SUPPLEMENTAL MATERIAL

METHODS

BCA protein assay. Total protein concentration in CSF samples was measured using BCA Protein Assay Kit (Pierce), according to manufacturer's instruction. Briefly, CSF samples were diluted by 1:20 in prepared BCA working reagent and incubated at 37°C for 30 min. Samples were allowed to cool at room temperature, and then absorbance was measured at 562 nm using a plate reader. Final protein concentrations were calculated using a standard curve generated by the absorbance of serially diluted BSA standards.

Isolation of synaptic vesicles. Synaptic vesicles were isolated using a synaptic vesicle kit (Sigma-Aldrich), and anti-synaptophysin antibody staining was used to confirm enriched synaptic vesicle fraction as described (51). The relative abundance of vesicular neuregulin-1 (NRG1) and chromogranin B (CHGB) was quantified by Western blotting for CHGB (diluted 1:1000; Abcam Inc.) and NRG1 (diluted 1:1000; Santa Cruz Biotechnology Inc. H-210) antibodies, followed by Ig-horseradish peroxidase conjugates (diluted 1:2000; Santa Cruz Biotechnology Inc.) with enhanced chemiluminescence detection (Amersham Bioscience) as previously described (14, 15). Equal amounts of protein (BCA Protein Assay) were loaded in each lane and quantitation was done using densitometry of film in the linear range.

CSF collection and storage procedures. For animal studies, CSF was collected immediately following anesthesia by inhalation of isoflurane (2-2.5 % atm) in 2min/L O_2, followed by *cardiac puncture and* thoracectomy. *Cardiac puncture* was performed to collect blood for body water measurements of ^2H_2O enrichments. Immediately after thoracectomy, the head was

positioned at an angle having the cisterna magna facing up. The first incision was made at the base of the brain stem and muscle layers were separated by blunt dissection. Residual muscles lying on top of the dura mater were wiped away with soft cotton gauze in order to expose a clear area of dura mater. The dura mater was then penetrated with a pre-made CSF collection system (30cm long micro-polyethylene tubing-[0.011 I.D X 0.025 O.D] with a 30G1/2 needle at the tip, connected to a 100 µl glass syringe). CSF was aspirated slowly and gently from the cisterna magna avoiding puncturing of the blood vessels (arteria dorsalis spinalis). The collected clear CSF samples (~25 µl per mouse) were without blood contamination and were immediately centrifuged at 400 *g* for 5 min at 4°C to remove cellular debris, then snap-frozen in liquid nitrogen after addition of protease inhibitors to prevent protein degradation **(**Calbiochem, 2.5 µl protease inhibitors cocktail in 25 µl CSF). Samples were stored at -80 °C and thawed on ice prior to use.

For the human study, CSF samples were collected through lumbar puncture at the L3-4 or L4-5 interspace. The first crystal clear 10-15 ml CSF were collected in a sterile polypropylene tube. To avoid adherence of proteins to the test tube walls, protease inhibitors were added and the sample was gently mixed to avoid gradient effects. At the same time, a plasma sample was taken. All CSF samples with more than 500 erythrocytes/ microliter were excluded. The CSF samples were centrifuged at 500 *g* for 10 minutes at 4°C to eliminate cells and other insoluble material. Aliquots of 1 mL were collected in cryogenic polypropylene tubes, fast frozen in liquid nitrogen and stored at −80°C after addition of protease inhibitors to prevent protein degradation **(**Calbiochem, 1mL protease inhibitors cocktail in 4mL CSF). Aliquots were prepared for single use in experiments in order to eliminate any freeze/thaw effects. CSF samples were freshly thawed on ice and centrifuged at 6,800 *g* for 10 minutes at 4°C immediately prior to biochemical

analysis and isolation of cargo proteins. The hemoglobin levels in CSF samples were measured by ELISA Quantitation Kit from Bethyl Lab Inc. according to the manufacturer's instructions. This was done to establish an index of the degree of red blood cell contamination of CSF.

