Reduced K^+ Channel Inactivation, Spike Broadening, and After-Hyperpolarization in Kvβ1.1-Deficient Mice with Impaired Learning

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Abstract

A-type K^+ channels are known to regulate neuronal firing, but their role in repetitive firing and learning in mammals is not well characterized. To determine the contribution of the auxiliary K^+ channel subunit Kvβ1.1 to A-type K^+ currents and to study the physiological role of A-type K^+ channels in repetitive firing and learning, we deleted the Kvβ1.1 gene in mice. The loss of Kvβ1.1 resulted in a reduced K^+ current inactivation in hippocampal CA1 pyramidal neurons. Furthermore, in the mutant neurons, frequency-dependent spike broadening and the slow afterhyperpolarization (sAHP) were reduced. This suggests that Kvβ1.1-dependent A-type K^+ channels contribute to frequency-dependent spike broadening and may regulate the sAHP by controlling Ca^{2+} influx during action potentials. The Kvβ1.1-deficient mice showed normal synaptic plasticity but were impaired in the learning of a water maze test and in the social transmission of food preference task, indicating that the Kvβ1.1 subunit contributes to certain types of learning and memory.

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Introduction

Fast-inactivating voltage-gated K^+ channels (A-type K^+ channels) are prominent in neurons throughout the animal kingdom (for review, see Rudy 1988). A-type K^+ (I_A) currents contribute to repolarization of the action potential (Storm 1987; Bardoni and Belluzzi 1993), and because of their characteristic fast inactivation, they can be central regulators for neuronal firing. For example, I_A currents gate action-potential propagation in axons (Debanne et al. 1997) and back propagation in dendrites (Hoffman et al. 1997). In addition, it was suggested that the inactivation of I_A currents contributes to spike broadening during repetitive firing (frequency-dependent spike broadening; Ma and Koester 1996; for review, see Roeper and Pongs 1996). A-type K^+ channel function has also been implicated in learning and memory. Thus, in *Hermissenda*, conditioning of phototactic behavior correlates with a reduction of I_A currents in photoreceptors, and the effect was found to be mediated by neuromodulator-activated second-messenger systems (for review, see Alkon 1984; Crow 1988). In *Drosophila*, a genetic alteration of *Shaker* A-type K^+ channel inactivation behavior resulted in learning impairments in olfactory conditioning (Cowan and Siegel 1986). In addition to A-type K^+ channels, other K^+ channels have been implicated in learning in invertebrates (for review, see Byrne et al. 1991) and mammals (Woody et al. 1991; Meiri et al. 1997).

Shaker-related voltage-gated K^+ (Kv) channels

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are complexes of pore-forming α-subunits and auxiliary β-subunits (for review, see Jan and Jan 1997). The recently discovered auxiliary Kvβ1 subunits (for nomenclature, see England et al. 1995a) confer fast inactivation on the otherwise noninactivating Kv1.1, Kv1.2, and Kv1.5 channels (Rettig et al. 1994; England et al. 1995a,b; Majumder et al. 1995; McCormack et al. 1995; Morales et al. 1995; Heinemann et al. 1996; Levin et al. 1996; Nakahira et al. 1996; Stephens et al. 1996; Accili et al. 1997). The Kvβ1 gene gives rise to three alternatively spliced isoforms, Kvβ1.1–Kvβ1.3 (Rettig et al. 1994; England et al. 1995a,b; Majumder et al. 1995; Morales et al. 1995; Leicher et al. 1996). These isoforms differ in their amino-terminal sequences and have different expression patterns. Kvβ1.2 and Kvβ1.3 are preferentially expressed in heart (England et al. 1995a,b; Morales et al. 1995), whereas Kvβ1.1 expression is restricted to the brain (Rettig et al. 1994). In brain, Kvβ1.1 is highly expressed in the hippocampal CA1 region and in striatum (Rettig et al. 1994; Rhodes et al. 1996; Butler et al. 1998).

In addition to the proposed function in inactivation of Kv1 channels, it has been suggested that the Kvβ1 subunits have a chaperone-like function, because they promote K^+ channel surface expression in heterologous expression systems (Shi et al. 1996; Accili et al. 1997).

To test the role of A-type K^+ channels in repetitive firing as well as mammalian learning, we decided to manipulate these in mice. Because the physiological study of A-type K^+ channels has been hampered by the lack of specific pharmacological tools, we used gene targeting. A-type K^+ channels can be disrupted by targeting either the pore-forming α- or the auxiliary β-subunits. Because the loss of A-type K^+ channel function can result in seizures (e.g., Gandolfo et al. 1989), we targeted the Kvβ1.1 isoform. The loss of Kvβ1.1 caused a reduction in K^+ current inactivation in hippocampal CA1 pyramidal neurons. This change in K^+ current inactivation was accompanied by a reduction of frequency-dependent spike broadening and attenuation of the slow afterhyperpolarization (sAHP), without any obvious change in synaptic plasticity. Furthermore, the loss of Kvβ1.1 led to abnormalities in the learning of a water maze test and in the social transmission of a food preference task.

Materials and Methods

GENERATION OF Kvβ1.1-DEFICIENT MICE

For cloning the Kvβ1.1 targeting construct, a genomic clone was isolated from a 129/Sv library, and the position of the first coding exon for the Kvβ1.1 isoform was mapped. The PGKneobpA cassette (*neo*; Soriano et al. 1991) was cloned into the *Srf*I site of the 3.5-kb genomic *Sac*I-fragment (first step). The *neo* was inserted into the twenty-fifth codon in the same transcriptional orientation as the Kvβ1 gene. In the second cloning step, the 7.5-kb genomic *Sac*I fragment was cloned upstream of *neo* by replacing a 43-bp *Srf*I–*Sac*I fragment. R1 embryonic stem (ES) cells (Nagy et al. 1993) were transfected with the *Sal*I-linearized Kvβ1.1 targeting construct. After selection with G418, 23 out of 59 clones were identified and characterized as proper targeted clones via Southern blot analyses (also no additional *neo* integrations observed). Chimeras were generated by injection of cells from these targeted clones into blastocysts. The male chimeras were mated with C57BL/6J females, and heterozygous mutants deriving from one targeted clone were intercrossed to obtain homozygous mutants. If not mentioned otherwise, the mice were studied in the 129B6F2 background. The Cold Spring Harbor Laboratory animal facility is fully accredited by the American Association for the Accreditation of Laboratory Animal Care, and the animals are maintained in accordance with the Animal Welfare Act and the Department of Health and Human Services (DHHS) guide.

MOLECULAR BIOLOGICAL ANALYSIS

A Kvβ1.1-specific probe (nucleotides 139–384, GenBank accession no. X83127) was generated by PCR and used for high-stringency hybridization of Northern blots with mRNA from adult mutant and wild-type brain. The control hybridization was performed with an actin probe (Clontech, no. 7764-1). Membrane protein from hippocampus, striatum, olfactory bulb, and total brain was isolated (Gordon-Weeks 1987) for immunoblot analysis. Affinity-purified polyclonal antibodies against Kvβ1.1 (Rhodes et al. 1996), or Kvβ1/Kvβ2 (see below), or Kv1.1 (Veh et al. 1995), or Kv1.2 (Veh et al. 1995), or Kv1.4 (Veh et al. 1995), or Kv1.5 (O. Pongs, unpubl.) were used. Antigen–antibody complexes were visualized with an ECL detection kit (Amersham). The anit-Kvβ antibodies were generated as follows: Rabbit polyclonal sera were raised against a glutathionyl-*S*-transferase Kvβ1 fusion protein expressed in *Escherichia coli*. The 381-bp *Bam*HI/*Eco*RI Kvβ1 cDNA fragment encoding the carboxy-terminal

amino acid residues 275–401 was cloned into the pGEX2T vector. The fusion protein was purified by preparative polyacrylamide gel electrophoresis. The immunization protocol and the affinity purification of the antibodies was described previously (Veh et al. 1995).

GROSS MORPHOLOGICAL ANALYSIS

Parasagittal sections from adult mutant and wild-type brain were Nissl stained.

