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Endogenous Amyloid-β is Necessary for Hippocampal Synaptic Plasticity and Memory

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Abstract

Objective—The goal of this study was to investigate the role of endogenous amyloid-β peptide (Aβ) in healthy brain.

Methods—Long-term potentiation (LTP), a type of synaptic plasticity that is thought to be associated with learning and memory, was examined through extracellular field recordings from the CA1 region of hippocampal slices, whereas behavioral techniques were used to assess contextual fear memory and reference memory. Amyloid precursor protein (APP) expression was reduced through small interfering RNA (siRNA) technique.

Results—We found that both antirodent Aβ antibody and siRNA against murine APP reduced LTP as well as contextual fear memory and reference memory. These effects were rescued by the addition of human $A\beta_{42}$, suggesting that endogenously produced Aβ is needed for normal LTP and memory. Furthermore, the effect of endogenous Aβ on plasticity and memory was likely due to regulation of transmitter release, activation of α7-containing nicotinic acetylcholine receptors, and $A\beta_{42}$ production.

Interpretation—Endogenous $A\beta_{42}$ is a critical player in synaptic plasticity and memory within the normal central nervous system. This needs to be taken into consideration when designing therapies aiming at reducing Aβ levels to treat Alzheimer disease.

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During the last 25 years, the amyloid-β peptide (Aβ) hypothesis has been the central theory of Alzheimer disease (AD) pathogenesis, because the peptide is the principal component of the amyloid plaque, a main pathological hallmark of AD. Extensive literature suggests that synaptic disorders, progressively involving larger areas of the brain, could be produced, at least in part, by an increase in Aβ levels.[1] Synaptic disorders, in turn, are likely to underlie subtle amnesic changes occurring at early disease stages.[2] High levels of Aβ have also been found to markedly reduce long-term potentiation (LTP), a type of synaptic plasticity that is thought to underlie learning and memory, and cause memory loss.[3–15] Moreover, several studies using transgenic human Aβ-producing mice have shown significant deficits in LTP and memory.[16] Taken together, these findings make a strong case in favor of a toxic role of Aβ in AD. Different Aβ species (eg, $A\beta_{42}$ and $A\beta_{40}$), however, are not only present in elevated amounts in disease state, but are also present in the brain in low levels throughout life, suggesting a possible physiological role for Aβ in normal healthy individuals.

Aβ peptides are generated from cleavage of the amyloid precursor protein (APP). APP is a type-1 transmembrane ubiquitous protein that resembles a cell surface receptor[17] and contains a large extracellular domain, a hydrophobic transmembrane domain, and a short intracellular domain.[18,19] APP cleavage by α- or β-secretases generates large, soluble, secreted fragments (sAPPα and sAPPβ) and membrane-associated carboxy-terminal fragments (CTFs). Sequential cleavage of APP by β-secretase followed by γ-secretase cleavage within the transmembrane domain leads to the production of Aβ peptides. APP has a structure similar to that of the APP-like proteins 1 and 2 (APLP1 and APLP2) and undergoes similar processing by secretases.[20,21] Only APP, however, contains an Aβ domain, supporting the hypothesis that Aβ may have a unique physiological function in vivo, which may have been created during evolution when its sequence was introduced into the APP gene.

Studies regarding the physiological function of Aβ peptides have been limited. Picomolar levels of exogenously applied $A\beta_{40}$ have been found to play a neurotrophic role in cell cultures,[22,23] and treatment of hippocampal neural stem cell progeny with $A\beta_{42}$ induces an increase in the number of newly generated neurons.[24] Aβ levels are likely to be regulated by synaptic activity in an endocytosis-dependent manner,[25–27] depressing synaptic function, as demonstrated in systems overexpressing familial AD-mutant APP.[26] Moreover, brain interstitial fluid concentration of Aβ seems to be correlated with neurological status, with Aβ concentrations increasing as neurological status improves and vice versa.[28] Recently, we have demonstrated that low picomolar amounts of exogenously applied $A\beta_{42}$ enhance synaptic plasticity and memory[29]; however, a role for endogenous Aβ in normal brain remains to be defined. To determine if Aβ has a function in normal brain, we examined synaptic plasticity and memory in wild-type mice in which endogenous Aβ was depleted using either an antirodent Aβ antibody or small interfering RNA (siRNA) directed against rodent APP.

Materials and Methods

Animals

C57BL/6, male, wild-type mice (3–4 months old) were obtained from a breeding colony housed in the animal facility at Columbia University. α7-nAChR knockout (KO) mice have been previously described.[29] APP KO mice were purchased from Jackson Laboratories (Bar Harbor, ME).

