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Increased Neuronal Excitability, Synaptic Plasticity, and Learning in Aged Kvβ1.1 Knockout Mice

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Summary

Background: Advancing age is typically accompanied by deficits in learning and memory. These deficits occur independently of overt pathology and are often considered to be a part of "normal aging." At the neuronal level, normal aging is known to be associated with numerous cellular and molecular changes, which include a pronounced decrease in neuronal excitability and an altered induction in the threshold for synaptic plasticity. Because both of these mechanisms (neuronal excitability and synaptic plasticity) have been implicated as putative cellular substrates for learning and memory, it is reasonable to propose that age-related changes in these mechanisms may contribute to the general cognitive decline that occurs during aging.

Results: To further investigate the relationship between aging, learning and memory, neuronal excitability, and synaptic plasticity, we have carried out experiments with aged mice that lack the auxiliary potassium channel subunit Kvβ1.1. In aged mice, the deletion of the auxiliary potassium channel subunit Kvβ1.1 resulted in increased neuronal excitability, as measured by a decrease in the post-burst afterhyperpolarization. In addition, long-term potentiation (LTP) was more readily induced in aged Kvβ1.1 knockout mice. Finally, the aged Kvβ1.1 mutants outperformed age-matched controls in the hidden-platform version of the Morris water maze. Interestingly, the enhancements in excitability and learning were both sensitive to genetic background: The enhanced learning was only observed in a genetic background in which the mutants exhibited increased neuronal excitability.

Conclusions: Neuronal excitability is an important determinant of both synaptic plasticity and learning in aged subjects.

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Introduction

Cognitive impairments, especially deficits in learning and memory, are the hallmark of normal aging; they often occur in the absence of neuropathology such as that associated with Alzheimer's disease [1]. Because of the central role that it plays in mammalian learning and memory, the hippocampus is thought to be responsible, at least in part, for the age-related cognitive decline that occurs during normal aging [2]. This idea gains support from behavioral experiments demonstrating that aged humans [3], rats [4], and mice [5] perform poorly in tasks that require spatial learning strategies, suggesting age-related impairments in hippocampal function.

The exact cause of these age-related deficits in hippocampal-dependent learning and memory is not currently known. However, in recent years several promising hypotheses have emerged. Prominent among them is the calcium dysregulation hypothesis of brain aging and neurodegeneration. This hypothesis asserts that a number of age-related changes in neuronal function, as well as neuronal dysfunction associated with Alzheimer's disease (AD), may be the result of a dysregulation of the homeostasis of cytosolic free calcium (for multiple reviews, see [6]).

There is significant experimental evidence for an age-related elevation in intracellular free calcium in pyramidal neurons in the hippocampus. The most direct evidence comes from calcium imaging studies that demonstrate a marked increase, in response to prolonged synaptic stimulation, in intracellular calcium levels in neurons from aged animals [7]. This age-related increase in intracellular calcium is thought to have a number of functional consequences. In the hippocampus, one of the better-characterized changes secondary to the age-related increase in intracellular calcium is a decrease in postsynaptic neuronal excitability. This age-related decrease in neuronal excitability is manifested by an increase in calcium-activated potassium currents. These currents underlie the slow afterhyperpolarization (sAHP) that follows bursts of action potentials in hippocampal pyramidal neurons. This age-related increase in the post-burst AHP is correlated with decreased performance in hippocampal-dependent learning tasks, such as trace eye blink conditioning (for review, see [8]). In addition, pharmacological manipulations (e.g., nimodipine, an L-type Ca^{2+} channel antagonist) that decrease the AHP have been shown to decrease age-related learning deficits [9, 10].

Similarly, there is experimental evidence demonstrating an age-related alteration in the induction threshold for synaptic plasticity (for review, see [11]). Specifically, there is an increase in the induction threshold for synaptic potentiation in hippocampal slices taken from aged

animals. Furthermore, it has been suggested that these changes in induction threshold reflect changes in the post-burst AHP because pharmacological agents (nifedipine and apamin) known to decrease the post-burst AHP in aged animals shift the induction threshold for plasticity [11]. Taken collectively, the data outlined above suggest a possible link between the age-related alterations in intracellular calcium, the post-burst AHP, synaptic plasticity, and learning in aged animals.

To explore how excitability affects plasticity and learning in aged animals, we have carried out a series of experiments with aged mice whose neuronal excitability has been genetically enhanced by the targeted deletion of the potassium channel subunit Kvβ1.1 [12]. The Kvβ1.1 subunit confers fast (A-type) inactivation on otherwise noninactivating mammalian Kv1 K^+ channels, and the expression of this modulatory subunit is confined to discrete brain areas, including pyramidal neurons in the hippocampal area CA1 [13–15]. Our studies with the Kvβ1.1 knockout mice show that increasing neuronal excitability can facilitate long-term potentiation (LTP) induction and improve learning and memory in aged mice.

Results

Spike Broadening and Post-Burst AHP

Deletion of the Kvβ1.1 subunit decreases the cumulative spike broadening that normally occurs during repetitive spiking in CA1 pyramidal neurons of the mouse hippocampus [12]. To determine whether this change persists into old age, we compared the amount of spike broadening in aged (over 18 months old) Kvβ1.1 knockout mice with spike broadening in their wild-type littermates. Consistent with our previous findings with young mice [12], deletion of the Kvβ1.1 subunit decreased spike broadening in aged knockout animals when compared to their age-matched wild-type littermates (Figures 1A and 1B). The width of the first spike in the train was not different in mutant and wild-type mice (Figures 1C and 1D), which is also consistent with our previous findings.

