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INCREASING AGE REDUCES EXPRESSION OF LONG-TERM DEPRESSION AND DYNAMIC RANGE OF TRANSMISSION PLASTICITY IN CA1 FIELD OF THE RAT HIPPOCAMPUS

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Abstract—Long-term depression, depotentiation and long-term potentiation of field excitatory postsynaptic potentials in the CA1 field of the hippocampus were studied in slices from two-, 12-, 24- and 36-week-old rats. Long-term potentiation was induced by stimulating afferent fibres for 1 s at 100 Hz. Long-term depression was induced either by stimulating the afferent pathways twice for 15 min at 1 Hz (protocol 1), giving in total 1800 pulses, or by stimulating the fibres at 5 min intervals twice at 1 Hz for 5 min followed by 5 min stimulation at 5 Hz (protocol 2), giving in total 2100 pulses. We found significant long-term depression in slices of all groups stimulated with protocol 1; however, the magnitude of long-term depression in slices from 24-and 36-week-old rats was significantly lower than that in slices from two- and 12-week old rats, although there was no such difference in the magnitude of long-term potentiation between slices. Stimulation protocol 2 induced long-term depression only in slices from two- and 12-week-old rats. Comparison of the dynamic range of transmission plasticity in slices from two- and 36-week-old rats, calculated as the difference between the nearly saturated long-term potentiation and nearly saturated depotentiation, revealed a significantly smaller dynamic range in slices from 36-week-old rats in comparison with slices from two-week-old animals. The decrease in the dynamic range in slices from 36-week-old rats was due to a diminished capacity to depotentiate the nearly saturated long-term potentiation and not due to a decreased long-term potentiation expression in these slices. In contrast to long-term depression, in which the slope of the field excitatory postsynaptic potentials consistently and significantly decreased below the baseline level, the nearly saturated depotentiation did not decrease below the original, pre-long potentiation baseline level.

The results demonstrate that increasing age reduces expression of long-term depression and the dynamic range of transmission plasticity. © 1998 IBRO. Published by Elsevier Science Ltd.

Key words: hippocampus, age, long-term depression, long-term potentiation, plasticity.

Long-term modification of synaptic strength is thought to be involved in the storage of information in the brain.[20] Two forms of long-term modification of synaptic strength have been demonstrated in the CA1 field of the hippocampus, a structure implicated in memory processes.[45] One of these is the well established and well-studied long-term potentiation (LTP) that was first described by Bliss and Lømo in 1973[7] in the synapse of the perforant path to granule cells of the gyrus and later found in the CA1 field of the hippocampus and in many other brain structures including the neocortex.[26,51] The other is long-term depression (LTD), which was originally seen in the CA1 field of the hippocampus as a heterosynaptic correlate of the homosynaptic LTP in this field.[34] Later, heterosynaptic LTD was also seen in the dentate gyrus,[1,30,31] and homosynaptic form of LTD was found in the CA1 field of the hippocampus[14,37] and elsewhere in the brain.[32]

Originally, LTP was induced by brief (1–2 s) high-frequency (50–100 Hz) stimulation (HFS) of afferent fibres.[7] However, other stimulation protocols, such as e.g., theta bursts[29] and primed burst stimulations[43] or a combination of low-frequency stimulation (LFS) with strong depolarization of the postsynaptic neuron,[19] appear to be equally efficient in inducing LTP in the hippocampus. LTD in the CA1 field of the hippocampus can be most readily elicited by stimulation at 1–5 Hz stimulation.[14,37] Stimulation at 10 Hz or higher has no effect or it elicits LTP.[14] LTD in the CA1 field of the hippocampus, like LTP in this field,[8] is input specific, and both LTP and LTD require activation of *N*-methyl-D-aspartate (NMDA) type glutamate receptors and Ca^{2+} influx through channels coupled to these receptors.[14,37,39] This suggests that the same receptors but different intracellular effectors are involved in these contrasting forms of synaptic plasticity.

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Abbreviations: ANOVAR, analysis of variance for repeated measurements; APV, D(−)-2-amino-phosphonovaleric acid; fEPSPs, field excitatory postsynaptic potentials; HFS, high-frequency stimulation; LFS, low-frequency stimulation; LTD, long-term depression; LTP, long-term potentiation; NMDA, *N*-methyl-D-aspartate.

The expression of LTP and LTD is typically measured as an increase or decrease, respectively, in the slope of the field excitatory postsynaptic potentials (fEPSPs), evoked by stimulation of the afferent pathway, relative to that of the baseline response to test stimulation, obtained in naive hitherto not-stimulated slices, before the application of the conditioning stimulation protocols.

