2 Materials and Methods

2.1 Laboratory animals

Seven-to-eight week old (approx. $250g$) male Wistar rats underwent electrode implantation. Animals were mostly obtained from an in-house breeding facility (Zentrale Tierversuchsanlage, Charité, Berlin). The animals were kept under standard housing conditions (Landesamt für Arbeitsschutz, Gesundheitsschutz und technische Sicherheit Berlin) with a $12h$ light/dark cycle (light on at 6 am) in a temperature ($22 \pm 2\,^{\circ}\mathrm{C}$) and humidity ($55 \pm 5\%$) controlled room and allowed free access to food and water. Before surgery animals were housed in groups of 6 per cage; after that, they were caged individually. All experimental procedures were carried out in the light phase of the cycle. The Principles of Laboratory Animal Care were followed.

2.2 Electrodes

For differentially amplified evoked potentials a ground and a reference electrode as well as a bipolar stimulation electrode and a monopolar recording electrode were used. Both stimulation and recording electrodes were referenced to stainless steel screws ($1.5mm$ Ø, Schließblockschrauben, Optotec, Germany) soldered to silver-coated

copper wires ($0.8mm$ Ø). Stretched polyurethane- coated stainless steel wire ($100\mu m$ Ø) attached to cardboard were used as recording and bipolar stimulating electrodes. The tips of the stimulating electrode were $1mm$ apart and cut to a length of 5–$6mm$. The free ends were exposed and fixed to a socket. The impedance of the recording electrode was approx. $9k\Omega$.

2.3 Electrode implantation

2.3.1 Anaesthesia and surgical preparation

The animals were anaesthetised with an initial dose of $52mg$ per kg animal weight i.p. of sodium pentobarbitone (Synopharm, Germany)/propylene glycol (Carl Roth, Germany) mixture (dispensing see paragraph 5.1.1). Depth of anaesthesia was checked during the whole preparation by tail and eyelid reflexes. If required, an additional dose of sodium pentobarbitone mixture was administered. After an appropriate anaesthetic level was obtained, the animals' forehead and neck was shaved and they were placed and fixed in a stereotactic frame (430005-series, TSE Systems, Germany). To protect eye dehydration panthenol ointment (Panthenol-Augensalbe, Jenapharm GmbH, Germany) was applied. To expose the skull, the skin was cut and the periosteum was removed. In addition to the anaesthesia, Lidocain (Xylocain® Pumpspray, Astra Zeneca GmbH, Germany) was applied locally. For cleaning and dehydration the skull was swabbed three times with 3% H_2O_2 and abraded with a sharp bone-spoon. For stereotactic calculations bregma was marked and referred subsequently to as the anterioposterior (AP) and mediolateral (ML) zero point. The angle of the skull was adjusted by allowing a negative slope of $1mm$ between bregma and a point $7mm$ posterior to bregma on the midline. The AP coordinates for the drill holes

were marked stereotactically onto the *margo sagittalis*. From there the ML positions were calculated and marked unilaterally onto the right *os parietale*.
Holes for the electrodes and the guide cannula were drilled with a trepan (High-speed micro-drill, Fine Science Tools GmbH, Germany). The drill heads (Bohrköpfe, Hager und Meisinger GmbH, Germany) used for the start of the drilling were $1.1mm$ in diameter (size 9) and were then exchanged for $1.3mm$ diameter (size 12) drillheads to finish the hole. The ground electrode hole was placed in the left *os frontale* approx. $4mm$ lateral and near the *margo frontalis*. The reference electrode hole was drilled at approx. $8mm$ posterior and $4mm$ lateral. The screws were inserted, fixed and sealed with cyanoacrylate glue and covered with dental acrylic (Paladur®, Heraeus Kulzer GmbH, Germany). The holes for the guide cannula and the electrodes were drilled in the same manner on the positions marked before. The dura was then gently pierced with a needle. The guide cannula ($5.6mm$ length, $0.8mm$ diameterm, Gündel Biomedical Instruments, Germany) was stereotactically inserted via a guiding needle at $0.5mm$ medial and $1.6mm$ lateral with an approximately depth of $4mm$ into the lateral cerebral ventricle. Subsequently the guide cannula was fixed by cyanacrylate glue and the guiding needle was removed.

