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Reelin and ApoE Receptors Cooperate to Enhance Hippocampal Synaptic Plasticity and Learning*

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Two apolipoprotein E (apoE) receptors, the very low density lipoprotein (VLDL) receptor and apoE receptor 2 (apoER2), are also receptors for Reelin, a signaling protein that regulates neuronal migration during brain development. In the adult brain, Reelin is expressed by GABA-ergic interneurons, suggesting a potential function as a modulator of neurotransmission. ApoE receptors have been indirectly implicated in memory and neurodegenerative disorders because their ligand, apoE, is genetically associated with Alzheimer disease. We have used knockout mice to investigate the role of Reelin and its receptors in cognition and synaptic plasticity. Mice lacking either the VLDL receptor or the apoER2 show contextual fear conditioning deficits. VLDL receptor-deficient mice also have a moderate defect in long term potentiation (LTP), and apoER2 knockouts have a pronounced one. The perfusion of mouse hippocampal slices with Reelin has no effect on baseline synaptic transmission but significantly enhances LTP in area CA1. This Reelin-dependent augmentation of LTP is abolished in VLDL receptor and apoER2 knockout mice. Our results reveal a role for Reelin in controlling synaptic plasticity in the adult brain and suggest that both of its receptors are necessary for Reelin-dependent enhancement of synaptic transmission in the hippocampus. Thus, the impairment of apoE receptor-dependent neuromodulation may contribute to cognitive impairment and synaptic loss in Alzheimer disease.

Apolipoprotein E (apoE)[1] is a component of lipoproteins that mediates the transport and receptor-mediated uptake of these particles by target tissues (1). ApoE is produced by several tissues in the body including glial cells in the brain, predominantly astrocytes (2, 3) in which the physiological significance of apoE secretion and its binding to cognate neuronal apoE receptors remains to be established.

ApoE occurs in three major isoforms in the general human population, apoE2, apoE3 and apoE4, with apoE3 being the most common isoform. In 1993 Schmechel *et al.* (4) reported that the apoE4 isoform is genetically associated with late onset Alzheimer disease, a debilitating neurodegenerative disorder that is characterized by the loss of synapses and neurons, the accumulation of amyloid plaques, and the occurrence of neurofibrillary tangles. The underlying biochemical mechanism by which apoE4 predisposes its carriers to this disease is not precisely known and is under debate. One model that has been proposed by us (5) suggests that members of a family of apoE receptors that are abundantly expressed on the surface of neurons may be involved in this pathological process.

Two members of this family of apoE receptors, the very low density lipoprotein receptor (VLDLR) and the apolipoprotein E receptor 2 (apoER2), have been shown to participate in a neuronal signaling pathway that governs the layering of the developing cortex (6). This pathway involves the signaling molecule Reelin, a large protein of ~400 kDa that is secreted by Cajal-Retzius neurons during the development of the brain (7, 8). The lack of Reelin (9), its receptors VLDLR and apoER2 (6), or the cytoplasmic adaptor protein Dab1 (10–12) all result in the same phenotype, which is characterized by cerebellar dysplasia and scrambling of the neuronal layers in the neocortex. Because VLDLR and apoER2 are partially redundant in transmitting the Reelin signal to migrating neurons, only mild neuroanatomical abnormalities that do not affect neuronal connectivity in the hippocampus are present in the brains of mice that lack only one or the other of these receptors (45).

After the fetal phase of brain development, Reelin-expressing Cajal-Retzius neurons in the subpial layer are largely replaced by Reelin-expressing GABA-ergic interneurons that are dispersed throughout the neocortex and in the hippocampus. The Reelin receptors apoER2 and VLDLR and the adaptor protein Dab1, which are all essential to Reelin signaling, remain expressed in the adult brain, but their function there remains a mystery. An association of Reelin with synapses has been reported (13), raising the possibility of a potential role in neurotransmission that might involve signaling through apoE receptors.

Here we show that mice lacking the Reelin/apoE receptors VLDLR and apoER2 have pronounced defects in memory for-

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[1] The abbreviations used are: apoE, apolipoprotein E; apoER2, apoE receptor 2; LDL, low density lipoprotein; VLDL, very low density lipoprotein; VLDLR, VLDL receptor; GABA, γ-aminobutyric acid; LTP, long term potentiation; CS, conditioned stimulus; EPSP, excitatory postsynaptic potential; pEPSP, population EPSP; GST, glutathione *S*-transferase; RAP, receptor-associated protein; WT, wild-type; PPF, paired pulse facilitation; TBS, θ-burst stimulation; HFS, high frequency stimulation; NMDA, *N*-methyl-D-aspartate.

TABLE I
Motor learning and general activity in VLDLR and ApoER2 knockouts

For Rotorod behavior the results shown are the average total time the animals remain on the rotating rod over two consecutive days. Four training trials were given each day. The increase in time the animal remained on the rod is taken as an index of motor learning. No significant differences were seen in rotorod performance for the VLDLR or apoER2 knockouts compared to wild-type controls. General activity levels for open field behavior are evaluated by measurements of horizontal activity (total distance traveled) and vertical activity (rearing) during a 15 min test session. The open field test also measures anxiety levels as assessed by the center distance to total distance ratio. ApoER2 mice exhibited no differences in open field testing. VLDLR knockouts showed a significant increase in total distance traveled or the center to total distance ratio between groups. Results are shown as mean ± S.E. * represents a p value of < 0.01.

	Rotorod test		Open field test		
	Day 1	Day 2	Total distance	Vertical activity	Center to total ratio
			cm		
VLDLR −/−	112.7 ± 20.89	178.1 ± 26.47	*4781 ± 307.1	*235.0 ± 38.4	*0.0450 ± 0.0074
Wild-type	137.9 ± 32.90	206.9 ± 29.39	2014 ± 366.1	49.67 ± 11.0	0.01000 ± 0.0025
ApoER2 −/−	176.9 ± 17.21	247.8 ± 17.85	1772 ± 296.3	41.92 ± 12.9	0.01667 ± 0.0079
Wild-type	157.0 ± 20.00	223.6 ± 20.26	1430 ± 178.9	38.00 ± 9.67	0.01692 ± 0.0061

mation and long term potentiation (LTP) and that Reelin greatly enhances LTP in hippocampal slice cultures. Our results thus reveal a role for apoE receptors and for Reelin in synaptic function and the formation of long term memory, which is likely to promote the stability and maintenance of synapses in the central nervous system. Taken together with other findings (14) that have shown apoE-induced memory impairment in transgenic mice, our data are consistent with a hypothetical model in which the promotion of memory dysfunction by apoE4 might involve an impairment of apoE receptor-dependent signaling pathways, thereby accelerating synaptic loss and the onset of dementia.

