Supplemental Material to manuscript

New HSF1 inducer as a therapeutic factor in a rodent model of Parkinson's disease

Irina V. Ekimova, Daria V. Plaksina, Yuri F. Pastukhov, Ksenia V. Lapshina, Vladimir F. Lazarev, Elena R.Mikhaylova, Sergey G. Polonik, Bibhusita Pani, Boris A. Margulis, Irina V. Guzhova*, and Evgeny Nudler*

Supplementary Figure 1S

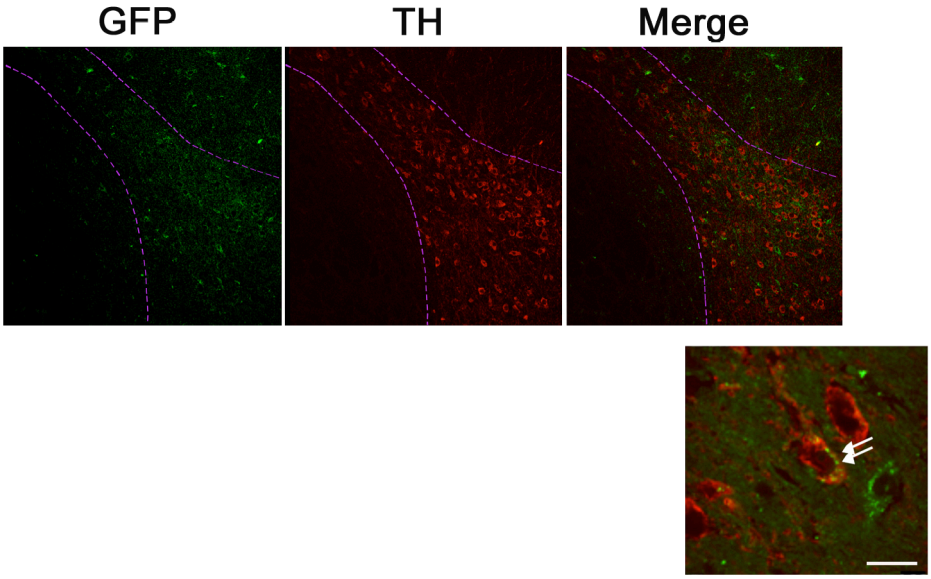


Figure 1S. Target delivery of a lentivirus expressing GFP into SNpc.

Rats were injected with a lentivirus that expresses GFP and slices of brain tissue were studied by optical (**A**) and confocal (**B**) microscopy. Slices in **B** were also stained with an antibody against TH (red) to verify the delivery of the lentivirus to specific neurons.

Supplementary Figure 2S

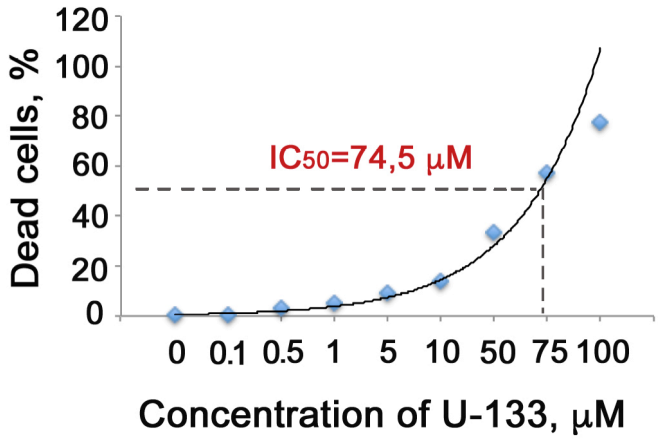


Figure 2S. Echinochrome derivative (U-133) is not toxic for 3T3 mouse fibroblasts. 3T3 cells seeded in the 96-well plate were treated with the indicated amount of U-133 the next day. Cell viability was measured with CytoTox96 according to the manufacture's protocol.

