Genetic deletion of *Nogo/Rtn4* ameliorates behavioral and neuropathological outcomes in *APP* transgenic mice

Eliezer Masliah[1,2], **Fang Xie**[1], **Shanna Dayan**[1], **Edward Rockenstein**[1], **Michael Mante**[1], **Anthony Adame**[1], **Christina M. Patrick**[1], **Andrea F. Chan**[1], and **Binhai Zheng**[1,*]

[1]Department of Neurosciences, University of California San Diego, School of Medicine, 9500 Gilman Drive, La Jolla, California 92093, U.S.A.

[2]Department of Pathology, University of California San Diego, School of Medicine, 9500 Gilman Drive, La Jolla, California 92093, U.S.A.

Abstract

The cognitive impairment in Alzheimer's disease (AD) is associated with synaptic loss, neuritic sprouting and altered neuroplasticity. Compensatory neuritic sprouting might be beneficial, while aberrant sprouting could contribute to the neurodegenerative process. Nogo (or Rtn4) is a major myelin-derived inhibitor of axonal sprouting in adult CNS. Recent evidence has implicated both the Reticulon family of proteins and a receptor for Nogo, NgR, in reducing amyloid-β production, a key step in AD pathogenesis. To test the hypothesis that Nogo, as an inhibitor of axonal sprouting, modulates disease progression in a mouse model of AD, we introduced an *APP* transgene (a human *APP* minigene carrying the Swedish and Indiana mutations under the *PDGFB* promoter) into a *Nogo* null background and characterized the behavioral and neuropathological consequences. We found that deleting *Nogo* ameliorates learning and memory deficits of *APP* transgenic mice in the Morris water maze at an early/intermediate stage of the disease. Furthermore, deleting *Nogo* restored the expression levels of markers for synapto-dendritic complexity and axonal sprouting including synaptophysin, MAP2, GAP43 and neurofilament that are otherwise reduced in *APP* transgenic mice. Other aspects of disease progression including neuronal loss, astrogliosis, microgliosis and, importantly, Aβ levels and amyloid deposits were not significantly altered by *Nogo* deletion. These data support the hypothesis that Nogo-mediated inhibition of neuritic sprouting contributes to the disease progression in an *APP* transgenic model of AD in a way that is mechanistically distinct from what has been proposed for Rtn3 or NgR.

Keywords

Alzheimer's disease; central nervous system; myelin-derived axon growth inhibitors; neuritic sprouting; amyloid deposition; Reticulon

Alzheimer's disease (AD) is characterized by widespread neurodegeneration throughout the association cortex and limbic system, with formation of the Amyloid-β (Aβ) plaques and neurofibrillary tangles being the neuropathological hallmarks of the disease (Selkoe, 2005). One interesting aspect of disease progression is the cycles of synapse loss and aberrant

*Corresponding author: Tel: 1-858-534-5807; fax: 1-858-822-1021. binhai@ucsd.edu..

sprouting (Troy et al., 1997; Hashimoto and Masliah, 2003). During the early stages of AD, the synapse loss in the limbic system appears to be partially compensated by sprouting of the cholinergic system in the neocortex and inner molecular layer of the hippocampus. However, as the disease progresses and synaptic damage is accentuated, aberrant sprouting of axons and dendrites may eventually contribute to the neurodegenerative process (Geddes and Cotman, 1991; Hashimoto and Masliah, 2003). Therefore, neuritic sprouting may be either beneficial or detrimental in disease progression. However, how neuritic sprouting modulates disease progression in AD remains poorly understood.

Axonal sprouting in the adult mammalian CNS has been extensively studied in the context of CNS injuries (Gonzenbach and Schwab, 2008). There is evidence that CNS myelin contains inhibitory molecules that restrict axonal sprouting following injury, thus limiting functional recovery. Nogo is a major myelin-derived inhibitor of axonal growth. The *Nogo* gene encodes three isoforms, Nogo-A, B and C, via alternative splicing and alternative promoter usage (Chen et al., 2000; GrandPre et al., 2000). The three isoforms share a C-terminal segment that contains two transmembrane domains separated by a 66 amino acid loop region (Nogo-66) that possesses potent inhibitory activity on neurite growth *in vitro* (GrandPre et al., 2000). The same C-terminal segment shares homology with a family of proteins called Reticulons (or Rtns), with Nogo (or Rtn4) being the 4th and last member of the family. Although Nogo alone cannot explain the very limited regeneration of injured axons in the CNS (Lee and Zheng, 2008; Lee et al., 2009), there is consensus for a role of Nogo in inhibiting compensatory sprouting of uninjured axons and in local axonal plasticity (Buffo et al., 2000; Raineteau et al., 2001; McGee et al., 2005; Cafferty and Strittmatter, 2006) Lee et al., in press.