INTRACELLULAR RECORDINGS IN CA1

Intracellular recordings in CA1 pyramidal cells were obtained in transverse hippocampal slices (400 μm thick) from adult mice. The slices were kept submerged in artificial cerebrospinal fluid (ACSF) containing 125 nM NaCl, 25 nM $NaHCO_3$, 1.25 nM KCl, 1.25 nM KH_2PO_4, 2.0 nM $CaCl_2$, 1.5 nM $MgCl_2$, 16 nM D-glucose and saturated with 95% O_2 and 5% CO_2. Whole-cell giga-seal voltage-clamp recordings were obtained at 23–26°C, in Ca^{2+}-free medium with 3 mM $MnCl_2$ and 0.5 μM tetrodotoxin (TTX) to block Ca^{2+}-dependent currents and Na^+ currents. The intracellular solution contained 140 mM potassium gluconate, 10 mM HEPES, 2 mM Mg.ATP, 1 mM $MgCl_2$, and 0.2 mM GTP (pipette resistance 5–7 MΩ). Series resistance compensation was not used. Currents were measured between 7 min and 15 min after the break-in and switch to Ca^{2+}-free medium, to ensure comparable conditions. Current-clamp recordings were obtained at 30–31°C (mutants: 30.8 ± 0.1°C, $n = 12$ cells; wild-type mice: 30.8 ± 0.1°C, $n = 17$ cells), using sharp microelectrodes filled with 2 M potassium acetate (resistance 70–90 MΩ) and connected to an amplifier in bridge current-clamp mode. The ''resting'' membrane potential was always adjusted to −60 mV by depolarizing DC current injection to facilitate sAHP measurements and comparisons of current-clamp responses between cells. All results were tested with two-tailed *t*-tests assuming unequal variances.

EXTRACELLULAR RECORDINGS IN CA1

Transverse hippocampal slices (400 μm thick) were placed in a submerged recording chamber perfused continuously at a rate of 1.5 ml/min with ACSF equilibrated with 95% O_2 and 5% CO_2 at 31°C. Extracellular field excitatory postsynaptic potentials (EPSPs) were recorded with an electrode filled with ACSF in CA1 stratum radiatum. In all experiments one bipolar melted-in-glass platinum electrode was used to stimulate Schaffer collateral/commissural afferents ~300 μm away from the recording pipette. A second electrode was used to evoke single EPSPs from a second control pathway. Stimulus duration was 100 μsec. ACSF contained 120 nM NaCl, 3.5 nM KCl, 2.5 nM $CaCl_2$, 1.3 nM $MgSO_4$, 1.25 nM NaH_2PO_4, 26 nM $NaHCO_3$, and 10 nM D-glucose.

BEHAVIORAL ANALYSIS

Animals homozygous for pink-eyed dilution were not analyzed, because these animals have impaired vision. All experiments were done blind to the genotype.

Thirteen Kvβ1.1-deficient mice and 12 control littermates were tested in an open field (Huang et al. 1995). The open field was a gray circular arena, 45 cm in diameter and 30 cm in height. It was divided into six peripheral and one central sector of equal area. Each mouse was placed into the center of the open field, and the crossings (total sector crossings and central crossings) were scored for 15 min.

If not mentioned otherwise, the mice were tested in the Morris water maze as described previously (Bourtchuladze et al. 1994). The animals were trained with two trials per day (1-min intertrial interval). The water temperature was 25 ± 2°C, and the walls of the pool were 20 cm higher than the water surface. At day 10 and day 14 the animals were given a transfer test. During a transfer test, there was no platform in the swimming pool, and the mouse was allowed to swim for 1 min. For the ''new-location'' test, the platform was placed in the opposite quadrant during days 15–18. At day 18 a further transfer test was undertaken. Twenty-two Kvβ1.1-deficient mice and 19 control littermates have been tested in two independent experiments. Because both experiments showed similar results, the data were combined. One wild-type and one mutant mouse were excluded from the analysis, because of extensive floating.

The mice were studied in contextual conditioning as described previously (Bourtchuladze et al. 1994). Eleven mutants and 11 control littermates were tested 24 hr after training. After an additional 10 days, these mice were tested again for contextual conditioning.

For the social transmission of food preference, Kvβ1.1-deficient mice with four crosses into C57BL/6J were used. The mice were tested as described previously (Kogan et al. 1997). Ten mutant and 10 wild-type mice were tested 21 hr after the interaction with the demonstrator mice in two independent experiments. In contrast to the 21-hr test (which showed food preference for the wild-type mice; see Results), 15 mutant and 18 wild-type mice tested immediately after the interaction with the demonstrator mice showed no preference for the cued food, neither among the wild-type mice nor the mutants. To exclude the possibility that this lack of preference results from a decreased food intake, we compared the consumed food during the immediate and the 21-hr testing. The mice of both genotypes ate more food at the immediate relative to the 21-hr test. The absent food preference immediately after the interaction with the demonstrator mice may be attributable to the genetic background in which the mice were studied. This hypothesis is consistent with the fact that mice in the 129B6F2 background (Kogan et al. 1997), but not mouse lines after different backcrossings into C57BL/6J, discriminate between the cued and noncued food immediately after interaction with demonstrator mice (data not shown).

The mice were tested in the bar hang test as described previously (Gerlai et al. 1996). Eight mutants and seven wild-type mice having four crosses into C57BL/6 were used.

All results were tested with a one-way or a two-way analysis of variance (ANOVA) with repeated measures.

Results

GENERATION OF Kvβ1.1-DEFICIENT MICE

The Kvβ1.1 targeting construct contained an insertion of a neomycin gene expression cassette in the first coding exon of Kvβ1.1 (Fig. 1A). ES cells were transfected with the linearized targeting construct and were tested for homologous recombination by Southern blots. Cells from targeted ES cell clones were injected into blastocysts to generate chimeras. Homozygous mutants were obtained (Fig. 1B) in a Mendelian ratio ($n = 249$; $\chi^2 = 0.024$; $P > 0.975$), indicating that the introduced mutation did not lead to lethality during embryonic or early postnatal development. A Northern blot analysis with mRNA from adult mutant and wild-type brain (Fig. 1C) showed that the mutation resulted in the loss of the Kvβ1.1 mRNA. Immunoblot analyses with an anti-Kvβ1.1 antibody and protein from adult brain further confirmed the loss of Kvβ1.1 and showed that Kvβ1.1 expression was reduced by ~50% in heterozygotes (Fig. 1D).

To test whether the introduced mutation affected Kvβ1.1 specifically, the expression of related and interacting molecules was analyzed. The expression of the structurally related Kvβ2 subunit (Scott et al. 1994) was normal in striatum, hippocampus, and olfactory bulb of the mutants (Fig. 1E). Furthermore, no expression of Kvβ1 isoforms was found in striatum and hippocampus of the mutants, the brain regions where Kvβ1.1 is highly expressed (Rettig et al. 1994; Rhodes et al. 1996), indicating that the other Kvβ1 isoforms did not compensate for the loss of Kvβ1.1 (Fig. 1E). The loss of Kvβ1.1 also did not lead to obvious changes in the expression of the interacting Kv1 α-subunits Kv1.1, Kv1.2, and Kv1.4 in hippocampus and striatum (Fig. 1E).

GROSS MORPHOLOGY AND BEHAVIOR

The loss of Kvβ1.1 did not result in alterations in adult brain morphology at the light microscope level (Fig. 1F). Accordingly, the Kvβ1.1-deficient mice showed normal body weight, normal grooming, no catalepsy, and no hints of ataxia. During 99 hr of observation we did not detect any behavioral seizures. In addition, four Kvβ1.1-deficient mice were chronically monitored by electrocorticographic recordings for 2-hr periods over three successive days, and no seizure activity was observed (J.L. Noebels, unpubl.).

In an open-field test (Huang et al. 1995), there was no difference in the exploratory behavior between mutant and wild-type mice. In the circular arena, the mutants showed 148 ± 21 total and 26 ± 3 central crossings, and the wild-type mice showed 160 ± 17 total and 27 ± 3 central crossings during 15 min of testing ($P > 0.42$).

In a test for motor strength and motor coordination, the bar hang test (Gerlai et al. 1996), there was also no difference between mutant and wild-type mice. The average hanging time on the bar was 39.8 ± 4.6 sec for the mutants and 37.0 ± 4.3 sec for the wild-type mice ($P = 0.67$).