Supplementary figure 3S

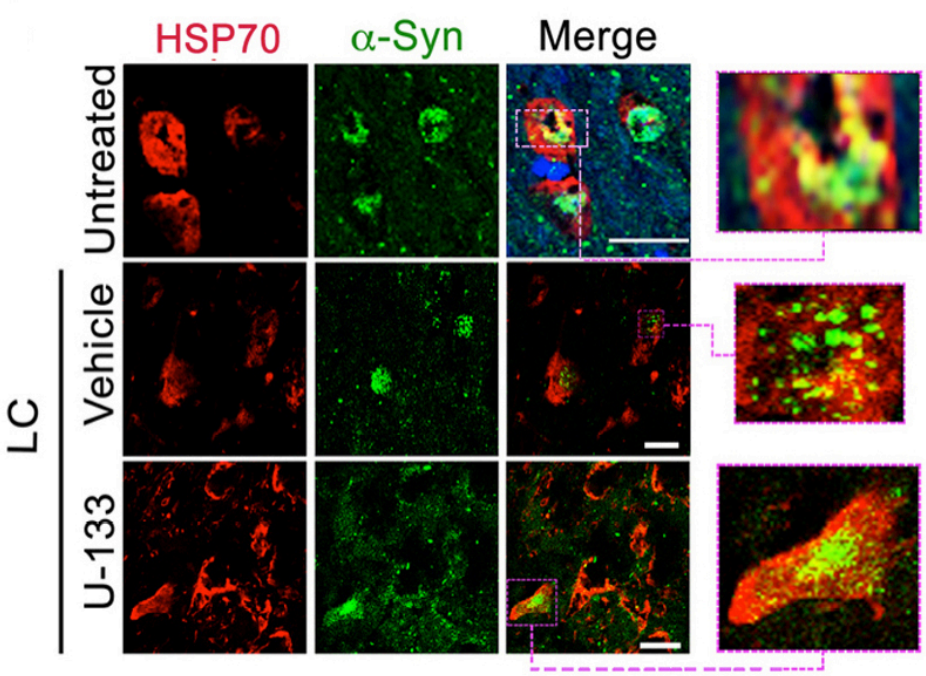


Figure 3S. Hsp70 is colocalizes with a-synuclein aggregates in dopaminergic neurons of SNpc.

Double immunofluorescence staining for Hsp70 (mouse, Clone 3C5) and α-synuclein (1:200; rabbit, Novus Biologicals, USA) was performed. As secondary anti-mouse antibody labeled with Alexa-555, or anti-rabbit antibody labeled with DyLight 488 were used. Fluorescent images were captured by the Leica TCS SP2 confocal system (Leica, Germany). To avoid possible cross interference among various fluorochromes, images for Alexa-555 and DyLight 488 were acquired using a sequential image recording method.