LTP of a specific set of synapses can readily be reversed by LFS. This reversal of LTP, reported originally by Barrionuevo and co-workers[3] and later confirmed by many others (e.g., see Ref. 15) has been termed depotentiation.[16] Depotentiation can be seen as a mechanism by which a potentiated synapse can regain its original or even a lower transmission strength, thereby making the synapse available for the next LTP event. Indeed, like the naive synapse, the synapse that is expressing LTD can be readily potentiated to express LTP if HFS is applied to the neuron during the LTD phase of transmission.[15,23] The magnitude of LTP and LTD and/or depotentiation can be seen as reflecting the dynamic range of long-term modulation of transmission strength in glutamatergic synapses.[5] This dynamic range may be of significance for information processing by the hippocampus.

Although the stimulation protocol used, the receptors involved, and the magnitude and duration of expression are similar for depotentiation and LTD,[5] it is still uncertain whether LTD and depotentiation are indeed the same process, mediated by a same intracellular effector system. In addition, up to 24 months-of-age there is no significant decline in the magnitude of LTP expression in the CA1 field of the rat hippocampus,[12,13,27] although difficulties in induction of LTP in slices from rats older than 12 weeks have been reported.[24] However, LTD can be reliably and readily elicited only in slices from young rats up to six weeks-of-age.[14,47] In fact, LTD expression in adult rats older than eight to 12 weeks has very seldom been reported.[2,21]

Here, we studied the effect of age (two, 12, 24 and 36 weeks) on the magnitude of LTP and LTD expression in the CA1 field of the rat hippocampus. LTP was induced by 1 s stimulation at 100 Hz. LTD was induced by two different protocols, one protocol, 1, consisted of stimulation for 15 min at 1 Hz, repeated twice within an interval of 15 min, giving in total 1800 pulses. We chose to repeat this original protocol[14,17] twice in order to exclude the possibility of failure of LTD induction as a result of insufficient number of low frequency stimuli that might have a cause of the failing LTD induction in other studies in slices from adult animals (e.g., see Ref. 24). The other protocol (2), in which a twice-repeated LFS for 5 min at 1 Hz was followed by stimulation at 5 Hz, a frequency used by some authors to elicit depotentiation.[46,56] We also compared the dynamic range of transmission plasticity in the CA1 field of the hippocampus measured as the difference in magnitude of the slope of the nearly fully potentiated and nearly fully depotentiated fEPSPs in slices from two- and 36-week-old rats. Some of the results have been reported earlier.[53]

EXPERIMENTAL PROCEDURES

Animals and preparation

Male Wistar male rats were used. The rats were housed two per home cage, maintained on a 12/12 h dark/light cycle, with water and food *ad libitum*. At age of two, 12, 24 and 36 weeks after birth, rats were anaesthetized by inhalation of Isoflurane^{R} and decapitated. The brains were rapidly taken out of the skull and put in ice-cold oxygenated (95% O_2, 5% CO_2) medium of the following composition (mMol): 124.0 NaCl, 3.3 KCl, 1.2 KH_2PO_4, 1.3 $MgSO_4$, 10.0 glucose, 20.0 $NaHCO_3$, and 2.5 $CaCl_2$. Transversal, 450 µm-thick slices were prepared as described elsewhere.[42] The slices were kept for at least 60 min in oxygenated medium at room temperature before being used.

Recording

After slices had been left undisturbed in the recording chamber for about 15 min, bipolar stainless steel electrodes, made from a stainless steel wire of 100 µm in diameter, insulated except for the tip, were placed on the afferent fibres of the stratum radiatum of the CA1 field of the hippocampus. Recordings of fEPSPs were recorded in the stratum radiatum were made by using glass microelectrodes with a tip diameter of approximately 2 µm, filled with the incubation medium. Individual fEPSPs were amplified, digitized at a sampling rate of 10 kHz and stored in a computer, using Spike2^{R} software (Cambridge Electronic Devices Ltd, U.K.). Each experiment started by determining the stimulus-intensity to elicit a threshold (I_1) and maximum (I_5) fEPSPs. Subsequently, stimulus–response relationship was determined for five stimulation intensities (I_1–I_5), starting from the threshold level (I_1) and increasing in proportional steps, calculated as (I_5–I_1)/4. The stimulus intensity eliciting half maximum fEPSPs, typically between 65–125 µA, was used as the test stimulus throughout the experiments. Only the slices in which the amplitude of the fEPSPs elicited with maximum stimulus was greater than 1 mV were used for further experiments.