2.3.2 Electrophysiologically-guided electrode insertion

In the perforant path – dentate gyrus preparation animals were implanted unilaterally on the right side with stimulating electrodes in the angular bundle of the medial perforant path and recording electrodes in the granule cell layer of the dentate gyrus, respectively. Perforant path stimulating coordinates were $6.9mm$ posterior and $4.1mm$ lateral relative to bregma, whereas dentate gyrus recording coordinates were $3.1mm$ posterior and $1.9mm$ medial relative

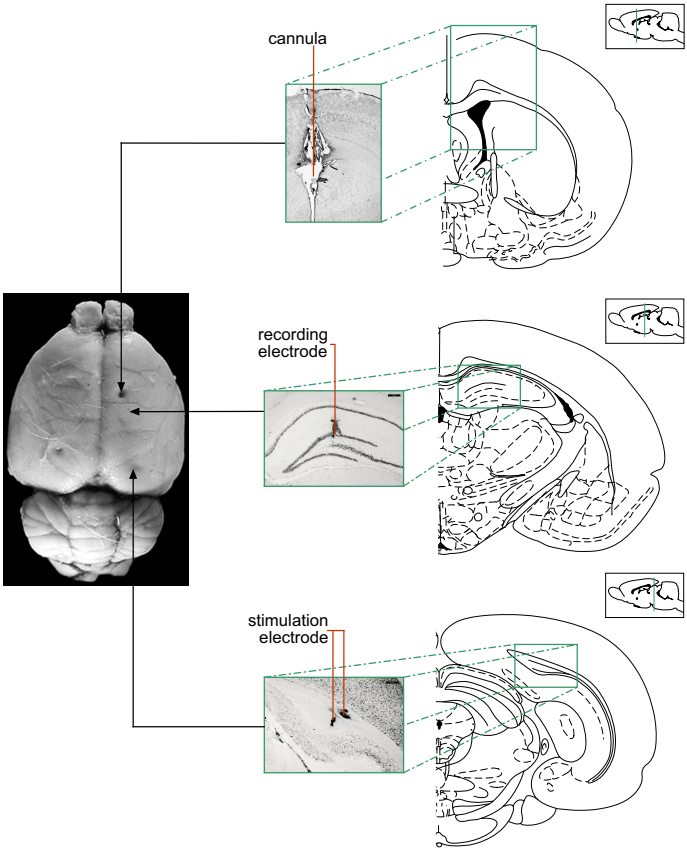

Figure 2.1: Implantation sites of the cannula and the electrodes
Left panel: Shows a photograph of a rat brain from above. The insertion sites of the cannula and the recording as well as the stimulation electrodes are still visible. **Middle panel:** The corresponding coronal sections in a Nissl staining show the exact positioning in depth. **Right panel:** For an overview, the schematic drawings adapted from [Paxinos & Watson, 2003] showing the corresponding AP coordinates.

to bregma (see Fig. 2.1).

The recording electrodes were fixed on the left stereotactic microma-

nipulator (advanced 3-dimensional model, 430005-series, TSE Systems, Germany). Therewith the recording electrode was placed in position. The dorsoventral zero point was set to the point were the electrodes touched the skull on the midline. The electrodes were then attached to an electrophysiological set up. The signals were passed through an amplifier (Differential Amplifier, Science Products GmbH, Germany) for 100× amplification. A further connection to an oscilloscope (HM407, Hameg Instr. GmbH, Germany) allowed the variable amplification and visualisation of the recorded signals. The bipolar stimulating electrode was fixed on the right stereotactic micromanipulator. The dorsoventral zero point was calculated in the same manner as for the recording electrode. With a pulse generator (TR-0361, EMG Pulse Generator, Hungary) the oscilloscope and an isolated stimulation unit (Institute for Physiology, Charité Berlin) could be manually triggered. The stimulation unit was attached to the stimulating electrode. The recording as well as the stimulating electrode was carefully lowered into the brain (beneath the drilled holes) to a starting position $3.5mm$ and $3.3mm$ below zero respectively.

