4 Discussion

4.1 Interim summary

The current study demonstrates the distinctly different impact of the group I mGlu, mGlu1 and mGlu5, on synaptic plasticity induced by afferent stimulation. Previous studies indicated a bidirectional regulation of synaptic plasticity by these receptors. Possible explanations of this phenomenon are a subtype-specific regulation or a regulation via the amount of activation. Here, it is demonstrated for the first time that the mGlu1 receptor is necessary for the induction of LTP as well as the induction of LTD in the dentate gyrus of freely moving rats. In contrast, the mGlu5 receptor is only required for the induction and maintenance of LTP. Taken together, the group I mGlu subtypes are differentially involved in synaptic plasticity in vivo. Whereas a bidirectional regulation underlies the mGlu1 receptor function, the mGlu5 receptor seems to be only involved in the regulation of LTP.

Intriguingly, in agreement with several in vitro studies [Camodeca et al., 1999, Huber et al., 2000, Huber et al., 2001] the activation of group I mGlu by DHPG, and more specifically (as shown in this thesis), the activation of mGlu5 receptor by CHPG results in the induction of chemical LTD in the dentate gyrus of freely moving rats. As opposed to stimulus-induced LTD, the LTD

induced by activation of metabotropic glutamate receptors is not dependent on mGlu1 receptor activation, indicating a requirement of mGlu5 receptors in processes involved in distinct forms of LTD.
The processes underlying LFS and chemically-induced LTD share some common features, and yet seem to be quite different. Recently, it was demonstrated that LTD in the CA1 region is dependent on protein synthesis, independent of the induction protocol [Manahan-Vaughan et al., 2000]. Similarly, DHPG-LTD elicited in vitro [Huber et al., 2000] in the CA1 region and DHPG-LTD induced in the dentate gyrus of freely moving rats is dependent on de novo protein synthesis. LTD induced via mGlu activation is not always protein synthesis dependent, however. Here it was demonstrated for the first time that the broad group III mGlu agonist, AP4, induces a stable, long lasting depression in the dentate gyrus of freely moving rats which does not require protein synthesis, but which is associated with a subtle deterioration of cell viability in the CA1 and the dentate gyrus. A different expression site, for this form of plasticity is discussed.
In the next sections a detailed discussion of obtained results will be followed by the discussion of the possible physiological significance as well as my answers of the main questions resulting from this thesis work.

4.2 Detailed discussion

4.2.1 LTP

Involvement of mGlu1 in LTP: regulation of induction and maintenance

High frequency tetanisation (HFT) at a frequency of 200Hz results in robust LTP in the dentate gyrus in vivo, as previously demonstrated by others [Manahan-Vaughan & Reymann, 1996, Manahan-Vaughan et al., 1998]. The induction of this LTP is dose-dependently impaired in the presence of LY367385. LY367385 is a highly selective antagonist of mGlu1 receptors. This compound antagonizes mGlu1α receptors in recombinant cells in a low micromolar range ($IC_{50}= 8.8\mu M$). It fails to interact with other mGlu subtypes up to $100\mu m$ [Clark et al., 1997].
LTP induction was unaffected in the lowest concentration tested ($4nmol/5\mu l$), but LTP maintenance was slightly inhibited by LY367385, with effects becoming apparent roughly $3h$ post-tetanisation. It is possible that this effect may have been mediated by an mGlu1 receptor-mediated disturbance of the facilitatory coupling between ryanodine receptors and L-type Ca^{2+}-channels [Chavis et al., 1996], which may be necessary for prolonged expression of LTP.
At the higher concentration ranges of LY367385 ($8nmol/5\mu l$ and $16nmol/5\mu l$) an inhibition of both the induction and expression of LTP became evident. The necessity for mGlu1 receptor activation during LTP induction is thus apparent. Consistent with this was the finding that antagonism of mGlu1 receptors *after* HFT had no effect on LTP expression. Although one cannot rule out the possibility that mGlu1 receptors triggered protein synthesis-dependent cascades during the application of HFT, the present findings suggest a protein

synthesis-independent regulation of synaptic plasticity by mGlu1 receptor activation.

The diminishment in the impairment of LTP by an even higher LY367385 concentration ($32nmol/5\mu l$) may have arisen due to unspecific drug effects. Thus, antagonists of mGlus have been shown to have direct effects on native and recombinant NMDA receptors, where amino acid contamination led to an unspecific co-activation on NMDA receptor currents while inhibiting the target receptors [Contractor et al., 1998]. On the other hand, it is also possible that a higher concentration of LY367385 antagonised a larger pool of mGlu1 receptors resulting in an imbalance of transduction cascades regulating LTP or LTD, and consequently causing a less potent blockade of LTP.

Evidence exists for mGlu1 receptor involvement in NMDA receptor response modulation [Skeberdis et al., 2001, Lan et al., 2001, Heidinger et al., 2002]. This may comprise a mechanism for LY367385 inhibition of LTP. During the tetanus, mGlu1 receptor antagonism with $8nmol/5\mu l$ and $16nmol/5\mu l$ LY367385 could have resulted in the inhibition of NMDA receptor currents, resulting in a less accomplished LTP [Skeberdis et al., 2001]. Blocking mGlu1 receptor activation will decelerate NMDA receptor trafficking [Lan et al., 2001] and therefore alter the magnitude of LTP induced by the tetanus. As always, both processes may contribute equally to the phenomenon observed and may explain the potent effects on LTP induction seen with LY367385 in the present study.

