Impaired synaptic plasticity in a rat model of tuberous sclerosis

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Zusammenfassung in deutscher Sprache:

Beeinträchtigung synaptischer Plastizität in einem Ratten – Modell der tuberösen Sklerose

Die tuberöse Sklerose (Synonym: Bourneville – Pringle - Syndrom) ist ein zur Krankheitsgruppe der Phakomatosen gehörendes, autosomal-dominant erbliches Fehlbildungssyndrom mit einer statistischen Häufigkeit von etwa 1:8000 - 1: 10.000 Neugeborenen. Klinisch zeigt sich ein multisystemisches Krankheitsbild mit Dysfunktionen verschiedener, dysplastisch veränderter Organe, wie zum Beispiel Herz, Niere und Zentralnervensystem. In Bezug auf die Funktionen des Zentralnervensystems kommt es im Verlauf der Erkrankung zu geistiger Retardierung, Verhaltensstörungen und Epilepsie.

Die tuberöse Sklerose wird durch genetische „loss of function – Mutationen" verursacht, welche die beiden Tumor – Suppressorgene, TSC1 (Genlocus: 9q34) und TSC2 (Genlocus: 16p13), in ihrer Funktion einschränken. Die TSC Gene kodieren für zwei miteinander interagierende Proteine, Hamartin und Tuberin. Die veränderte Funktion dieser beiden Proteine hat eine veränderte Aktivierung zweier intrazellulärer Transduktionskaskaden, (mTor- ; p44/42 MAPK - Kaskade) zur Folge. Diese beiden Kaskaden sind in charakteristischer Weise an den Mechanismen neuronaler Plastizität beteiligt. Neuronale Plastizität, also aktivitätsabhängige Modulation neuronaler Effizienz, ist ein Schlüsselmechanismus der Informationskodierung und -speicherung. So bildet neuronale Plastizität die Grundlage für kognitive Funktionen, wie beispielsweise Lernen und Gedächtnis.

Zusammen betrachtet legt die Betrachtung dieser physiologischen Zusammenhänge die These nahe, dass in Lebewesen, in denen Mutationen der TSC-Gene beobachtet werden, plastische Eigenschaften neuronaler Transmission verändert sind.

Diese experimentelle Theorie wurde in der vorliegenden Arbeit in einem Tiermodell (Eker Ratte) der tuberösen Sklerose untersucht. Die Eker Ratte trägt eine spontane, autosomal-dominante, heterozygote Keimzell-Mutation des TSC2 ($TSC2^{+/-}$) Gens und zeigt dadurch eine gestörte Funktion des Tuberins. In der dieser Arbeit wurden männliche $TSC2^{+/-}$ Ratten benutzt, um zu untersuchen, ob und wie Mutationen im Tuberin Gen, synaptische Plastizität im Hippocampus beeinflussen.

Nach der Züchtung wurden die Tiere genotypisiert und im Alter von 20 bis 40 Tagen den elektrophysiologischen Experimenten zugeführt. Die Versuchstiere wurden anästhesiert, das Gehirn wurde freigelegt und die Hippocampusregion wurde präpariert. Für die Untersuchung der synaptischen Phänomene der neuronalen Kurzzeit- und Langzeitplastizität wurden exzitatorische postsynaptische Summenpotentiale (fEPSPs) der hippocampalen Schaffer Kollateral-CA1 Synapse durch bestimmte Paradigmen induziert und abgeleitet. Solche Paradigmen stellen etablierte Protokolle der Untersuchung neuronaler Phänomene dar. In allen Experimenten der hier vorliegenden Arbeit wurde der Genotyp der Tiere erst im Nachhinein entschlüsselt. Die Resultate der Experimente wurden für $TSC2^{+/-}$ und Wild-Typ Versuchstiere miteinander verglichen.

Input-output Kurven, welche durch Auftragen der Amplitude des fEPSPs gegen die Stärke der Stimulation generiert werden, gelten als Maßstab für basale synaptische Transmission. Es zeigte sich kein Unterschied in den beiden experimentellen Gruppen. Im Anschluss wurde die synaptische Kurzzeitplastizität untersucht. Die Fazilitierung zweier unmittelbar aufeinander folgender Stimuli (Paar-Puls-Fazilitierung, PPF) wurden miteinander verglichen. $TSC2^{+/-}$ Ratten zeigten eine signifikant höhere PPF als die Wild-Typ Ratten. Auch nach Blockade der GABAergen Inhibition änderte sich dieser Sachverhalt nicht.