In addition to decreasing spike broadening, deletion of the Kvβ1.1 subunit in young animals reduces the amplitude of the AHP that follows a burst of action potentials; this is presumably due to a decrease in calcium influx during the burst of action potentials [12]. To determine whether this alteration is maintained in aged mutant mice, we measured the post-burst AHP in two different ways. In both cases, a depolarizing current step was applied to the current clamp head stage to elicit spiking in the soma. In the first series of experiments, the current level was kept constant, and the duration of the pulse was successively increased (Figures 2A and 2B). In the second, the duration was fixed, and the current was adjusted to elicit at least five spikes (Figure 2C). In both cases the post-burst AHP was significantly smaller in Kvβ1.1 knockout mice than in wild-type animals. These results demonstrate that the spike broadening and post-burst AHP alterations seen in the young mutant mice persist into old age.

NEURON Simulations

Under voltage clamp conditions, neurons in the CA1 region of the hippocampus in Kvβ1.1 knockout mice exhibit significant alterations in an I_A-type macroscopic K^+ current [12]. In particular, there is a significant reduction in the rapidly inactivating portion of the current as well as an increase in the sustained outward current [12]. To further examine the relationship between alterations in voltage-sensitive K^+ currents and the reduction in spike broadening and post-burst AHP, we carried out simulations with the NEURON simulation environment with a reconstructed CA1 cell [16] (Figure 3A; see Experimental Procedures). The inactivation exponential (h^3) of the I_A in the model was set to 0 so that it mimicked the reduction in the rapidly inactivating portion of the macroscopic potassium currents recorded in vitro. The contribution of the delayed rectifier K^+ current in the model was doubled so that it mimicked the observed increase in the sustained current (see Figure 2 in [12]). Consistent with data obtained under current clamp conditions ([12] and current study), decreasing the inactivation of I_A and increasing the sustained current in the model had little effect on the shape of a single spike (Figure 3B) but significantly reduced spike broadening (Figure 3D). In addition, the resulting post-burst AHP in simulations run under these conditions was significantly reduced (Figure 3E). Data from these simulations support the hypothesis that alterations in A-type K^+ currents can contribute to spike broadening changes, which in turn decrease the post-burst AHP.

Deletion of Kvβ1.1 Reduces the Threshold for LTP Induction

To determine the impact of altering neuronal excitability on synaptic plasticity, we examined what effect deleting the Kvβ1.1 subunit in aged mice had on LTP that was induced in hippocampal area CA1 by several different induction protocols. In the first series of experiments, a perithreshold tetanus (2 theta burst) was used (see Experimental Procedures). Consistently with previous reports with similar stimulation protocols [17–20], slices prepared from aged wild-type mice exhibited a minimal amount of sustained LTP (Figure 4A). On the other hand, in slices prepared from aged Kvβ1.1 knockout mice, the same stimulation protocol produced significant LTP that lasted for at least 40 min after the tetanus (Figure 4A). In a subset of slices that received the 2 theta tetanus, a second, much more robust stimulation (10 theta; see Experimental Procedures) was delivered 40–50 min after the first tetanus (Figure 4B). Slices prepared from both aged wild-type and aged knockout mice exhibited equivalent levels of LTP, demonstrating that the LTP enhancement in the mutant mice was specific to the minimal stimulation protocol.

To address the hypothesis that the Kvβ1.1 null mutation lowered the threshold for LTP induction, we carried out experiments with a stimulation protocol consisting of 900 pulses at 1 Hz. After a transient depression, the slope of the field excitatory postsynaptic potentials (fEPSP) returned to baseline in slices prepared from aged wild-type mice. In contrast, this stimulation protocol resulted in significant potentiation in slices from Kvβ1.1 knockout mice (Figure 4C), confirming that this mutation altered the threshold for LTP induction in aged mice. These results are consistent with previous findings

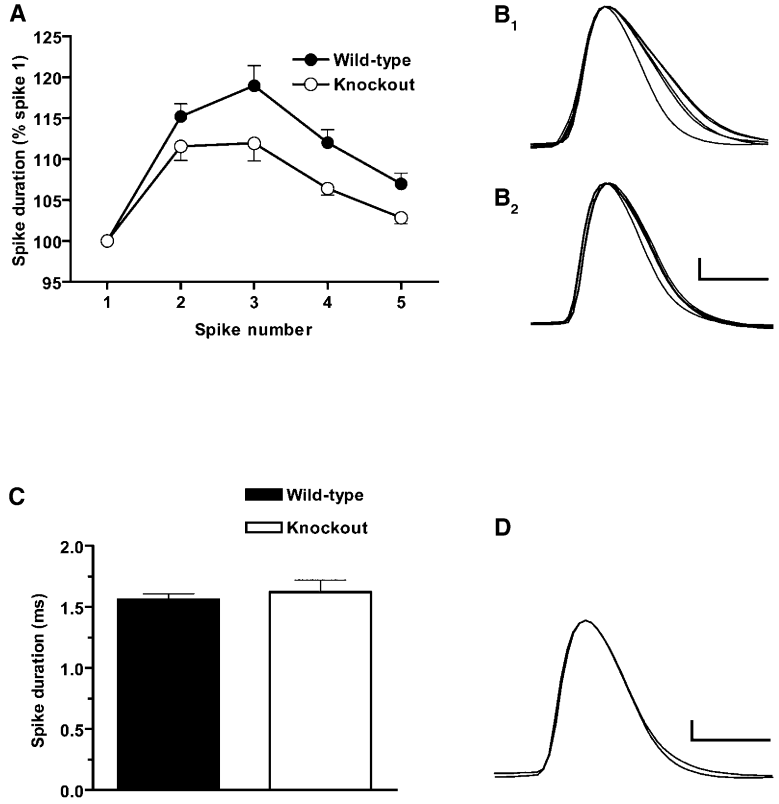

Figure 1. Deletion of Kvβ1.1 Decreases Spike Broadening in Aged Animals

Spike broadening was measured in aged wild-type mice (15 cells/7 animals) and Kvβ1.1 knockout mice (20 cells/6 animals) in response to a 0.20 nA current step from the resting membrane potential. In all figures, error bars represent the mean ± the standard error of the mean.