Experimental protocol

Expression of long-term depression and long-term potentiation with increasing age. LTD expression was studied in slices prepared from rats aged two weeks (n=6), 12 weeks (n=7), 24 weeks (n=8) and 36 weeks (n=8). LTD was induced by two different protocols. Protocol 1 consisted of stimulation for 15 min at 1 Hz followed by a 15 min rest period after which another 15 min stimulation at 1 Hz was administered, giving a total of 1800 pulses. Protocol 2 consisted of 5 min stimulation at 1 Hz, 5 min rest, 5 min stimulation at 1 Hz, followed by 5 min stimulation at 5 Hz, giving a total of 2100 pulses. LTP was studied in slices from rats aged two weeks (n=7), 12 weeks (n=8), 24 weeks (n=9) and 36 weeks (n=9). Stimulation for 1 s at 100 Hz was used to induce LTP. Test stimuli in all experiments were administered at a frequency of 0.05 Hz; fEPSPs were recorded for at least 60 min after the LTD- or LTP-inducing stimulations unless specified otherwise.

*Input specificity and N-methyl-*D*-aspartate receptor dependence of long-term depression in the CA1 field of the hippocampus*. The input-specificity of LTD was studied in three slices from 12-week-old rats by stimulating two sites on either side of the recording electrode with S_1 and S_2 intensities. These experiments started by determining the

stimulus–response relationship for S_1 and S_2 stimuli of increasing intensity, followed by a 15 min recording of baseline fEPSPs elicited by S_1 and S_2 test stimuli administered alternatively at a frequency of 0.05 Hz. At 0 min, the LFS protocol 1 (15 min, 1 Hz) was applied to the S_1 input. The fEPSPs responses to stimulation at S_1 and S_2 were recorded for 60 min after LTD induction. NMDA glutamate receptor dependence was tested in one slice from a 12-week-old rat. Baseline fEPSPs were recorded for 15 min and then a solution containing 50 μmol NMDA receptor antagonist D(−)-2-amino-5-phosphonovaleric acid (APV) was applied for 30 min. At the end of antagonist application, the LFS protocol 1 was applied. The antagonist was washed out of the preparation by superfusion with the control medium during recording of the EPSPs elicited by test stimuli. After a 40 min washout period, LFS of the protocol 1 was again applied and fEPSPs were recorded for another 60 min.

The dynamic range of plasticity in hippocampus of two- and 36-week-old rats. The dynamic range of transmission plasticity was studied in slices from two-week- (n=6) and 36-week-old (n=6) rats by comparing the difference in the slope of the fEPSPs in the maximally potentiated and maximally depotentiated state. After a 15 min period of recording baseline fEPSPs, HFS was applied three times at 20 min intervals in order to obtain nearly maximum LTP. 20 min after the last stimulation, depotentiation started by stimulating slices five times at 1 Hz for 5 min at 20 min intervals. fEPSPs were recorded throughout the stimulation period. The experiment was terminated 20 min after the last LFS.

Statistical analysis

. The average slope of baseline fEPSPs was computed and set at 100%. The slope of the fEPSPs during LTD and LTP is expressed as the percent change from baseline. Means (±S.E.M.) of the slope of fEPSPs were plotted. The means of stimulus–response relations of the fEPSPs for different age groups and stimulus intensities were compared using an analysis of variance for repeated measurements (ANOVAR). The presence of LTP and LTD in individual age groups were determined by comparing the average slope of the fEPSPs at baseline with that at 60 min after stimulation, using Wilcoxon's matched-pair signed-ranks test. A depression of 15% or more in the slope of fEPSPs, compared to the baseline, was considered to reflect LTD in individual slices. The differences in expression of LTD and LTP in slices from rats of different ages were compared by using an ANOVAR with a *post hoc* Duncan's multiple range test to identify age groups that had a different response. The difference in the mean dynamic range of plasticity between two-week- and 36-week-old rats was determined in two-tailed t-test for independent samples. The mean depression in the slope of the fEPSPs 5 min after either LTD protocol was used was compared in a two-tailed test for independent samples.

RESULTS

Figure 1 illustrates the mean (±S.E.M.) slope of fEPSPs, expressed as a percentage of baseline, for all stimulus intensities. A nearly linear increase was found in the relative slope of the fEPSPs evoked by a proportional increase in the stimulus intensity. ANOVAR comparison revealed no difference between age groups. However, the mean absolute slope of the baseline fEPSPs in slices from two-week-old rats was significantly greater than that of the slices from older rats (see Table 1).