CSF total protein isolation. For total protein isolation, 0.8mL of -20°C Acetone was added to 0.2 mL of albumin/immunoglobulins depleted CSF sample. The sample was vortexed and incubated -20°C for 3 days. The sample was then centrifuged at 14,000g at 4°C for 30 min and the resulting pellet was washed with 1 volume of -20°C Acetone. After centrifugation at 14,000g at 4°C for 20 min, the pellet was dried under vacuum.

Identification of proteins by mass spectrometry. Cargo protein solution was adjusted to 8M urea, subjected to carboxyamidomethylation of cysteines, and digested with trypsin. The sample was then desalted using a c18 spec tip (Varian). A nano LC column, consisting of 10 cm of Polaris C18 5 μm packing material (Varian) was packed in a 100 μm inner diameter glass capillary with an emitter tip. The column was loaded by use of a pressure bomb and washed with buffer A (see below). The column was then directly coupled to an electrospray ionization source mounted on a Thermo-Fisher LTQ XL linear ion trap mass spectrometer. An Agilent 1200 HPLC equipped with a split line so as to deliver a flow rate of 30 nl/min was used for chromatography. Peptides were eluted using a gradient from 100% Buffer A to 40%Buffer A/60% Buffer B. Buffer A was 5% acetonitrile/ 0.02% heptaflurobutyric acid (HBFA); buffer B was 80% acetonitrile/ 0.02% HBFA. The programs SEQUEST and DTASELECT were used to identify peptides and proteins from the rodent and human database (52, 53). Xcorr cutoffs of 1.8, 2.2 and 3.5 were used for inclusion of peptides with +1, +2 and +3 charge states, respectively. These cutoffs have been

shown to yield peptide identifications with less than a 1% false positive rate (54). Two peptides meeting these statistical criteria were required to consider a protein as positively identified.

Measurement of ^2H_2O enrichment in body water. Measurement of ^2H_2O enrichment in body water was measured using a modification of previously described procedure (55). Briefly, plasma samples were centrifuged at 500 *g* for 10 minutes at 4°C to eliminate cells and other insoluble material. Aliquots of 1 mL were collected in cryogenic polypropylene tubes, fast frozen in liquid nitrogen and stored at −80°C. Aliquots of plasma were freshly thawed on ice, diluted 1:100 and placed into the caps of inverted sealed screw-capped vials for overnight distillation at 80°C. Protons from plasma water were transferred to acetylene by reaction with calcium carbide. Acetylene samples were then analyzed using a Series 3000 cycloidal mass spectrometer (Monitor Instruments), which was modified to record ions at m/z 26 and 27 (M0 and M1) and calibrated against a standard curve prepared by mixing 99.9% ^2H_2O with unlabeled water.