Zudem wurde die hippocampale Langzeitpotenzierung (LTP) mittels eines für diese Synapse spezifischen Induktionsprotokolls (theta - burst stimulation) induziert. $TSC2^{+/-}$ Ratten zeigen deutlich reduzierte Amplituden von LTP. Auch nach Blockade GABAerger Inhibition fand sich dieser Unterschied.

Nachfolgend wurde hippocampale Langzeitdepression (LTD) durch zwei verschiedene Induktionsprotokolle induziert. Auch hier zeigte sich in beiden Versuchsansätzen, dass das Potential zur Ausbildung von LTD in den $TSC2^{+/-}$ Versuchstieren deutlich reduziert ist.

Die elektrophysiologischen Untersuchungen der hippocampalen synaptischen Kurz- und Langzeitplastizität zeigen, dass, bei grundlegend unveränderter, basaler synaptischer Transmission sowohl die Kurzzeitplastizität, als auch LTP und LTD als Formen von synaptischer Langzeitplastizität in den $TSC2^{+/-}$ Versuchstieren substantiell reduziert ist. Die Resultate belegen einen gravierenden Verlust der Fähigkeit von $TSC2^{+/-}$ Versuchstieren, aktivitäts- abhängige synaptische Modifikation auszubilden.

Mögliche zugrundeliegende Mechanismen sind vielfältig, mit Hilfe der verwendeten Methoden aber nicht genau zu klären. Eine veränderte Aktivierung der durch Tuberin modulierten Transduktionskaskaden in Form einer Überaktivierung der Kaskade, könnte

einen solchen Verlust an synaptischem Plastizitätspotential erklären. In manchen Krankheitsmodellen sind LTP Defizite auf veränderte GABAerge Inhibition zurückzuführen. In der vorliegenden Arbeit wurde dieser potentielle Mechanismus durch die Integration von Experimenten unter Blockade GABAerger Inhibition und deren Resultate ausgeschlossen.
Ein Hauptmechanismus der Informationsspeicherung des ZNS ist somit in den $TSC2^{+/-}$ Versuchstieren nicht funktionstüchtig. Schon lange Zeit wurde postuliert und bereits für viele andere neurologische Krankheiten, wie zum Beispiel Epilepsie oder Morbus Alzheimer gezeigt, dass die Einschränkung kognitiver Funktionen oder Gedächtnisverluste auf veränderte, fehlerhafte oder gar nicht funktionierende Mechanismen der synaptischen Plastizität zurückzuführen sind.

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Impaired synaptic plasticity in a rat model of tuberous sclerosis

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Abstract

Tuberous sclerosis complex (TSC) is a common hereditary disorder caused by mutations in either the TSC1 or TSC2 genes, and characterized by severe epilepsy, cerebral hamartomas and mental retardation. We have used rats that are heterozygous for an autosomal-dominant germline mutation in the TSC2 gene ($TSC2^{+/-}$ rats) to examine the consequences of TSC2 mutations for hippocampal synaptic plasticity. While basal synaptic transmission in the Schaffer collateral–CA1 synapse was not altered, paired-pulse plasticity was significantly enhanced in $TSC2^{+/-}$ rats (interpulse intervals 20–200 ms). Moreover, $TSC2^{+/-}$ rats exhibited a marked reduction of different forms of synaptic plasticity. Long-term potentiation (LTP) elicited following high-frequency tetanization of Schaffer collaterals was significantly decreased from 1.45 ± 0.05-fold potentiation to 1.15 ± 0.04 (measured after 60 min). This difference in LTP levels between $TSC2^{+/-}$ and wild-type rats also persisted in the presence of the γ-aminobutyric acid $(GABA)_A$ receptor antagonist bicuculline. In addition to changed LTP, the level of long-term depression (LTD) elicited by different forms of low-frequency stimulation was significantly less in $TSC2^{+/-}$ rats. These results suggest that TSC2 mutations may cause hippocampal synapses to lose much of their potential for activity-dependent synaptic modification. An understanding of the underlying molecular pathways may suggest new therapeutic approaches aimed at inhibiting the development of the profound mental retardation in TSC.

Introduction

Tuberous sclerosis complex (TSC) is a severe neurological disorder that affects about 1 of 8000 children. It manifests in early childhood with symptoms including severe epilepsy and pronounced mental retardation (Roach *et al*., 1998; Curatolo, 2003). On a neuropathological level, patients with tuberous sclerosis display developmental malformations characterized by disturbed cortical organization and the presence of dysplastic neurons and giant cells (Crino *et al*., 1996; Vinters *et al*., 1999). TSC is caused by mutations in either of two genetic loci, TSC1 (9q34) and TSC2 (16p13), with the latter being about four times more frequent in the affected patient population (Jones *et al*., 1999; Dabora *et al*., 2001). While the clinical phenotype is similar for both mutations, some differences exist. For instance, severe mental retardation appears to be more strongly associated with TSC2 mutations (Jones *et al*., 1997; Dabora *et al*., 2001).