Figure 2 summarizes the results of the experiment in which LTD was elicited with protocol 1 (Fig. 2A)

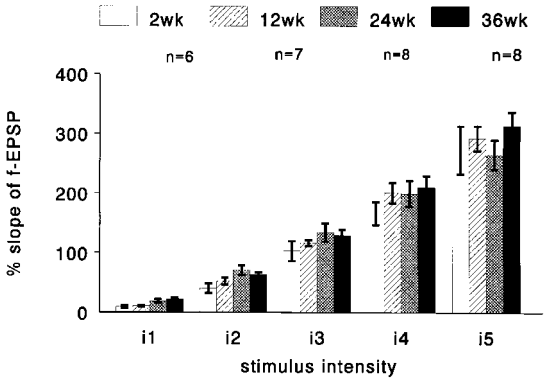

Fig. 1. Stimulus–response relation of the slope of fEPSPs elicited by stimulation with five increasing intensities ranging from about threshold (I_1) to maximum response stimulation (I_5) in slices from two-, 12-, 24- and 36-week-old rats. ANOVAR comparison revealed no significant differences between the groups. The average response to the test stimuli, approximately 50% of the maximum response (between I_2 and I_3), was set to 100%.

and protocol 2 (Fig. 2B) in slices from rats of different ages. In all slices in which LTD was elicited with protocol 1 (1 Hz), the slope of the fEPSPs at 60 min after the LTD-inducing stimulation was significantly different (Wilcoxon's test) from that at baseline, indicating that LTD was induced in all groups (typical example is on the inset to Fig. 2). LTD in slices from two- and 12-week-old was similar, amounting to 62.5±3.4% of the baseline (P=0.027; n=6) and 64.62±4.17% (P=0.018; n=7), respectively. LTD in slices from 24- and 36-week-old rats 60 min after LFS was less, reaching only 84.8±4.2% of the baseline (P=0.035; n=8) and 88.7±2.3% (P=0.018; n=7). ANOVAR comparison of the slope of the fEPSPs after the conditioning stimulation showed a highly significant difference between age groups (see Fig. 2A). The subsequent *post hoc* Duncan's test indicated that the magnitude of LTD in slices from two- and 12-week-old rats significantly differed from that in slices from 24- and 36-week-old rats, in which LTD was comparable.

Stimulation protocol 2 (1 Hz+5 Hz stimulation) induced LTD only in slices from two- and 12-week-old rats. At 60 min after the conditioning stimulation, the slope of the fEPSPs in slices from two-week-old rats was 65.8±5.3% of the baseline (P=0.027 in Wilcoxon's test; n=6) and 80.16±11.77% of the baseline (P=0.018; n=7), respectively (Fig. 2B). In slices from 24- and 36-week-old rats, the slope of the fEPSPs was 92.0±3.5% of the baseline (n.s.; n=8) and 92.5±3.7% (n.s.; n=8), respectively.

The average initial depression of the slope of the fEPSPs 5 min after the termination of stimulation slices from two- and 12-week-old rats was significantly (ANOVA) greater than that in slices from 24- and 36-week-old rats, irrespective of the stimulation protocol used. However, the depression following stimulation with protocol 2 was markedly greater

Table 1. Summary of the baseline slope of field excitatory postsynaptic potentials and the expression of long-term depression in slices from rats of different age

		Protocol 1 (1 Hz)			Protocol 2 (1 Hz+5 Hz)		
Group	Weight in g	Number	Mean (±S.E.M.) of the slope EPSP in mV/s	LTD no.(%)	Number	Mean (±S.E.M.) of the slope EPSP mV	LTD no.(%)
Two weeks	70	6	0.664±0.08*	6(100%)	6	0.518±0.08	5(83.3%)
12 weeks	300	7	0.426±0.04	7(100%)	7	0.371±0.05	5(71.4%)
24 weeks	500	8	0.428±0.02	5(62.5%)	8	0.404±0.02	1(12.5%)
36 weeks	550	7	0.466±0.05	3(42.9%)	8	0.419±0.03	1(12.5%)

no., number of animals expressing LTD; *significantly different from the remaining groups in ANOVA comparison ($F_{3,24}$=5.060; P=0.0074; *post hoc* Duncan's test indicated that group two weeks was different from the remaining groups).

than that seen after the stimulation with protocol 1 (see Fig. 2A,B). Thus, the slope of the fEPSPs in slices from two-week-old rats after stimulation with protocol 1 (1 Hz) decreased to 46.3±1.6% of the baseline, while it was 23.9±8.8% of the baseline with protocol 2 (P<0.05 in a two-tailed t-test). Also, in slices from 12-week-old rats was this depression 5 min after stimulation with protocol 2 significantly (P<0.05) greater than that after stimulation with protocol 1. In slices from 24-week-old rats, the mean slope of the fEPSPs at this time point after protocol 1 was 80.5±4.7% of baseline, while it was 64.1±3.9% of baseline after stimulation with protocol 2 (Fig. 2A,B). The initial depression in slices from 36-week-old rats was not significantly different from that of slices from 24-week-old rats.