K^+ CURRENTS IN HIPPOCAMPAL CA1 PYRAMIDAL NEURONS

In the CNS of wild-type mice, Kvβ1.1 mRNA is prominently expressed in striatal and hippocampal

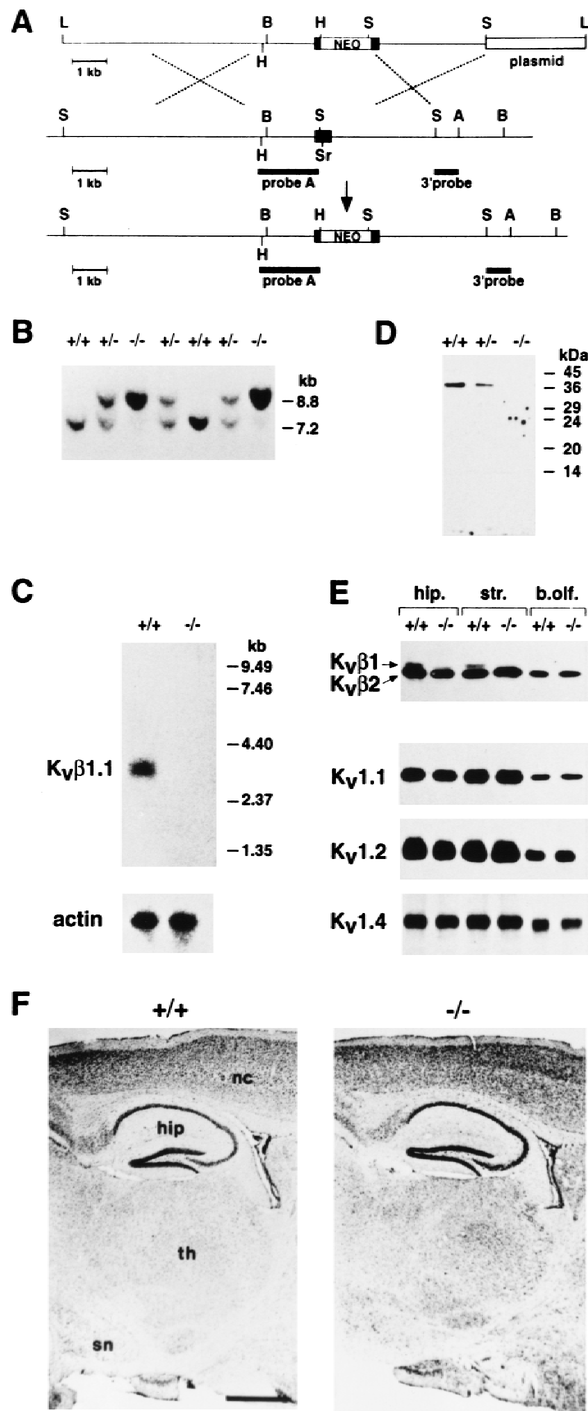

Figure 1: Disruption of the Kvβ1.1 isoform from the Kvβ1 gene. (*A*) The Kvβ1.1 targeting strategy. The targeting construct (*top*), a partial map of the genomic locus (*middle*), and the resulting targeted locus (*bottom*) are illustrated. Possible recombination forks (broken lines) and the Southern probes (thick lines) are shown. The first coding exon of the Kvβ1.1 isoform is shown as a black box. The following restriction sites are shown: (A) *Acc*I; (B) *Bam*HI; (H) *Hin*dIII; (L) *Sal*I; (S) *Sac*I; (Sr) *Srf*I. (*B*) Southern blot analysis of *Bam*HI-digested genomic DNA with the 3′ probe. (+/+) Wild-type; (+/−) heterozygous; (−/−) homozygous. (*C*) Northern blot analysis with poly(A) RNA from adult wild-type and mutant brain using a Kvβ1.1-specific probe and an actin probe as control. (*D*) Immunoblot analysis of Kvβ1.1 expression in homozygous, heterozygous, and wild-type brain. At the bottom of the gel there was a 20% gel such that small peptides could not run out of the gel and would have been detected. (*E*) Immunoblot analyses of the expression of Kvβ1, Kvβ2, Kv1.1, Kv1.2, and Kv1.4 in hippocampus, striatum, and olfactory bulb from wild-type and Kvβ1.1-deficient mice. Total protein was also tested for Kvβ1, Kvβ2, Kv1.1, Kv1.2, and Kv1.4 expression, and the same result as for *E* was obtained (data not shown). The expression of Kv1.5 in brain could not be detected (data not shown). (*F*) Nissl-stained sections in sagittal planes from adult wild-type and Kvβ1.1-deficient brain. (hip) Hippocampus; (nc) neocortex; (sn) substantia nigra; (th) thalamus. Scale bar, 1 mm.

neurons (Rettig et al. 1994; Rhodes et al. 1996; Butler et al. 1998). To test whether disrupting the Kvβ1.1 gene expression reduced the amplitude of I_A current in hippocampal CA1 pyramidal neurons, we patch-clamped somata of CA1 pyramidal neurons in acute hippocampal slices from adult wild-type and Kvβ1.1-deficient mice. Leak conductances (mutants: 9.7 ± 0.8 nS; wild-type mice: 10.1 ± 0.7 nS; $P = 0.71$) and resting membrane potentials (current-clamp conditions; mutants: −67.7 ± 1.4 mV; wild-type mice: −67.8 ± 1.6 mV; $P = 0.97$) of CA1 pyramidal neurons from mutant mice were indistinguishable from the ones of wild-type mice. Voltage-activated K^+ currents were recorded typically showing a rapidly inactivating (I_A) and a sustained current (I_{SO}) component during

the 500-msec depolarizing test pulses (Fig. 2A). The primary current characteristics were similar to previously described rat CA1 K^+ currents (for review, see Storm 1990). I_A time courses could be fitted with a single exponential (Fig. 2B). Inactivation time constants (τ_i) for I_A in wild-type ($\tau_i = 26 \pm 3$ msec, $n = 6$ mice, 14 cells) were like the ones in Kvβ1.1-deficient mice ($\tau_i = 27 \pm 4$ msec, $n = 6$ mice, 13 cells). However, I_A amplitudes were markedly reduced in the CA1 pyramidal neurons of Kvβ1.1-deficient mice (292 ± 52 pA, $n = 6$ mice) as compared with wild-type mice (485 ± 38, $n = 6$ mice, 14 cells; $P < 0.05$) (Fig. 2C). Thus, K^+ currents recorded from CA1 pyramidal neurons of the Kvβ1.1-deficient mice were characterized by a smaller ratio of I_A/I_{SO} amplitudes (0.36 ± 0.07) than those of wild-type control littermates (0.61 ± 0.03; $P < 0.05$) (Fig. 2D).

