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Subcutaneous Nogo Receptor Removes Brain Amyloid-β and Improves Spatial Memory in Alzheimer's Transgenic Mice

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The production and aggregation of cerebral amyloid-β (Aβ) peptide are thought to play a causal role in Alzheimer's disease (AD). Previously, we found that the Nogo-66 receptor (NgR) interacts physically with both Aβ and the amyloid precursor protein (APP). The inverse correlation of Aβ levels with NgR levels within the brain may reflect regulation of Aβ production and/or Aβ clearance. Here, we assess the potential therapeutic benefit of peripheral NgR-mediated Aβ clearance in APPswe/PSEN-1ΔE9 transgenic mice. Through site-directed mutagenesis, we demonstrate that the central 15–28 aa of Aβ associate with specific surface-accessible patches on the leucine-rich repeat concave side of the solenoid structure of NgR. In transgenic mice, subcutaneous NgR(310)ecto-Fc treatment reduces brain Aβ plaque load while increasing the relative levels of serum Aβ. These changes in Aβ are correlated with improved spatial memory in the radial arm water maze. The benefits of peripheral NgR administration are evident when therapy is initiated after disease onset. Thus, the peripheral association of NgR(310)ecto-Fc with central Aβ residues provides an effective therapeutic approach for AD.

Key words: **Alzheimer's disease; β-amyloid; Nogo-66 receptor; axon; therapy; radial arm water maze; degeneration; amyloid precursor protein**

Introduction

Neurodegeneration in Alzheimer's disease (AD) is accompanied by amyloid plaques and neurofibrillary tangles (Glenner et al., 1984; Hardy and Selkoe, 2002). The amyloid plaques are composed primarily of a 40–43 aa amyloid-β (Aβ) peptide that is derived from proteolytic cleavage of amyloid precursor protein (APP) (Li et al., 1995; Sinha et al., 1999; Vassar et al., 1999). Potential therapies include decreasing β production (Lanz et al., 2003) with secretase inhibitors, increasing β degradation (Frautschy et al., 1992; Qiu et al., 1998; Bertram et al., 2000; Iwata et al., 2001; Yasojima et al., 2001), and promoting Aβ-specific immunity (Morgan et al., 2000; Lee, 2001; Younkin, 2001). However, problems with toxicity and clearing the blood–brain barrier (BBB) have hampered efforts to treat AD (Birmingham and Frantz, 2002; Orgogozo et al., 2003).

The Nogo-66 receptor (NgR) participates in limiting injury-induced axonal growth and experience-dependent plasticity in the adult brain (Fournier et al., 2001; McGee and Strittmatter, 2003; McGee et al., 2005). In this role, it serves as a receptor for three myelin inhibitor proteins, Nogo, MAG, and OMgp, signaling to activate Rho GTPase in axons (Fournier et al., 2001, 2003; Liu et al., 2002; Wang et al., 2002; McGee and Strittmatter, 2003). Recently, we found that brain NgR interacts with APP through its Aβ domain (Park et al., 2006). Moreover, increased levels of brain NgR result in reduced Aβ load, whereas loss of endogenous NgR elevates Aβ. Parallel changes in Aβ and secreted APPα plus APPβ suggest that at least a portion of the *in vivo* effects of brain NgR on Aβ levels is mediated by blockade of α/β-secretase activity. However, the high affinity of NgR for Aβ and the presence of NgR in plaques imply that NgR might also regulate the clearance of Aβ (Park et al., 2006).

Immunological methods have been successful in decreasing Aβ plaque burden, as reviewed by Schenk (2002). Both active and passive immunizations have promoted efflux, inhibited influx, or activated microglia-induced antibody (Ab) degradation (Schenk et al., 1999; Morgan et al., 2000; Weiner and Selkoe, 2002). Active immunization with Aβ(1–42) plus adjuvant in transgenic mutant APP reduced Aβ plaque pathology (Schenk et al., 1999). Bard et al. (2000) demonstrated that humoral immunity is sufficient to reduce plaque burden by triggering Ab trafficking across the BBB. In contrast, DeMattos et al. (2001) demonstrated that an Aβ Ab reduces Alzheimer pathology without Ab passage across the BBB, implicating a peripheral sink mechanism for anti-Aβ reductions in Alzheimer's pathology. Different Aβ Abs recognizing various epitopes have significantly different actions *in vivo*. The N-terminal Aβ residues serve as robust antigens for a humoral immune response, and passive administration of some (but not all) N-terminal-directed Abs effectively remove Aβ from

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the brain (Bard et al., 2000). Passive transfer of one midportion Aβ Ab is highly effective in removing Aβ from the mouse brain (DeMattos et al., 2001). As antigens, the C-terminal residues of Aβ appear to yield Abs that are less effective in reducing Aβ burden (Levites et al., 2006).

In a range of studies, reducing Aβ burden in the brain by immunological means has been associated with improved spatial memory performance in Alzheimer model transgenic mice. However, in several reports, behavioral improvements occurred acutely, before any change in plaque density, suggesting the Ab association with particular soluble Aβ species is responsible for improved function. Two non-Ig proteins, RAGE and gelsolin, have been shown to bind Aβ and, when administered peripherally, to decrease brain Aβ load (Deane et al., 2003; Matsuoka et al., 2003; Arancio et al., 2004). This supports the peripheral sink hypothesis for passive immunization against Aβ. Whether Aβ reduction by peripheral non-Ab Aβ-binding proteins is associated with improved cognitive and memory function has not been tested.

Because previous studies suggest that Aβ burden can be reduced by peripheral administration of Aβ-binding agents, we hypothesized that the high affinity of NgR for Aβ should also reduce CNS Aβ levels through a peripheral sink mechanism. In this report, we define the amino acid residue requirements for the Aβ–NgR interaction. We demonstrate that subcutaneous NgR(310)ecto-Fc reduces plaque burden after disease onset in APPswe/PSEN-1ΔE9 transgenic mice. Most critically, NgR(310)ecto-Fc treatment improves short-term memory of Alzheimer transgenic mice in the radial arm water maze (RAWM) task.

