

3 Results

3.1 Effects of Group I mGlu antagonists on 200 Hz LTP

It was previously shown that group I metabotropic glutamate receptors (mGlu_s) have been implicated in long-term potentiation. The group I mGlu agonist DHPG, facilitates short-term potentiation into LTP in vivo [Manahan-Vaughan & Reymann, 1996]. Application of the group I mGlu antagonist, (S)-4-Carboxyphenylglycine (4CPG), blocks the maintenance of LTP in the dentate gyrus of freely moving rats [Manahan-Vaughan et al., 1998]. Interestingly, the agonist as well as the antagonist is able to block depotentiation in the dentate gyrus in freely moving animals [Kulla & Manahan-Vaughan, personal communication]. In this study, we tested the subtype specific involvement on long-term potentiation in the dentate gyrus of freely moving rats implanted with chronic hippocampal electrodes.

3.1.1 mGlu₅, a receptor subtype of group I mGlu_s

The involvement of mGlu₅ on LTP was tested by application of the highly selective antagonist at mGlu₅ 2-Methyl-6-(phenylethynyl)-pyridine hydrochloride (MPEP) [Gasparini et al., 1999].

Antagonist of mGlu5 has no effect on basal synaptic transmission

Basal synaptic transmission in the presence of the vehicle was stable with regard to both PS amplitude and fEPSP slope over the 25h period monitored. When MPEP was injected into the lateral

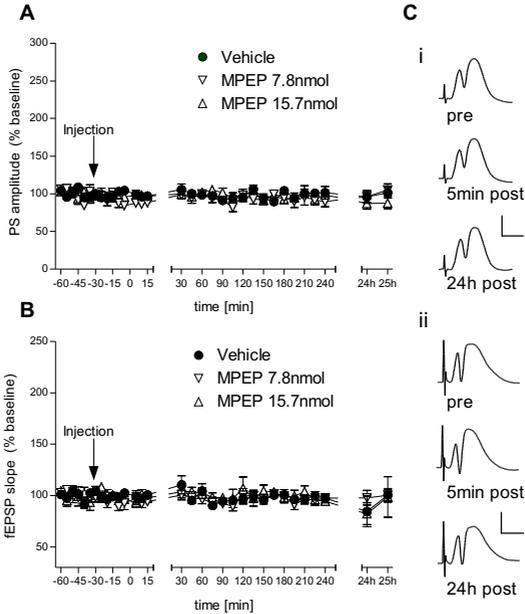


Figure 3.1: Antagonism of mGlu5 has no effect on basal synaptic transmission

A, B: Test-pulse stimulation when given in the presence of the mGlu5 antagonist, MPEP, ($7.8\text{nmol}/5\mu\text{l}$, $n=12$ or $15.7\text{nmol}/5\mu\text{l}$, $n=4$) does not affect basal PS amplitude (A) or fEPSP (B) compared to vehicle-injected controls ($n=7$). **C:** Original traces showing the field potentials evoked from the dentate gyrus pre injection, 5min and 24h following injection of (i) vehicle or (ii) MPEP ($15.7\text{nmol}/5\mu\text{l}$). Vertical scale-bar corresponds to 5mV , horizontal scale-bar to 4ms . Line breaks indicate change in time scale.

cerebral ventricle as 7.8nmol in a $5\mu\text{l}$ injection volume, no effect on synaptic transmission was seen over the 25h monitoring period ($n=12$, compared to controls $n=7$, confirmed by ANOVA: between-

factor, $F(1,31)=0.53$, $p=0.985$ for PS, $F(1,31)=1.79$, $p=0.1821$ for fEPSP, see Fig. 3.1). Similarly no effect was seen when $15.7\text{nmol}/5\mu\text{l}$ MPEP was injected ($n=4$) (confirmed by ANOVA: between-factor, $F(1,31)=0.74$, $p=0.391$ for PS, $F(1,31)=0.28$, $p=0.5980$ for fEPSP, see Fig. 3.1). Analysis of input/output curves obtained 30min follow-

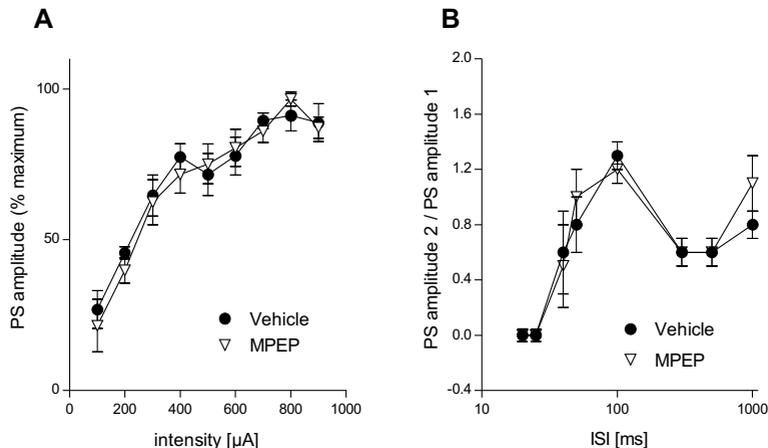


Figure 3.2: Antagonism of mGlu5 has no influence on I/O curve and paired pulse characteristics

A: Input/output curves obtained 30min following application of vehicle ($n=6$) or MPEP ($15.7\text{nmol}/5\mu\text{l}$, $n=8$) revealed no differences in the profile of responses. **B:** Paired pulse stimulation following application of vehicle ($n=6$) or MPEP ($15.7\text{nmol}/5\mu\text{l}$, $n=8$) showed no differences in the responses obtained.

ing vehicle ($n=6$) or MPEP ($15.7\text{nmol}/5\mu\text{l}$, $n=6$) injection revealed no differences in the responsiveness to stimulation intensities varying from 100 through $900\mu\text{A}$ (confirmed by paired t-test: $p=0.3503$, see Fig. 3.2 A). To confirm that MPEP did not produce changes in basal transmission, responses to paired pulse stimulation were also examined. Responses to paired pulse stimulation with interpulse intervals (IPIs) ranging from 20ms to 1000ms were recorded 30min after vehicle ($n=6$) and MPEP ($n=5$, $15.7\text{nmol}/5\mu\text{l}$) injections. The paired

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t-test revealed no significant differences in paired pulse responsiveness ($p=0.4758$, see Fig. 3.2B).

Antagonism of mGlu5 dose-dependently impairs long-term potentiation

Two hundred Hertz high frequency tetanisation (HFT=10 bursts of 15 stimuli, 0.2ms stimulus duration) resulted in robust LTP in the dentate gyrus in vivo (see Fig. 3.3). MPEP (7.8nmol/5 μ l, n=6) when applied 30min prior to HFT did not significantly alter the initial magnitude of potentiation (5min post-HFT) when compared to vehicle-injected animals (n=6). Approximately 2h after tetanisation the fEPSP slope and the PS amplitude had significantly decreased compared to LTP levels in vehicle-injected animals. For PS amplitude the response was significantly different from controls from 165min post-HFT ($p>0.05$). A similar decrease was also seen in fEPSP, with a significant difference from controls appearing at 165min post-HFT ($p>0.05$, see Fig. 3.3). A complete return of evoked potentials to pre-HFT levels did not occur in the initial four-hour monitoring period, however. Four-hours post-HFT a significantly ($p>0.01$) higher magnitude of PS amplitude and fEPSP slope was detected compared to animals, which received MPEP in the absence of HFT (baseline controls, see Fig. 3.1). Thus, in comparison to vehicle-injected controls where 165min post-HFT PS amplitude values were $228.9 \pm 16.1\%$ and fEPSP slope values were $178.6 \pm 8.7\%$, a reduction to PS amplitude value of $162.8 \pm 17.1\%$ and fEPSP values of $142.8 \pm 9.5\%$ could be seen when 7.8nmol/5 μ l MPEP was injected 30min prior to HFT. Twenty-four hours post-HFT, LTP was still evident in control animals, but was significantly inhibited in MPEP treated animals (see Fig. 3.3).

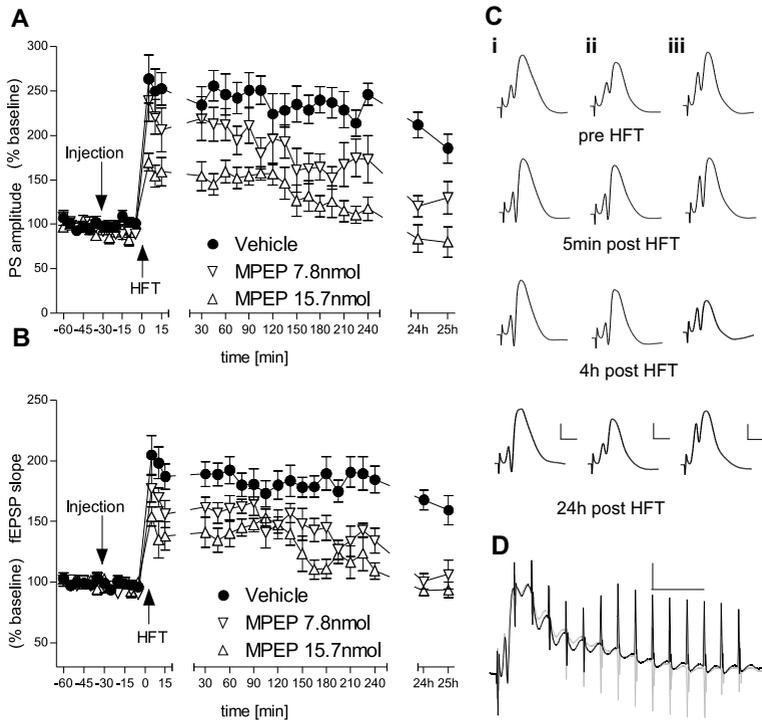


Figure 3.3: Antagonism of mGlu5 dose-dependently impairs long-term potentiation

A, B: 200Hz HFT in the presence of vehicle ($n=6$) results in a robust long-term potentiation of both PS (A) and fEPSP (B). Administration of the mGlu5 antagonist MPEP ($7.8nmol/5\mu l$, $n=6$) or ($15.7nmol/5\mu l$, $n=5$) dose-dependently inhibits LTP compared to vehicle-injected controls. Effects become evident approximately 2.5h post-HFT in $7.8nmol/5\mu l$ treated animals. Animals treated with $15.7nmol/5\mu l$ MPEP show a significant reduction in LTP immediately after HFT. **C:** Original analog traces showing the field potentials evoked from the dentate gyrus pre-HFT, 5min, 4h and 24h following HFT in the presence of (i) vehicle, (ii) MPEP ($7.8nmol/5\mu l$) or (iii) MPEP ($15.7nmol/5\mu l$). Vertical scale-bar corresponds to $5mV$, horizontal scale-bar to $4ms$. **D:** Application of MPEP ($7.8nmol/5\mu l$) 30min prior to the tetanus had no effect on the fEPSP. Original analog traces showing the fEPSP response during 200Hz tetanization (grey traces, MPEP; black trace, vehicle). Vertical scale-bar corresponds to $5mV$, horizontal scale bar $5ms$ for MPEP, vertical scale-bar corresponds to $6.1mV$, horizontal scale bar $5ms$ for vehicle-treated animals. Line breaks indicate change in time scale.

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Thus, both PS amplitude and fEPSP slope had returned to basal levels when compared to animals which received MPEP in the absence of HFT. Twenty-four hours post-HFT, PS amplitude and fEPSP values decreased from $211.9 \pm 14.5\%$ and $168.0 \pm 8.2\%$ respectively in vehicle treated animals to $120.4 \pm 11.8\%$ and $100.4 \pm 6.5\%$ in $7.8\text{nmol}/5\mu\text{l}$ MPEP-treated animals. MPEP ($7.8\text{nmol}/5\mu\text{l}$) had no effect on the fEPSP properties during tetanus ($n=6$), indicating the absence of NMDA receptor impairment [Mannaioni et al., 2001, Lee et al., 2002] in this concentration. Raising the concentration of MPEP to $15.7\text{nmol}/5\mu\text{l}$ ($n=5$) did not alter the general profile of response to HFT. However, 4h after HFT evoked potentials had returned to basal levels compared to baseline controls (see Fig. 3.3). Furthermore, the PS amplitude immediately following application of HFT was statistically lower than in $7.8\text{nmol}/5\mu\text{l}$ MPEP-injected animals ($p>0.05$, $t=5\text{min}$ post-HFT). For value comparison see Tab. 3.1.

Table 3.1: Post-HFT values

treatment		5min	4h	24h
vehicle	PS[%]	252.5 ± 18	246.3 ± 12	211.9 ± 15
HFT	fEPSP[%]	187.0 ± 11	185 ± 11.3	168.0 ± 8
15.7nmol	PS[%]	169.8 ± 10	117.6 ± 13	98.8 ± 4
MPEP-HFT	fEPSP[%]	154.2 ± 8	109.2 ± 7	92.8 ± 4
15.7nmol	PS[%]	98.0 ± 7	98.5 ± 4	87.3 ± 8
MPEP control	fEPSP[%]	93.3 ± 5	94.5 ± 14	82.3 ± 11

ANOVA confirmed the statistical significance between the control and $7.8\text{nmol}/5\mu\text{l}$ MPEP-treated LTP groups, as well as between the two groups which received different concentrations of MPEP. Comparing the PS values of the control and $7.8\text{nmol}/5\mu\text{l}$ MPEP groups, the statistical results for the between-factor analysis were $F(1,31)=72.29$, $p<0.0001$. For fEPSP values the between-factor analysis yielded $F(1,31) = 119.09$, $p<0.0001$. Comparing the $7.8\text{nmol}/5\mu\text{l}$

MPEP group with the $15.7\text{nmol}/5\mu\text{l}$ MPEP group results in the between-factor analysis yielded $F(1,31)=65.29$, $p<0.0001$ for the PS and in $F(1,31)=27.85$, $p<0.0001$ for the fEPSP values.

Application of the mGlu5 antagonist 5min post-HFT results in a significant impairment of late-LTP

The effect of MPEP in the lower dose ($7.8\text{nmol}/5\mu\text{l}$) on late-LTP could have occurred through modulation of LTP induction by HFT. To examine this possibility, MPEP was applied 5min after HFT. In this case, a significant impairment of LTP with regard to both PS amplitude and fEPSP slope was seen which became evident 24h after MPEP was applied ($p<0.005$, see Fig. 3.4). Thus, in comparison to vehicle-injected controls where 24h post-HFT PS amplitude values were $173.0 \pm 6.3\%$, fEPSP slope: $144.6 \pm 11.2\%$ a reduction to PS amplitude $107.3 \pm 9.5\%$ and fEPSP slope $106.3 \pm 9.3\%$ could be seen when $7.8\text{nmol}/5\mu\text{l}$ MPEP was injected 5min after HFT. This result was confirmed by ANOVA. The statistical results for the between-factor analysis was $F(1,29)=4.00$, $p=0.047$ for PS amplitude and $F(1,29)=19.14$, $p<0.0001$ for fEPSP slope values. These data support a modulation by MPEP of LTP maintenance corresponding to an inhibition of late-LTP.

