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Age-related influence of the HDL receptor SR-BI on synaptic plasticity and cognition

Eric H. Chang^{a,b}, Attilio Rigotti^c, and Patricio T. Huerta^{a,*}

^a *Burke/Cornell Medical Research Institute, Department of Neurology and Neuroscience, Weill Medical College of Cornell University, White Plains, NY 10605, USA*

^b *Center for Neural Science, New York University, New York, NY 10003, USA*

^c *Departamento de Gastroenterología, Facultad de Medicina, Pontificia Universidad Católica, Santiago, Chile*

Abstract

Dysregulated cholesterol metabolism is a major risk factor for atherosclerosis and other late-onset disorders, such as Alzheimer's disease. The scavenger receptor, class B, type I (SR-BI) is critical in maintaining the homeostasis of cholesterol and α -tocopherol. SR-BI binds high density lipoproteins (HDL) and mediates the selective transfer of cholesteryl esters and α -tocopherol from circulating HDL to cells. SR-BI is also involved in reverse cholesterol transport from peripheral tissues into the liver. Previous studies using SR-BI genetic knockout mice indicated that the deletion of SR-BI resulted in an accelerated onset of atherosclerosis. We hypothesized that SR-BI dependent lipid dysregulation might disrupt brain function leading to cognitive impairment. Here, we report that very old SR-BI knockout mice show deficient synaptic plasticity (long-term potentiation) in the CA1 region of the hippocampus. Very old SR-BI KO mice also display selective impairments in recognition memory and spatial memory. Thus, SR-BI influences neural and cognitive processes, a finding that highlights the contribution of cholesterol and α -tocopherol homeostasis in proper cognitive function.

Keywords

cholesterol; HDL; α -tocopherol; scavenger receptors; lipid metabolism; atherosclerosis; late-onset Alzheimer's disease; LTP; recognition memory; spatial memory

1. Introduction

Cholesterol is an essential component of the plasma membrane of cells throughout the body, including brain cells. Cholesterol dysregulation has emerged as a prominent risk factor for late onset human diseases, such as atherosclerosis and Alzheimer's disease (AD). In the case of atherosclerosis, it has been established that elevated levels of low density lipoprotein (LDL)

*Correspondence should be addressed to Patricio T. Huerta, Burke/Cornell Medical Research Institute, 785 Mamaroneck Ave, White Plains, NY, 10605, USA. Email: pato.huerta@gmail.com. Telephone: (914) 597-2459. Fax: (914) 597-2796.

Disclosure Statement

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cholesterol as well as reduced levels of high density lipoprotein (HDL) cholesterol in the blood plasma are clear risk signals for the disease [21]. In the case of AD, the evidence extends from epidemiological and clinical studies in patients to *in vitro* and *in vivo* studies in animals models [30,50,75]. Patients with cardiovascular disease show an increased risk of developing AD [67]. There is a higher prevalence of senile plaques in cognitively intact individuals with heart disease compared to age-matched controls with no cardiovascular related ailments [68]. Cholesterol augments A β production in hippocampal neurons [65] and has been shown to increase plaque burden in transgenic mice overexpressing AD-related mutations [39]. Moreover, there is evidence that abnormal cholesterol homeostasis is sufficient for causing many pathological signs of AD, including excessive tau phosphorylation [67], amyloid plaque formation [56,58,68], neurodegeneration [19], and cholinergic and synaptic disruptions [10, 34]. Thus, studying how cholesterol dysfunction affects neurophysiology in mouse models with genetic manipulation of cholesterol metabolism has obvious implications for AD [10, 57].

Among several metabolic pathways, cholesterol is transported through the blood plasma from the body tissues to the liver and steroidogenic tissues for subsequent processing or storage. HDL cholesterol, a major player in this transport process [59], is metabolized by the lipoprotein receptor SR-BI which mediates high affinity HDL binding as well as selective cholesterol uptake into cells. As a consequence of its role in HDL cholesterol metabolism, SR-BI is critical in reverse cholesterol transport from peripheral tissues into the liver. Recently, it has been shown that SR-BI facilitates the cellular uptake and metabolism of HDL α -tocopherol in mice [41]. Therefore, SR-BI plays a physiologically important role in whole-body cholesterol and α -tocopherol homeostasis *in vivo*. It is likely that SR-BI manipulation would be valuable for understanding a variety of lipid-related human ailments [1,37,59]. Indeed, deletion of the *srbi* gene, by null mutation in mice, results in dysregulations of plasma cholesterol and α -tocopherol [42,60], which might underlie an accelerated onset of atherosclerosis in an apolipoprotein E-deficient background [70,73].

It has been proposed that fine-tuning of neural cholesterol dynamics is essential for basic synapse function, plasticity and behavior [35]. In addition, vitamin E has a central role in maintaining neurological structure and function [49,64]. Given these potential roles of cholesterol and α -tocopherol, we set out to test whether the SR-BI KO mouse model might also exhibit pathophysiological changes and cognitive deficits similar to those observed in mouse models for early, familial AD and late onset AD. To our knowledge, this is the first examination of the role of SR-BI function in synaptic physiology and cognition.