Materials and Methods

NgR mutagenesis and ligand-binding assays. AP-Aβ and AP-Nogo proteins were produced as described previously (Fournier et al., 2001; Park et al., 2006). To generate AP-Aβ mutant constructs, Aβ fragments were amplified, ligated into the pAP5tag vector (GenHunter, Nashville, TN), and sequenced. Recombinant proteins were confirmed by immunoblotting. The binding of AP fusion proteins to transfected COS-7 cells has been described previously (Fournier et al., 2001). COS-7 cells were transfected with p75-NTR and RAGE, membrane proteins reported to bind Aβ (Deane et al., 2003) (Yaar et al., 1997). Synthetic Aβ(1–28) was used to assess AP-Aβ(1–28) and AP-Nogo-66(1–33) displacement from immobilized NgR(310)ecto-Fc in an ELISA format.

NgR mutagenesis has been described previously (Fournier et al., 2003; Hu et al., 2005). Bound AP was stained and measured using NIH Image software. Mutants of human NgR were detected immunofluorescently on the surface of transfected COS-7 cells. Whole COS-7 cell lysates expressing NgR mutants were subjected to SDS-PAGE and blotted with anti-NgR Abs.

Treatment of transgenic mice with NgR. To administer rat NgR(310)ecto-Fc protein, APPswe/PSEN-1ΔE9 mice (Park et al., 2006) from The Jackson Laboratory (Bar Harbor, ME) were anesthetized with isoflurane and oxygen, and an Alzet (Cupertino, CA) osmotic pump 2004 was subcutaneously inserted over the scapula and allowed to rest between fascia. The 200 μl pump delivered 0.25 μl/h for 28 d of a 1.2 μg/μl solution of rat NgR(310)Ecto-Fc or rat IgG in PBS. Pumps were replaced after 28 d for a total treatment duration of 12 weeks. The anti-Aβ (6E10) Ab was from Chemicon (Temecula, CA). DAB staining reagents were from Vector Laboratories (Burlingame, CA).

Biochemical and histological analyses. Brains from subcutaneously treated APPswe/PSEN-1ΔE9 transgenic mice were homogenized in PBS plus a protease inhibitor mixture (Roche Products, Welwyn Garden City, UK), followed by centrifugation at 100,000 × *g* for 20 min. Secreted APPα (sAPPα) was measured by immunoblot analysis with 6E10 anti-Aβ-(1–17) Ab (Park et al., 2006) (Chemicon). Alternatively, the brain extract was subjected to Protein A/G Plus Sepharose (Pierce, Rockford, IL) immunoprecipitation and analyzed by anti-NgR polyclonal Ab (AF1440; R & D Systems, Minneapolis, MN). Aβ ELISAs were performed according to the manufacturer's protocol (Biosource, Camarillo, CA), as described for formic acid extracts of the brain (Park et al., 2006). Aβ plaques in parasagittal sections were fixed by paraformaldehyde and labeled with anti-Aβ-(1–17) 6E10 Ab after 0.1 M formic acid treatment (Park et al., 2006). Plaque area was quantitated as a percentage of total cerebral cortical area for two sections from each animal using NIH Image software. Neuritic dystrophy and reactive astrocytosis were visualized by staining with monoclonal anti-synaptophysin GA-5 (Sigma, St. Louis, MO) and monoclonal anti-GFAP SY38 (Chemicon) in parasagittal paraffin-embedded sections. The area of the cerebral cortex and hippocampus occupied by clusters of dystrophic neurites and reactive astro-