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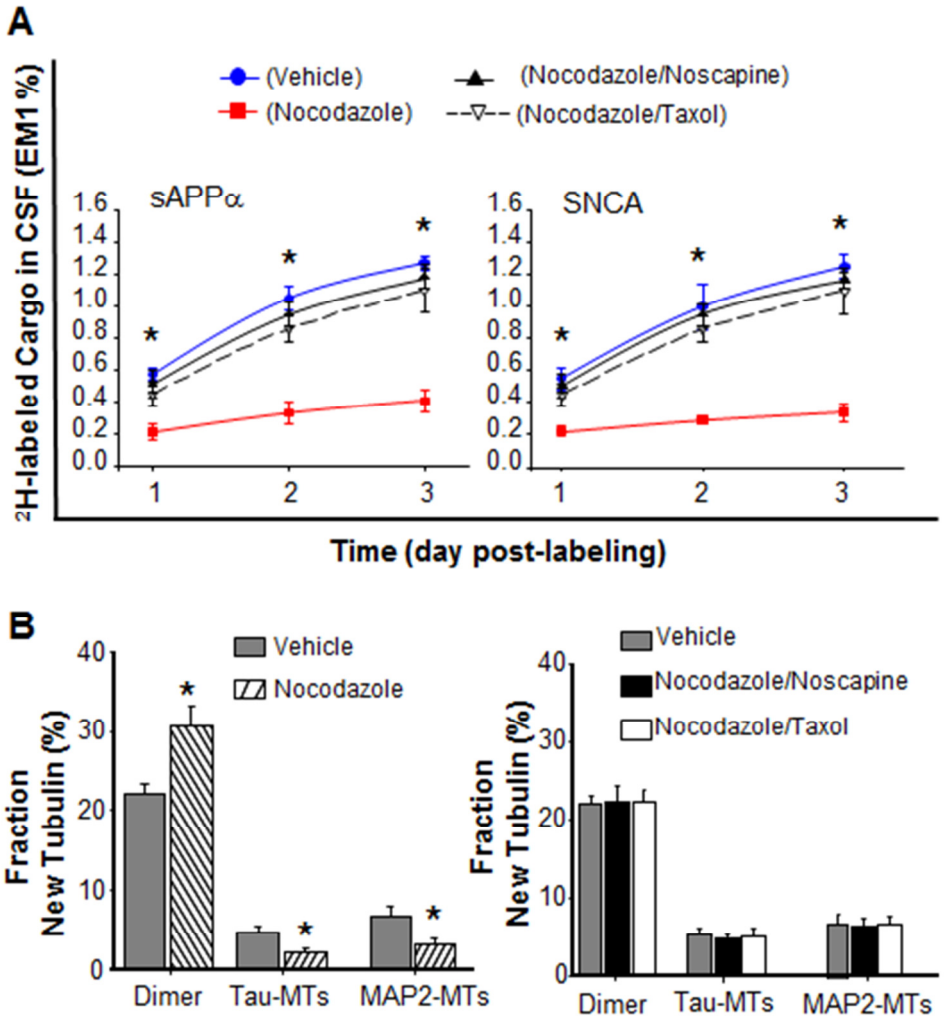
