

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

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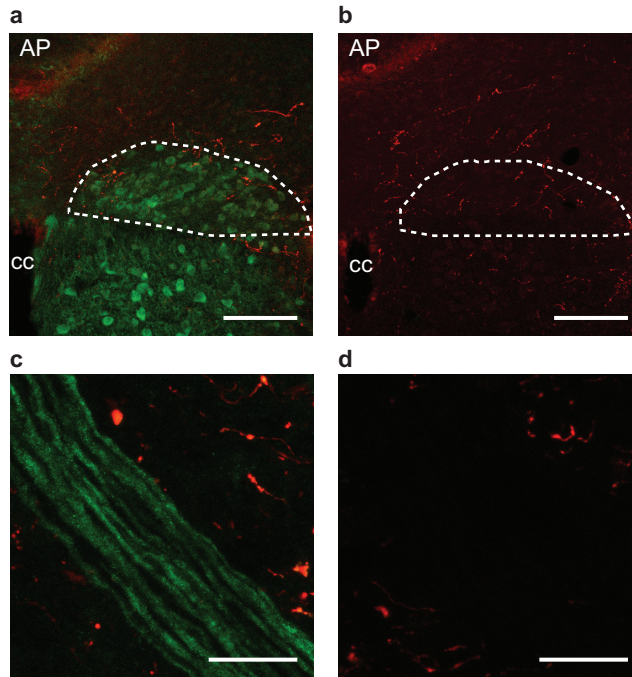
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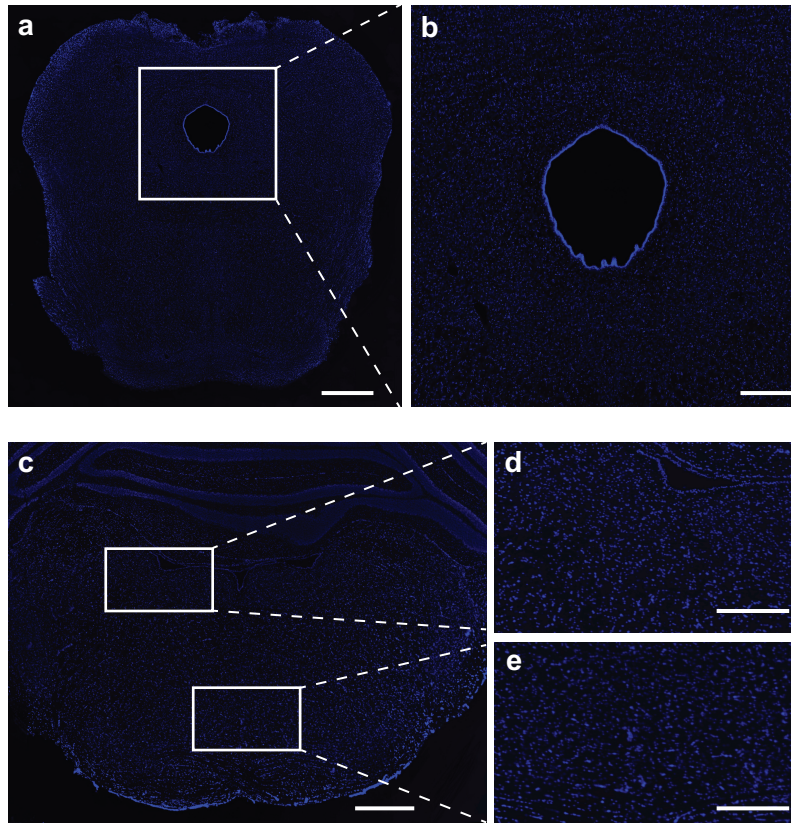
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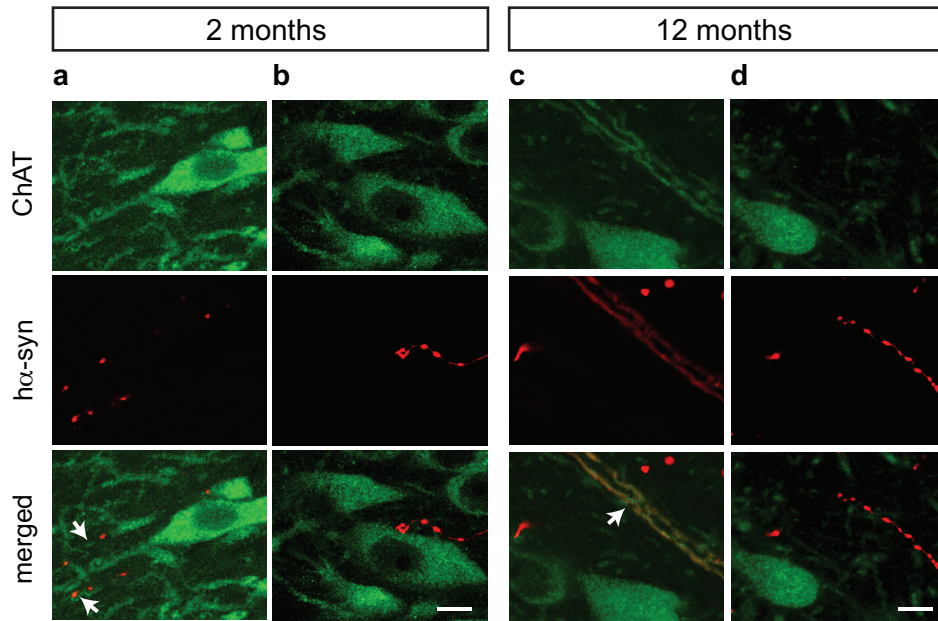
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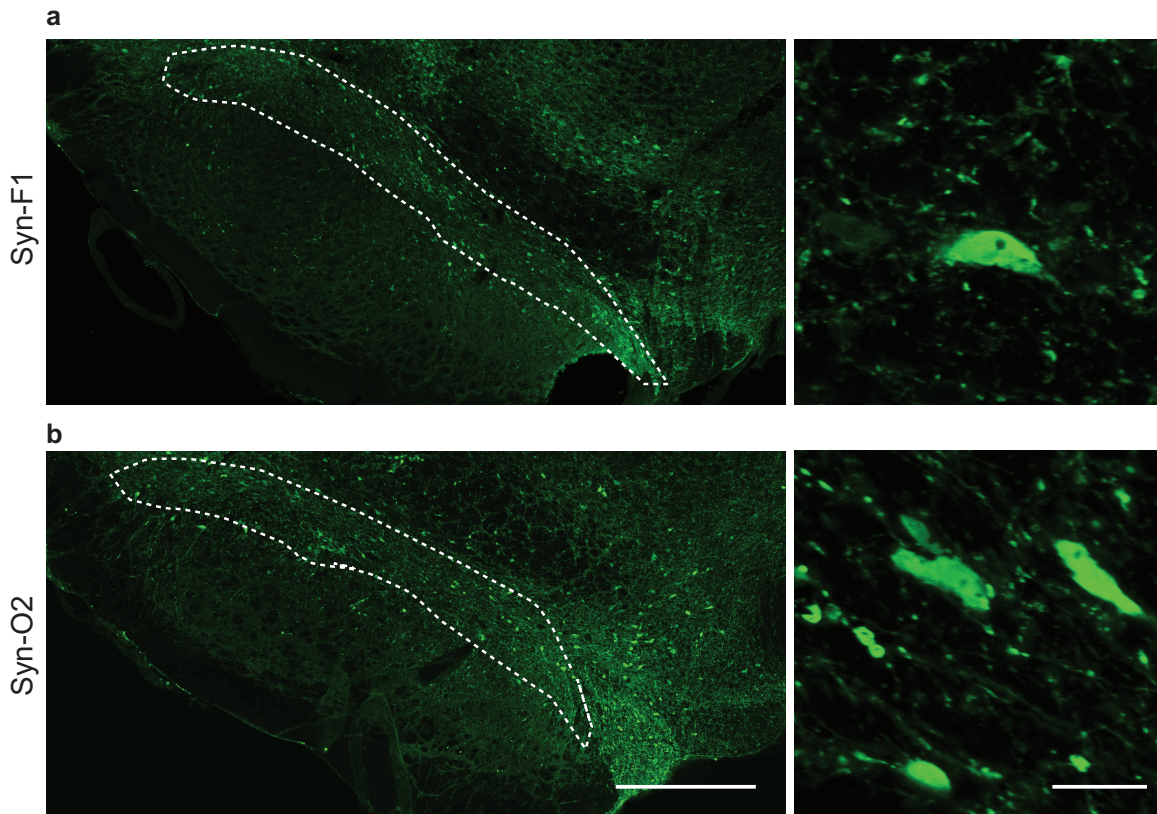
Supplementary Fig. 1 Rats ($n = 3$) received a single midbrain injection of h α -synuclein-AAVs and were killed at 6 months post-treatment. **a, b** Consecutive sections of the medulla oblongata containing the DMnX were double-stained with anti-h α -synuclein (red) and anti-ChAT (green) (**a**) or incubated with anti-h α -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 μ m. **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-h α -synuclein and anti-ChAT (**c**) or incubated with anti-h α -synuclein in the absence of ChAT primary antibody (**d**). Scale bars = 10 μ m. Lack of labeling in (**b**) and (**d**) confirms specificity of the ChAT labeling.