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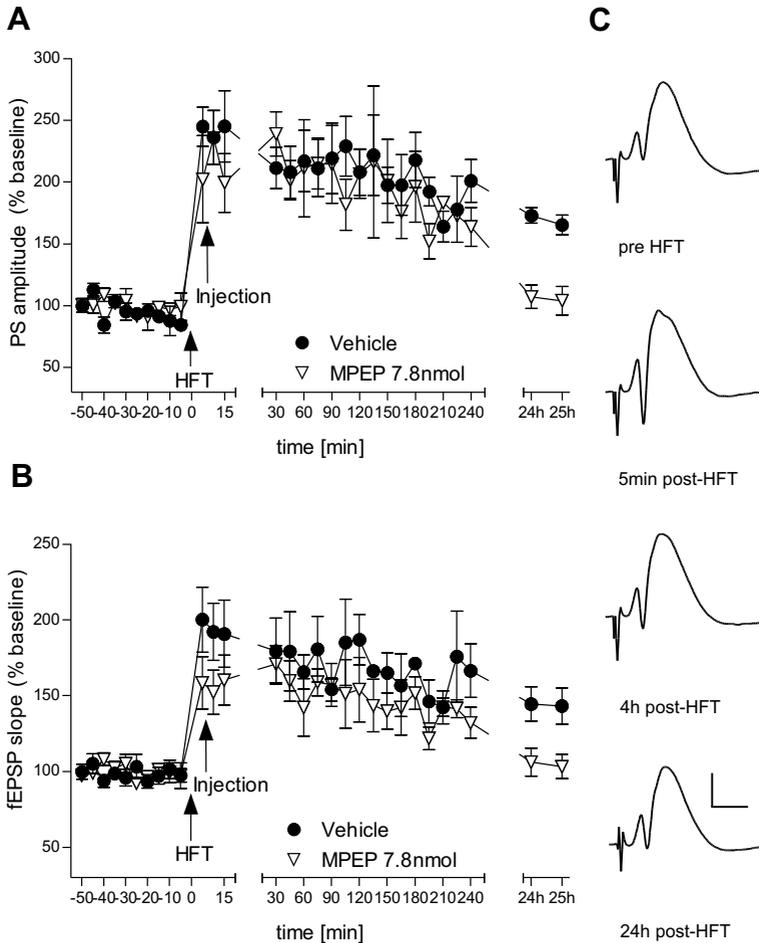


Figure 3.4: Application of the mGlu5 antagonist 5min post-HFT results in a significant impairment of late-LTP

A, B: Vehicle ($n=5$) applied 5min after 200Hz HFT results in a robust LTP of both PS amplitude (A) and fEPSP slope (B). Administration of the mGlu5 antagonist MPEP (7.8nmol/5 μ l, $n=4$) 5min post-HFT produces a significant inhibition of late-LTP. Effects become evident approximately 24h post-HFT. **C:** Original analog traces showing the field potentials evoked from the dentate gyrus pre-HFT, 5min, 4h and 24h following HFT where MPEP (7.8nmol/5 μ l) was given 5min after HFT. Vertical scale-bar corresponds to 5mV, horizontal scale-bar to 4ms. Line breaks indicate change in time scale.

3.1.2 mGlu1, a receptor subtype of group I mGlu

The involvement of mGlu1 on LTP was tested by application of the highly specific mGlu1 antagonist (S)-(+)- α -Amino-4- carboxy-2-methylbenzeneacetic acid (LY367385) [Clark et al., 1997].

Antagonism of mGlu1 has no effect on basal synaptic transmission

When LY367385 was injected into the lateral cerebral ventricle in concentrations of $4\text{nmol}/5\mu\text{l}$ (n=5), $8\text{nmol}/5\mu\text{l}$ (n=6), $16\text{nmol}/5\mu\text{l}$ (n=5) or $32\text{nmol}/5\mu\text{l}$ (n=4), in a $5\mu\text{l}$ injection volume, no effect on synaptic transmission was seen over a 25h monitoring period (compared to controls n=11, see Fig. 3.5, for ANOVA see Tab. 3.2).

Table 3.2: Baseline values–ANOVA results

concentration		between factor	
		F(1,31)	p=
<i>4nmol</i>	PS amplitude	2.74	0.0985
	fEPSP slope	1.38	0.2403
<i>8nmol</i>	PS amplitude	1.56	0.2121
	fEPSP slope	0.55	0.4603
<i>16nmol</i>	PS amplitude	1.64	0.2010
	fEPSP slope	0.96	0.3268
<i>32nmol</i>	PS amplitude	1.41	0.2353
	fEPSP slope	1.89	0.1699

The mGlu1 subtype is involved in presynaptic regulation of glutamate release

To determine if LY367385 produce changes in basal transmission, responses to paired pulse stimulation were examined. Responses

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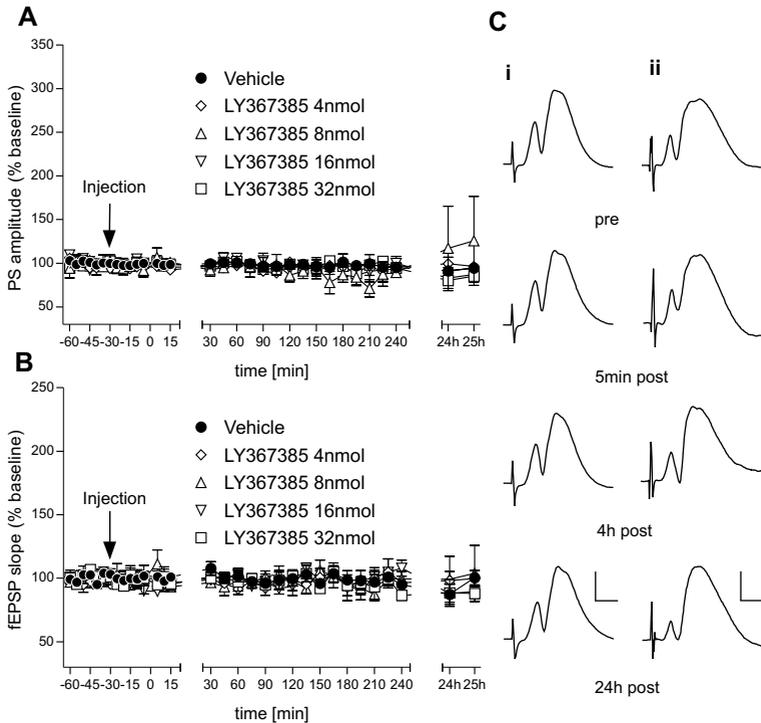


Figure 3.5: Antagonism of mGlu1 has no effect on basal synaptic transmission

A, B: Test-pulse stimulation when given in the presence of the mGlu1 antagonist, LY367385, in concentrations of $4\text{nmol}/5\mu\text{l}$ ($n=5$), $8\text{nmol}/5\mu\text{l}$ ($n=6$), $16\text{nmol}/5\mu\text{l}$ ($n=5$) and $32\text{nmol}/5\mu\text{l}$ ($n=4$), does not affect basal PS amplitude (A) or fEPSP slope (B) compared to vehicle-injected controls ($n=6$). **C:** Original analog traces showing the field potentials evoked from the dentate gyrus pre-injection, 5min, 4h and 24h following injection in the presence of (i) vehicle or (ii) LY367385 ($16\text{nmol}/5\mu\text{l}$). Vertical scale-bar corresponds to 5mV , horizontal scale-bar to 4ms . Line breaks indicate change in time scale.

to paired pulse stimulation with interpulse intervals (IPIs) ranging from 20ms to 1000ms were recorded 30min after vehicle ($n=8$) and LY367385 ($n=10$, $16\text{nmol}/5\mu\text{l}$) injections (see Fig. 3.6). Responses obtained in the IPI range of $20\text{--}50\text{ms}$ reflect the activation of

GABAergic interneurons and subsequent inhibition of dentate gyrus granule cells corresponding to paired pulse [Albertson & Joy, 1987, Halasy & Somogyi, 1993, Moser, 1996]. Responses obtained when paired pulses are given in the 50–300ms IPI range reflect the selective increase of the NMDA receptor-mediated response to further release of glutamate from the vesicle pool within the presynaptic bouton corresponding to inhibition of paired pulse facilitation [Albertson & Joy, 1987]. Responses obtained in the 500–1000ms IPI range reflect the opening of calcium-dependent potassium channels which suppress presynaptic glutamate release and thus correspond to a second phase of paired pulse depression [Thalmann & Ayala, 1982]. LY367385 had no effect on paired pulse responses in the 20–25ms or

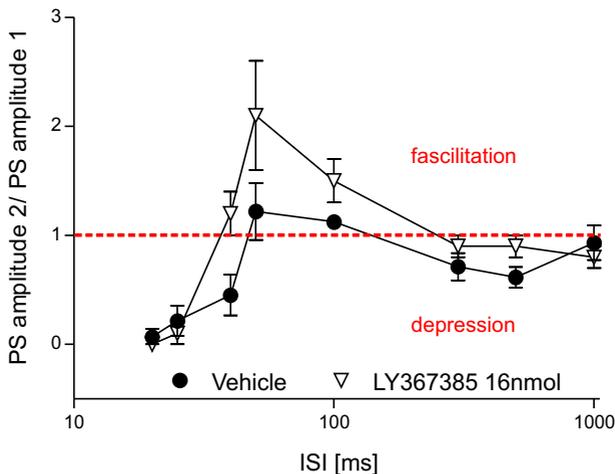


Figure 3.6: Antagonism of mGlu1 elicits a small effect on paired pulse depression

Paired pulse stimulation given 30min following application of vehicle (n=8) resulted in a characteristic depression (20–50ms intervals), followed by a facilitation (50–300ms intervals) and second depression (500–1000ms intervals). Application of LY367385 (16nmol/5μl, n=10) resulted in an facilitation of PS amplitude responses in the 40ms range whereas all other pulse interval responses were unaffected.

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50–1000ms IPI ranges. However, single value analysis in the case of 40ms IPI showed a significant facilitation instead of the paired pulse depression seen in vehicle tested animals (t-test, $p=0.0165$). The paired pulse PS amplitude ratio increased from 0.5 ± 0.2 in vehicle-treated animals to 1.2 ± 0.2 in LY367385 treated animals, suggesting that a suppression of GABAergic inhibition may have occurred.

Antagonism of mGlu1 dose-dependently impairs long-term potentiation

Two hundred Hertz HFT resulted in robust LTP in the dentate gyrus in freely moving rats. LY367385 in a $4nmol/5\mu l$ ($n=4$) concentration when applied 30min prior to HFT did not significantly change the initial magnitude of potentiation (5min post-HFT) when compared to vehicle-injected animals ($n=8$, see Fig. 3.7). However, roughly

Table 3.3: LTP values – ANOVA results

concentration		between factor		within factor	
		F(1,31)	p=	F(1,31)	p=
4nmol	PS amplitude	18.50	0.0001	5.26	0.0001
	fEPSP slope	7.54	0.0064	10.16	0.0001
8nmol	PS amplitude	178.60	0.0001	7.23	0.0001
	fEPSP slope	109.69	0.0001	12.01	0.0001
16nmol	PS amplitude	18.43	0.0001	2.27	0.0003
	fEPSP slope	7.58	0.0063	1.30	0.1375
32nmol	PS amplitude	12.54	0.0005	1.63	0.0226
	fEPSP slope	11.77	0.0007	1.46	0.0639

three hours after tetanisation PS amplitude had visibly decreased compared to LTP levels in vehicle-injected animals, although fEPSP slope seemed to remain unchanged (see Fig. 3.7, for ANOVA see Tab. 3.3). Though there was a significant difference between PS amplitude and fEPSP values obtained in $4nmol/5\mu l$ treated animals

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compared to controls by means of between-factor ANOVA, there was no significant difference between the treated and untreated group in terms of single value comparison by paired t-test.

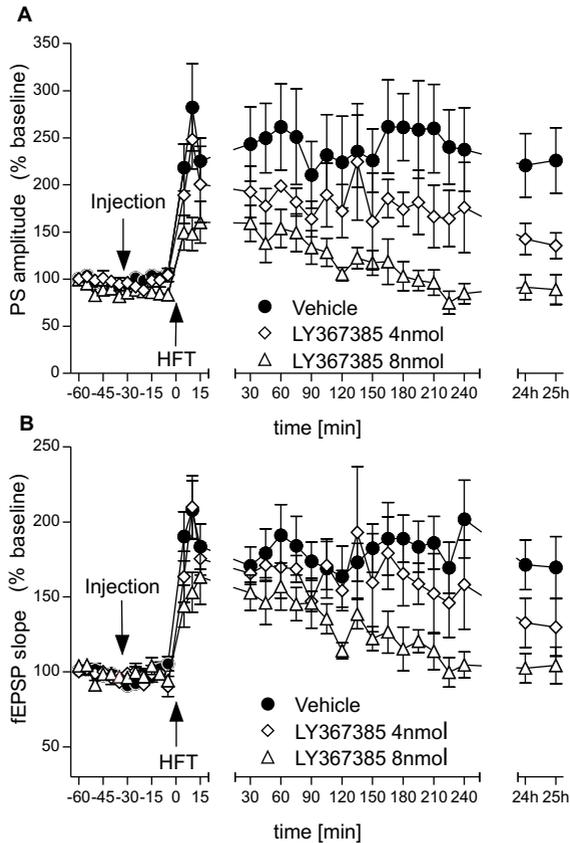


Figure 3.7: Antagonism of mGlu1 dose-dependently impairs LTP
A, B: 200Hz HFT in the presence of vehicle (n=8) results in a robust LTP of both PS amplitude (A) and fEPSP slope (B). Administration of the mGlu1 antagonist LY367385 (4nmol/5 μ l, n=4) or (8nmol/5 μ l, n=9) dose-dependently inhibits LTP compared to vehicle-injected controls. LTP is affected approx. 3h post-HFT in 4nmol/5 μ l treated animals. Animals treated with 8nmol/5 μ l LY367385 show a significant reduction in LTP immediately after HFT. Line breaks indicate change in time scale.

