SUPPLEMENTARY INFORMATION:

Supplementary Methods.

Pathology and image analysis.

Toluidene blue-stained semi-thin sections were imaged using a x 100 objective on an Axiovert 200M with an Axiocam HR camera (Carl Zeiss). In order to capture the whole area of the medullary gracile nucleus and the lumbar spinal tracts up to 200 pictures were taken and stitched together using Axiovision MosaiX software (Carl Zeiss). The compiled image was converted to JPEG format and view at 100% in Photoshop CS4 Extended (Adobe). Lesions are identified then counted using the Count Tolol in CS4. The criteria for typing axonal lesions (swollen or degenerating; with the latter originally sub-divided into dense-body and vacuolated types) are summarized in Results describing Fig 2A and depended on both the size and the morphological appearance. Swollen axons generally had diameters > 14 μm. Some abnormal axons had features of both dense-body and vacuolated types. A small area of one of the stitched images of the gracile nucleus with the lesions typed as either swollen or degenerating is shown in Supplementary Fig S1 as an illustrative example of the method.

Assay of PtdCho and cholesterol in mouse brain tissue.

PtdCho was determined essentially as described by Murai *et al* (2004). Briefly, tissue was homogenized in 0.4M $HClO_4$, lipids were extracted in $CHCl_3$, and then PtdCho was hydrolysed to choline (and phosphatidic acid) by heating (90°C; 1h) in 1M $HClO_4$/0.5% Triton-X100. After neutralization, aliquots were taken for assay of choline and (as an internal control) cholesterol by enzyme-linked assays using Amplex kits (Molecular Probes). For the data in Fig 4, values obtained for individual cerebral hemispheres were expressed relative (%) to the mean value for vehicle-dosed control or WT control within the same assay; % values from separate experiments were then aggregated to yield the final data. Statistical significance was assessed by Student t-test.

Secretion of reelin and APP from CGN.

Cerebellar granule neurons were isolated from NTE-cKO and litter-mate WT mice at post-natal day 7 and plated at 0.5 x 10^6 cells per well in 24-well plates in Neueobasal medium with B27 and 25mM KCl (Read et al, 2007). Medium was aspirated from CGN at 7 days *in vitro*; the cells were washed three times with PBS, then covered with 0.5ml fresh medium

and re-incubated. For assay of APP secretion, a potent, cell-permeable inhibitor of β-secretase (Inhibitor IV; Calbiochem) was added to give a final concentration of 2 μM, 2 - 3 h before removing the original medium and was thereafter added, at the same concentration, to the fresh medium. (In assays with APP^{NFEV}-expressing HEK293 cells, Inhibitor IV has an IC_{50} of 29 nM; Stachel et al, 2004). This was to minimize cleavage of APP by β-secretase so that the great majority of the APP fragment detected in the medium is derived from cleavage by α-secretase; ie, sAPPα. We adopted this approach with Inhibitor IV because we could not detect murine sAPPα in CGN-conditioned medium using a monoclonal antibody, 2B3 (Immuno-Biological Labs Company) putatively specific for this fragment. (In fact, we found that 2B3 reacted on Western blots with a polypeptide of ~100kDa present in the B27 component of un-conditioned medium). After various times (up to 4h) medium was again removed and saved, the cells again washed three times with PBS and then harvested by trituration in 0.5ml RIPA buffer (50mM Tris-HCl, pH 7.4 (at 20°C) with 150mM NaCl, 1mM EDTA, 0.5% (w/v) Triton X-100, 0.125% (w/v) sodium deoxycholate, 1% (w/v) protease inhibitor cocktail (Pierce)). Both the cell lysate and the conditioned medium were centrifuged (16,000xg; 4 min) to remove debris and an aliquot of the supernatants was mixed with 10X-concentrated SDS-PAGE sample buffer (10% (w/v) SDS, 10% (w/v) glycerol, 5% (w/v) dithiothreitol). Aliquots of the cell lysate and corresponding conditioned medium were run in adjacent lanes on 4-20% gradient gels (Lonza) then transferred (16h; 25V) onto a nitrocellulose membrane. The blot was probed with mouse monoclonal antibodies to either reelin (G10; Abcam; 1:1000), or APP (epitope at residues 66-81; MAB348; Millipore; 1:4000). Subsequently blots were incubated with peroxidase-labelled anti-mouse IgG (1:1000), then enhanced chemiluminescence reagents (Amersham). Bands on the resulting films were quantified by densitometry. A secretion index for reelin (the sum of the 400kDa and 180kDa forms) or APP (the sum of the ~100 kDa and ~80 kDa forms) was expressed as the ratio of reelin or APP in medium to that in the corresponding cells for cultures in individual wells. For **Fig 4D**, in each of 6 separate experiments, 3 – 4 wells of both WT and NTE-cKO CGN were harvested at 2h and analysed. Reelin secretion indices for individual samples were expressed relative (%) to the mean secretion index of WT samples in the same experiment. Aggregate data (**n = 20** for both WT and NTE-cKO) were used to compute the final result: WT = 100 $\pm$ 3.2 %; cKO = 82.4 $\pm$ 4.1 % (mean and standard error): P = 0.00148 (Student t-test). For **Fig 4F**, in each of three separate experiments, 6 - 8 wells of both WT and NTE-cKO CGN were harvested at each of 3 time points (1, 2, 4h). APP secretion indices for individual samples were expressed relative (%) to the mean secretion index of WT samples at that time point in each experiment. Aggregate data from

all 3 time points (**n = 59** for both WT and NTE-cKO) were used to compute the final result: WT = 100 $\pm$ 3.1 %; cKO = 79.7 $\pm$ 3.0 % (mean and standard error): P = 4.936E-07 (Student t-test).