2. Methods

2.1. Animals

The generation of SR-BI KO mice was described previously [60]. We studied 35 male SR-BI KO mice and 34 male wild-type (WT) littermates. Mice were maintained on a 1:1 C57BL/6 X 129/Sv genetic background by crossing heterozygous *srbi* mutant females with heterozygous *srbi* mutant males; previous studies showed that pure C57BL/6 female SR-BI KO mice are infertile [24,42,44,70]. Homozygous *srbi* mutant (SR-BI KO) and control wild-type mice were screened by PCR. Animals were maintained according to NIH guidelines. Most mice underwent behavioral assessment followed by electrophysiological examination. Every experiment was performed in blind format with the researchers unaware of the animals' genotype.

2.2. Hippocampal electrophysiology

The electrophysiological procedures were described previously [14,26,72]. Mice were anaesthetized with isoflurane in a closed container, then immediately decapitated. The brain was extracted, bisected, and both hemispheres were mounted onto a block with ethyl cyanoacrylate glue. The block was placed in a brain slicer (Vibratome 1000 Plus, St. Louis, MO) that was used to prepare transverse hippocampal slices (350 μm thick). The slicer was filled with ice-cold ($< 2^{\circ}\text{C}$) artificial cerebral spinal fluid (ACSF) which contained (in mM): 126 NaCl, 26 NaHCO_3 , 10 glucose, 2.5 KCl, 2.4 CaCl_2 , 1.3 MgCl_2 , 1.2 NaH_2PO_4 . Kynurenic acid (1 mM), which is a non-specific blocker of excitatory amino acid receptors, was added to the ACSF only during slice cutting. Slices were incubated (35 min in 35°C ACSF followed by ~ 2 hr in 20°C ACSF) and transferred to a recording chamber continuously perfused with 30°C ACSF. We studied 1–3 viable slices from each mouse brain. Picrotoxin (100 μM) was added to block GABA_A -mediated activity and a cut was made between CA3 and CA1 to prevent epileptic activity. Field excitatory postsynaptic potentials (fEPSP) were recorded with borosilicate glass electrodes (2–3 $\text{M}\Omega$ tip resistance) placed in CA1's *stratum radiatum* at the midpoint between two stimulating electrodes (bipolar Pt-Ir electrodes, Frederick Haer & Co, Bowdoinham, ME) that were placed ~ 100 μm from *stratum pyramidale* to activate the Schaeffer collateral/commissural axons. This setup allowed for the recording of two independent pathways (test and control) in the same slice. The initial slope of the fEPSP was used as a measure of the postsynaptic response. Responses were amplified (AM Systems 1800), digitized (10 kHz), and stored on PC running custom software (written with AxoBasic, Axon Instruments, Union City, CA). For obtaining input-output (I-O) functions, the stimulation was reduced to a value at which no fEPSP was evoked. The stimulation was then increased incrementally (exact increment varied by the strength of the response) to evoke steeper and larger fEPSPs. This was done until the appearance of a population spike, which reflected action potentials generated by CA1 pyramidal cells and defined the final point of the I-O function.

The protocol for paired-pulse facilitation (PPF) involved activating the afferent axons with two stimulating pulses within a short (< 1 sec) inter-pulse interval (IPI). The IPIs were (in msec): 20, 30, 40, 50, 80, 100, 150, 200, and 300. The paired-pulse ratio was calculated as the slope of the second fEPSP divided by the slope of the first fEPSP.

For long-term potentiation (LTP) experiments, a stable baseline was obtained for at least 15 min. The baseline intensity was set to obtain a fEPSP slope that was half-maximal, as determined by I-O curves. LTP was induced by either a tetanus (100 Hz for 1 sec) or theta-burst stimulation (TBS) for 5 sec. TBS consisted of 10 trains (4 pulses at 100 Hz), with 200 msec between trains. The tetanus or TBS were delivered at the baseline stimulus intensity. In the few cases in which the fEPSPs of the control pathway changed $>15\%$ from the mean value for the initial 15 min, the experiment was discarded. A detailed analysis of the temporal summation that occurred during the TBS was used to indirectly measure the activation of N-methyl-D-aspartate (NMDA) receptors. Using analysis software (Origin, OriginLab, Northampton, MA), the total depolarization area was calculated by integrating the area of each fEPSP response during TBS stimulation.

2.3. Behavioral assessment

The behavioral procedures have been described previously [36,72]. The preliminary behavioral screen consisted of a total of 38 measurements recorded for each mouse [16,29]. Assessment started with observation in a cylindrical glass flask (height 15 cm, diameter 11 cm) followed by transfer to an arena (55×33 cm^2). This was continued with manipulations using tail suspension for measuring visual acuity, grip strength, body tone and reflexes. Subsequently, the mouse was restrained in supine position to record autonomic responses of skin color, limb tone and abdominal tone. Salivation and provoked biting were also recorded. The screen was

completed by measuring the righting reflex and negative geotaxis. Throughout the procedure, incidences of abnormal behavior, irritability and vocalizations were recorded. Anxiety was measured with the elevated plus maze task. The apparatus was shaped as a + sign and was placed 70 cm from the floor. It consisted of two opposite arms with walls (27 cm long, 8 cm wide, 30 cm high, painted black) and two opposite open arms (30 cm long, 4 cm wide, 0.5 cm high, painted white). A mouse was placed at the end of an enclosed arm and observed for 5 min. We measured the initial latency to enter an open arm (with four paws), the number of entries and the total time spent in the open arms because these parameters are anxiety-related [15].

