Oligomeric assemblies of amyloid-β (Aβ) peptide (Aβo) in the brains of individuals with Alzheimer’s disease (AD) are toxic to neuronal synapses. More than a dozen Aβ receptor candidates have been suggested to be responsible for various aspects of the molecular pathology and memory impairment in mouse models of AD. A lack of consistent experimental design among previous studies of different receptor candidates limits evaluation of the relative roles of these candidates, producing some controversy within the field. Here, using cell-based assays with several Aβ species, including Aβo from AD brains obtained by autopsy, we directly compared the Aβ-binding capacity of multiple receptor candidates while accounting for variation in expression and confirming cell surface expression. In a survey of 15 reported Aβ receptors, only cellular prion protein (PrPC), Nogo receptor 1 (NgR1), and leukocyte immunoglobulin-like receptor subfamily B member 2 (LilrB2) exhibited direct binding to synaptotoxic assemblies of synthetic Aβ. Both PrPC and NgR1 preferentially bound synaptotoxic oligomers rather than nontoxic monomers, and the method of oligomer preparation did not significantly alter our binding results. Hippocampal neurons lacking both NgR1 and LilrB2 exhibited a partial reduction of Aβo binding, but this reduction was lower than in neurons lacking PrPC under the same conditions. Finally, binding studies with soluble Aβo from human AD brains revealed a strong affinity for PrPC, weak affinity for NgR1, and no detectable affinity for LilrB2. These findings clarify the relative contributions of previously reported Aβ receptors under controlled conditions and highlight the prominence of PrPC as an Aβ-binding site.

The pathophysiology responsible for the clinical signs and symptoms of AD has been studied since Alois Alzheimer first described the characteristic senile plaques and neurofibrillary tangles in the brain of a patient who suffered a deteriorating psychological condition that included sleep disturbances, memory impairment, and confusion. Today, AD is the leading cause of dementia worldwide and carries with it a tremendous economic burden projected to exceed 2 trillion United States dollars by the year 2030. Despite more than a century of research, AD remains without a treatment that is capable of curing, preventing, or slowing the progression of disease. The role of senile plaques and neurofibrillary tangles in the etiology of Alzheimer’s disease continues to be investigated and contested within the field. In 1998, Lambert et al. described the self-assembly of synthetic β-amyloid monomers into soluble, multimeric, nonfibrillar aggregates dubbed Aβo. These oligomers were potentially neurotoxic and capable of inducing cell death, and they inhibited long-term potentiation in organotypic hippocampal slices. Aβo are immunologically distinct from monomers or fibrils, induce synapse loss, and are correlated with disease progression. Similar species of Aβo were identified in brains from human AD patients in 2003. The observations that synthetic and AD brain–derived Aβo bound to neurons in a trypsin-sensitive manner gave rise to the search for cell surface receptors capable of binding extracellular Aβo and transducing their neurotoxic signal intracellularly. More than a dozen proteins have been reported as responsible for mediating the deleterious effects of Aβo on neurons (reviewed in Ref. 26). These studies have been highly disparate in both the quality and nature of evidence used to qualify a candidate as a receptor for Aβ. Variation in Aβ preparations, experimental design, and model systems have led to a call for systematic and standardized comparison of reported Aβ receptors for sufficiency, affinity, and Alzheimer’s disease relevance.

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This article contains Fig. S1.

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2 The abbreviations used are: AD, Alzheimer’s disease; Aβ, amyloid-β; Aβo, amyloid-β oligomer(s); PrPc, cellular prion protein; NgR1, Nogo receptor 1; LilrB2, leukocyte immunoglobulin-like receptor subfamily B member 2; RAGE, advanced glycosylation end product-specific receptor; PGRMC1, membrane-associated progesterone receptor component 1; EphA1, ephrin type-A receptor 1; FcγR, low-affinity immunoglobulin γ receptor; FcγRIIIb; SorLA, sortilin-related receptor; p75NTR, tumor necrosis factor receptor superfamily member 16; NLG1, neuroligin 1; EphB2, ephrin type-B receptor 2; FZD, frizzled-5; GluR5, metabotropic glutamate receptor 5; Epha4, ephrin type-A receptor 4; CRMP2, collapsin response mediator protein 2; NRP1, neuropilin-1; BAβi, biotin amyloid-β; BAβo, BAβ oligomers; Aβg, amyloid-β globulomer(s); Aβim, amyloid-β monomer(s); Pirb, paired immunoglobulin-like receptor b; NARChR7, neuronal acetylcholine receptor subunit α7; GluN2B, glutamate receptor ionotropic NMDA 2B; hPrPc, human PrPC; SEC, size-exclusion chromatography; HMW, high-molecular weight; RIPA, Radioimmunoprecipitation assay; TBS, Tris-buffered saline; HFIP, 1,1,1,3,3,3-hexafluoroisopropanol.