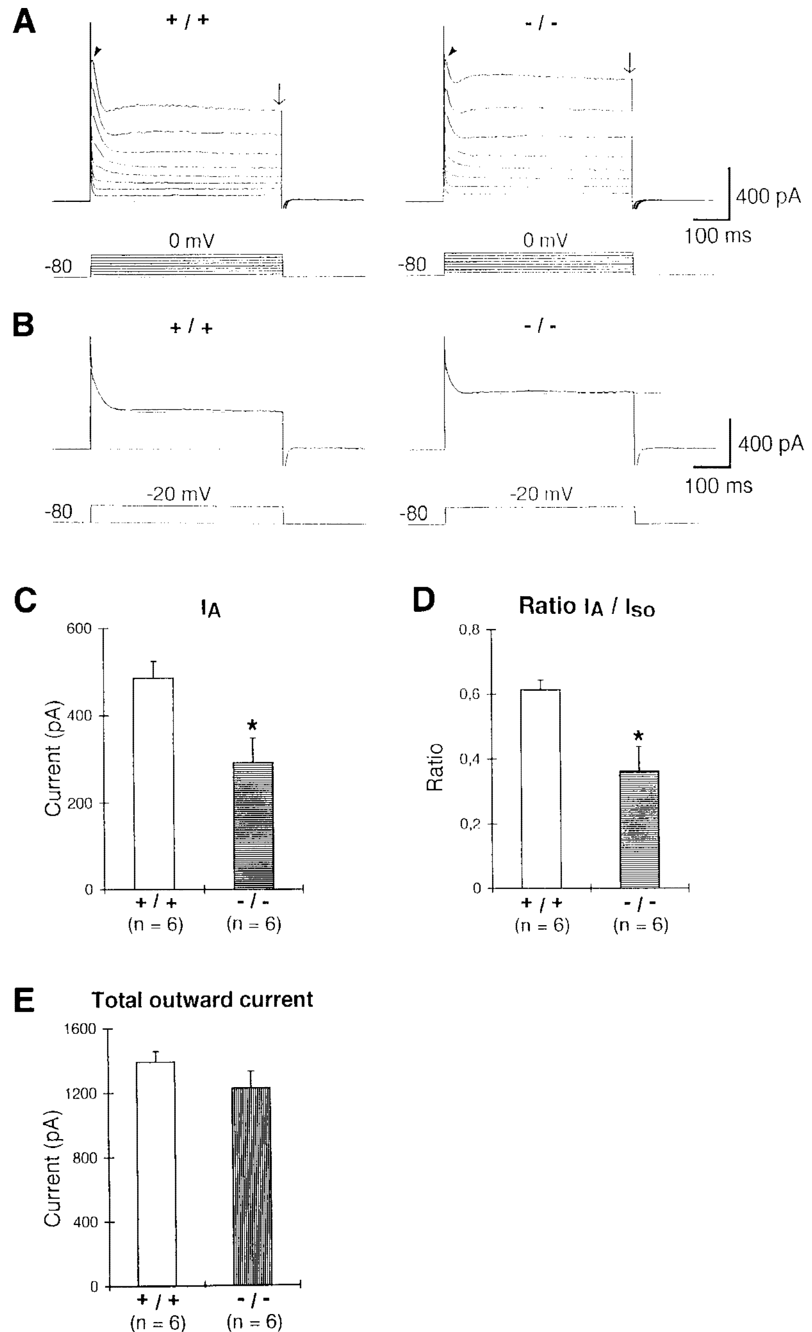

Figure 2: Intrinsic K^+ currents of hippocampal CA1 pyramidal neurons in Kvβ1.1-deficient and wild-type mice. Bar diagrams illustrate means ± S.E.M. (*A*) K^+ current families elicited by 500-msec-long voltage commands (−70 to −10 mV, in 10 mV increments) from a holding potential of −80 mV. Arrowheads indicate the peak of the fast-inactivating I_A current; arrows indicate where the I_{SO} current was measured, at the end of the 500-msec-steps. (*B*) K^+ currents elicited by steps from −80 mV to −20 mV fitted with a single exponential. (*C*) The I_A current, measured as the current that inactivated within 100 msec, was significantly smaller in the mutant mice ($n = 6$ mice, 13 cells) as compared with wild-type mice ($n = 6$ mice, 14 cells; $P < 0.05$). (*D*) The ratio between the amplitudes of I_A and I_{SO} was significantly smaller for the mutant mice ($n = 6$ mice, 13 cells) than for wild-type mice ($n = 6$ mice, 14 cells; $P < 0.05$). (*E*) The initial total outward current, measured at the peak of the early transient current, showed no significant difference between mutant ($n = 6$ mice, 13 cells) and wild-type mice ($n = 6$ mice, 13 cells; $P = 0.40$). (*C–E*) All currents were measured during steps from −80 mV to −20 mV.

Because Kvβ1.1 may confer rapid inactivation to otherwise noninactivating Kv channels, the null mutation of the Kvβ1.1 gene might have produced in parallel to the loss of rapidly inactivating Kv channels a gain in noninactivating Kv channel activity. We determined from whole-cell patch-clamp experiments the amplitudes of the total initial K^+ current (measured at the peak of I_A, including both transient and persistent components) and of I_{SO} at the end of the depolarizing test pulses. The mean amplitude of the total initial K^+ current in CA1 pyramidal neurons of Kvβ1.1-deficient mice (1277 ± 104 pA; $n = 6$ mice, 13 cells) was smaller than the one of wild-type mice (1387 ± 65 pA; $n = 6$ mice, 14 cells). Conversely, mean I_{SO} amplitudes in CA1 pyramidal neurons of Kvβ1.1-deficient mice (905 ± 136 pA; $n = 6$ mice, 13 cells) were larger than the ones in wild-type mice (794 ± 51 pA; $n = 6$ mice, 14 cells). Despite the relatively large variability in I_{SO} amplitudes in the mutant mice, the data suggest than an increase in I_{SO} compensates to some extent for the reduced I_A amplitude in the Kvβ1.1-deficient mice. This is in agreement with our observation that the mean duration of single action potentials elicited in CA1 pyramidal neurons of Kvβ1.1-deficient mice was 1.31 ± 0.12 msec ($n = 8$ mice, 12 cells) versus 1.20 ± 0.04 msec ($n = 10$ mice, 17 cells; $P = 0.34$) in wild-type control littermates (Fig. 3A). Apparently, single action potentials were repolarized in CA1 pyramidal neurons of mutant and wild-type mice with a similar, if not identical, efficiency, although I_A amplitudes were reduced in the Kvβ1.1-deficient mice.

FREQUENCY-DEPENDENT SPIKE BROADENING IN HIPPOCAMPAL CA1 PYRAMIDAL NEURONS

We then tested whether the reduced ratio of I_A/I_{SO} amplitudes in the Kvβ1.1-deficient mice affected trains of action potentials. Spike trains consisting of five spikes were elicited by current injection in CA1 pyramidal neurons using sharp microelectrodes. We analyzed frequency-dependent spike broadening relative to the first spike and found a significant difference between wild-type and mutant mice (Fig. 3B,C). Thus, during 50-msec long bursts consisting of five action potentials, the fourth spike showed 38.6 ± 4.8% broadening in wild-type controls ($n = 10$ mice, 17 cells) (Fig. 3B). In the Kvβ1.1-deficient mice, the broadening of the fourth spike was only 23.1 ± 3.9% ($n = 8$ mice, 12 cells; $P < 0.05$). The more robust action-potential repolarization in the mutants was also seen for the fifth action potential but not for the second and third spike (Fig. 3C).

THE sAHP IN HIPPOCAMPAL CA1 PYRAMIDAL NEURONS

The spike broadening most likely reflects the inactivation of rapidly inactivating Kv channels that contribute to action-potential repolarization. The time course of spike repolarization is particularly important because action-potential duration is an important determinant of activity-dependent Ca^{2+} entry (Llinás et al. 1982; Jackson et al. 1991; Borst and Sakmann 1996) and regulation of downstream Ca^{2+}-dependent processes, for example, the sAHP of spike trains that is mediated by Ca^{2+}-activated K^+ channels (for review, see Storm 1990; Sah 1996). We found that the sAHP amplitudes following the five-spike bursts were smaller in CA1 pyramidal neurons of Kvβ1.1-deficient mice (5.39 ± 0.40 mV; $n = 8$ mice, 12 cells) than in the ones of wild-type control littermates (8.40 ± 0.77 mV; $n = 10$ mice, 17 cells; $P < 0.01$) (Fig. 4). A classical role of I_A in invertebrates is to prolong the afterhyperpolarization and, thereby, to slow down the firing rate of action potentials (Connor and Stevens 1971). Interestingly, the Kvβ1.1 mutation resulted in a reduction of I_A and of the sAHP.

HIPPOCAMPUS-DEPENDENT LEARNING

Because the deletion of the Kvβ1.1 subunit altered the firing properties of hippocampal pyramidal neurons in hippocampus, it might be expected to affect hippocampus-dependent learning. Therefore, we studied learning of the Kvβ1.1-deficient mice in several behavioral paradigms. The mutants were tested in the hidden platform version of the Morris water maze, a hippocampus-dependent task (Morris et al. 1982) (Fig. 5). The mutants ($n = 21$) reached the platform as quickly as wild-type mice ($n = 18$) during training, and they searched as selectively as wild-type mice during a transfer test at the end of training (Fig. 5A,B). These results show that the loss of Kvβ1.1 did not lead to significant impairments in spatial learning, as tested in this version of the water maze task. These results also indicate that the mutants had normal motivation, motor coordination, and vision, as needed to find the hidden platform. Consistent with the water maze results, we also found