The TSC1 and TSC2 genes encode for the proteins hamartin and tuberin, respectively, which are widely expressed in the human brain (Menchine *et al*., 1996; Johnson *et al*., 1999). A multitude of recent studies has begun to unravel the functional relevance of these molecules in intracellular signalling cascades. Hamartin and tuberin are known to interact (Van Slegtenhorst *et al*., 1998; Nellist *et al*., 1999), and have been shown to affect the mTOR cascade (Gao *et al*., 2002; Inoki *et al*., 2002; El-Hashemite *et al*., 2003). It is thought that loss-of-function mutations in either TSC1 or TSC2 may cause enhanced activity of the mTOR cascade (Manning & Cantley, 2003; Zhang *et al*., 2003), which in turn induces transcriptional regulation of specific genes. In addition to the mTOR cascade, tuberin functionally downregulates several ras-related G-proteins, including rap1 and rab5 (Wienecke *et al*., 1995; Xiao *et al*., 1997; Yoon *et al*., 2004), essential signalling molecules in the Ca^{2+}-cAMP/PKA–p44/42-MAPK pathway involved in the transcriptional activation of plasticity-associated genes (Waltereit *et al*., 2001).

In contrast to the emerging pathogenetic models linking TSC mutations to the malformations found in TSC (Kenerson *et al*., 2002; Baybis *et al*., 2004), the pathogenesis of the severe cognitive decline of patients with TSC has remained a mystery. A major putative mechanism of learning and memory is activity-dependent synaptic plasticity. Thus, one underlying cellular mechanism for cognitive impairment could be disturbed synaptic plasticity in brain regions important for specific cognitive functions, as hypothesized for other neurological disorders (see, i.e. Jiang *et al*., 1998; Beck *et al*., 2000; Goussakov *et al*., 2000; Costa *et al*., 2001; Oddo *et al*., 2003; Weeber *et al*., 2003; Bear *et al*., 2004) or during ageing (i.e. Auerbach & Segal, 1997). Indeed, both the mTOR (Tang *et al*., 2002; Cammalleri *et al*., 2003; Kelleher *et al*., 2004) and the p44/42-MAPK signalling cascades (Morozov *et al*., 2003; see Sweatt, 2004 for review) that are modulated by tuberin are also profoundly involved in the regulation of synaptic efficacy. Collectively, these data suggest an impact of TSC mutations on synaptic plasticity.

We have examined this issue in a rat model carrying a spontaneous TSC2 mutation (Eker & Mossige, 1961; Hino *et al*., 1995; Kobayashi *et al*., 1995; Xiao *et al*., 1995). The Eker rat is an autosomal-dominant germline mutation of TSC2 truncating the GTPase-activating domain, thereby disrupting the function of tuberin (Yeung *et al*., 1994, 1995; Hino *et al*., 1995; Xiao *et al*., 1995). The Eker rat has some similarities to human TSC. For instance, as in humans, the homozygous $TSC2^{-/-}$ rat is embryonically lethal due to disrupted neuroepithelial growth (Rennebeck *et al*., 1998). The heterozygous Eker rat ($TSC2^{+/-}$)

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develops kidney tumours and, at higher age (≥ 2 years) and lower frequency develops cerebral hamartomas resembling those in human patients with TSC (Yeung *et al.*, 1997; Wenzel *et al.*, 2004). We have used $TSC2^{+/-}$ rats to study how loss-of-function mutations in the tuberin gene affect activity-dependent synaptic plasticity in the hippocampus. We find that both long-term potentiation (LTP) and long-term depression (LTD) are severely impaired in $TSC2^{+/-}$ rats.

Materials and methods

Animals

A breeding colony of the Long–Evans strain carrying the Eker mutation (Eker & Mossige, 1961) was transferred from the M.D. Anderson Cancer Center, University of Texas, to the Department of General Neurology, University of Tübingen. Eker ($TSC2^{+/-}$) and wild-type ($TSC2^{+/+}$) genotypes were determined by polymerase chain reaction (PCR; Rennebeck *et al.*, 1998). Experiments with Eker and wild-type rats were interleaved; in all cases the investigator was blinded to the genotype of the animals.