MATERIALS AND METHODS

Mice—ApoER2 and VLDL receptor knockout mice have been described (6, 15). Control mice were either littermates or age- and sex-matched non-mutant mice of the same strain background. Mice were housed on a 12-h light/dark schedule. All experiments were performed in compliance with the Baylor College of Medicine Institutional Animal Care and Use Committee and national regulations and policies.

General Activity and Motor Learning—The accelerating Rotorod test was utilized to assess overall balance and motor coordination. The test was performed on an accelerating Rotorod apparatus (Ugo Basile) with a 3-cm diameter rod starting at an initial rotation of 4 rpm and accelerating to 40 rpm over 5 min. Mice were tested for the time spent on the rod during each of four trials per day for two consecutive days. The open field domain consisted of a square area (43 × 43 cm) surrounded by Plexiglas walls with a field lighted by four overhead lights (75 watts) in a room with otherwise standard lighting. Through the use of eight photoreceptor beams on each side of the test arena, the field was divided into 16 quadrants in which the activity of an animal was determined and recorded with a personal computer-controlled Digiscan optical animal activity system (model RXYZCM-8, Omnitech Electronics). The animal was released in the center of the field and allowed to roam the open field for 30 min. Activity was recorded from the number of photobeam disruptions in each quadrant to give the total distance traveled and the vertical activity (rearing). Also, the center to total distance ratio was determined by dividing the center distance by the total distance.

Fear Conditioning—For the two-pairing, fear conditioning paradigm, animals were placed in the fear conditioning apparatus for 3 min, then a 30 s acoustic conditioned stimulus (CS) (white noise, 70 dB) was delivered with a 0.5-mA shock (unconditioned stimulus) applied to the floor grid during the last 2 s of the CS. Training consisted of two mild shocks paired with two conditioned stimuli with a 2-min interval between each. The stimulus strength and number of training shocks were chosen based on pilot experiments to optimize learning. For short term memory testing, mice were placed in the isolation chamber and exposed for 6 min to the same context as that used for the training (~40 min following training). Immediately after the contextual test, mice were placed into a novel context and exposed to the CS for 3 min (~1 h following training). For long term memory testing, both the context and the cue were performed the next day ~24 h following training performed as described above. Learning was assessed by measuring freezing behavior (*i.e.* motionless position) every 5 s. The scorer of the behavioral experiments was blind in reference to animal genotype.

Hippocampal Slice Preparation and Electrophysiology—Adult mice were sacrificed by decapitation, and the brains were rapidly removed and briefly submerged in ice-cold cutting saline (110 mM sucrose, 60 mM NaCl, 3 mM KCl, 1.25 mM NaH_2PO_4, 28 mM $NaHCO_3$, 0.5 mM $CaCl_2$, 5 mM D-glucose, and 0.6 mM ascorbate). All solutions were saturated with 95% O_2 and 5% CO_2. Whole brains were then dissected on cutting solution-soaked filter paper and mounted on a glass platform resting on ice. Hippocampal slices (400 μm) were prepared on a vibratome and allowed to equilibrate in a 50% cutting saline and 50% artificial cerebrospinal fluid solution (125 mM NaCl, 2.5 mM KCl, 1.24 mM NaH_2PO_4, 25 mM $NaHCO_3$, 10 mM D-glucose, 2 mM $CaCl_2$, and 1 mM $MgCl_2$) at room temperature for a minimum of 30 min. Slices were transferred to an interface chamber supported by a nylon mesh and allowed to recover for a minimum of 1 h prior to recording. Extracellular field recordings were obtained from area CA1 stratum radiatum. Stimulation was supplied with a bipolar Teflon-coated platinum electrode, and a recording was obtained with the use of a glass microelectrode filled with artificial cerebrospinal fluid (resistance 1–4 mΩ). Tetani used to evoke CA1 LTP consisted of either 100 Hz high frequency stimulation or θ-burst stimulation (TBS). The 100-Hz stimulation protocol consisted of two trains of 100-Hz frequency stimulation for 1 s with each train separated by a 20-s interval. The θ-burst stimulation consisted of five trains of four pulses at 100 Hz with an interburst interval of 20 s. Stimulus intensities were adjusted to give pEPSPs (population excitatory postsynaptic potentials) with slopes that were ≤50% that of the maximum determined from an input/output curve. The calculated 50% maximum stimulus intensity was used for both LTP-inducing protocols. Potentiation was measured as the normalized increase of the mean pEPSP following tetanic stimulation normalized to the mean pEPSP for the duration of the baseline recording. Experimental results were obtained from those slices that exhibited stable baseline synaptic transmission for a minimum of 30 min prior to the delivery of the LTP-inducing stimulus. Reelin or control medium was diluted in oxygenated artificial cerebrospinal fluid, and slices were perfused at 1 ml/min.

Production of Recombinant Proteins—Reelin-conditioned medium was harvested from cells (293 line) that had been stably transfected with a Reelin expression construct (9). Cells were grown in Dulbecco's modified Eagle's medium containing 10% fetal calf serum. After reaching confluence, the medium was replaced with Dulbecco's modified Eagle's medium containing 0.2% bovine serum albumin. Conditioned medium was collected after 2 days, fresh medium was added, and Reelin-conditioned medium was collected again 2 days later. The medium was concentrated ~50-fold on an Amicon concentrator under nitrogen pressure prior to use. The concentration of Reelin used in the perfusion experiments was estimated to be ~5 nM (*i.e.* ~8 × K_d). Control medium was prepared in an identical manner from untransfected 293 cells. Recombinant GST-RAP was produced in *Escherichia coli* as described previously (16).