(A) Group data for the first five spikes in the train (data normalized to the first spike). Deletion of the Kvβ1.1 subunit reduced the spike width for spikes 3, 4, and 5 ($p = 0.057$, 0.013, and 0.024, respectively, compared to wild-type mice).

(B) Representative recordings from wild-type (B_1) and Kvβ1.1 knockout (B_2) mice.

(C and D) There was no difference in the width of the first spike in the train ($p > 0.05$). The scale bars represent 10 mV/1 ms.

that manipulations known to reverse the age-related increase in the post-burst AHP can produce increases in synaptic efficacy in response to a variety of stimuli [21].

Morris Water Maze

To examine the cognitive effects of genetically reducing the post-burst AHP in aged mice, we trained aged wild-type and aged Kvβ1.1 knockout mice in the hidden platform version of the Morris water maze (see Experimental Procedures). Deletion of Kvβ1.1 did not affect average escape latency—a crude measure of spatial (and non-spatial) learning—during training (Figure 5A). After 5 days of training, mice were given a single probe trial, in which the hidden platform was removed. Although the percentage of time spent in the training quadrant was significantly greater than 25% in both groups, the Kvβ1.1

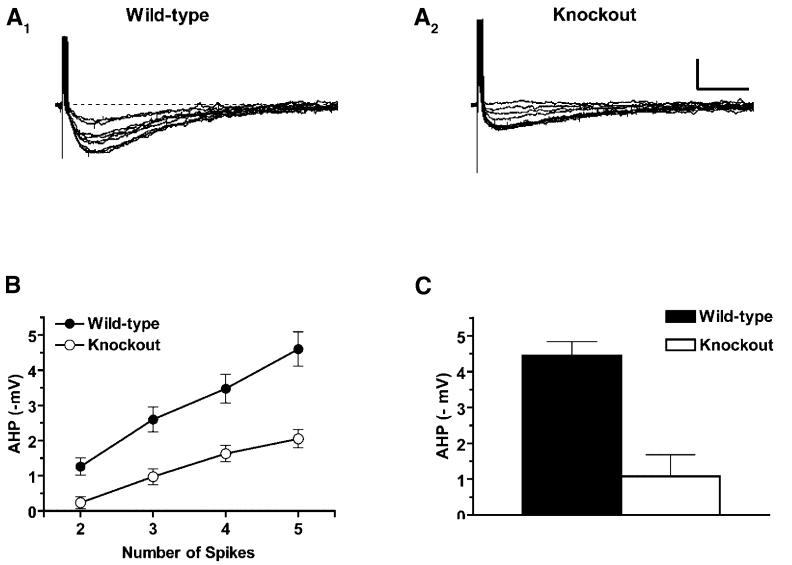

Figure 2. Deletion of Kvβ1.1 Decreases Post-Burst AHP Amplitude in Aged Animals

The post-burst AHP was measured in two different ways.

(A and B) In the first series of experiments, cells were held at −55 mV, and a depolarizing step (0.2 nA) was injected with an increasing duration (for 10–100 ms in 10 ms increments). Traces in (A) are representative recordings (average of five sweeps per time interval) for both genotypes. The scale bar represents 3 mV/500 ms. The group data for this experiment is presented in (B), which plots the average AHP that followed bursts of 2–5 spikes (measured at the maximal AHP) for wild-type (15 cells/5 animals) and Kvβ1.1 knockout (20 cell/5 animals) mice. The resulting AHP was smaller in the mutant mice for all spike numbers ($p < 0.01$).

(C) In the second series of experiments, cells were held at −55 mV, and the AHP was measured after injection of a current pulse of fixed duration (either 50 or 100 ms) and of sufficient amplitude to elicit at least five spikes. The post-burst AHP (as measured 200 ms after the end of the current step) was significantly reduced in the aged knockout mice when compared to that in age-matched wild-type mice (1.08 ± 0.60mV [12 cells/4 animals] versus 4.45 ± 0.38 mV [20 cells/6 animals], respectively; $p < 0.01$).