Electrophysiology

Male $TSC2^{+/-}$ and wild-type rats (30–50 days) were deeply anaesthetized by ketamine hydrochloride (100 mg/kg i.p.) and xylazine hydrochloride (80 mg/kg i.p.), and heart-perfused with 25 mL of cold artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl, 125; KCl, 3; $NaHCO_3$, 26; NaH_2PO_4, 1.25; $CaCl_2$, 2.5; $MgCl_2$, 1.3; D-glucose, 13 (95% O_2, 5% CO_2, pH 7.4). Following perfusion, animals were decapitated; brains were removed and immediately immersed into cold dissection ACSF (composition as above, except for $CaCl_2$, 0.25 mM and $MgCl_2$, 5 mM). Transverse hippocampal slices of 400 μm were prepared using a vibratome (WT 1000S, Leica, Wetzlar, Germany) and transferred to a submerged holding chamber where they were equilibrated for at least 1.5 h. Slices were subsequently directly transferred one at a time into an interface chamber perfused with carbogenated ACSF (2.5 mL/min, 35 °C). Experiments were begun after an equilibration time of > 0.5 h. For LTP experiments in bicuculline, a surgical cut was made between CA3 and CA1 regions to eliminate the propagation of burst discharges generated in the CA3 region. In this set of LTP experiments, slices were incubated in ACSF containing 5 μM bicuculline for ∼ 1.5 h before initiating recording.

Osmolality was adjusted to 306–316 mosmol/kg with sucrose in all solutions. Recording was started after 1 h of recovery in the interface chamber. Recording of field excitatory postsynaptic potentials (fEPSPs) was performed with borosilicate glass recording electrodes (∼ 5 MΩ) filled with ACSF. Monopolar stimulation was performed with low-resistance borosilicate glass electrodes (∼ 1 MΩ). Stimulation and recording electrodes were placed in the stratum radiatum of the CA1 region. Stimuli were applied with a stimulus isolator (WPI, Sarasota, USA). Analogue data were digitized (Digidata 1200, Axon Instruments, USA) and stored for offline analysis (pClamp software, Axon Instruments, USA). Baseline stimulation strength was adjusted to 60% of the maximal fEPSP amplitude. LTP was induced with a tetanus consisting of 10 brief trains of five stimuli at 100 Hz, separated by 200-ms intervals. The duration of each individual stimulus was always 100 μs. During the tetanus, stimulation intensity was increased twofold compared with the baseline stimulation intensity. Two different LTD induction protocols were used. A first set of experiments was carried out with application of 900 stimuli at 1 Hz, in a second set LTD was induced by two trains of 1 Hz stimulation (600 stimuli each, 10 min apart). Full bars represent mean values and error bars the standard error of the mean. Statistical comparison was carried out with Student's unpaired two-tailed *t*-test with the level of significance set to $P < 0.05$. Significant differences are indicated with asterisks in all figures.

Results

Altered short-term synaptic plasticity in $TSC2^{+/-}$ rats

We first examined basal synaptic transmission and short-term synaptic plasticity in $TSC2^{+/-}$ rats compared with wild-type littermates at the Schaffer collateral–CA1 synapse (see Materials and methods). When the stimulation intensity was systematically enhanced, fEPSP amplitudes increased to a saturating value, which was not different in $TSC2^{+/-}$ rats (1.50 ± 0.17 mV, $n = 9$) compared with wild-type littermates (1.50 ± 0.29, $n = 7$). Input–output curves generated by plotting the fEPSP amplitude vs. stimulation strength did not reveal a shift in the input–output relation at the Schaffer collateral–CA1 synapse (Fig. 1A and B). We then examined short-term synaptic plasticity by applying two closely spaced stimulations to the Schaffer collaterals (interpulse interval 10–500 ms, example traces in Fig. 1C with interstimulus interval of 50 ms). Wild-type littermate controls displayed paired-pulse facilitation (PPF) at interpulse intervals of 20–200 ms (Fig. 1D, empty bars). $TSC2^{+/-}$ rats showed significantly more PPF at all these interpulse intervals (filled bars, asterisks in Fig. 1D). We asked whether this significantly different PPF is altered because of altered γ-aminobutyric acid (GABA)ergic inhibition. The PPF ratio was tested with two stimuli given 40 ms apart from each other. $TSC2^{+/-}$ rats (1.35 ± 0.04, $n = 4$; see Fig. 1E and filled bars in Fig. 1F) displayed significantly more PPF than wild-type littermate controls (1.21 ± 0.01, $n = 5$; see Fig. 1E and empty bars in Fig. 1F), while PPF values were not significantly altered by applying 5 μM bicuculline ($TSC2^{+/-}$: 1.33 ± 0.04, $n = 7$; wild-type: 1.21 ± 0.03, $n = 6$, Fig. 1F).