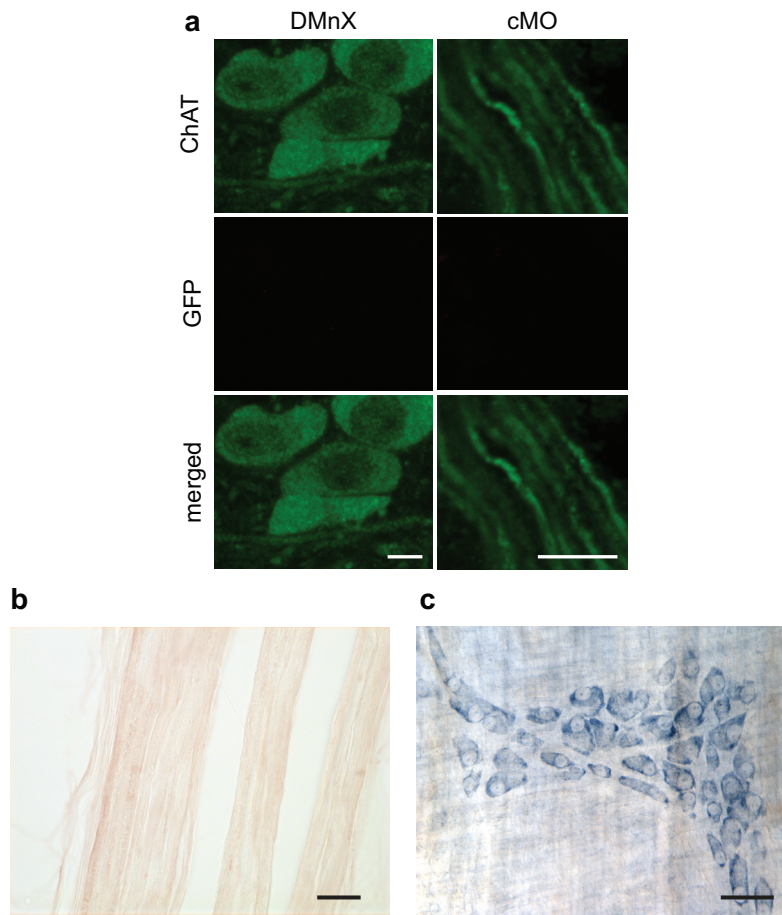
Supplementary Fig. 2 Rats ($n = 3$) received a single midbrain injection of α -synuclein-AAVs and were killed at 2 months post-treatment. **a-e** Sections from the lower midbrain (representative images in **a** and **b**) and from the pons (representative images in **c-e**) were processed for fluorescent *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 μ m in (**b**, **d** and **e**).



Supplementary Fig. 3 a-d Rats ($n \geq 5$ /time point) received a single midbrain injection of h α -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-h α -synuclein (h α -syn). Representative confocal images show DMnX axons co-labeled with h α -syn and ChAT (**a** and **c**; white arrows in the merged panels), or immunoreactive for h α -syn but not ChAT (**b** and **d**). Scale bars = 10 μ m.



Supplementary Fig. 4 a, b Rats ($n = 5$) were killed at 6 months after a single midbrain injection of h α -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 μ m (lower magnification) and 20 μ m (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 .