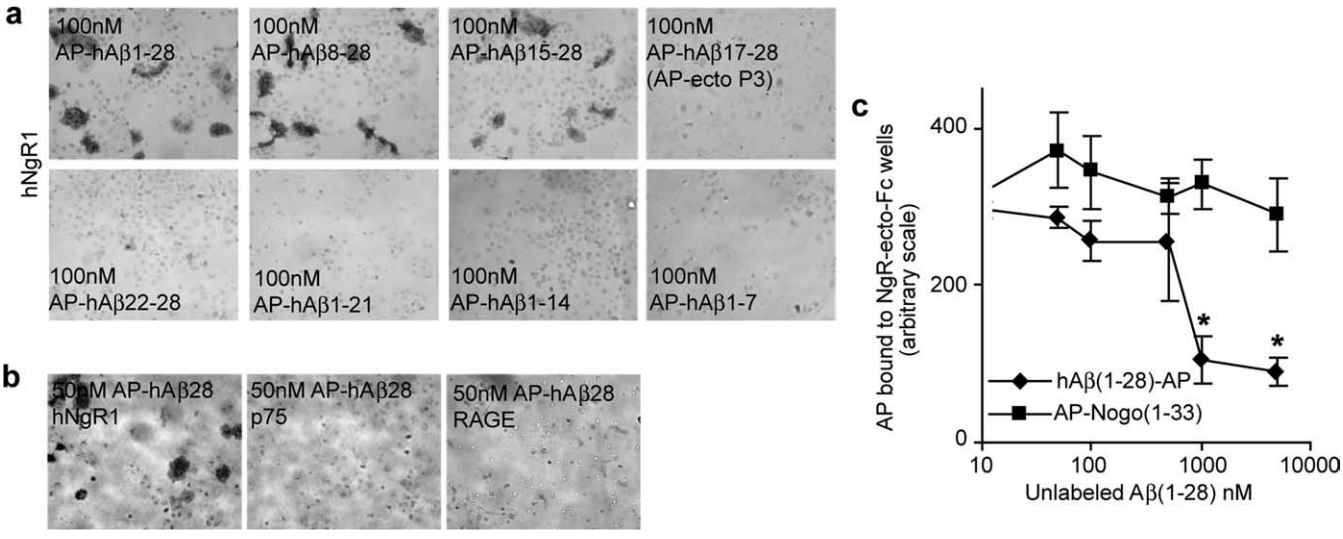

Figure 1. Residues 15–28 in Aβ(1–28) are essential for binding to NgR. ***a***, Deletion mapping of the AP-Aβ(1–28) region required for binding to COS-7 cells expressing wild-type NgR. The 8–28 construct demonstrates strong binding, 15–28 shows weaker binding, but the 1–21, 1–14, 1–7, 22–28, and 17–28 fragments do not support AP binding to NgR-expressing cells. ***b***, AP-Aβ(1–28) does not bind to COS-7 cells expressing p75-NTR or RAGE under conditions that allow binding to cells expressing NgR. ***c***, Displacement of AP-Aβ(1–28) but not AP-Nogo-66(1–33), from NgR by Aβ(1–28). A concentration of 250 nM soluble AP-Aβ(1–28) or AP-Nogo-66(1–33) was allowed to bind to wells coated with purified NgR(310)ecto-Fc in the presence of the indicated concentrations of free Aβ(1–28). In this cell-free assay, avidity for NgR is reduced compared with the cell-based binding system, and the measured K_i for Aβ(1–28) is 700 nM. Data are means ± SEM from four experiments. *$p < 0.05$, significant inhibition by Aβ(1–28) (ANOVA).

cytes were measured as a percentage of total area by the same method as Aβ plaque load (Park et al., 2006). DAB staining reagents were from Vector Laboratories.

RAWM testing. A modified RAWM testing protocol was based on a communication with D. Morgan (Wilcock et al., 2006). The maze consisted of a circular pool 1 m in diameter with six swim alleys 19 cm wide that radiated out from a 40 cm open central area, and a submerged escape platform was located at the end of one arm. Spatial cues were presented on the walls and at the end of each arm. The behaviorist was blind to the treatment and genotype. To control for vision, motivation, and swimming, mice were tested in an open-water visual-platform paradigm for up to 1 min, and latency times were recorded. Next, mice were placed in a random arm according to the following: Excel function = MOD[$CELL+RANDBETWEEN(1,5),6], where $CELL is the location of the hidden platform. Each mouse was allowed to swim for up to 1 min to find the escape platform. After entering an incorrect arm (all four paws within that swim alley) or failing to select an arm after 20 s, the mouse was pulled back to the start arm and charged an error. All mice spent 30 s on the platform after each trial before beginning the next trial. Thereafter, the mouse was tested four more times, constituting a learning block. Mice were allowed to rest for 30 min between learning blocks. In total, mice were tested over three learning blocks of five trials over the first day and then three blocks of five trials on the subsequent day. Through the group of 30 trials, the hidden platform position was kept constant for each mouse.

Statistical comparisons were made using ANOVA tests and SPSS 11 software (SPSS, Chicago, IL).

Results

Before assessing NgR as a therapeutic agent in Alzheimer model systems, we extended our characterization of the specificity of Aβ interaction with NgR. First, we sought to determine whether a linear subsegment of Aβ(1–28) might interact with NgR in a cell-binding assay. We created deletion constructs containing various portions of the Aβ ectodomain fused to AP. The region of Aβ responsible for NgR interaction localizes to residues 15–28, the central residues of Aβ(1–40) (Fig. 1*a*).

We also compared the binding of AP-Aβ(1–28) to NgR with other reported partners, p75 and RAGE (Yaar et al., 1997; Deane et al., 2003). Under conditions in which AP-Aβ(1–28) binding to NgR is readily detectable, p75 and RAGE do not exhibit a significant interaction with Aβ fusion protein (Fig. 1*b*).

NgR was identified by virtue of its affinity for Nogo-66, so we considered whether Aβ and Nogo-66 compete for binding to NgR. Competition was assessed in binding assays of AP-Aβ(1–28) or AP-Nogo66(1–33) to immobilized, purified NgR protein. Synthetic Aβ(1–28) disrupts the ability of NgR to interact with the Aβ ligand but not the Nogo-66 ligand (Fig. 1*c*). Thus, the two ligand-binding sites of NgR are distinguished by this assay.

To probe the NgR domains that interact with Aβ and Nogo-66, a strategy based on the crystal structure of NgR was used. A number of human NgR surface-accessible residues were mutated to Ala either individually or as groups of adjacent residues (Table 1), and resultant ligand-binding characteristics were assessed. The expression of each mutant NgR protein was verified by immunohistochemical detection at the surface of cells transfected with expression vector (Fig. 2*a*). For each of the mutants with altered binding characteristics, expression of immunoreactive NgR protein with electrophoretic mobility similar to wild type was also confirmed by immunoblot analysis (Fig. 2*c*). The mobility of each mutant was indistinguishable from wild-type NgR, except for the mutations in N-linked glycosylation sites (N82 and N179).

The binding of both AP-Aβ(1–28) and AP-Nogo-66 ligands to cells expressing this collection of NgR mutant proteins was assessed at concentrations equal to the predetermined K_D of the ligands (Fig. 2*b*,*d*). A large group of amino acids are unnecessary for the binding of either ligand (Table 1; Fig. 2*e*, green); this includes all of the residues examined from the convex side of the NgR leucine-rich repeat (LRR) domain. Another subset of amino acids is essential for the binding of both Aβ(1–28) and Nogo-66