The stimulation protocols also differed in terms of the number of slices that expressed LTD, especially in slices from older rats (see Table 1). With protocol 1, the slope of fEPSPs decreased by 15% or more in all slices from two- (n=6) and 12-week-old (n=7) rats, whereas protocol 2 elicited such a decrease in five out of six slices from two-week-old rats and in five out of seven slices from 12-week-old rats. In slices from 24-week-old rats, protocol 1 depressed the slope of the fEPSPs in five out eight slices, whereas protocol 2 caused such a depression in only one out eight slices.

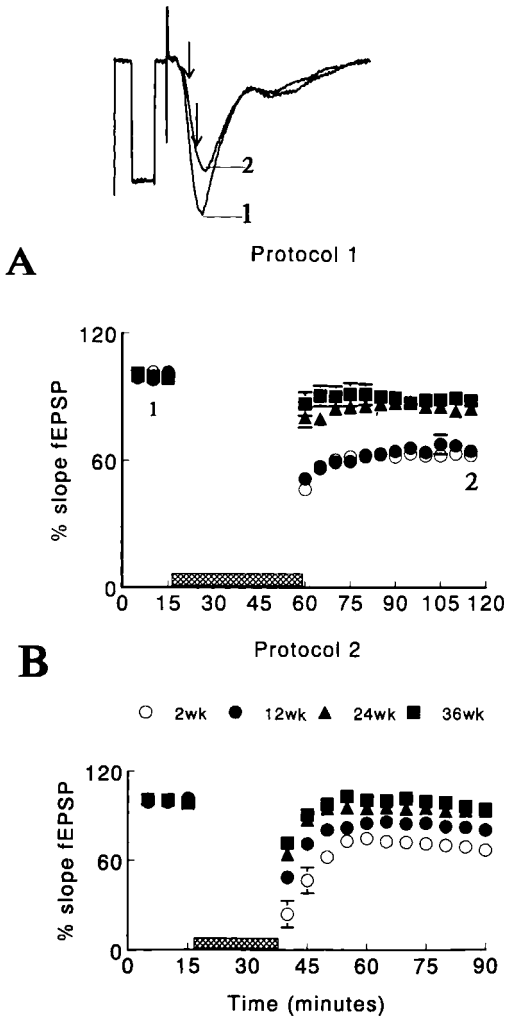

Fig. 2. The expression of LTD induced in slices from two-, 12-, 24- and 36-week(wk)-old rats with the low frequency stimulation (LFS) protocol 1 (A) and 2 (B), applied immediately after the third baseline stimulation. The bar indicate the duration of the stimulation protocol (bar). Plotted are the means (±S.E.M.) of the relative slopes, measured between arrows in the inset, of fEPSPs before and after stimulation. ANOVAR comparison of LTD in A revealed significant difference between the age groups ($F_{3,24}$=13.190; P<0.0001). The *post hoc* Duncan's test indicated that slices from 24- and 36-week-old rats differed significantly from those from two-and 12-week-old rats. ANOVAR comparison of LTD in B revealed significant difference between the age groups ($F_{3,25}$=8.4771; P<0.005). The *post hoc* Duncan's test indicated that slices from 36-week-old rats were significantly different from those rats from other ages. Inset: an example of the average (15 sweeps) of fEPSPs, evoked in the stratum radiatum by stimulation of the afferent fibres, before (1) and 60 min (2) after LFS, obtained in slices from two-week-old rats. The initial fEPSPs depression 5 min after LFS of slices from two- and 12-week-old rats differed significantly from that of slices from 24- and 36-week-old rats, regardless the protocol used (Protocol 1: ANOVA: $F_{3,24}$=17.6323, P<0.0001, in the *post hoc* Duncan's test slices from two- and 12-week-old rats differed significantly (P<0.05) from group 24- and 36-week-old rats; Protocol 2: ANOVA: $F_{3,25}$=14.9156; P<0.0001, in the Duncan's test slices from two- and 12-week-old rats significantly (P<0.05) differed from those from 24- and 36-week-old rats).