The presence of neuritic sprouting in AD pathology raised the possibility that inhibitors of CNS axonal sprouting such as Nogo may influence the disease outcome. Interestingly, recent studies indicate that both Reticulons (especially Rtn3) and NgR (also known as NgR1), a receptor for the Nogo-66 inhibitory domain (Fournier et al., 2001), can reduce the production of Aβ by interacting with BACE1 and APP respectively (He et al., 2004; Park et al., 2006). However, the proposed mechanisms by which Reticulons or NgR influences neurodegeneration appear to be independent of the role of Nogo/Rtn4 as an inhibitor of axonal sprouting. Indeed, whether Nogo/Rtn4 plays a role in mouse models of AD remains unknown. We explored this possibility by generating and characterizing mice that express a mutated human *APP* transgene in a *Nogo* null background. We found that deleting *Nogo* ameliorates disease progression in this *APP* transgenic model of AD as assessed by behavioral and neuropathological measures. In particular, *Nogo* deletion restored the reduced expression of markers for axonal sprouting and synapto-dendritic complexity in *APP* transgenic mice. These date support the hypothesis that Nogo modulates disease progression in AD as an inhibitor of neuritic sprouting.

EXPERIMENTAL PROCEDURES

Experimental mice

The *PDGFB-hAPP* transgene with Swedish (K670N/M671L) and Indiana (V717F) mutations (Line J9) (Mucke et al., 2000) was crossed with homozygous *Nogo* null mice (Lee et al., 2009). *APP tg*, *Nogo+/−* mice were then crossed to *Nogo−/−* mice to obtain the experimental mice of four different genotypes (see Results) as littermates in a C57BL/6, 129S7 and DBA mixed genetic background. All experimental procedures were approved by the Institutional Animals Care and Use Committee at UCSD.

Behavioral studies

Mice were tested in the Morris water maze as described (Rockenstein et al., 2006) at 8–10 months of age. For this purpose, a pool was filled with opaque water and mice were first trained to locate a visible platform (days 1–3) and then a submerged hidden platform (days 4–7) in three daily trials 2–3 min apart. Mice that failed to find the hidden platform within 90 seconds were placed on it for 30 seconds. The platform location was fixed. The entry point into the water for each mouse was alternated randomly between two points at an equal distance from the platform. On day 8, another visible platform trial was performed to exclude differences in motivation and fatigue, and a probe trial was performed when there was no platform present to calculate percent time spent in the target quadrant. Time to reach the platform and path length were recorded with a Noldus Instruments EthoVision video-tracking system (San Diego Instruments).

Tissue processing

Mice were euthanized by deep anesthesia and brains removed and divided sagittally. One hemibrain was post-fixed in 4% paraformaldehyde at 4°C for 48 hrs and sectioned at 40 μm with a Vibratome 2000 (Leica), while the other hemibrain was snap frozen and stored at −70°C for protein analysis.

Immunohistochemistry

As described (Rockenstein et al., 2006), blind-coded Vibratome sections were immunostained with monoclonal antibodies against synaptophysin (1:20, Chemicon), MAP2, (1:40, Chemicon), NeuN (1:1000, Chemicon), GFAP (1:500, Chemicon), Iba-1 (1:1000, Wako Chemicals), GAP43 (1:100, Chemicon), neurofilament (SMI312, 1:1000, Abcam), APP (Mucke et al., 2000), and rabbit anti-Nogo (1:500) (Zheng et al., 2003). After overnight incubation with the primary antibodies, sections were incubated with fluorescein isothiocyanate (FITC)-conjugated IgG secondary antibodies, transferred to SuperFrost slides and mounted under glass coverslips with anti-fading media. Alternatively, sections were detected with peroxidase reaction with standard techniques. All sections were processed under the same standardized conditions. The immunostained blind-coded sections were serially imaged with the laser scanning confocal microscope (MRC1024, BioRad) and analyzed with the Image 1.43 program (NIH). For each mouse, a total of three sections were analyzed and for each section, four fields in the frontal cortex and hippocampus were examined. The average of individual measurements was used to calculate group means. For GAP43, MAP2 and synaptophysin, results were expressed as percent area of the neuropil occupied by immunoreactive axons/dendrites/terminals; for neurofilament, GFAP and Iba-I, levels were expressed as optical density. For NeuN, the mean neuronal density was estimated using the optical disector method (Chana et al., 2003); briefly, for stereology, immunostained sections were counterstained with 1% cresyl violet and four 100-μm-wide fields from at least three sections (180-μm interval) per animal were analyzed and results averaged and expressed as total number per mm^3. The extent of the Aβ deposits were detected using the mouse monoclonal antibody 4G8 (1:600, Senetek) as described (Rockenstein et al., 2006) and percent area occupied by Aβ deposits plotted.