After the brain tissue was allowed to recover for $30min$, the final depth positioning of all electrodes was done under electrophysiological control, and was set to optimize the response from the implanted pathway. This was done by taking recordings of evoked field potentials via the implanted electrodes by means of online stimulation and recording. The procedure of exact electrode positioning was conducted as follows: the recording electrode start position was located in the outer dendritic layer (see Fig. 2.2). A test stimulus (monophasic square wave, $0.2ms$, 4–$8mV$) evoked a negative-going excitatory postsynaptic field potential (fEPSP). Stepwise (0.2–$0.4mm$) lowering of the stimulating electrode increased the resulting fEPSP and revealed a positive-going population spike (PS). Once the PS and

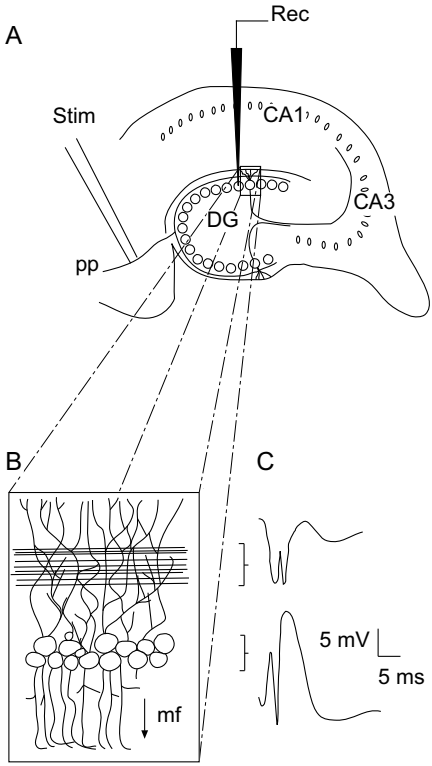

Figure 2.2: Schematic electrode positioning
A: Schematic drawing of a parasagittal section through the hippocampal formation. The stimulation electrode is located in the perforant path (pp). The recording electrode is located in the granule cell layer of the dentate gyrus (DG). **B:** Shows in inset rectangle, in A, in amplification. Here you can see the dendritic field of the granule cells, as well as the somatic field with the ascending mossy fibres (mf). **C:** The population response evoked in the synaptic layer and the cell body layer by a strong perforant path volley. (Figure adapted from: [Bliss & Lomo, 1973]).

the fEPSP reached maximum size, the recording electrode was lowered in steps of $0.2mm$– $0.4mm$. By penetrating the recording electrode through different layers of the dentate gyrus the polarity of the

evoked field potential changed, and the resulting fEPSP reversed. Further lowering brought the electrode near to the 'sink', the granule cell somata, of the fEPSP, mirrored by an increasing positive-going fEPSP with an superimposed negative going PS. Subsequently brain tissue was allowed to equilibrate for 15–20min. The optimal electrode positioning was adjusted by slight and careful up- and down movements of both electrodes, yielding a positive-going fEPSP with a width of max. 12ms and a superimposed negative going PS with a latency of max. 5ms and an amplitude of minimum 7mV. No changes were made for 20min to allow a stable level to be reached. To ensure that the responses remained maximal, fEPSPs were frequently checked and the positioning of the electrodes readjusted if they were not. When stable fEPSPs were obtained, electrodes were permanently fixed.

For experiments in which the calcium chelator BAPTA-AM was used an additional intrahippocampal cannula was implanted next to the recording electrode. For this procedure the recording electrode was fixed to a modified injection cannula (0.9mm diameter, stainless steel) serve as guide cannula with the tip of the electrode extending 1–3mm beneath the cannula. A stylus (modified injection cannula, 0.55mm diameter) was inserted to prevent leakage of fluids or blockade of the guide cannula. The implantation procedure was the same as described above.

In both preparation types liquid and blood was removed and cyanoacrylate glue was applied to the points of electrode insertions. The electrodes were fixed to the skull with dental cement and the electrodes were disconnected from the electrophysiological set up. After gently removing the electrodes from the micromanipulators the sockets were fixed and sealed with dental cement. To avoid wound infection an antibiotic powder (Chlorhexidin Puder, Riemser Arzneimittel AG, Germany) was applied. The animals were allowed to recover

from surgery for 7–10 days.

2.4 Electrophysiological recordings

All the recordings were performed after the animals were placed for acclimatisation into the recording chambers on the evening before the experiment (purpose-designed boxes, $40cm \times 40cm$). Throughout the experiment, the animals were allowed to move freely with *ad libitum* access to food and water.