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Increasing the concentration of LY367385 to $8\text{nmol}/5\mu\text{l}$ ($n=9$) significantly reduced the LTP immediately after HFT (PS amplitude: $149.2 \pm 18.4\%$, fEPSP slope: $144.0 \pm 14.0\%$) compared to vehicle-injected controls ($n=8$, PS amplitude: $218.5 \pm 24.9\%$, fEPSP slope: $190.5 \pm 16.4\%$). By 210min post-HFT, evoked potentials had returned to pre-HFT levels. PS amplitude and fEPSP slope responded similarly, thus PS amplitude and fEPSP values reduced in vehicle-treated animals from $259.9 \pm 46.9\%$ and $186.1 \pm 17.5\%$, respectively, to $96.1 \pm 13.5\%$ and $113.9 \pm 12.3\%$ in $8\text{nmol}/5\mu\text{l}$ LY367385 treated animals (see Fig. 3.7, for ANOVA see Tab. 3.3).

Application of $16\text{nmol}/5\mu\text{l}$ LY367385 ($n=7$) did not increase the impairment of LTP as elicited by $8\text{nmol}/5\mu\text{l}$ LY367385 (see Fig. 3.8, for ANOVA see Tab. 3.3). Five minutes after HFT PS amplitude values were 149.7 ± 5.8 compared to 218.5 ± 24.8 in controls ($p < 0.05$), fEPSP slope values were 145.9 ± 9.5 compared to 190.5 ± 16.4 in controls ($p < 0.05$). Evoked potentials returned to pre-HFT levels by 180min post-HFT.

LY367385 in a concentration of $32\text{nmol}/5\mu\text{l}$ was less effective in impairing LTP. With this concentration an inhibition of LTP expression but not induction was seen (see Fig. 3.8, for ANOVA see Tab. 3.3). This effect was similar in profile to the response elicited with $4\text{nmol}/5\mu\text{l}$ LY367385. Although an impairment of LTP became evident 180min after HFT had been given ($p < 0.05$).

The dose-response curves (see Fig. 3.9) for PS amplitude and fEPSP slope showed, with increasing drug concentration, an U-shaped characteristic of the 4h post-HFT values. Compared to vehicle-injected controls $4\text{nmol}/5\mu\text{l}$ LY367385 reduced PS amplitude values to 74.0% and fEPSP slope values to 78.0% (not significant), $8\text{nmol}/5\mu\text{l}$ reduced PS amplitude values to 35.7% ($p < 0.005$) and fEPSP slope values to 51.8% ($p < 0.005$).

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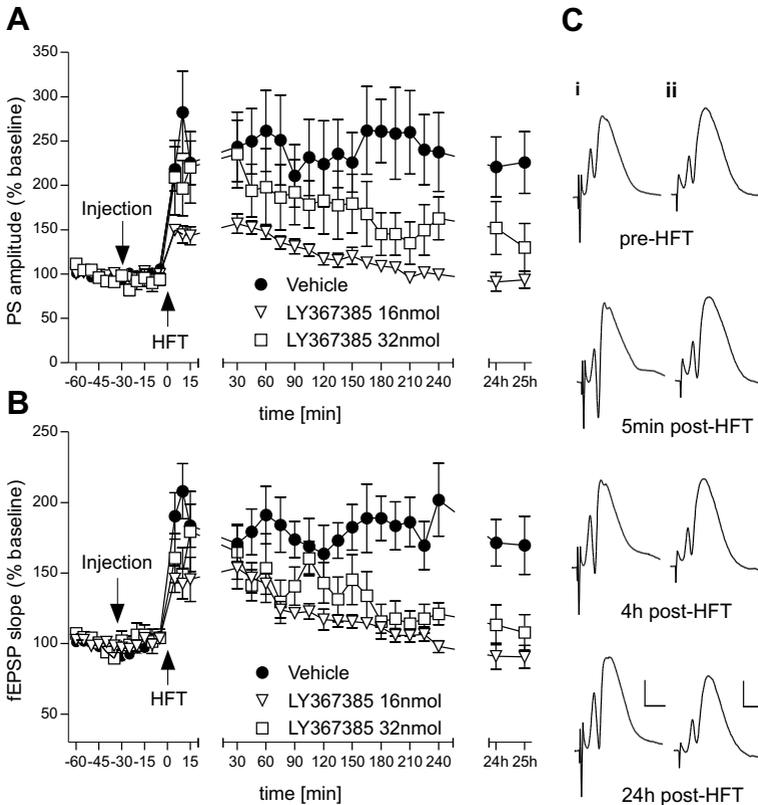


Figure 3.8: Antagonism of mGlu1 dose-dependently impairs LTP
A, B: 200Hz HFT in the presence of vehicle ($n=8$) results in a robust LTP of both PS amplitude (A) and fEPSP slope (B). Administration of the mGlu1 antagonist LY367385 (16nmol/5 μ l, $n=7$) or (32nmol/5 μ l, $n=8$) dose-dependently inhibits LTP compared to vehicle-injected controls. Effects become evident immediately after HFT in 16nmol/5 μ l treated animals. Animals treated with 32nmol/5 μ l LY367385 show a significant reduction in LTP which become evident 150min after HFT. **C:** Original analog traces showing the field potentials evoked from the dentate gyrus pre-HFT, 5min, 4h and 24h following HFT in the presence of (i) vehicle or (ii) LY367385 (16nmol/5 μ l). Vertical scale-bar corresponds to 5mV, horizontal scale-bar to 4ms. Line breaks indicate change in time scale.

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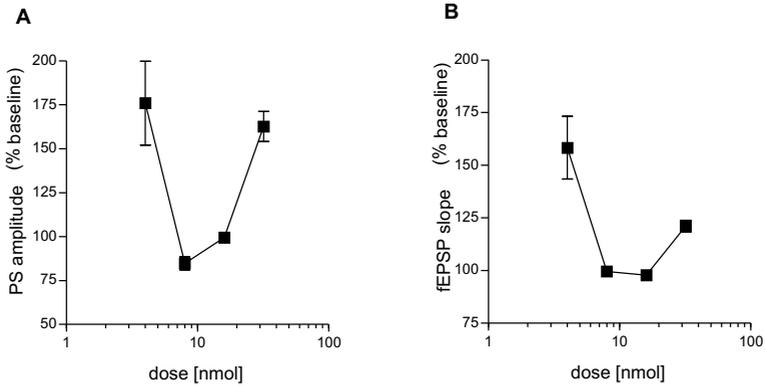


Figure 3.9: LY367385 dose-dependently impairs LTP in an U-shaped manner

Dose-response curve for the inhibitory effect of LY367385 on LTP in the dentate gyrus in freely moving rats. The values represent PS amplitude (A) and fEPSP slope (B) values observed at 4h post-HFT.

Upon increasing the concentration to $16\text{nmol}/5\mu\text{l}$, no decrease was seen in PS amplitude and fEPSP values compared to $8\text{nmol}/5\mu\text{l}$. However, in comparison with vehicle treated controls a significant reduction to 41.9% ($p=0.021$) for PS amplitude values and to 48.5% for ($p=0.0018$) fEPSP values was seen.

Further increasing the LY367385 concentration to $32\text{nmol}/5\mu\text{l}$ decreased the reduction of both values. For PS amplitude to 68.5% (not significant) and for fEPSP slope to 60% ($p=0.007$).

Application of the mGlu1 antagonist 5min post-HFT results in an unaffected LTP

The effect of LY367385 ($16\text{nmol}/5\mu\text{l}$) on LTP could have occurred through modulation by LTP induction during HFT. To examine this possibility, LY367385 was applied 5min after HFT. In this case, no significant changes of LTP with regard to both PS amplitude and fEPSP slope was seen (see Fig. 3.10).

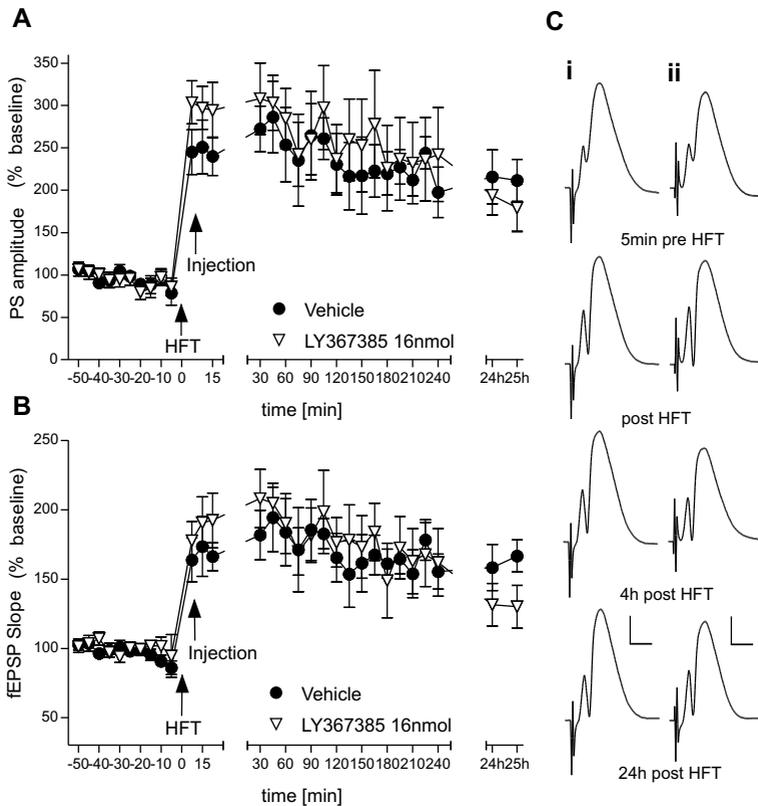


Figure 3.10: Application of the mGlu1 antagonist 5min post-HFT results in an unaffected LTP

A, B: Vehicle ($n=5$) applied 5min after 200Hz HFT results in a robust LTP of both PS amplitude (A) and fEPSP slope (B). Administration of the mGlu1 antagonist LY367385 (16nmol/5 μ l, $n=7$) 5min post-HFT produces no significant variation in LTP. **C:** Original analog traces showing the field potentials evoked from the dentate gyrus pre-HFT, 5min, 4h and 24h following HFT where LY367385 (16nmol/5 μ l) was given 5min after HFT. Vertical scale-bar corresponds to 5mV, horizontal scale-bar to 4ms. Line breaks indicate change in time scale.

This result was confirmed by ANOVA. The statistical results for the between-factor analysis were $F(1,29)=2.59$, $p=0.1087$ for PS amplitude and $F(1,29)=1.11$, $p=0.2932$ for fEPSP slope values. These data support a modulation by LY367385 of LTP corresponding to an inhibition of the LTP induction.

3.1.3 Subtype specific involvement of group I mGlu in LTP

Whereas both, the mGlu1 antagonist LY367385 and the mGlu5 antagonist MPEP, showed, in the higher concentration range, a decreased induction of PS amplitude and fEPSP slope values after HFT, only the mGlu5 antagonist showed an impaired late-LTP without affecting the induction phase in the lower concentrations used (see Fig. 3.11). This results are equivalent to the results obtained in the experiments where the antagonist were injected 5min after HFT (see Fig. 3.10 on page 63 and Fig. 3.4 on page 54). Application of the mGlu5 antagonist MPEP, but not the application of the mGlu1 antagonist LY367385, 5min post-HFT resulted in a significant impairment of late-LTP.

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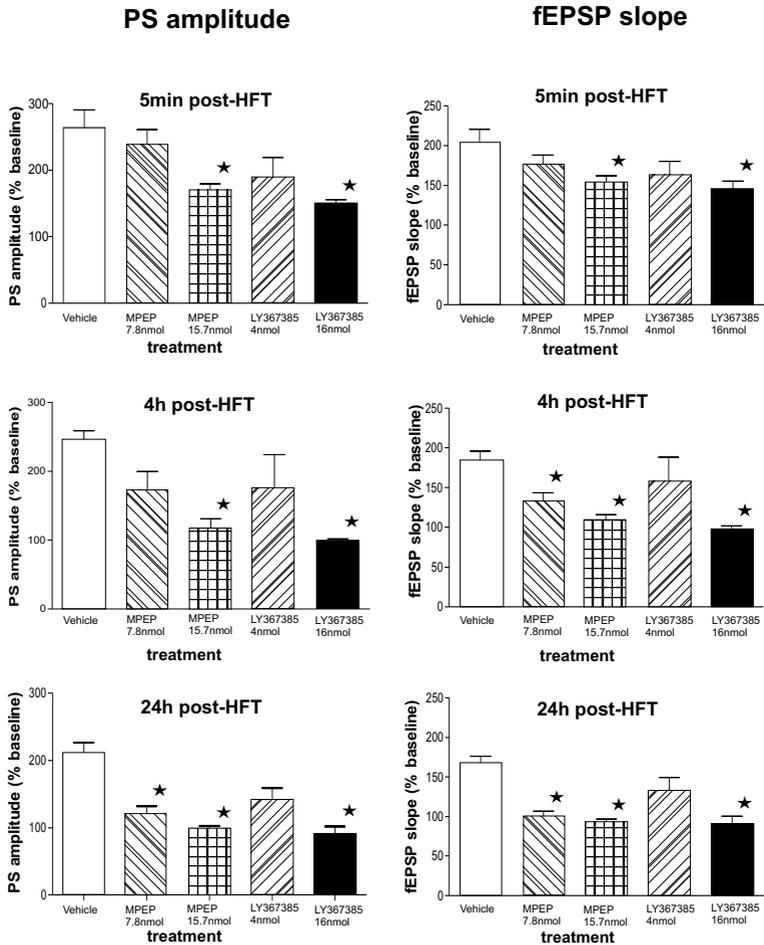


Figure 3.11: Subtype specific involvement of group I mGlu in LTP PS amplitude (left panel) and fEPSP slope (right panel) values of vehicle (n=14), MPEP (7.8nmol n=6, 15.7nmol n=5) and LY367385 (4nmol n=4, 16nmol n=7) treated animals 5min, 4h and 24h post-HFT. The stars indicate significant differences compared to control.

3.2 Group I mGlu and LTD

In the previous section it was shown that group I mGlu antagonists are subtype-specifically involved in the induction and maintenance of 200Hz LTP. We therefore investigated whether these receptor subtypes are differentially involved in the expression of LTD induced by 1Hz low frequency stimulation (LFS=1Hz, 900 pulses) in the dentate gyrus of freely moving animals. In previous studies it was shown that group I mGlu are critically involved in the induction and maintenance of LTD, although it was not possible to induce a homosynaptic LTD in in vivo perforant path – dentate gyrus preparations (for review see [Kemp & Bashir, 2001]).