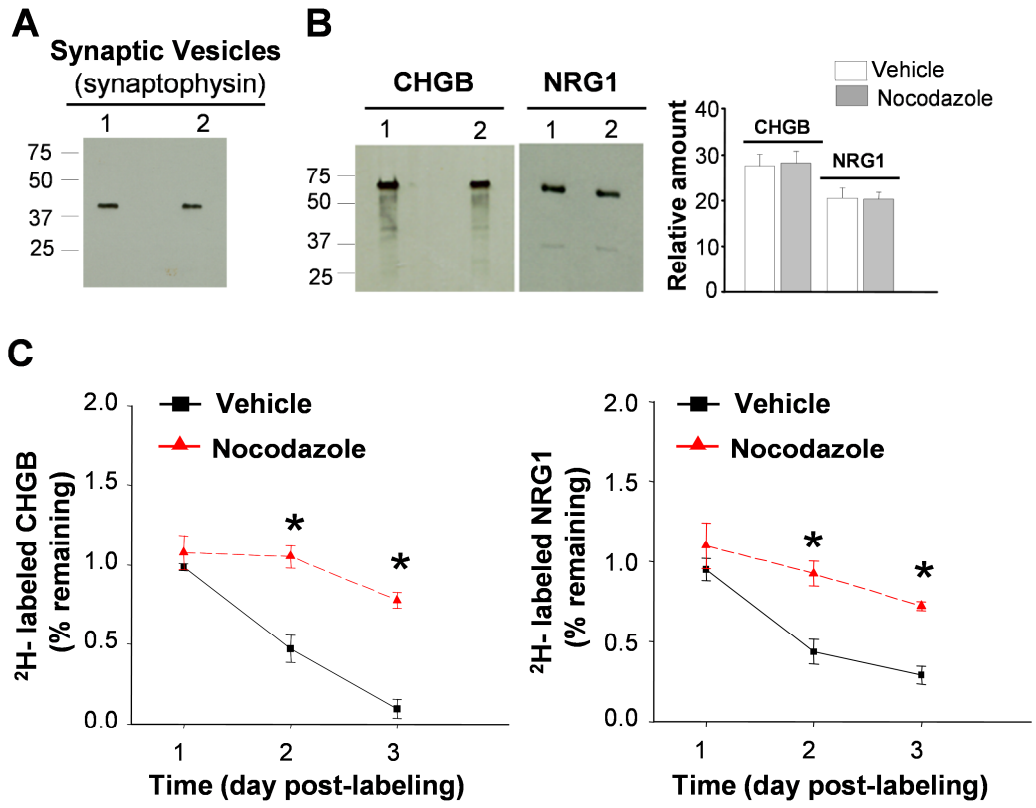
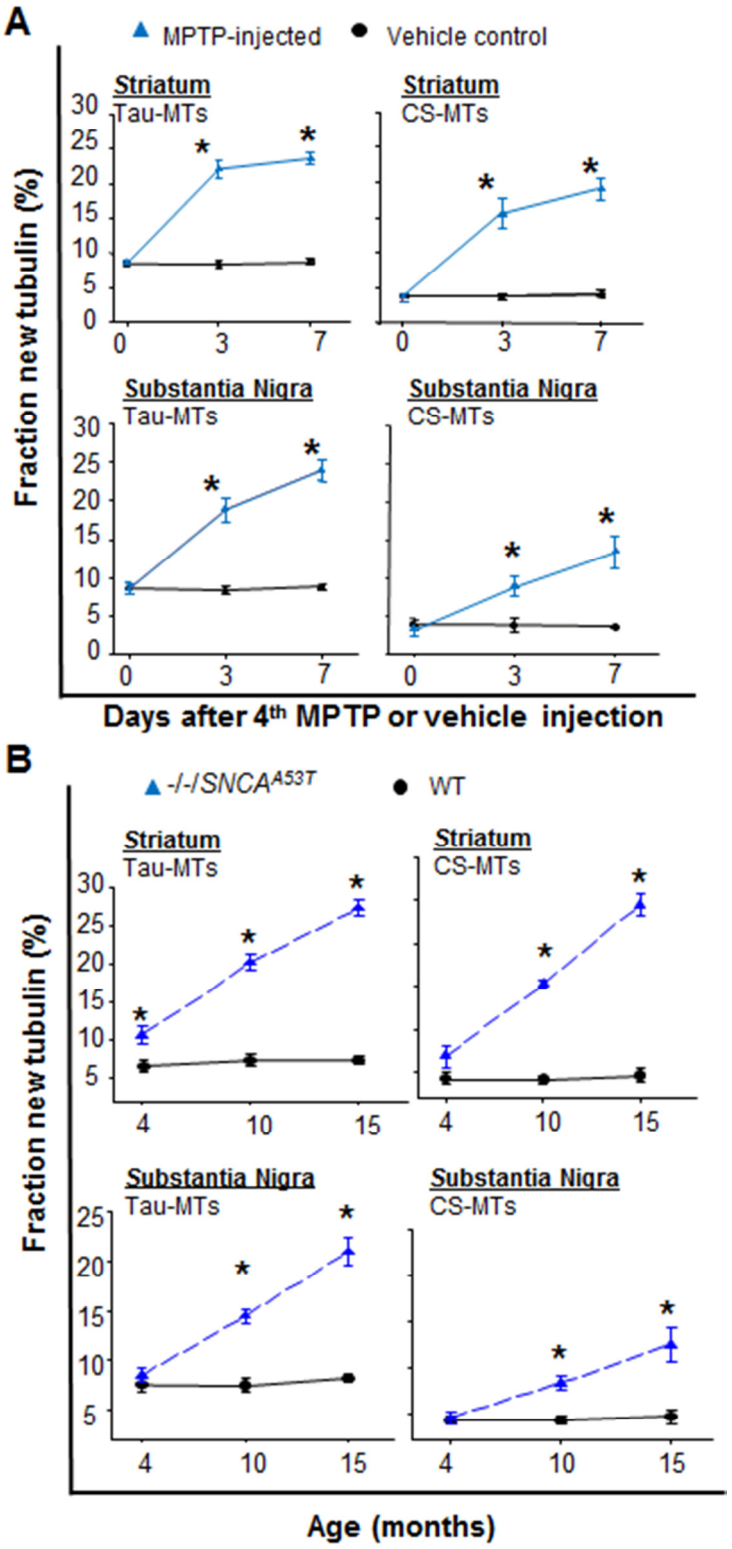