Involvement of mGlu1 in LTP: role of pre- and postsynaptic mGlu1 receptors

The selective blockade of mGlu1 receptors produces neuroprotection by enhancing GABAergic transmission [Cozzi et al., 2002]. An

altered GABA release by selective mGlu1 receptor blockade using LY367385 could thus result in impaired LTP induction. To examine this possibility a paired pulse paradigm was performed in the dentate gyrus.

Paired pulse responses, in the form of synaptic depression obtained in the range of 10–40ms, reflect the activation of $GABA_A$ fast inhibitory post-synaptic potentials (IPSPs) and subsequent inhibition of dentate gyrus granule cells [Moser, 1996, DiScenna & Teyler, 1994]. Paired pulse facilitation elicited with pulse intervals of 40–120ms reflect the selective increase of NMDA-mediated responses to further release of glutamate from the presynaptic bouton [McNaughton, 1982, Albertson & Joy, 1987] and may also be mediated by activation of $GABA_B$ receptors [Kahle & Cotman, 1993]. The synaptic depression elicited with pulse intervals of 200–2000ms may also reflect activation of late $GABA_B$ IPSPs [Rausche et al., 1989, Rausche et al., 1988], with the facilitatory and inhibitory effects of these receptors depending tightly on the interstimulus interval of the paired pulses [Kahle & Cotman, 1993].

The application of LY367385 30min before paired pulse stimulation resulted in an inhibition of the generally depressant responses at 40ms. At this interstimulus interval, LY367385 rather caused a facilitation of responses. This effect suggests either that antagonism of mGlu1 receptors may directly inhibit $GABA_A$ receptor regulation of excitability in the dentate gyrus or alternatively that antagonism of mGlu1 receptors facilitates $GABA_B$ enhancement of responses. This latter finding agrees with reports, which suggest that mGlu1 receptor antagonists enhance GABAergic neurotransmission in ischaemic hippocampal slice cultures [Cozzi et al., 2002, Pellegrini-Giampietro, 2003]. The observation, however, that no significant impairment of paired pulse responses occurred at any other time interval implies that LY367385 mediates its impairment of LTP

by predominantly postsynaptic mechanisms.
A postsynaptic regulation by mGlu1 receptors of synaptic plasticity is supported by several immunocytochemical studies showing group I mGlu concentrated in the perisynaptic zone at the periphery of postsynaptic densities at excitatory glutamatergic synapses [Baude et al., 1993, Lujan et al., 1997]. However, activation of presynaptic group I mGlu activation has been shown to result in the facilitation of short-term potentiation into LTP [Riedel et al., 1995, Manahan-Vaughan & Reymann, 1996, Manahan-Vaughan et al., 1999b].

Involvement of mGlu5 in LTP: regulation of induction and maintenance Although mGlu1 and mGlu5 receptors utilize similar signal transduction pathways and share nearly identical synaptic localisations, the antagonism of mGlu5 receptor prior to HFT results in an impairment of both LTP induction and expression.
mGlu5 receptor antagonism using methyl-6-(phenylethynyl) pyridine (MPEP), a highly selective antagonist at mGlu5 receptor, has no effect on basal synaptic transmission, paired pulse responses and input/output curves but dose-dependently inhibits LTP. MPEP exhibits an IC_{50} of $36nM$ at mGlu5 receptor with no activity at any other mGlu subtype [Gasparini et al., 1999]. The higher concentration ($15.7nmol/5\mu l$) used, significantly inhibits the induction and the following expression of LTP. This reduction of early LTP is consistent with studies in various other brain regions that identified the mGlu5 receptor as uniquely responsible for NMDA receptor potentiation [Doherty et al., 1997, Doherty et al., 2000, Jia et al., 1998, Mannaioni et al., 2001, Pisani et al., 1997, Lee et al., 2002]. Although the higher concentration of MPEP may have elicited LTP impairment through inhibition of NMDA receptor currents, the lower concentration of MPEP impaired late-LTP in an NMDA receptor in-

dependent manner. Thus, the fEPSP response during the tetanus was unaffected by this concentration of MPEP. In addition, application of MPEP after the tetanus successfully impaired late-LTP.

Following induction of LTP in the dentate gyrus an upregulation of mGlu5 receptors and the receptor anchoring protein Homer 1a has been reported [Manahan-Vaughan et al., 2003, Brakeman et al., 1997, Xiao et al., 2000]. The interactions of mGlu5 receptor and Homer are multifarious [Xiao et al., 2000]. Next to receptor coupling [Fagni et al., 2002] and trafficking a role in spatial targeting is discussed that might result in the interaction of NMDA receptors and ryanodine or IP_3 receptors. Furthermore, mGlu5 can regulate NMDA receptor mediated currents, in that agonists enhance NMDA currents and antagonists reduces these currents [Harvey & Collingridge, 1993, Attucci et al., 2001]. Taken together, a receptor interaction between mGlu5 and NMDA receptor after HFT cannot be ruled out.