Anti-Aβ Antibodies

The antirodent Aβ monoclonal antibody, JRF/rAb2, was generated at Janssen Pharmaceutica (Beerse, Belgium). Balb/c mice were immunized with rodent Aβ-derived peptide (amino acids 1–15) coupled to keyhole limpet hemocyanin (KLH) according to the manufacturer's instructions (Pierce, Rockford, IL). Mice were boosted every 2 weeks with 100μg KLH-coupled peptide, first in complete Freund adjuvant, and subsequently in incomplete Freund adjuvant. Mouse spleen cells were fused with Sp2/0 cells by a modified procedure of Kohler and Milstein. The hybridomas were seeded in 96-well plates and screened after 10 days in a direct enzyme-linked immunosorbent assay (ELISA) on bovine serum albumin-coupled rodent Aβ peptide and confirmed on noncoupled rodent Aβ peptide. Positive cells on free rodent Aβ1–15 were immediately subcloned, and positive clones were frozen in liquid nitrogen. All hybridomas were grown in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum (Hyclone Europe, Edegem, Belgium), 2.5% ESG Hybridoma supplement (Elscolab, Kruibeke, Belgium), 2% HT media supplement (Sigma-Aldrich, St Louis, MO), 1mM sodium pyruvate, and 2mM L-glutamine and penicillin (100U/ml) and streptomycin (50mg/ml) (Life-Technologies, Paisley, UK). Cells were incubated in a humidified 8% CO_2 air incubator. This antibody recognizes an epitope within residues 1–15 of the rodent Aβ sequence, a region that contains 3 amino acid differences compared to the human sequence. This antibody binds synthetic and endogenous, brain-derived rodent Aβ (both $Aβ_{40}$ and $Aβ_{42}$) with high affinity by ELISA[30] and immmunoprecipitation,[30,31] and completely fails to recognize human Aβ (Supplementary Fig 1). In addition, in an aged APP transgenic mouse with robust β-amyloid deposition, which allows for detection of the abundantly deposited Aβ, JRF/rAb2 monoclonal antibody (mAb) detects codeposited murine Aβ by immunohistochemistry.[30] No cytotoxicity has been seen when neuronal primary mouse cultures as well as mouse N2a cells have been grown in the presence of 1μg/ml JRF/rAb2 mAb-containing media for 24 hours, nor has intraperitoneal injection of 0.5 mg JRF/rAb2 mAb daily over 1 week shown any toxic effects in wild-type or human APP transgenic mice (data not shown). The working concentration of 4μg/ml used in the electrophysiology experiments was based upon the concentration of JRF/rAb2 mAb typically employed at Janssen Pharmaceutica for immunoprecipitation, and the amount injected into mice (4μg/hippocampus) was calculated from this. As a control, we used an IgG2a isotype-matched control antibody that does not bind to any rodent proteins (termed *control mAb*, Tepnel Life Sciences, Manchester, UK).

Aβ preparation, electrophysiological studies, infusion technique, and behavioral studies have been previously described[29] (see also Supplementary Material and Methods for a detailed description).

siRNA Preparation and Testing

siRNAs were designed according to published guidelines with dTdT 3′ overhangs[32] (see Supplementary Material and Methods for a detailed description). The sequence for the sense strand of the central 19-nucleotide double-stranded region was GCAC-TAACTTGCACGACTATG. Short hairpin RNA (shRNA) with the same sequence has been previously shown to acutely knock down APP function.[33] The control siRNA sequence used was AAUGGUGUGGCCAUUGACCCC.

Aβ Measurement

Following euthanasia, mouse hippocampi were quickly removed and frozen at given time points (along with the control regions of the cerebellum). The specimen was homogenized in 1ml of HEPES-sucrose buffer (20mM HEPES/NaOH pH 7.4, 1mM ethylenediaminetetraacetic acid, 1mM ethyleneglycoltetraacetic acid, 250mM sucrose) supplemented with protease inhibitors. The tissue homogenates were treated with diethanolamine to extract soluble $A\beta_{42}$. Endogenous murine Aβ levels were determined by sandwich ELISAs using the mouse $A\beta_{x\text{-}42}$ kit from Covance (Princeton, NJ) or the $A\beta_{1–42}$ kit from Invitrogen (Carlsbad, CA), according to the manufacturer's protocol. For rescue experiments of siRNA effect on LTP, 8 hippocampal slices were pooled together and homogenized prior to performing ELISA measurements.

Statistical Analyses

Experiments were performed in blind. Results were expressed including standard error of the mean with level of significance set at $p < 0.05$. Results were analyzed by Student *t* test (pairwise comparisons) or analysis of variance for repeated measures (multiple comparisons) with treatment condition as main effect. Planned comparisons were used for post hoc analysis. For analysis of behavior tests, experiments were designed in a balanced fashion, and mice were trained and tested at each of the different conditions in 3 or 4 separate experiments.

Results

Depletion of Endogenous Aβ Impairs Hippocampal LTP

To investigate the role of Aβ in synaptic plasticity, we blocked endogenously produced Aβ with the monoclonal antibody JRF/rAb2. This antibody recognizes a rodent-specific epitope within the first 15 amino acids of rodent $A\beta_{40}$ and $A\beta_{42}$ with high affinity under multiple conditions (nondenaturing, denaturing, monomeric, oligomeric/fibril)[30,31] (see Supplementary Fig 1). When hippocampal slices were perfused with JRF/rAb2 mAb (4μg/ml, for 20 minutes prior to tetanizing Schaffer collateral fibers), LTP was dramatically reduced (Fig 1A). By contrast, the IgG2a isotype-matched negative control antibody (4μg/ml; termed *control mAb*) did not affect LTP (see Fig 1A). Nontetanized slices treated with JRF/rAb2 mAb alone or with control mAb alone showed no change in basal synaptic transmission (data not shown). To clarify whether Aβ acted on the LTP maintenance phase or induction, we applied the antibody after tetanization. However, perfusion with JRF/rAb2

mAb 20 minutes post-tetanus did not affect LTP (Supplementary Fig 2), suggesting that LTP maintenance is not affected by depletion of endogenous Aβ.

Because the JRF/rAb2 mAb might have effects independent of Aβ depletion, such as binding to APP, βCTF, sAPPα, or other nonspecific targets, our next goal was to determine whether the impairment of LTP was specifically mediated by Aβ. We performed rescue experiments with human $A\beta_{42}$, which is not recognized by the antibody (see Supplementary Fig 1). Addition of an aged preparation of human $A\beta_{42}$ (200pM) (Supplementary Fig 3) concurrent with the JRF/rAb2 mAb rescued the LTP deficit (see Fig 1B). Furthermore, a higher concentration of $A\beta_{42}$ (300pM) in addition to JRF/rAb2 increased LTP by ~50% (see Fig 1B), resembling the enhancement of LTP that we had previously demonstrated in slices perfused with 200pM $A\beta_{42}$ alone.[29] JRF/rAb2 mAb paired with $A\beta_{42}$ without tetanization did not affect baseline transmission (data not shown). Scrambled $A\beta_{42}$ peptide was used as a control for any nonspecific effects of $A\beta_{42}$ due to potential changes in osmolarity of the bath solution and failed to rescue the reduction in LTP by JRF/rAb2 mAb (~30% less of tetanized vehicle-treated slices; data not shown). Finally, a preparation enriched in monomers was not capable of rescuing LTP in slices that were concomitantly treated with mAb (Supplementary Fig 4), suggesting that oligomeric forms of Aβ are likely to be critical for normal synaptic plasticity.