Supplemental Figure 1. Secretion kinetics of sAPPα or SNCA and cortical MT turnover are affected in nocodazole-infused mice. (**A**) Delays in the time of appearance, T_{max} and disappearance of ^{2}H-labeled sAPPα and ^{2}H-labeled SNCA were observed in CSF from nocodazole-infused mice as compared to age-matched vehicle controls (n=10, males aged 8 weeks per each time point; in duplicate; time of appearance, T_{max} and disappearance mean ± SD. *$P < 0.001$). Treatments with noscapine and taxol normalized MT-mediated transport rates of ^{2}H- sAPPα and ^{2}H-SNCA to the levels observed in CSF of age-matched vehicle controls (n=10, males aged 10 weeks per each time point; time of appearance, T_{max} and disappearance, in duplicate mean ± SD. *$P < 0.001$). (**B**) ^{2}H-label incorporation of cortical tubulin in dimers and MTs was compared in the indicated experimental groups. In nocodazole-infused mice an increase in ^{2}H-free tubulin dimers correlated with a reduction in ^{2}H-labeling of MTs associated with tau and MAP2 (n=3 mice per group; mean ± SD; * $P< 0.001$). The nocodazole/noscapine and nocodazole/taxol treated groups showed ^{2}H-labeling of tau-associated and MAP2-associated MTs similar to vehicle controls (n=4 per group; males aged 10 weeks, mean ± SD).