Using the lower concentration of MPEP results in an impairment of LTP roughly $150min$ after LTP induction. Interestingly, the application of protein synthesis inhibitors in vivo also causes an impairment of late LTP [Frey et al., 1988]. In addition, the induction of dentate gyrus LTP in vivo is associated with an increase in the expression of mGlu5 receptors [Manahan-Vaughan et al., 2003] and calcium sensor proteins [Braunewell et al., 2003]. This impairment of late-LTP may therefore be related to an inhibition of the synthesis of new proteins essential for the maintenance of LTP. This possibility is supported by the observation that application of MPEP after tetanisation resulted in a significant inhibition of late-LTP.

Interim conclusion

In conclusion, the results of this study demonstrate that subtype specific antagonists of both group I mGlu results in an impairment of LTP in the dentate gyrus of freely moving rats.
Whereas mGlu1 receptors seem to be highly involved in the induction of LTP, possibly via NMDA or GABA receptors, the involvement of mGlu5 receptor in LTP appears to be more intricate. The inhibition of LTP induction corresponds perhaps to an NMDA receptor inhibition, whereas the late-phase inhibition may be related to mGlu5-triggered protein synthesis.

4.2.2 LTD

This aspect of the thesis reveals for the first time, that persistent and robust LTD can be elicited following LFS or following chemical activation of group I mGlu in the dentate gyrus of freely moving rats. Whereas LFS-induced LTD is mGlu1 dependent, chemical-LTD is dependent upon activation of mGlu5 receptors. Both forms of LTD require elevations of intracellular calcium for their induction. However, whereas activation of mGlu1 during LFS is necessary for LFS-induced LTD, mGlu5 activation does not influence the profile of responses during LFS. Interestingly, the elevations of calcium elicited by LFS and mGlu5 activation can summate to enable induction of LTP. These data suggest that LFS-induced and chemically-induced LTD incorporate different cellular mechanisms. This may enable these forms of LTD to encode different types of synaptic information.

Subtype specific regulation

The existence of LTD in dentate gyrus of freely moving rats has been the subject of much debate (for review see: [Kemp & Bashir, 1999]). This thesis demonstrates now the reliable induction of homosynaptic LTD at medial perforant path-dentate gyrus synapses in freely moving wistar rats following $1Hz$ afferent low frequency stimulation (LFS, 900 pulses). The study further presents a critical role for mGlu1 in LFS-induced LTD, demonstrated by the effective inhibition by the mGlu1 antagonist LY367385 of this form of LTD. Conversely, the mGlu5 antagonist MPEP failed to prevent LTD. Interestingly, LY367385 was not able to disturb LTD after induction by LFS, showing that this receptor may play a role in LTD induction rather than expression. These results differ from those obtained in the CA1 region of freely moving animals where it was shown that group I mGlu activation after LFS is critical factor in the expression of LTD [Manahan-Vaughan, 1997]. In that study, application of the group I mGlu antagonist (S)-4-carboxyphenylglycine $5min$ after LFS effectively blocked LTD. This finding highlights sub region-dependent differences in the regulation of LTD by group I mGlu in the hippocampus. Particularly interesting is the corollary that LFS-induced *and* chemically-induced LTD in the CA1 region may be mGlu5 dependent, as mGlu5 but not mGlu1 is expressed in this region [Lujan et al., 1996]. Also, in the CA1 region a clear mGlu5 receptor dependency for chemical-LTD was shown [Huber et al., 2001, Fitzjohn et al., 1999, Faas et al., 2002, Rush et al., 2002].

Two forms of LTD coexist in the dentate gyrus

In the hippocampal CA1 region two forms of LTD have been identified in early postnatal rats [Oliet et al., 1997]. One form is induced by low-frequency stimulation via an NMDA receptor dependent mech-

anism [Bear & Abraham, 1996], the other depends on the activation of mGlus [Oliet et al., 1997, Bolshakov & Siegelbaum, 1994]. The present data provide evidence that similar phenomena also coexist in the dentate gyrus in vivo. To ascertain if these forms of plasticity share common induction mechanisms an occlusion experiment was conducted. The principle of occlusion dictates that one form of synaptic plasticity will not become further strengthened by subsequent induction of the other from of plasticity if both forms share similar induction pathways. The attempted enhancement of DHPG induced LTD by subsequent LFS application failed. These findings agree with in vitro studies where occlusion of DHPG-induced and LFS-induced LTD in the dentate gyrus was reported [Camodeca et al., 1999]. In contrast, studies in the CA1 region [Palmer et al., 1997, Tan et al., 2003] have shown that DHPG-LTD is not occluded by prior LFS. In the CA1 region only mGlu5 is expressed [Lujan et al., 1996], whereas both mGlu1 and mGlu5 are expressed in the dentate gyrus. DHPG activates both receptor types [Brabet et al., 1995]. Thus the occlusion effect seen when DHPG and LFS were applied concurrently in the present study may have derived from activation of mGlu1 by DHPG. The finding that sole activation of mGlu5 by CHPG did not respond in the same way to LFS supports this possibility. Under these circumstances, complete reversal of LTD occurred.