RESULTS

Contextual Fear-conditioned Learning Deficits in VLDLR and apoER2 Knockout Mice—To explore the functional significance of Reelin and its receptors apoER2 and VLDLR in the adult mouse brain, we first subjected mice lacking either of these two receptors to a variety of behavioral tests. In contrast to Reelin- or Dab1-deficient mice, which are severely ataxic because of the failure of cerebellar development (10–12), mice

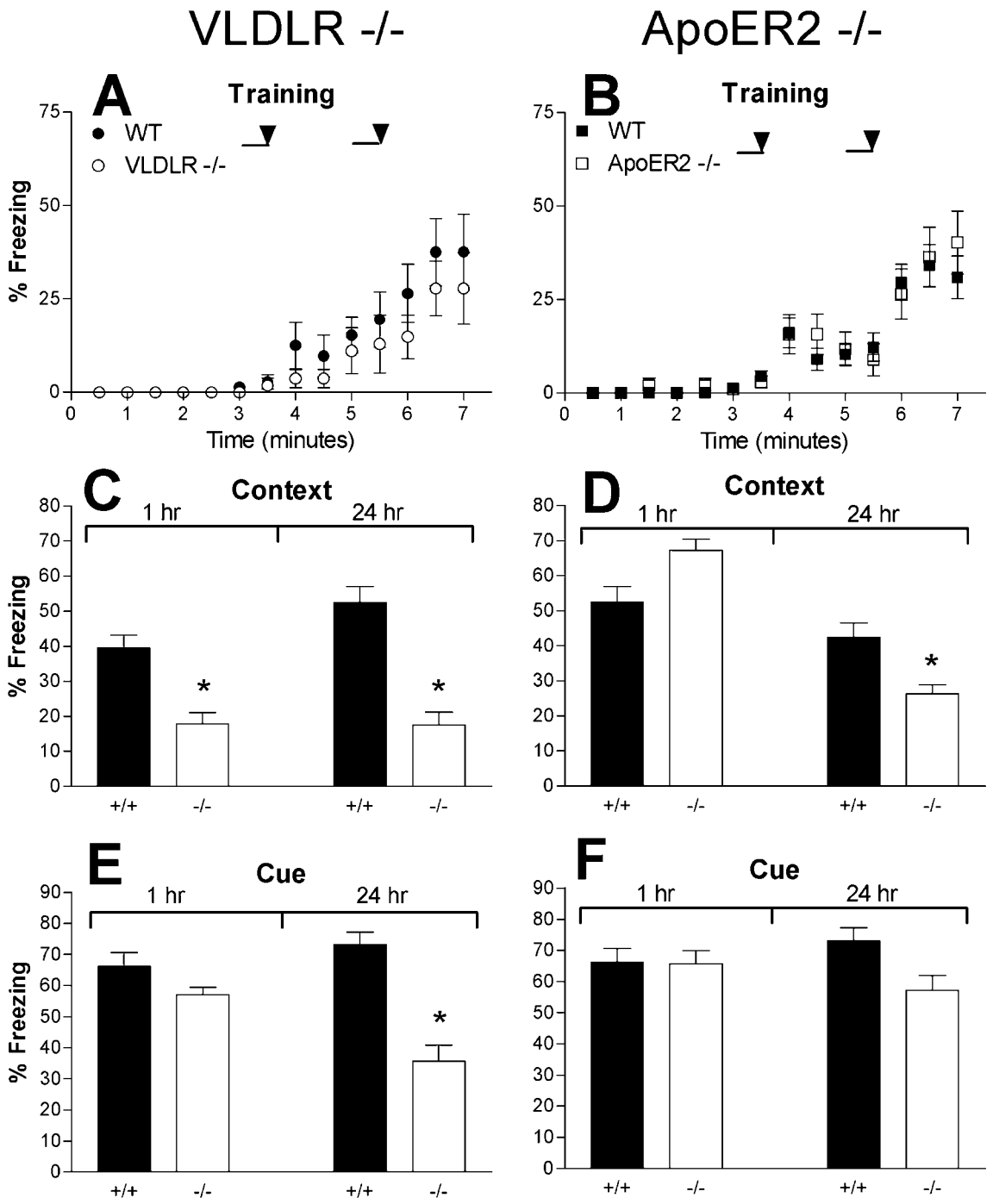

FIG. 1. **Impairment of fear-conditioned memory formation in apoER2 and VLDL knockouts.** *A* and *B* depict fear conditioning. VLDL receptor knockout mice (*open circles*) and apoER2 knockout mice (*open squares*) are compared with littermate wild-type mice (*closed symbols*). A tone (*solid bar*) was paired with a foot shock (*arrowhead*) between 3–4 and 5–6 min. Freezing behavior is shown on the day of training for VLDLR- (*A*) and apoER2-deficient (*B*) or wild-type mice and is comparable in all groups. *Panels C* and *D* show results from the contextual fear response test. During this test, animals were reintroduced to the context in which they were trained. VLDLR-deficient mice showed significantly reduced freezing 1 and 24 h following training (*C*). apoER2 knockout mice showed a reduced freezing response only when tested 24 h following training (*D*). *Panels E* and *F* show the freezing response measured during reintroduction to the cue component (white noise) when the animal was placed in a novel context. VLDLR deficient mice showed normal freezing 1 h following training, but revealed a significant decrease in the freezing response at 24 h post-training (*E*). ApoER2-deficient mice showed normal freezing to the cue component at both time points, compared with littermate controls (*F*). *Black bars*, wild-type mice; *clear bars*, mutants. *Asterisk* represents $p < 0.01$. Results are shown as mean ± S.E.

lacking only apoER2 (6) or VLDLR (15) have been reported to have apparently normal coordination and motor functions.

We undertook a broad based behavioral characterization designed to determine whether mice lacking the neuronal apoE receptors apoER2 or VLDLR have memory defects. The general activity and overall motor learning ability of the animals were monitored to exclude the possibility that any perceived learning deficit could be due to a physical disability such as muscle weakness or coordination defects. Coordination and motor skill acquisition were analyzed using the Rotorod test. The amount of time an animal can stay on a rotating rod is an index of its general level of coordination. Mice also improve their performance with training, which is an indicator of motor learning (17). VLDLR and apoER2 mutants exhibited normal motor learning