Reduced LTP in $TSC2^{+/-}$ rats

Following the demonstration of altered short-term synaptic plasticity, we asked whether long-term synaptic plasticity might also be affected in $TSC2^{+/-}$ rats. In wild-type animals, application of a theta-burst stimulation (TBS) to Schaffer collaterals caused an initial post-tetanic potentiation (2.19 ± 0.25-fold potentiation compared with pre-tetanus baseline), with a subsequent robust and stable long-term potentiation of the fEPSP slope (1.45 ± 0.05-fold potentiation, measured 1 h after TBS, $n = 12$, empty symbols in Fig. 2A). $TSC2^{+/-}$ rats exhibited pronounced post-tetanic potentiation that was not significantly different from control animals (1.96 ± 0.11-fold potentiation, Fig. 2C). However, $TSC2^{+/-}$ rats exhibited significantly reduced levels of LTP (1.15 ± 0.04-fold potentiation after 1 h, $n = 12$, filled symbols in Fig. 2A, and summary in Fig. 2D, $P < 0.05$).

Reduced LTP in $TSC2^{+/-}$ rats in the presence of $GABA_A$ receptor inhibition

To assess whether the reduced LTP in $TSC2^{+/-}$ rats is due to altered GABAergic inhibition, we performed LTP experiments with stimulus protocol identical to those described above in the presence of 5 μM bicuculline, a specific $GABA_A$ receptor antagonist. In wild-type animals, TBS resulted in initial post-tetanic potentiation (2.81 ± 0.21-fold potentiation compared with pre-tetanus baseline) with robust and stable long-term potentiation of the fEPSP slope (1.42 ± 0.08-fold potentiation measured 1 h after TBS, $n = 7$, empty symbols in

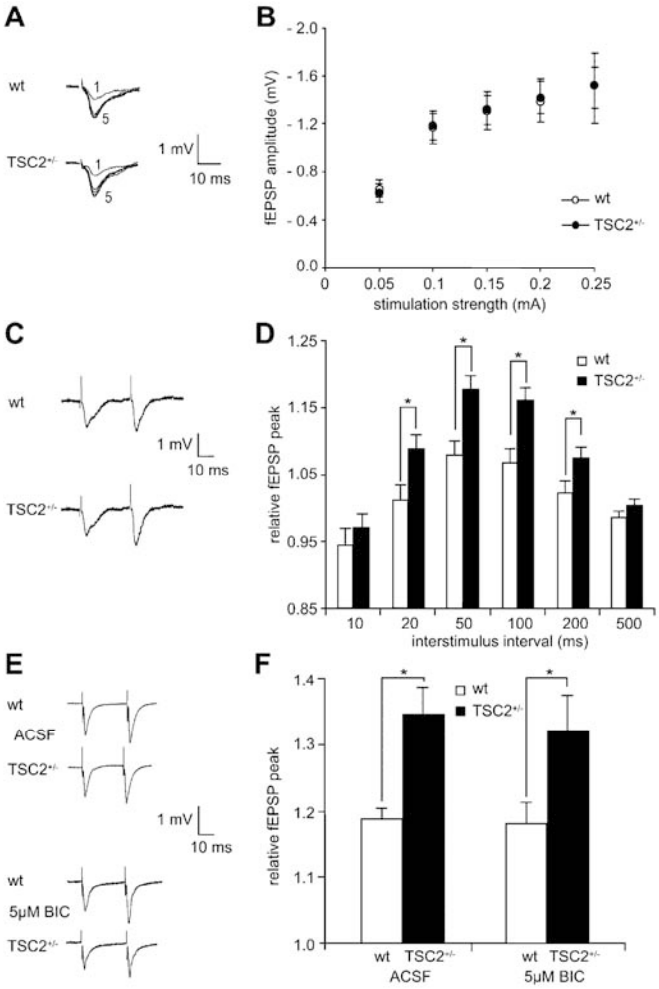

FIG. 1. Input–output relation and PPF in wild-type and $TSC2^{+/-}$ rats. (A and B) The input–output relation was tested by applying five consecutive stimulations, 30 s apart, each with a different stimulation strength, enhanced from 0.05 to 0.25 mA (representative traces shown in A for each stimulation strength, 1 shows the trace with stimulation of 0.05 mA, 5 shows the trace with stimulation of 0.25 mA). (B) Summary of input–output relation that shows absolute values of field excitatory postsynaptic potential (fEPSP) peak amplitudes for both wild-type (empty circles) and $TSC2^{+/-}$ rats (filled circles). (C and D) PPF was tested by applying two consecutive synaptic stimulations 10–500 ms apart (representative traces shown in C for 50 ms interstimulus interval). PPF was quantified as the ratio of the second to the first fEPSP peak amplitude. (E and F) PPF with 40 ms interstimulus interval in control conditions (ACSF) and in conditions of GABAergic inhibition with 5 µM bicuculline (BIC) (representative averaged traces shown in E, summary in F). $P < 0.05$.