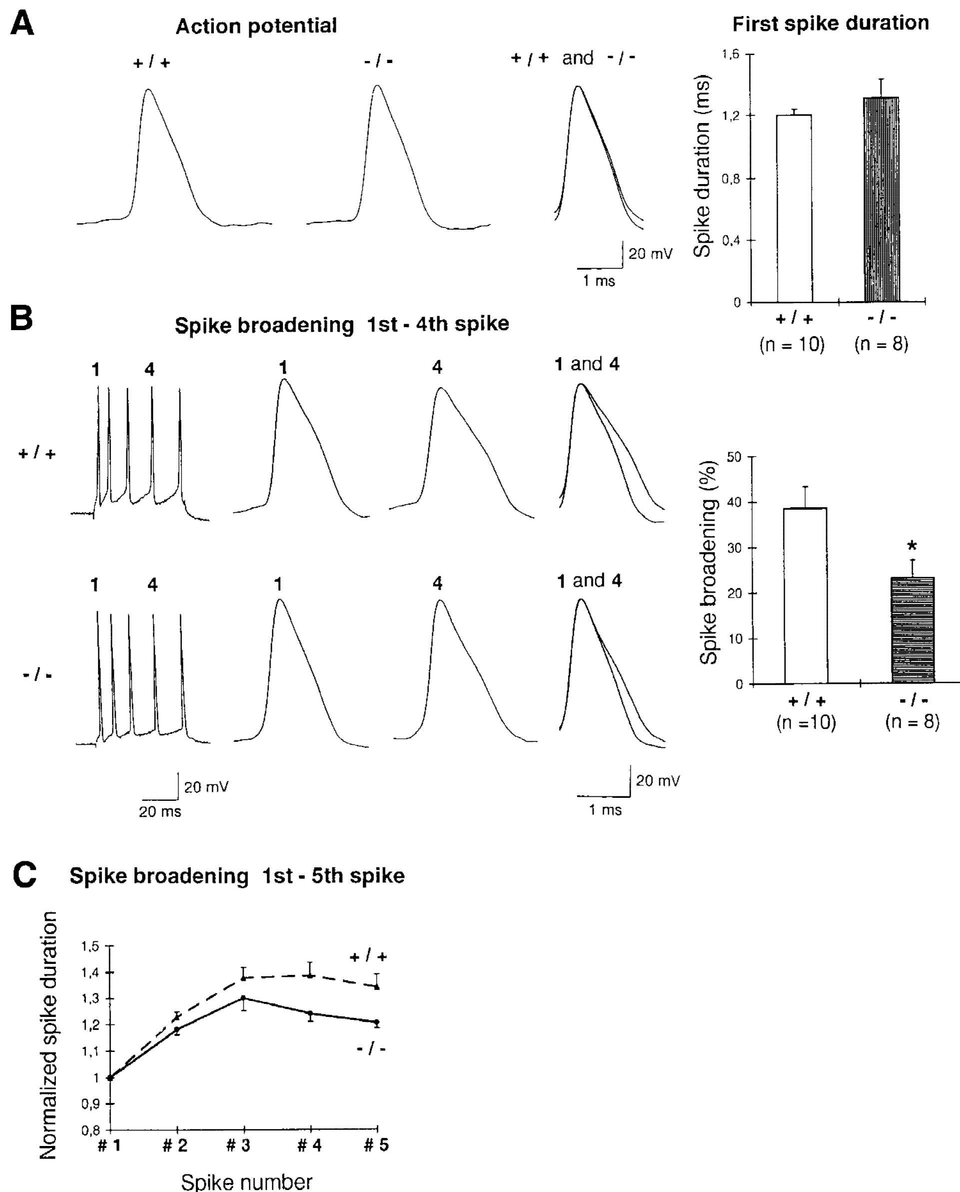

Figure 3: Reduced frequency-dependent spike broadening in hippocampal CA1 pyramidal neurons in Kvβ1.1-deficient mice. Bar diagrams illustrate means ± s.e.m. (*A*) Single spike duration. There was no significant difference in duration of single action potentials between mutant ($n = 8$ mice, 12 cells) and wild-type mice ($n = 10$ mice, 17 cells; $P = 0.34$). The spike amplitude was also normal (amplitudes of initial spikes measured from threshold to peak in cells with "resting potential" adjusted to −60 mV; mutants: 79 ± 2 mV; wild-type mice: 82 ± 2 mV; $P = 0.33$). All data are from intracellular recordings with sharp microelectrodes from CA1 pyramidal cells (see Materials and Methods). Action potentials were elicited by injecting a 50-msec depolarizing current pulse, once every 10 sec. Representative examples of the first spike during each pulse are shown, singly and superimposed (aligned on the peak). (*B*) Frequency-dependent spike broadening was reduced in the mutant mice. A burst of five action potentials was elicited once every 10 sec by injecting 50-msec-long depolarizing current pulses (*left*). Representative examples of the first (1) and fourth (4) spike are compared. The spike duration was measured 20 mV above the threshold level (defined by the intercept between two straight lines, fitted to the last 0.5 msec of the prespike trajectory and the upstroke of the spike, respectively). The spike broadening from the fourth spike to the first spike (in percent of the first spike duration) was significantly smaller in the mutant ($n = 8$ mice, 12 cells) than in the wild-type mice ($n = 10$ mice, 17 cells; $P < 0.05$). (*C*) Spike broadening for all spikes in the five-spike bursts (normalized with respect to the first spike duration). Mutant ($n = 8$ mice, 12 cells) vs. wild-type mice ($n = 10$ mice, 17 cells) showed a significantly smaller spike broadening for the fourth and fifth spikes ($P < 0.05$) but not for the second and third ($P = 0.058$ and $P = 0.13$, respectively), indicating a selective reduction in late spike broadening in the mutants.

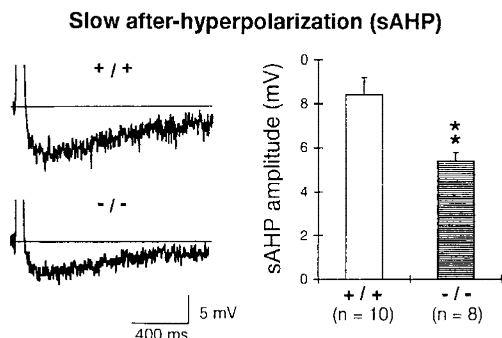

Figure 4: Reduced sAHP in hippocampal CA1 pyramidal neurons in Kvβ1.1-deficient mice. Bar diagrams illustrate means ± S.E.M. The amplitude of the sAHP following 50-msec-long five-spike bursts (see Fig. 3) was significantly smaller in the mutant ($n = 8$ mice, 12 cells) than in the wild-type mice ($n = 10$ mice, 17 cells; $P < 0.01$). Two cells that showed exceptionally large sAHPs, one wild-type cell with a 17-mV sAHP and one mutant cell with a 15-mV sAHP (i.e., 12 and 25 × S.E.M. from the mean, respectively), were considered abnormal and excluded from the analysis of sAHPs and spikes.

no abnormalities in contextual conditioning, also a hippocampus-dependent task (Kim and Fanselow 1992; Phillips and LeDoux 1992), in mutants tested 1 day and 11 days following training (e.g., 24 hr after training, the mutants froze 40.3 ± 7.8%, $n = 11$, of the time and the wild-type mice froze 43.5 ± 8.2%, $n = 11$, of the time; $P = 0.78$).

In contrast, the mutant mice showed impairments in another version of the water maze task (Fig. 5A,C). In this test, previously trained mice had to find the hidden platform in a new location. The mutants needed more time than control littermates to reach the platform ($P < 0.05$) despite normal swimming speed (Fig. 5A). In addition, in a transfer test, the mutants spent less time than wild-type mice searching for the platform located in the new target quadrant (search time in target quadrant: mutants, 31.9 ± 2%; wild-type mice, 40.7 ± 2%; $P < 0.001$). During this transfer test, the mutants also crossed the exact site where the platform was located less frequently than control littermates (crossings: mutants, 3.0 ± 0.5; wild-type mice, 4.8 ± 0.5; $P < 0.05$). Thus, in three different measures we found evidence that the loss of Kvβ1.1 led to learning impairments.

We also tested the Kvβ1.1-deficient mice in the social transmission of food preference task, another hippocampus-dependent test (Bunsey and Eichenbaum 1995). This test is based on the fact that mice develop a preference for novel foods that they recently smelled on the breath of other mice (“demonstrator” mice). Twenty-one hours after the interaction with the demonstrator mice, the Kvβ1.1-deficient mice ($n = 10$) did not show any preference for the cued food ($P = 0.51$), whereas control littermates did ($n = 10$; $P < 0.001$) (Fig. 6). The mutants ate 0.29 ± 0.06 grams of cued food versus 0.24 ± 0.05 grams of noncued food, and the wild-type mice ate 0.36 ± 0.04 grams of cued food versus 0.16 ± 0.02 grams of noncued food. The total amount of eaten food was the same for mutant and wild-type mice (mutants: 0.53 ± 0.05 grams; wild-type mice: 0.52 ± 0.04 grams; $P = 0.92$). This result confirms that the loss of Kvβ1.1 leads to learning impairments.