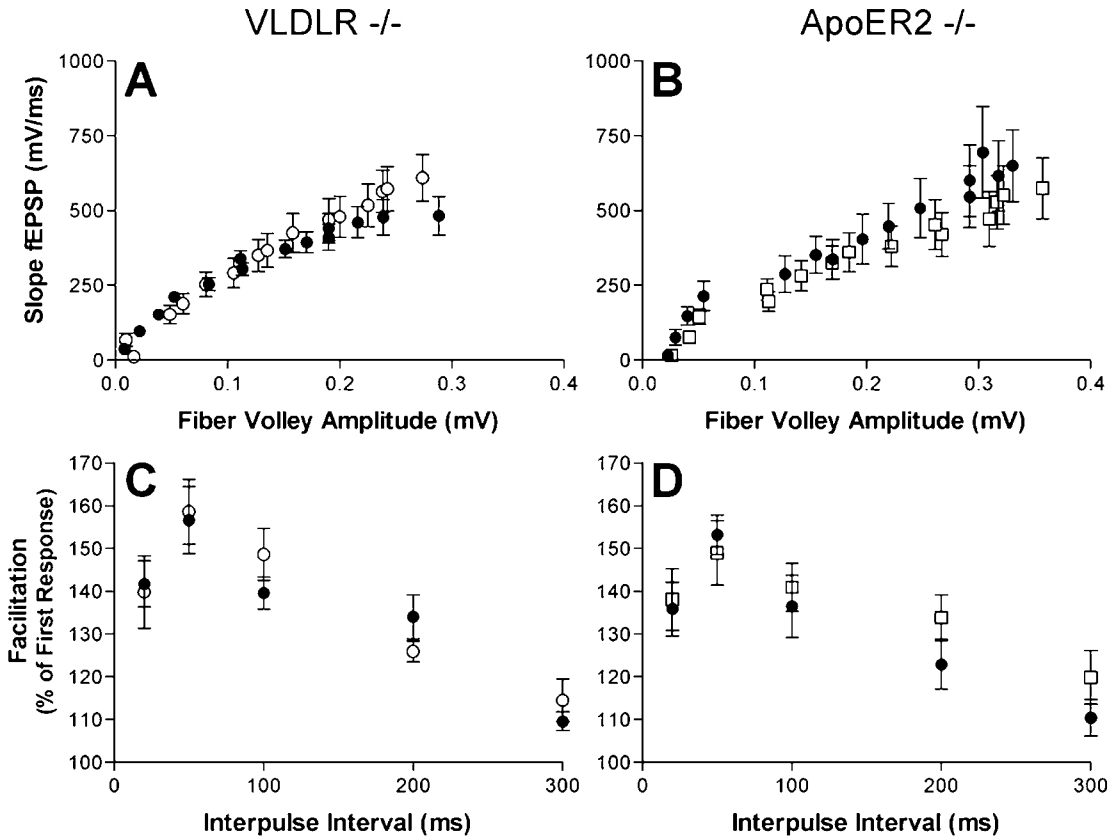

FIG. 2. **Electrophysiologic responses at Schaffer collateral synapses in area CA1 of hippocampus.** *A* and *B*, the loss of VLDLR (*open circles*) or apoER2 (*open squares*) had no effect on the baseline synaptic transmission in the stratum radiatum of the CA1 region of the hippocampus measured at 25 °C compared with that in wild-type mice (*closed circles*). *C* and *D*, short term plasticity assessed by PPF was also unaffected in either knockout strain. Results are shown as mean ± S.E.).

over 2 days of training. No differences in total Rotorod performance were observed compared with wild-type control animals (Table I).

An open field test was used to evaluate the behavior of apoER2-deficient mutants and littermate controls upon placement into a novel environment. General locomotor activity and exploratory behavior was evaluated by determining the distance traveled and the amount of rearing of the animal. Moreover, open field testing can be used to calculate the center to total distance ratio, revealing the center field exploration latency of the animal, which is an index of overall animal anxiety. This analysis exploits the natural tendency of mice to avoid open areas. No differences were observed for total distance traveled, vertical activity, or center to total distance between apoER2 mutants and control animals; however, VLDLR knockout mice showed increased activity in these measurements (Table I). Therefore, absence of the VLDLR results in hyperactivity but does not apparently cause the excessive anxiety in which the animals avoid the center portion of the open field altogether.

We next utilized a conditioning paradigm that elicits robust associative learning using a procedure that tests both hippocampus- and amygdala-dependent learning (18, 19). For these experiments, an aversive stimulus (in this case, a mild foot shock) is paired two times with an acoustic component (CS; white noise) in a novel context. When tested, mice exhibit marked fear in response to re-presentation of either the context or the CS when it is presented in a novel context different from the training context. The amount of fear an animal exhibits is generally measured by the freezing behavior of the animal. Enhanced freezing behavior upon replacement into the context or re-presentation of the CS is taken as an index of the animal having learned to associate the context or CS with the foot shock.

We used the associative fear-conditioned learning paradigm to evaluate both short and long term memory in our knockout animals. We found that both mutant animal groups displayed similar response to training, exhibiting freezing behavior in response to a shock equal to that of the wild-type control (WT, $n = 19$; apoER2 −/−, $n = 17$; VLDLR −/−, $n = 16$) (Fig. 1, *A* and *B*). Testing in the context 1 h following training revealed that only the VLDLR mutants exhibited significantly less freezing than littermate controls (WT, 39 ± 3.75%, $n = 10$; VLDLR −/−, 18 ± 3.2%, $n = 10$, $p = 0.003$). However, both the VLDL receptor (WT, 52 ± 4.5%, $n = 19$; VLDLR −/−, 17 ± 3.7%, $n = 16$, $p < 0.0001$) and apoER2 (WT, 43 ± 3.5%, $n = 19$; apoER2 −/−, 26 ± 2.5%, $n = 17$, $p = 0.001$) mutant animals exhibited significant deficits in contextual associative fear-conditioned learning when tested 24 h following training (Fig. 1, *C* and *D*). In contrast, associative learning to the cue component 1 h following training was normal in both sets of animals, and only VLDLR-deficient mice exhibited less freezing behavior at the 24-h time point (WT, 73 ± 4.1%, $n = 19$; VLDLR −/−, 35 ± 5.1%, $n = 16$, $p = 0.001$) (Fig. 1, *E* and *F*). The consequences observed in the short *versus* the long term memory of our VLDLR- or apoER2-deficient mice suggest that each receptor appears to play a differential role in associative memory formation. The lack of a prominent deficit to the cue component is consistent with a derangement in hippocampal function (20, 21). Thus, the contextual fear conditioning deficits in the VLDLR- and apoER2-deficient mice are compatible with a role for these receptors in modulating the synaptic plasticity that underlies long term hippocampus-dependent associative learning.