Western blot

Antibodies used: Rtn3 (R458, 1:1000) a generous gift from Dr. R. Yan, Cleveland, OH, USA (He et al., 2004), NgR (1:1000, a generous gift from Dr. B. L. Tang, Singapore), LC3 (1:1000, MBL), Beclin 1 (1:1000, Novus Biologicals), Caspase 3 (1:1000 Cell Signaling), Aβ (6E10, 1:1000, Covance).

Statistical Analysis

Values in the figures were expressed as means ± SEM. To determine the statistical significance, values were compared by using the one-way ANOVA with post-hoc Dunnet when comparing the *Non tg* to the *Nogo* null or *App tg* mice. Additional comparisons were done using Tukey-Krammer or Fisher post-hoc tests.

RESULTS

In order to evaluate the functional effects of deleting *Nogo* on disease progression in an *APP* transgenic model of AD, an *APP transgenic* line (*PDGFB-hAPP* with Swedish and Indiana mutations) (Mucke et al., 2000) were crossed with *Nogo* null mice. Unlike other *Nogo* mutants reported in the literature, the *Nogo* null mutant used in this study is fully viable and lacks the expression of all known Nogo isoforms including Nogo-A,B,C (Lee et al., 2009). The *PDGFB-hAPP* transgenic line (referred to as *APP tg* here) was chosen because this line has been extensively characterized for neuritic sprouting (Chin et al., 2004). Four groups of mice were generated as littermates: 1) non-transgenic Nogo+/− controls (referred to as *Non tg*), 2) *Nogo* null mutants (Nogo−/−), 3) *APP* transgenic, *Nogo+/−* (*APP tg*); 4) *APP* transgenic, *Nogo−/−* (*APP tg*, *Nogo−/−*).

To determine whether *Nogo* deletion alters the disease progression in *APP tg* mice, we first tested the behavior of all four groups of mice in a Morris water maze at 8–10 months of age. This time line was chosen because it has been shown that at this age behavioral deficits and axonal pathology become apparent in the *PDGFB-hAPP* transgenic line (Chin et al., 2004). During the training portion of the test with the visible platform, *APP tg* mice and *APP tg*, *Nogo−/−* mice spent a longer time to reach the platform when compared with *Non tg* or *Nogo−/−* mice. (Fig. 1*A*, *B*). During the spatial learning portion with the platform submerged, as expected, the *APP tg* mice had significant deficits compared to the *Non tg* controls. *Nogo−/−* mice did not differ significantly from the *Non tg* controls. In contrast, *APP tg*, *Nogo−/−* mice showed significant improvement over time as compared with *APP tg* mice, with their time (or latency) to reach the platform approaching that of *Non tg* or *Nogo −/−* mice towards the end of the 7-day training/testing period (Fig. 1*A*, *B*). Consistent with this finding, in the final probe portion of the test, *APP tg*, *Nogo−/−* mice expended more time in the target quadrant than *APP tg* mice (Fig. 1*C*). Thus, deleting *Nogo* ameliorated a learning and memory deficit in the *APP tg* mice at an early/intermediate stage of the disease as assessed by the Morris water maze test.

To verify *APP* transgene expression and *Nogo* deletion, we examined the expression of APP and Nogo-A in the brain of various genotypes by immunohistochemistry. In *Non tg* mice or *Nogo−/−* mice, APP immunoreactivity was detected at background levels (Fig. 2*A*, *B*). In contrast, a high level of APP immunoreactivity was detected in both *APP tg* mice and *APP tg*, *Nogo−/−* mice (Fig. 2*C*, *D*). The expression pattern is consistent with transgenic human APP protein being primarily expressed by neurons. Nogo-A is also predominantly expressed by neurons in the hippocampus in *Non tg* and *APP tg* mice and absent in *Nogo−/−* or *APP tg*, *Nogo−/−* mice (Fig. 2*E–H*). There does not appear to be any cross regulation between *APP* transgene and endogenous Nogo-A expression. These results were corroborated with Western blot analysis on brain protein extracts (Fig. 2*I*). Because NgR and Rtn3 have been implicated for a role in mouse models of AD (Park et al., 2006; Shi et al., 2009), we also examined the expression levels of these two proteins by Western blot analysis and found no appreciable differences among the genotypes (Fig. 2*J*).