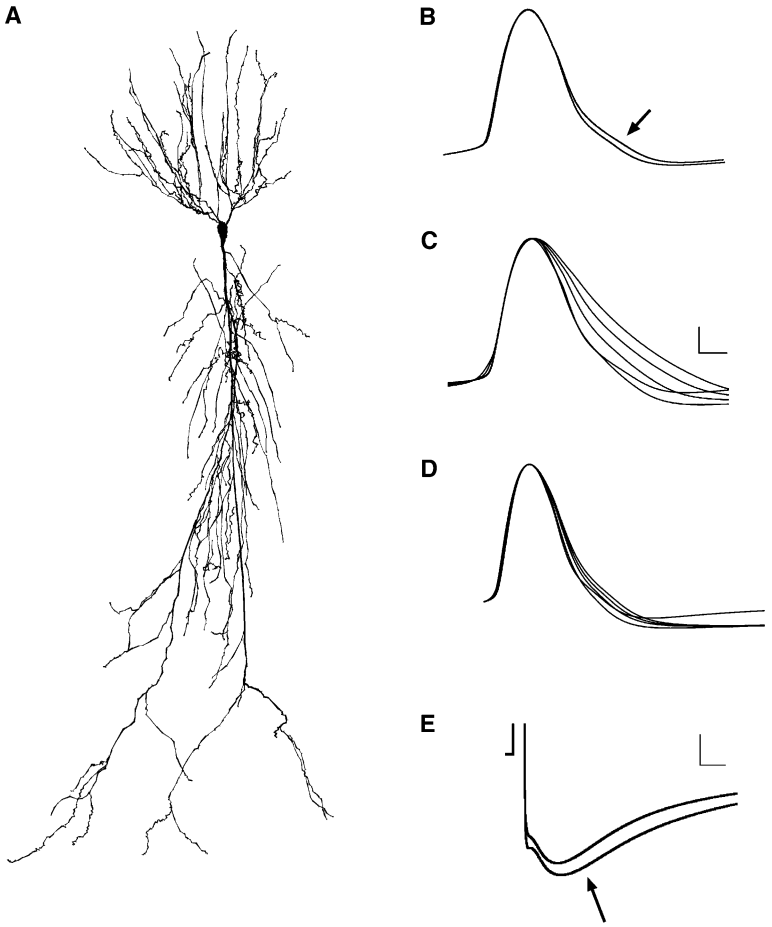

Figure 3. NEURON Simulations

(A) Two-dimensional reconstruction of CA1 pyramidal neuron n184 (see Experimental Procedures).

(B) First action potential in train under normal conditions (arrow) and with decreased inactivation and increased sustained K^+ currents (see text).

(C) Action potentials 1–5 (superimposed) under normal conditions.

(D) Action potentials 1–5 (superimposed) with modified K^+ currents.

(E) Post-burst AHP under normal conditions (arrow) and with modified currents. The scale bar represents 13 mV/5 ms (B, C, and D) and 2 mV/200 ms (E).

mutant mice spent on average more time in the training quadrant than the wild-type mice did (Figure 5B). In fact, almost all (9 out of 11) of the aged knockout mice spent over 45% of the probe trial searching in the training quadrant. This is in marked contrast to the aged wild-type mice, in which only 2 out of 11 subjects performed at this level (Figure 5C). Performance on the visible platform was the same for both groups, as was swim speed during the probe trial, suggesting that motor or motivation/visual impairments do not account for the difference in water maze performance between the two groups (Figure 5D). This enhanced performance in this task was specific to the aged Kvβ1.1 mutant mice. Young adult mutants and wild-type mice (3–5 months of age) performed at nearly identical levels (% time in target quadrant: wild-type $= 54.5 \pm 4.3$, knockout $= 49.4 \pm 5.8$; $t = .71$, $p > 0.05$; $n = 8$ for both groups; see Figure S1, available with this article's Supplemental Data online).

Impact of Genetic Background on Neuronal Excitability and Cognition

There is currently a large body of experimental evidence demonstrating the impact of genetic background on many phenotypes, including those for learning in mutant mice [22]. Not surprisingly, there is evidence that genetic background plays a similar role in cognitive senescence (for review, see [23]). The experiments described above were carried out with mice in an N4 genetic background (see Experimental Procedures). To examine the possible interaction between deletion of the Kvβ1.1 subunit and genetic background, we carried out a series of experiments similar to those described above in mice that were in a C57Bl/6 background (N7/8; see Experimental Procedures). In contrast to the case of the N4 background, the deletion of the Kvβ1.1 subunit in the N7/8 background failed to produce a decrease in the post-burst AHP (Figures 6A and 6B). Accordingly, in this background, deletion of the Kvβ1.1 subunit does not improve performance in the hidden platform version of the Morris water maze (Figures 6C and 6D). After 5 days of training, both wild-type and Kvβ1.1 knockout mice perform at chance during the probe trial. A probe trial given after an additional 5 days of training shows that although both groups can learn the task, their performances were indistinguishable; both groups spent equivalent percentages of time searching in the training quadrant (Figures 6E and 6F). Thus, in the N7/8 genetic background, the deletion of Kvβ1.1 failed both to decrease the post-burst AHP and to improve learning; this result is consistent with the hypothesis that the decrease in the post-burst AHP in N4 Kvβ1.1 knockout mice accounts for their improved hippocampal learning. Furthermore, these findings also illustrate how the wealth of genetic variability between mutant mouse strains can be exploited to