Supplementary figure 4S

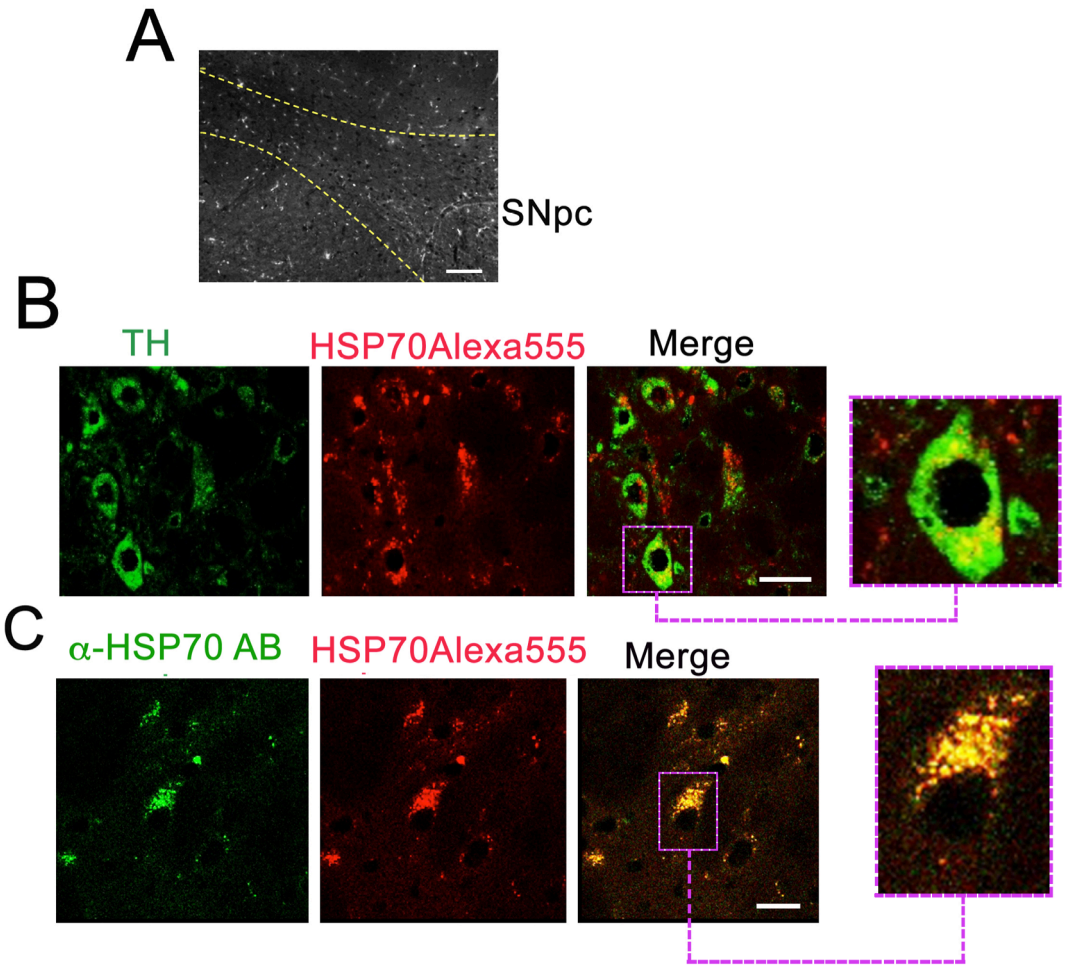


Figure 4S. Intranasally injected HSP70 penetrates into the SNpc and is internalized by the dopaminergic neurons in the LC model, mimicking the clinical stage of PD. HSP70 labeled with Alexa555 (red) was injected intranasally (n=3) and brain slices were studied by optical (A) and confocal microscopy (B and C). Slices were also stained with antibody against TH (B, green). Scale bars: 100 μm for A and 20 μm for B and C.