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for a sharing of materials and validation of results between laboratories (26–28).

To address these discrepancies and better understand the relative contributions of each putative receptor to Aβ neurotoxicity, we compared the potential of each receptor to confer Aβ binding capacity to heterologous cells and neurons, the ability of each candidate to discriminate between nontoxic monomers and toxic oligomers, and the effect of different oligomer preparations on the binding profile. To determine whether synthetic preparations of Aβ faithfully recapitulate the binding profile of Aβ found in the brains of patients with AD, we also compared the ability of candidate receptors to bind soluble Aβ extracted from the brains of patients diagnosed with AD. These insights are critical to clarifying the roles of these receptors in AD pathogenesis and their therapeutic value to drug development. Preventing the interaction of neurotoxic Aβo with its receptors is an attractive drug target, and clinical trials targeting advanced glycosylation end product–specific receptor (RAGE), membrane-associated progesterone receptor component 1 (MRGC1), and tumor necrosis factor receptor superfamily member 16 (p75NTR) are under way (NCT00141661, NCT00566397, NCT02916056, NCT02083064, NCT03522129, NCT03507790, and NCT03069014) (29–32).

**Results**

*PrPc*, **Lilrb2**, and **NgR1** bind oligomeric **Aβ**

Few descriptions of candidate receptors for Aβ have included a demonstration of sufficiency for conferring Aβ binding to live cells. To examine this attribute, we compiled a panel of putative receptors for Aβ and cloned the cDNA of each into expression vectors encoding a Myc epitope at the cytoplasmic terminus of transmembrane proteins or at the mature N terminus of glycosylphosphatidylinositol-anchored proteins, which included PrPc, NgR1, PGRMC1, and sortilin receptor superfamily member 16 (p75NTR) are under way (NCT00141661, NCT00566397, NCT02916056, NCT02083064, NCT03522129, NCT03507790, and NCT03069014) (29–32).

The concentration of oligomeric Aβ in AD patient brains has been reported to be ~2.6 nM monomer equivalent (7). To examine the ability of candidate receptors to bind neurotoxic oligomers of Aβ, transfected cells were incubated with 1 μM monomer equivalent (~5 nM oligomer) biotinylated Aβ oligomers (BAβo) at 4 °C to allow binding but minimize receptor internalization following binding. A high concentration was utilized so as to allow detection of Aβ binding even to low-affinity receptors. Following fixation and staining, the receptor expression–normalized Aβ binding was calculated and normalized to that of human PrPc (hPrPc). Fig. 2 (A and B) shows that PrPc, Lilrb2, and NgR1 exhibit Aβo binding capacity. Among these, PrPc binds Aβo more than either Lilrb2 or NgR1 (Fig. 2B). None of the other receptors demonstrated detectable Aβo binding with a lower limit of detection equal to 3% of hPrPc binding.

**PrPc** and **NgR1** preferentially bind synaptotoxic Aβ species

An important characteristic of a receptor capable of transducing pathological signaling in response to Aβo is an ability to discriminate between neurotoxic oligomers and nontoxic monomeric Aβ. Aβ monomers spontaneously and rapidly assemble into high-molecular weight species in physiological buffers. Samples incubated overnight yield preparations enriched for oligomeric assemblies, which can be resolved using size-exclusion chromatography (SEC) (Fig. 3A). Analysis of the relative abundance of oligomeric Aβ in different preparations demonstrates that Aβ freshly prepared in Ham’s F12 medium is 7% high-molecular weight (HMW) Aβo. Oligomeric preparations are 21% Aβo. Oligomerization can be inhibited by the use of nonphysiological buffers. When Aβ is dissolved in 0.1 n NaOH, only 3% of the preparation is HMW Aβo (Fig. 3B). Incubating transfected cells with freshly prepared Aβ in F12 is intended to provide a context in which monomeric Aβ is more readily available for binding; however, the preparation cannot be considered purely monomeric. Fig. 3C shows the expression-normalized monomeric Aβ signal for each receptor compared with oligomeric Aβ binding to hPrPc. None of the receptors bind this monomeric preparation of Aβ to a great extent (Fig. 3C). By comparing oligomeric and monomeric binding, we examined each receptor’s ability to discriminate Aβ species. PrPc and NgR1 demonstrate an ability to distinguish between oligomeric Aβ and monomeric Aβ. Lilrb2 binds oligomeric and monomeric Aβ to similar extents (Fig. 3D).
Neither temperature nor Aβ oligomerization method alters the binding profile of the receptor panel