3.2.1 Group I mGlu antagonists disclose a subtype specific involvement in dentate gyrus LTD

LFS at 1Hz, in the presence of vehicle, produced a long-lasting, slowly increasing depression of both PS amplitude ($50.0 \pm 6.0\%$, $t=24h$) and fEPSP values ($58.8 \pm 7.3\%$, $t=24h$) which stabilized to values of around 60% in fEPSP and 45% in PS amplitude by 120min post-LFS (n=10, see Fig. 3.12).

mGlu5 is not critically involved in LTD

The administration of the mGlu5 antagonist MPEP, in a concentration which significantly impairs late-LTP ($7.8nmol/5\mu l$, see paragraph 3.1.1) elicited no reduction in induction and maintenance of LTD (n=9) compared to vehicle-injected controls (n=10, see Fig. 3.12). For value comparison: fEPSP slope values obtained 5min post-LFS in controls were $70.8 \pm 4.4\%$ compared to $72.1 \pm 3.0\%$ in MPEP-injected animals ($p>0.05$). The PS amplitude values obtained 5min post-LFS behave similarly ($63.6 \pm 5.2\%$ in vehicle-injected ani-

mals compared to $55.3 \pm 7.3\%$ in the MPEP-treated group, $p > 0.05$). No significant difference between the control- and MPEP-group was evident 24h post-LFS ($p > 0.05$, fEPSP slope: $68.6 \pm 5.7\%$ and PS amplitude: $45.6 \pm 8.2\%$ in control animals, compared to $50.0 \pm 6.0\%$ and $58.8 \pm 7.3\%$).

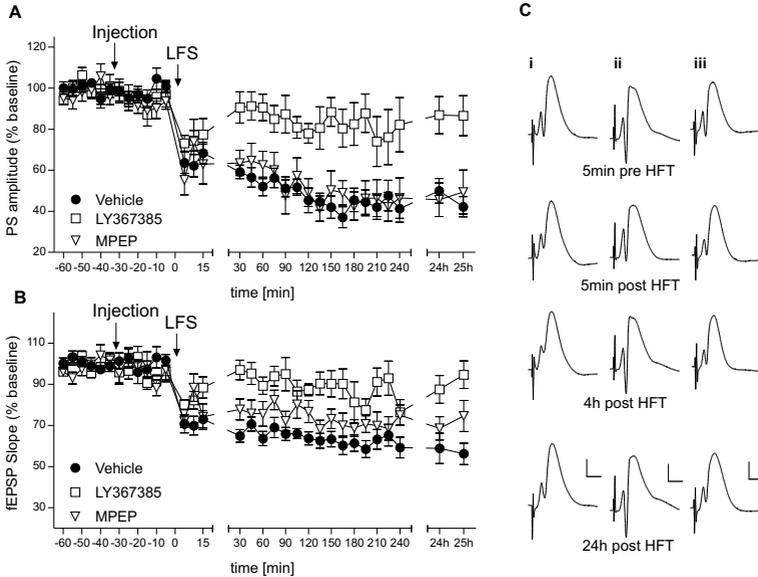


Figure 3.12: Subtype specific involvement of group I mGluR in LTD **A, B**: 1Hz LFS in the presence of vehicle ($n=10$) results in a robust LTD of both PS amplitude (A) and fEPSP slope (B). Administration of the mGlu1 antagonist LY367385 ($16\text{nmol}/5\mu\text{l}$, $n=6$) inhibits LTD compared to vehicle-injected controls. Effects become evident 30min after LFS. Animals treated with $7.8\text{nmol}/5\mu\text{l}$ MPEP show no significant alteration in LTD compared to vehicle-injected controls. **C**: Original analog traces showing the field potentials evoked from the dentate gyrus pre-HFT, 5min, 4h and 24h following HFT in the presence of (i) vehicle, (ii) LY367385 ($16\text{nmol}/5\mu\text{l}$), or (iii) MPEP ($7.8\text{nmol}/5\mu\text{l}$). Vertical scale-bar corresponds to 5mV , horizontal scale-bar to 4ms . Line breaks indicate change in time scale.

mGlu1 is critically involved in LTD

In Paragraph 3.1.2 an important role for mGlu1 activation during LTP induction using the highly specific antagonist LY367385 is demonstrated. To test the involvement of mGlu1 in LTD the antagonist was applied 30min prior to LFS, in a concentration which significantly impairs LTP (16nmol/5μl, n=6). The administration of LY367385 (16nmol/5μl, n=6), 30min prior to LFS, had no significant effect on the initial magnitude of the induced depression with regard to both PS amplitude ($73.1 \pm 3.9\%$, $t=5min$ post-LFS) and fEPSP slope ($80.1 \pm 2.5\%$, $t=5min$ post-LFS) compared to control-injected animals (PS amplitude: $63.6 \pm 5.2\%$, fEPSP slope: $70.8 \pm 4.4\%$, see Fig. 3.12). In contrast to the MPEP-treated animals, 30min post-LFS the depression in LY367385 treated animals reversed to PS amplitude and fEPSP slope values near baseline level ($90.6 \pm 7.7\%$, $97.0 \pm 4.8\%$), but was still evident in vehicle-injected controls ($t=30min$, $p < 0.001$ for PS amplitude and $p < 0.0001$ for fEPSP slope). This significant difference remained for at least 24h. ANOVA confirmed the inhibition of LTD expression in LY367385 treated animals compared to the controls (between-factor analysis: $F(1,31)=177.3$, $p < 0.0001$ for PS amplitude and $F(1,31)=149.8$, $p < 0.0001$ for fEPSP slope). These data show that, the mGlu1 antagonism by LY367385, but not mGlu5 antagonism by MPEP, blocks LTD expression in the dentate gyrus of freely moving rats.

The mGlu1 antagonist (LY367385) does not block the expression of LTD when given after completion of low frequency stimulation.

The inhibitory effects of LY367385 (16nmol/5μl) on LTD could have been mediated by a regulation of LTD induction mechanisms during LFS. To examine this possibility, LY367385 was applied 5min after

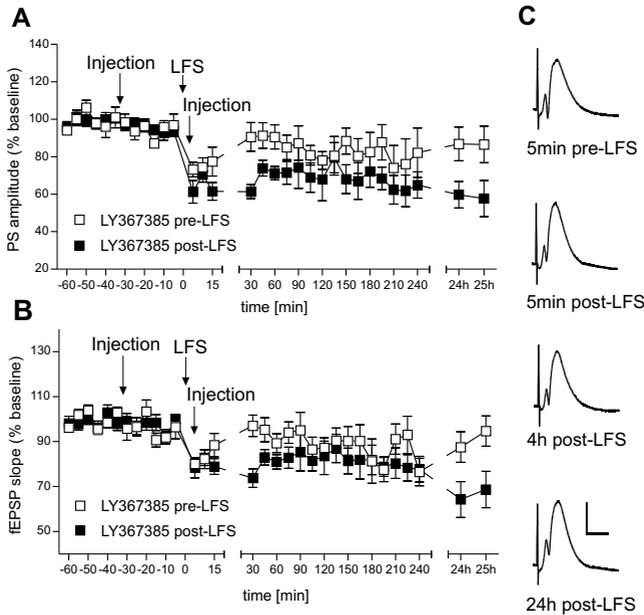


Figure 3.13: The mGlu1 antagonist (LY367385) does not block the expression of LTD when given after completion of low frequency stimulation

In contrast to the ability of LY367385 ($16\text{nmol}/5\mu\text{l}$, $n=6$) to fully block the expression of LTD when applied prior to LFS, LY367385 were ineffective on LTD when applied after its induction, with regard to both PS amplitude (**A**) and fEPSP slope (**B**) ($n=10$). **C**: Original analog traces showing the field potentials evoked from the dentate gyrus pre LFS, 5min, 4h and 24h following LFS in the presence of LY367385 ($16\text{nmol}/5\mu\text{l}$) when applied after LTD induction. Vertical scale-bar corresponds to 5mV, horizontal scale-bar to 6ms. Line breaks indicate change in time scale

LFS ($n=10$). Here, it could be demonstrated that the mGlu1 antagonist did not block the expression of LTD (see Fig. 3.13). ANOVA confirmed a significant difference between LY367385 application before and after LFS, with regard to PS amplitude (between-factor analysis: $F(1,31)=30.47$, $p<0.0001$) and fEPSP slope (between-factor analysis: $F(1,31)=16.01$, $p<0.0001$).

These data support that LTD, in the dentate gyrus of freely moving rats, depends on mGlu1 activation during LFS.

DHPG-induced chemical LTD occludes LFS-induced LTD

The administration of DHPG, a selective agonist of mGlu1 and mGlu5, in a concentration of $15\text{nmol}/5\mu\text{l}$ ($n=8$) induced robust LTD which persisted throughout the 24h monitoring period ($p<0.05$, see Fig. 3.14). ANOVA confirmed the DHPG-induced LTD when compared to vehicle-injected controls (between-factor analysis: $F(1,31)=821.23$, $p<0.0001$ and $F(1,31)=519.44$, $p<0.0001$ for PS amplitude and fEPSP slope respectively, within-factor analysis: $F(1,31)=9.71$, $p<0.0001$ for PS amplitude and $F(1,31)=4.76$, $p<0.0001$ for fEPSP slope).

To test if chemically- and LFS-induced LTD share some common induction mechanisms, LFS was applied 30min after DHPG injection. Interestingly, DHPG-induced depression was not further enhanced by subsequent LFS ($p>0.05$, see Fig. 3.14, $n=8$). To summarize, low-frequency stimulation results in a long-lasting depression of both PS amplitude and fEPSP slope, which can be occluded by prior activation of group I mGlu, indicating that both forms of LTD share some mechanistic similarities.

Pharmacological activation of mGlu5 induces chemical LTD

Studies obtained in the CA1 region demonstrated a predominant role for mGlu5 in DHPG-induced LTD [Palmer et al., 1997, Huber et al., 2001]. It was then investigated if LTD could be induced when a highly selective mGlu5 agonist was applied. (RS)-2-chloro-5-hydroxyphenylglycine (CHPG) is completely inactive at mGlu1 but selectively activates mGlu5 [Doherty et al., 1997]. Test-pulse stimulation in the presence of CHPG ($n=8$, $2.5\text{nmol}/5\mu\text{l}$) elicited

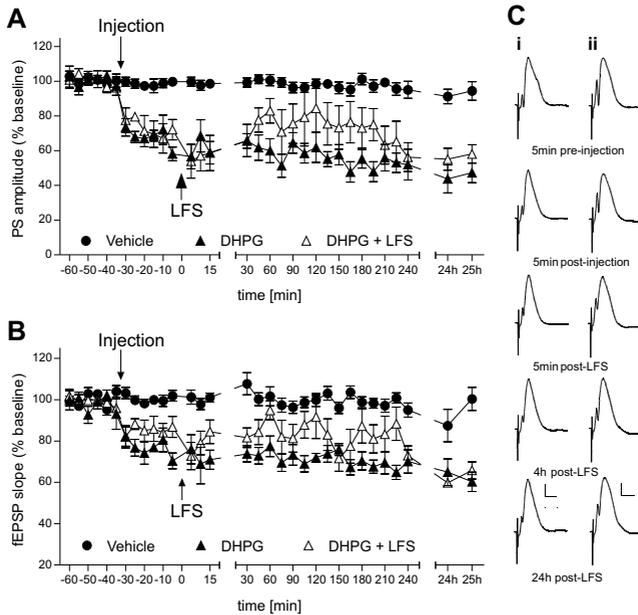


Figure 3.14: DHPG-induced chemical LTD occludes LFS-induced LTD

DHPG ($15\text{nmol}/5\mu\text{l}$, $n=8$) induces robust LTD in the perforant path dentate gyrus synapse *in vivo* which occludes subsequent LFS-LTD ($n=8$) with regard to both PS amplitude (**A**) and fEPSP slope (**B**). **C**: Original analog traces showing the field potentials evoked from the dentate gyrus pre DHPG injection and 5min post injection as well as 5min, 4h and 24h following (i) LFS or (ii) the time where the LFS was applied in the comparable group. Vertical scale-bar corresponds to 3mV, horizontal scale-bar to 6ms. Line breaks indicate change in time scale.

a marked depression of basal synaptic transmission (see Fig. 3.15). The time-course of this chemically-induced LTD was not different to LTD induced by LFS or DHPG. For example, 24h after application of CHPG, PS amplitude and fEPSP slope values did not significantly differ (PS amplitude: $60.3 \pm 16.7\%$ in CHPG-treated animals compared to $50.0 \pm 6.0\%$ in LFS-treated and $43.8 \pm 8.1\%$ in DHPG-treated animals, fEPSP slope: $78.3 \pm 15\%$ for CHPG-injected animals and

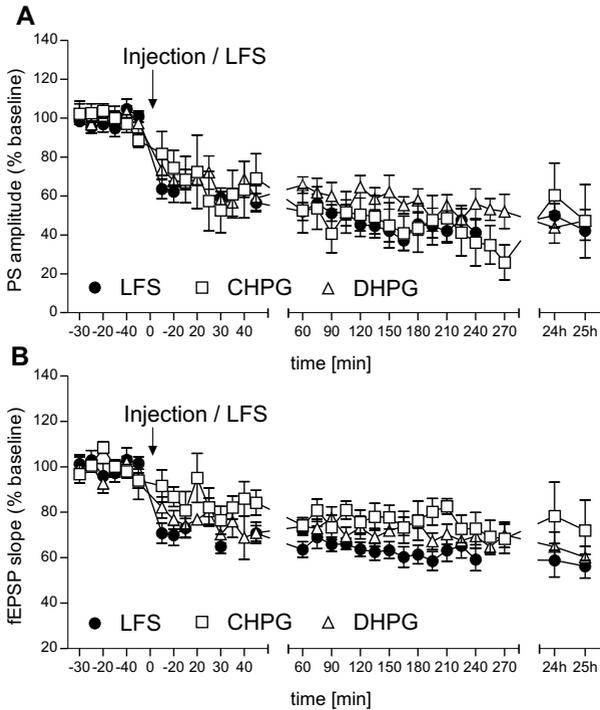


Figure 3.15: Pharmacological activation of mGlu5 induces chemical LTD

The injection of CHPG, a highly selective agonist of mGlu5 induced a stable LTD for the 24h monitoring period ($2.5\text{nmol}/5\mu\text{l}$, $n=8$) with regard to both PS amplitude (**A**) and fEPSP slope (**B**). This LTD is comparable to the depression evoked by LFS ($n=10$) or DHPG injection ($n=8$). Line breaks indicate change in time scale

$58.8 \pm 7.3\%$ for LFS-treated and $64.9 \pm 6.5\%$ for DHPG-treated animals). DHPG activates both mGlu5 and mGlu1 with an EC_{50} of $6.6\mu\text{M}$ at mGlu1 and $1.9\mu\text{M}$ at mGlu5 [Brabet et al., 1995]. Taken together with the data presented in here, it thus seems likely that predominantly mGlu5 activation caused the LTD seen in DHPG-injected animals.