Another factor that should be considered is that the ostensible occlusion of DHPG- and LFS-LTD may not in fact have truly comprised occlusion. In our study we found that the intensity of afferent activity during LFS had an impact on the induceability of homosynaptic LTD. Thus, LFS failed to induce LTD in control animals where basal synaptic transmission had been deliberately reduced (by reduction of the stimulation intensity) to chemical LTD levels. Similar results have been reported in CA1 slice preparations

[Dunwiddie & Lynch, 1978, Staubli & Ji, 1996] where LTD only occurred reliably when baseline response levels were above spiking threshold. These findings raise the question as to whether the occlusion effects observed in this and other studies may have been derived from a subthreshold level of synaptic activation, or caused by sharing of common cellular mechanisms by the forms of plasticity assessed.

Intracellular calcium and NMDA receptor activation

Intriguingly, whereas DHPG-induced LTD was simply unaltered by LFS, CHPG-induced LTD was reversed by LFS. This may have occurred due to a summation of intracellular calcium elevation induced by CHPG and LFS. According to the ABS rule, the postsynaptic calcium concentration determinates the direction of synaptic modification [Artola et al., 1990]. Activation of glutamatergic synapses and the subsequent change in intracellular calcium results either in LTP, in LTD or in no change. Thus, in principle the same stimulation paradigm can result in either LTP, LTD or no change, dependent upon the concomitant intracellular calcium concentration [Cho et al., 2001]. One could postulate that the de-depression seen after LFS application in CHPG-treated animals is a consequence of the attainment of a calcium concentration near to LTP induction requirements.

BAPTA-AM is a cell-permeable fast-acting calcium chelator [Pethig et al., 1989, Tsien, 1980]. Intracellular BAPTA concentrations above $5\mu M$ block LTP [Jouvenceau et al., 2002], but seem to have no influence on LTD expression [Fitzjohn et al., 2001, Gubellini et al., 2001]. This may derive from the fact that relatively low calcium concentrations are sufficient for induction of LTD. In the hippocampal CA1 region it was shown that LTD occurs at calcium concentrations between $180nM$ and $500nM$, whereas

more than $500nM$ is required to induce LTP [Cormier et al., 2001]. On the other hand, buffering calcium concentrations to near normal resting concentrations (of $100nM$) blocks LTD in CA1 neurons [Hsia et al., 1995]. In the present study, the de-depression (elicited by giving LFS to CHPG-treated animals) is blocked with $2.5\mu M/0.5\mu l$ BAPTA-AM. This suggests that the combination of LFS with CHPG evoked an elevation of intracellular calcium, which approached the levels required for LTP induction. Consistent with this possibility we found that a higher BAPTA-AM concentration induced an increase in the CHPG-induced depression suggesting that the concentration of intracellular calcium elicited by CHPG application was already in the upper ranges.

The distinct regulation of NMDA receptor activation by group I mGlu subtypes has been investigated by Benquet and colleagues (2002) . They and others [Lu et al., 1999, Huang et al., 2001] described one pathway, depending on G-protein activation by mGlu1 and mGlu5 receptors leading to an activation of PKC via PLC and DAG which further activates NMDA receptor currents via tyrosine kinase. The other, G-protein independent pathway seems to be involved in NMDA receptor activation via mGlu1 receptor dependent tyrosine kinase phosphorylation. Despite those up-regulating pathways, the calcium release from intracellular stores in response to G-protein activation via group I mGlu results in a depression of NMDA receptor responses. This interaction of group I mGlu activation and NMDA receptor modulation seems to be one factor as to whether group I mGlu stimulation results in potentiation or depression of excitatory transmission in the hippocampus. Recently, Kotecha et al. (2003) obtained similar results in CA1 neurons. They demonstrated an increase of excitatory transmission due to CHPG application. Blocking a possible NMDA receptor component by co-application of an NMDA receptor antagonist results in depression of excitatory

transmission. In the present experiments in vivo the potentiation of the potentials depressed by CHPG application may also be a consequence of NMDA receptor co-activation due to the LFS application. In agreement with the observed inhibition of de-depression by BAPTA-AM application, an additional calcium concentration threshold must be achieved for NMDA-mGlu5 receptor co-activation regulated via tyrosine kinase phosphorylation [Kotecha et al., 2003].
To examine this possibility further, evoked potentials during LFS were assessed to determine a possible modulation of NMDA-receptor currents by mGlu5 activation. Analysis of LTD induction *during* LFS revealed that synaptic depression, equivalent to that sustained after LFS was complete, had already been reached by the 200^{th} LFS pulse. No effect on the development of LTD during LFS was apparent when LFS applied to vehicle-injected controls, with LFS given to animals treated with CHPG and BAPTA were examined.
On the other hand, application of the mGlu1 antagonist LY367385 after LFS did not result in LTD-inhibition suggesting that mGlu1-mediated LTD is elicited through modulation of mechanisms stimulated during LFS. These data suggest that mGlu1 and mGlu5 activation employ different cellular mechanisms to elicit LTD.