Table 1. Summary of human NgR mutants: list of residues mutated to alanine

No binding	Binding to AP-Ng-66 and AP-Aβ28	Differential binding
163	61	210
82, 179	92	256, 259, 284
133, 136	108	
158, 160	122	
182, 186	127	
211, 213	131	
232, 234	138	
111, 113, 114	139	
182, 186, 210	151	
111, 113, 114, 138	176	
182, 186, 158, 160	179	
189, 191, 211, 213	227	
211, 213, 237, 256, 259, 284	237	
171, 172, 175, 176, 196, 199, 220, 223, 224, 250	250	
67, 68, 71	259	
67, 68, 71, 89, 90, 92	108, 131	
87, 89, 133, 136	114, 117	
Negative control	127, 151	
	127, 176	
	143, 144	
	189, 191	
	196, 199	
	202, 205	
	256, 259	
	267, 269	
	277, 279	
	114, 117, 139	
	189, 191, 237	
	189, 191, 284	
	202, 205, 227	
	202, 205, 250	
	220, 223, 224	
	237, 256, 259	
	296, 297, 300	
	171, 172, 175, 176	
	292, 296, 297, 300	
	196, 199, 220, 223, 224	
	171, 172, 175, 176, 196, 199	
	196, 199, 220, 223, 224, 250	
	108, 131, 61	
	36, 38	
	36, 38, 61	
	61, 131, 36, 38	
	63, 65	
	78, 81	
	87, 89	
	89, 90, 114, 117	
	95, 97	
	95, 97, 117, 119, 120, 188, 189	
	95, 97, 122	
	Wild type	

Ala-substituted human NgR mutants were tested for their binding to AP-Aβ(1–28) and AP-Nogo-66. There are three categories, as follows: (1) NgR mutants that lose binding to both ligands, (2) mutants that maintain binding to all NgR ligands, and (3) differential-binding mutants that bind AP-Nogo-66 but not AP-Aβ(1–28).