Conducting binding assays at 4 °C affords convenience but decreases the fluidity of the cell membrane and impacts the thermodynamics of receptor–ligand interactions. To determine whether temperature influences the binding profile of the receptor panel, we incubated transfected cells with 1 μM monomer-equivalent BAg at 37 °C. As with the 4 °C incubation, only PrPC, LilrB2, and NgR1 bind BAg at 37 °C (Fig. 4A). In comparing binding at both temperatures, we found no effect of temperature on BAg binding (Fig. 4B).

Multiple methods for the generation of oligomeric Aβ assemblies from monomeric synthetic peptides have been reported (10, 33, 34). Differences in preparation lead to unique profiles with regard to the types and relative abundance of multimeric Aβ assemblies as assessed under native conditions using SEC. Oligomers prepared in F12 culture medium, incubated overnight, and separated from insoluble fibrillar species by centrifugation generate larger species of oligomers with masses in the range of 100 kDa and larger (7, 10). To account for differences in oligomeric species generated, we utilized a second preparation of soluble multimeric Aβ termed globulomers (Ag) that generates primarily a 60-kDa species by SEC (33). Atomic force microscopy confirms differences in sizes of soluble oligomers generated by the different preparations (Fig. 4C). Incubation of transfected cells with Ag demonstrates a binding profile similar to that observed with the oligomeric preparations (Fig. 4D). When comparing binding of BAg and Ag to the binding-competent receptors, there is no effect of preparation on the binding (Fig. 4E).

NgR1 and LilrB2 are minor contributors to Aβ binding to neurons

Our survey of putative receptors has revealed that of those reported, only PrPC, LilrB2, and NgR1 are sufficient for binding
to neurotoxic preparations of synthetic Aβ. PrP<sup>C</sup> has been demonstrated to be responsible for 50% of Aβo binding to hippocampal neurons (10). To examine the contribution of the two additional Aβo receptors confirmed here (11, 12), we examined the role of NgR1 and the murine homolog of LilrB2, Pirb, in double-knockout neurons under the same conditions used for PrP<sup>C</sup> (10). Loss of NgR1 and Pirb results in a 20% decrease in BAβo binding to double-knockout neurons (Fig. 5, A and B).

**PrP<sup>C</sup> is the highest-affinity receptor for Aβo**

To better understand the relative affinities of the positive receptors, we implemented plate-based assays of Aβo binding to purified protein. This assay is more sensitive and quantitative than the cell-based assays and allows for measurement of binding kinetics. We again utilized oligomeric, globulomeric, and monomeric Aβ. Fig. 6 (A–F) shows the binding curves and Scatchard plots for Aβo, Aβg, and Aβm, respectively. As measured in the cell-based assays, hPrP<sup>C</sup>, LilrB2, and NgR1 bound neurotoxic oligomers and globulomers (Fig. 6). The plate assay faithfully recapitulated effects of Aβ preparations on binding profiles. We observed highly similar binding of Aβo and Aβg to hPrP<sup>C</sup> and NgR1 (Fig. 6, G and H) and a preference of LilrB2 for Aβo over Aβg (Fig. 6I) as was also observed in Fig. 4E. Binding of Aβm to the receptors was minimal as was observed in the cell-based assay (Fig. 6, E and F). The dissociation constants and B<sub>max</sub> extracted from Fig. 6 are reported in Table 1. PrP<sup>C</sup> is the highest-affinity receptor for neurotoxic Aβo and Aβg, with affinities of 1.4 and 1.5 nM, respectively. The affinity of NgR1 for...
these preparations was ~3–4-fold lower than that of hPrPC. LilrB2 demonstrated the lowest affinity, with $K_D$ of 42.3 and 207.2 nM. To quantify each receptor’s ability to discriminate between neurotoxic and nontoxic preparations of Aβ, we calculated a discrimination factor by dividing each receptor’s $K_D$ for Aβ by that of the indicated species. hPrPC was highly discriminate in its preference for oligomeric and globulomeric Aβ with discrimination factors of 68.5 and 63.9, respectively. NgR1 also discriminated in binding, although to a lesser degree than hPrPC. LilrB2 exhibited a modest preference for Aβo compared with monomers and bound Aβg with the same affinity as for Aβm (Table 1).