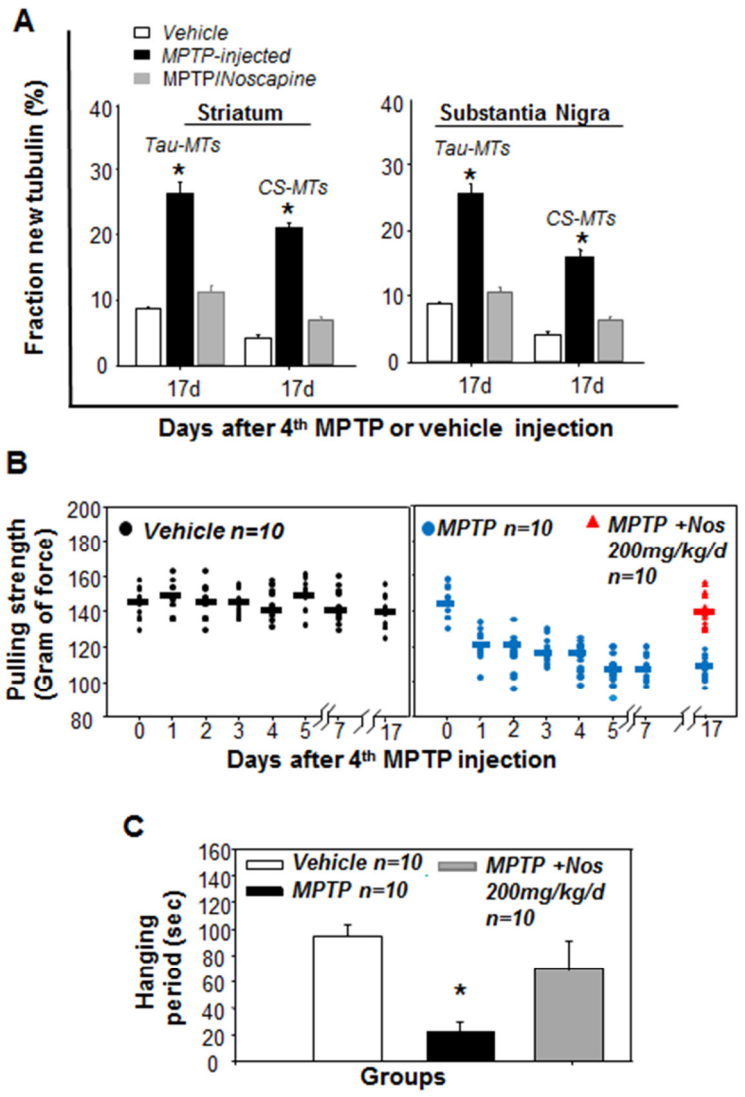


Supplemental Figure 2. Rates of MT-mediated transport of synaptic vesicles are affected in cortical axons of nocodazole-infused mice. Anti-synaptophysin (**A**), anti-CHGB and anti-NRG1 (**B**) Western blots of synaptic vesicles isolated from the cortex of vehicle (lane 1) and nocodazole treated (lane 2) mice (*n*=4, per group) showed equal quantitative isolation of CHGB and NRG1. Positions of *molecular weight* protein *markers* in kiloDaltons *are shown on left*-hand *side* of *each* panel. (**C**) Pulse $^{2}H_{2}O$ labeling revealed marked differences (persistence of labeled cargo) in the rate of MT-mediated transport of synaptic vesicles in cortical axons of nocodazole as compared to vehicle treated mice (*n*=4 per each time point; in duplicate; mean ± SD; **P*< 0.001).

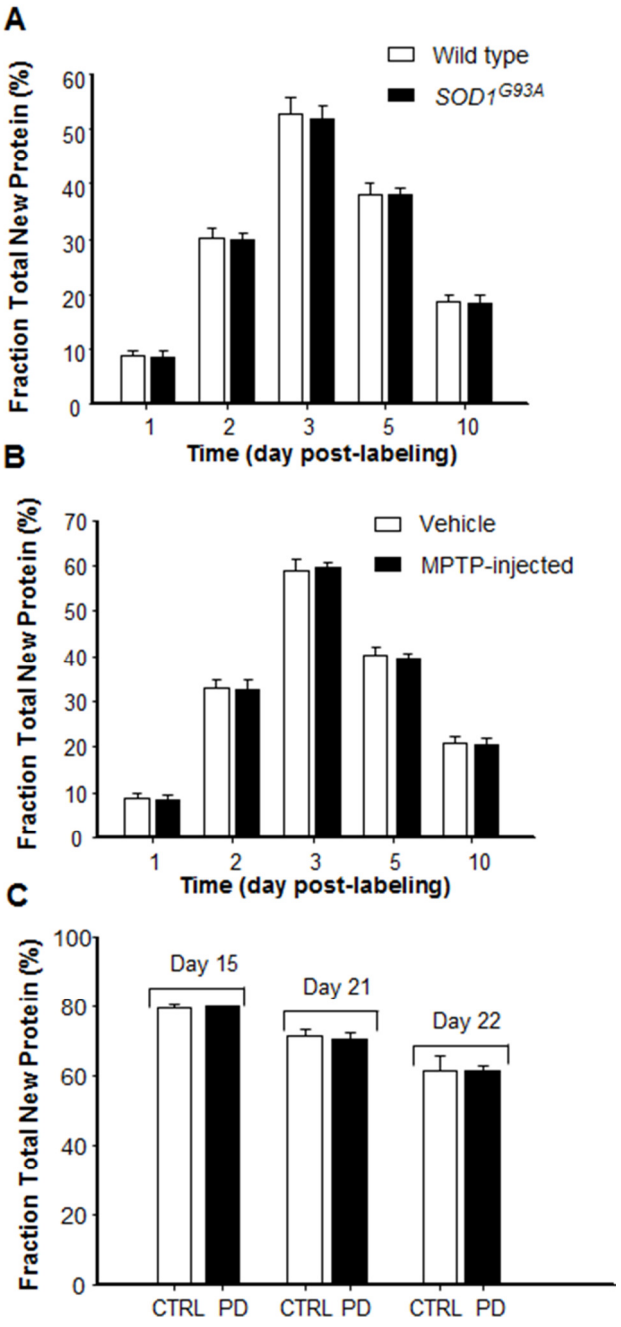


Supplemental Figure 3. Alteration of microtubule turnover in MPTP-injected and PD -/-/*SNCA*^{A53T} mice. (**A**) 8wk old C57bl/6 males that received an acute MPTP intoxication (four injections of 20 mg/kg on 1 day, at 2 h interval) show hyperdynamic tau- associated and cold-