References for Supplementary Information:

Murai, S., Saito, H., Shirato, R., Tamura, H., Yamada, A. and Kato, H. (2004) An improved method for assaying phosphatidylcholine in mouse tissue. *J. Pharmacol. Toxicol. Methods* **50**, 223-229.

Stachel, S.J, Coburn, C.A., Steele, T.G., Jones, K.G., Loutzenhiser, E.F., Grego, A.R., *et al* (2004) Structure-based design of potent and selective cell-permeable inhibitors of human beta-secetase. *J. Med. Chem.* **47**, 6447-6450.

Read, D.J., Langford, L., Barbour, H.R., Forshaw, P.J. and Glynn, P. (2007) Phospholipase B activity and organophosphorus compound toxicity in cultured neural cells. *Toxicol. Appl. Pharmacol.* **219**, 190-195.

Supplementary Fig S1: Typing of axonal lesions: an illustrative example.

A small area of a stitched image covering the medullary gracile nucleus from a 3 month NTE-cKO mouse showing assignment of abnormal axons to either the degenerating (red) or swollen (green) types.

Supplementary Fig S2: Massively swollen axon in medullary gracile nucleus.

Taken from a 6 month–old NTE-cKO mouse. Scale bar, 10 μm. Cross-sectional area of this swollen axon is ~2000 μm^2.

Supplementary Video: Progressive hind-limb dysfunction in NTE-cKO mice.

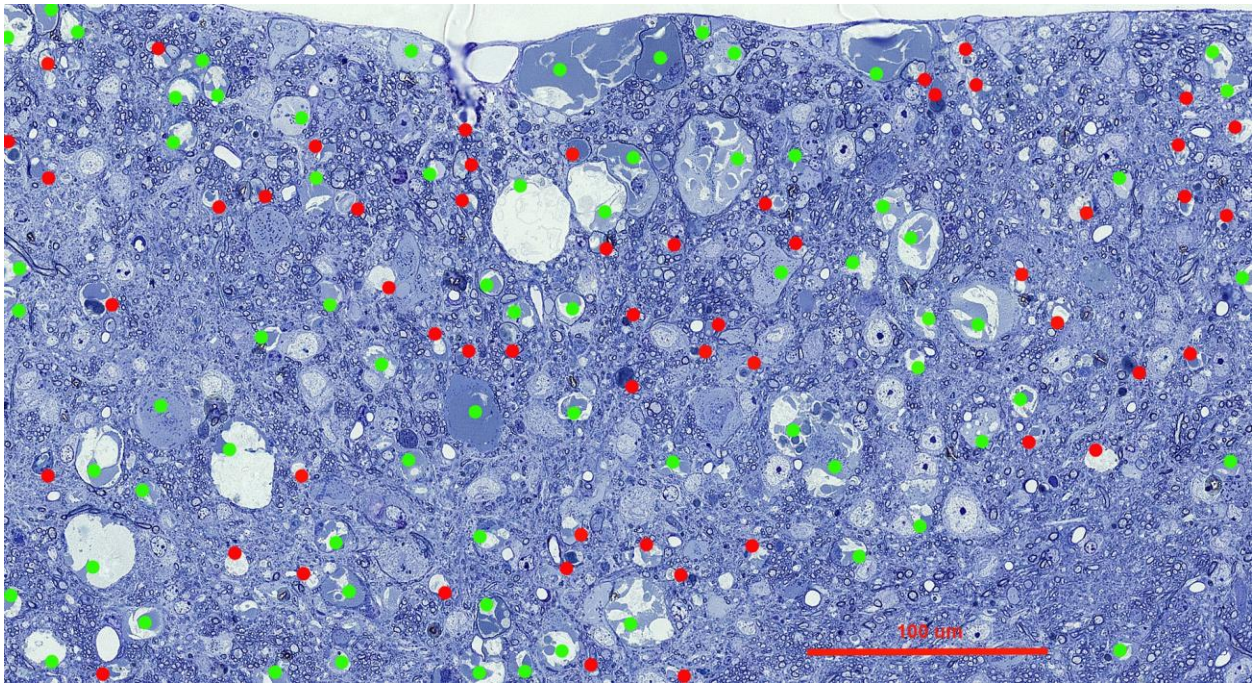
Age- and sex-matched pairs of *nestin-cre-:NTEfl/fl* (WT) and *nestin-cre+:NTEfl/fl* (NTE-KO) are shown either moving freely in open-field or traversing a 2cm-diameter dowel beam. Representative pairs are shown at ages: 14-month KO and 16-month WT; 7-month KO and WT; 3.7-month WT and KO; 2.8-month WT and KO. Note the KO mouse is generally slightly smaller than the WT: this confirms the observations of Akassoglou et al (2004) on mice at ages up to 4 months.

Open-field movement: KO mice at 7 and 14 months walk with hind-limb digits bunched together while corresponding WT mice generally splay these digits. Locomotion of the 14-

month KO mouse is noticeably abnormal (although not seriously impaired). KO mice at 3.7 and 2.8 months were indistinguishable from WT in this test.

Dowel beam traverse: KO mice at 7 and 14 months cannot lift lower body by action of hind limbs and use tail in attempt to grip beam, while WT mice cross beam with lower body well supported by hind-limbs. KO mouse at 3.7 months more able than 7 month KO, but hind-limb weakness apparent near end of traverse. KO mouse at 2.8 months was indistinguishable from WT in this test.

Supplementary Fig S1



Supplementary Fig S2