Interim conclusion

In conclusion, the subtype specific involvement of group I mGlu in LTD in the dentate gyrus of freely moving rats was investigated.
It was revealed that two distinct forms of mGlu-dependent LTD coexist in this sub region, both of which endure for at least $24h$. LFS-induced LTD in the dentate gyrus critically requires mGlu1, whereas chemically induced LTD depends on mGlu5. Concurrent induction of chemical (mGlu5 induced)-LTD and LFS-induced LTD does not result in enhanced LTD, but rather causes reversal of LTD. This phe-

nomenon appears to depend on the intracellular calcium concentrations and indicate different mechanisms for induction of both forms of LTD.

4.2.3 Protein-synthesis dependency

As it was shown in the previous section, agonist induced activation of group I mGlu leads to a stable LTD in the dentate gyrus of freely moving rats. But group I mGlu are not the only receptors capable of inducing chemical LTD. Activation of the group I, group II or group III mGlu produced a long-lasting depression of synaptic transmission [Camodeca et al., 1999, Manahan-Vaughan & Reymann, 1995a, Manahan-Vaughan & Reymann, 1995c]. In this thesis, a comparison was made between LTD induced by activation of group I mGlu and that induced by activation of group III mGlu.

Group I mGlu induced LTD

The depressant action of DHPG is similar to that demonstrated in slice preparations of the dentate gyrus [Camodeca et al., 1999] and CA1 region [Fitzjohn et al., 1999, Palmer et al., 1997]. Both a pre- and a postsynaptic locus for this form of LTD have been postulated [Fitzjohn et al., 2001, Faas et al., 2002, Watabe et al., 2002, Xiao et al., 2001, Snyder et al., 2001, Huber et al., 2000].
Several immunohistochemical studies have provided evidence for an exclusively postsynaptic localization of group I mGlu in the rat hippocampus, which would rather support a postsynaptic locus for DHPG-LTD [Lujan et al., 1996, Shigemoto et al., 1997, Takumi et al., 1999]. In contrast other reports support a presynaptic existence of phospholipase C-coupled mGlus [Rodriguez-Moreno et al., 1998, Sistiaga et al., 1998, Romano et al., 1995, Manahan-Vaughan et al., 1999b]. Under in

vivo electrophysiological conditions, a direct and reliable determination of expression site of synaptic events is very difficult. Using the paradigm of paired pulse stimulation can give some insight, however; therefore, the locus of chemical LTD was investigated using paired pulse stimulation. This in vivo study, confirmed the findings of several in vitro studies [Fitzjohn et al., 2001, Faas et al., 2002], with the DHPG induced depression of evoked potentials leading to an enhanced paired pulse facilitation at ISIs of $40ms$ and $50ms$, indicating a presynaptic locus for mGlu LTD.

One can only speculate what interactions might have accompanied the paired pulse facilitation at $40ms$ and $50ms$. A modulation of NMDA [Albertson & Joy, 1987, McNaughton, 1982] or $GABA_B$ receptors [Kahle & Cotman, 1993] mediated responses are possible. In either case a presynaptic component to DHPG-induced chemical LTD, in the dentate gyrus in vivo, is evident.

This thesis work supports an additional postsynaptic locus for DHPG-induced LTD in the dentate gyrus. Thus, in agreement with reports for the CA1 region [Huber et al., 2001] it is demonstrated here that DHPG-induced LTD in the dentate gyrus is protein synthesis dependent.

These results suggest, that similar to the CA1 region [Watabe et al., 2002], DHPG-LTD depends upon presynaptic effects on transmitter release as well as postsynaptically triggered plasticity.

Faas et al (2002) proposed, that the initial phase of mGlu dependent LTD is not dependent on protein synthesis. The present data indicate however an influence of the translation inhibitor, Anisomycin on the initial phase of DHPG-LTD. This observations are in contrast to the general presumption that protein synthesis is required for the late phase of synaptic plasticity but not for the early phase [Frey & Morris, 1997, Frey et al., 1988]. Huber et al (2000) suggests

that the synthesis of proteins required for the induction of mGlu mediated LTD in hippocampal neurons occurs within minutes after LTD induction. In agreement with these results is the hypothesis, that proteins required for the induction of LTD are constitutively present with an unusually high turnover rate against fast degradation. The application of Anisomycin $2h$ before DHPG injection may have mediated a run down of plasticity-related proteins such that the early phase of DHPG-LTD was impaired. The effects of Anisomycin thus appears to be two-fold: an impairment of the early development of DHPG-LTD in the presence of the translation inhibitor, but by $30min$ following DHPG-injection, LTD nonetheless develops to control levels. Six hours after DHPG injection, however, a significant inhibition of DHPG-LTD becomes apparent with evoked potentials returning by $7h$ post-DHPG injection to basal levels. This observation may suggest that distinct protein pools contribute to the early and late development of DHPG-induced LTD.
To exclude possible effects on cell viability and to investigate the 'permanency' of the chemical LTD, HFT was applied subsequently to a fully developed LTD ($2h$ post agonist injection). HFT elicits LTP in control animals. In contrast, HFT application subsequent to DHPG injection results in a reduced LTP whose magnitude recovered by $24h$, following DHPG injection, to control LTD levels. This effect can be explained by a LTD-promoting cascade activated by the application of DHPG, which results in an irreversible preservation of LTD. This effect may be enabled by the protein synthesis induction generated by DHPG.