The behavioral deficits in the *APP tg* mice have been associated with synaptic deficits (Mucke et al., 2000). Since *Nogo* deletion ameliorated the behavioral deficits in the *APP tg* mice, we investigated the effects of deleting *Nogo* on axonal, dendritic and synaptic markers

in the hippocampus of these mice. GAP43, a marker for new axonal sprouts, were reduced in the inner molecular layer of the dentate gyrus in *APP tg* mice (Fig. 3*A*, *C*, *I*), as expected (Chin et al., 2004). Deleting *Nogo* restored the level of GAP43 expression in the inner molecular layer (Fig. 3*D*, *I*). As a control, deleting *Nogo* alone did not significantly alter GAP43 expression in the inner molecular layer (Fig. 3*B*, *I*) (A trend for a slight increase in GAP43 immunoreactivity in *Nogo* mutants as compared with *Non tg* controls did not reach statistical significance). Similarly, the expression of neurofilament (a pan-axonal marker) in the molecular layer was reduced in *APP tg* mice but restored in *APP tg*, *Nogo−/−* mice (Fig. 3*E–H*, *J*). Likewise, a modest but significant reduction in the expression of MAP2 (a dendritic marker) and synaptophysin (a synaptic marker) in the molecular layer of the hippocampal dentate gyrus of *APP tg* mice were restored to control levels by *Nogo deletion* (Fig. 3*K*, *L*). Thus, *Nogo* deletion restored the expression level of axonal, dendritic and synaptic markers that was reduced in the dentate gyrus region of the *APP tg* mice, including a marker for new axonal sprouts.

To determine whether the effect of *Nogo* deletion is specific to neuronal processes or more general in reversing *APP tg*-associated neuropathology, we examined the expression of markers for neurons, astrogliosis, microgliosis, autophagy, apoptosis, amyloid β levels and plaque deposition. *APP tg* mice between 8–10 months of age displayed a significant loss of neurons in both the frontal cortex and hippocampus as assessed with NeuN immunohistochemistry (Fig. 4*A*, *C*, *E*, *F*). *Nogo* deletion did not significantly alter the loss of neurons in *APP tg* mice (Fig. 4*D*, *E*, *F*), suggesting that the effect of *Nogo* deletion on disease progression is not due to its effect on neuronal loss. GFAP immunoreactivity, which indicates levels of reactive astrogliosis, was upregulated in the frontal cortex and hippocampus of *APP tg* mice (Fig. 4*G*, *I*, *K*, *L*), as expected (Mucke et al., 2000). This astroglial response was not significantly altered by *Nogo* deletion (Fig. 4*J*, *K*, *L*). Similarly, microgliosis in *APP tg* mice, as assessed by the elevated expression of the Iba-1 microglia marker, was not significantly altered by *Nogo* deletion (Fig. 4*M*). As a control, *Nogo* deletion alone did not significantly alter the expression of NeuN, GFAP or Iba-1 (Fig. 4*B*, *E*, *F*, *H*, *K*, *L*). Western blot analysis on brain protein extracts indicated that there were no appreciable differences in the ratio of LC3II/LC3I or the level of Beclin 1, both markers of autophagy, among the different genotypes (Fig. 4*N*). *APP tg* was associated with a modest increase in the level of active Caspase 3, which was not further altered by *Nogo* deletion (Fig. 4*N*).

Finally, to determine whether Nogo modulates disease progression by altering Aβ metabolism or amyloid deposition, we assessed Aβ levels and amyloid plaque formation in the neocortex and hippocampus of these mice by Western blot and immunohistochemistry respectively. *APP tg* was associated with high levels of full-length APP and Aβ, which was not significantly altered by *Nogo* deletion (Fig. 4*O*, *P*). Likewise, *APP tg* mice displayed a substantial amount of amyloid plaques in the frontal cortex and hippocampus, which was not significantly altered by *Nogo* deletion (Fig. 4*Q*). Take together, these data indicate that *Nogo* deletion did not significantly alter Aβ metabolism or amyloid deposition.

DISCUSSION

It has long been hypothesized that neuritic sprouting plays a role in disease progression of AD (Geddes and Cotman, 1991; Masliah et al., 1992). Neuritic sprouting may be beneficial, compensating for the lost synaptic contacts, or detrimental if it becomes increasingly aberrant in nature. However, to our knowledge the hypothesis that neuritic sprouting modifies AD-like phenotypes in a mouse model of AD has never been directly tested. The availability of mice lacking inhibitors of CNS axon sprouting offers an opportunity to examine the effect of altered axonal sprouting in the disease progression of AD-like

phenotypes. To test this hypothesis, we introduced a *PDGFB-hAPP* transgene carrying human mutations (abbreviated as *APP tg* here), which causes AD-like phenotypes, into a *Nogo* null background that lacks the expression of all known Nogo isoforms. We found that deleting *Nogo* ameliorates behavioral and neuropathological outcome at an early/ intermediate stage of the disease in the *APP tg* line. These results support the hypothesis that Nogo modulates disease progression in an *APP* transgenic model of AD as an inhibitor of axonal sprouting through a mechanism that is distinct from what has been proposed for Rtn3 or NgR, the Nogo-66 receptor.