**PrPC and NgR1 bind Aβ present in the brains of patients with AD**

Despite the ability of preparations of synthetic Aβ to generate neurotoxic species with masses similar to those found in the brains of patients with AD and to cause neurotoxicity *in vitro*, *ex vivo*, and *in vivo*, it is critical to examine each receptor’s disease relevance using pathological Aβ present in disease (10, 33, 35–37). Having validated the concordance of our plate-based assay with the observations made in living cells, we quantified binding of Aβ present in TBS-soluble extracts of brains from 10 patients with autopsy-confirmed AD and compared it with signal from brains of 11 cognitively normal patients. The mean anti-Aβ signal from wells coated with hPrPC was highest and significantly greater than that from cognitively normal patients. LilrB2 exhibited no binding to Aβ present in brains of patients with AD and no difference between AD and control brains. NgR1 modestly bound Aβ present in AD patient brains, and this signal was significantly greater than that in brains from cognitively normal patients (Fig. 7).

**Discussion**

Here, a panel of putative Aβ receptors was expressed on the surface of nonneuronal cells and examined for sufficiency to mediate Aβ binding. By accounting for differences in receptor expression using a common epitope tag across the receptors, we made direct comparisons of Aβ affinity. Importantly, binding took place in living cells rather than cell-free systems. This allows for potential binding events to take place at the cell surface and in a physiological context with respect to the lipid and protein environment. Our experiments demonstrate that most of the reported receptors for Aβ are not sufficient to confer Aβ binding capacity to cells using three different preparations of synthetic Aβ. It is possible that those receptors that failed to bind Aβ in our experiments require a coreceptor that is not present in COS-7 cells. Whereas our cell surface biotinylation experiments demonstrate successful transport through the secretory system, which is typically associated with proper protein folding, they do not definitively exclude misfolding that could impact ligand binding. Whereas these negative proteins

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*Figure 3. PrPC and NgR1 preferentially bind neurotoxic Aβo. A, SEC trace of 50 μM BAβo showing absorbance at 280 nm. Alternate vertical columns indicate fractions collected for analysis. Yellow fraction, HMM Aβ. Elution times of various standards (kDa) are indicated by arrows. V0, void volume as determined by blue dextran, 2,000 kDa. B, detection and quantification of the effect of the preparative method on the distribution of BAβ in different SEC fractions by dot blot analysis. C, quantification of expression-normalized Aβ signal from COS-7 cells transfected with the indicated candidate receptor and incubated with 1 μM monomeric biotin Aβ at 4 °C. Values are normalized to oligomeric biotin Aβ binding to hPrPC. One-sided t test comparing with an expected value of 100. n = 3–5 experiments. D, binding of receptors to monomeric Aβ compared with Aβo from Fig. 2B. Shown are multiple t tests with Holm–Sidak correction for multiple comparisons. Individual data points indicate different experiments. Error bars, S.D. n.s., not significant; *, p < 0.05; **, p < 0.01; ***, p < 0.001.*
may have Aβ affinity under some experimental conditions, in a cellular context, their binding affinity is clearly much less than that of the three positive receptors. By including an analysis of binding to monomeric Aβ, we revealed that PrPC and NgR1 discriminate between pathological and nontoxic forms of Aβ. It should be noted that neither oligomeric nor monomeric preparations are exclusive to a single species but that these designations are meant to reflect the assemblies for which the preparation is enriched. It is possible that the reduced but persistent binding of monomer preparations to PrPC and NgR1 may reflect the decrease in the abundance of Aβo. It is clear, however, that upon enrichment for monomeric Aβ, none of the candidates that failed to bind Aβo showed an increase in binding. We also found that LilrB2 does not differentiate in binding these oligomeric and monomeric Aβ preparations. The promiscuity of LilrB2 may indicate that an epitope present in both species of synthetic Aβ mediates this interaction. Recently, Cao et al. demonstrated that LilrB2 can bind minimal peptides comprising Aβ amino acids 1–21, 15–35, and a tandem repeat of 16–21 without a specific oligomerization procedure (38). In contrast, PrPC has been shown to undergo structural changes specifically in the presence of oligomeric Aβg (39).
Neuronal acetylcholine receptor subunit α-7 (nAChRα7) and NMDA receptor subunits 1 and 2B have also been reported to bind Aβ (40–42). We sought to include these in our investigation; however, efficient transport to the cell surface as determined by cell surface biotinylation experiments was insufficient to make comparisons with the rest of the panel. Despite these expression levels, and because we have observed that even very low expression of the high-affinity receptor PrP^C can result in robust Aβ binding, nAChRα7 with and without the chaperone RIC-3 (resistance to inhibitors of cholinesterase 3) and GluN2B with and without GluN1 (glutamate receptor ionotropic NMDA 1) were included in our cell-binding experiments. No detectable Aβ-binding signal was observed in nAChRα7 or GluN2B experiments (data not shown).