To confirm our findings obtained using JRF/rAb2 mAb, we designed a siRNA specific for murine APP linked to Penetratin 1 (Pen1-APP-siRNA) peptide for intraneuronal delivery based on an shRNA sequence previously shown to acutely knock down APP expression.[33] In preliminary experiments, we demonstrated by quantitative Western blot analysis that full-length APP was reduced by approximately 60% in primary hippocampal cultures that were exposed to Pen1-APP-siRNA (80nM) for 24 hours prior to cell collection (Supplementary Fig 5), whereas a control siRNA did not affect APP expression. Next, we implanted cannulas bilaterally into mouse dorsal hippocampi and after 6 to 8 days injected siRNA (80nM in a final volume of 2μl over 1 minute, 24 hours prior to investigating LTP). APP suppression dramatically reduced LTP, whereas control siRNA failed to reduce LTP (see Fig 1C). Also, the effect of APP suppression by siRNA on synaptic plasticity was due, at least in part, to $A\beta_{42}$ deficiency, because the addition of 200pM $A\beta_{42}$ ameliorated LTP (see Fig 1C). Finally, we excluded the possibility that siRNA- or antibody-induced reduction in LTP was offset by the LTP-enhancing effects of Aβ via independent pathways. When we performed experiments in 2-month-old APP null mice exposed to JRF/rAb2 mAb or APP-siRNA, we found that neither the antibody nor APP-siRNA affected LTP (Supplementary Fig 6). Thus, our combined biochemical and genetic approaches indicate that $A\beta_{42}$ is required for the induction of LTP in the hippocampus.

Our next goal was to determine the threshold of $A\beta_{42}$ needed for normal synaptic plasticity. In these experiments, APP-siRNA was injected into hippocampi, after 24 hours mice were sacrificed, and slices were cut from the hippocampi. Following treatment with vehicle or human synthetic $A\beta_{42}$ at different concentrations (100, 200, 300pM), LTP was measured. Levels of endogenous $A\beta_{42}$ after siRNA treatment were also measured and found to be equal to 79.35 ± 3.11pM. Given that a complete rescue of potentiation was observed with

300pM $A\beta_{42}$ (Supplementary Fig 7), it is likely that the threshold of $A\beta_{42}$ needed for normal synaptic plasticity is ~380pM.

Endogenously Produced Aβ Is Required for the Induction of Memory

Given that LTP is thought to be associated with learning and memory,[34] we next assessed the effects of endogenous Aβ on hippocampal-dependent memory. JRF/rAb2 mAb, control mAb (4μg/μl, in a final volume of 1μl over 1 minute) or vehicle was bilaterally infused through cannulas, and after 15 minutes animals were tested for reference and contextual fear memory. We first tested reference memory by using the Morris water maze (MWM), a widely used spatial learning test known to require hippocampal function and assessing reference memory.[35] Mice treated with JRF/rAb2 mAb prior to each of the 6 sessions revealed a longer latency to find the hidden platform compared to vehicle-infused mice (Fig 2). The probe trial performed after removal of the platform demonstrated that JRF/rAb2 mAb-treated mice spent less time than vehicle-treated mice in the target quadrant (TQ), where the platform had been located during training. Infusion with control mAb or JRF/rAb2 mAb immediately after training did not affect mouse performance during the hidden platform and the probe trials. A visible platform trial performed after the probe trial did not reveal any significant difference in the time to reach the platform among the 4 groups tested (data not shown). Memory impairment was specifically mediated by Aβ, because application of 200pM $A\beta_{42}$ in addition to JRF/rAb2 mAb rescued the behavioral deficits during the hidden and probe tasks. Infusion with a higher concentration of $A\beta_{42}$ (250pM but not 300pM) and JRF/rAb2 mAb revealed faster learning compared to vehicle-infused mice, and planned comparisons showed a significant difference from the second to the fourth sessions. Given that the probe trial performed after the sixth session did not reveal further memory enhancement in the rescue experiments with 250pM $A\beta_{42}$, we postulate that excessive training may have obscured any difference in the time spent in the TQ. Thus, we performed additional experiments in which mice infused with 250pM $A\beta_{42}$ and JRF/rAb2 mAb, with JRF/rAb2 mAb alone, or with vehicle received only 3 training sessions with the hidden platform and then were tested in the probe trial. This experimental paradigm revealed a difference between mice treated with 250pM $A\beta_{42}$ and JRF/rAb2 mAb and those treated with vehicle and between mice treated with JRF/rAb2 mAb alone and those treated with vehicle during the hidden platform task (Supplementary Fig 8a) and the probe trial performed after the third hidden-platform session (see Supplementary Fig 8b). These findings were confirmed by infusing mice with Pen1-APP-siRNA 24 hours prior to performing the MWM test. APP suppression severely impaired reference memory, whereas control siRNA did not affect cognitive performance. Moreover, addition of 200pM $A\beta_{42}$ ameliorated the memory deficits. Thus, our combined biochemical and genetic approaches indicate that $A\beta_{42}$ is necessary for reference memory in hippocampus.

