Group x Sex: $F_{(1,42)}$=0.002, p=0.968 | Group: $F_{(1, 41)}$=48.77, p<0.001<br>Sex: $F_{(1, 41)}$=2.82, p=0.100<br>Group x Sex: $F_{(1,41)}$=0.90, p=348 |
| **TH+ labelling** (Figure S3c) | | | **dSTR** | | Group: $F_{(1, 16)}$=82.04, p<0.001<br>Sex: $F_{(1, 16)}$=0.04, p=0.847<br>Group x Sex: $F_{(1,16)}$=0.44, p=0.517 |
| | | | **NAc** | | Group: $F_{(1, 16)}$=17.54, p<0.001<br>Sex: $F_{(1, 16)}$=0001, p=0.973<br>Group x Sex: $F_{(1,16)}$=0.90, p=0.358 |
| **TH+ labelling** (Figure S3c) | | | **SNpc** | | Group: $F_{(1, 16)}$=1504, p<0.001<br>Sex: $F_{(1, 16)}$=3.18, p=0.093<br>Group x Sex: $F_{(1,16)}$=4.25, p=0.060 |
| | | | **VTA** | | Group: $F_{(1, 16)}$=5.66 p<0.05<br>Sex: $F_{(1, 16)}$=1.17, p=0.296<br>Group x Sex: $F_{(1,16)}$=0.60, p=0.448 |
| **TH+ labelling** (Figure S3a) | | **dSTR** | **Vehicle** | | $t_{(16)}$=1.77, p=0.0954 |
| | | | **6-OHDA** | | $t_{(20)}$=8.28, p<0.001 |
| | | **NAc** | **Vehicle** | | $t_{(16)}$=1.32, p=0.204 |
| | | | **6-OHDA** | | $t_{(20)}$=3.12, p<0.01 |
| **TH+ cells** (Figure S3a) | | **SNpc** | **Vehicle** | | $t_{(16)}$=1.15, p=0.268 |
| | | | **6-OHDA** | | $t_{(20)}$=14.39, p<0.001 |
| | | **VTA** | **Vehicle** | | $t_{(16)}$=1.12, p=0.279 |
| | | | **6-OHDA** | | $t_{(20)}$=3.17, p<0.01 |
| **Neurochemical analysis** (Figure S4b) | | | | **dSTR** | **NAc** |
| | **DA** | **Vehicle** | | $t_{(8)}$=0.50, p=0.633 | $t_{(8)}$=0.54, p=0.601 |
| | | **6-OHDA** | | $t_{(12)}$=8.19, p<0.001 | $t_{(12)}$=5.11, p<0.001 |
| | **DOPAC** | **Vehicle** | | $t_{(8)}$=0.06, p=0.957 | $t_{(8)}$=0.50, p=0.631 |