Supplementary Figure 5S

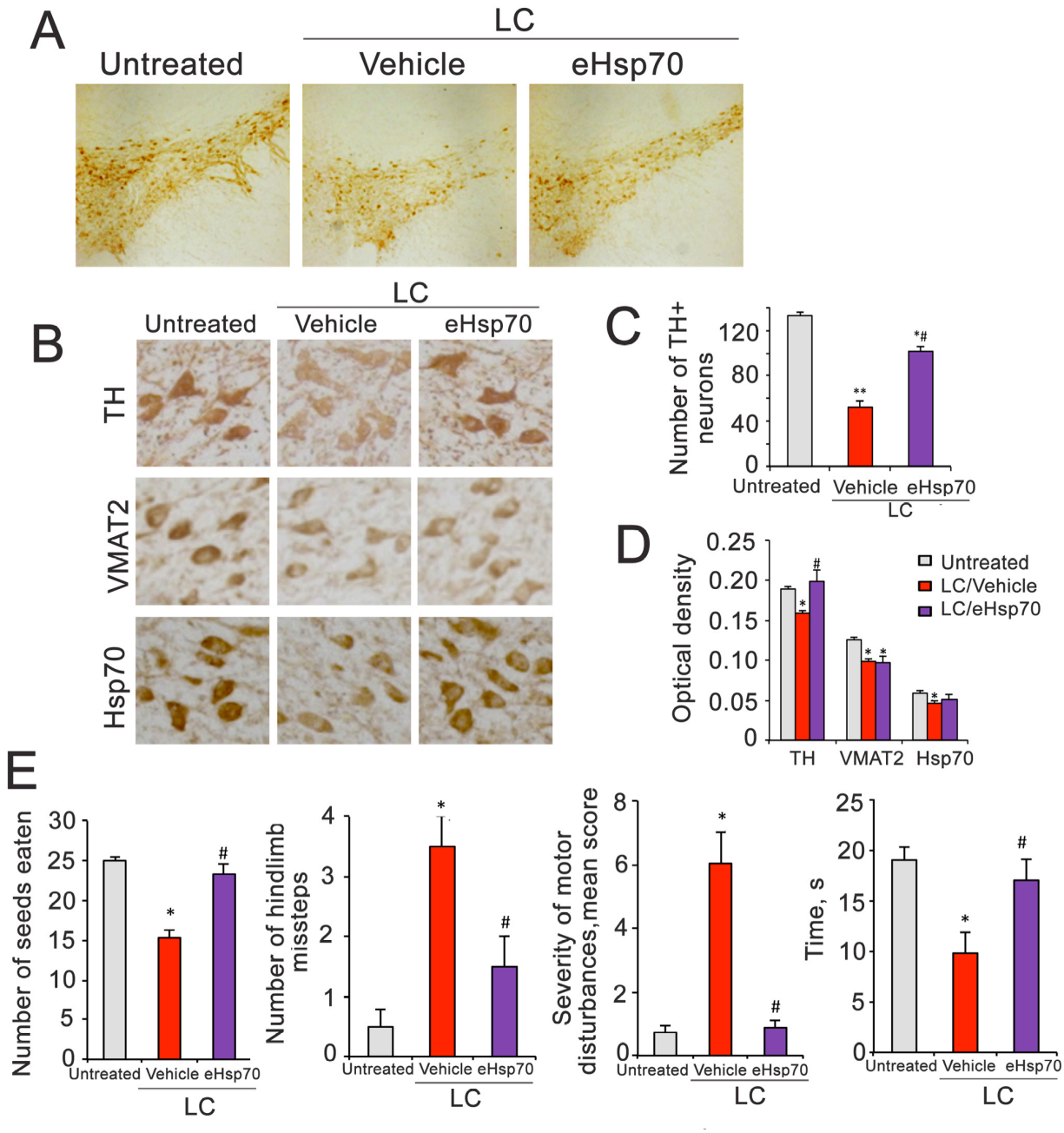


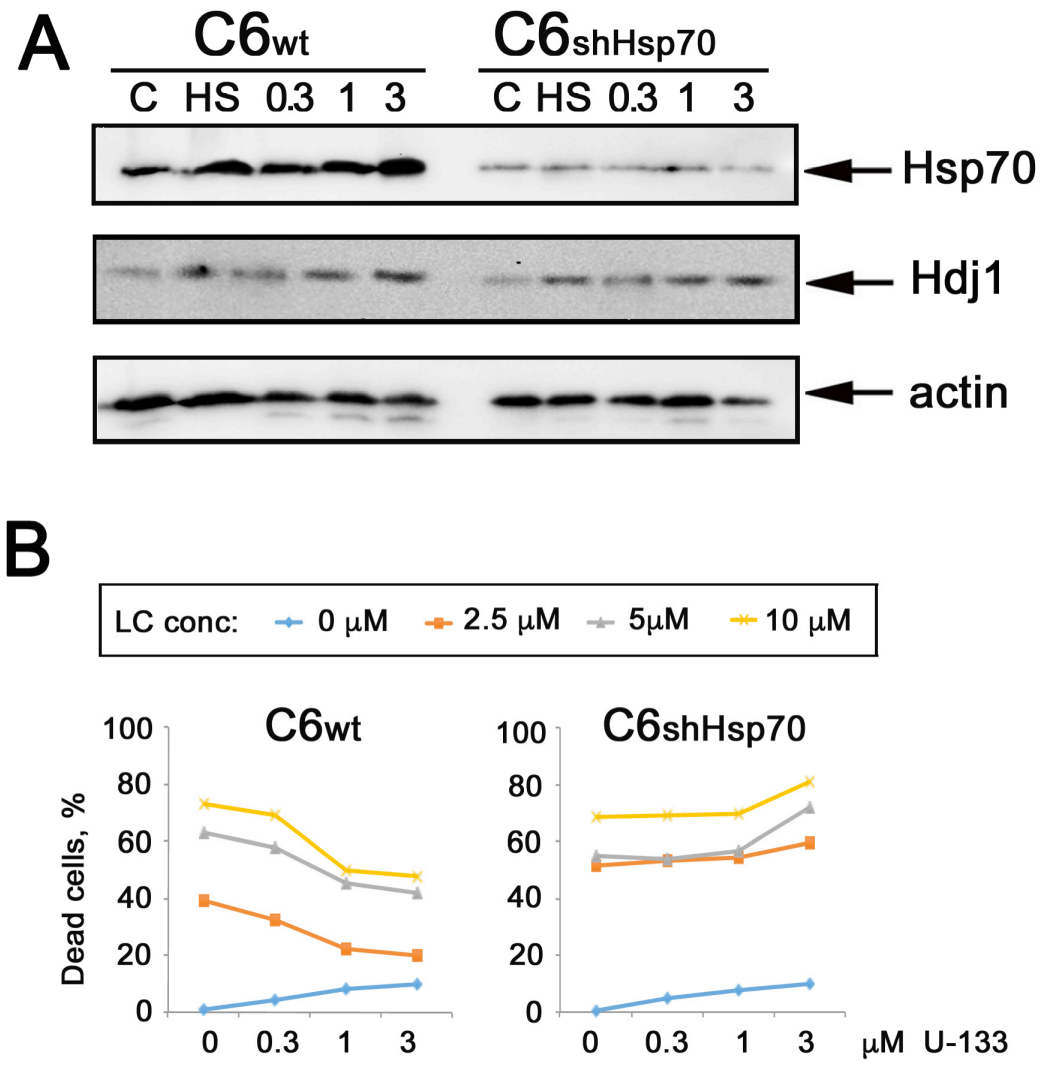
Figure 5S. Treatment of PD-like animals with HSP70 attenuates neurodegeneration in SNpc, stimulates the compensatory mechanisms directed at maintaining the dopamine level in DA neurons, and prevents motor dysfunction.