Working memory was measured with the T-maze force-choice alternation task. The apparatus had three arms, each of which was 30 cm long, 8 cm wide, 30 cm high. The floor was made of white plastic, with a layer of bedding over it, and the walls were made of clear plastic. The maze was situated in a room with numerous distal visual cues. Initially, mice were familiarized (4 sessions of 5 min) to run for a reward (50 μ l of 25% sucrose solution in distilled water) which was placed in a cup recessed into the floor bedding at the end of each goal arm. For the task proper, each mouse received four trials per day, over 4 days, with a minimum intertrial interval of 1 hr. At the start of each trial, both goal arms were baited. A trial consisted of two runs; in the first one, a removable partition blocked either the left or the right arm and the mouse traveled from the start point (back of the stem arm) to the final point (end of the open arm). After it entered the open arm, the animal was confined there by blocking the entrance. It was allowed to drink the reward before being returned to the start point by the experimenter. The second run consisted in the mouse running from the start point and simply choosing between the left and right arms which were both open. At this point, only the unvisited arm contained reward. A choice was defined as the mouse entering the arm with the whole body. Memory was scored by the number of alternations over blocks of 4 trials.

Two-object recognition memory was tested in a plastic chamber (25 cm long, 25 cm wide and 60 cm high) with open ceiling and illuminated from the top (40 W yellow bulb). A video camera was mounted on top and was used for videotaping behavior. The floor was covered with a thin layer of bedding. Before formal testing, each mouse was familiarized with the empty chamber for three sessions (5 min each over 2 days). A single trial consisted of 3 phases: sample, delay, and choice. For the sample phase, mice were allowed to explore two identical objects for 5 min. For the delay phase, mice were placed in their home cage for 1 h. During this interval, sample objects were replaced by test objects. One of them was identical to those in the sample phase ("familiar object") whereas the other was different ("novel object"). Care was taken in placing the objects in exactly the same positions occupied by the sample objects. For the choice phase, mice were allowed to explore for 5 min. Object exploration was scored when the mouse touched the object with the face (mouth, whiskers, and nose). After the experiment, videotapes of the trials were reviewed and the time (in sec) spent on each of the objects was obtained. We defined A1 and A2 as the times exploring the sample objects, A3 as the time exploring the familiar object, and B1 as the time exploring the novel object. To determine recognition memory, we measured the exploration ratio in the sample phase (run 1) which was defined as $[(A1+A2)/300]$, the exploration ratio in the test phase (run 2) which was defined as $[(A3+B1)/300]$, the preference index which was defined as $[B1-A3]$, and the discrimination ratio which was defined as $[(B1-A3)/(B1+A3)]$.

Spatial memory was measured with the Morris water maze task. A pool (160 cm diameter) was filled with water (18–20°C, made opaque with a mix of white and blue paint) in which a hidden platform was submerged (0.5 cm). The maze was surrounded by salient distal objects (focally illuminated, mounted on walls). Mice were trained to find the location of the platform, starting from one of four points near the edge of the pool. During phase 1 (first 12 trials), a "large" platform (24 cm diameter) was used. For phases 2 and 3, a "small" platform (16 cm diameter)

was used. For phase 3 (last 12 trials), the platform was moved to a novel location. Performance was based upon latency to reach the platform (maximum allotted time, 90 sec). The amount of perseveration was measured by comparing the latency of the last 4 trials in phase 2 (L1) versus the latency of the initial 4 trials in phase 3 (L2) with a perseveration ratio, defined as $[(L2-L1)/(L2+L1)]$. Memory for the platform location was tested with probe trials in which the platform was removed and the mice swam during 60 sec. The pool was then divided into 4 imaginary sectors and the time spent in each sector was measured. We defined T as the percent time in the trained ("target") sector and U as the mean percent time in the untrained sectors. To determine spatial memory, we measured the spatial index which was defined as $[T - U]$, and the spatial memory ratio which was defined as $[(T-U)/(T+U)]$, to perform normalized comparisons across ages.

2.4. Statistical Analyses

Statistical comparisons were done with parametric tests (analysis of variance, ANOVA, or Student's *t*-test) with the use of appropriate software (Statistica and Origin).