To extend our conclusions to an additional type of memory, we examined whether depletion of Aβ using JRF/rAb2 mAb (15 minutes prior to the foot shock) affects freezing in the contextual fear paradigm. Freezing was found to be unaffected following treatment with mAb during the training phase of the fear conditioning test (Fig 3). However, when contextual fear learning was assessed 24 hours later, JRF/rAb2 mAb-treated mice showed less freezing than vehicle-treated mice. By contrast, mice infused with control mAb or JRF/

rAb2 mAb post-training showed normal freezing. In interleaved experiments, 200 or 300pM $A\beta_{42}$ concurrent with JRF/rAb2 mAb re-established normal freezing; however, 250pM $A\beta_{42}$ further enhanced freezing compared to vehicle-injected mice, resembling the enhancement of fear memory that we had previously demonstrated in mice infused with 200pM $A\beta_{42}$ alone.[29] The genetic approach confirmed that $A\beta_{42}$ is required for induction of associative memory. Indeed, when we infused mice with Pen1-APP-siRNA (24 hours prior to performing behavioral analysis for contextual memory), contextual fear memory was impaired, whereas perfusion with control siRNA did not affect the cognitive performance. Moreover, infusion with human synthetic $A\beta_{42}$ (200pM) rescued the memory deficit. Thus, these findings strongly support the hypothesis that, similar to LTP and reference memory, $A\beta_{42}$ is required for induction of associative memory.

Hippocampal Aβ42 Production Is Enhanced during Memory Induction

We measured hippocampal levels of $A\beta_{x\text{-}42}$ in mice that were trained for contextual fear learning and sacrificed at different intervals after the electric shock. Mice sacrificed at 1 minute (but not at 5 and 30 minutes) showed a significant increase in peptide levels compared to a naive control group (Fig 4). We obtained similar results when we measured $A\beta_{42}$ levels using a different antibody that recognizes $A\beta_{1\text{-}42}$. By contrast, cerebellar $A\beta_{x\text{-}42}$ levels were not modified at 1 minute after training. Interleaved experiments testing different conditions, including a group exposed to the context without tone and foot shock, a group exposed to the tone alone, and a group exposed to foot shock without context association showed no changes in $A\beta_{x\text{-}42}$ levels, with the exception of the cohort exposed to the tone plus foot shock. These findings strongly support the hypothesis that hippocampal $A\beta_{42}$ production is enhanced during memory induction.

Depletion of Endogenous Aβ Impairs Hippocampal PTP

Given that Aβ levels are regulated by synaptic activity[25,26] and that $A\beta_{42}$ enhances transmitter release during tetanus,[29] we examined post-tetanic potentiation (PTP), a type of synaptic plasticity that reflects a tetanus-induced enhancement of transmitter release. As previously demonstrated,[29] $A\beta_{42}$ (200pM) enhanced PTP, whereas JRF/rAb2 mAb (4μg/ml, 20 minutes prior to tetanus) reduced it (Fig 5A). Most importantly, the impairment of PTP due to the effect of the mAb was rescued by concomitant application of 200pM $A\beta_{42}$, with 300pM $A\beta_{42}$, producing a more pronounced enhancement similar to 200pM $A\beta_{42}$ alone (see Fig 5B). Taken together, these findings strongly support the hypothesis that endogenously produced Aβ is critical for normal transmitter release during tetanus.

The Effect of Endogenous Aβ on Synaptic Plasticity Involves α7-Nicotinic Acetylcholine Receptors

We have previously shown that α7-nicotinic acetylcholine receptors (nAChRs) are involved in synaptic plasticity and memory and play a role in enhancement upon the addition of exogenous $A\beta_{42}$.[29] Here, we determined whether α7-nAChRs are involved in the reduction of LTP by depletion of $A\beta_{42}$ levels. When hippocampal slices from α7-nAChR KO mice were perfused with JRF/rAb2 mAb (4μg/ml, 20 minutes prior to tetanus), LTP was no longer reduced (Fig 6). By contrast, JRF/rAb2 mAb reduced LTP in slices from wild-type littermates. Consistent with these findings, PTP was not affected by JRF/rAb2 mAb in slices

from α7-nAChR KO mice, whereas the mAb was capable of impairing PTP in slices from wild-type littermates. Thus, involvement of α7-nAChRs is likely to be necessary for the effect of endogenous $A\beta_{42}$ on transmitter release.

Discussion

Our data provide novel robust evidence that endogenously produced Aβ is critical for normal synaptic plasticity and memory. Understanding the physiological role of APP and its fragments, including Aβ, is a highly complex task. Most of these studies have been performed using KO animals, which have been shown to have a complex phenotype characterized by weight reduction, agenesis of the corpus callosum, hypersensitivity to seizures, defects in copper and lipid homeostasis, and impaired grip strength, locomotor activity, exploratory activity, cognition, and LTP.[36–40] Yet many aspects of APP function are not clear, most likely due to limitations of the KO approach. LTP was found to be reduced only in aging KO mice or when potentiation was evoked through a theta-burst stimulation.[38] In addition, muscle weakness,[41] hypersensitivity to seizures,[42] different genetic background,[43] and developmental issues[33] confound the interpretation of the behavioral experiments assessing cognitive function in APP-depleted animals. Therefore, due to these challenges, we have chosen 2 alternative strategies by which to block endogenous murine Aβ function: (1) by utilization of a rodent-specific monoclonal antibody named JRF/rAb2 and (2) by means of a siRNA against APP.

Experiments in both *Caenorhabditis elegans*[44] and mice[45] have shown that secreted, non-Aβ-containing APP products are capable of rescuing the effects of APP loss-of-function mutations. Hence, one might raise concerns about the uniqueness of Aβ as a molecule responsible for rescuing deficits by APP suppression. However, the *C. elegans* study could not demonstrate a function for Aβ, because the organism does not express Aβ naturally. In mice, memory rescue was tested at a young age, when cognition is still normal in KO animals[45]; LTP, due to the advanced age of the animals, was already reduced in wild-type mice; thus, it is possible that the LTP investigated in the rescue experiments with knockin mice was independent of the mechanisms that affect it as a consequence of suppression of APP function.[45] Due to the limitations of using genetically modified animals, through our approaches with JRF/rAb2 or APP-siRNA, we have unraveled a novel function for Aβ as a molecule that is indispensable for synaptic plasticity and memory within the normal central nervous system (CNS).