SYNAPTIC PLASTICITY IN THE HIPPOCAMPAL CA1 REGION

The learning impairments of the Kvβ1.1-deficient mice could be owing to the observed changes in firing properties of CA1 pyramidal neurons. Alternatively, the loss of Kvβ1.1 may have affected synaptic plasticity in the CA1 region, which is known to be involved in learning (e.g., Tsien et al. 1996). Therefore, we tested various forms of synaptic enhancement and depression in the Kvβ1.1-deficient mice, using extracellular field recordings in the CA1 region. We did not observe any abnormalities in the mutant mice (Fig. 7). Neither paired-pulse facilitation (PPF) nor long-term potentiation [LTP, induced by either 10 Hz for 6 sec, or by 10 θ bursts (Larson and Lynch 1986; Chapman et al. 1995)] nor depotentiation (by 900 pulses at 1 Hz) of 10 θ burst-induced LTP showed any measurable difference between the Kvβ1.1-deficient and wild-type mice.

Discussion

A-type K^+ channels are known to be important regulators for neuronal firing (Ma and Koester 1996; Debanne et al. 1997; Hoffman et al. 1997), but their role in repetitive firing and learning in mammals is not well characterized. To determine the contribution of the auxiliary K^+ channel subunit Kvβ1.1 to I_A currents and to study the physiological role of I_A currents in repetitive firing and learning, we have deleted the Kvβ1.1 subunit gene in mice. Our results indicate that Kvβ1.1 confers fast inactivation to a portion of the A-type K^+ channels in hippocampal CA1 pyramidal neurons. Fur-

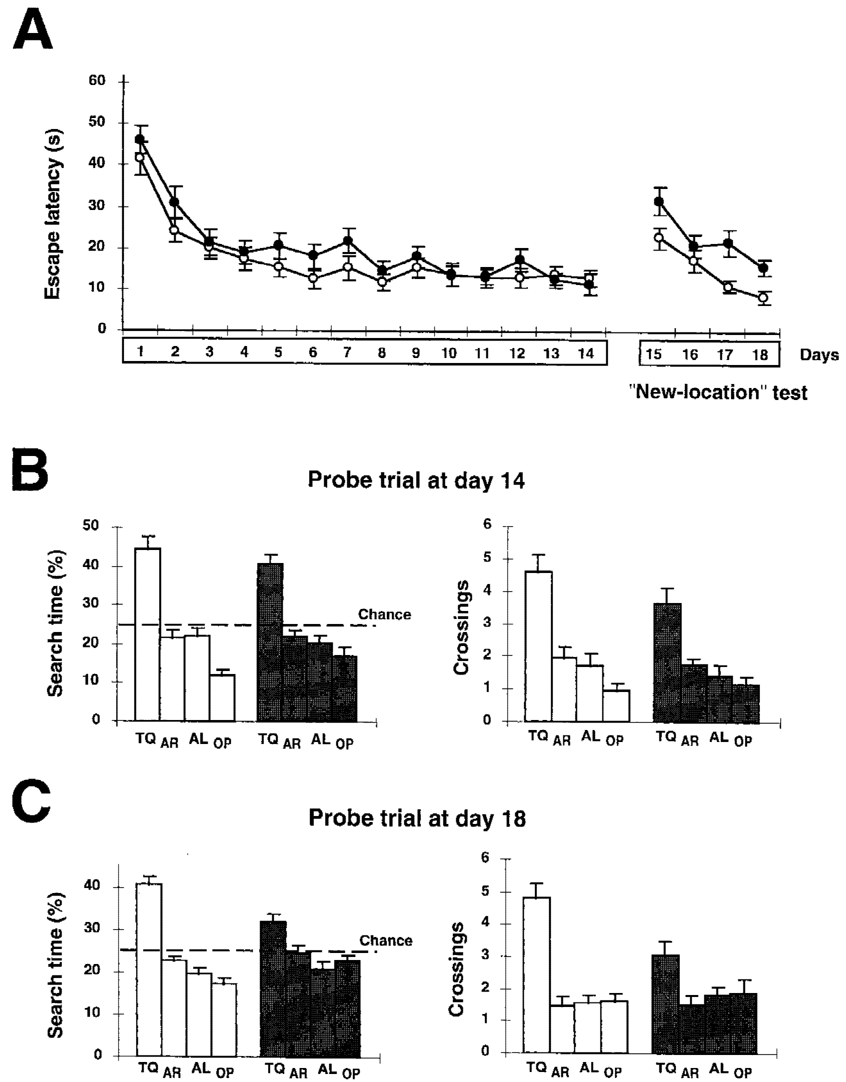

Figure 5: Learning impairments of Kvβ1.1-deficient mice in the Morris water maze. Means ± S.E.M. are illustrated. (*A*) The initial-training and a new-location test is shown for mutant and wild-type mice in the hidden-platform version of the water maze. The average time to reach the platform was plotted vs. training days. The Kvβ1.1-deficient mice ($n = 21$) and control littermates ($n = 18$) performed equally well during initial-training [effect of genotype: $F_{(1,37)} = 2.37$, $P = 0.13$; effect of training: $F_{(13,481)} = 22.32$, $P < 0.001$; interaction of genotype × training: $F_{(13,481)} = 0.65$, $P = 0.82$], whereas the Kvβ1.1 mutants were impaired in a new-location test [effect of genotype: $F_{(1,37)} = 6.77$, $P < 0.05$; effect of training: $F_{(3,111)} = 20.95$, $P < 0.001$; interaction of genotype × training: $F_{(3,111)} = 1.25$, $P = 0.29$]. (○) +/+; (●) −/−. (*B*) A transfer test was given at the end of initial-training test (day 14). The search time and the platform crossings are plotted for each of the quadrants: (TQ) quadrant where the platform was located or target quadrant; (AR) adjacent right; (AL) adjacent left; (OP) opposite to the target quadrant. There was no significant difference between mutant and wild-type mice in respect to search time [comparison of time in TQ between genotypes: $F_{(1,37)} = 0.81$, $P = 0.37$; comparison of time in TQ vs. in the other three quadrants for the mutants, which represents selectivity: $F_{(3,80)} = 23.63$, $P < 0.001$] and platform crossings [comparison of crossings in TQ between genotypes: $F_{(1,37)} = 1.70$, $P = 0.20$; comparison of crossings in TQ vs. in the other three quadrants: $F_{(3,80)} = 9.67$, $P < 0.001$]. Furthermore, a transfer test at day 10 revealed no abnormalities for the Kvβ1.1 mutants (data not shown). (*B,C*) (Open bars) +/+; (solid bars) −/−. (*C*) A further transfer test was given at the end of new-location test (day 18). Note that TQ here was OP for the transfer test at day 14. The mutants were able to search selectively in TQ [comparison of time in TQ vs. in the other three quadrants: $F_{(3,80)} = 6.32$, $P < 0.001$] but spent less time in TQ than wild-type mice [comparison of time in TQ between genotypes: $F_{(1,37)} = 9.83$, $P < 0.001$]. In addition, the mutants did not show preferential platform crossings in TQ [$F_{(3,80)} = 2.48$, $P > 0.05$] and had less platform crossings in TQ than wild-type mice [$F_{(1,37)} = 7.26$, $P < 0.05$]. There was no difference in swimming speed for mutant and wild-type mice during transfer tests [e.g., $F_{(1,37)} = 1.76$, $P = 0.19$ at day 18].

thermore, the results strongly suggest that Kvβ1.1-dependent A-type K^+ channels contribute to frequency-dependent spike broadening and regulate the sAHP by controlling Ca^{2+} influx during action potentials. Finally, the Kvβ1.1 subunit does not appear to be required for synaptic plasticity but is necessary for solving certain learning tasks.