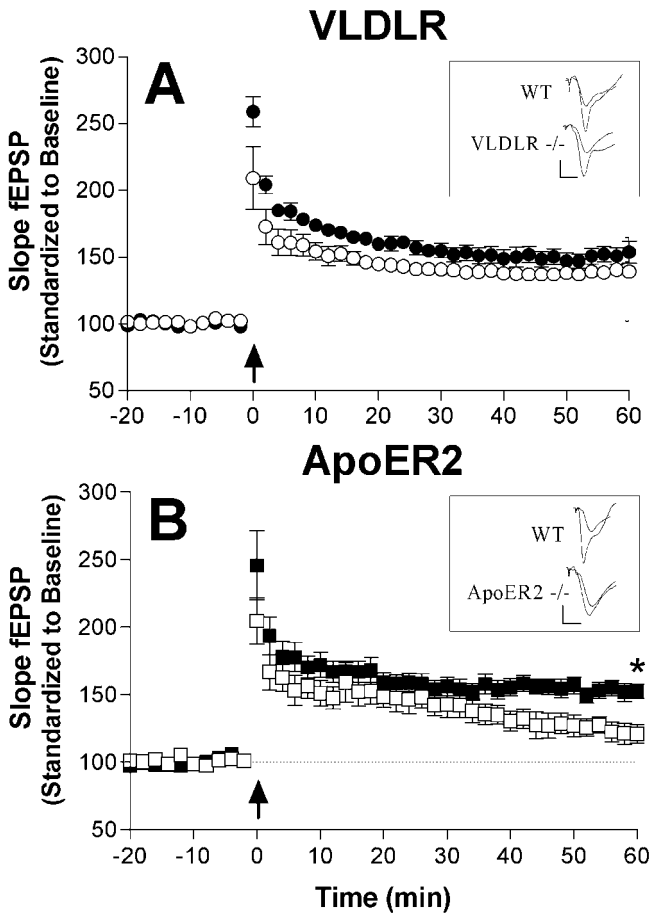

FIG. 3. **LTP induction is modestly reduced in VLDLR and severely perturbed in apoER2 mutants.** LTP induced with two trains of a 1-s long, 100-Hz stimulation separated by 20 s (represented by an *arrow*) is shown for mice deficient in VLDLR or apoER2 and compared with that of wild-type mice (*closed symbols*). LTP induction is mildly impaired in VLDLR knockout mice (*open circles*) (*A*) and severely perturbed apoER2 knockouts (*B*; *open squares*) where it rapidly decays to near baseline response within 60 min. *Inset,* representative traces (mean of six successive EPSPs) are shown for baseline and 60 min post-tetanic stimulation (*scale bars* represent 1 mV and 10 ms). Results are shown as mean ± S.E. In this and the following figures, data are normalized to the average of the initial 20-min baseline value (defined as 100%). *Asterisks* indicate a value significantly different from the baseline ($p < 0.05$, Student's t test).

Synaptic Transmission and Short Term Plasticity Are Normal—We therefore extended our studies to directly measure synaptic function using hippocampal slices *in vitro*. We used a population field recording technique to record EPSPs from area CA1 in hippocampal slices to assess synaptic transmission and short and long term potentiation in VLDLR and apoER2 mutants. The absence of VLDLR (VLDLR −/−, $n = 16$; WT, $n = 11$) or apoER2 (apoER2 −/−, $n = 14$; WT, $n = 11$) did not affect baseline synaptic transmission at Schaffer collateral synapses, because the input-output functions for the stimulation of area CA1 were not different between wild-type and knockout mice (Fig. 2, *A* and *B*). Thus, the synaptic connectivity in area CA1 in which these measurements were taken appeared to remain unaffected despite the subtle morphologic changes of hippocampal anatomy in apoER2-deficient mice (6).

Paired pulse facilitation (PPF) is a form of short term synaptic plasticity that is commonly held as a presynaptic phenomenon due to the residual calcium augmentation of neurotransmitter release (22). As with baseline synaptic transmission, PPF was normal in the VLDLR (VLDLR −/−, $n = 16$; WT, $n = 11$) (Fig. 2*C*) and apoER2 knockouts (apoER2 −/−, $n = 14$; WT, $n = 11$) (Fig. 2*D*), indicating that neither receptor is a component of the machinery underlying PPF at Schaffer collateral synapses. These data also suggest that short term synaptic plasticity mechanisms such as those underlying PPF are unperturbed in VLDLR- and apoER2-deficient animals.

Hippocampal LTP Induction Is Defective in ApoER2 Knockout Mice—We next tested whether the hippocampal-dependent contextual fear conditioning memory defects seen in the VLDLR and apoER2 mutant mice were accompanied by a reduction in the area CA1 LTP at Schaffer collateral synapses. The time course of synaptic potentiation following two trains of 100 Hz, 1-s stimulation in the mutant strains is shown in Fig. 3. We observed a modest decrease in post-tetanic potentiation in our VLDLR deficient mice (209 ± 23%, $n = 18$; WT, 258 ± 11%, $n = 17$, $p = 0.051$); however, the overall magnitude of LTP of the VLDLR mutants (139 ± 3%, $n = 18$) was nearly identical to that of the wild-type mice (154 ± 8%, $n = 17$, $p = 0.12$, 60 min post-tetanus). In contrast, apoER2 mutants showed a strong impairment in LTP induction. In these animals there was a decay of LTP to nearly that of baseline responses (apoER2 −/−, 120 ± 6%, $n = 26$; WT 152 ± 5%, $n = 19$, $p = 0.001$, 60 min post-tetanus). Thus, apoER2-deficient mice exhibit an apparently normal baseline synaptic function but have a deficit in long lasting forms of synaptic plasticity. The induction and maintenance of LTP is generally regarded as an important factor in the formation and retention of memories. There are numerous examples (23–26) in which altered LTP has been shown to be associated with hippocampal learning deficits in mouse models for human neurodegenerative and cognitive disorders. Thus, these results suggest that the LTP deficit in area CA1 could contribute to the memory deficits in the apoER2 knockout mice and that the modest post-tetanic potentiation decrease that is present in the VLDL receptor-deficient animals may also account at least in part for the fear conditioning deficit seen in this strain.

The Extracellular Domains of ApoE Receptors Are Necessary for LTP Formation—In the first part of our studies we used genetic ablation of two apoE receptors, the VLDL receptor and the apoER2, to reveal a role for these receptors in synaptic plasticity and memory formation. As an additional control, we also used recombinant receptor associated protein (RAP), a universal inhibitor of ligand binding to LDL receptor family members. RAP is a chaperone for LDL receptor family members that prevents the premature binding of co-expressed ligands in the endoplasmic reticulum (27, 28). It also blocks the binding of virtually all receptor ligands at the cell surface (16, 29). RAP has previously been reported (30) to block LTP induction and maintenance in hippocampal slices from wild-type mice. In agreement with these previous findings, which used a different LTP induction protocol, we found that a RAP-mediated inhibition of LDL receptor family members in wild-type mice had no effect on baseline synaptic transmission but almost completely blocked LTP induction (60 min post-tetanus, GST-RAP, 124 ± 6%, $n = 8$; GST, 209 ± 33%, $n = 7$, $p = 0.02$) (Fig. 4).