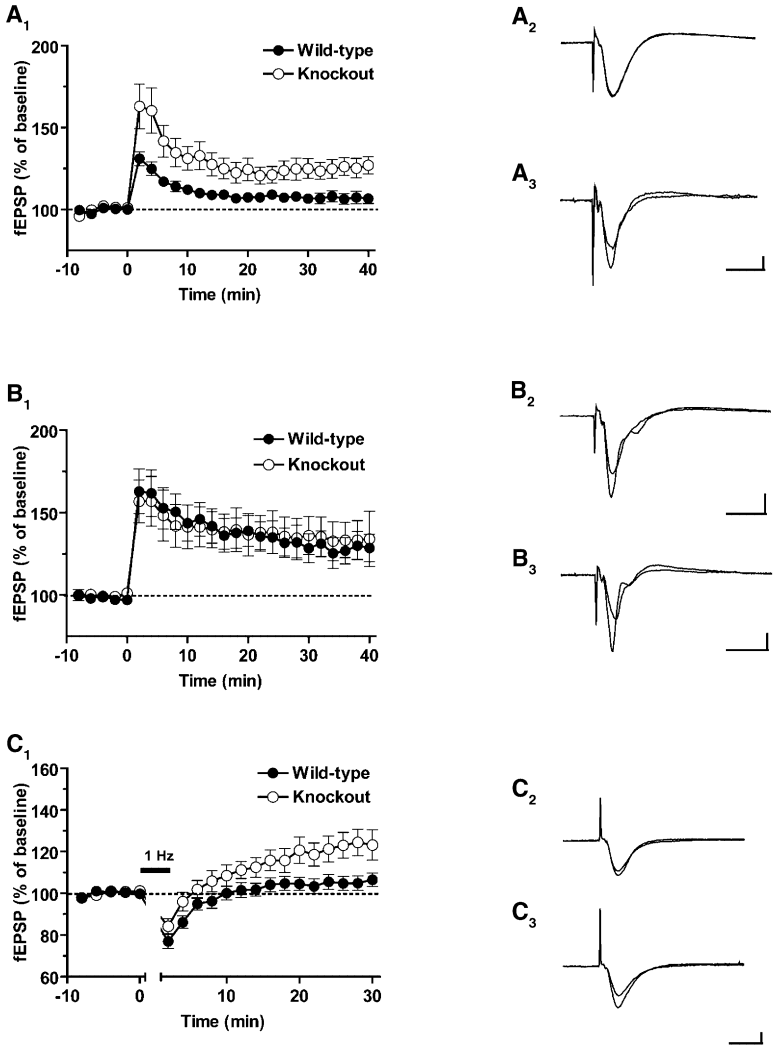

Figure 4. Deletion of Kvβ1.1 Enhances Synaptic Plasticity in Aged Mice

(A_1) Plot of field potential EPSP slope as a percentage of the baseline (first 10 min). Slices prepared from wild-type mice (14 slices/9 animals) and Kvβ1.1 knockout mice (9 slices/6 animals) were subjected to a theta burst stimulation protocol in which 2 theta bursts were delivered after 10 min of baseline recording (see Experimental Procedures). Although slices from wild-type mice exhibited a modest level of potentiation 30–40 min after the tetanus ($t = 2.17$; $p = 0.049$ compared to 100%), this potentiation was significantly less than the potentiation that resulted from the same tetanus in slices from the Kvβ1.1 knockout mice ($t = 2.84$; $p < 0.01$).

(A_2 and A_3) Representative recordings from slices prepared from wild-type and knockout mice (respectively). Traces presented here (and in [B] and [C]) are the average of five pre-tetanus sweeps (10 min of baseline) compared to recordings made during the last 10 min of the experiment (average of six sweeps).

(B) In a subset of slices from knockout mice (5 slices/5 animals) and wild-type mice (6 slices/5 animals), an additional tetanus of ten theta bursts was delivered 40–50 min after the first tetanus. This stimulation protocol produced an equivalent amount of potentiation in slices prepared from both groups ($t = 0.36$; $p > 0.05$).

(B_2 and B_3) Representative recordings from slices prepared from wild-type and knockout mice, respectively.

(C_1) In a separate series of experiments, a low-frequency (1 Hz) tetanus was delivered for 15 min (indicated by the black bar). Slices prepared from knockout mice (14 slices/8 animals) exhibited a transient depression, which was followed by substantial LTP 20–30 min after the tetanus ($t = 3.49$; $p < 0.01$ compared to 100%). On the other hand, in slices from wild-type mice (9 slices/5 animals) no potentiation was observed ($t = 1.46$; $p > 0.05$ compared to 100%).

(C_2 and C_3) Representative recordings from slices prepared from wild-type and knockout mice (respectively). The scale bars for all traces represent 200 μV/10 ms.

investigate the connections between biological phenomena.

Discussion

Previous findings have suggested that decreases in neuronal excitability contribute to age-related cognitive decline [24–26]. Here, we show that aged Kvβ1.1 mutants, with enhanced neuronal excitability, outperformed age-matched controls in the hidden-platform version of the Morris water maze. In addition, hippocampal slices prepared from aged Kvβ1.1 mutants showed a marked decrease in the threshold for LTP induction compared to those taken from control mice. These results show that neuronal excitability is an important determinant of both synaptic plasticity and learning in aged subjects.

Deletion of the Kvβ1.1 gene reduces cumulative spike broadening and the post-burst AHP in young mice [12], and now we show that this alteration persists into old age (Figure 1 and 2). The modeling data presented here (Figure 3; see also reference 27) provides an explanation for how alterations in A-type K^+ currents can affect the post-burst AHP: a decrease in the inactivation of the A-type K^+ current, coupled with an increase in the delayed rectifier, decreases cumulative spike broadening, which in turn decreases calcium influx [27, 28] and ultimately leads to a reduction in the post-burst AHP.

Since there is evidence that genetic factors can play a significant role in learning and specifically in age-related cognitive decline in rodents, we used genetic background to study the relationship between neuronal excitability and cognitive decline in aged animals. Reducing the post-burst AHP in aged animals in the N4 genetic background improved the performance in the hidden-platform version of the Morris water maze (Figure 5). In the N7/8 genetic background, however, the post-burst AHP was similar in mutants and controls. Importantly, their behavioral performances also did not differ. The lack of a difference between mutants and controls in the N7/8 genetic background was not due to either a