3. Results

3.1. Synaptic parameters and short-term synaptic plasticity are normal in SR-BI KO mice

To study whether deletion of the *srbi* gene produced detectable alterations in synaptic parameters, SR-BI KO mice ($n = 30$) and WT littermates ($n = 30$) were examined electrophysiologically. Since the effects of abnormal cholesterol metabolism on brain function typically do not bear until late in life, mice were examined at three advanced ages: 6–14 months ("adult"), 15–19 months ("old"), and 20–28 months ("very old"). We recorded fEPSPs in *ex vivo* slices of the hippocampus, a brain area that is critically involved in spatial and temporal memories [27,46,51]. Basal synaptic transmission was measured with input-output (I-O) functions (Fig. 1), which were similar between genotypes across all age groups. The I-O functions were compared using ANOVA, with fiber volley amplitude as the repeated measure. This test showed that there were no differences between SR-BI KO and WT mice (adult, $F = 0.48$, $P = 0.72$; old, $F = 10.27$, $P = 0.23$; very old, $F = 0.59$, $P = 0.67$). Moreover, applying a linear regression on each I-O function allowed us to determine the mean slope of the I-O functions for each genotype by age (Fig. 1B). Statistical analysis revealed no differences in this parameter of synaptic transmission (mean slope of I-O curves: adult, $t = 0.95$, $P = 0.35$; old, $t = 0.69$, $P = 0.5$; very old, $t = 1.53$, $P = 0.14$, *t* test). These results suggested that the elevated plasma cholesterol and brain α -tocopherol levels, due to abnormal HDL metabolism, did not affect the strength of basal transmission across the population of hippocampal synapses.

We then tested paired-pulse facilitation (PPF) in slices from SR-BI KO mice using the paired-pulse paradigm (Fig. 2). This paradigm is designed to detect changes among the population of presynaptic terminals. It has been established as an assay for short-term plasticity [78]. PPF profiles were compared using ANOVA, with inter-pulse interval as the repeated measure. This test showed that there were no differences between SR-BI KO and WT mice (adult, $F = 1.87$, $P = 0.07$; old, $F = 0.64$, $P = 0.75$; very old, $F = 1.49$, $P = 0.16$). Thus, short-term plasticity was unaffected in SR-BI KO mice across all tested ages.

3.2. LTP is impaired in very old SR-BI KO mice

We examined long-term synaptic plasticity by inducing LTP in brain slices from SR-BI KO mice. LTP is an electrophysiological correlate of memory within the brain [7] and has been linked with hippocampal-dependent memory [47,72]. Slices from adult and old SR-BI KO mice exhibited robust LTP comparable to WT mice (Fig. 3, LTP level 45 min after induction, mean $\pm$ SEM: adult, KO, $139 \pm 2\%$; WT, $139 \pm 1\%$, $t = 0.044$, $P = 0.97$; old, KO, $143 \pm 1\%$,

WT, $141 \pm 2\%$, $t = 0.93$, $P = 0.37$, t test). In contrast, very old SR-BI KO mice had a significant LTP deficit (Fig. 3, KO, $110 \pm 1\%$; WT, $141 \pm 1\%$, $t = 19.5$, $P < 0.0001$, t test).

Measurements of short-term potentiation (STP) indicated that only very old SR-BI KO mice exhibited significant deficits when compared to age-matched WT mice (Fig. 3C, KO, $131 \pm 2\%$; WT, $144 \pm 1\%$, $t = 7.1$, $P < 0.0001$, t test). Therefore, very old SR-BI KO mice had deficits in long-term synaptic plasticity, despite having normal basal transmission at this late age (Fig. 1).

3.3. Burst analysis reveals decreased total depolarization during LTP induction in very old SR-BI KO mice

Since the synaptic responses of NMDA receptors have a long duration ($>100\text{ms}$), they summate effectively when the excitatory synapses are stimulated with 'high frequency' bursts (larger than 10Hz). The total depolarization value is a measure of this NMDA receptor response summation. An analysis of responses during the LTP-inducing TBS showed that the mean total depolarization in very old SR-BI KO mice was decreased compared to very old WT mice (Fig. 4, KO, $235 \pm 16 \text{ mV} \times 10^2$; WT, $333 \pm 32 \text{ mV} \times 10^2$, $t = 2.75$, $P < 0.01$). Total depolarization for the adult and old ages did not differ significantly (Fig. 4A).

3.4. Many behaviors are normal in SR-BI KO mice

To study whether the deletion of the *srbi* gene produced detectable alterations in behavior, SR-BI KO mice ($n = 30$) and WT littermate controls ($n = 30$) were subjected to a battery of behavioral tests in a longitudinal design. We found that SR-BI KO and WT mice performed similarly at every age in a variety of behavioral tests that examined their basic neurological reflexes as well as perceptual abilities. Both groups displayed normal feeding with no difference in average body weight (Table 1) and normal grooming. SR-BI KO mice showed normal anxiety levels when tested in the elevated plus maze (Fig. 5A). None of the anxiety measures reached statistical significant differences ($P > 0.05$ for all measures, t test) when comparing SR-BI KO and WT mice. Moreover, there was no difference between groups in a locomotion task (Fig. 5B, $F = 0.66$, $P = 0.42$, ANOVA). To assess working memory, SR-BI KO and WT groups were tested in a T-maze task for spatial working memory (Fig. 5C). There were no differences in the working memory abilities of SR-BI KO mice when compared to WT controls ($F = 1.39$, $P = 0.25$, ANOVA with alternations as the repeated measure).