Reelin Enhances LTP in Wild-type Mice—Because RAP blocks LTP, presumably by inhibiting ligand binding to LDL receptor family members, and LTP was significantly reduced in the apoER2 knockout, we decided to conduct the converse experiment. We asked whether Reelin, which is a ligand for both the VLDL receptor and the apoER2, might actually enhance LTP induction and maintenance. In these experiments we utilized two distinct LTP-inducing protocols: 1) θ-burst stimulation consisting of four short trains of 100-Hz stimulation; and 2) the commonly used two pairings of a 1-s long, 100-Hz stim-

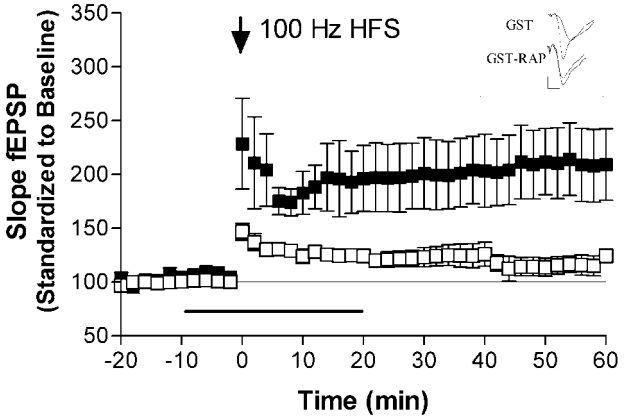

FIG. 4. **LTP is blocked by RAP.** GST-RAP (*open squares*) or GST (*closed squares*) was added to the perfusion medium (indicated by a *bar*) prior to HFS (2 × 100 Hz/s). *Inset*, representative traces (mean of six successive EPSPs) are shown for baseline synaptic responses in the presence of 10 μg/ml GST-RAP or GST control and 60-min post-tetanic stimulation (scale bars represent 1 mV and 10 ms). Results are shown as mean ± S.E.

ulation (100 Hz/s). We chose to utilize both LTP-inducing protocols because of the potential differences in the mechanisms of synaptic plasticity elicited by each protocol (31, 32). Moreover, TBS more accurately mimics the natural neuronal firing that occurs in the mammalian brain.

To examine the effects of Reelin on LTP induction, hippocampal slices were perfused with either recombinant Reelin or control medium applied via the perfusion media for 10 min prior to and 20 min following HFS (as indicated by the bar in Fig. 5, *panels A* and *B*). The application of Reelin had no effect on baseline synaptic transmission but caused a trend toward enhanced LTP induction using the 100-Hz, 1-s HFS protocol (control medium, 161 ± 14%, $n = 13$; Reelin 199 ± 11%, $n = 15$, $p = 0.053$, 60 min post-tetanus) (Fig. 5*A*). However, Reelin produced a significant increase in synaptic potentiation (control medium, 147 ± 11%, $n = 13$; Reelin, 220 ± 21%, $n = 15$, $p = 0.005$) 60 s post-tetanus and 1 h post-tetanus (control medium, 144 ± 10%, $n = 13$; Reelin, 181 ± 15%, $n = 15$, $p = 0.020$) using the TBS protocol (Fig. 5*B*).

Reelin Fails to Enhance LTP in VLDL Receptor and ApoER2 Knockouts—To test whether Reelin remained effective in enhancing LTP in the absence of the VLDL receptor and apoER2, we performed LTP experiments using hippocampal slices from both receptor-deficient mutant strains. Reelin was applied to slices from VLDL receptor-deficient (60 min post-tetanus, Reelin, 139 ± 3%, $n = 10$; control medium, 137 ± 3%, $n = 11$, $p = 0.56$) (Fig. 6*A*) or apoER2-deficient mice (60 min post-tetanus, Reelin, 120 ± 4%, $n = 12$; control medium, 124 ± 4%, $n = 9$, $p = 0.54$) (Fig. 6*B*), and the TBS protocol was used to obtain the maximum amount of Reelin-dependent augmentation of LTP induction. Genetic ablation of either of the Reelin receptors abrogated the LTP-inducing effect of Reelin; neither in the VLDL receptor-deficient mice nor in the apoER2 knockouts did Reelin show any enhancement of LTP induction. Thus, this effect was indeed specific for Reelin and not due to a contaminating compound in the Reelin preparation, because it was dependent on the presence of the Reelin receptors. These findings also suggest that VLDLR and apoER2 work together in a non-redundant fashion and that Reelin, which is expressed by interneurons dispersed throughout the neocortex and the hippocampus in the adult brain, may play a potentially memory-enhancing role by modulating synaptic plasticity *in vivo*.

In additional control experiments, we tested whether VLDLR and apoER2 knockouts exhibited similar deficits in

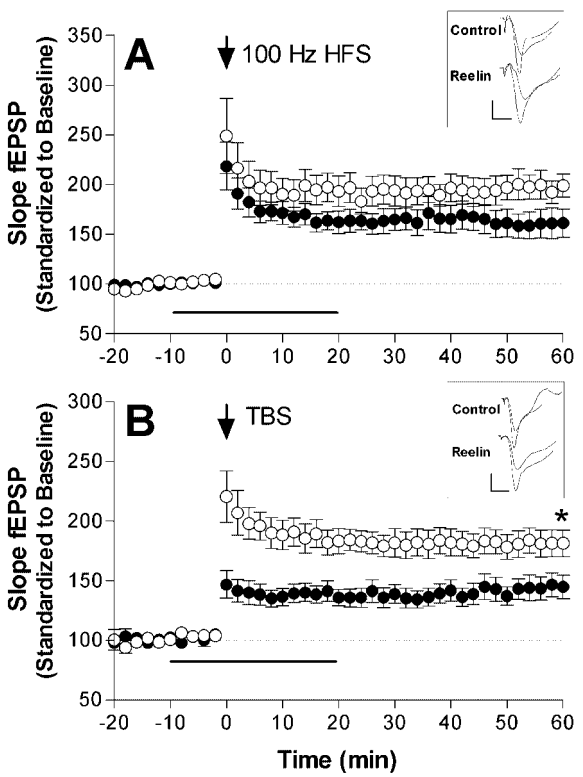

FIG. 5. **Reelin stimulates LTP induction.** Perfusion with ~5 nM Reelin (*open circles*) enhances LTP induction and maintenance compared with perfusion with control medium (*closed circles*) for both 100 Hz (*A*) and θ burst stimulation (*B*) protocols, indicating that the stimulation of apoE receptor-dependent signaling enhances neurotransmission. *Inset*, representative traces (mean of six successive EPSPs) are shown for baseline synaptic responses in the presence of control medium or Reelin-containing medium and at 60-min post-tetanic stimulation (*scale bars* represent 1 mV and 10 ms). The application of Reelin or control medium is indicated by *horizontal lines*. Results are shown as mean ± S.E. *Asterisks* indicate a value significantly different from the baseline ($p < 0.05$, Student's t test).

response to TBS compared with that observed from 100-Hz HFS. TBS-induced LTP in VLDLR-deficient mice was comparable with that in wild-type mice (60 min post-tetanus, VLDLR −/−, 149 ± 6%, $n = 10$; WT, 150 ± 5%, $n = 10$, $p = 0.89$) (Fig. 6*C*), whereas apoER2 mutants showed a deficit in LTP induction (60 min post-tetanus, apoER2 −/− 121 ± 5%, $n = 16$; WT 149 ± 6%, $n = 14$, $p = 0.005$) (Fig. 6*D*). This deficiency in TBS-induced LTP in the apoER2 mutant mice is similar to that seen using the 100-Hz stimulation protocol (Fig. 3*B*) and provides further evidence of a role for Reelin-dependent modulation of hippocampal synaptic plasticity.