Fig. 2B). Again, $TSC2^{+/-}$ rats showed levels of post-tetanic potentiation that were not significantly different from control animals (2.63 ± 0.14-fold potentiation, filled symbols in Fig. 2B, summary in Fig. 2C). In the absence of $GABA_A$ receptor-mediated inhibition, however, LTP was again dramatically reduced in $TSC2^{+/-}$ rats (1.13 ± 0.09-fold potentiation after 1 h, $n = 9$, filled symbols in Fig. 2B, and summary in Fig. 2D, $P < 0.05$). This indicates that loss of LTP in $TSC2^{+/-}$ rats is not caused by altered GABAergic inhibition. It should be noted that the decay of the amplitude of fEPSPs following the initial post-tetanic potentiation was slowed markedly in the presence of bicuculline, both in $TSC2^{+/-}$ rats and wild-type animals, but this interesting phenomenon was not further investigated.

Reduced LTD in $TSC2^{+/-}$ rats

We subsequently examined changes in hippocampal LTD at the same synaptic location (Fig. 3). LTD was induced with two different low-frequency stimulation protocols. Following application of 900 stimuli at

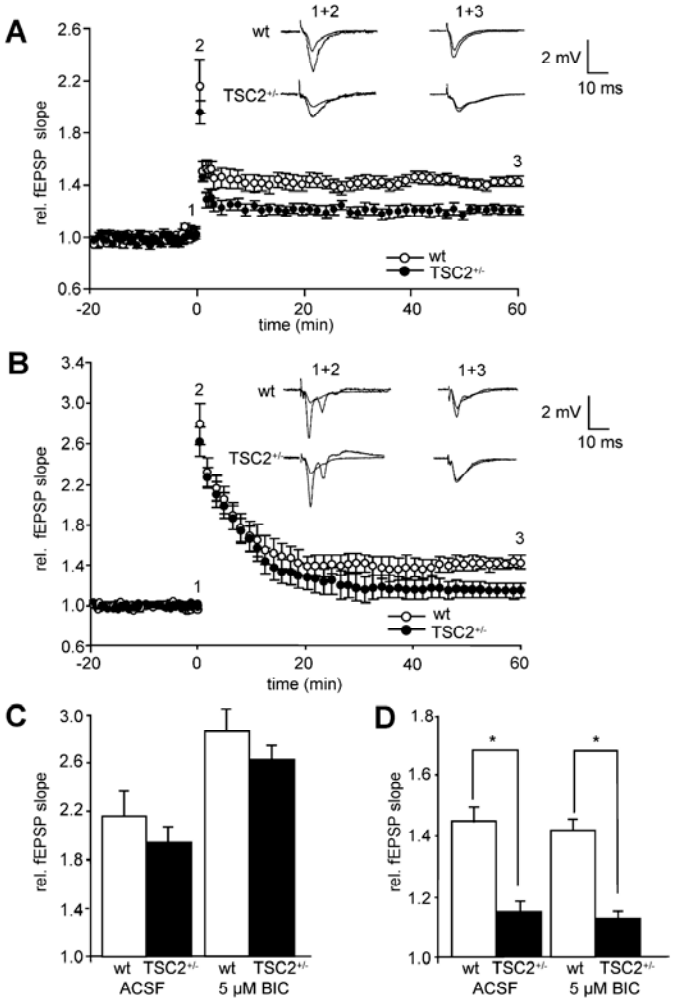

FIG. 2. (A and B) Post-tetanic potentiation and LTP in control (ACSF) and after $GABA_A$ receptor inhibition with bicuculline (BIC), respectively. Averaged time course of LTP induced with tetanic stimulation (see Materials and methods) applied at time point 0, in both wild-type (empty symbols) and $TSC2^{+/-}$ rats (filled symbols) in control condition (ACSF; A) and after $GABA_A$ receptor inhibition (B), respectively. Representative traces were obtained at the time points indicated by the numbers. (C) Summary of the amount of post-tetanic synaptic potentiation in both wild-type (empty bars) and $TSC2^{+/-}$ rats (filled bars) in control (ACSF) and after $GABA_A$ receptor inhibition (BIC). (D) Summary of the amount of synaptic potentiation after 1 h in both wild-type (empty bars) and $TSC2^{+/-}$ rats (filled bars) in control condition (ACSF) and after $GABA_A$ receptor inhibition (BIC), respectively. The mean values were expressed as the ratio of field excitatory postsynaptic potential (fEPSP) slope immediately after tetanization (C), respectively, 1 h after tetanization (D) to the fEPSP slope immediately before tetanization. $P < 0.05$.