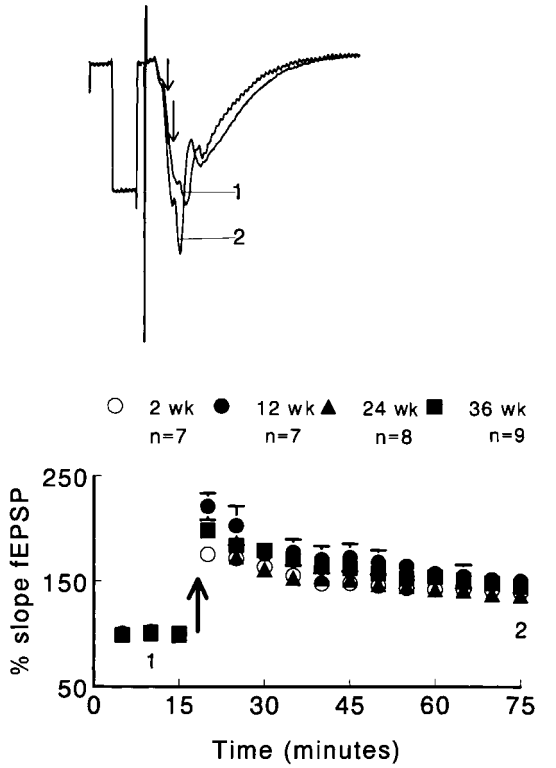

Fig. 3. Expression of LTP induced in slices from two-, 12-, 24- and 36-week(wk)-old rats by using high frequency stimulation (HFS, 100 Hz for 1 s), starting at time 0 min (arrow). Plotted are the means (±S.E.M.) of the relative slope of fEPSPs, measured between arrows in the inset, before and after HFS (thick arrow). ANOVAR comparison revealed no significant difference between slices from rats of different age ($F_{3,27}$=0.6963; not significant). Inset: an example of the average (15 sweeps) of fEPSPs, evoked in the stratum radiatum by stimulation of the afferent fibres in slices from 36-week-old rats before (1) and 60 min (2) HFS.

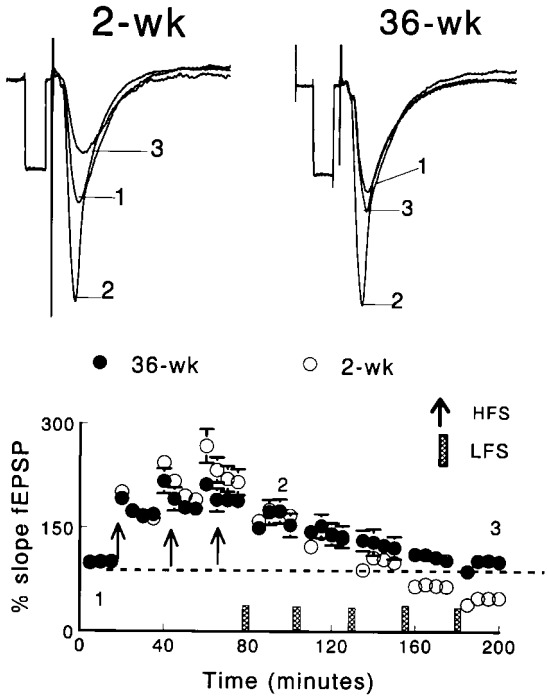

Fig. 4. Dynamic range of transmission plasticity in slices from two- and 36-week(wk)-old rats. Plotted are the means (±S.E.M.) of the relative slope of fEPSPs after high frequency stimulation (3×1 s at 100 Hz (arrows) and low frequency stimulation (5×5 min at 1 Hz) at the time indicated by vertical bars. Insets: examples of the average (15 sweeps) of fEPSPs, evoked in the stratum radiatum by stimulation of afferent fibres, recorded in slices from a two-week-old and a 36-week-old rat at the times indicated by numbers.

In slices from 36-week-old rats, protocol 1 elicited LTD in three out of eight slices, whereas protocol 2 induced LTD in only one out eight slices.

Figure 3 illustrates the magnitude of LTP in slices from two-, 12-, 24-and 36-week-old rats. In all the age groups, the slope of the fEPSPs 60 min after HFS increased significantly ($P \geq 0.02$ in Wilcoxon's test), to 139.8±3.34%, 150.3±8.69%, 136.8±3.8% and 145.4±4.6% of baseline in the two-, 12-, 24- and 36-week-old rats, respectively. There were no significant differences (ANOVA) between the age groups.

Input specificity

The input specificity of LTD in the CA1 field was tested in three slices from 12-week-old rats by alternately applying test stimuli to S_1 and S_2 inputs at a frequency of 0.05 Hz. LFS (1 Hz for 15 min) was applied to S_1 input only. After 60 min the slope of the fEPSPs had decreased by 60.4±5.5% (P<0.05 in Wilcoxon's test), indicating LTD induction. Responses elicited by stimulation at S_2 changed little (data not shown).

N-*methyl*-D-*aspartate receptor dependence*

The NMDA receptor dependence of LTD in the CA1 field was determined in one experiment in which LFS at 1 Hz for 15 min, (protocol 1) was applied to the slice from 12-week-old animals superfused with 50 µmol APV and then again after the antagonist had been washed out of the preparation. After application of APV for 30 min LFS failed to decrease the slope of the fEPSPs and thus failed to induce LTD. After a 40 min wash-out period, LFS resulted in a marked decrease in the slope of the fEPSPs which lasted for 60 min, indicating that LTD was induced by LFS after washout of the NMDA receptor antagonist (data not shown).