Group III mGlu induced LTD

In contrast to the results obtained for DHPG-LTD, the AP4-mediated LTD seems to be predominantly presynaptic: LTD was not protein

synthesis dependent and significant changes in paired pulse responses were elicited by AP4. Thus, this data demonstrates that the depression of synaptic transmission induced by the pharmacological activation of group III mGlu is associated with a reduced paired pulse inhibition with 40ms ISI, indicating a presynaptic mechanism. Application of HFT 2h after AP4 injection reverses the AP4-mediated synaptic depression and results in a synaptic potentiation comparable to vehicle injected animals suggesting that AP4-LTD is not as robust as DHPG-LTD.

An autoreceptor function for group III mGlu has been proposed [Gereau & Conn, 1994, Macek et al., 1996]. Group III mGlu are critically involved in hippocampal long-term depression in vivo however [Manahan-Vaughan, 2000] which would support that these receptors use a mechanism in addition to their autoreceptor function: mediated perhaps by postsynaptically localized group III mGlu [Bradley et al., 1996].

It seems to be really unlikely that AP4 remained bound to the group III receptor over the 24h-monitoring period, thereby causing prolonged depression and thereby not an authentic LTD. This is supported by the finding that administration of HFT 30min after application of AP4 results in complete inhibition of LTP induction and expression [Manahan-Vaughan & Reymann, 1995c] whereas administration of HFT 2h after the same concentration of AP4 results only in impairment of LTP maintenance. This suggests that AP4 removal from the receptors is already well established as early as 2h after injection. This furthermore supports that LTD is genuinely induced by application of AP4.

Interim conclusion

Taken together, LTD can be induced chemically by activation of phospholipase C-coupled group I, and adenylyl cyclase coupled group III mGlu in the dentate gyrus of freely moving rats. Interestingly, both types of chemically induced LTD are quite distinct. Whereas both appear to comprise a presynaptic component, only LTD elicited by the group I mGlu agonist, DHPG, is protein synthesis dependent suggesting that this form of LTD has an additional postsynaptic locus. AP4-induced LTD can be reversed by tetanisation whereas DHPG-LTD can't, suggesting that DHPG-LTD is the more robust from of plasticity. This difference may be explained by the fact that Anisomycin did not affect AP4-mediated LTD. The absence of effect of Anisomycin on AP4-LTD furthermore suggests that stable LTD in the dentate gyrus in vivo does not necessarily require dendritic protein synthesis.

4.2.4 Cell viability

The group III mGlu agonist AP4 has been shown to reduce glutamate release and thereby suppress synaptic transmission [Trombley & Westbrook, 1992]. This depressant effect was confirmed previously [Manahan-Vaughan & Reymann, 1995c] in both the dentate gyrus as well as the CA1 region in freely moving rats. The present study replicated and confirmed these results. As discussed above, AP4-LTD seems to be a solely presynaptic effect, without the need for protein synthesis.
Another chemically-induced type of synaptic plasticity, known as slow-onset potentiation, has been shown to occur in the dentate gyrus and CA1 region after ACPD injection, probably due to group I mGlu activation [Manahan-Vaughan & Reymann, 1997, Manahan-Vaughan & Reymann, 1995a,

Manahan-Vaughan & Reymann, 1995b]. This type of synaptic plasticity could be strongly related to neuronal cell death [Manahan-Vaughan et al., 1999a]. Several studies indicate AP4 as a potent protection against neuronal-programmed cell death as well as nitrite oxide-induced neuronal injury, and therefore seems to offer robust therapeutic strategies for neurodegenerative disease [Vincent & Maiese, 2000, Maiese et al., 2000].
Surprisingly, the present data demonstrate that the depression of synaptic transmission induced by pharmacological activation of group III mGlu is associated with a subtle deterioration of cell viability in both hippocampal regions.

Magnitude of demolition

The group III mGlu were consistently described as presynaptic autoreceptors for glutamate [Koerner & Cotman, 1981, Gereau & Conn, 1994, Macek et al., 1996]. Their critical involvement in hippocampal long-term depression in vivo however [Manahan-Vaughan, 2000] support that these receptors use a mechanism in addition to their autoreceptor function: mediated perhaps by postsynaptically localised group III mGlu [Bradley et al., 1996].
The finding that AP4-mediated synaptic depression is coupled with an increase in cell death in the hippocampus is surprising. Previous work demonstrated how the induction of chemical potentiation using agonists of group I mGlu elicited potent neurodegenerative effects in the hippocampus in vivo [Manahan-Vaughan et al., 1999a]. These effects are likely to be mediated by changes in intracellular calcium levels induced by group I mGlu activation. In the present study, the obvious prediction was that chemical induction of synaptic depression should cause either no alteration in, or improved cell viability in the hippocampus. The cell deterioration observed is thus