1 Hz, the fEPSP slope was initially strongly depressed in wild-type rats (post-tetanic depression, 0.70 ± 0.01 of pre-tetanus baseline, empty symbols in Fig. 3A), with stable long-term depression after 60 min (0.92 ± 0.02, $n = 5$). $TSC2^{+/-}$ rats exhibited a marked reduction in post-tetanic depression and loss of LTD (post-tetanic depression: 0.96 ± 0.03; LTD: 0.98 ± 0.01, $n = 9$, filled symbols in Fig. 3A and D). Similar findings were obtained following stimulation with two consecutive trains of 1 Hz stimulation (600 stimuli, 10 min apart, Fig. 3B). In wild-type rats, significant LTD was observed (0.83 ± 0.05, empty symbols, $n = 9$), whereas $TSC2^{+/-}$ rats showed a marked decrease in the amount of depression (0.94 ± 0.02, filled symbols, $n = 5$, $P < 0.05$, Fig. 3D for summary). Interestingly, post-tetanic depression was not different following this latter LTD induction protocol (0.78 ± 0.05 vs. 0.75 ± 0.05 in wild-type vs. $TSC2^{+/-}$ animals, Fig. 3B, and Fig. 3C for summary). Taken together, these experiments show that both LTP and LTD are markedly impaired in $TSC2^{+/-}$ rats, indicating

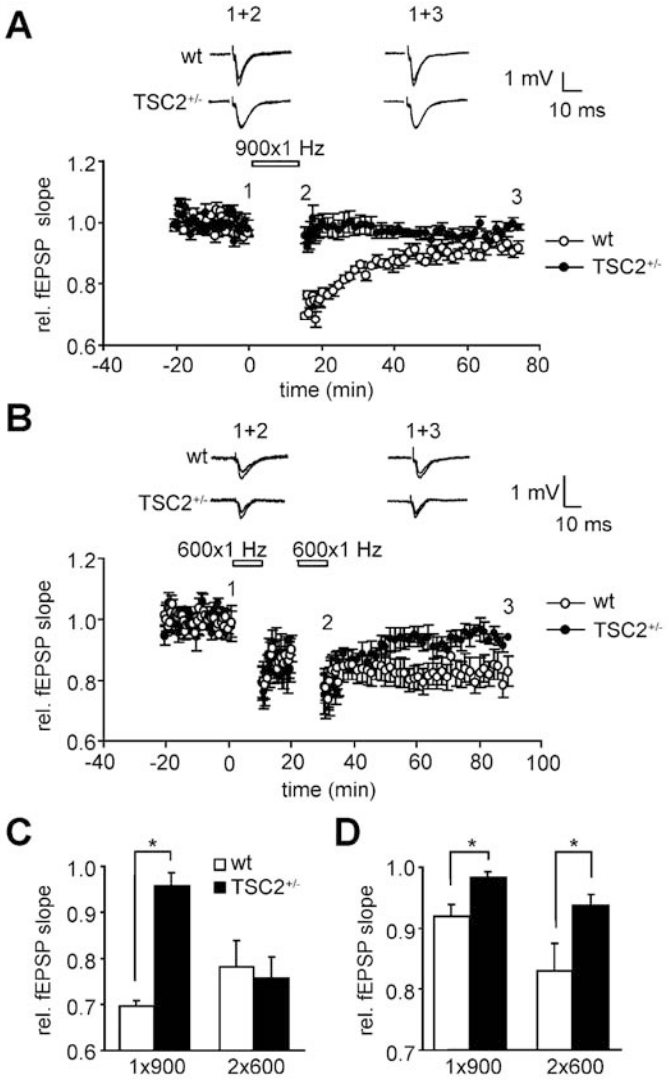

FIG. 3. (A and B) LTD in wild-type and TSC2$^{+/-}$ rats. (A) Averaged time course of LTD induced by a single train of 900 stimuli (1 Hz, indicated by the horizontal bar). (B) Time course of LTD induced by two consecutive trains of 600 stimuli (1 Hz, 10 min apart). Representative traces were obtained at the time points indicated by the numbers. (C) Amount of post-tetanic depression of field excitatory postsynaptic potential (fEPSP) slope observed immediately after LTD induction with two different stimulation patterns. (D) Amount of LTD measured 1 h after the end of the inducing stimulus train, expressed as the ratio of fEPSP slope following LTD to fEPSP slope immediately preceding LTD induction. $P < 0.05$.

that hippocampal synapses may have lost much of their normal capacity for activity-dependent synaptic modification.