stable (CS) MTs in striatum and substantia nigra at 3 and 7 days after the last (fourth) MPTP-injection (n=4 per group; males aged 8 weeks; mean ± SD; *P< 0.001). (**B**) In vivo MT dynamics was measured in subtantia nigra and striatum at presymptomatic (4 and 10 months) and symptomatic (15 months) disease stages in -/-/$SNCA^{A53T}$ mice. Tau- associated and CS- MTs were extraordinarily dynamic in these mice well prior to symptoms, and dynamicity continued to increase as disease advanced (n=3 per group; mean ± SD; *P< 0.001).



Supplemental Figure 4. Noscapine normalizes microtubule turnover and reverses symptoms in MPTP-injected mice. (**A**) Treatment with noscapine reduced hyperdynamic MTs (*n*= 4 per group, males aged 8 weeks, mean ± SD.). Noscapine was administered at 7 days after the last MPTP-injection, treatment was for 10 days and a pulse $^{2}H_2O$ labeling was administered the last day of treatment (17 days after the last, fourth, MPTP injection). (**B, C**) Treatment with noscapine (Nos) reverses symptoms in MPTP-injected mice. Noscapine was administered at 7 days after the last MPTP-injection, treatment was for 10 days and a pulse $^{2}H_2O$ labeling was administered the last day of treatment (17 days after the last (fourth) MPTP injection). Neuromuscular strength was measured using the grip strength test (**B**) and the grid hang test (**C**). Grip strength test measured the tension peak of pull (gram of force) by the forepaws and it was performed 1 day before (day 0), 1, 2, 3 4, 5, 7 and 17 days after the last (fourth) MPTP-injection (10♂ per each time point). The hang test was performed 17 days after the last (fourth) MPTP-injection and percentage of success was recorded as maximum time hanging/300 sec X 100 (*n*=10, males aged 8 weeks per each time point; in duplicate, time of appearance, T_{max} and disappearance; mean ± SD; **P* < 0.001).



Supplemental Figure 5. Turnover rate of newly synthesized total proteins in CSF of symptomatic *SOD1*^{G93A} mice, MPTP-injected mice and PD subjects compared to healthy controls. (**A**, **B**) Fractional synthesis (turnover) of total proteins is unchanged in the CSF of symptomatic 13 week old *SOD1*^{G93A} mice (*n*=5male/5female per each time point; in duplicate, mean ± SD.) and in CSF of symptomatic MPTP-injected mice (*n*=10, males per each time point; in duplicate, mean ± SD) as compared to age-matched WT and vehicle controls. (**C**) Fractional synthesis (turnover) of total proteins is unchanged in the CSF of ten PD subjects as compared to healthy controls (CTRL), mean ± SD was generated by technical replicates of repeated preparations and analyses.

Supplemental Table 1. Levels of phosphorylated tau in mouse brain homogenates.

	Vehicle controls	**MPTP-injected**
Striatum Total Tau (ng/mL)	0.58± 0.2 (*n*=10)	0.57± 0.2 (*n* =10)
Striatum P-Tau (Ser 262) (ng/mL)	0.014± 0.01 (*n* =10)	0.28± 0.1 (*n* =10)
Substantia Nigra Total Tau (ng/mL)	0.64± 0.2 (*n* =10)	0.62± 0.2 (*n* =10)
Substantia Nigra P-Tau (Ser 262) (ng/mL)	0.017± 0.1 (*n* =10)	0.31± 0.1 (*n* =10)
Cerebellum Total Tau (ng/mL)	0.78± 0.2 (*n* =10)	0.77± 0.2 (*n* =10)
Cerebellum P-Tau (Ser 262) (ng/mL)	0.014± 0.1 (*n* =10)	0.013± 0.1 (*n* =10)

Total tau and phosphorylated tau (P-Tau) at serine 262 were quantitated by ELISA. Values represent mean ± SD, three replicates.