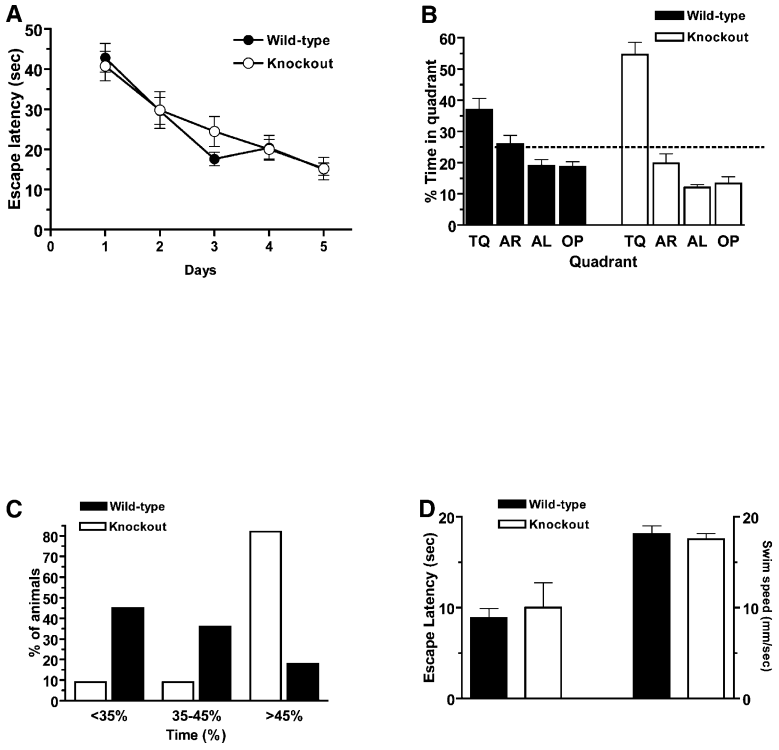

Figure 5. Water Maze Performance Is Significantly Enhanced in Aged Kvβ1.1 Knockout Mice

(A) Average escape latencies (in seconds) during training are plotted for days 1–5. There was no significant difference between knockout ($n = 11$) and wild-type ($n = 11$) mice.

(B) At the end of training, a probe trial was conducted in which the platform was removed. The average percentages of time spent in the various quadrants (TQ, training; AR, adjacent right; AL, adjacent left; OP, opposite) are plotted for both genotypes. Both knockout and wild-type mice spent a significant amount of time in the TQ when compared to chance (dashed line; $p < 0.01$ for both groups). However, knockout mice spent a greater percentage of time in the TQ than wild-type mice ($t = 3.25$; $p < 0.01$).

(C) Distribution of performance during probe trial for wild-type and knockout mice. Performance is plotted as percentage of animals in each genotype that spent less than 35%, 35%–45%, or more than 45% of the probe trial in the TQ. The majority (9/11) of knockout mice spent more than 45% of the probe trial in the TQ, whereas only 2 out of the 11 wild-type animals performed at this level.

(D) On the left side of the graph, the average escape latency during visible platform test (two trials averaged for each animal after hidden platform training) is plotted for wild-type and knockout mice. The average swim speed during the probe trial is plotted on the right side of the graph. There was no significant difference between the two groups for either measure ($p > 0.05$).

floor or ceiling effect, since with extended training both groups showed similar intermediary performance levels (Figure 6). These results demonstrate that in the absence of the increased neuronal excitability the Kvβ1.1 mutation does not improve learning.

At present, it is not known what gene or genes are responsible for the divergent phenotypes seen in the two different genetic backgrounds. One possibility is that the reduced AHP in the N4 background (as well as in the original F2 background in [12]) is the result of a "flanking" gene effect. In this scenario, a linkage disequilibrium arises because the original chimeras were generated from 129Sve stem cells, and limited backcrossing into the C57BL/6 strain would result in a null mutation flanked by two 129-derived alleles, whereas the wild-type litter mates would have C57BL/6 alleles at those same loci [29]. To explore this possibility, we have generated so-called reverse F2 (RF2) mice. This was accomplished by crossing heterozygous Kvβ1.1 (N10) with 129Sve mice and then intercrossing the subsequent heterozygous offspring (nonsiblings) to generate Kvβ1.1 knockout and wild-type mice. This breeding scheme would be expected to result in homozygous mutants and wild-type mice with an overall genetic background resembling the original F2 hybrids, but with a flanking region surrounding the Kvβ1.1 gene that is predominantly homozygous for 129Sve alleles [29]. As was the case in both the N4 background and the original F2 hybrids, deletion of Kvβ1.1 produced a significant reduction in the post-burst AHP when compared to wild-type littermates sAHP (4.2 ± .45 mV and 6.5 ± .49 mV, respectively; $t = 3.32$, $p = 0.0019$; see Figure S2). On the grounds of these data, we have ruled out the possibility of a flanking gene effect.

Finally, in the C57BL/6 background, there may be an additional gene or genes that act to modify the magnitude of the AHP reduction that results from the deletion of the Kvβ1.1 auxiliary subunit. To our knowledge, there are no reports that identify genes modifying potassium channel function or expression. However, a gene has recently been identified and mapped that is specific to the C57BL/6 background and modifies the severity of the phenotype in mice with mutations in the gene that encodes the $Na_V1.6$ sodium channel [30]. The existence of a similar modifier gene or genes would be consistent with the data presented here, and may help to explain the individual differences in cognitive abilities among aged populations.

It has been proposed that changes in neuronal excitability, such as decreases in the post-burst AHP, affect LTP [11], an experimental model of the synaptic changes thought to underlie learning and memory [31]. Indeed, in aged mice, deletion of the Kvβ1.1 subunit specifically decreases the threshold for induction of LTP (Figure 4): Minimal stimulation reveals a difference in LTP levels between mutants and controls, whereas strong stimulation protocols occlude this difference. It is interesting to note that the minimal stimulation protocol (2 theta) used to study LTP relies on two brief bouts of stimulation with a 200 ms pause between them. This delay places the second burst near the maximal level of hyperpolarization produced by the first bout of stimulation. Therefore, the enhanced LTP in the slices prepared from aged Kvβ1.1 mutants may be due to an increase in the amount of postsynaptic depolarization during the tetanus. In fact, mutants and controls express comparable levels of potentiation when a more extensive tetanus (10 theta) is used (Figure 4B). It is possible that because the addi-

