Brain-to-stomach transfer of α-synuclein via vagal preganglionic projections

Ayse Ulusoy,1 Robert J. Phillips,2 Michael Helwig,1 Michael Klinkenberg,1 Terry L. Powley2 and Donato A. Di Monte1

1German Center for Neurodegenerative Diseases (DZNE), Sigmund-Freud-Strasse 27, 53127 Bonn, Germany

2Department of Psychological Sciences, Purdue University, 703 Third Street, West Lafayette, IN 47907-2081, USA

Correspondence to: Donato A. Di Monte, German Center for Neurodegenerative Diseases (DZNE), Sigmund-Freud-Strasse 27, 53127 Bonn, Germany

E-mail: donato.dimonte@dzne.de

Phone: +49 228 43302650

Fax: +49 228 43302689
Supplementary Fig. 1 Rats (n = 3) received a single midbrain injection of hα-synuclein-AAVs and were killed at 6 months post-treatment. a, b Consecutive sections of the medulla oblongata containing the DMnX were double-stained with anti-hα-synuclein (red) and anti-ChAT (green) (a) or incubated with anti-hα-synuclein in the absence of ChAT primary antibody (b). The right DMnX is delineated by dashed lines, and the area postrema (AP) and central canal (cc) are indicated. Scale bars = 200 μm. c, d Consecutive sections of the medulla oblongata at the level where intramedullary fibers of the right vagus nerve are formed were double-stained with anti-hα-synuclein and anti-ChAT (c) or incubated with anti-hα-synuclein in the absence of ChAT primary antibody (d). Scale bars = 10 μm. Lack of labeling in (b) and (d) confirms specificity of the ChAT labeling.
Supplementary Fig. 2 Rats (n = 3) received a single midbrain injection of hα-synuclein-AAVs and were killed at 2 months post-treatment. a-e Sections from the lower midbrain (representative images in a and b) and from the pons (representative images in c-e) were processed for fluorescent in situ hybridization to detect WPRE mRNA (white). Sections were also counterstained with DAPI (blue). The square box in (a) encompasses the periaqueductal gray shown at higher magnification in (b). The rectangular boxes in (c) encompass the right locus coeruleus / parabrachial nucleus and the pontine reticular nucleus that are shown at higher magnification in (d) and (e), respectively. No white signal (i.e. no WPRE hybridization) was detected in any of the sections from these brain regions. Scale bars = 1 mm in (a and c) and 500 μm in (b, d and e).
Supplementary Fig. 3 a-d Rats (n ≥ 5/time point) received a single midbrain injection of hα-synuclein-AAVs and were killed at 2 and 12 months post-treatment. Sections of the medulla oblongata were double-stained with anti-ChAT and anti-hα-synuclein (hα-syn). Representative confocal images show DMnX axons co-labeled with hα-syn and ChAT (a and c; white arrows in the merged panels), or immunoreactive for hα-syn but not ChAT (b and d). Scale bars = 10 µm.
Supplementary Fig. 4 a, b Rats (n = 5) were killed at 6 months after a single midbrain injection of hα-synuclein-AAVs. Sections of the midbrain containing the substantia nigra pars compacta (delineated by dashed lines in the left panels) were stained with an antibody (Syn-F1) that specifically recognizes mature α-synuclein fibrils (a) or an antibody (Syn-O2) that recognizes oligomeric and fibrillar forms of α-synuclein (b). Higher magnification images (right panels) show nigral cell bodies and neuronal projections containing aggregated α-synuclein. Scale bars = 250 µm (lower magnification) and 20 µm (higher magnification).
Supplementary Fig. 5 Rats (n = 5) received a single injection of GFP-AAVs in the right midbrain and were killed at 12 months. a Sections of the right medulla oblongata were double-stained with anti-ChAT (green) and anti-GFP (false-colored in red). ChAT-positive neurons and neuronal projections are shown in the DMnX (left panels); intramedullary fibers of the vagus nerve were also labeled for ChAT in caudal sections of the medulla oblongata (cMO; right panels). Please note the absence of GFP co-labeling. Scale bars = 10 µm. b Longitudinal sections of right vagus nerves were stained with anti-GFP. The representative image shows no specific immunoreactivity. Scale bar = 10 µm. c Stomach whole mounts were stained with anti-GFP and counterstained with Cuprolinic Blue. The representative image shows ganglionic cells of the myenteric plexus. No specific GFP immunoreactivity was detected. Scale bar = 50 µm.