2.4.1 Experimental set-up

The head-stage of the rats were connected by a ribbon cable via a swivel connector. The cable allowed the rats to move freely in the recording box (see Fig. 2.3). Evoked responses were generated by a single biphasic square wave pulses of $0.2ms$ duration and manually specified stimulation intensity of $100\mu A$–$900\mu A$ using a constant current stimulation unit (A385, WPI, USA). The signals from the recording electrodes were amplified using an A-M-Systems 1700 differential amplifier (100×), filtered ($0.1Hz$–$10kHz$ bandpass) and digitised at $10kHz$ through a DA/AD converter (CED 1401-plus, micro CED, Cambridge Electronic Design, UK). Waveforms, displayed and analysed on a PC with data acquisition software (PWin, Leibniz-Institute Magdeburg), were stored in the hard disk online. The cortical electroencephalogram (EEG) was monitored throughout the course of each experiment with an biomonitor (RFT Biomonitor, GDR) used as an oscilloscope.

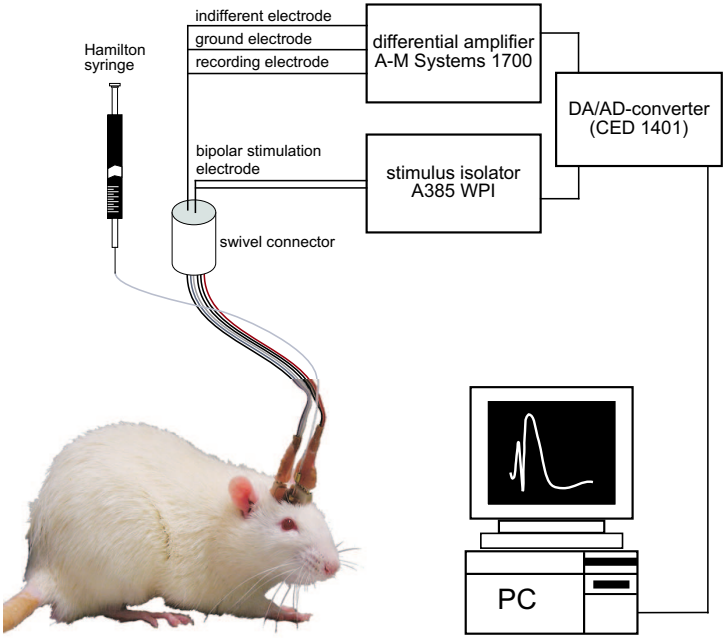

Figure 2.3: Set-up for electrophysiological recordings in freely moving rats

The implanted bipolar stimulating and the monopolar recording electrode are connected to the set-up via a flexible cable and a swivel connector. A PC supported stimulus is applied via a stimulation isolator into the in the perforant path located stimulation electrode. Subsequently it generates a potential in the granule cell layer of the dentate gyrus. This evoked potential is differentially amplified by an amplifier and digitised by an AD converter. The data were recorded and online analysed by computer.

2.4.2 Analysis of evoked potentials

The characteristic field response of the dentate gyrus granule cells to perforant path stimulation consisted of a positive-going fEPSP with a superimposed negative going PS (see Fig. 2.4). The fEPSP slope and PS amplitude were measured for each response. The slope function of the fEPSP was calculated by the slope between the first minimum

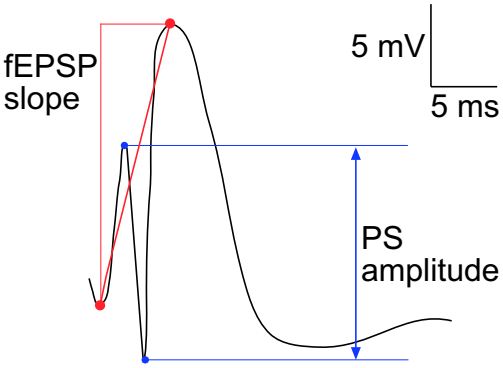

Figure 2.4: Analysis of evoked potentials
The figure shows the population response evoked by a strong perforant path volley in the granule layer of the dentate gyrus. The blue horizontal lines mark the peaks between which the amplitude of the population spike was measured. This value reflects the number and synchrony of granule cells firing. The synaptic potential, the fEPSP corresponds to the depolarisation of the subjacent dendrites. The slope function between the first minimum and the maximum (marked in red) of the potential reflects this depolarisation.

and the second maximum of the potential. This value reflects the granule cell depolarisation. The PS amplitude was measured as the absolute value between the first maximum of the evoked potential and the first following minimum in mV. This amplitude reflects the number of granule cells firing, as well as the synchrony with which they fire.