Whereas the ability to bind Aβ is a crucial characteristic for a receptor, we found it important to interrogate the necessity of confirmed receptors for Aβ binding to neurons. Pirb is the mouse homolog of LilrB2 and was shown to bind Aβ (11). By generating double-knockout mice deficient in NgR1 and Pirb, we found that up to 20% of binding of synthetic Aβ to neurons is mediated by these two receptors.

Preparations of synthetic Aβ peptide provide a convenient source of neurotoxic Aβ with species representative of those found in the brains of AD patients. When investigating the relevance of a potential receptor to human disease, the gold standard should be an ability to bind Aβ present in the brains of patients diagnosed with AD. We first validated our plate-based assay by replicating the experiments done in mammalian cells and found the two to be in close agreement. We determined that hPrP^C is the high-affinity receptor for Aβ (K_D = 1.4), followed by NgR1 (K_D = 3.9) and LilrB2 (K_D = 42.3). We then utilized this assay for the detection of binding to soluble Aβ found in extracts of brains from patients diagnosed with AD. We found that hPrP^C and NgR1 bound Aβ present in AD patient brains and that hPrP^C did so to the greatest extent. Finally, we found that despite binding synthetic Aβ from multiple preparations and in cell-based and purified protein assays, LilrB2 did not detectably bind the Aβ species present in AD patient brains.

The experiments described here provide much needed clarity to the field regarding the nature of the interaction of neurotoxic Aβ in the extracellular space with the cell surface. The observation made here that NgR1 and LilrB2 account for up to 20% of Aβ binding to hippocampal neurons, coupled with previous observations that PrP^C is responsible for 50% of Aβ binding to neurons and the knowledge that Aβ binds neurons in a trypsin-sensitive manner, suggests that at least one additional receptor for Aβ remains to be identified (3, 10). Future characterizations of novel Aβ receptors should include experiments testing the necessity and sufficiency for cellular Aβ binding. It will also be necessary to examine the disease relevance of observations made with synthetic peptides and transgenic animals by using materials obtained from patients with AD.

Experimental procedures

Tissue culture

COS-7 or HEK293T cells were grown at 37 °C with 5% CO2 on 100-mm tissue culture dishes (Fisher, 08-772E) in Dulbecco’s modified Eagle’s medium (Gibco, 11965) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin (Gibco, 15140). For Aβ-binding experiments, COS-7 cells were seeded into 8-well chamber slides (Lab-Tek, 154941) or pSecTag2A (Thermo, V90020). Accession numbers for cDNA sequences are as follows: hPrP (NM_000311.3), mPrP (NM_011170.3), LilrB2 (XM_006726139.1), NgR1 (NM_023004.5), EphA1 (BC_130291.1), FcγRIIB (BC031992.1), SorLA (NM_003105.5), Sortilin (NM_002959.5), p75^NTR (NM_002507.3), PGRMC1 (NM_006667.4), NLGN1 (NM_014932.3), RAGE (AB036432.1), EphB2 (BC146296.1), FZD5 (BC172518.1), mGluR5 (NM_017012.1), EphA4 (BC026327.1), CRMP2 (NM_001386). The construct encoding NR1P1 has been described previously (43). Constructs were verified by DNA sequencing.
Cell surface biotinylation and immunoprecipitation