Low-frequency stimulation applied concurrently with mGlu5 activation leads to reversal of chemical LTD

To evaluate if chemically- and LFS-induced LTD share similar induction mechanisms, LFS was applied 30min after CHPG injection (see Fig. 3.16). Interestingly, in this case LFS reversed the CHPG-induced LTD. Here, PS amplitude and fEPSP values returned to baseline lev-

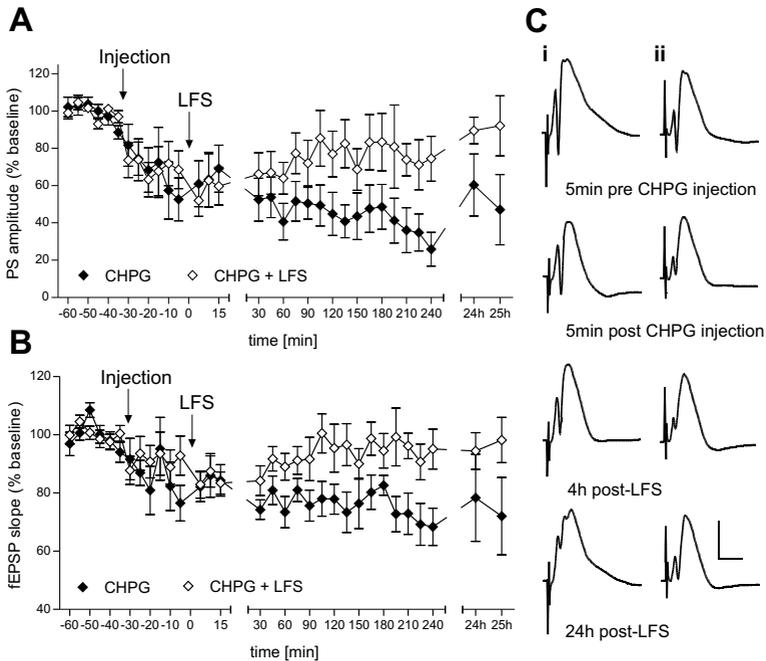


Figure 3.16: Low frequency stimulation applied concurrently with mGlu5 activation leads to reversal of chemical LTD

LTD induced by the application of the mGlu5 agonist CHPG ($n=8$) was reversed by subsequent LFS stimulation ($n=12$) with regard to both PS amplitude (**A**) and fEPSP slope (**B**). The traces (**C**) show fEPSPs 5min prior to CHPG injection and 5min after injection as well as 5min and 4h following (i) LFS or (ii) the time where the LFS was applied in the comparable group. Vertical scale-bar corresponds to $3mV$, horizontal scale-bar to $6ms$. Line breaks indicate change in time scale.

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els starting 75min post-LFS ($p>0.05$, see Fig. 3.17). ANOVA confirmed the differences between CHPG-only and CHPG-LFS groups (between-factor analysis: $F(1,31)=49.4$, $p<0.0001$ for fEPSP slope and $F(1,31)=32.53$, $p<0.0001$ for PS amplitude).

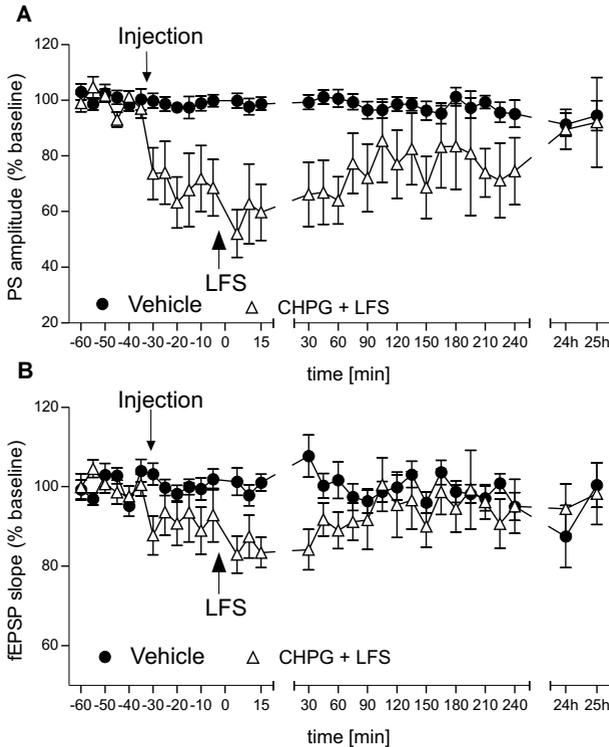


Figure 3.17: Low frequency stimulation induces redepression
LTD induced by the application of the mGlu5 agonist CHPG was reversed by subsequent LFS stimulation ($n=12$). PS amplitude (**A**) and fEPSP slope (**B**) values were statistically comparable ($p>0.05$) to baseline controls ($n=10$) from 75min and 45min post-LFS, respectively. Line breaks indicate change in time scale.

Low-frequency stimulation fails to induce LTD in animals with depressed basal synaptic transmission

In contrast to studies obtained in hippocampal slice preparations which examined LTD in the CA1 region [Palmer et al., 1997], both the CHPG-LTD and the DHPG-LTD were not further strengthened by a subsequent LFS. This raised the question as to whether LTD induced by LFS or group I mGlu activation was already in the maximal range. Therefore, level of basal synaptic transmission was reduced to LTD levels by lowering the stimulation intensity. The reduction of stimulation intensity was graded to mimic the time course of the DHPG induced LTD (n=5, see Fig. 3.18). Under these conditions it is impossible to induce a normal LTD by LFS in freely moving animals.

Interestingly, the PS amplitude values of both the mimic animals and the DHPG-treated animals which received subsequent LFS, showed a nearly identical profile. However, comparison of the values obtained from both groups with the CHPG-LFS-treated animals disclosed a significant difference in the 24h values ($p < 0.005$). Thus, whereas the mimic and the DHPG-LFS-treated group demonstrated PS amplitude values of $44.9 \pm 3.9\%$ and $55.2 \pm 6.4\%$ respectively, the CHPG-LFS-treated group expressed PS amplitude values of 89.6 ± 7.1 (see Fig. 3.18). These data suggest that in the dentate gyrus, it is not possible to induce LTD with LFS under conditions of reduced synaptic transmission, regardless of whether the reduction in transmission was elicited by chemical means (DHPG) or by reducing stimulation intensity. However, strong and selective activation of the mGlu5 by CHPG prior to application of LFS appears to block the functional mechanisms leading to LTD under normal conditions.

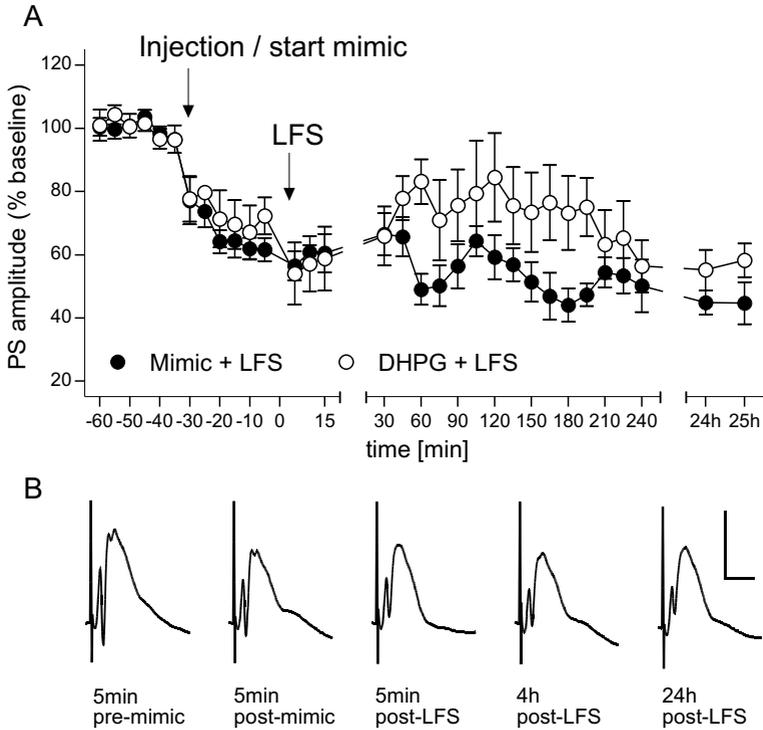


Figure 3.18: Low frequency stimulation fails to induce LTD in animals with depressed basal synaptic transmission

A: Test-pulse stimulation when given in the presence of DHPG ($15\text{nmol}/5\mu\text{l}$, $n=8$) significantly depresses synaptic transmission. LFS given 30min after injection did not result in the further (additional) induction of LTD. Instead of drug injection, vehicle was injected ($n=5$) and the depression of basal synaptic transmission by DHPG was mimicked by decreasing the stimulation intensity. Under these conditions LFS also failed to induce persistent LTD. **B:** Original analog traces showing the field potentials evoked from the dentate gyrus 5min before and 5min after the mimicked depression as well as 5min , 4h and 24h post LFS. Vertical scale-bar corresponds to 5mV , horizontal scale-bar corresponds to 5ms . Line breaks indicate change in time scale.

LFS induced reversal of CHPG-LTD is mediated by a calcium dependent mechanism

There is wide agreement that both LTP and LTD, are triggered by different $[Ca^{2+}]$ levels [Bear & Malenka, 1994, Malenka, 1994, Mulkey & Malenka, 1992]. In agreement with the theory proposed by Lisman (1989), Artola and Singer (1993) demonstrated that a moderate rise in intracellular $[Ca^{2+}]$ leads to a predominant activation of phosphatases, resulting in LTD, whereas a strong increase in cytosolic $[Ca^{2+}]$ favors activation of kinases and subsequent LTP. More recently it was shown that LTD is induced with a stimulation paradigm that normally results in LTP, if calcium levels are buffered appropriately [Cho et al., 2001].

The following postulation was made: the CHPG-induced depression may result from an increase of intracellular $[Ca^{2+}]$ mediated by mGlu5 activation, whereas LFS may produce a similar elevation in intracellular $[Ca^{2+}]$ to result in LFS-induced LTD. In the context where CHPG application was followed by LFS, the resulting inhibition of LTD may have arisen because a summation of intracellular $[Ca^{2+}]$ elevation by CHPG and LFS occurred which raised intracellular $[Ca^{2+}]$ to a level sufficient for LTP to be induced. To test this hypothesis, intrahippocampal injections of the membrane-permeable calcium chelator BAPTA-AM were conducted. The chelator was injected exactly beside the recording site in the dentate gyrus granule cell layer 10min after the CHPG injection in the lateral cerebral ventricle ($2.5\mu M/0.5\mu l$, $n=4$, see Fig. 3.19 and Fig. 3.20). This concentration, as well as the higher concentration of $5\mu M/0.5\mu l$ (data not shown) had no influence on basal synaptic transmission. The CHPG-induced depression was unchanged in the presence of BAPTA-AM ($p>0.05$, $n=4$). However LFS application in the presence of CHPG resulted in normal LTD when BAPTA-AM was previously

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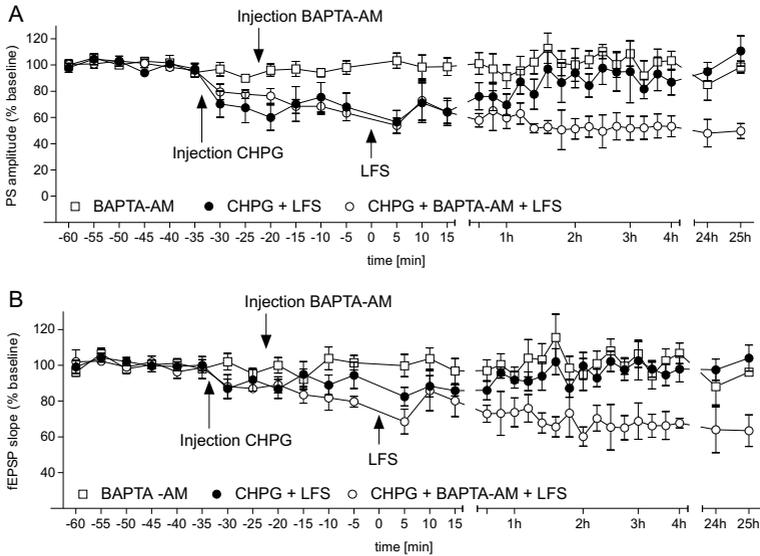


Figure 3.19: LFS induced reversal of CHPG-LTD is mediated by a calcium dependent mechanism

Injection of the calcium chelator BAPTA-AM ($2.5\mu\text{M}/0.5\mu\text{l}$, $n=4$) did not influence basal synaptic transmission over the 25h monitoring period. LFS applied 30min prior to CHPG injection ($2.5\text{nmol}/5\mu\text{l}$, $n=11$) causes reversal of this chemically induced LTD with regard to both PS amplitude (A) and fEPSP slope (B). Injection of BAPTA-AM intrahippocampally 10min after CHPG injection ($n=4$) prevents the LFS induced dedepression. Line breaks indicate change in time scale.

applied (see Fig. 3.19 and Fig. 3.20). The results were confirmed by ANOVA (between-factor: $F(1,31)=27.75$, $p<0.0001$ for PS amplitude and $F(1,31)=76.54$, $p<0.0001$ for fEPSP slope) when CHPG-LFS was compared with BAPTA-AM-CHPG-LFS.

These data suggest that CHPG elicits LTD by causing an increase in $[Ca^{2+}]$, this calcium increase can summate with intracellular $[Ca^{2+}]$ levels elicited by LFS to achieve a sufficient $[Ca^{2+}]$ concentration for the induction of LTP.