2.4.3 Recording protocol

For each time-point measured during the experiment, an average value was calculated from five, or, for input/output (I/O) curve three, successive stimuli with $40ms$ intervals between each stimulus. The main experimental designs are shown in Fig. 2.5.

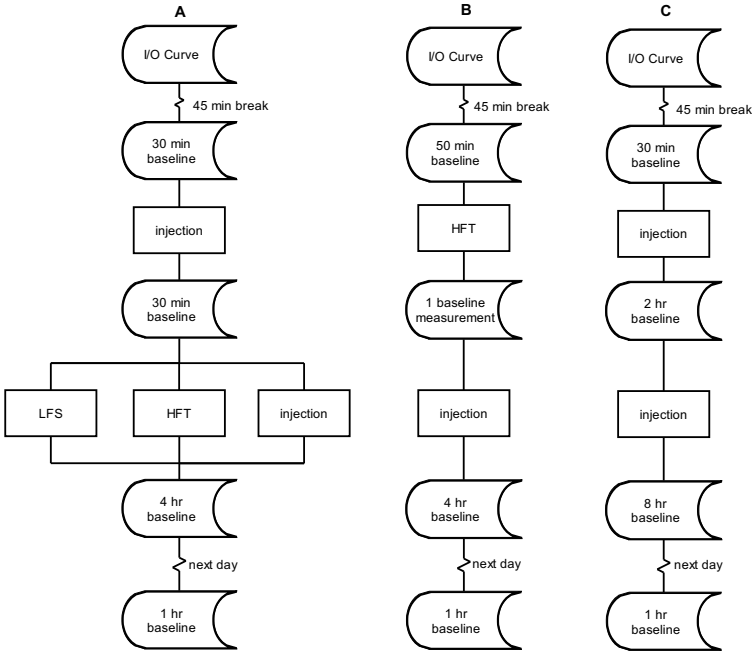

Figure 2.5: Experimental design
The flowcharts visualizes the main experimental designs used in this work. **A:** Protocol A – regular plasticity experiments. **B:** Protocol B – drug administration after synaptic plasticity-inducing protocol. **C:** Protocol C – Extended experiments on transcription and translation.

Input/output curve generation

I/O curves were generated with the use of varying stimulation current intensities (100–900μA) to establish the test intensity used in subsequent experiments. The stimulus intensity was increased stepwise by 100μA, from below threshold to saturation. Maximum PS amplitude was determined as 100% and the stimulus intensity for a 40% output was calculated. All further potentials were evoked with this stimulus intensity.

Initial baseline and injection

Following the I/O curve the animals were allowed to rest for $45min$. Then a $30min$ baseline was recorded with test-pulses every $5min$. At each time point 5 sweeps were recorded and values averaged. After the 5^{th} time point the internal cannula for drug or vehicle injections into the lateral cerebral ventricle was inserted (see paragraph 2.5). After the 6^{th} time point a volume of $5\mu l$ was injected within a period of 5–$6min$ via a Hamilton syringe.

Regular plasticity experiments – Protocol A

After injection the drug or vehicle was allowed to diffuse for $30min$. During this period normal baseline recordings were obtained. Thirty minutes after injection a synaptic plasticity-inducing protocol (see paragraph 2.6) was applied and followed by $4h$ of subsequent recordings. In control experiments, the experimental design was identical but no synaptic plasticity-inducing protocol was applied. The first three time-points were recorded with test stimuli every five minutes followed by $15min$ intervals.
Twenty-four hours after the plasticity protocol measurements were taken for a further $60min$.
In case of intrahippocampal drug injection the protocol A was followed expect of the intrahippocampal injection between the 2^{nd} and 3^{rd} measurement after the first injection into the lateral cerebral ventricle.