DISCUSSION

In this study we have investigated the roles of the VLDL receptor and the apoER2 in LTP and the formation of memory. Both proteins are members of the LDL receptor gene family, a group of lipoprotein receptors that are expressed on the surface of neurons where they are thought to bind apoE and cholesterol complexes secreted by glial cells. However, the VLDL receptor and apoER2 are also receptors for Reelin (6, 9, 33), a large protein that is secreted by Cajal-Retzius neurons during embryonic development of the brain, in which it controls cortical lamination (7, 8). In the adult brain, Reelin is secreted by a subset of interneurons in the adult brain (13, 34). Here we have shown that Reelin and the receptors to which it binds modulate hippocampal synaptic plasticity and, by this mechanism, are likely to contribute to hippocampus-dependent memory in the adult mouse brain.

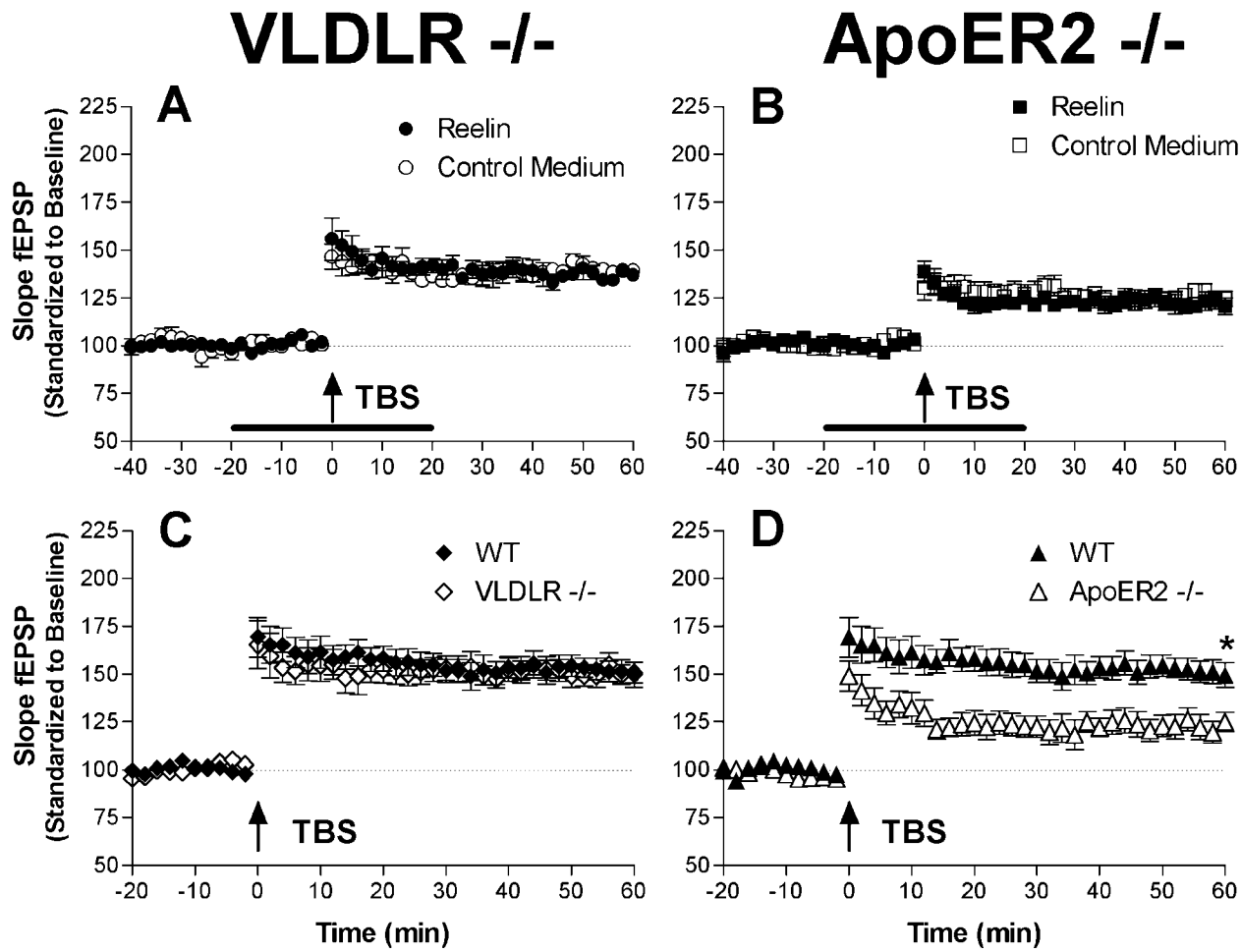

FIG. 6. **Reelin has no effect on LTP in mice lacking VLDL receptor or apoER2.** LTP induction using the TBS protocol in the VLDLR knockout is not enhanced in the presence of Reelin (*closed circles*) compared with perfusion with control medium (*open circles*) (*A*). The LTP deficit in apoER2 knockouts cannot be rescued by Reelin. Hippocampal slices perfused with Reelin (*closed squares*) or control medium (*open squares*) showed indistinguishable LTP induction (*B*). LTP induction using TBS in the VLDLR-deficient mice (*open diamonds*) is normal compared with that in wild-type (*closed symbols*) (*C*). Similar to the LTP deficits seen using 100-Hz stimulation, apoER2-deficient mice (*open triangles*) show a significant decrease in LTP induction (*D*). Note that slices from mutant mice that were not treated with cell culture media showed a similar amount of potentiation as did slices from mutant mice perfused with Reelin or control medium (~150%) (compare *panel A* to *panel C* and *panel B* to *panel D*). Thus, application of the control medium had no affect on LTP induction in either mutant. TBS stimulation is represented by an *arrow*. In *panels A* and *B* the application of Reelin or control medium is indicated by *horizontal lines*; results are shown as mean ± S.E. *Asterisks* indicate a value significantly different from the baseline ($p < 0.05$, Student's *t* test).