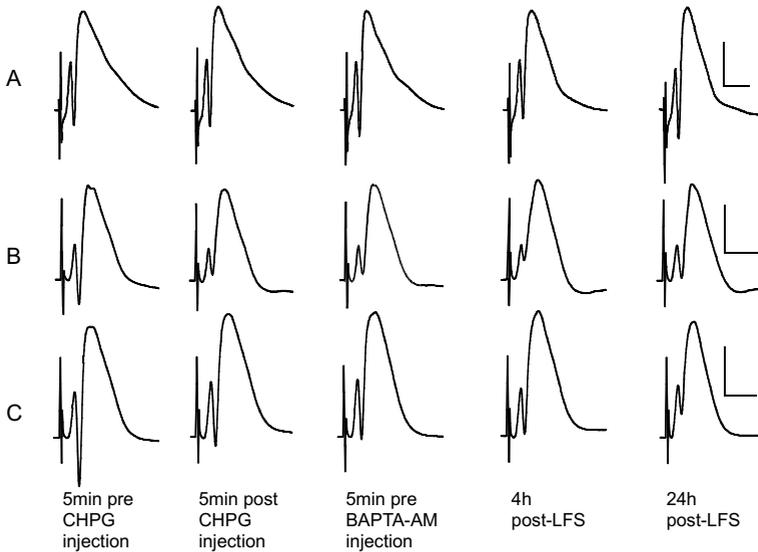


Figure 3.20: LFS induced reversal of CHPG-LTD is mediated by a calcium dependent mechanism

Original analog traces showing the field potentials evoked from the dentate gyrus before ($t=-35$) and after ($t=-30$) CHPG injection, after BAPTA-AM injection ($t=-20$), as well as *5min* and *4h* after LFS application in animals which received (C) CHPG and BAPTA-AM injections as well as LFS. The traces obtained from animals which received only (A) BAPTA-AM injections or only (B) CHPG injection with subsequent LFS were taken at analog time points. Vertical scale-bar corresponds to $3mV$, horizontal scale-bar corresponds to $6ms$.

Calcium chelation in the presence of CHPG does not alter the profile of LTD induction during LFS

In order to evaluate if BAPTA-AM injections causes differences in the profile of LTD induction, a comparison of LTD induction *during* LFS was made between vehicle-treated animals ($n=8$) and those which received CHPG with BAPTA-AM. Thus, PS amplitude and fEPSP values were assessed during the 900 LFS pulses at $1Hz$. CHPG given alone caused in a significant depression of both PS amplitude and fEPSP values as described earlier.

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For value comparability the data were expressed as mean % pre-LFS baseline reading \pm standard error of the mean using the last three pre-LFS values. Therefore the PS amplitude and fEPSP values of both groups were not significantly different when obtained 5min pre-LFS. Thus, fEPSP values were $104.8 \pm 1.9\%$ in the vehicle injected animals and $101.2 \pm 2.0\%$ in the animals which received CHPG and BAPTA-AM. PS amplitude was $102.2 \pm 2.5\%$ and $104.0 \pm 3.1\%$ in the vehicle and CHPG/BAPTA-AM treated group respectively. Both, PS amplitude and fEPSP slope values showed no significant differences ($p > 0.05$) when the vehicle- and drug-group were compared during the course of the LFS (see Fig. 3.21). For example, the first 10 stimuli resulted in PS amplitude values of $85.4 \pm 10.8\%$ and $89.8 \pm 19.4\%$ in vehicle- and drug-treated animals respectively, the last 10 stimuli resulted in PS amplitude values of $40.1 \pm 10.5\%$ in vehicle treated animals and $44.1 \pm 11.3\%$ in CHPG/BAPTA-AM treated animals. The fEPSP values reduced by 63% in the CHPG/BAPTA-AM group and 69% in the vehicle group and the PS amplitude values reduced by 47% and 49% respectively, during the 1Hz stimulation.

However, the first test pulse evoked 5min after LFS showed significantly ($p < 0.05$) different PS amplitude values, with $59.0 \pm 6.9\%$ in vehicle treated animals and $83.7 \pm 5.2\%$ in CHPG/BAPTA-AM treated animals. The fEPSP slope values did not reach significance with values of $74.3 \pm 3.8\%$ in vehicle and $86.8 \pm 6.2\%$ in CHPG/BAPTA-AM treated animals.

Taken together, evaluation of evoked responses during LFS stimulation in CHPG and BAPTA-AM treated animals revealed no significant difference compared to vehicle-injected controls. However the synaptic depression post-LFS with regard to PS amplitude, obtained in CHPG/BAPTA-AM-treated animals was significantly decreased compared to vehicle injected controls.

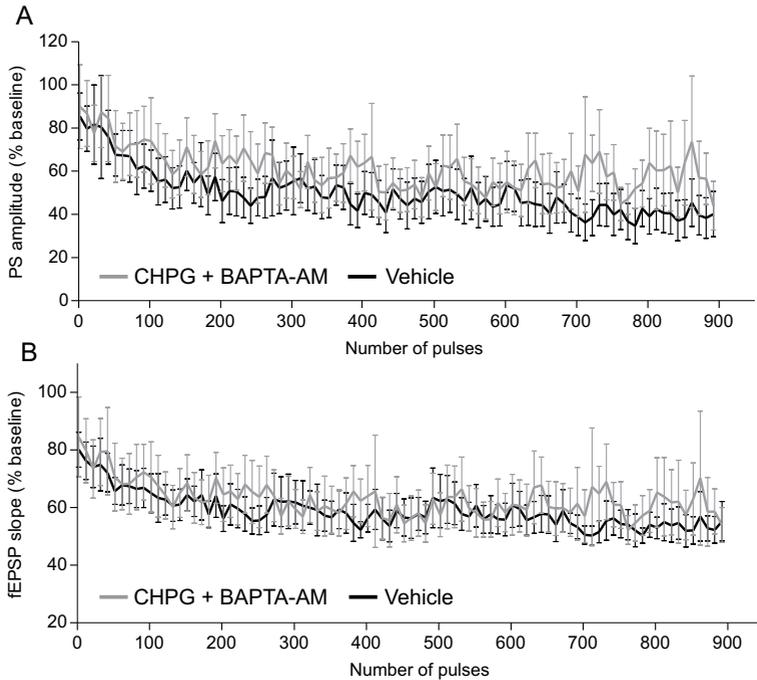


Figure 3.21: BAPTA-AM does not alter the profile of LTD induction during LFS

PS amplitude (A) and fEPSP slope (B) responses to each of 900 stimuli given at 1Hz were recorded and presented as groups of 10 stimuli (mean % pre-LFS baseline). In the vehicle treated group (n=8) as well as in the group which received CHPG (2.5nM/5μl) together with BAPTA-AM (2.5μM/0.5μl, n=4) no significant difference in the change of PS amplitude and fEPSP slope during LFS application could be observed.

3.2.2 Protein synthesis dependency of chemically induced LTD

LTD induced by Group I and III mGlu activation

Test-pulse evoked potentials in control animals ($n=9$) were stable over a 24h monitoring period. Administration of DHPG a general agonist of mGlu1 and mGlu5 in a concentration of $15\text{nmol}/5\mu\text{l}$ ($n=10$, see Fig. 3.22) induced a robust LTD in which PS amplitude values were depressed 5min after injection to $42.1 \pm 9.4\%$ of control ($p<0.05$) and fEPSP slope values to $83.8 \pm 4.1\%$ of control. DHPG-

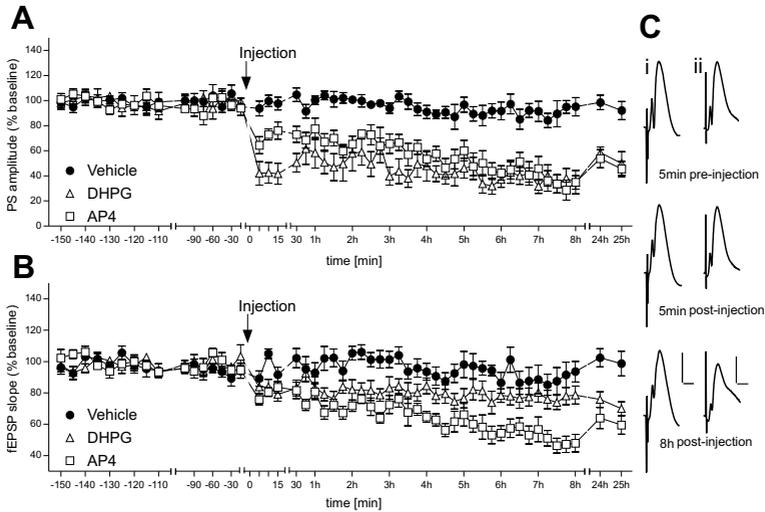


Figure 3.22: Application of DHPG and AP4 induce robust LTD **A, B:** Administration of DHPG ($n=9$) or AP4($n=10$) induced a robust, long-lasting LTD in the dentate gyrus of freely moving animals with regard to both PS amplitude (A) and fEPSP slope (B). **C:** Original analog traces showing the field potentials evoked from the dentate gyrus pre-injection, 5min and 8h post-injection from DHPG (i) and AP4 (ii) treated animals. Vertical scale-bar corresponds to 3mV, horizontal scale-bar to 4ms. Line breaks indicate a change in time-scale.

LTD was still present after 24h. ANOVA confirmed this chemically

induced LTD compared to vehicle-injected controls (between-factor analysis: $F(1,51)=509.27$, $p<0.0001$ and $F(1,51)=94.86$, $p<0.0001$ for PS amplitude and fEPSP slope respectively; within-factor analysis: $F(1,51)=6.86$, $p<0.0001$ for PS amplitude and $F(1,51)=2.22$, $p<0.0001$ for fEPSP slope). The administration of a highly selective agonist of group III mGluR, AP4, in a concentration of $400\text{nmol}/5\mu\text{l}$ ($n=10$) induced robust LTD in the dentate gyrus of freely moving rats (see Fig. 3.22) in which PS amplitude values were depressed 5min after injection to $64.47 \pm 6.8\%$ of control ($p<0.05$) and fEPSP slope values to $75.75 \pm 3.3\%$ of control. AP4-LTD was still present after 24h. ANOVA confirmed this chemically induced LTD compared to vehicle-injected controls (between-factor analysis: $F(1,51)=505.07$, $p<0.0001$ and $F(1,51)=399.49$, $p<0.0001$ for PS amplitude and fEPSP slope respectively; within-factor analysis: $F(1,51)=8.79$, $p<0.0001$ for PS amplitude and $F(1,51)=7.45$, $p<0.0001$ for fEPSP slope).

Protein synthesis dependency

In the CA1 region of freely moving animals it was shown that Anisomycin the mRNA translation inhibitor significantly inhibit LFS-LTD [Manahan-Vaughan et al., 2000]. In this study we wanted to show the influence of protein translation in DHPG- and AP4-LTD. Anisomycin ($2.4\text{mg}/5\mu\text{l}$, $n=4$) had no significant effect on basal synaptic transmission when compared to vehicle-injected animals ($n=9$, see Fig. 3.23).

When Anisomycin ($2.4\text{mg}/5\mu\text{l}$) was applied 2h prior to DHPG ($15\text{nmol}/5\mu\text{l}$) injection, a significant inhibition of LTD occurred ($n=5$). In contrast to control animals where the depression elicited by DHPG was apparent immediately following DHPG injection, DHPG induced a slowly developing depression (see Fig. 3.24). This depression was still significant compared to vehicle-injected controls,

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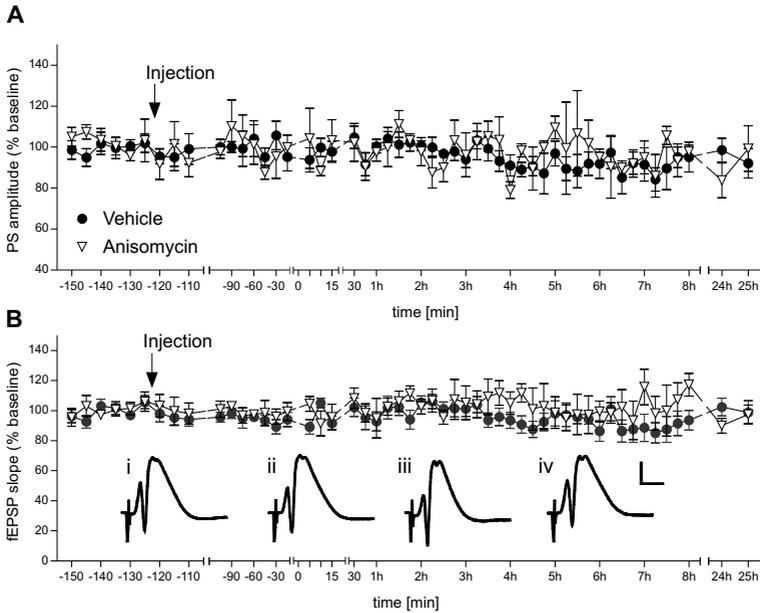


Figure 3.23: Anisomycin has no effect on basal synaptic transmission **A, B:** Administration of Anisomycin ($2.4\text{mg}/5\mu\text{l}$) has no effect on basal synaptic transmission compared to vehicle-injected controls in the dentate gyrus of freely moving animals with regard to both PS amplitude (A) and fEPSP slope (B). **Inserts:** Original analog traces showing the field potentials evoked from the dentate gyrus (i) 5min pre-injection (ii) 5min post-injection, (iii) 4h and (iv) 24h post-injection. Vertical scale-bar corresponds to 3mV , horizontal scale-bar to 4ms . Line breaks indicate change in time scale.

but was of lesser magnitude (for value comparison see Tab. 3.4). Thirteen min after DHPG-injection no significant difference was apparent between the LTD levels of the Anisomycin-treated and untreated groups. Effects were maintained in the Anisomycin/DHPG group until $t=6\text{h}$ following DHPG-injection. After this point a significant recovery of PS amplitude values in the Anisomycin group was noted ($p<0.05$). ANOVA confirmed the differences between the Anisomycin treated and untreated DHPG groups. Thus, the between-

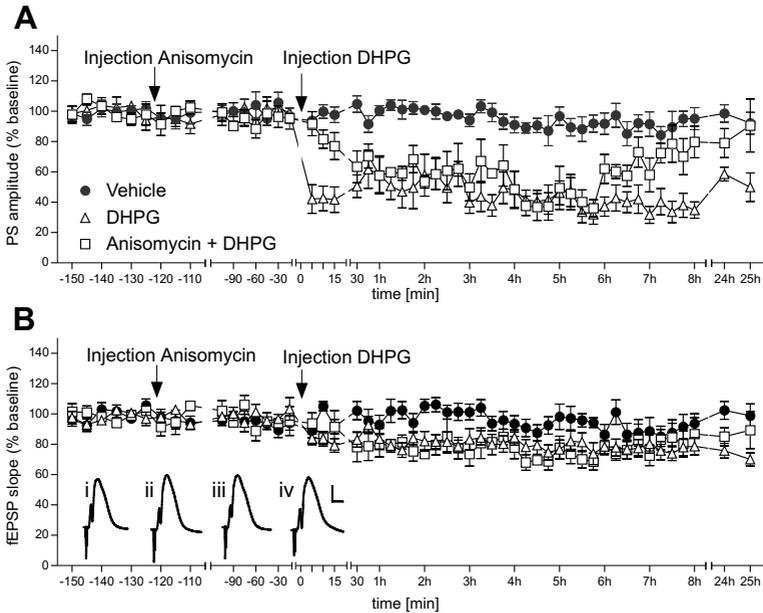


Figure 3.24: Anisomycin reduces the initial magnitude of depression and inhibits the expression of DHPG-LTD