Drug administration after plasticity induction – Protocol B

In some experiments the time-window of drug action was tested by applying the drug $5min$ after stimulus administration. During this $5min$ a further baseline measurement was obtained. The time course

of the experiment was nearly identical with the one discribed for protocol A (see paragraph 2.4.3), except that a prolonged initial baseline measurement was carried out.
On the following day $1h$ of additional recordings at $15min$ intervals were made, referred to as $24h$ measurement

Extended experiments on translation – Protocol C

Drug or vehicle administration was followed by a $2h$ period of observation of basal synaptic transmission (three $5min$ time points continued with $15min$ time points). A second drug was injected in all experiments. Evoked responses were then monitored for another $8h$. As for protocol B a $24h$ measurement was carried out by means of $1h$ of additional recordings on the following day.

2.5 Drug treatment

For drug administration, an internal cannula ($0.9mm$ length, $0.8mm$ diameter, Gündel Biomedical Instruments, Germany) was connected to a Hamilton Syringe (Hamilton 84301, Hamilton Comp., USA) via a polythene tube ($0.4mm$ ID, $0.8mm$ OD, SIMS Portex Ltd., UK). The tube was filled and the internal cannula was inserted into the implanted guide cannula after the 5^{th} initial baseline measurement. To exclude secondary effects due to the insertion of the internal cannula another test stimuli was recorded. Following this measurement $5\mu l$ of either drug or vehicle were injected into the lateral cerebral ventricle. In case of intrahippocampal injections, the applied volume was $0.5\mu l$. The injection took place within a time range of 5–$6min$. The drugs were dissolved in the appropriate vehicle and the doses were referred to as total drug weight applied per rat in a $5\mu l$ volume. The following table 2.1 displays all used drugs and their dispensing.

Table 2.1: Compounds used

Drug selectivity	**Drug name**	**Compound name**	**Supplier**	**Dissolved in**
mGlu5 antagonist	MPEP	2-Methyl-6-(phenylethynyl) pyridine hydrochloride	Tocris Cookson Ltd., Bristol, UK	NaCl (0.9%)
mGlu1a antagonist	LY367385	(S)-(+)-a-Amino-4-carboxy--2-methyl benzeneacetic acid	Tocris Cookson Ltd., Bristol, UK	90% NaCl (0.9%) + 10% NaOH ($0.1N$)
Group I mGlu agonist	(RS)-3,5-DHPG	(RS)-3,5-Dihydroxyphenylglycine	Tocris Cookson Ltd., Bristol, UK	NaCl (0.9%)
mGlu5 agonist	CHPG	(RS)-2-Chloro-5-hydroxyphenylglycine	Tocris Cookson Ltd., Bristol, UK	70% NaCl (0.9%) + 30% NaOH ($1N$)
Group III mGlu agonist	AP4	L-(+)-2-Amino-4-phosphonobutyric acid	Tocris Cookson Ltd., Bristol, UK	NaCl (0.9%)
Translation inhibitor	Anisomycin	(2R,3S,4S)-2-(4-Methoxy benzyl)-3,4-pyrrol idinediol-3-acetate 2-[(4-Methoxy phenyl) methyl]-3,4-pyrrol idinediol 3-acetate	Sigma-Aldrich, Munich, Germany	$2.4mg$ in $15\mu l$ HCl ($1N$), treated with NaOH ($1N$) to create a pH of 7, made up to a $50\mu l$ volume with NaCl (0.9%)
Calcium chelator	BAPTA-AM	1,2-Bis(2-aminophenoxy) ethane-N,N,N´,N´-tetraacetic acid tetrakis (acetoxymethyl ester)	Sigma-Aldrich, Munich, German	DMSO <0.1% NaCl 0.9%

2.6 Stimulus paradigms

After the initial baseline recording a stimulation protocol was applied to induce long-term potentiation (LTP) with high frequency tetanisation or long- term depression (LTD) with low frequency stimulation.

2.6.1 High frequency tetanisation

LTP was induced by high frequency tetanisation (HFT) at $200Hz$. Ten bursts of 15 stimuli with a pulse duration of $0.2ms$ were applied with an interstimulus interval of $5ms$ and interburst interval of $10s$.

2.6.2 Low frequency stimulation

LTD was induced by a low frequency stimulation (LFS) at $1Hz$. Nine hundered stimuli with a pulse duration of $0.2ms$ were applied.