Recombinant human HSP70, at dose 5 μg in a volume 10 μl of sterile PBS was applied to each nostril 4 h and 24 h after each LC microinjection and 7 days after the last LC microinjection. Intranasal administration of HSP70 was accomplished via a micropipette at

a flow rate of 3 µl/min. Each rat (n=7) was laid on its back so that the head was at an angle of about 45^{o} above the horizontal plane. This angle allows the intranasally injected solution to reach the olfactory epithelium and prevent drips into the nasopharynx. Intranasal administration of recombinant HSP70 protein conjugated with Alexa-555 fluorochrome was carried out to analyze the location of exogenous HSP70 in different brain regions (n=3).

(**A**) Brain slices of LC-animals treated with eHsp70 or vehicle or untreated were stained with TH (**A** and **B**, upper panel) or VMAT2 and HSP70 antibodies (**B**). (**C**) Quantitative analysis of TH-positive neurons in SNpc. (**D**) Optical density of TH, VMAT2, or HSP70 immunoreactivity in individual nigral cells. Scale bars - 50 µm. * $p < 0,05$, ** $p < 0,01$ vs. the vehicle injected group (n=7); # $p < 0,01$ vs. the LC group (n=7). (E) Data from the Sunflower seed, Suok and Inverted horizontal grid tests are presented. * $p < 0,01$ vs. the vehicle injected group; # $p < 0,01$ vs. the LC group.

Supplementary Figure 6S



Supplementary Figure 6S. Downregulation of Hsp70 with by specific shRNA abolishes the protective effect of U-133 against LC toxicity.

(**A**). C6 glioma cells of wild type ($C6_{wt}$) and C6 with downregulated Hsp70 ($C6_{shHsp70}$) were treated with heat shock (HS, 43°C, 30 min) or with the indicated amount (μM) of U-133. Cells were collected 24 hours after induction and Western blotting was performed with antibodies against Hsp70 or Hdj1. Anti-actin antibodies were used for loading control. (**B**) C6wt and C6shHsp70 cells were treated simultaneously with U-133 in concentrations 0.3, 1 or 3 μM and with LC in concentrations 2.5, 5 or 10 μM. Cell viability was measured using the CytoTox96 assay.

Supplemental Figure 7S.