Dishes of transfected HEK293T cells were washed three times with ice-cold PBS, pH 8, and biotinylated with 10 ml of 0.48 mg/ml Sulfo-NHS-LC-LC-Biotin (Thermo Fisher 21338) in PBS, pH 8, and incubated at room temperature for 30 min. Cells were washed once with 50 mM Tris, pH 8, and then twice with ice-cold PBS, pH 8. Cells were harvested in 1 ml of ice-cold PBS and gently scraped to collect. Collected cells were transferred to a new tube and centrifuged at 500 g for 3 min at 4 °C to pellet them. The cell pellet was resuspended in 1 ml of RIPA lysis buffer (Millipore 20-188) with PhosSTOP (Roche Diagnostics, 04906837001) and Complete Mini protease inhibitors (Roche Diagnostics, 11836170001) and sonicated. After sonication, samples were centrifuged at 100,000 g, 4 °C for 30 min. Supernatants were transferred to c-Myc beads (Pierce 20169) and incubated at 4 °C with endo-over-end mixing for 1 h. Beads were washed three times with RIPA buffer. Proteins were eluted with 4 Laemmli sample buffer (Bio-Rad, 161-0747) and analyzed by immunoblotting using anti-Myc (Cell Signaling, catalog no. 2276, 1:1,000), donkey anti-mouse IRDye 680LT (LI-COR Biosciences, 926-68022, 1:20,000), and streptavidin IRDye 800CW (LI-COR Biosciences, 926-32230, 1:20,000) and imaged with the Odyssey infrared imaging system (LI-COR Biosciences).

Synthetic Aβ preparations

Lyophilized synthetic β-amyloid (residues 1–42) was purchased from The ERI Amyloid Laboratory, LLC. Vials of pep-
Table 1

Binding affinities for positive receptor candidates and preparations of synthetic Aβ

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Aβ0</th>
<th>Aβg</th>
<th>Aβm</th>
</tr>
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<tbody>
<tr>
<td>KD (nM)</td>
<td>1.4 ± 0.2</td>
<td>1.5 ± 0.2</td>
<td>95.9 ± 6</td>
</tr>
<tr>
<td>Bmax (AU)</td>
<td>6.27 × 10^6</td>
<td>5.9 × 10^6</td>
<td>6.46 × 10^6</td>
</tr>
<tr>
<td>Discrimination factor</td>
<td>68.5</td>
<td>63.9</td>
<td></td>
</tr>
</tbody>
</table>

Figure 7. hPrPc and NgR1 bind soluble Aβ present in brains of AD patients. Shown is binding of Aβ in TBS-soluble fraction of brains of AD patients or control patients (Ctrl) to wells coated with hPrPc, NgR1, or LirrB2. Each dot represents an individual patient brain. Shown are multiple t tests with Holm-Sidak correction for multiple comparisons. Error bars, S.D. **, p < 0.01; ***. p < 0.001. AFU, arbitrary fluorescence unit.

Immunocytochemistry and heterologous cell-binding assays

COS-7 cells were cultured in 8-well chamber slides as described above. For Aβ binding experiments, the culture medium was removed from wells, leaving a volume sufficient to cover the cells, and washed with F12, and the indicated Aβ preparation was added. Slides were then incubated for 2 h at the indicated study temperature. Wells were washed three times with PBS and fixed with 3.7% formaldehyde (J.T. Baker, 2106) in PBS with 6% sucrose (AmericanBio, AB01900) for 30 min at room temperature. The fixative was then removed, and cells were permeabilized by incubation in 0.2% Triton X-100 (Fisher Scientific, 42235-5000) in PBS for 30 min. After permeabilization, cells were blocked by incubation with 5% normal donkey serum (Jackson ImmunoResearch, 017-000-121) for 1 h at room temperature. After blocking, cells were incubated with the indicated primary antibodies for anti-Myc (Cell Signaling, catalog no. 2276, 1:5,000) or anti-Aβ (Cell Signaling, catalog no. 8243, 1:1,000), diluted in 1% BSA (Sigma, catalog no. A9647) at 4 °C overnight. The next day, cells were washed three times with PBS with incubation periods of 0, 3, and 5 min. The indicated appropriate secondary antibodies diluted in 1% BSA was used. For atomic force microscopy and SEC, NaOH monomer samples were generated by dissolving HFIP-treated Aβ in 440 μl of 0.1 M NaOH, resulting in a 250 μM stock, and processed for atomic force microscopy or SEC immediately.
normalized by background-subtracted Myc signal and expressed as a percentage of that signal in hPrP<sup>C</sup>-transfected cells, which were included in every experiment for comparison. Limit of detection was calculated as the mean background-subtracted Aβ signal in cells transfected with cytoplasmic enhanced GFP, expressed as a percentage of that in hPrP-transfected cells, plus two S.D. values.