Is it possible that other fragments play a role in synaptic plasticity and memory? For instance, a putative physiological function of sAPPα in synaptic plasticity and memory has been suggested[46] (but also see Furukawa et al[47]). Intriguingly, sAPPα belongs to the extracellular domain of APP and shares 16 amino acids with Aβ. Thus, an attractive hypothesis is that amino acids 1 to 16 of Aβ, which are found at the C-terminal end of sAPPα, are responsible for the effect on synaptic plasticity and memory. Nevertheless, our foremost conclusion that Aβ itself is a critical positive modulator of synaptic plasticity and memory within the normal CNS remains unchallenged.

An interesting observation from our experiments is that JRF/rAb2 blocked LTP and memory when it was administered prior to tetanus in electrophysiological experiments or training in behavioral experiments. Application of the antibody immediately after tetanus or training, however, did not affect plasticity and memory, supporting the hypothesis that Aβ is required for the induction phase of synaptic plasticity and memory, but not for plasticity maintenance and memory consolidation. Consistent with this idea is the observation that hippocampal endogenous Aβ levels are increased immediately after training in the contextual fear test and the finding that Aβ secretion is increased following synaptic or neuronal activity,[25,26] as well as inhibition of PTP by JRF/rAb2. Taken together, these experiments suggest a model in which newly formed endogenous Aβ is necessary for the release of neurotransmitter from the presynaptic terminal, which underlies mechanisms of plasticity that occur during learning.

To demonstrate specificity of our approaches against Aβ, we performed rescue experiments in which Aβ was applied concomitantly with JRF/rAb2 or APP-siRNA. Human $A\beta_{42}$, which differs by 3 amino acids from rodent Aβ and is not recognized by the antibody, was used to avoid any problems in the interpretation of the results that may have arose from interference of JRF/rAb2 mAb with putative functions of APP and its fragments. Nevertheless, it is not a trivial task to accurately control for the Aβ species responsible for the restoration of normal synaptic plasticity and memory, because Aβ can easily change its conformation by the time it reaches the brain tissue after its initial preparation.[29] Interestingly, when we used a solution enriched in monomers, we did not find any rescue of LTP impairment by depletion of endogenous Aβ, suggesting that oligomers are likely to be responsible for the rescue effect. Further experiments that are beyond the scope of the present work will be necessary to determine which oligomeric form(s) are responsible for the function of endogenous Aβ.

It is interesting to note that the effect of antibodies and siRNA was not always profound. Such a finding is compatible with a partial reduction of Aβ levels following our manipulations, as suggested by the experiments in which Aβ levels in animals treated with APP-siRNA were partially reduced. Alternatively, another explanation is linked to the possibility that Aβ acts as a modulator of ongoing neuronal synaptic transmission instead of directly causing it, and therefore serves as a memory modulator instead of a memory permitting molecule. To this end, a recent work has suggested that the interaction between the nicotinic receptor complex and $A\beta_{42}$ on the presynaptic nerve terminal probably plays an important neuromodulatory role in synaptic dynamics.[48]

The presence of α7-nAChRs was necessary for the effects of endogenous Aβ on synaptic plasticity. This is consistent with the observation that α7-nAChRs are involved in synaptic plasticity and memory, boosting hippocampal LTP induction, and enhancing transmitter release in several brain regions including the hippocampus.[49] A link between Aβ and α7-nAChRs has also been demonstrated in studies showing that Aβ is likely to bind to α7-nAChRs at picomolar concentrations,[50] possibly through binding with membrane lipids[51] such as lipid rafts.[48] This link might either act in a nootropic fashion in physiological conditions (see for instance data showing that picomolar concentration of Aβ activates α7-nAChRs at presynaptic nerve endings of synaptosomes,[52] and enhances synaptic plasticity and memory[29]), or reduce memory during pathology (see for instance data showing that α7-

nAChRs are highly expressed in the brains of patients with sporadic AD,[53] and targeting α7-nAChRs might reduce AD symptoms[54]). In conclusion, our principal observations from these studies are that endogenous Aβ is required for synaptic plasticity and memory and this effect is mediated via α7-nAChRs. These results do not contradict previous studies demonstrating that high concentrations of Aβ impair LTP[14] and memory.[11] Indeed, we consider Aβ as a Dr. Jekyll/Mr. Hydelike molecule that might be involved in the production or blockage of memory depending on its concentration. These opposing aspects are intricately balanced and rely upon Aβ homeostasis at the synapse. Because most current therapeutic strategies for AD target the reduction of Aβ levels, these findings should be taken into consideration to design safer, more effective therapeutics against the disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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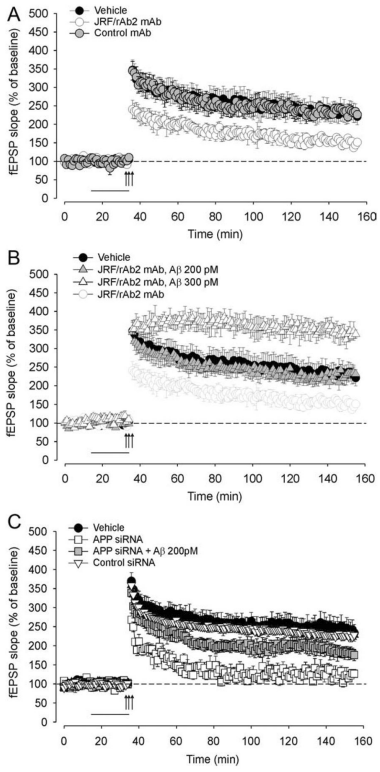

FIGURE 1.