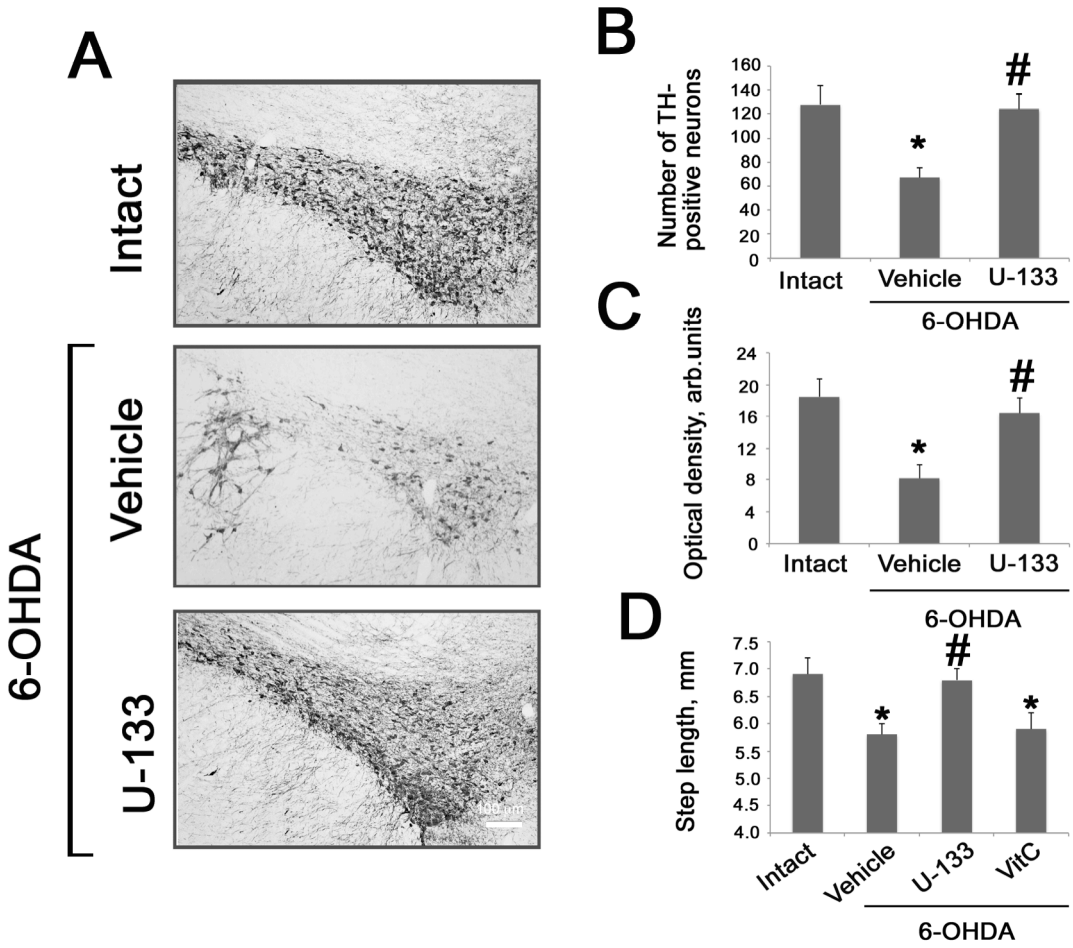


Figure 7S. The U-133 inducer of HSP70 is effective in the 6-hydroxydopamine (6-OHDA) model, mimicking the clinical stage of PD in rats.

Microinjections of 6-OHDA (20 mg/4 ml 0,2% ascorbate saline) were performed bilaterally into the striatum (n=6). The control group (n=6) received 0,2% ascorbate saline as vehicle at the same volume. U-133 was injected intraperitoneally at a dose of 5 mg/kg on the 2nd and 7th days after the 6-OHDA treatment (n=6). Twenty one days after the 6-OHDA injection animals from each experimental group were subjected to the Step test, anaesthetized with Nembutal (50 mg/kg, i.p.), and rapidly transcardially perfused with 50 ml of 0,1 M phosphate buffer and 200 ml 4% paraformaldehyde in 0,1 M phosphate buffer

(pH 7,4). Brains were removed, postfixed in the same fixative, infiltrated with 30% sucrose and then rapidly frozen in cold isopentane (- 42°C) and stored at – 80°C.

A. Brain slices were stained with antibody against TH and (B) the number and optical density (C) of TH-positive neurons were estimated by PhotoM freeware. D. The step length test was performed on the 13th day after 6-OHDA administration. Scale bars: 100 μm. Statistical significance is indicated as *$* p < 0,01$* for comparison with the untreated group; #$p < 0,01$ for comparison with the 6-OHDA treated group.