**Size-exclusion chromatography**

SEC was performed on the described preparations of biotinylated Aβ using a Superdex 200 Increase 10/300 GL column (GE Healthcare, 28990944) and Akta Pure 25 M1 (GE Healthcare) with an F9-C fraction collector. SEC was performed at 4 °C. 1.5-ml fractions were collected in 2-ml deep 96-well plates (USA Scientific, 1896–2110). For the representative trace, 50 µM BAβo in Ham’s F12 medium was loaded into a 500-µl sample loop. For dot blot analysis, 1 µM samples were loaded. Samples prepared in Ham’s F12 were run using Ham’s F12 as the mobile phase. NaOH monomer samples were run in a mobile phase of 0.25× PBS, pH 7.4. Gel filtration standards used were blue dextran (Sigma, D4772), thyroglobulin, bovine γ-globulin, chicken ovalbumin, equine myoglobin, and vitamin B12 (Bio-Rad, 1511901). Fractions were concentrated using centrifugal filters with a 3,000 nominal molecular weight limit (Amicon UFC500324). Dot blot analysis was performed by applying 80% of concentrate volume to a nitrocellulose membrane using a UFC500324). Fractions were concentrated using centrifugal filters with a 3,000 nominal molecular weight limit (Amicon UFC500324). Dot blot analysis was performed by applying 80% of concentrate volume to a nitrocellulose membrane using a Bio-Rad Bio-Dot apparatus. Samples were incubated on the membrane for 4 h at room temperature, washed twice with TBS, blocked for 1 h at room temperature with Rockland blocking buffer for fluorescent Western blotting (Rockland, MB070010TF), and probed overnight at 4 °C with streptavidin Alexa Fluor 488 donkey anti-rabbit IgG (1:1,000, Life Technologies, Inc., A21206), and biotinylated Aβ was visualized with streptavidin Alexa Fluor 568 (1:500, Life Technologies, S11226). z-Stacks were collected at ×40 using a spinning disc confocal microscope. z-Stacks were then max-projected using National Institutes of Health ImageJ (47). Quantification was performed using CellProfiler (44). Integrated punctate Aβ signal was calculated after background subtraction and thresholding. Integrated signal was then normalized to the area positive for MAP2. When representing these data graphically, each data point represents a single region of interest from 5–7 dishes. The experiment was repeated four times.

**Plate-based assay**

384-well MaxiSorp plates (Thermo, 460372) were coated overnight with 20 µl/well of the indicated protein at 250 ng in 100 mM BupH carbonate-bicarbonate coating buffer (Thermo 28382) at 4 °C. Plates were washed twice with PBST (PBS, 0.05% Tween 20) and blocked with 25 µl of Protein-Free T20 PBS Blocking buffer (Pierce 37573) for 4 h at room temperature. After washing three times with PBST, 20 µl of either TBS fraction of human brain samples or synthetic Aβ was applied to wells and incubated overnight at 4 °C. Aβ binding was detected using D54D2 anti-Aβ antibody (Cell Signaling, catalog no. 8243, 1:2,000) in PBSTB (PBS, 0.05% Tween 20, 0.5% BSA) for 2 h. After four washes with PBST, 20 µl of Eu-N1 goat anti-rabbit IgG (PerkinElmer Life Sciences, AD105, 1:4,000) was diluted in DELFIA assay buffer (PerkinElmer Life Sciences) and incubated for 1 h at room temperature. After four washes with PBST, 20 µl of DELFIA Enhancement Solution (PerkinElmer Life Sciences) was applied, and time-resolved europium fluorescence was measured with a Victor 3V plate reader (PerkinElmer Life Sciences).