THE Kvβ1.1 MUTATION DID NOT DISRUPT OTHER K^+ CHANNEL SUBUNITS

To disrupt the Kvβ1.1 isoform in mice, we have inserted a *neo* gene into the twenty-fifth codon of the Kvβ1.1-specific exon. In principle, this mutation allows the formation of a 26-amino-

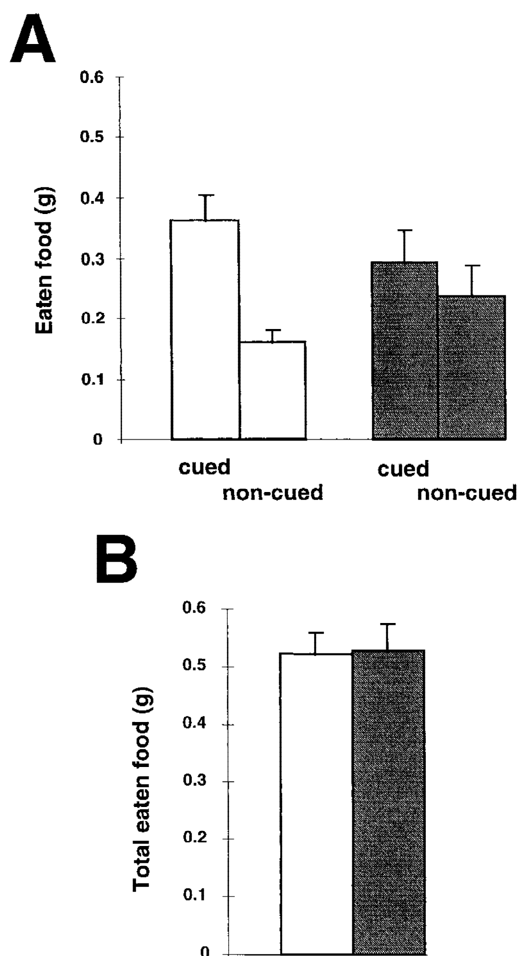

Figure 6: Impairments of the Kvβ1.1-deficient mice in the social transmission of food preference task. Bar diagrams illustrate means ± S.E.M. (Open bars) +/+; (solid bars) −/−. (*A*) Twenty-one hours after the interaction with the demonstrator mice, the mutants ($n = 10$) did not prefer the cued food [$F_{(1,18)} = 0.46$, $P = 0.51$], whereas the wild-type mice ($n = 10$) did [$F_{(1,18)} = 17.6$, $P < 0.001$]. (*B*) The total amount of eaten food was the same between mutant and wild-type mice [$F_{(1,18)} = 0.01$, $P = 0.92$].

acid peptide containing the 25 amino-terminal amino acids of Kvβ1.1. It was shown previously that this peptide does not confer A-type inactivation on Kv1 α-subunits (Stephens et al. 1996); only a mutated version of this peptide at high concentrations confers A-type inactivation on Kv1 α-subunits (Rettig et al. 1994). Just much longer amino-terminal Kvβ1.1 peptides (>40 amino acids) confer A-type inactivation at low concentrations (20 nM) in vitro (R. Falker, pers. comm.). A probe that detects Kvβ1.1 and *neo* fusion transcripts was used to study Kvβ1.1 mRNA expression. No Kvβ1.1 transcript was detected. Immunoblot analysis with a specific anti-Kvβ1.1 antibody confirmed the com-

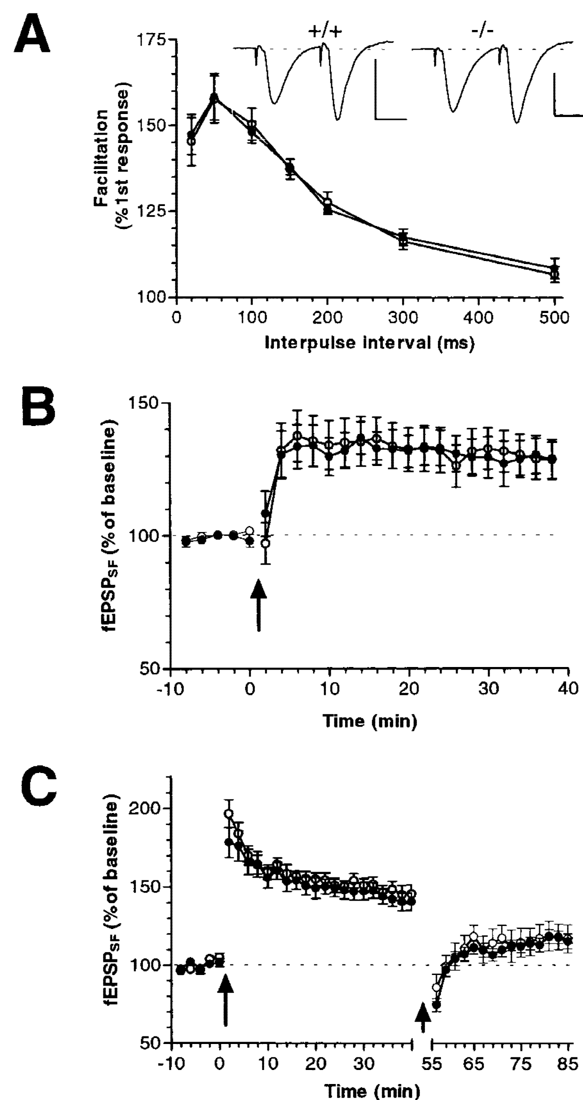

Figure 7: Normal synaptic plasticity in the hippocampal CA1 region of the Kvβ1.1-deficient mice. Means ± S.E.M. are illustrated. (*A*) No abnormalities in paired-pulse facilitation were found between mutant ($n = 7$ mice) and wild-type mice ($n = 7$). Representative traces are shown; calibration bars, 1 mV, 10 msec. (●) +/+; (○) −/−. (*B*) LTP induced by a 10-Hz tetanus (60 pulses) was the same for mutant ($n = 10$ mice) and wild-type mice ($n = 6$ mice). This tetanus is close to the threshold of LTP generation, because in some slices no LTP was obtained after the tetanus. The tetanus is indicated by the arrow. (*C*) Ten-θ-burst-induced LTP (long arrow) was indistinguishable between mutant ($n = 9$ mice) and wild-type mice ($n = 8$ mice). Depotentiation (short arrow) induced by a 1-Hz stimulation for 15 min was also normal in the mutants.

plete loss of Kvβ1.1. In agreement with the Northern blot results, the immunoblots gave no indication of possible expression of an altered or truncated Kvβ1.1 polypeptide. In addition, the immunoblots showed that the expression of the structurally related Kvβ2 subunit (Scott et al. 1994) was not altered in striatum and hippocampus of the mutants, that is, the brain regions where Kvβ1.1 is preferentially expressed (Rettig et al. 1994; Rhodes et al. 1996; Butler et al. 1998). The expression of the pore-forming Kv1 α-subunits (Kv1.1, Kv1.2, and Kv1.4), which are known to associate with Kvβ1.1, was also normal in these brain regions of the mutants.

In heterologous expression studies, Kvβ1.1 was shown to have a chaperone-like function, because it promoted the surface expression of Kv1 α-subunits (Shi et al. 1996; Accili et al. 1997). Similarly, in the *Drosophila*, *Hyperkinetic* β-subunits aid the cell surface expression of *Shaker* α-subunits (Chouinard et al. 1995; Wang and Wu 1996). Our studies suggest that the Kvβ1.1 subunit is not required for the surface expression of Kv1 α-subunits that contribute to somatodendritic K^+ current in hippocampal CA1 pyramidal neurons. After depolarization, the initial total K^+ current was not significantly reduced, and the single action-potential duration did not differ significantly between Kvβ1.1 mutants and wild-type mice. It is possible that the more abundant Kvβ2 subunit compensated the Kvβ1.1 deficiency (Rhodes et al. 1996; Shi et al. 1996). Therefore, mutant and wild-type mice may have a similar surface expression of Kv1 channels. Alternatively, it is possible that our analysis may not have been sensitive enough to detect a small decrease in the total initial K^+ current nor in the action potential duration in the mutants.