Nogo is a member of the Reticulon family of proteins and has three isoforms: Nogo-A,B,C (or Rtn4-A,B,C). Previous studies indicate that Reticulons, and in particular Rtn3 and Rtn4-B (or Nogo-B), biochemically interact with BACE1 (the enzyme responsible for β-secretase activity) and inhibits Aβ production *in vitro* (He et al., 2004; Murayama et al., 2006). From these studies it was anticipated that an excess of Reticulons (e.g. Rtn3) would lead to reduced Aβ levels and consequently reduced amyloid deposition and decelerated disease progression. However, the *in vivo* validation of this hypothesis has been complicated by the surprising finding that Rtn3 overexpression leads to Rtn3 aggregation and the formation of Rtn3 immunoreactive dystrophic neurites in the hippocampus, which was associated with impairments in spatial learning and memory (Hu et al., 2007). Because such Rtn3-immunoreactive dystrophic neurites also exist in human AD patients and in mice expressing mutant APP, it was proposed that Rtn3 aggregation contributes to AD pathogenesis by inducing neuritic dystrophy. Crossing the Rtn3 overexpression transgene into an APP/PS1 (Presenilin 1) double transgenic line reduced amyloid deposition in the cortex, the hippocampus CA3 region and dentate gyrus, as expected if Rtn3 were to inhibit Aβ production by interacting with BACE1; however, in the CA1 region preformed Rtn3 immunoreactive dystrophic neurites appeared to counteract with the beneficial effect of Rtn3 overexpression (Shi et al., 2009). Thus, Rtn3 may simultaneously have two opposite effects on neurodegenerative phenotypes in AD: beneficial as an antagonist of BACE1 activity and promoting the formation of dystrophic neurites that contribute to neurodegeneration when overexpressed. Whether endogenous Rtn3 plays a role in an animal model of AD awaits loss-of-function analysis. Furthermore, it is not known whether the aggregate-promoting effect of Rtn3 overexpression is shared with other Reticulons such as Nogo/Rtn4.

Interestingly, NgR, a receptor for the Nogo-66 inhibitory domain, has been found to physically interact with APP and overexpressing NgR in culture leads to reduced Aβ production (Park et al., 2006). As predicted from these *in vitro* data, genetically deleting *NgR* leads to increased Aβ levels, amyloid deposition and dystrophic neurites in an APPswe/ PSEN-1(ΔE9) double transgenic model of AD (Park et al., 2006). Conversely, administration of a soluble NgR ectodomain reduces Aβ levels, amyloid deposition and dystrophic neurites in the same animal model. Thus, it appears that NgR can bind to APP, blocking it from being processed by β-secretase (Park et al., 2006). Such a mechanism would presumably not involve the Nogo-NgR inhibitory signaling pathway, since such an involvement would predict that soluble NgR ectodomain treatment and *NgR* deletion would elicit a similar effect, while the opposite was observed (Park et al., 2006). In our study, Aβ levels and amyloid deposition were not significantly altered in *APP tg*, *Nogo−/−* mice as compared with *APP tg* mice, further arguing against a mechanism where Nogo modulates disease progression through the effect of NgR on Aβ production.

Consistent with our hypothesis that Nogo modulates disease progression as an inhibitor of neurite growth, the reduced expression of several markers indicative of synaptic and dendritic complexity and axonal sprouting is restored by *Nogo* deletion in the *APP tg* mice. In contrast, other neurodegeneration-associated phenotypes such as astrogliosis, microgliosis and neuronal loss was not significantly affected by *Nogo* deletion. Nor was the expression of

markers for autophagy and apoptosis examined. Most notably, Aβ levels and amyloid plaque formation in *APP tg* mice were not reduced by *Nogo* deletion. Thus, it appears that Nogo modulates disease progression without directly affecting Aβ metabolism or amyloid deposition, but rather, through its inhibitory effect on compensatory neuritic sprouting that occurs during disease progression, especially at an early/intermediate stage of the disease. Such a mechanism would be distinct from what has been proposed for Rtn3 or NgR on Aβ production, or Rtn3 overexpression on dystrophic neurites. Finally, both NgR and its ligands Nogo-66 and OMgp have been shown to regulate activity-dependent synaptic plasticity in the hippocampus (Lee et al., 2008) (R. J. Giger, University of Rochester, personal communication). It remains to be seen whether this functional aspect of the myelin inhibitory pathway may also contribute to – perhaps even provide an alternative explanation for – the improved behavioral outcome in the *Nogo*-deleted, *APP* transgenic mouse model of AD described here.