Recombinant hexahistadine-tagged hPrP<sup>C</sup> was generated using a method described previously (7). The extracellular domain of LilrB2 is encoded by amino acids 22–461. We subcloned cDNA encoding this region into pSecTag2A (Invitrogen) to include a C-terminal hexahistidine tag. HEK293T cells were transfected with this ecto-LilrB2 construct, and 24 h later, the medium was replaced with serum-free medium. The next day, the conditioned medium was collected and purified using the same method described for hPrP<sup>C</sup>. Human NgR-Fc decoy protein encodes amino acid residues 1–310 of human NgR1 with the C266A and C309A substitutions fused to the Fc domain of human IgG1. NgR(310)-Fc protein was produced as described previously and supplied by ReNetX Bio (48).

**Human brain samples**

Post-mortem human tissue was collected in accordance with institutional review board protocols approved by Yale University. Samples of brain tissue were microscopically analyzed to

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*Standardized comparison of reported Aβ receptors*

**Atomic force microscopy**

AFM samples were prepared by placing 10 µl of 0.25 mg/ml Aβ preparations on freshly cleaved mica and allowing it to adsorb for 2 min. The sample was washed twice with 200 µl of Milli-Q water and dried carefully. Images where taken in a Bruker Dimension Fastscan AFM in a tapping mode using silicon nitride cantilevers.

**Mouse breeding and care**

Mice were cared for by the Yale Animal Resource Center, and all experiments were approved by Yale’s institutional animal care and use committee. NgR<sup>–/–</sup> (Rtn4r) and Pirb<sup>–/–</sup> mice have been described previously and were maintained on a C57/B6 background (45, 46). Pirb<sup>–/–</sup> mice were a generous gift of Dr. Toshiyuki Takai. NgR<sup>–/–</sup> and Pirb<sup>–/–</sup> mice were bred together to generate double-heterozygous and eventually double-knockout mice.

**Neuron cultures and neuronal binding assays**

Mouse hippocampal neurons from WT and Rtn4R<sup>−/−</sup>, Pirb<sup>−/−</sup> double knockout animals were isolated from embryonic day 17 to postnatal day 0 pups and cultured on MatTek dishes (P35G-1.5-14-C) coated with poly-d-lysine (MP Bio, 0215017580) in Neurobasal medium (Gibco, 10888-022) supplemented with B-27 (Gibco, 17504-044), sodium pyruvate (Gibco, 11360-070), and Glutamax (Gibco, 35050-061), with 1% penicillin and streptomycin (Gibco, 15140). Binding experiments were performed at 18–20 days in vitro. After washing, neurons were incubated with 500 nM monomer-equivalent (~2.5 nM oligomer) biotinylated Aβ for 1 h at 4 °C. Following fixation, permeabilization, and blocking, dendrites were visualized using anti-MAP2 (1:2,000, Millipore, AB5622) and Alexa Fluor 488 donkey anti-rabbit IgG (1:1,000, Life Technologies, Inc., A21206), and biotinylated Aβ was visualized with streptavidin Alexa Fluor 568 (1:500, Life Technologies, S11226). z-Stacks were collected at ×40 using a spinning disc confocal microscope. z-Stacks were then max-projected using National Institutes of Health ImageJ (47). Quantification was performed using CellProfiler (44). Integrated punctate Aβ signal was calculated after background subtraction and thresholding. Integrated signal was then normalized to the area positive for MAP2. When representing these data graphically, each data point represents a single region of interest from 5–7 dishes. The experiment was repeated four times.
confirm the clinical diagnosis of AD (Braak Stage V or higher). Samples from neurologically healthy controls were required to have no or minimal histopathological signs of AD (Braak 0–II). Frozen prefrontal cortex was stored at −80 °C until used. Human brain was weighed and Dounce-homogenized in 3 volumes of TBS, pH 7.4 (Bio-Rad, catalog no. 170-6435) supplemented with Complete Mini Protease Inhibitors (Roche Diagnostics, 1183617001) and PhosStop phosphatase inhibitors (Roche Diagnostics, 04906837001). Samples were centrifuged at 100,000 × g at 4 °C for 1 h, and the supernatant was collected, flash-frozen in liquid nitrogen, and stored at −80 °C until assayed. The supernatant was referred to as the TBS-soluble fraction. Patient demographics for brains used are described in Table 2.

Statistics

All results are presented as mean ± S.D. unless otherwise stated. Prism version 7 was used for statistical analysis, and specific tests used are indicated in the figure legends.

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References