Kvβ1.1 CONFERS INACTIVATION ON I_A CURRENTS IN THE BRAIN

In heterologous expression studies, Kvβ1.1 confers fast inactivation on the otherwise noninactivating Kv1 α-subunits (Rettig et al. 1994; Heinemann et al. 1996). The Kvβ1.1 isoform binds to the amino terminus of the α-subunits (Sewing et al. 1996; Yu et al. 1996). In accordance with the ''ball-and-chain model'' (Zagotta et al. 1990), the amino-terminal ''ball'' domain of Kvβ1.1 can rapidly block the internal mouth of the channel when it opens in response to depolarization. Consistent with the function of Kvβ1.1 in heterologous expression studies, we found that I_A amplitude and the ratio of I_A/I_{SO} amplitudes were reduced by the loss of Kvβ1.1 in hippocampal CA1 pyramidal neurons. Despite of the relatively large variability of I_{SO} amplitudes in the mutant mice, the data suggest that an increase in I_{SO} compensates to some extent for the reduced I_A amplitude in the Kvβ1.1-deficient mice. The fact that I_{SO} in CA1 pyramidal cells is probably composed of several components (for review, see Storm 1990) may render it too variable to allow reliable detection of a change in just one of the current components (i.e., the one with Kvβ1.1-dependent fast inactivation). Taken together, the changes in the K^+ currents and the reduced frequency-dependent spike broadening in the mutants suggest that some A-type K^+ channels in the Kvβ1.1-deficient mice have lost their fast inactivation subunits, thus being transformed into noninactivating, delayed rectifier-type K^+ channels.

In hippocampal CA1 pyramidal neurons, the Kv α-subunits Kv1.2, Kv1.4, Kv2.1, Kv2.2, and Kv4.2 are expressed at relatively high levels (Kues and Wunder 1992; Weiser et al. 1994; Maletic-Savatic et al. 1995; Tsaur et al. 1997). Our results show that there are Kvβ1.1-dependent and -independent I_A currents in the somatodendritic region of hippocampal CA1 pyramidal neurons. The residual I_A current could be mediated by α-subunits that are independently capable of fast inactivation. For example, heterologous expression studies showed that the Kv4.2 subunit, which is also expressed in the somatodendritic region of CA1 pyramidal neurons (Sheng et al. 1992), can form A-type K^+ channels on its own (Baldwin et al. 1991).

SPIKE BROADENING DEPENDS ON Kvβ1.1

During a spike train, the inactivation of K^+ currents can produce progressive broadening of the spikes. This frequency-dependent spike broadening has been found in neurons from a variety of species (Aldrich et al. 1979; Jackson et al. 1991; Ma and Koester 1996). Because the loss of Kvβ1.1 reduced I_A amplitude, we determined whether frequency-dependent spike broadening was altered in hippocampal CA1 pyramidal neurons in the mutant mice. We focused our analysis on short spike trains because they are characteristic of CA1 neurons during learning. For example, CA1 place cells normally fire bursts of two to six spikes in their place fields (for review, see Lisman 1997).

The loss of Kvβ1.1 resulted in a reduction of frequency-dependent spike broadening in the so-

matodendritic region of CA1 pyramidal neurons. This reduction in spike broadening in the mutants could result directly from the reduction in inactivation of I_A. Normally, the cumulative inactivation of A-type K^+ channels during a spike train should reduce the repolarizing current in later spikes and, therefore, increase their duration. Consistent with this model, it was recently shown that blocking the inactivation of I_A currents with dynamic clamp (electronic manipulation of currents) eliminated frequency-dependent spike broadening in *Aplysia* neurons (Ma and Koester 1996). On this background, it seems reasonable to conclude from the present data that Kvβ1.1-dependent A-type K^+ channels contribute to frequency-dependent spike broadening.

A-TYPE K^+ CHANNEL ACTIVITY INFLUENCES THE sAHP

Following a spike train, the sAHP amplitude is reduced by ~36% in the Kvβ1.1 mutants. This result could be explained by the reduction of frequency-dependent spike broadening observed in the mutants. Such spike broadening may control Ca^{2+} influx during a spike train (Jackson et al. 1991). Because Ca^{2+} influx can occur during the falling phase of the spike (Llinás et al. 1982; Borst and Sakmann 1996), even slight spike broadening may enhance Ca^{2+}-dependent processes (Klein et al. 1982). Ca^{2+} influx during a spike train activates Ca^{2+}-dependent K^+ channels that are responsible for the sAHP (for review, see Storm 1990; Sah 1996). Therefore, our results suggest that Kvβ1.1-dependent A-type K^+ channels modulate the sAHP by regulating frequency-dependent spike broadening.

LEARNING IMPAIRMENTS IN Kvβ1.1-DEFICIENT MICE

LTP impairments in the hippocampal CA1 region have been correlated with hippocampus-dependent learning defects (Silva et al. 1997). In addition to LTP, there may be further mechanisms underlying learning and memory. Previous studies have proposed that neurotransmitters, such as acetylcholine or serotonin, modulate learning and memory by regulating the sAHP (Hasselmo and Bower 1993; Buhot 1997). Computer modeling suggests that regulation of the sAHP can be used as a filtering device for information storage (Hasselmo and Bower 1993; Lisman and Idiart 1995) and can stabilize learned information (Berner 1991). In addition, in rabbits, trace eye-blink conditioning—a hippocampus-dependent form of learning—alters the sAHP in CA1 hippocampal neurons (for review, see Disterhoft et al. 1996). Because the Kvβ1.1-deficient mice had an abnormal sAHP, but synaptic plasticity appeared normal, this mutant seems appropriate for studying the role of the sAHP in learning and memory. The mutants were impaired in two behavioral tasks: a version of the Morris water maze and the social transmission of food preference.

Studies of the mutants in the hidden-platform version of the Morris water maze showed seemingly normal performance during initial training (''initial-training'' test). However, trained mutants were impaired in their ability to learn a new location for the hidden platform (new-location test). These two water maze tests place different demands on the mice. In the initial-training test, mice have to learn the general requirements of the water maze task (e.g., the presence of an escape platform, the use of distal cues, etc.) and the fixed spatial location of the platform. In contrast, in the new-location task, the mice are already familiar with the task and have to learn to ignore the former location and search for the platform in a new place. Therefore, the new-location test requires that the mice use spatial information flexibly. Our results suggest that these two tasks tap into distinct learning processes: an initial Kvβ1.1-independent learning and a Kvβ1.1-dependent flexible learning. Consistent with this idea, another task that requires the flexible use of learned information (the social transmission of food preference) was also affected by the loss of Kvβ1.1.

The social transmission of food preference task consists of three parts. First, demonstrator mice are given a distinctively scented food. Second, the ''demonstrators'' are allowed to interact with the mice to be tested (''observer'' mice). During this interaction, the observer mice can smell the odor of the scented food on the breath of the demonstrator mice. Third, the observer mice are given a choice between the scented food they smelled in the breath of the demonstrator mice and another scented food. Training and testing are fundamentally different in this task. During training the mice are not taught to choose between two foods, as they are expected to do during testing. They are simply exposed to the demonstrator mice, and during testing they have to apply the

learned information in a novel situation. As in the new-location test in the water maze, this demands the flexible use of learned information.

In contrast to the learning tasks described above, the mutants were not impaired in a number of other behavioral tests. The bar hang test, locomotion, and swimming tests indicated normal motor strength and coordination in the mutants. The open field test showed normal exploratory behavior and normal responses to stress. Conditioning experiments indicated normal processing and expression of fear responses. Shock-reactivity experiments also indicated that nociception was unaltered in the mutants. These results demonstrate that the Kvβ1.1 mutation spared a large number of behavioral systems in the mutant mice.

The Kvβ1.1-deficient mice were impaired in two learning tests (Schöpke et al. 1991; Bunsey and Eichenbaum 1995; Devan et al. 1996), which are especially sensitive to hippocampal lesions (Cohen and Eichenbaum 1993), suggesting that physiological alterations in the hippocampus underlie the behavioral deficits. However, it is possible that physiological and/or anatomical alterations (which were not detectable at the gross neuroanatomical level) in other brain structures also contribute to the learning impairments of the mutants. For example, abnormalities in the striatum or parietal cortex could account for deficits in the new-location test of the water maze (Kolb et al. 1983; Devan et al. 1996). In addition, alterations in the olfactory cortex could result in the impairments in the social transmission of food preference task observed in the mutants.

CONCLUSION

The number and frequency of spikes is thought to encode information in the brain (for review, see Rieke et al. 1997). Therefore, mechanisms regulating the properties of spike trains could have an impact on learning. Here, we have addressed this hypothesis by deleting an auxiliary A-type K^+ channel subunit and found that mice with reduced frequency-dependent spike broadening and sAHP, two key properties of spike trains, have learning impairments.

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