Our findings indicate an important physiological role for apoE receptors in neurotransmission and memory formation in the adult central nervous system. Several lines of evidence support this conclusion. First, in agreement with earlier findings (30), the general inhibition of ligand binding to LDL receptor family members by the receptor-associated protein RAP largely abolishes LTP in hippocampal slices from wild-type mice (Fig. 4). More specifically, however, knockout mice lacking the apoER2 exhibit normal baseline synaptic transmission, but a profound deficit of LTP is present in these mice (Fig. 3). Furthermore, Reelin, a ligand and agonist for the VLDL receptor and apoER2, significantly augments LTP induction in hippocampal slices from wild-type mice (Fig. 5) but not in slices obtained from either VLDLR- or apoER2-deficient animals (Fig. 6). Both strains of knockout mice also exhibit significant defects in contextual fear conditioning (Fig. 1), a behavioral test that is generally accepted as one measure of hippocampus-dependent memory induction and retention (18, 19).

A role for apoE receptors in the modulation of the electrophysiological processes that are thought to underlie the formation and consolidation of memories (*i.e.* LTP) raises the intriguing possibility that a differential functional impairment of neurotransmission, for instance by the receptor ligand apoE4, might be at least in part responsible for the effect of apoE genotype on late onset Alzheimer disease (4). A molecular basis for such a hypothetical mechanism might lie in the different physicochemical properties of the apoE isoforms. apoE2 binds poorly to LDL receptor family members, in contrast to apoE3 and apoE4 (1), because it lacks a positively charged amino acid that participates in receptor binding by the other isoforms. ApoE4 differs from apoE3, the most common isoform in the human population, by a single amino acid substitution, which nevertheless profoundly alters the three-dimensional structure of apoE4. As a consequence of this structural change, apoE4 preferentially associates with larger lipoprotein particles than does apoE3 (35), and these particles effectively compete for the binding of LDL to the LDL receptor in the liver. Although it is currently unknown whether apoE4 also forms such particles in the interstitial space in the brain, they would likely also be effective competitors for the binding of other physiological ligands, *e.g.* Reelin, to LDL receptor family members on neurons. Results from an *in vitro* stimulation assay with Reelin in cultured neurons in which apoE4 was more effective in dampening the Reelin signal than apoE3 (36) support such a model. Also

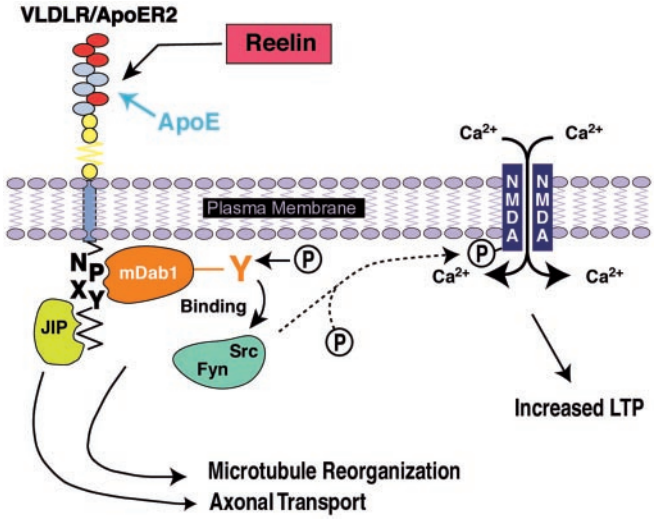

FIG. 7. **Hypothetical model of the actions of Reelin in LTP induction.** Reelin binding to apoER2 and the VLDL receptor stimulates intraneuronal tyrosine kinase activity and induces Dab tyrosine phosphorylation. Non-receptor tyrosine kinases of the Src family are thus activated and may stimulate NMDA receptor activity, thereby increasing Ca^{2+} influx and LTP. ApoE can compete for Reelin binding to the extracellular domains of apoER2 and the VLDL receptor and thereby suppress tyrosine phosphorylation of Dab1 (36). ApoER2 binds members of the JIP family of scaffolding proteins on its cytoplasmic tail and thus indirectly interacts with the microtubule-associated molecular motor kinesin (39, 40). *Blue ovals* designate alternatively spliced ligand binding repeats in apoER2.

consistent with an apoE competition model is the observation that the presence of the apoE2 isoform, a weak receptor ligand and thus a poor competitor, tends to be protective against late onset Alzheimer disease when compared with apoE3 (4).

The importance of Reelin for neuronal migration and cortical lamination during the embryonic phase of brain development has been extensively studied. In contrast, little has been known about the role of this neuronal signaling protein in the adult brain. The production of Reelin by a subset of GABA-ergic interneurons and its association with postsynaptic densities in the vicinity of apical dendritic spines has been reported, and a relationship between the reduction of dendritic spines and neuropil in bipolar disorders has been speculated upon (13, 37). Our results now indicate a direct role for Reelin in the modulation of hippocampal synaptic plasticity in the adult brain.

What may be the biochemical mechanisms by which Reelin, the VLDL receptor, and apoER2 modulate synaptic plasticity? From earlier studies (38–40) of the Reelin signaling complex on primary embryonic neurons, we know that Reelin signaling induces tyrosine phosphorylation of the adaptor protein Dab1 and that the normal strength of the Reelin signaling input is necessary to prevent abnormal phosphorylation of the microtubule-associated protein τ (33). Thus, Reelin signaling controls the activity of tyrosine kinases as well as serine/threonine kinases in the neuronal cytoplasm. Although these kinases have not yet been positively identified, they are likely to include members of the Src family of tyrosine kinases (41) and the τ kinases Cdk5 and GSK-3β (42). According to the hypothetical model shown in Fig. 7, the activation of similar kinase cascades in the adult brain may directly or indirectly alter the phosphorylation state of postsynaptic NMDA receptors or of proteins that regulate the activity of NMDA receptors, thereby modulating the likelihood and magnitude of LTP induction. Although this model is speculative at present, it is a parsimonious explanation for our findings based on known mechanisms for regulating NMDA receptor function.

Reelin signaling may also regulate the axonal transport of synaptic components by regulating the association of apoER2 and the VLDL receptor with molecular motors, kinesin in particular. ApoER2 binds on its cytoplasmic tail members of the JIP family of scaffolding proteins for which functional interaction with kinesin has been shown in a genetic model system in *Drosophila* (43) as well as in cultured neuronal cells of mammalian origin (44).

At present it is as yet unknown whether Reelin signaling in the adult brain uses the same kinase pathways as in embryonic neurons or whether the biochemical machinery by which it modulates LTP induction is different. Although this crude model is therefore at present still highly speculative, it should provide a useful basis on which the functions of Reelin in the mature central nervous system and its potential role in neurological and neurodegenerative disorders can be investigated.

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