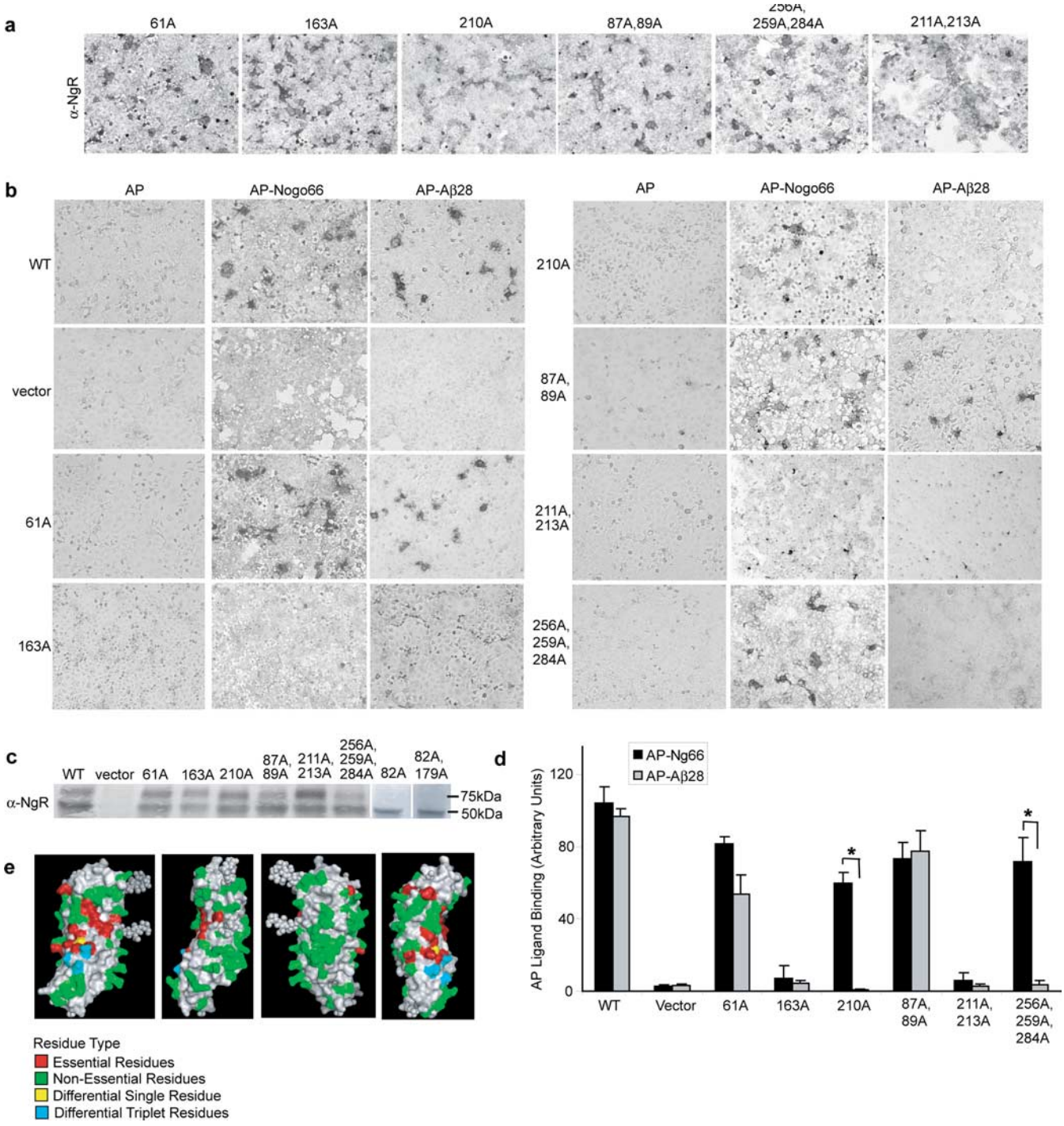

Figure 2. Specific residues in NgR support binding to AP-Aβ(1–28). ***a***, Mutant NgR proteins at the surface of transfected COS-7 cells were detected by immunostaining with rabbit anti-NgR Ab recognized by anti-rabbit-AP. ***b***, Binding of AP or AP-fused NgR ligands to COS-7 cells expressing NgR mutants displaying differential binding. The ligand concentrations were as follows (in nM): 30 AP, 5 AP-Nogo-66, and 50 AP-Aβ(1–28). ***c***, Cell lysate of COS-7 cells expressing NgR and mutants were immunoblotted with anti-NgR Ab to ascertain molecular weight and expression levels. ***d***, Quantitation of AP binding of NgR ligands to NgR mutants expressed as a percentage of wild-type NgR. After incubation with AP-fused ligands, AP bound to COS-7 cells expressing NgR were stained and measured. Data are mean ± SEM from four independent samples. *$p < 0.01$, significant difference for binding of the two ligands. ***e***, The molecular surface of NgR is illustrated with residues essential for binding AP-Nogo-66 and AP-Aβ(1–28) labeled red, residues not required for ligand binding labeled blue, and residues required for differential binding labeled yellow. Pictures were created using PyMol. The molecules are positioned with their N termini pointing up and their C termini pointing down. WT, Wild type.

(Table 1; Fig. 2*e*, red). Because these amino acids do not alter the localization or molecular size of NgR protein and are clustered in close proximity on the concave surface, we hypothesize that they form a core ligand-binding site. This is consistent with the observation that for other LRR proteins, such as the follicle-stimulating hormone receptor, ligand binding predominantly occurs on the concave side (Fan and Hendrickson, 2005). Without structural studies, the possibility that these mutations prevent native NgR protein folding cannot be excluded. Most interesting are a third group of amino acids, for which Ala substitution results in NgR binding of Nogo-66 but not Aβ(1–28) (Table 1; Fig. 2*e*, blue or yellow). Because AP-Nogo-66 binding is indistin-

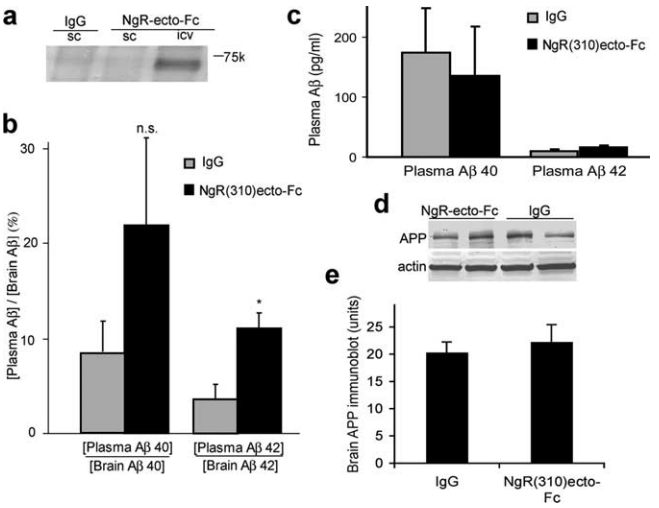

Figure 3. NgR(310)ecto-Fc treatment acts peripherally to alter the plasma/brain Aβ ratio. ***a***, An anti-NgR immunoblot of protein A/G binding proteins in brain lysate of APPswe/PSEN-1ΔE9 transgenic mice treated subcutaneously (sc) with rat IgG, subcutaneously with NgR(310)ecto-Fc, or intracerebroventricularly (icv) with NgR(310)ecto-Fc. The dose of each protein was 0.27 mg/kg/d. ***b***, The ratio of plasma versus brain Aβ level in APPswe/PSEN-1ΔE9 transgenic mice at 10 months of age is plotted as a percentage. In each sample, levels of Aβ(1–40) and Aβ(1-42) were assessed by ELISA. After 3 months of subcutaneous treatment, there is a significant increase in the plasma/brain Aβ(1–42) ratio. $^*p < 0.05$ (ANOVA). n.s., Not significant. ***c***, The absolute level of plasma Aβ level in APPswe/PSEN-1ΔE9 transgenic mice at 10 months of age is plotted. The differences are not significant. ***d***, An anti-APP (6E10) immunoblot of brain lysate of APPswe/PSEN-1ΔE9 transgenic mice treated subcutaneously with rat IgG or subcutaneously with NgR(310)ecto-Fc from 7 to 10 months of age. The dose of each protein was 0.27 mg/kg/d, by continuous infusion. ***e***, The level of anti-APP immunoreactivity in brain lysates from an experiment as in ***c*** is plotted. Data are mean ± SEM from $n = 4–5$ mice.

guishable from wild-type NgR, aberrant protein folding is unlikely to be the basis for reduced Aβ binding. Instead, NgR amino acids 210, 256, 259, and 284 are likely to contribute selectively to Aβ but not Nogo-66 interaction.

Although endogenous NgR plays a role in limiting Aβ production and deposition, the affinity of NgR for the central domain of Aβ suggests that it might promote peripheral clearance if delivered outside of the CNS. To examine whether rat NgR(310)ecto-Fc administered subcutaneously enters the brain of mouse, we assayed the presence of NgR(310)ecto-Fc in brain lysates. The NgR(310)ecto-Fc fusion protein or control rat Ig was concentrated by protein A/G affinity chromatography. Although intracerebroventricular administration leads to easily detected NgR(310)ecto-Fc levels in brain tissue, no NgR(310)ecto-Fc is detected centrally after subcutaneous treatment (Fig. 3*a*). This is consistent with the hypothesis that NgR(310)ecto-Fc cannot pass the BBB to an appreciable degree in APPswe/PSEN-1ΔE9 mice.