3.5. Recognition memory is impaired in very old SR-BI KO mice

The two object task was used to test for proper recognition memory (Fig. 6). Each mouse was initially exposed to two objects that were made to look as equal as possible. After a delay (1 h), one object was replaced by a new one and the mouse was re-exposed to the objects. Mice naturally tend to explore the novel object for a longer time than the familiar object. Adult and old mice displayed the expected behavior without any differences across genotype (statistical comparison for discrimination ratios; adult, $t = 0.94$, $P = 0.36$, old, $t = 0.63$, $P = 0.54$, t test). However, the very old SR-BI KO group showed poorer performance in their ability to recognize the novel object ($t = 2.13$, $P < 0.05$, t test). Thus, very old SR-BI KO mice exhibited a clear deficit in recognition memory.

3.6. Spatial memory is impaired in very old SR-BI KO mice

Spatial memory for a location was assessed with the Morris water maze task [14,46,47]. The task was implemented in three phases (each consisting of 12 trials): phase 1, in which the mice were required to find a large hidden platform (24 cm diameter); phase 2, in which the platform was made smaller (16 cm diameter) but remained in the same location; and phase 3, in which the small platform was placed in a different location (this maneuver is sometimes called

“reverse learning”). Mice of both genotypes behaved equally during phases 1 and 2 (Fig. 7). For phase 3 an analysis of variance, with the 12 last trials as the repeated measure, showed no significant difference between genotypes in the adult group ($F = 0.187$, $P = 0.67$), but clear differences in the old group ($F = 14.76$, $P < 0.005$) and the very old group ($F = 20.3$, $P < 0.005$).

An analysis centered on the change of location of the platform (from phase 2 to 3) showed an age-related increase in the perseveration ratio for SR-BI KO mice (Fig. 8A), revealing a deficit in memory flexibility. Two probe trials were implemented to test for spatial memory (probe 1 was after the initial 24 trials, and probe 2 was at the end of training). Adult and old SR-BI KO mice behaved similarly to WT mice in both probe trials (Fig. 8). However, very old SR-BI KO mice displayed a reduced spatial bias to their trained quadrant when compared to age-matched WT mice (Fig. 8). This deficit was reflected in their spatial indexes (mean $\pm$ SEM, probe 1, KO, 9.88 ± 4.64 ; WT, 30.1 ± 4.12 , $t = 3.26$, $P < 0.005$; probe 2, KO, 4.2 ± 3.38 ; WT, 23.23 ± 6.53 , $t = 2.42$, $P < 0.05$, t test), as well as their spatial memory ratios (Fig. 8C, mean $\pm$ SEM, probe 1, KO, 0.15 ± 0.07 ; WT, 0.45 ± 0.05 , $t = 3.39$, $P < 0.005$; probe 2, KO, 0.06 ± 0.06 ; WT, 0.33 ± 0.08 , $t = 2.51$, $P < 0.05$, t test). Thus, SR-BI KO mice exhibited several age-related deficits in cognitive abilities, as reflected in their deterioration of recognition memory, memory flexibility, and spatial memory across ages. As SR-BI KO mice aged, they found it increasingly harder to learn a spatial task and to adapt to novel platform locations.

4. Discussion

Previous studies have established that SR-BI is critically involved in HDL cholesterol metabolism and protects against atherosclerosis in mice. Our results show that SR-BI deletion is associated with age-related disruptions of hippocampal LTP and cognitive impairment. These results support our hypothesis that SR-BI KO mice could exhibit subtle impairments in memory acquisition [41,42] and are consistent with a role of SR-BI in synaptic plasticity, further expanding its importance in normal physiology. There are several possible mechanisms by which the severely abnormal cholesterol homeostasis found in SR-BI KO mice might result in the abnormal brain physiology and behavioral performance observed.

Cholesterol is a multifaceted molecule that is an essential component of cellular membranes, a cofactor for signaling molecules, and a precursor for steroid hormones. Although about 25% of the total body cholesterol present in humans is localized in the brain [18], the current knowledge on cholesterol metabolism and regulation in the central nervous system (CNS) is less well understood than cholesterol homeostasis in the rest of the body [6]. Most of the brain cholesterol originates from local neosynthesis, suggesting significant apolipoprotein-dependent cholesterol recycling, with little flow into the systemic circulation. The major pathway for brain cholesterol elimination is the enzymatic conversion of CNS cholesterol to 24S-hydroxycholesterol, an oxysterol that does not recycle, but enters the blood and is transported to the liver as an alternative mechanism for maintaining brain cholesterol balance [6,9]. SR-BI deletion may impair the transport of cholesterol itself, or 24S-hydroxycholesterol species, from the brain into the liver. Besides affecting cholesterol uptake from plasma HDL into the liver, SR-BI deficiency may also decrease cholesterol efflux from cells [59]. These dysfunctions could lead to retrograde cholesterol accumulation in selective cerebral regions and cholesterol-related neuronal dysfunction.