2.7 Data analysis

During the initial baseline recordings, six consecutive measurements were obtained. At each time point, 5 sweeps were recorded and the fEPSP slope and PS amplitude values were averaged. The mean values of the averaged sweeps were obtained and set to 100%. Each averaged fEPSP slope and PS amplitude value were then plotted as the percentage of their respective mean values. Data of rats undergoing the same treatment were pooled, and values for every time point were shown as the mean percentage ± the standard error of the mean (s. e. m.). Statistical analysis was performed by two-way analysis of variance (ANOVA) to estimate the differences between the means of the groups. The unpaired Student's t-test was applied for further confirmation. In either case, level of significance was set to $p< 0.05$.

PC based software was used for data processing (MS Excel, Graph Pad Prism, SPSS).

2.8 Histology

To control the electrode positioning, standard histological procedures were carried out on randomly selected animals. Brains were carefully removed out of the cranium and fixed in alcohol/formalin (37%)/acetic acid solution (7:2:1) for $2h$. The tissue was then dehydrated stepwise to ensure antifreeze by putting them in 10% and 20% glycerol. Afterwards the brains were shock-frozen in isopentan and stored at $-70\,^{\circ}\mathrm{C}$. The brain tissue was then cut frontally on a cryostat into $50\mu m$ slices. The slices were kept in $0.1m$ phosphate buffer (see paragraph 5.1.2) and placed onto 4% potassium chrome alum-gelatine mounted slides in 45% NaCl solution. After 7 days of equilibration the tissue was completely dry for staining. Slides were stirred for $45min$ in a 1:1 mixture of chloroform and 96% ethanol, then washed in distilled water and stained in 0.5% cresyl violet. The staining was then differentiated in 50% ethanol with three drops of acetic acid. The slices were dehydrated in an ascending alcohol series, and degreased in xylene. Bubble-free embedding was done with DePex (Serva GmbH, Germany). For histological examination a light microscope without filters in the translucent mode was used (Zeiss Axioskop, Zeiss, Germany). If necessary digital images with a digital camera and a data acquisition software were made (Kappa DX30, Kappa Image base, Kappa Messtechnik GmbH, Germany).

2.9 Paired pulse experiments

In order to investigate the effect of different drugs on the efficiency of recurrent inhibition in the perforant path – dentate synapses, paired pulses were applied.

2.9.1 Paired pulse stimulation

Paired pulse facilitation and depression was tested as follows. First, the maximum PS amplitude was determined for each individual animal by means of I/O curve determination. All potentials were evoked with the lowest stimulation intensity to yield the maximum PS amplitude. This was approximately 70–80% of the maximum PS amplitude. Vehicle injections were made as described earlier and the vehicle was allowed to diffuse for $30min$. Five single test stimuli with an interval of $40s$ and a pulse duration of $0.2ms$ were made to confirm stability. Afterwards paired stimuli were delivered with interstimulus intervals of $20ms$, $25ms$, $40ms$, $50ms$, $100ms$, $300ms$, $500ms$ and $1000ms$ in ascending order with $40s$ interpair interval. Five micro litre of the examined drug was applied via the implanted guide cannula as described earlier. The drug was allowed to diffuse for $30min$. Again paired pulses were delivered as described for the vehicle conditions. Afterwards, the standard I/O-curve paradigm was performed.

2.9.2 Data analysis

Paired pulse ratios were measured between the second PS amplitude and the first one. A ratio $= 1$ reflects no changes between the PS amplitudes evoked by the two stimuli. Ratio < 1 reflects a paired pulse facilitation, and a ratio > 1 a depression. The statistical significance of the differences between the groups was estimated using a one-way

analysis of variance (ANOVA). The probability level interpreted as statistically significant was $p < 0.05$.

2.10 Toxicological Analysis

The aim of this experiment was to examine toxicity mediated by the group III mGlu agonist AP4. The study was designed in two steps. The first step was to quantify cell death by histological quantifications. The aim of the next step was to evaluate the histological results by electrophysiological methods. For the histological quantifications, preparatory work was done by D. Manahan-Vaughan.