Dynamic range on the transmission plasticity

The dynamic range, maximum potentiation-depotentiation, was studied in slices from two-week-old (n=6) and 36-week-old (n=6) rats. The results are summarized in Fig. 4. HFS (100 Hz for 1 s)

applied three times elicited a nearly maximal increase in the slope of the fEPSPs, which in slices from two-week-old rats was 216.1 ± 18.8% of the baseline ($P < 0.05$ in Wilcoxon's test; $n=6$) and 188.7 ± 12.6% ($P < 0.05$; $n=6$) in slices from 36-week-old rats. The slope of the fEPSPs 20 min after the last HFS in slices from 36-week-old rats rose by only approximately 10%, indicating that LTP was nearly saturated. Although the slope of the fEPSPs after the third HFS in slices from two-week-old rats was still on average 20% higher than that 20 min after the second HFS, also in these slices LTP was nearly saturated. The subsequent LFS (1 Hz for 5 min), repeated five times at 20 min intervals, resulted in a marked decrease in the slope of fEPSPs, which dropped in slices from 36-week-old rats 20 min after the last LFS to 101.3 ± 4.8% of baseline. In slices from two-week-old rats the slope fEPSPs 20 min after the last LFS was not more than 48.9 ± 16.4% of the original baseline before the start of the experiment. This relatively small decrease of approximately 15% in the slope of the fEPSPs after the fifth LFS, compared to that seen after the fourth LFS, indicates that depotentiation was nearly saturated. The total dynamic range of the slope of the fEPSPs slope, measured as the difference between the mean potentiation 20 min after the last HFS and the maximal depotentiation 20 min after the last LFS, in slices from two-week-old rats was 167.3 ± 16.7% whereas it was only 87.3 ± 5.9% in slices from 36-week-old rats, the difference being highly significant ($P < 0.0001$ in two-tailed *t*-test; $n=6$). There was no significant difference between both groups in the magnitude of the near maximal LTP ($P=0.133$ in two-tailed *t*-test); however, there was a highly significant ($P < 0.001$) difference in the magnitude of depotentiation between both groups. Thus, the significantly smaller dynamic range of the transmission plasticity in slices from 36-week-old rats was predominantly due to a decrease in depotentiation capacity of these slices. Interestingly, the magnitude of the depotentiation in slices from two-week-old rats was similar to that of LTD induced with 1 Hz 15 min stimulation (see Fig. 2A for comparison), although the stimulation protocol was not identical in these two experiments. In contrast, depotentiation of slices from 36-week-old rats did not decrease below baseline although these slices exhibited clear LTD when LFS protocol 1 was used (see Fig. 2A for comparison).

DISCUSSION

The principal finding of this study was that LTD can readily be elicited in slices from rats aged up to 36 weeks, using a 1 Hz LFS protocol. However, slices from 24-week-old rats and older expressed significantly less LTD than slices from younger animals. There was no significant difference in the expression of LTP. In addition, the slices from 36-week-old rats exhibit a significantly smaller dynamic range of transmission plasticity than did slices from 12-week-old animals, mainly due to a significantly lower expression of depotentiation of the nearly maximally expressed LTP.

LTD in this study showed input specificity and NMDA receptor dependence, and in this it was comparable to the LTD in this field reported by others.[14,37] Most of the studies on LTD have demonstrated LTD expression in slices from rats aged two to six weeks, using the original protocol of 15 min stimulation at 1 Hz.[14] This type of LFS, consisting in total of 900 stimuli, results in an approximately 20–40% decrease in the slope of the fEPSPs compared to baseline.[14,15,25,37,38,46,54] The mean LTD decrease of 32–38% below baseline found in slices from two- and 12-week-old rats is in agreement with this, being close to the maximal magnitude of LTD expression reported by others[38,46] and suggests that the viability of our slices was comparable to that of others. However, several groups have failed to induce LTD in the CA1 field in slices from rats older than six weeks when stimulating with 900 pulses at 1 Hz.[4,54,56] The significant LTD in slices from 24- and 36-week-old rats reported here demonstrates that the hippocampus essentially does not lose its capacity for LTD expression with increasing age, although the mechanisms of LTD in older mature rats may become more susceptible to experimental conditions.