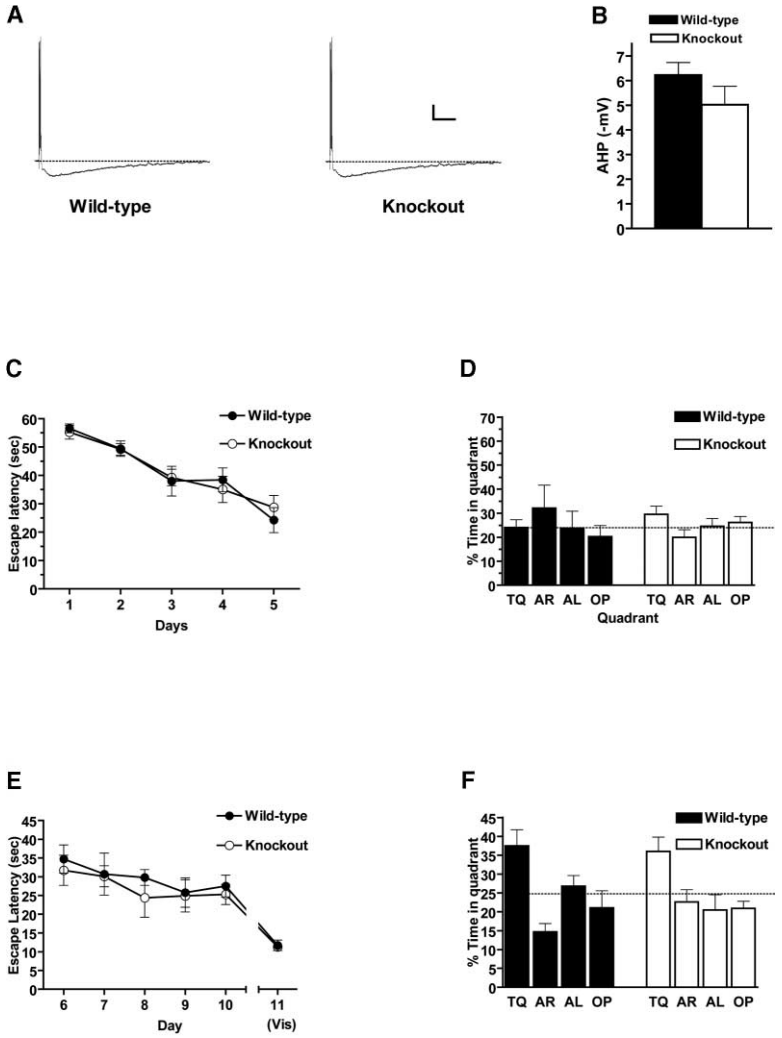

Figure 6. Impact of Genetic Background on Age-Related Changes in Neuronal Excitability and Cognition

All data presented in this figure are from animals in the N7/8 genetic background (see Experimental Procedures).

(A) Cells were held at 5 mV below action potential threshold, and the post-burst AHP was measured 200 ms after the termination of a 50 ms current pulse of sufficient amplitude to elicit at least five spikes. The scale bar represents 10 mV/500 ms.

(B) The post-burst AHP in Kvβ1.1 knockout mice (13 cells/5 animals) was modestly reduced when compared to that in wild-type mice (11 cells/4 animals). However, this trend was not significant ($t = 1.26$; $p = 0.25$).

(C) Average escape latencies (in seconds) during training are plotted for days 1–5. There was no significant difference between wild-type ($n = 7$) and knockout ($n = 9$) mice.

(D) At the end of training day 5, a probe trial was conducted in which the platform was removed. The average percentage of time spent in the various quadrants is plotted for both genotypes. Both wild-type and knockout mice failed to exhibit a selective search strategy. In fact, mice in both groups performed essentially at chance with respect to 25% time spent in the TQ ($t = -0.31$ and $t = 0.85$ for wild-type and knockout mice, respectively, and $p > 0.05$ for both groups). Furthermore, there was no significant difference between the two groups ($t = -1.14$; $p > 0.05$).

(E and F) Animals were further trained for 5 more days (6 trials a day). There was no decrease in escape latency with further training, nor was there a significant difference between the two groups. Similarly, there was no significant difference between the groups during the visible-platform test (see Experimental Procedures). (F) After an additional 5 days of training, a probe trial was conducted on day 10 after the last training trail. Both wild-type and knockout mice exhibited a selective search strategy. Both groups spent significantly more time in the TQ than would be predicted by chance ($t = 2.89$, $p = 0.028$ and $t = 2.85$, $p = 0.021$ for wild-type and knockout mice, respectively). However, there was no significant difference between the two groups ($t = 0.260$; $p > 0.05$).

tional bursts in the 10 theta protocol provide strong cumulative depolarization, the difference in neuronal excitability between mutants and controls is not a factor in determining the levels of LTP.

Previous reports have suggested that slices prepared from aged rats have a lower threshold for the induction of LTD when they are compared to slices prepared from adults [32, 33] (but see also [34]). Our inability to induce LTD in aged wild-type slices may be due to the fact that we used mice in our experiments (cf. rats as in [32] and [33]) or that our artificial cerebral spinal fluid (aCSF) contained a different ratio of calcium/magnesium, as has been previously suggested [35]. Nonetheless, our results support the idea that decreasing the post-burst AHP alters the threshold for induction of LTP.

One reasonable hypothesis that could account for our observations is that the learning impairments that accompany aging are caused by an increase in intracellular free calcium, which produces an increase in the AHP that ultimately results in a decrease in neuronal excitability. This decrease in neuronal excitability could in turn increase the threshold for LTP induction, thereby altering the ability of the hippocampus to encode information.

Conclusions

The results presented here show that neuronal excitability is an important determinant of both synaptic plasticity and learning and memory in aged subjects. These findings indicate that interactions between neuronal excitability and synaptic plasticity play a role in learning, and they suggest that manipulations of either of these two parameters could be used to ameliorate age-related cognitive decline.