To the extent that NgR(310)ecto-Fc functions as a peripheral sink for Aβ, the ratio of plasma to brain Aβ should be elevated, as shown for anti-Aβ treatment. Levels of Aβ40 and Aβ42 were assessed by ELISA in brain and plasma samples from peripherally treated mice (Fig. 3*b*). Subcutaneous treatment with NgR(310)ecto-Fc increases plasma to brain ratios for Aβ by more than twofold. The increase in this ratio is primarily attributable to a decrease in brain levels with NgR(310)ecto-Fc treatment (see brain analysis below) and not to a change in plasma levels (Fig. 3*c*). Previously, we noted that central, intracerebroventricularly administered NgR(310)ecto-Fc reduces levels of sAPPα and sAPPβ protein in the brain (Park et al., 2006). If subcutaneous NgR(310)ecto-Fc acts to alter Aβ clearance in the periphery, and not to alter Aβ production centrally, then similar changes in APP should fail to occur with peripheral treatment. Indeed, brain APP levels are not altered by subcutaneous NgR(310)ecto-Fc (Fig. 3*d*,*e*).

The restriction of subcutaneous NgR(310)ecto-Fc to the periphery allows an assessment of its effect as a "sink" on central Aβ burden. Treatment of APPswe/PS-1ΔE9 transgenic mice was initiated at 7 months of age when the mice have become symptomatic, as judged by Aβ deposition in brain and by reduced spatial memory function (see below). After 3 months of subcutaneous treatment with 0.27 mg/kg/d NgR(310)ecto-Fc versus IgG, the brain was examined. The total Aβ(1–40) and Aβ(1–42) levels as well as Aβ plaque are decreased significantly by NgR(310)ecto-Fc, to a level ~50% less than the control (Fig. 4*a*,*d*,*e*). In parallel, dystrophic neurites detected by antisynaptophysin staining are decreased by peripheral NgR(310)ecto-Fc treatment (Fig. 4*b*,*f*). Astrogliosis detected by anti-GFAP staining intensity was also reduced significantly by therapy with peripheral NgR(310)ecto-Fc (Fig. 4*c*,*g*). Thus, delayed subcutaneous administration of NgR(310)ecto-Fc suppresses histologic evidence of Aβ-associated disease in transgenic mice.

The ability of subcutaneous NgR(310)ecto-Fc therapy to reduce Aβ plaque is encouraging, but cognitive performance is the relevant symptom in clinical AD. To assess APPswe/PSEN-1ΔE9 transgene-related impairments in spatial memory and learning over a 24 h period, we used a modified RAWM paradigm (Wilcock et al., 2006). If performance on this task reflects spatial learning and not alterations in motivation, motor coordination, stamina or vision, then the latency to reach a visible escape platform should not differ between groups. Such latencies are indistinguishable between littermate-matched wild-type and APPswe/PS-1ΔE9 mice (38 ± 2 s vs 42 ± 5 s, respectively; mean ± SEM; $n = 8–10$ mice). Learning deficits in the 24 h spatial memory task are apparent in APPswe/PSEN-1ΔE9 versus wild-type littermate mice by 4 months of age (Fig. 5*a*). By 13 months of age, wild-type mice perform less well at this task than do young mice, whereas APPswe/PSEN-1ΔE9 transgenic mice are completely unable to learn the task in our training paradigm illustrating disease progression (Fig. 5*b*). As a control, loss of NgR expression (in *ngr*−/− mice) does not significantly alter RAWM performance (Fig. 5*c*).

The number of swim errors made by APPswe/PS-1ΔE9 mice after 20 training trials increases steadily at 8, 9, and 10 months when mice receive control IgG therapy subcutaneously for 1, 2, or 3 months. In contrast, mice treated with subcutaneous NgR(310)ecto-Fc exhibit a halt in disease progression and show a trend toward improved performance after 3 months, by 10 months of age (Fig. 5*d*). RAWM errors are significantly reduced after 2 and 3 months of subcutaneous NgR(310)ecto-Fc treatment compared with rat IgG-treated mice (ANOVA, $p < 0.05$ and 0.02, respectively). These differences are related to improved memory function rather than altered vision, motivation, or motor capacity because no significant difference was observed in visible platform escape latencies between these groups (supplemental Fig.1, available at www.jneurosci.org as supplemental material). The improved performance of the NgR(310)ecto-Fc-treated mice is not present during initial swim trials but develops during the 24 h training period (supplemental Fig. 2, available at www.jneurosci.org as supplemental material). There is a positive correlation between the average RAWM errors and the density of Aβ-immunoreactive deposits across the two groups (Fig. 5*e*).

Discussion

In the present study, we have defined the molecular requirements for Aβ interaction with NgR and exploited the affinity of these

polypeptides to demonstrate a subcutaneous method for alleviating memory impairments in AD model mice. The 15–28 central residues of Aβ bind to a pocket on the concave surface of NgR that can be distinguished from the surface required for Nogo-66 binding. When NgR is administered subcutaneously, the protein does not enter the brain but can functionally shift Aβ from the brain to plasma. The net result of this shift is a lessening of AD-related pathology. Most critically, peripheral NgR(310)ecto-Fc improves memory performance even when treatment is initiated after symptoms develop. Thus, NgR therapy provides an alternative to immune-based Aβ therapies for AD.

In previous work, we had administered NgR(310)ecto-Fc intracerebroventricularly rather than subcutaneously to transgenic AD mice (Park et al., 2006). Similar reductions in Aβ were detected, but memory deficits were not assessed. Interestingly, the central administration of NgR(310)ecto-Fc reduced brain sAPPα as well as Aβ. Furthermore, the elevated Aβ and sAPPα in mice lacking NgR indicated that endogenous neuronal NgR serves to limit Aβ production. Thus, the binding of excess NgR to both APP and Aβ has the potential to reduce Aβ production as well as to enhance Aβ clearance. The predominant action of subcutaneous NgR is the acceleration of Aβ clearance.