| | | | | |
|---|---|---|---|---|
| | | **6-OHDA** | $t_{(12)}$=4.90, p<0.001 | $t_{(12)}$=3.66, p<0.01 |
| | **NE** | **Vehicle** | $t_{(8)}$=0.38, p=0.715 | $t_{(8)}$=0.56, p=0.594 |
| | | **6-OHDA** | $t_{(12)}$=1.68, p=0.119 | $t_{(12)}$=0.16, p=0.875 |
| | **5-HT** | **Vehicle** | $t_{(8)}$=0.42, p=0.685 | $t_{(8)}$=0.41, p=0.694 |
| | | **6-OHDA** | $t_{(12)}$=0.39, p=0.704 | $t_{(12)}$=0.55, p=0.591 |
| | **5-HIAA** | **Vehicle** | $t_{(8)}$=0.63, p=0.545 | $t_{(6)}$=0.80, p=0.461 |
| | | **6-OHDA** | $t_{(12)}$=0.18, p=0.864 | $t_{(12)}$=0.69, p=0.506 |
| | **DOPAC/DA** | **Vehicle** | $t_{(8)}$=0.61, p=0.557 | $t_{(8)}$=0.15, p=0.884 |
| | | **6-OHDA** | $t_{(12)}$=3.66, p<0.01 | $t_{(12)}$=0.39, p=0.701 |
| **Glial reaction analysis** (Figure S5) | | | **dSTR** | **SNpc** |
| | **GFAP+ area** | **Vehicle** | $t_{(10)}$=1.41, p=0.189 | $t_{(8)}$=0.45, p=0.663 |
| | | **6-OHDA** | $t_{(10)}$=3.71, p<0.01 | $t_{(10)}$=3.06, p<0.05 |
| | **IBA1+ area** | **Vehicle** | $t_{(10)}$=0.09, p=0.933 | $t_{(8)}$=0.89, p=0.397 |
| | | **6-OHDA** | $t_{(10)}$=0.75, p=0.469 | $t_{(10)}$=1.01, p=0.339 |
| | **IBA1+ cells** | **Vehicle** | $t_{(10)}$=0.02, p=0.983 | $t_{(8)}$=1.26, p=0.243 |
| | | **6-OHDA** | $t_{(10)}$=4.58, p<0.01 | $t_{(10)}$=3.17, p<0.01 |
| | **IBA1 Total Branch Length** | **Vehicle** | $t_{(10)}$=0.08, p=0.936 | $t_{(8)}$=0.81, p=0.444 |
| | | **6-OHDA** | $t_{(10)}$=2.26, p<0.05 | $t_{(10)}$=2.03, p<0.05 |

**Table S3.** Perioperative health assessment and procedures after 6-OHDA injection in mice (adapted from [1])

| Type | Identification | Treatment |
|---|---|---|
| General health | Animal's behavior into the home cage: interaction with the environment, cage mates and nest building | - |
| Dehydration | Reduced skin turgor; recessed eyes and fuzzy fur | Fluid replacement through subcutaneous or intraperitoneal administration of sterile saline (0.9%) |
| Hypothermia | Body temperature below 36.5°C; animals are cool to the touch | Increase housing room temperature; administration of warm fluids (e.g., warm sterile saline injections) |
| Aphagia and adipsia | Measurement of food and water consumption; Body weight monitoring | Easy access to palatable food supplementation; hydration complemented with glucose solution |
| Penile prolapse | Swollen and distended penis | Lubrification; soft bed; local analgesia |
| Spontaneous turning behavior | Animals spontaneously rotate to the ipsilateral side (unilateral lesion) | Animals adapt to rotational movement |

**Table S4.** Confocal microscopy for glial reaction: acquisition paramaters

| Region | Antigen | PMT Voltage (V) | Laser Transmissivity (%) | Emission Wavelength (nm) | Detection Wavelength (nm) | Detector Gain | Detector Offset (%) |
|---|---|---|---|---|---|---|---|
| **SNpc Mosaics** | DAPI | 410 | 1.01 | 461 | 430-470 | 1.25 | 8.0 |
| | TH | 430 | 1.80 | 520 | 500-540 | 1.0 | 10.0 |
| | GFAP | 600 | 6.49 | 618 | 570-620 | 1.0 | 11.0 |
| | IBA-1 | 570 | 4.88 | 671 | 650-750 | 1.0 | 10.0 |
| **Striatum Images** | DAPI | 410 | 1.30 | 461 | 430-470 | 1.0 | 12.0 |
| | TH | 460 | 2.0 | 520 | 500-540 | 1.0 | 12.0 |
| | GFAP | 650 | 7.0 | 618 | 570-620 | 1.0 | 14.0 |
| | IBA-1 | 595 | 5.0 | 671 | 650-750 | 1.0 | 13.0 |

**References**


1. Masini, D.; Plewnia, C.; Bertho, M.; Scalbert, N.; Caggiano, V.; Fisone, G. A Guide to the Generation of a 6-Hydroxydopamine Mouse Model of Parkinson's Disease for the Study of Non-Motor Symptoms. *Biomedicines* **2021**, *9*, 598, doi:10.3390/biomedicines9060598.