A, B: Injection of the protein translation inhibitor Anisomycin ($2.4\text{mg}/5\mu\text{l}$) 2h before DHPG injection ($n=5$) reduces the initial magnitude of depression and inhibits the expression of LTD from 6h post-DHPG injection in PS amplitude values (A). There is no significant difference between the Anisomycin treated and untreated group in comparison of fEPSP slope values obtained (B). **Inserts:** Original analog traces showing the field potentials evoked from the dentate gyrus (i) pre-injection, (ii) 5min, (iii) 4h and (iv) 24h post-injection from animals treated with Anisomycin and DHPG. Vertical scale-bar corresponds to 3mV , horizontal scale-bar to 4ms . Line breaks indicate change in time scale.

factor analysis yielded $F(1,51)=31.77$, $p<0.0001$ for PS amplitude values. The fEPSP values obtained show just a slight depression after DHPG-injection. Therefore, there was no significant difference between the LTD obtained in Anisomycin treated and untreated animals. When Anisomycin ($2.4\text{mg}/5\mu\text{l}$) was applied 2h prior to AP4 ($400\text{nmol}/5\mu\text{l}$) injection ($n=7$), no significant changes in comparison

Table 3.4: PS amplitude values obtained from Anisomycin-treated and -untreated animals in the presence of DHPG

treatment	10min post-DHPG	1h post-DHPG	8h post-DHPG	24h post-DHPG
untreated[%]	42.5 ± 8.2	58.4 ± 12.2	34.8 ± 5.4	58.5 ± 14.6
treated[%]	81.6 ± 6.1	57.6 ± 12.1	79.8 ± 10.4	79.0 ± 9.6

to animals which did not receive Anisomycin (n=10) were detected (see Fig. 3.25). ANOVA confirmed this lack of effect. Thus, the between-factor analysis yielded $F(1,51)=0.16$, $p>0.5$ for PS amplitude values and $F(1,51)=2.92$; $p>0.5$ for fEPSP slope.

Chemically induced LTD can be reversed by HFT

As it was shown in paragraph 3.1, HFT at 200Hz typically elicits robust LTP in the dentate gyrus of freely moving animals. To examine the mechanism of mGlu-mediated LTD, the response to HFT was evaluated.

The depression of basal synaptic transmission evoked by the group III mGlu agonist AP4 (n=6) resulted in PS amplitude values of $56.2 \pm 8.7\%$ approx. 2h post-injection. The DHPG induced depression (n=5) resulted in a similar reduction ($p>0.3$) with PS amplitude values of $66.2 \pm 4.0\%$ 2h after drug injection. Application of HFT 2h following drug injection resulted in a complete reversal of synaptic depression (see Fig. 3.26). Moreover, a synaptic potentiation was elicited by this tetanus paradigm which was persistent and still present at the termination of the experiment at 25h post-injection. PS amplitude values reversed to $147.3 \pm 21.2\%$ at 5min post-HFT and were still at $122.6 \pm 25.3\%$ of control 24h after tetanisation. In contrast, the DHPG treated group potentiated to baseline levels at 5min after the HFT (PS amplitude value: $107.8 \pm 25, 7\%$) and stayed there for further 2h, but returned to pre-stimulated levels 24h after HFT

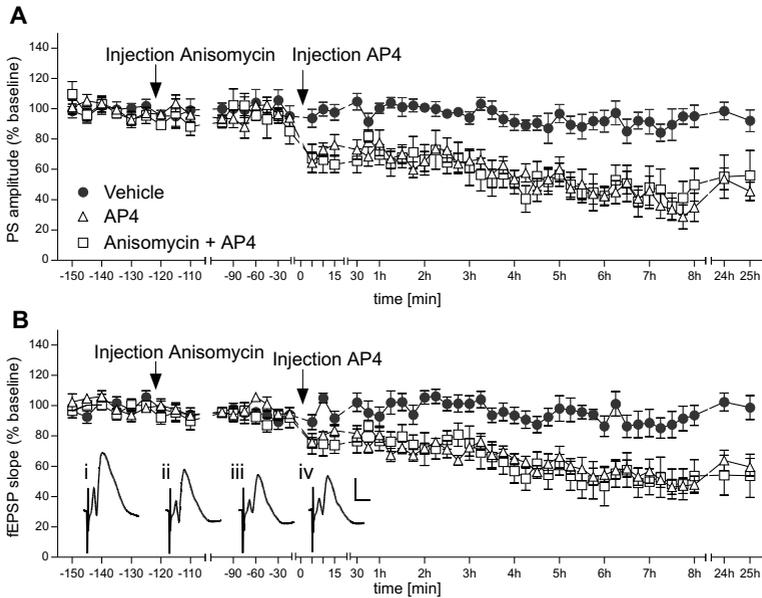


Figure 3.25: Anisomycin has no influence on AP4-LTD

A, B: Injection of the protein translation inhibitor Anisomycin ($2.4\text{mg}/5\mu\text{l}$) 2h before AP4 ($n=7$) injection do not significantly change the mGlu mediated depression with regard to both PS amplitude (A) and fEPSP slope (B). **Inserts:** Original analog traces showing the field potentials evoked from the dentate gyrus (i) pre-injection, (ii) 5min, (iii) 8h and (iv) 24h post-injection from an animal treated with Anisomycin and AP4. Vertical scale-bar corresponds to 3mV , horizontal scale-bar to 4ms . Line breaks indicate change in time scale.

application (PS amplitude value: $68.4 \pm 18.1\%$; $p > 0.9$).

To exclude an influence of the drugs on the LTP elicited, mimic experiments were performed. In this case we reduced the synaptic transmission after vehicle injection ($n=7$) by slowly decreasing the stimulation intensity to a level where PS amplitude values reached approx. 50%. A subsequent application of HFT resulted in a synaptic potentiation which was persistent over the 25h monitoring period (see Fig. 3.26). Five minutes after HFT was applied PS amplitude

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values reversed to $216.9 \pm 20.1\%$ of baseline level. Whereas the potentiation obtained in the fEPSP slope values of the AP4 treated group were significantly less than in the mimic controls in the first 2h after the HFT, the 24h values did not significantly differ ($p > 0.05$). In contrast, DHPG values were significantly different from mimic controls (ANOVA between-factor analysis: $F=29.33$; $p < 0.0001$).

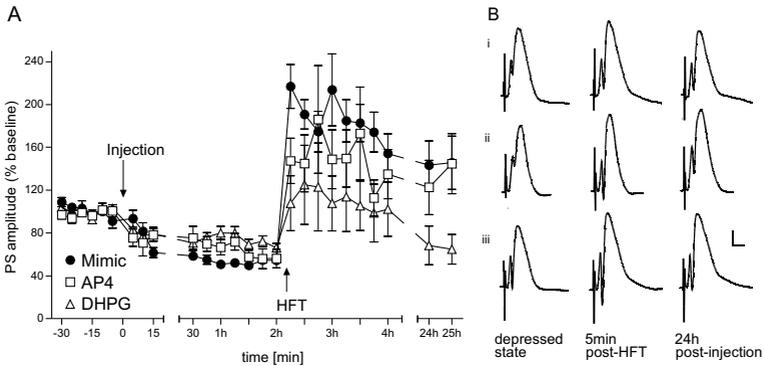


Figure 3.26: Metabotropic glutamate receptor mediated depression of basal synaptic transmission is reversed by HFT

A: Test-pulse stimulation when given in the presence of AP4 ($400\text{nmol}/5\mu\text{l}$, $n=6$) or DHPG ($15\text{nmol}/5\mu\text{l}$; $n=5$) significantly depresses synaptic transmission. HFT given 2h after AP4 injection significantly reverses the synaptic depression induced by application of the agonist. A significant potentiation of evoked responses occurred in the AP4 treated group which was still present 24h after injection. The HFT given to the DHPG treated group resulted in a repotentialization to baseline levels which decline to pre-HFT levels within 24h. Instead of drug injection, vehicle was injected ($n=7$) and the depression of basal synaptic transmission was mimicked by decreased stimulation intensity. In this case, HFT could elicit a stable potentiation over the 25h monitoring period. **B:** Original analog traces showing the field potentials evoked from the dentate gyrus before HFT, 5min post-HFT, 24h following application of (i) vehicle ($n=7$), (ii) AP4 ($400\text{nmol}/5\mu\text{l}$, $n=6$), or (iii) DHPG ($15\text{nmol}/5\mu\text{l}$, $n=5$). Vertical scale-bar corresponds to 3mV , horizontal scale-bar corresponds to 4ms . Line breaks indicate change in time scale.

Presynaptic induction of chemical LTD

Due to the controversy in the literature as to the expression site of DHPG-induced LTD, the locus of mGlu-induced LTD were investigated by means of a paired pulse paradigm. In control animals ($n=12$), the second synaptic response was consistently reduced in comparison to the first at short latency ($40ms$ ISI, paired pulse ratio= 0.4 ± 0.11) and long latency ($500ms$ ISI paired pulse ratio= 0.6 ± 0.06) paired pulse depression (see Fig. 3.27). The intermediate ISIs

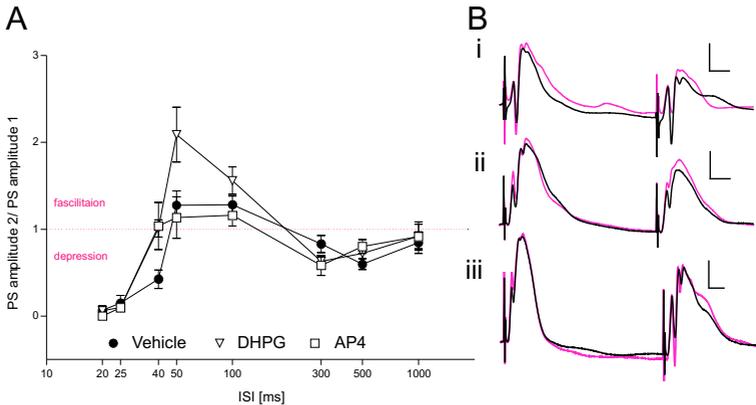


Figure 3.27: Inhibition of paired pulse facilitation due to mGlu activation

A: Paired pulse stimulation given 30min following application of vehicle ($n=12$) resulted in a characteristic depression ($20\text{--}40ms$ intervals), followed by a facilitation (50 and $100ms$ intervals) and second depression ($300\text{--}1000ms$ intervals). Application of (i) AP4 ($n=11$) and (ii) DHPG ($n=12$) resulted in a facilitation of responses in the $40ms$ range. In contrast to AP4, DHPG induced also an enhanced paired pulse facilitation at $50ms$ ISI (iii) whereas all other pulse interval responses were unaffected. Black analogs correspond to vehicle treatments, red analogs to agonist treatments. Vertical scale-bar corresponds to $3mV$, horizontal scale-bar corresponds to $5ms$.

($50ms$ and $100ms$) produce a paired pulse facilitation in the control animals ($50ms$, ISI paired pulse ratio= 1.33 ± 0.16). Application of the group I mGlu agonist ($n=12$) resulted in an inhibition of paired

pulse depression at 40ms ISI (ratio= 1.0 ± 0.1 ; $p < 0.001$) and in an enhanced paired pulse facilitation at 50ms ISI (ratio= 2.1 ± 0.3 , $p < 0.03$, see Fig. 3.27). Activation of group III mGlu by AP4 resulted only in an inhibition of paired pulse depression at 40ms ISI (ratio= 1.0 ± 0.3 ; $p < 0.05$, see Fig. 3.27).

3.3 AP4-mediated LTD is associated with cell death

Application of the group III mGlu agonist AP4 in a concentration of 400nmol/5 μ l induces significant depression of basal synaptic transmission in the dentate gyrus, as seen in the previous section, and CA1 region of the hippocampus in freely moving rats [Klausnitzer & Manahan-Vaughan, personal communication]. As a next step, the relation between chemically-induced LTD and cell viability will to be determined.

3.3.1 The group III mGlu agonist, AP4, both acutely and chronically impairs cell viability in the CA1 region

An earlier study revealed that chemical LTP induced by strong activation of group I mGlu elicits cell death in the hippocampus [Manahan-Vaughan et al., 1999a], here we examined if AP4 induced plasticity has consequences for cell viability. It was previously shown that vehicle injection elicited no significant effects on cell viability in the hippocampus [Manahan-Vaughan et al., 1999a]. Here, the effect of AP4 (400nmol/5 μ l) application was compared with vehicle application.

3.3 AP4-mediated LTD is associated with cell death

Four hours after injection a significant difference in the percentage of dead cells was noted when AP4 and vehicle treated animals were compared (see Fig. 3.28, Fig. 3.29 A). Thus the percentage of dead cells was significantly higher in AP4 treated animals ($p < 0.0001$). Live cell percentage was also significantly lower between both groups ($p = 0.001$, see Fig. 3.29 B). These effects were very subtle however. Thus, cells were predominantly healthy with $97.46 \pm 0.34\%$ live cells

Table 3.5: Values to compare cell viability in AP4 or vehicle treated animals 4h after injection

Analysis	Vehicle 4h	AP4 4h
dead cells[%]	2.54 ± 0.34	6.17 ± 0.3
live cells[%]	97.46 ± 0.34	93.8 ± 0.3
area dead cells[%]	0.07 ± 0.03	0.11 ± 0.01
ratio dead to live cells[%]	0.54 ± 0.05	0.59 ± 0.05
dead cells area[px]	499 ± 38	795 ± 67
live cells area[px]	96297 ± 3729	138117 ± 2474
total cell area[px]	96637 ± 3727	138913 ± 2475
dead cell density	0.54 ± 0.05	0.58 ± 0.04
live cell density	13.37 ± 0.52	19.18 ± 0.34

occurring in the vehicle treated and $93.8 \pm 0.3\%$ live cells present in the AP4 treated groups. The percentage area filled by dead cells ($(Pd/Pl + Pd) \times 100$) was neither significantly different ($p > 0.05$, see Fig. 3.29 C) nor was the ratio of dead to live cells (Pd/Pl) significantly altered (see Fig. 3.29 D). These observations may be explained by the fact that both dead cell area (Pd) and live cell area (Pl) were increased ($p < 0.05$ and $p < 0.001$ respectively, see Fig. 3.29 E, F), thus, the increase in dead cell number was not reflected by a change in dead cell to live cell ratio or increase in the percentage of total area filled by dead cells. This likelihood is corroborated by the observation that the total cell area ($Pl + Pd$) was increased in AP4 treated animals

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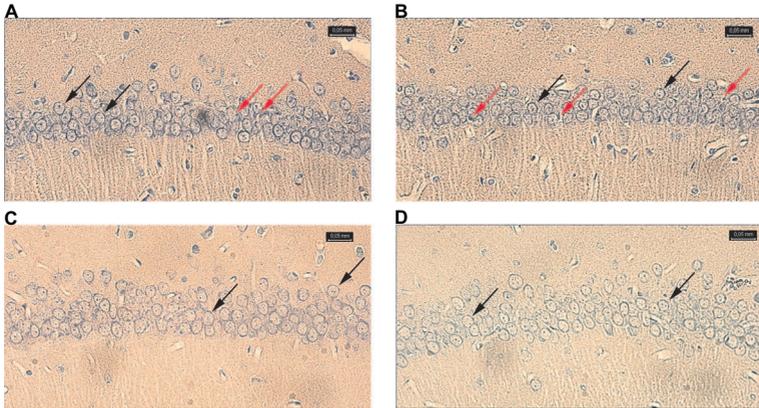


Figure 3.28: The effect of vehicle or AP4 injection on cell viability in the CA1 region

Application of AP4 ($400\text{nmol}/5\mu\text{l}$) increases the number of dead cells in the CA1 region when examined **A: 4h** and **B: 7d** following injection compared to vehicle treated controls **4h (C)** and **7d (D)** after injection. The black arrows indicate examples of healthy cells. The red arrows indicate typical examples of dead cells. Examples represent hippocampal sections from the hemisphere into which the drug or vehicle solutions were injected. Scale bar corresponds to 0.05mm .