unexpected. One has to emphasise however, that the effects seen is subtle. The changes in the dentate gyrus represent changes in a total cell population of less than 1%, which can be considered negligible. However changes in cell density and area are elicited which suggest that the effects are more profound than superficially represented by percent changes in the population of live and dead cells. The neurotoxic effects in the CA1 region involved a doubling of the population of dead cells (from ca. 2.5% to ca. 6%) coupled with marked changes in cell area and density. The effects on cell density and area in this hippocampal sub field are more potent than those seen in the dentate gyrus suggesting that the CA1 region is far more vulnerable to AP4-mediated neurotoxicity than the dentate gyrus. A regional difference concerning the susceptibility to AP4 administration has been already described [Manahan-Vaughan & Reymann, 1995c].
Interestingly, the effects obtained are enduring. Comparisons of tissue taken $4h$ and $7d$ after AP4 administration reveals that a long-lasting alteration of cell viability is elicited, particularly in the CA1 region. However, the duration of the induced depression was only monitored for at least $24h$, therefore no evidence can be given concerning the connection between LTD duration and cell deterioration.

Modulation of GABA release

It has been described that intracerebrally injected concentrations of AP4, which are higher than $500nmol$, have proconvulsant effects in the hippocampus [Chapman et al., 2001]. In present study toxic effects following AP4 application were seen with a concentration of $400nmol$. No changes in EEG activity or ostensible changes in behaviour were observed in association with application of this concentration which would exclude the possibility that seizure activity gave rise to the increase of cell death seen.

It has not been checked so far, if the group III mGlu activation on its own, or the resultant depression led to the observed cell deterioration. In support of this possibility it has been shown that AP4 suppresses neuronal GABA release [Cartmell & Schoepp, 2000] and that mGlu4 is present on GABAergic synapses [Bradley et al., 1996]. Furthermore, activation of mGlu7 by AP4 has been shown to inhibit GABA release and thereby potentate NMDA-induced neurotoxicity [Lafon-Cazal et al., 1999]. The effect of AP4 as a partial NMDA receptor agonist in a higher molecular range has already been a point of interest [Contractor et al., 1998].
A dissociation between the AP4-mediated synaptic depressions observed in this study and the mild neurotoxic effects of AP4 might have occurred. A suppression of presynaptic glutamate release via activation of group III mGlu autoreceptors may have coincided with mGlu III receptor mediated alterations of GABAergic transmission. The potent synaptic depression caused by autoreceptor activation may have masked subtle changes in excitability, which altered the integrity and subsequent viability of a small population of hippocampal cells. Consistent with this possibility is the observation that application of high frequency tetanisation not only reversed AP4-mediated synaptic depression but elicited synaptic potentiation which was significantly greater than that elicited in controls. Regrettably, no data exists on cell viability after HFT mediated de-depression of AP4 induced depression.

Interim conclusion

In conclusion, this data demonstrates that synaptic depression, elicited by activation of group III mGlu is associated with an enduring decrease in cell viability within the hippocampus. These findings may reflect the role of group III mGlu in regulating cell excitability

and viability in the hippocampus and may reflect long-term alterations in inhibitory neurotransmission caused by activation of these receptors.

4.3 Physiological significance

4.3.1 Involvement of mGlus in processes underlying memory formation

The mechanisms of short-term memory are different from those of long-term memory. The former do not include gene expression or protein synthesis [Izquierdo et al., 2002, Bourtchouladze et al., 1998]. In terms of spatial memory, the animals ability to hold information online (working memory) is related to short-term memory processes, whereas their ability to retain information over prolonged periods (reference memory) seems to be more related to processes underlying long-term memory formation.
Group I metabotropic glutamate receptors are critically important for hippocampus-based learning [Balschun et al., 1999]. Using targeted gene deletion, mice lacking mGlu5 show deficits in spatial learning [Jia et al., 1998, Lu et al., 1997]. Daily application of the mGlu5 antagonist MPEP has also been shown to result in an impairment of both spatial working and spatial reference memory [Naie & Manahan-Vaughan, 2003]. A group I mGlu subtype specific regulation underlying memory processes has also been demonstrated: reference but not working memory performance is impaired after daily application of the mGlu1 antagonist LY367385 [Naie & Manahan-Vaughan, unpublished observations].

mGlu5

In case of mGlu5, one could speculate that the impairments in working memory might have been caused by a modulation of NMDA currents by mGlu5 antagonism whereas reference memory was impaired by an inhibition in protein synthesis caused by MPEP application. In support of this latter possibility, behavioural studies which examined the effects of protein synthesis inhibitors have found learning and memory deficits in different paradigms [Bourtchouladze et al., 1998, Kogan et al., 2000, Izquierdo et al., 2002].
Physiologically, activation of mGlu5 leads to a marked oscillatory response [Kawabata et al., 1996, Nakanishi et al., 1998] which may regulate gene expression [Berridge et al., 1998], or modulate the amount of kinase activity with CaMKII function as a frequency decoder [Koninck & Schulman, 1998]. The agonist induced protein synthesis demonstrated in this and other studies [Huber et al., 2000] further support this idea. The impairment of learning caused by the mGlu5 antagonist may therefore have been associated with an inhibition of mGlu5-stimulated protein synthesis and therefore a disturbance in information storage.
The working memory impairments reported are possibly related to NMDA currents modulation. In support of this idea, targeted molecular engineering which results in NMDA receptor (subunit) deletion also results in impairments of spatial learning [Tsien et al., 1996, Sakimura et al., 1995]. Activation of mGlu5 has also been demonstrated to contribute to processes leading to NMDA receptor enhancement [Attucci et al., 2001].