Human amyloid-β peptide $(Aβ)_{42}$ rescues the reduction of long-term potentiation (LTP) caused by the depletion of endogenous Aβ. (A) Perfusion of hippocampal slices with antirodent monoclonal Aβ antibody (JRF/rAb2 mAb) (4μg/ml) for 20 minutes prior to a θ-burst stimulation dramatically reduced LTP (JRF/rAb2 mAb, n = 9; vehicle, n = 8; $F_{1,15}$ = 8.23, p = 0.012). Perfusion with an IgG2a isotype-matched control antibody (control mAb; 4μg/ml) did not affect LTP (n = 6, $F_{1,12}$ = 0.01, p = 0.923). The horizontal bar indicates the period during which mAb was added to the bath solution in this and the following figures. (B) Perfusion of hippocampal slices with human $Aβ_{42}$ (200pM) for 20 minutes prior to a θ-burst stimulation ameliorated LTP in slices concurrently treated with 4μg/ml JRF/rAb2 mAb (n = 7, $F_{1,13}$ = 0.156, p = 0.699). A higher concentration of $Aβ_{42}$ (300pM) in addition to JRF/rAb2 mAb produced an enhancement of LTP compared to vehicle-treated slices (n = 8, $F_{1,12}$ = 10.35, P = 0.007). These experiments were interleaved with those shown in panel A. (C) Amyloid precursor protein (APP)-small interfering (siRNA) (80nM in a final volume of 2μl over 1 minutes, 24 hours before examination of LTP) severely impaired LTP (n = 7 per group, $F_{1,12}$ = 32.88, $p < 0.0001$). Two hundred picomolars $Aβ_{42}$ was beneficial against the reduction of LTP (n = 7, $F_{1,12}$ = 4.46, p = 0.065). Control siRNA (80nM in a final volume of 2μl over 1 minute, 24 hours before studying LTP) did not affect LTP (n = 7, $F_{1,12}$ = 0.41, p = 0.532).

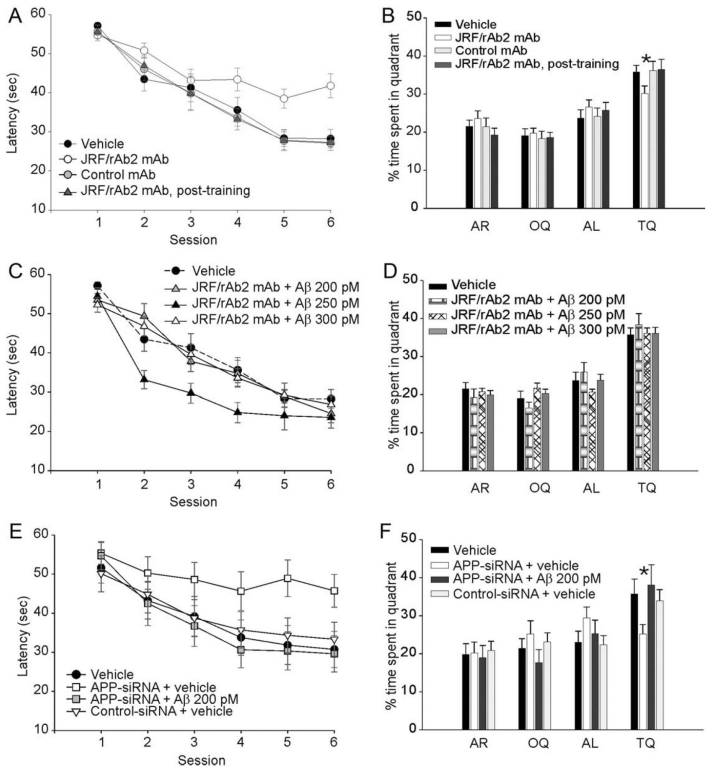

FIGURE 2.

Human amyloid-beta $(A\beta)_{42}$ rescues the reduction of reference memory by depletion of endogenous Aβ. (A) Bilateral injections of JRF/rAb2 mAb (4μg/μl, in a final volume of 1 μl over 1 minute), but not IgG2a isotype matched control mAb, into dorsal hippocampi, 15 minutes prior to the session increased the time to reach the hidden platform in the Morris Water Maze (MWM) (JRF/rAb2 mAb, n = 12; vehicle, n = 16; $F_{1,26} = 8.5$, $p = 0.007$; Control mAb, n = 12; $F_{1,26} = 8.5$, $p = 0.768$). When JRF/rAb2 mAb was administered immediately after the individual sessions, the performance was not affected (n = 10, $F_{1,24} = 0.055$, $p = 0.816$). (B) The probe trial showed a reduction in time spent in target quadrant (TQ) in mice infused with JRF/rAb2 mAb ($t_{26} = 2.12$, $p = 0.04$), but not in mice infused with control mAb ($t_{26} = 0.13$, $p = 0.895$) or those that received JRF/rAb2 mAb immediately after the hidden session ($t_{24} = 0.208$, $p = 0.837$). OQ = opposite quadrant, AL = alternate quadrant to the left, AR = alternate quadrant to the right. (C) Bilateral injections of $A\beta_{42}$ (200–300 pM) with JRF/rAb2 mAb (4 λg/μl) in a final volume of 1 μl over 1 minute, 15 minutes prior to the session, showed normal memory as the mice searched for the hidden platform (200 pM: n = 10, $F_{1,24} = 1.46$, $p = 0.706$; 250 pM: n = 10, $F_{1,24} = 0.65$, $p = 0.427$; 300 pM: n = 15, $F_{1,29} = 0.19$, $p = 0.658$ compared to vehicle-infused mice). These experiments were interleaved with those shown in panel A. (D) The probe test performed after the experiments shown in C showed normal memory in mice infused with $A\beta_{42}$ with JRF/rAb2 mAb (200 pM: n = 10, $t_{21} = 0.799$, $p = 0.433$; 250 pM: n = 10, $t_{21} = 0.151$, $p = 0.881$; 300 pM: n = 15, $t_{26} = 0.131$, $p = 0.897$). (E) Intrahippocampal injections of amyloid precursor protein (APP) small interfering RNA (siRNA) (80 nM in a final volume of 2 μl over 1 minute, daily until the end of behavioral testing, starting 24 hours before the first session of the hidden platform test) prolonged the time needed to reach the hidden platform