It is likely that the alterations in brain cholesterol and 24S-hydroxycholesterol levels via the potential mechanisms discussed above could disrupt synaptic physiology, especially given the importance of sterols in membrane fluidity, protein function, protein trafficking, and the ion-gating activity of neurotransmitter receptors [34,40]. Aging is associated with a significant increase in the level of cholesterol in neuronal membranes, leading to greater membrane rigidity and decreased fluidity, especially in hippocampal and cortical areas [2,52]. This opens the

possibility that abnormal cholesterol homeostasis can affect the stability and function of receptors within neuronal membranes and may be responsible for the decreased amount of depolarization during LTP induction that we observed in the very old SR-BI KO mice (Fig. 4A). The decreased temporal summation of multiple fEPSPs during the tetanus likely contributes to the LTP deficit at this age, as similar LTP induction impairments have been shown in the hippocampi of comparably aged rats [62]. The LTP deficit subsequently leads to memory impairment on tasks requiring proper spatial memory.

Another potential mechanism for the synaptic and cognitive impairments in very old SR-BI KO mice is that increased brain cholesterol levels promoted the formation of A β from APP [56,57,65]. There is abundant evidence from epidemiological, cell culture, and *in vivo* animal studies that cholesterol directly affects the amount of A β secreted from neurons through modulation of APP secretase complexes [75]. Interestingly, 24S-hydroxycholesterol has also been shown to regulate APP processing [9] and has been linked to increased A β deposition in the medial temporal lobe of late-onset AD patients [53]. The increase of A β levels within specific areas of the brain would in turn lead to a number of neurophysiological dysfunctions, including deficits in synaptic plasticity and basal transmission [25,63] similar to those observed in very old SR-BI KO mice.

Recent clinical, epidemiological and experimental results have suggested that cholesterol exchange between the plasma and the cerebrospinal fluid may exist, suggesting an interaction between the different pools of cholesterol [32,58]. Rodent studies have reported cognitive impairments as a consequence of dietary lipid changes alone [22,45]. In transgenic mice overexpressing amyloid-precursor protein (APP), a docosahexanoic acid-depleted diet results in impaired performance in the Morris water maze task [12]. Statins, a class of compounds that reduce the levels of plasma cholesterol by inhibiting its *de novo* synthesis, markedly reduce the risk for developing late-onset AD [11,31]. When applied to cultures of hippocampal neurons, statins reduce the levels of both intracellular and extracellular A β [20]. When administered through the diet, statins are able to reduce levels of cerebral A β *in vivo* [20,54]. This is worth noting because some statins are unable to cross the blood-brain barrier, indicating that they exert their beneficial effects (for AD) without even penetrating the central nervous system. For example, simvastatin decreased A β levels in the cerebral spinal fluid (CSF) and the brain, even though it did not increase brain cholesterol [75]. This lack of a measurable decrease in brain cholesterol may be explained by the long 2–4 month half-life of brain cholesterol [5], making it difficult to precisely monitor fluctuations of brain cholesterol levels. This is consistent with the interpretation that simvastatin reduced *de novo* synthesis of cholesterol in the brain.

Impaired SR-BI expression and/or function may also have significant implications for human neurodegenerative diseases. For instance, many of the pathogenic mechanisms that promote atherosclerosis are also associated with late-onset AD. In fact, it has been suggested that late-onset AD may result from atherosclerosis of extra- or intra-cranial blood vessels [23,61] or discrete brain infarction [66]. Support for shared causal factors between atherosclerosis and late-onset AD has emerged from studying apolipoprotein E (apoE), one of the major apolipoproteins in the plasma and the principal cholesterol carrier in the cerebrospinal fluid [33,55]. ApoE has been implicated in both diseases by human association studies and animal gene manipulation. Individuals with a specific variant allele of the apoE gene (APOE- ϵ 4) have elevated levels of circulating plasma cholesterol and increased atherogenesis [17]. Interestingly, the APOE- ϵ 4 allele is the most significant known genetic risk factor linked with late-onset AD [69]. Mice with genetic knockout of the apoE gene (APOE KO mice) spontaneously develop atherosclerotic lesions, which closely resemble those observed in humans, around 3 months of age [8,77]. These APOE KO mice exhibit many pathophysiological characteristics, including compromised synaptic density, loss of

cholinergic function and altered synapto-dendritic integrity [43,55]. These mice also exhibit cognitive deficits, being impaired in the Morris water maze task at both 2 and 6 months of age [38]. Meanwhile, APOE knock-in mice expressing the APOE-ε4 allele have deficient LTP with normal basal synaptic transmission [71]. Thus, the available evidence on mice with alterations in lipoproteins and cholesterol homeostasis, such as APOE KO and SR-BI KO mice, indicates that synaptic plasticity and memory appear to be particularly sensitive to manipulations of cholesterol regulation.