CONCLUSION

Our data provide evidence that axonal/neuritic sprouting is functionally relevant to disease progression in AD-like phenotypes, thus linking a major neurodegenerative disease and a major mechanism for axonal plasticity and central nervous system repair, two important areas of clinical neuroscience. Together with published reports, our study indicates that Nogo/Rtn4, other Reticulons (e.g. Rtn3) and the Nogo-66 Receptor have independent and complex roles in modulating AD pathogenesis.

Acknowledgments

Abbreviations

Rtn	Reticulon
Rtn3	Reticulon 3
Rtn4	Reticulon 4 (also known as Nogo)
AD	Alzheimer's disease
APP	amyloid precursor protein
APP tg	*APP* transgenic
Non tg	Non transgenic
Aβ	Amyloid-β
CNS	central nervous system
PDGFB	platelet-derived growth factor subunit B
GAP43	Growth Associated Protein 43
MAP2	microtubule-associated protein 2
GFAP	glial fibrillary acidic protein
BACE1	beta-site APP cleaving enzyme 1

NgR   Nogo Receptor (also known as NgR1)

DG   dentate gyrus

REFERENCES

Buffo A, Zagrebelsky M, Huber AB, Skerra A, Schwab ME, Strata P, Rossi F. Application of neutralizing antibodies against NI-35/250 myelin- associated neurite growth inhibitory proteins to the adult rat cerebellum induces sprouting of uninjured purkinje cell axons. J Neurosci. 2000; 20:2275–2286. [PubMed: 10704503]

Cafferty WB, Strittmatter SM. The Nogo-Nogo receptor pathway limits a spectrum of adult CNS axonal growth. J Neurosci. 2006; 26:12242–12250. [PubMed: 17122049]

Chana G, Landau S, Beasley C, Everall IP, Cotter D. Two-dimensional assessment of cytoarchitecture in the anterior cingulate cortex in major depressive disorder, bipolar disorder, and schizophrenia: evidence for decreased neuronal somal size and increased neuronal density. Biol Psychiatry. 2003; 53:1086–1098. [PubMed: 12814860]

Chen MS, Huber AB, van der Haar ME, Frank M, Schnell L, Spillmann AA, Christ F, Schwab ME. Nogo-A is a myelin-associated neurite outgrowth inhibitor and an antigen for monoclonal antibody IN-1. Nature. 2000; 403:434–439. [PubMed: 10667796]

Chin J, Palop JJ, Yu GQ, Kojima N, Masliah E, Mucke L. Fyn kinase modulates synaptotoxicity, but not aberrant sprouting, in human amyloid precursor protein transgenic mice. J Neurosci. 2004; 24:4692–4697. [PubMed: 15140940]

Fournier AE, GrandPre T, Strittmatter SM. Identification of a receptor mediating Nogo-66 inhibition of axonal regeneration. Nature. 2001; 409:341–346. [PubMed: 11201742]

Geddes JW, Cotman CW. Plasticity in Alzheimer's disease: too much or not enough? Neurobiol Aging. 1991; 12:330–333. discussion 352–335. [PubMed: 1961362]

Gonzenbach RR, Schwab ME. Disinhibition of neurite growth to repair the injured adult CNS: focusing on Nogo. Cell Mol Life Sci. 2008; 65:161–176. [PubMed: 17975707]

GrandPre T, Nakamura F, Vartanian T, Strittmatter SM. Identification of the Nogo inhibitor of axon regeneration as a Reticulon protein. Nature. 2000; 403:439–444. [PubMed: 10667797]

Hashimoto M, Masliah E. Cycles of aberrant synaptic sprouting and neurodegeneration in Alzheimer's and dementia with Lewy bodies. Neurochem Res. 2003; 28:1743–1756. [PubMed: 14584828]

He W, Lu Y, Qahwash I, Hu XY, Chang A, Yan R. Reticulon family members modulate BACE1 activity and amyloid-beta peptide generation. Nat Med. 2004; 10:959–965. [PubMed: 15286784]

Hu X, Shi Q, Zhou X, He W, Yi H, Yin X, Gearing M, Levey A, Yan R. Transgenic mice overexpressing reticulon 3 develop neuritic abnormalities. EMBO J. 2007; 26:2755–2767. [PubMed: 17476306]

Lee H, Raiker SJ, Venkatesh K, Geary R, Robak LA, Zhang Y, Yeh HH, Shrager P, Giger RJ. Synaptic function for the Nogo-66 receptor NgR1: regulation of dendritic spine morphology and activity-dependent synaptic strength. J Neurosci. 2008; 28:2753–2765. [PubMed: 18337405]

Lee JK, Zheng B. Axon regeneration after spinal cord injury: insight from genetically modified mouse models. Restor Neurol Neurosci. 2008; 26:175–182. [PubMed: 18820409]

Lee JK, Chan AF, Luu SM, Zhu Y, Ho C, Tessier-Lavigne M, Zheng B. Reassessment of corticospinal tract regeneration in Nogo-deficient mice. J Neurosci. 2009; 29:8649–8654. [PubMed: 19587271]

Lee JK, Geoffroy CG, Chan AF, Tolentino KE, Crawford MJ, Leal MA, Kang B, Zheng B. Assessing spinal axon regeneration and sprouting in Nogo, MAG and OMgp deficient mice. Neuron. in press.