The magnitude of LTD and the number of slices in which LTD was elicited appear to be dependent, amongst others, on the intensity of the test stimulation. In slices from 150–300 g rats, Staubli and Ji[47] obtained LTD of approximately 40% in only 21% of the slices examined, using the test stimuli of an intensity that elicited fEPSPs in the stratum radiatum with little or no contamination by the population spike. The appearance of a population spike in the fEPSPs was associated with a reduction in the magnitude of LTD of approximately 10–20%, although LTD was then observed in 88% of the slices examined. The fEPSPs in this study contained little or no population spike (see examples in Figs 2 and 4), and we observed LTD of 20–50% in more than 70% of slices from the rats of 12-week-old rats (250–300 g), suggesting that experimental conditions other than the test stimulus intensity may affect the ability to induce LTD of this magnitude. The failure to induce LTD in slices from 24- and 36-week-old rats with stimulation at 1 and 5 Hz (protocol 2) is consistent with this.

The initial depression seen after LFS (either protocol) in slices from two- and 12-week-old rats was significantly larger than that seen in slices from older animals. The mechanisms of this initial depression of the fEPSPs are not understood. Several types of presynaptic depression of glutamate-mediated fEPSPs in the CA1 field of the hippocampus have been demonstrated (for review see Ref. 49). Thus, activation of presynaptic $GABA_B$, adenosine A_1 and

metabotropic glutamate receptors depress the fEPSPs in the CA1 field of the hippocampus. The $GABA_B$ and adenosine A_1 receptor-induced presynaptic inhibition of EPSPs is of a rapid onset and fully reversible within 5–10 min after agonist application.[22,57] The depression of the fEPSPs produced by activation of the presynaptic metabotropic glutamate receptor, however, lasts more than 15 min after the termination of agonist application,[5,10,35] which is comparable to that elicited by LFS (see Fig. 2 for comparison). The LFS-induced decrease in fEPSPs could have also been caused by a temporary decrease in excitability of the postsynaptic membrane by an increased GABA receptor-mediated inhibition, which would reduce the magnitude of EPSPs[11,36,52] and/or by increased outward potassium conductance[17,40,48] (for review see Ref. 44). Currently we are investigating this initial depression in more detail as it may provide a clue for the poor LTD expression in slices from elderly rats.

The depotentiation in the CA1 field resembles LTD in that it is synapse specific, requires activation of NMDA receptors and calcium influx, either through NMDA receptor channels or through voltage-dependent Ca^{2+} channels,[12] has a similar magnitude and duration as LTD, and, like LTD, can be reversed into LTP by HFS of afferent fibres (for review see Ref. 33). Both may thus be mediated by the same mechanisms. However, the fact that depotentiation in this field requires the synapse studied to be potentiated first, and the fact that depotentiation can be readily demonstrated in slices in which LTD cannot be induced,[4,9,16,55] and the differential effect of GABA receptor antagonists on LTD and depotentiation[54] support the idea that LTD and depotentiation are not mediated by identical mechanisms. We found significant LTD, i.e. significant depression of the slope of fEPSPs compared to baseline, in slices from 24- and 36-week-old rats, using two times repeated stimulation at 1 Hz for 15 min, but failed to depotentiate slices from these rats to below baseline with the repeated 1 Hz stimulation, which seems to support the difference in mechanisms between LTD and depotentiation.

We investigated the effect of age on the dynamic range of synaptic transmission by comparing the magnitude of nearly saturated LTP with that of nearly saturated depotentiation in slices from two- and 36-week-old rats, and by comparing the differences between the slope of fEPSPs associated with saturated LTP and that associated with saturated depotentiation of fEPSPs in slices from two-week-old and 36-week-old rats. The results showed a significantly smaller dynamic range of transmission plasticity in slices from 36-week-old rats than in slices from two-week-old rats. Compared to slices from two-week-old rats, there was only a slight, insignificant decrease in the magnitude of the saturated LTP in slices from 36-week-old rats, demonstrating that the ability to express LTP is not significantly changed at this age. Although earlier studies have suggested a deficit in LTP expression in aged rats,[27–29,50] later experiments have clearly shown that 24-month-old rats express the same magnitude of LTP during the first 30 min after HFS as do two-month animals.[13] This contrasts with the results of others,[23] who reported difficulties in inducting LTP in slices from rats older than 12 weeks. We have no explanation for the latter results, except that differences in the viability of the preparation might have been the cause for the reduced LTP expression in slices from rats of this age. The magnitude of depotentiation and the full dynamic range of transmission plasticity were both significantly lower in slices from 36-week-old rats than in slices from two-week-old rats. This demonstrates, for the first time, that plasticity in the CA1 field of the hippocampus significantly decreases with increasing age. Although the behavioural significance of this finding cannot be determined from these experiments, it is possible that the age-related decrease in the range of transmission plasticity in the hippocampus is related to the impaired performance of spatial learning tasks seen in aging rats.[18]

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