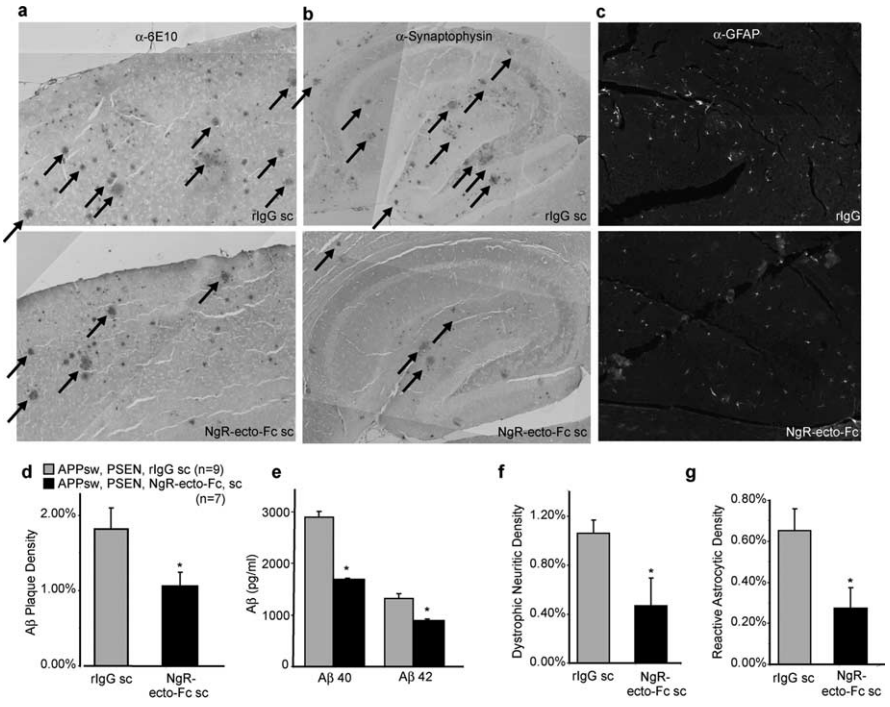

Figure 4. Reduction in Aβ plaque load, neuritic dystrophy, and astrocytosis in NgR(310)ecto-Fc-treated APPswe/PSEN-1ΔE9 mice. Sections of cerebral cortex from APPswe/PSEN-1ΔE9 transgenic mice treated with rat IgG or NgR(310)ecto-Fc are shown. Each mouse subcutaneously received 0.6 mg of protein over 3 months (0.27 mg/kg/d) beginning at 7 months of age. At 10 months of age, brain tissue was analyzed by immunohistochemistry and ELISA. Data are means ± SEM from nine mice in the rat IgG-treated group and from seven mice in the NgR(310)ecto-Fc-treated group. ***a***, Arrows indicate examples of anti-Aβ-immunoreactive plaque deposits in the cerebral cortex of control and NgR(310)ecto-Fc-treated transgenic mice. ***b***, Arrows indicate examples of anti-synaptophysin-immunoreactive plaque deposits in the hippocampus. ***c***, Anti-GFAP immunoreactivity in the hippocampus. ***d***, The percentage of area occupied by Aβ plaque were quantified from images as in ***a***. The decrease in plaque density is significant. $p < 0.05$ (ANOVA). ***e***, Aβ(1–40) and Aβ(1–42) levels were assessed by ELISA and are significantly different ($p < 0.05$, ANOVA) between NgR(310)ecto-Fc and rat IgG groups. ***f***, The area occupied by anti-synaptophysin-immunoreactive dystrophic neurites is reported for images as in ***b***. The decrease in neuritic dystrophy density is significant. $p < 0.05$ (Student's *t* test). ***g***, The percentage of area occupied by anti-GFAP immunoreactivity was measured from images as in ***c***. The decrease in anti-GFAP immunoreactivity is significant. $p < 0.05$ (ANOVA).

The reduction in both Aβ and memory deficits with a nonimmunologic NgR protein provides additional evidence for the amyloid hypothesis of AD and also for the peripheral sink hypothesis of anti-Aβ Ab function. Several previous studies demonstrate that peripheral administration of Abs directed against Aβ reduces AD pathology. The Aβ residues recognized by NgR match most closely to the Aβ(13–28) epitope reacting with the m266 Ab. Like NgR(310)ecto-Fc, the m266 Ab reduces plaque burden (DeMattos et al., 2001; Dodart et al., 2002). As opposed to active or passive immunologic reagents, NgR is an endogenous protein and is not predicted to activate or to require cell-mediated mechanisms to reduce Aβ levels and Aβ-associated cognitive deficits. Two other non-Ig proteins, RAGE and gelsolin, have been shown to bind Aβ and to decrease brain Aβ load (Deane et al., 2003; Matsuoka et al., 2003; Arancio et al., 2004) when administered peripherally. However, the effect of these proteins on memory function has not been examined, and shifts in Aβ are complicated by the fact that endogenous gelsolin is cytoplasmic and that RAGE alters BBB transport (Deane et al., 2003).

Several recent studies indicate that specific Aβ isoforms, such as soluble oligomers including Aβ*56, are better correlated with memory impairment than the total Aβ load in the brain (McLaurin et al., 2006). In our study, errors in RAWM performance and total brain Aβ deposition are directly correlated. This suggests that NgR(310)ecto-Fc does not distinguish between Aβ isoforms. The time course of improvement in memory performance after NgR(310)ecto-Fc is slow. After 1 month, the differences are minimal, whereas they are clear at 2 months and dramatic at 3 months of treatment. This suggests that NgR is not binding acutely to a small pool of a function-blocking species of Aβ to promote rapid reversal of cognitive impairment but, rather, is leading to a shift in the steady-state levels of multiple Aβ forms in the brain through slow equilibration with species bound peripherally to the infused NgR protein. Future studies are required to directly assess NgR affinity for different Aβ forms and their regulation by subcutaneous NgR(310)ecto-Fc infusion.