Masliah E, Mallory M, Hansen L, Alford M, DeTeresa R, Terry R, Baudier J, Saitoh T. Localization of amyloid precursor protein in GAP43-immunoreactive aberrant sprouting neurites in Alzheimer's disease. Brain Res. 1992; 574:312–316. [PubMed: 1386275]

McGee AW, Yang Y, Fischer QS, Daw NW, Strittmatter SM. Experience-driven plasticity of visual cortex limited by myelin and Nogo receptor. Science. 2005; 309:2222–2226. [PubMed: 16195464]

Mucke L, Masliah E, Yu GQ, Mallory M, Rockenstein EM, Tatsuno G, Hu K, Kholodenko D, Johnson-Wood K, McConlogue L. High-level neuronal expression of abeta 1–42 in wild-type human amyloid protein precursor transgenic mice: synaptotoxicity without plaque formation. J Neurosci. 2000; 20:4050–4058. [PubMed: 10818140]

Murayama KS, Kametani F, Saito S, Kume H, Akiyama H, Araki W. Reticulons RTN3 and RTN4-B/ C interact with BACE1 and inhibit its ability to produce amyloid beta-protein. Eur J Neurosci. 2006; 24:1237–1244. [PubMed: 16965550]

Park JH, Gimbel DA, GrandPre T, Lee JK, Kim JE, Li W, Lee DH, Strittmatter SM. Alzheimer precursor protein interaction with the Nogo-66 receptor reduces amyloid-beta plaque deposition. J Neurosci. 2006; 26:1386–1395. [PubMed: 16452662]

Raineteau O, Fouad K, Noth P, Thallmair M, Schwab ME. Functional switch between motor tracts in the presence of the mAb IN-1 in the adult rat. Proc Natl Acad Sci U S A. 2001; 98:6929–6934. [PubMed: 11381120]

Rockenstein E, Torrance M, Mante M, Adame A, Paulino A, Rose JB, Crews L, Moessler H, Masliah E. Cerebrolysin decreases amyloid-beta production by regulating amyloid protein precursor maturation in a transgenic model of Alzheimer's disease. J Neurosci Res. 2006; 83:1252–1261. [PubMed: 16511867]

Selkoe DJ. Defining molecular targets to prevent Alzheimer disease. Arch Neurol. 2005; 62:192–195. [PubMed: 15710846]

Shi Q, Prior M, He W, Tang X, Hu X, Yan R. Reduced amyloid deposition in mice overexpressing RTN3 is adversely affected by preformed dystrophic neurites. J Neurosci. 2009; 29:9163–9173. [PubMed: 19625507]

Troy CM, Stefanis L, Greene LA, Shelanski ML. Mechanisms of neuronal degeneration: a final common pathway? Adv Neurol. 1997; 72:103–111. [PubMed: 8993689]