(vehicle, n = 12; APP-siRNA + vehicle, n = 10; $F_{1,20} = 20.686$, $p < 0.0001$), whereas control-siRNA did not affect memory (n = 10, $F_{1,20} = 0.277$, $p = 0.604$). $A\beta_{42}$ (200 pM) rescued the siRNA-induced impairment of reference memory (n = 10, $F_{1,20} = 0.151$, $p = 0.702$). (F) The probe test performed after the experiments shown in panel E demonstrated a beneficial effect by $A\beta_{42}$ against the reduction of reference memory caused by APP-siRNA in the target quadrant (APP-siRNA + vehicle, $t_{20} = 2.848$, $p = 0.010$; APP-siRNA + $A\beta_{42}$, $t_{20} = 0.441$, $p = 0.664$; control-siRNA + vehicle, $t_{20} = 0.481$, $p = 0.635$). Asterisks indicate statistical significance.

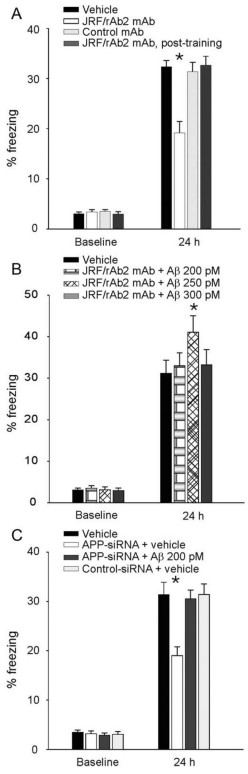

FIGURE 3.

Human $A\beta_{42}$ rescues the reduction of contextual memory by depletion of endogenous Aβ. (A) Bilateral injections of antirodent monoclonal Aβ antibody (JRF/rAb2 mAb) (4μg/μl, in a final volume of 1μl over 1 minute), but not IgG2a isotype-matched control mAb, into dorsal hippocampi 15 minutes prior to training, reduced freezing in mice exposed to the context after 24 hours (vehicle, n = 15; JRF/rAb2 mAb, n = 16, $t_{29} = 5.08$, $p = 0.0001$; control mAb: n = 12, $t_{25} = 0.42$, $p = 0.672$). When JRF/rAb2 mAb was administered immediately after the training, the performance was not affected (n = 13, $t_{26} = 0.11$, $p = 0.912$). (B) Bilateral injection of human $A\beta_{42}$ (200pM) into dorsal hippocampi, 15 minutes prior to training, improved contextual conditioning performance in mice concurrently infused with JRF/rAb2 mAb (n = 12, $t_{24} = 0.40$, $p = 0.693$). A higher concentration of 250pM $A\beta_{42}$ concurrent with JRF/rAb2 mAb further enhanced performance compared to vehicle-injected mice (n = 13, $t_{25} = 2.21$, $p = 0.036$). (C) Amyloid precursor protein (APP)-small interfering (siRNA) (80nM in a final volume of 2μl over 1 minute, 24 hours before the electric shock) dramatically impaired contextual fear memory (vehicle, n = 15; APP-siRNA + vehicle, n = 14, $t_{27} = 3.93$, $p = 0.001$), whereas control siRNA did not affect memory (n = 14, $t_{27} = 0.02$, $P = 0.982$). $A\beta_{42}$ rescued the siRNA-induced impairment of contextual memory (n = 15, $t_{28} = 0.26$, $p = 0.797$). Asterisks indicate statistical significance.

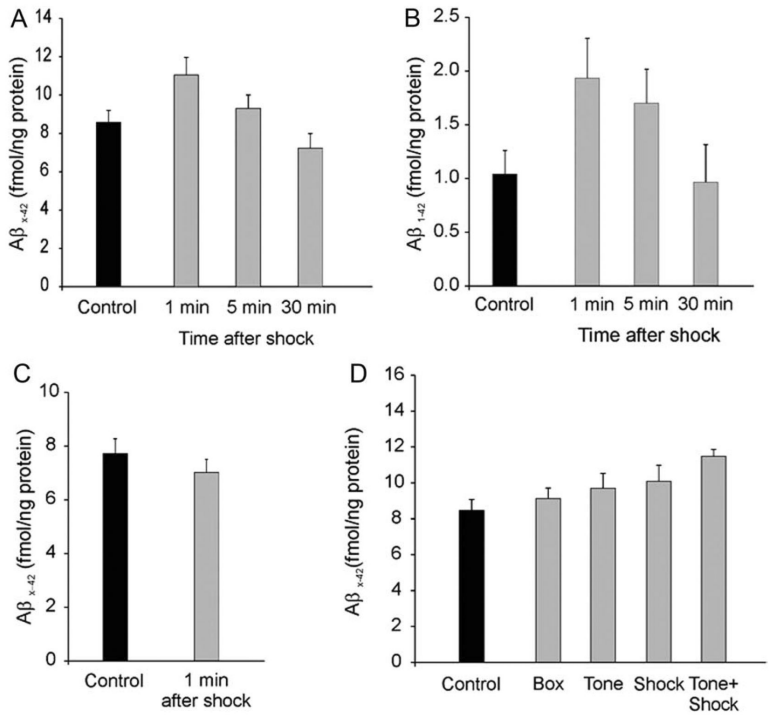

FIGURE 4.