Aside from its effect on plasma HDL cholesterol levels and overall cholesterol homeostasis, SR-BI mediates selective HDL α-tocopherol uptake into cells [41]. α-tocopherol is an isoform of vitamin E with antioxidant properties that may be effective for treating or preventing AD. SR-BI KO mice show a profound impairment in α-tocopherol homeostasis, as indicated by increased HDL α-tocopherol levels in the blood plasma and decreased α-tocopherol levels in selected tissues such as the brain, lungs, and gonads [42]. The reduced brain α-tocopherol levels in SR-BI KO mice may predispose them to oxidative stress-related neurodegenerative disorders, such as AD, and impair LTP via an increase in free radicals [76]. Additional studies with APP overexpression in SR-BI deficient mice should provide new insights on the relevance of this lipoprotein receptor as a modulator of neuropathological processes [28].

It has long been known that increasing HDL levels is anti-atherogenic because HDL particles promote the efflux of cholesterol from peripheral tissues to the liver [4]. When SR-BI is overexpressed by transgenesis, there is increased selective uptake of HDL cholesterol in the liver, increased biliary cholesterol content, and a decreased risk for atherosclerosis [3,74]. Such findings indicate that increased expression and/or function of SR-BI can have therapeutic benefits in humans. Importantly, *in vitro* work on the regulation and distribution of the human SR-BI suggests that it may play a similar role in controlling plasma HDL levels [37] as its murine counterpart. Human SR-BI has tissue expression similar to mice, exhibits comparable ligand binding properties, and can mediate selective cholesterol uptake via HDL [13,49]. Therefore, the SR-BI KO mouse is a valuable model for studying both the genetic and environmental determinants of atherosclerosis. And given the role of cholesterol and other lipids in the pathophysiology of AD, the disruption of lipid homeostasis in the SR-BI KO mouse also makes it a rare learning tool for investigating the role of cholesterol in the etiology of late-onset AD.

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Figure 1.

Synaptic parameters in SR-BI KO mice. **(A)** Input-output (I-O) functions are used to measure basal excitatory transmission. At left, the plots for each age group show fEPSP slopes (mean $\pm$ SEM) against fiber volley (FV) amplitudes, binned every 0.1 mV. At right, traces from SR-BI KO mice, at each age group, show representative FVs (arrows) followed by fEPSPs; scale bars, 1 mV (y-axis), 10 msec. **(B)** The plot displays the mean slopes of the I-O functions for each genotype by age. The mean slopes were obtained from the raw data, not the binned results. Statistical analysis reveals no differences in basal transmission ($P > 0.5$ for all age-paired groups, t test); n indicates number of slices.

Figure 2.

Short-term synaptic plasticity in SR-BI KO mice. (A) Representative fEPSPs from paired-pulse stimulation. Arrows show points of stimulation at 0, 30, 60, and 100 msec; scale bars, 0.5 mV (y-axis), 30 msec. (B) At left, paired pulse profile (mean $\pm$ SEM) for mice at the very old age. At right, plot of paired pulses ratios, at the 50-msec inter-pulse interval, for each genotype by age. Ratios > 1 indicate paired pulse facilitation (PPF). ANOVA with inter-pulse interval as the repeated measure showed no differences between genotypes for any age group ($P > 0.07$ for all age-paired groups), suggesting that short-term plasticity was unaffected in SR-BI KO mice.; n indicates number of slices.

Figure 3.

Age-related impairment of LTP in SR-BI KO mice. (A) Levels of LTP were not different between genotypes in the adult group. Values represent the initial slope of the fEPSP (mean $\pm$ SEM). Inset, illustrative fEPSPs (from a single experiment) taken immediately before (*i*) and 45 min after (*ii*) tetanic stimulation; scale bars, 1 mV (y-axis), 10 msec (x-axis). (B) LTP was impaired in very old SR-BI KO mice ($t = 19.5$, $P < 0.0001$, t test), symbols as in (A). (C) Short-term potentiation (STP) and LTP across ages. Deficits in STP and LTP were observed at the very old age (*, $P < 0.001$, t test); n indicates number of slices.

Figure 4.

Analysis of tetanic bursts. **(A)** Total depolarization during tetanic stimulation did not differ at the adult and old ages ($P > 0.34$ for both groups), but was decreased in the SR-BI KO mice at the very old age ($P < 0.01$, t -test). **(B)** Representative fEPSP examples of a single theta-burst stimulation event during LTP induction. Gray-filled space shows the total depolarization area calculated from the fEPSP baseline; scale bars, 1.0 mV (y-axis), 20 msec.

Figure 5.

Behavioral tests in SR-BI KO mice. **(A)** Levels of anxiety measured in the elevated plus maze task are comparable between very old SR-BI KO and WT mice. **(B)** Test for locomotion (three sessions of 5 min inside a small arena) in very old mice reveals that both groups spend similar amounts of time moving. Bar graphs (at right) show comparable behavior in session 3 for mice of all ages. **(C)** The T-maze task reveals that both groups show similar learning curves for working memory.

Figure 6.