Zheng B, Ho C, Li S, Keirstead H, Steward O, Tessier-Lavigne M. Lack of enhanced spinal regeneration in Nogo-deficient mice. Neuron. 2003; 38:213–224. [PubMed: 12718856]
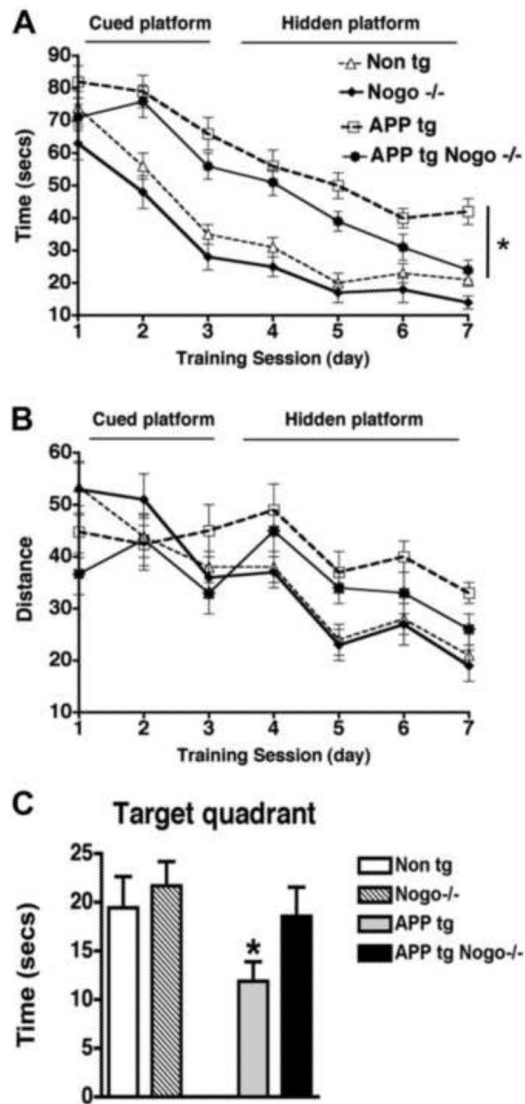
Figure 1.
Effects of *Nogo* deletion on the behavioral performance of *APP tg* mice in the Morris water maze. *A*, *B*, Average time taken and distance traveled for mice of various genotypes to reach the platform during the cued and hidden platform phases of the test. *C*, Percentage of time spent in the target quadrant by mice of various genotypes on day 8. *P* < 0.05 compared with *Non tg*, n = 8/group, Error bar = SEM.
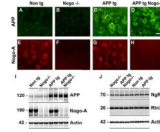
Figure 2.
Expression of APP tg, Nogo-A, NgR and Rtn3 in crosses of *APP tg* and *Nogo* null mice. *A–H*, APP and Nogo-A immunoreactivity in the hippocampus from representative mice of various genotypes. Genotype is indicated on the top; antigen is indicated on the left. APP, green; Nogo-A, red. Scale bar = 15 μm. *I*, Western blot analysis of transgenic APP protein and Nogo-A along with Actin control on representative mice of various genotypes. *J*, Western blot analysis of NgR and Rtn3 on representative mice of various genotypes.
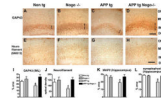
Figure 3.
Effects of *Nogo* deletion on patterns of axonal, dendritic and synaptic markers in the hippocampus of *APP tg* mice. Representative images of GAP43 (*A–D*) and neurofilament (*E–H*) immunoreactivity in hippocampal regions from mice of various genotypes are shown. OML, outer molecular layer; MML, middle molecular layer; IML, inner molecular layer; DG, dentate gyrus. Scale bar = 50 μm. *I–L*, Quantification of GAP43 (*I*), neurofilament (*J*), MAP2 (*K*) and synaptophysin (*L*) immunoreactivity. *$P < 0.05$ compared with *Non tg*, n = 8/group.
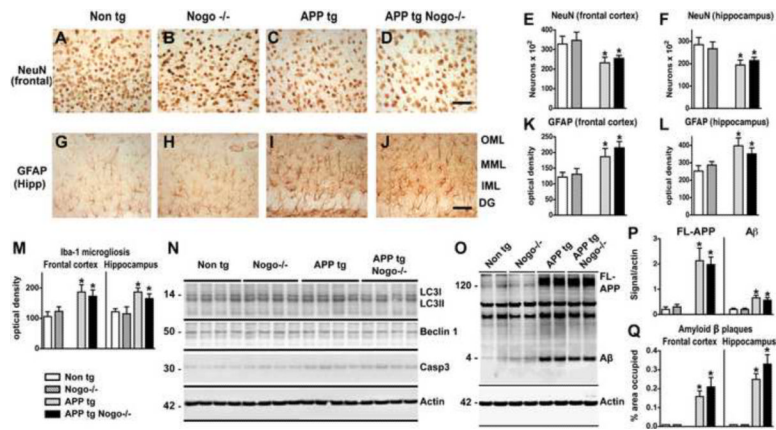
Figure 4.
Lack of effect for *Nogo* deletion on other aspects of *APP tg*-associated neuropathology. *A*–*D*, *G*–*J*, Representative images of NeuN (*A*–*D*) and GFAP (*G*–*J*) immunoreactivity in the frontal cortex and hippocampus respectively from mice of various genotypes. OML, outer molecular layer; MML, middle molecular layer; IML, inner molecular layer; DG, dentate gyrus. Scale bar = 50 μm. *E*, *F*, *K*, *L*, *M*, Quantification of NeuN *(E, F)*, GFAP *(K*, *L)* and Iba-1 (*M*) immunoreactivity. *N*, *O*, Western blot analysis on LC3I, LC3II, Beclin 1, Caspase 3 (Casp3) (*N*), Full-length APP (FL-APP) and Aβ (*O*) in brain protein extracts of various genotypes. *P*, *Q*, Quantification of FL-APP and Aβ levels (*P*) and amyloid plaque formation in the frontal cortex and hippocampus (*Q*). *$P < 0.05$ compared with *Non tg*, n = 8/group.