Discussion

Activity-dependent changes in the efficacy of synapses are key mechanisms for information storage that enable connections between neurons to retain a trace of prior activity. These activity-dependent changes can appear as LTP and LTD. In the present study, we find a significant reduction in the potential to exhibit synaptic plasticity in the hippocampus of TSC2$^{+/-}$ rats, a model of TSC. This applies both to LTP and LTD elicited by different protocols, suggesting that Schaffer collateral–CA1 synapses have lost much of their potential for bi-directional activity-dependent synaptic modification.

What might be the cellular mechanism underlying altered synaptic plasticity? Tuberin functionally affects two major cascades that are involved in synaptic plasticity: the p44/42-MAPK cascade and the mTOR cascade. Such an altered activation appears likely because tuberin functionally downregulates several ras-related G-proteins, notably rap1 (Wienecke *et al*., 1995; Yoon *et al*., 2004). Rap1, in turn, is an essential component of the Ca^{2+}-cAMP/PKA–p44/42-MAPK pathway, which is required for several forms of hippocampal LTP (English & Sweatt, 1997; Coogan *et al*., 1999; Kanterewicz *et al*., 2000). In the $TSC2^{+/-}$ rats, we would expect the converse: loss of inhibitory control over rap1 would lead to overactivation of the MAPK pathway. Indeed, such an overactivation has been shown in $TSC2^{+/-}$ animals (Waltereit *et al*., 2004). Both the MAPK and mTOR signalling cascades have been shown to be involved in protein synthesis-dependent late LTP (Tang *et al*., 2002; Cammalleri *et al*., 2003).

Another mechanism that may account for altered synaptic plasticity might be altered trafficking of AMPA receptors. Rap1 activation appears to mediate LTD by removing AMPA receptors during LTD expression, while ras activation has the converse effect (Zhu *et al*., 2002). Constitutive activation of rap1 due to loss of tuberin in $TSC2^{+/-}$ animals may therefore also disturb AMPA receptor trafficking to and from the postsynaptic density.

In some disease models, deficits in LTP are due to secondary effects of gene mutations on GABAergic inhibition. For instance, mice carrying a heterozygous null mutation of the NF1 gene encoding for neurofibromin display upregulated ras-p44/42-MAPK signalling (Ingram *et al*., 2001), resulting in impaired LTP via enhanced GABAergic inhibition (Costa *et al*., 2002). This is particularly interesting because mutations in the NF1 gene give rise to neurofibromatosis type 1 in humans, which is an inherited disorder associated with mental retardation like TSC (Kandt, 2003). In our study, however, we show that, unlike NF1 mutant mice, the mechanism leading to decreased LTP in $TSC2^{+/-}$ rats is not due to enhanced GABAergic inhibition.

Regardless of the mechanism involved, a major mechanism for information storage in the CNS appears to be dysfunctional. Given the importance of different forms of synaptic plasticity as a candidate mechanism for memory processes, it has long been thought that impaired synaptic plasticity might underlie impaired memory performance in several neurological diseases. Findings of decreased synaptic plasticity in models of Alzheimer's disease (e.g. Nalbantoglu *et al*., 1997; Oddo *et al*., 2003), epilepsy (Beck *et al*., 2000; Goussakov *et al*., 2000), fragile X syndrome (Godfraind *et al*., 1996), neurofibromatosis (Costa *et al*., 2001) or during ageing (i.e. Auerbach & Segal, 1997) are in line with this view. In the specific case of TSC, altered synaptic plasticity even occurred before $TSC2^{+/-}$ rats generally exhibit brain pathology (Takahashi *et al*., 2004). Moreover, the deficit in synaptic plasticity observed could be even more pronounced following the onset of recurrent seizures, which also reduce the potential for hippocampal synaptic plasticity at different synapses (Beck *et al*., 2000; Goussakov *et al*., 2000; Kirschstein & Beck, 2004). Such a mechanism may contribute to the clinical observation that the onset of convulsions has a devastating effect on the intellectual capabilities of patients with TSC.

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Abbreviations

ACSF, artificial cerebrospinal fluid; fEPSP, field excitatory postsynaptic potential; GABA, γ-aminobutyric acid; LTD, long-term depression; LTP, long-term potentiation; PPF, paired-pulse facilitation; TBS, theta-burst stimulation; TSC, tuberous sclerosis complex.

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