Experimental Procedures

Mice

Aged mice used in both electrophysiological and behavioral experiments were 18 months or older. In all experiments, comparisons were made between mutants and wild-type littermates that were age-matched. Animals in the N4 background were F2 progeny from heterozygotes crossed four consecutive times into the C57BL/6 background. Animals in the N7/8 background (Figure 6) were F2 progeny from heterozygotes crossed 7–8 consecutive times into

the C57BL/6 background. All behavioral and electrophysiological experiments were conducted with the experimenter blind to the genotype of the mice. All experiments were conducted with the approval of the UCLA Animal Research Committee of the Chancellor's Office of Protection of Research Subjects and under continuous supervision of the campus veterinarian.

Electrophysiology

Animals were placed under deep halothane anesthesia prior to decapitation. Whole-brain (minus the cerebellum) sagittal sections (400 μm) were made, and rapid microdissection of the hippocampi was completed in ice-cold aCSF that was saturated with a mixture of 95% O_2 and 5% CO_2 and contained the following (in mM): 120 NaCl, 20 $NaHCO_3$, 3.5 KCl, 1.25 NaH_2PO_4, 2.5 $CaCl_2$, 1.3 $MgSO_4$, and 10 d-glucose.

Hippocampal slices were then transferred to a holding chamber to rest at room temperature for at least 1 hr prior to the start of the experiment. All recordings were made in a submerged chamber perfused continuously with oxygenated aCSF at a rate of $\sim$2 ml/min at 31°C.

All current clamp recordings were made in CA1 pyramidal neurons with an Axoclamp 2B amplifier (Axon Instruments, Foster City, CA) in bridge mode. In the N4 background, whole-cell patch electrodes were used (blind method); they contained (in mM): 135 potassium gluconate, 5 Hepes, 2 Mg-ATP, 5 $MgCl_2$, 0.3 GTP, and 0.05 EGTA. In these experiments, we used electrodes with fairly high resistance (7–11 MΩ), and recordings were made within 1 min of rupturing to minimize the "run-up" of the post-burst AHP [36, 37].

The later recordings done with mice in the N7/8 background were made with sharp microelectrodes (70–110 MΩ) containing 3 M potassium acetate. Although the absolute magnitude of the post-burst AHP was different between the two recording methods, the failure to find a difference between the two genotypes in the N7/8 background persisted regardless of recording method used (data not shown). All data were acquired and analyzed with pClamp 7.0 (Axon Instruments). Spike broadening data were further analyzed with Mini Analysis (Synaptosoft, Decatur, GA). Spike width was determined at 20 mV above threshold (as determined with the third derivative). Statistical comparisons were made with the number of animals. When multiple cells within an animal were used, they were averaged to a single value for each animal. Statistical comparisons were made with Student's t tests or Fisher's PLSD tests when making multiple comparisons between groups.

Extracellular recordings of fEPSP were made in the stratum radiatum of CA1 with Pt/Ir electrodes (FHC, Bowdoinham, ME) with resistance of $\sim$2 MΩ. Field EPSPs were evoked by stimulating the Schaffer afferent fibers with bipolar platinum electrodes. All test stimuli and tetanus pulses were 100 μs in duration and 1/2–2/3 maximal stimulation strength. Long-term potentiation was induced with two different induction protocols. In the first series of experiments (Figures 4A and 4B), LTP was induced with a "theta burst" protocol, in which two or ten bursts were delivered with an inter-burst interval of 200 ms. Each burst consisted of four pulses at 100 Hz. In the second series of experiments (Figure 4C), low frequency stimulation was used (1 Hz for 15 min—900 pulses) to induce synaptic plasticity. Statistical comparisons were made with the number of slices. Statistical comparisons were made between groups with Student's t tests on the last 10 min of data (averaged).

Modeling

Modeling data were generated with the NEURON simulation environment [38] run on a Gateway Solo 9500 laptop computer with Windows 98 at 900 MHz with a time step of 1 μs (Figures 3B–3D) and 10 μs (Figure 3E). Simulations utilized a modified version of the Borg-Graham working model [39, 40]. The working model was extended to a reconstructed CA1 cell [16] (cell n184) acquired from the Duke/Southampton archive (http://www.cns.soton.ac.uk) and converted to NEURON hoc code with CVAPP (Robert Cannon; http://www.compneuro.org). All active conductances were confined to the soma, with the dendrites as passive structures. Passive properties of the cell were as follows: $R_a = 200\ \Omega$ cm; $C_m = 0.7\ \mu F/cm^2$; and $R_m = 25$ and 40 k $\Omega\ cm^{-2}$ for the soma and dendrites, respectively. Five spikes were elicited by a 0.47 nA, 50 ms pulse applied to the cell soma. Spikes presented in Figure 3 are scaled, superimposed, and peak aligned.

Morris Water Maze

Spatial learning was assessed with the hidden platform version of the Morris water maze [41], as described previously [42]. Briefly, animals were trained with six trials (in blocks of two trials, 1 min inter-trial intervals and 1 hr inter-block intervals) per day for 5–10 days. Acquisition data are presented as the group averages, in which escape latencies across all six trials in a single day were averaged for each animal so that each animal contributed a single latency score for each of the 5 days. At the end of the training, the mice received a probe trial with the platform removed from the pool. On the day following the final training/probe trial, animals received two additional trials, in which the platform was clearly marked (visible platform test). Escape latencies for both trials during the visible-platform test are averaged for each animal. Statistical comparisons were made between groups (% time in training quadrant) with Student's t tests. In addition, the percentage of time spent in the training quadrant was compared to that of a chance performance (25%) with a single group comparison. A selective search strategy was indicated if animals performed significantly above chance.

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