Hippocampal amyloid-β peptide $(Aβ)_{42}$ production is enhanced during memory induction. (A) Training for contextual fear learning leads to an increase in hippocampal $Aβ_{x-42}$ levels at 1 minute after the electric shock (control, n = 9; 1 minute, n = 10, $t_{17} = 2.223$, $p = 0.040$; = minutes, n = 10, $t_{17} = 0.797$, $p = 0.454$; 30 minutes, n = 10, $t_{17} = 1.342$, $p = 0.197$). (B) Mice sacrificed at 1 minute (but not at = and 30 minutes) after foot shock showed a significant increase in peptide levels measured using an antibody that recognizes $Aβ_{1–42}$. (C) Cerebellar $Aβ_{x-42}$ levels were not modified at 1 minute after training. (D) No changes in $Aβ_{x-42}$ levels were detected in a group exposed to the context without tone and foot shock (Box), a group exposed to the tone alone, or a group exposed to foot shock without context association. An increase of $Aβ_{x-42}$ levels was only detected in a group exposed to the tone plus foot shock.

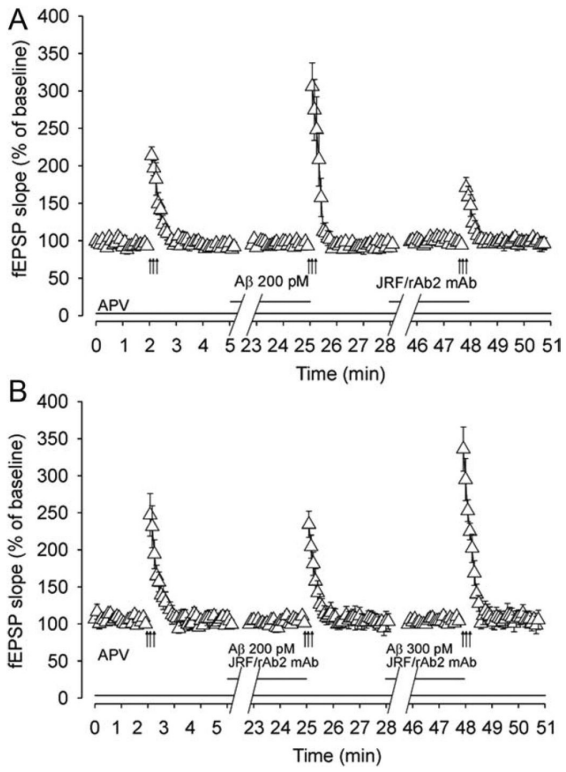

FIGURE 5.

Human amyloid-β peptide $(Aβ)_{42}$ counteracts the impairment of post-tetanic potentiation (PTP) by depletion of endogenously produced Aβ. (A) Perfusion of hippocampal slices with $Aβ_{42}$ (200pM) for 20 minutes prior to a θ-burst stimulation enhanced PTP (n = 7, t_{12} = 2.275, p = 0.042), whereas JRF/rAb2 mAb (4μg/ml) reduced it (t_{12} = 2.465, p = 0.030). The horizontal bar indicates the period during which antibodies and Aβ were added to the bath solution. These experiments were performed in the presence of the N-methyl-d-aspartate antagonist D-APV (50μM) to block LTP inductive mechanisms. (B) Perfusion of hippocampal slices with human $Aβ_{42}$ (200pM) for 20 minutes prior to a θ-burst stimulation produced normal PTP in slices concurrently treated with JRF/rAb2 mAb (n = 7, t_{12} = 0.616, p = 0.549). A higher concentration of $Aβ_{42}$ (300pM) with JRF/rAb2 mAb produced an enhancement of PTP (t_{12} = 1.922, p = 0.079).

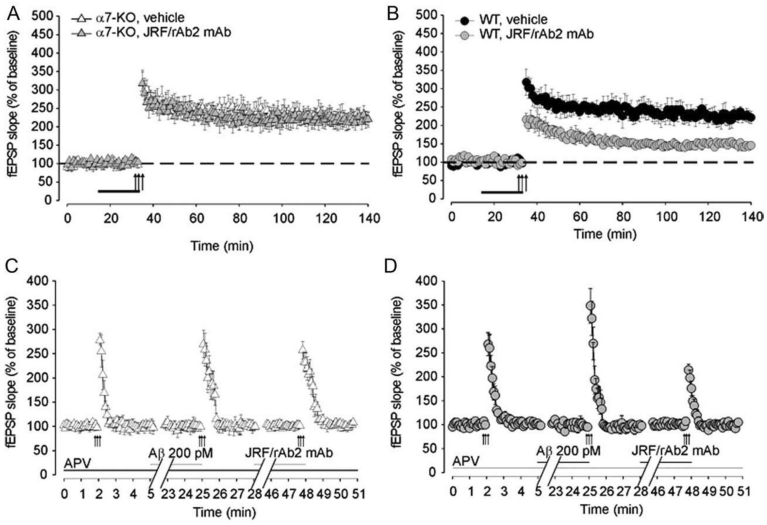

FIGURE 6.

α7-nicotinic acetylcholine receptor (nAChR) knockout (KO) mice do not show changes in synaptic plasticity induced by depletion of endogenous amyloid-b peptide. (A) Perfusion of hippocampal slices from α7-nAChR KO mice with antirodent monoclonal Aβ antibody (JRF/rAb2 mAb) (4μg/ml) for 20 minutes prior to a θ-burst stimulation did not affect post-tetanic potentiation (PTP) (n = 9, $t_{16} = 0.696$, $p = 0.497$). (B) JRF/rAb2 mAb (4μg/ml) reduced PTP in slices from wild-type (WT) littermates (n = 9, $t_{16} = 5.031$, $p < 0.0001$). (C) Perfusion of hippocampal slices from α7-nAChR KO mice with JRF/rAb2 mAb (4μg/ml) for 20 minutes prior to a θ-burst stimulation did not affect LTP (n = 6, $F_{1,10} = 0.352$, $p = 0.628$). (D) JRF/rAb2 mAb (4μg/ml) reduced LTP in slices from WT littermates (n = 6, $F_{1,10} = 52.166$, $p < 0.0001$).