2.10.1 Histology

The following preparation was done by D. Manahan-Vaughan. Nineteen animals with implanted guide cannulae received $400nmol$ AP4 or, for control, 0.9% NaCl in a $5\mu l$ volume into the right ventricle. The animals were anaesthetised and underwent cardiac perfusion to fix the brain after $4h$ and $7d$. Their brains were removed and placed in 4% formalin for $24h$, followed by immersion in 70% alcohol and underwent a paraffin embedding procedure. Hippocampal slices ($10\mu m$) were cut on a microtome and live/dead staining was determined according to standard methods [Victorov et al., 2000, Manahan-Vaughan et al., 1999a, Bock, 1989]. My work was to analyse the brain specimens. Ten to thirteen slices per animal were analysed. Image acquisition were performed using a digital camera (Kappa DX 30, Kappa Messtechnik GmbH, Germany) mounted on an inverted microscope (Zeiss Axioskop, Zeiss, Germany) with a 20× objective. The CA1 region and the dentate gyrus of the stained slice were identified and imaged using the 10 bit digitising image analysing system Kappa Image Base Metreo (Kappa Messtechnik

GmbH, Germany). The images were stored in the BMP format and transferred into *Cellcount*, a reason designed software (H. Sigmund, Johannes Müller Institute for Physiology, Berlin) to manually determine the live/dead cell area as well as the amount of live/dead cells. For analysing, a characteristic piece of the region under acquisition was determined with an expanse of $1200 \times 600px$ corresponding to an area of $800 \times 600\mu m$. Within this area all following analyses were made. The software calculated the area of one characteristic dead and live cell per slice by a hand-made outline. The live and dead cells were marked by mouse-click and counted by the software.

Data analysis

The data obtained from all specimens treated in the same way were pooled. The following values were calculated:

$$\% \text{ Deadcells} = \frac{nDeadcell}{nLivecell+nDeadcell} \times 100 \tag{2.1}$$

$$\% \text{ Livecells} = \frac{nLivecell}{nLivecell+nDeadcell} \times 100 \tag{2.2}$$

$$\text{Livecell Area [px] (Pl)} = LivecellSize[px] \times nLivecell \tag{2.3}$$

$$\text{Deadcell Area [px] (Pd)} = DeadcellSize[px] \times nDeadcell \tag{2.4}$$

$$\% \text{ Area Deadcells} = \frac{Pd}{Pd+Pl} \times 100 \tag{2.5}$$

$$\text{Ratio Dead to Livecells} = \frac{Pd}{Pl} \tag{2.6}$$

$$\text{Total Cell Area (Pa)} = Pd + Pl \tag{2.7}$$

$$\text{Dead Cell Density} = \frac{Pd}{Pa} \tag{2.8}$$

$$\text{Live Cell Density} = \frac{Pl}{Pa} \tag{2.9}$$

Pl, the live cell area as well as Pd, the dead cell area was determined by the counted cell numbers and the measured cell size by the outline.

The population density, relative to the cell free area, of live cells (2.9), as well as the population density of dead cells (2.8) were measured. To compare the values of dead neurons to the whole cell area, (2.5) was calculated. This formula discribes the direct relationship of the number of pixels occupied by the living and dead neurons to the sum of pixels within the whole cell area (2.7). The values obtained are thus independent from the image size and the ratio of the active cell elements to the cell-free region [Manahan-Vaughan et al., 1999a]. For statistical comparison between control and AP4 treated groups the nonparametric Mann-Whitney test was performed.

2.10.2 Electrophysiology

The experiment was commenced with an input/output curve to determine the maximum PS amplitude. After the initial $30min$ baseline measurement AP4 ($400nmol/5\mu l$) or 0.9% NaCl was administered into the lateral cerebral ventricle. In the case of the AP4 injection a further $2h$ of baseline measurement was made, followed by a HFT stimulation (see paragraph 2.6) and continued with $2h$ of basal stimulation. On the following day $1h$ of additional recordings were made $24h$ following HFT. In the control group (the NaCl treated group) the stimulation intensity was stepwise decreased after the injection to evoke an PS amplitude of appr. 50% of the initial baseline measurement, to mimick the AP4-effect. This stimulation intensity was kept for the subsequent HFT stimulation (see paragraph 2.6) and for a further $2h$ of baseline acquisition, as well as for the $24h$ measurement. Data analysis was made as described in paragraph 2.7 on page 41.