($p < 0.001$, see Fig. 3.29 G). In addition, the density of both dead cells (Pd/Pa) (see Fig. 3.29 H) and live cells (Pl/Pa) (see Fig. 3.29 I) was significantly higher in AP4 treated animals ($p < 0.05$ and $p < 0.0001$ respectively, for single value comparison see Tab. 3.5).

The relative scale of these effects was small however, given that the total percentage of dead cells was in the range of 6% following AP4 treatment. Thus, over 90% of cells in the CA1 region were unaffected by acute treatment with AP4. At the same time, the level of dead cells was in the range of $2.54 \pm 0.34\%$ in vehicle treated animals, whereupon application of AP4 brought about more than a doubling of cell death to $6.17 \pm 0.33\%$ in the CA1 region.

3.3 AP4-mediated LTD is associated with cell death

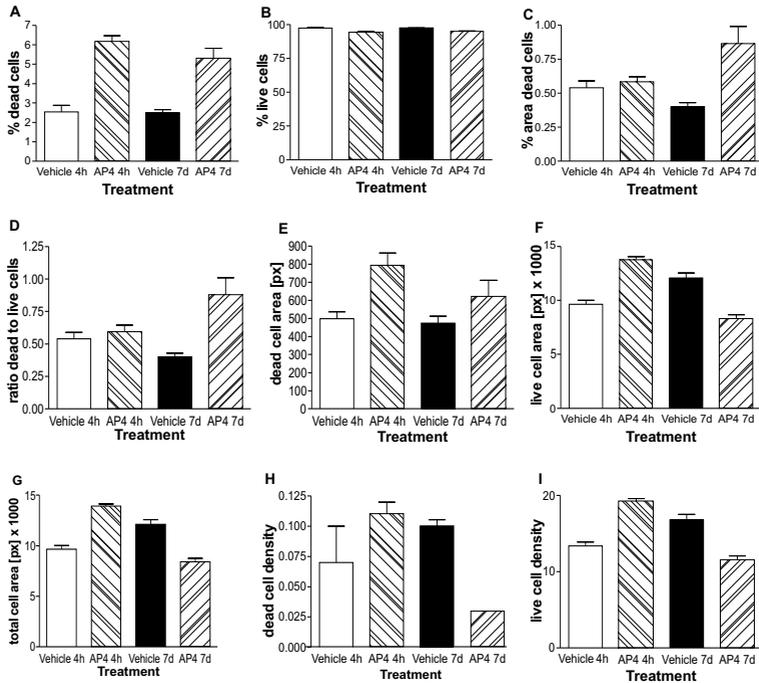


Figure 3.29: Barcharts representing the effect of application of vehicle or AP4 on cell viability in the CA1 region in vivo
A: Percentage number of dead cells. **B:** Percentage number of live cells. **C:** Percentage area of dead cells. **D:** Ratio of dead to live cells. **E:** Total area filled by dead cells. **F:** Total area filled by live cells. **G:** Total tissue area filled by cells. **H:** Density of dead cells. **I:** Density of live cells.

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Table 3.6: Values to compare cell viability in AP4 or vehicle treated animals 7d after injection

Analysis	Vehicle 7d	AP4 7d
dead cells[%]	2.5 ± 0.15	5.28 ± 0.54
live cells[%]	97.5 ± 0.15	94.7 ± 0.5
area dead cells[%]	0.1 ± 0.005	0.03 ± 0
ratio dead to live cells[%]	0.4 ± 0.03	0.88 ± 0.13
dead cells area[px]	474 ± 39	622 ± 90
live cells area[px]	120681 ± 4807	83419 ± 3453
total cell area[px]	121077 ± 4816	84081 ± 3427
dead cell density	0.4 ± 0.03	0.86 ± 0.13
live cell density	16.8 ± 0.7	11.59 ± 0.48

Analysis of the CA1 region 7d after injection revealed that AP4 treated animals exhibited a significantly higher percentage of dead cells compared to vehicle injected controls ($p < 0.0001$, see Fig. 3.29 A, for single value comparison see Tab. 3.6). Live cell percentage was significantly lower ($p < 0.0001$, see Fig. 3.29 B). The percentage area filled by dead cells ($Pd/(Pl + Pd) \times 100$) (see Fig. 3.29 C) and the ratio of dead to live cells (Pd/Pl) (see Fig. 3.29 D) was substantially higher in AP4 treated animals ($p < 0.001$ and $p < 0.01$, respectively). Dead cell area, (Pd), was not significantly altered in AP4 treated animals (see Fig. 3.29 E), whereas live cell area, (Pl), was reduced ($p < 0.0001$, see Fig. 3.29 F). The total cell area ($P + Pd$) was significantly lower in AP4 treated animals ($p < 0.0001$, see Fig. 3.29 G). In addition, the density of dead cells (Pd/Pa) and of live cells (Pl/Pa) was significantly lower ($p < 0.0001$, $p < 0.001$ respectively, see Fig. 3.29 H, I).

3.3.2 The group III mGlu agonist, AP4, mildly impairs cell density and area in the dentate gyrus

When the effect of AP4 application ($400\text{nmol}/5\mu\text{l}$) on cell viability was evaluated histologically some subtle but significant differences in cell density and area were found in AP4 treated animals compared to vehicle treated controls (see Fig. 3.30 and Fig. 3.31, for single value comparison see Tab. 3.7). Four hours after AP4 treatment a significant reduction in the percentage number of dead cells was seen compared to vehicle treated controls ($p < 0.001$, see Fig. 3.31 A). A slight increase in the percentage number of live cells was also noted in the AP4 treated group ($p < 0.001$, see Fig. 3.31 B). The percentage area filled by dead cells ($Pd/(Pl + Pd) \times 100$) was significantly increased however ($p < 0.0001$, Fig. 3.31 C), as was the ratio of dead to live cells (Pd/Pl) ($p < 0.001$, see Fig. 3.31 D) and the dead cell area, (Pd , $p = 0.004$, see Fig. 3.31 E). Live cell area, (Pl) and total

Table 3.7: Values comparing cell viability in AP4 or vehicle treated animals 4h after injection

Analysis	Vehicle 4h	AP4 4h
dead cells[%]	0.52 ± 0.1	0.24 ± 0.07
live cells[%]	99.48 ± 0.1	99.76 ± 0.07
area dead cells[%]	0.02 ± 0	0.04 ± 0.01
ratio dead to live cells[%]	0.07 ± 0.01	0.03 ± 0.07
dead cells area[px]	118 ± 14	340 ± 71
live cells area[px]	175598 ± 6372	114443 ± 2783
total cell area[px]	175652 ± 63720	114488 ± 2782
dead cell density	0.07 ± 0.01	0.32 ± 0.07
live cell density	24.39 ± 0.88	15.89 ± 0.39

cell area ($Pl + Pd$) were significantly less in AP4 treated animals ($p < 0.01$, see Fig. 3.31 F, G). The density of dead cells was higher

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($p=0.004$) whereas the live cell density was lower in AP4 treated animals ($p<0.001$, see Fig. 3.31 H, I). These findings reflect the like-

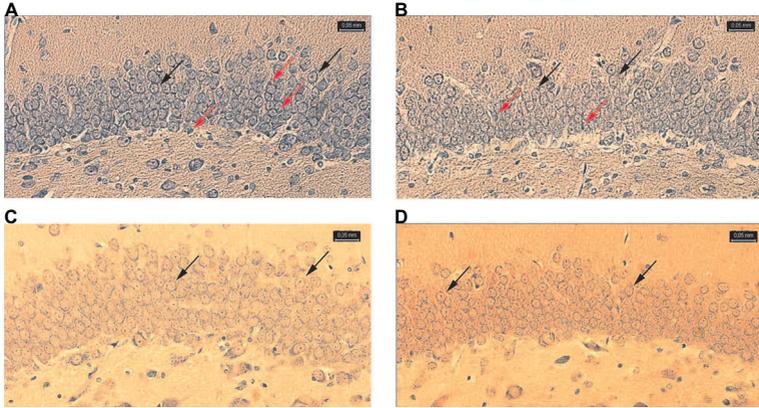


Figure 3.30: The effect of vehicle or AP4 injection on cell viability in the dentate gyrus region

Application of AP4 ($400\text{nmol}/5\mu\text{l}$) reduced the number of dead cells in the dentate gyrus when examined 4h (A) and 7d (B) following injection compared to vehicle treated controls 4h (C) and 7d (D) after injection. The black arrows indicate examples of healthy cells. The red arrows indicate typical examples of dead cells. Examples represent hippocampal sections from the hemisphere into which the drug or vehicle solutions were injected. Scale bar corresponds to 0.05mm .

likelihood that although the decrease in percentage number of both dead and live cells following treatment with AP4, was accompanied by a reduction in the quantity of cells present. This alteration is reflected by the reduction in live cell density seen following application of AP4. The relative scale of the effects was small however, given that the total percentage of dead cells was less than 1% following AP4 treatment.

Seven days after administration of AP4 a significant reduction of the percentage number of dead cells could still be noted ($p<0.05$, see Fig. 3.31 A, for single value comparison see Tab. 3.8). Interestingly a small but significant reduction of live cell percentage could also be

3.3 AP4-mediated LTD is associated with cell death

seen ($p < 0.05$, see Fig. 3.31 B). The percentage area filled by dead cells ($Pd/(Pl + Pd) \times 100$) was not significantly different in AP4 treated animals (see Fig. 3.31 C). The ratio of dead to live cells (Pd/Pl) was unchanged (see Fig. 3.31 D). Dead cell area, (Pd) was also not sig-

Table 3.8: Values comparing cell viability in AP4 or vehicle treated animals 7d after injection

Analysis	Vehicle 7d	AP4 7d
dead cells[%]	0.33 ± 0.08	0.19 ± 0.01
live cells[%]	99.67 ± 0.08	99.81 ± 0.09
area dead cells[%]	0.03 ± 0	0.02 ± 0.001
ratio dead to live cells[%]	0.12 ± 0.02	0.16 ± 0.04
dead cells area[px]	242 ± 35	236 ± 35
live cells area[px]	215009 ± 9440	177023 ± 6056
total cell area[px]	215068 ± 9442	177259 ± 6054
dead cell density	0.12 ± 0.02	0.16 ± 0.04
live cell density	29.86 ± 1.3	24.62 ± 0.84

nificantly changed in AP4 treated animals (see Fig. 3.31 E) whereas live cell area, (Pl), was significantly less ($p < 0.05$, see Fig. 3.31 F). The total cell area ($Pl + Pd$) was significantly lower in AP4 treated animals ($p = 0.003$, see Fig. 3.31 G). The density of dead cells was not changed (see Fig. 3.31 H) whereas the live cell density was lower ($p = 0.003$, see Fig. 3.31 I). The effects of AP4 in this hippocampal subfield were minimal, with less than 1% of cells being affected by application of the agonist.

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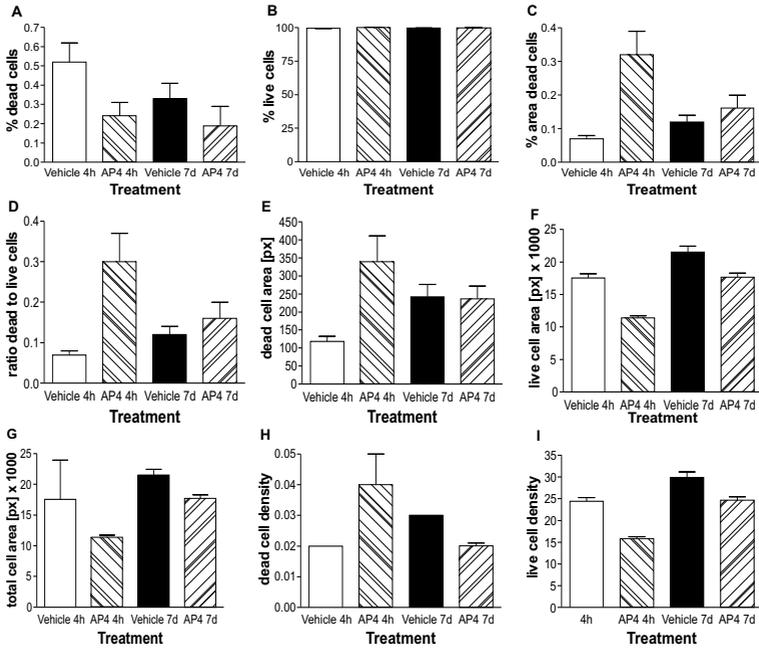


Figure 3.31: Barcharts representing the effect of application of vehicle or AP4 on cell viability in the dentate gyrus region in vivo

A: Percentage number of dead cells. **B:** Percentage number of live cells. **C:** Percentage area of dead cells. **D:** Ratio of dead to live cells. **E:** Total area filled by dead cells. **F:** Total area filled by live cells. **G:** Total tissue area filled by cells. **H:** Density of dead cells. **I:** Density of live cells.