In the current study, NgR affinity for Aβ has been defined and exploited for therapeutic benefit. CNS myelin also contains ligands for the NgR. The interaction of myelin with NgR limits experience-dependent plasticity in the cerebral cortex (McGee et al., 2005) and injury-induced regenerative axonal growth (GrandPre et al., 2002; Li and Strittmatter, 2003; Kim et al., 2004; Li et al., 2004, 2005). The influence of Aβ with the myelin/NgR within the brain remains to be defined. Because human Aβ binding to human NgR exhibits a substantially higher affinity than mouse Aβ for mouse NgR (Park et al., 2006), and because Aβ levels are low without disease, the Aβ–NgR interaction may function only in the pathological AD state. Alternatively, Aβ regulation of NgR-dependent axonal plasticity may contribute to impaired cognitive function in AD.

The LRR protein family is involved in multiple protein–protein interactions. LRR domains contribute to the ectodomain

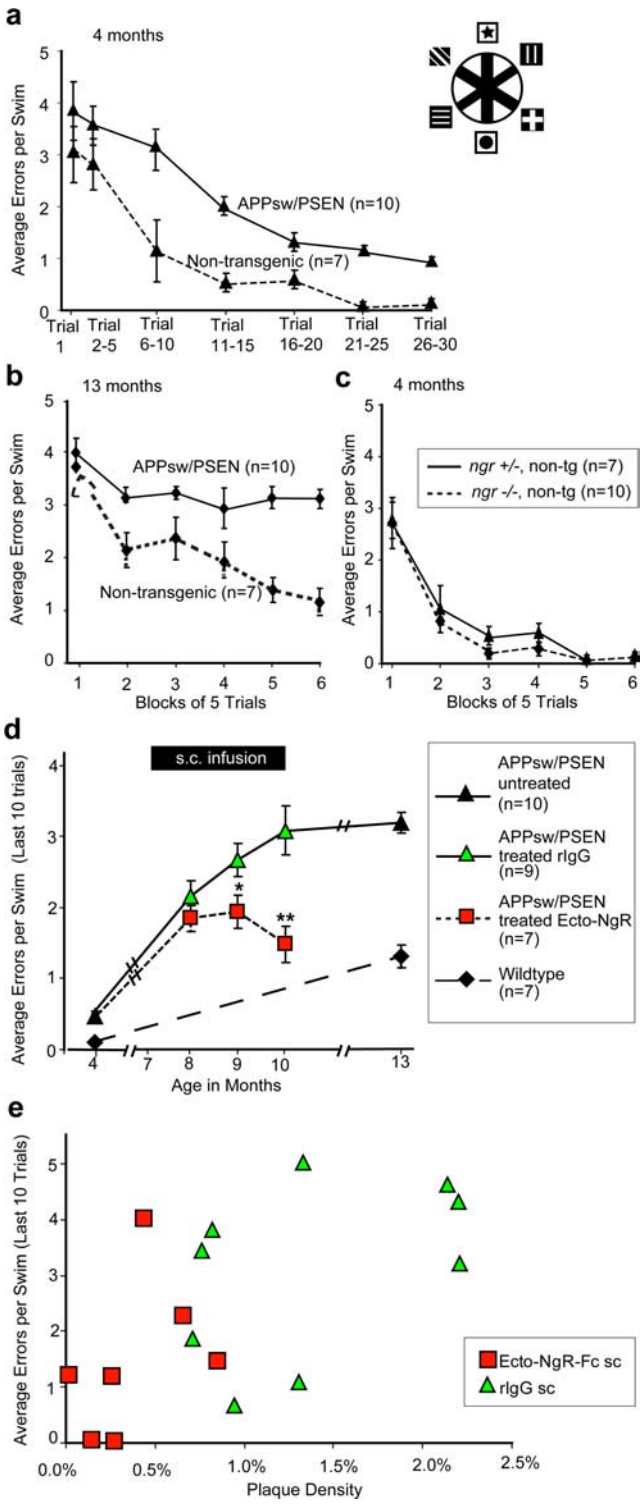

Figure 5. Subcutaneous treatment of NgR(310)ecto-Fc improves RAWM performance in APPswe/PSEN-1ΔE9 transgenic mice. ***a***, Average number of errors for the indicated swim trials in a six-arm radial water maze for APPswe/PSEN-1ΔE9 and wild-type littermates at 4 months of age. ***b***, Average number of errors in a six-arm radial water maze for APPswe/PSEN-1ΔE9 and wild-type littermates at 13 months of age. ***c***, Average number of errors in a six-arm radial water maze for $ngr^{+/-}$ and $ngr^{-/-}$ mice. ***d***, Subcutaneous (s.c.) treatment of NgR(310)ecto-Fc improved performance in APPswe/PSEN-1ΔE9 mice from months 7 to 10 and were tested at months 8–10. The data for 4 and 13 months are from a separate cohort of mice that did not receive treatment. $*p < 0.05$, significantly less than the IgG group at the same age (ANOVA); $**p < 0.02$ (ANOVA). ***e***, Scatter plot between plaque density and average errors per swim for the last 10 trials for each mouse. All data are mean ± SEM for the indicated numbers of mice in each group.

segment of a range of cell-surface receptors, including NgR, Trk receptors, Toll-like receptors, follicle-stimulating hormone/luteinizing hormone and related G-protein-coupled receptors, and platelet GP1bα (Kobe and Deisenhofer, 1994). Like the NgR, the platelet GP1bα interacts with unrelated protein ligands, von Willebrand's factor, and thrombin. Although Aβ, Nogo-66, MAG, and OMgp share no sequence similarity, they each bind to NgR. For Aβ and Nogo-66, binding sites defined by mutagenesis appear closely apposed along the midportion of the concave surface. Structural studies of ligand/receptor will be required to further illuminate NgR binding and to explore the possibility of small molecule NgR ligand compounds as therapeutic agents. In summary, we show that subcutaneous NgR(310)ecto-Fc binds Aβ in the periphery to reduce Aβ and improve spatial memory in a mouse model of AD.

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