Oral and intravenous transmission of α-synuclein fibrils to mice

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Online Resource 2 Deposits of phosphorylated α-synuclein accumulate in the motor cortex of diseased TgM83$^{+/-}$ mice

Immunohistochemical staining with an antibody to α-synuclein phosphorylated at Ser129 revealed that diseased animals had accumulated deposits of phosphorylated α-synuclein in neuronal cell somata (arrow heads) and neurites (arrows) of the motor cortex (**a**). To assess pathology in the motor cortex in dependence on the route of inoculation, we separately counted the number of somata (**b**) and neurites (**c**) with α-synuclein pathology in each cortical layer (I, II-III, IV, V, VI). In the motor cortex, pathology was most pronounced after intracerebral inoculation and weakest after intraperitoneal inoculation, and intermediate after oral or intravenous challenge. Cell body pathology was absent in layer I and most pronounced in layer V. Neurites were sparsely present in layer I but frequent in all other layers. Data are presented as mean ± standard deviation and are derived from three to four animals per inoculation group. WM: white matter. Scale bars = 200 µm.

Online Resource 3 Deposits of phosphorylated α-synuclein colocalize with ubiquitin in the spinal cord of diseased TgM83$^{+/-}$ mice

Immunofluorescence staining of tissue sections of the spinal cord show that phosphorylated α-synuclein (red), detected with the EP1536Y antibody, and ubiquitin (green) colocalize in affected neurons of diseased mice. We observed similar results regardless of the route of challenge. Neurons of BSA-challenged mice, for which only merged images are shown, did not accumulate any excessively phosphorylated α-synuclein or ubiquitinated protein deposits. Nuclear staining with DAPI is shown in blue. Scale bar = 20 µm.

Online Resource 4 Deposits of phosphorylated α-synuclein colocalize with p62 in the spinal cord of diseased TgM83$^{+/-}$ mice

Immunofluorescence staining of tissue sections of the spinal cord show that phosphorylated α-synuclein (red), detected with the pSyn#64 antibody, and p62 (green) colocalize in affected neurons of diseased mice. We observed similar results regardless of the route of challenge. Neurons of BSA-challenged mice, for which only merged images are shown, did not accumulate any excessively phosphorylated α-synuclein or p62-tagged protein deposits. Nuclear staining with DAPI is shown in blue. Scale bar = 20 μm.

Online Resource 5 Astrogliosis in the spinal cord of diseased TgM83$^{+/-}$ mice

Immunofluorescence staining of spinal cord tissue sections with an antibody against GFAP, a marker for astrocytes, shows that neurons of diseased TgM83$^{+/-}$ mice with deposits of phosphorylated α-synuclein (red), which were detected with the pSyn#64 antibody, were frequently surrounded by reactive astrocytes (green). In contrast, no excessive abundance of phosphorylated α-synuclein or reactive astrocytes was observed in the spinal cord of BSA-challenged, healthy control mice. Nuclear staining with DAPI is shown in blue. Scale bar = 20 μm.

Online Resource 6 Microgliosis in the spinal cord of diseased TgM83$^{+/-}$ mice

Immunofluorescence staining of spinal cord tissue sections with an antibody against IBA-1, a marker for microglia, shows that neurons of diseased TgM83$^{+/-}$ mice with deposits of phosphorylated α-synuclein (red) , which were detected with the pSyn#64 antibody, were frequently surrounded by activated microglia (green) with an amoeboid morphology. In contrast, no excessive abundance of phosphorylated α-

synuclein or activated microglia was observed in the spinal cord of BSA-challenged, healthy control mice. Nuclear staining with DAPI is shown in blue. Scale bar = 20 μm.