Age-related impairment of recognition memory in SR-BI KO mice. **(A)** The two-object recognition task was used to study recognition memory. The exploration ratios (time exploring the objects over the total time) in the Sample and Choice phases are similar for both groups. However, the preference index (time exploring the novel object minus time exploring the familiar one) is decreased in very old SR-BI KO mice (*, $P < 0.05$, t test). **(B)** Discrimination ratios (preference index over total exploration time) are similar for adult and old groups, whereas very old SR-BI KO mice show a slight but significant decrease when compared to age-matched WT littermates (*, $P < 0.05$, t test).

Figure 7.

Spatial learning in SR-BI KO mice. (A) The Morris water maze task was used to assess spatial learning. The diagrams explain the three phases of training: phase 1 with a large platform (gray circle) in location 1, phase 2 with a small platform in the same location and phase 3 with a small platform in location 2. (B) Adult mice of both genotypes show comparable performance throughout the task. (C, D) SR-BI KO mice in the old (C) and very old (D) groups display a clear deficit during phase 3 (last 12 trials), when the platform is switched to a novel location. ANOVA with the 12 last trials as the repeated measure showed no difference between genotypes for adult mice ($P = 0.67$), but a significant difference for old ($P < 0.005$) and very old mice ($P < 0.005$).

Figure 8.

Abnormal perseveration and reduced spatial bias in SR-BI KO mice. **(A)** At left, a detailed analysis of the change of location (from location 1, trial 24, to location 2, trial 25) shows that, after the switch, the SR-BI KO mice continue searching at the previous platform location. This behavior is quantified, at right, by the perseveration ratio, which is the difference between the latencies to find the new platform (in location 2) and the old platform (in location 1) over the sum of the latencies. The ratio shows a gradual increase for the SR-BI KO mice across ages (*, $P < 0.005$, t test), suggesting lack of memory flexibility. **(B)** Analysis of the probe tests across age groups shows that the very old SR-BI KO mice have a reduced spatial bias to the target quadrant (*, $P < 0.005$, t test), whereas adult and old mice display normal spatial bias.

Dotted lines represent chance level of spatial selectivity. Abbreviations for water maze sectors, T, target; O, opposite; L, left; R, right. (C) The spatial memory ratio, which is the difference between the percent time in the target quadrant minus the average for the other quadrants over the sum of both terms, is significantly lower for very old SR-BI KO mice (*, $P < 0.05$, t test) in both probe tests.

Table 1
Behavioral screen of SR-BI KO mice and WT littermates

Measurement	SR-BI KO, mean (SEM)	WT, mean (SEM)	P value
Body weight (g)	25.09 (.46)	24.79 (.37)	.62
Body length (cm)	18.61 (.16)	18.29 (.33)	.57
Body position	4.37 (.13)	4.29 (.14)	.68
Spontaneous activity	4.77 (.16)	4.68 (.21)	.73
Respiratory rate	4.3 (.17)	4.54 (.15)	.31
Transfer arousal	3.9 (.24)	4.21 (.26)	.37
Locomotion	3.9 (.43)	3.82 (.34)	.89
Palpebral closure	0	.15 (.15)	
Hair erection	.11 (.08)	.69 (.41)	.21
Pelvic elevation	3.44 (.15)	3.42 (.21)	.51
Tail elevation	2.44 (.18)	2.69 (.26)	.52
Finger approach	1.39 (.33)	1.69 (.47)	.62
Finger withdrawal	1.78 (.27)	2.23 (.28)	.48
Touch escape	.89 (.23)	.56 (.12)	.09
Hypotonic gait	.28 (.14)	.38 (.27)	.89
Positional passivity	4.44 (.66)	4.31 (.72)	.33
Visual placing	6.28 (.46)	6.15 (.61)	.39
Grip strength	4.94 (.36)	5.31 (.47)	.63
Body tone	5.06 (.17)	5.08 (.08)	.42
Ear reflex	1.89 (.36)	1.69 (.41)	.55
Corneal reflex	4.33 (.44)	3.15 (.41)	.09
Toe pinch	2.12 (.6)	3 (.88)	.13
Wire maneuver	1.39 (.45)	.6 (.46)	.38
Skin color	6.11 (.32)	5.08 (.35)	.11
Limb tone	3.18 (.19)	2.92 (.4)	.87
Abdominal tone	4.77 (.16)	5 (0)	.07
Irritability	2.28 (.46)	3.09 (.44)	.02
Salivation	.44 (.2)	.54 (.39)	.94
Provoked biting	1.11 (.31)	1.46 (.46)	.71
Tail pinch	.56 (.3)	.08 (.08)	.36
Righting reflex	.06 (.06)	0	
Negative geotaxis	.33 (.14)	.31 (.13)	.85
Vocalizing	.56 (.12)	.77 (.12)	.41

Note: The screen included 38 measurements which were scored with *ad hoc* scales (range 0–8), except body weight and length. Five measurements (tremors, bizarre movements, twitches, ataxic gait and aggression) gave null score for all animals, and are thus not listed in the table. The measurements do not show statistically significant differences between groups ($P > 0.05$, ANOVA), except for irritability ($P < 0.05$). SR-BI KO animals are less irritable than WT mice.