mGlu1

The prediction of the data of this thesis is that mGlu1 receptors should be more involved in short-term memory processes, given the

observation that mGlu1 receptor antagonism blocks LTP induction. Surprisingly, LY367385 application has been shown to result in reference , but not working, memory deficits [Naie & Manahan-Vaughan, unpublished observations]. The effects observed in the memory task took several days to become evident and could thus result as a cumulative effect of subtle but cumulative short-term memory effects. Of course, a functional difference of mGlu1 receptor in LTP and memory could not be excluded.
If one postulates that mGlu1 receptor activation is more related to short-term processes, one might predict a role in gain-control which would be strongly related to processes leading to the facilitation of different types of synaptic plasticity. This property could perhaps be quite useful in novelty acquisition [Manahan-Vaughan & Braunewell, 1999].

4.3.2 Involvement of mGlus in processes leading to pathology

It has previously been reported that brief seizure episodes are able to induce long-lasting changes in synaptic transmission reminiscent of LTP [Ari & Represa, 1990].
In addition, there is evidence that specific mGlu subtypes contribute to excitotoxicity and are implicated in the mechanisms that lead to neurodegeneration in models of ischemia, as well as to pathomechanisms contributing to epileptogenises or seizure. In contrast to group I mGlu, which seems to be more implicated in pathways leading to enhanced neurotoxicity, group II and III mGlus have been proposed as potential targets for neuroprotective drugs in various disease models.

Group I mGlu

In animal models of epilepsy, an initial upregulation of mGlu1 mRNA together with a slight downregulation of mGlu5 mRNA in the dentate gyrus has been demonstrated [Akbar et al., 1996, Blumcke et al., 2000]. Whereas the mGlu1 mRNA levels returned to control levels within four weeks, mGlu5 mRNA showed a persistent change [Akbar et al., 1996]. In models of cerebral ischemia an mGlu1 involvement was also demonstrated. An implication of mGlu1 in pathways leading to neuronal death, resulting from a postulated reduced inhibitory transmission due to negative controlled GABA release, has also been proposed [Pellegrini-Giampietro, 2003].

Massive activation of group I mGlu produces hyperexcitatory effects that lead to seizures and neuronal damage [Camon et al., 1998, Manahan-Vaughan et al., 1999a]. In context of plasticity, mGlu1 has been demonstrated to enhance NMDA activity [Benquet et al., 2002, Lan et al., 2001] and stimulates cAMP formation [Balazs et al., 1998]. One could conclude that mGlu1 is highly involved in the initial LTP-phase, resulting in the required intracellular calcium level for kinase activation to take place, and thus for the induction of a stable LTP [Lisman, 1994]. The additional reduction of GABA release due to mGlu1 activation resulting in enhanced neurotransmission [Pellegrini-Giampietro, 2003] highlights the involvement of this receptor in short-term processes.

Thus, mGlu1 receptors seem to be involved in processes altering the balance of neuronal interactions in that they change presynaptic glutamate release and assist in the induction phase by regulating intracellular calcium levels for initiate a stable plasticity to occur. Unbalancing of this process may subsequently result in the overbalance of the regulatory mechanisms, disrupting the calcium

homeostasis and leading not only to potentiation but also to cell death [Pellegrini-Giampietro, 2003].
On the other hand, as clearly demonstrated in this study, electrically induced LTP is dependent on mGlu5 receptor activation especially in terms of perpetuation of the late phase. This late phase synaptic plasticity has been shown to be strongly correlated to de novo protein synthesis. In addition to the observed receptor upregulation $28d$ after experimentally induced epilepsy, the mGlu5 seems to be more related to processes resulting in a persistent change. An alteration of mGlu5 receptor expression may also underlie pathological alterations in excitatory processing: changes in expression or functioning of this receptor contributes to neuropathic pain [Urban et al., 2003], hyperalgesia [Hudson et al., 2002, Walker et al., 2001] and convulsant activity [Chapman et al., 2000].

Group III mGlu

Surprisingly, the proposed neuroprotective effect of group III mGlu activation [Vincent & Maiese, 2000, Maiese et al., 2000] could not be confirmed by the results obtained in this study. Rather a pathological effect was demonstrated.
In case of AP4 mediated toxicity, as demonstrated in this study, the strong agonist-mediated group III mGlu activation might have resulted in a disregulation of excitatory and inhibitory neurotransmitter [Schoepp, 2001] at certain synapses. This could have resulted in net excitation of a small neuronal population due to inhibition of GABA release and a subsequent potentiation of NMDA currents thus leading to neurotoxicity [Lafon-Cazal et al., 1999], while in the majority of neurons the group III mGlu activation led to the synaptic depression observed.
The exclusively presynaptically located group III mGlu have been

shown act as modulators of the amount of plasticity that occurs. One mechanism for depression comprises a depression of transmitter release. Thus, group III mGlu activation should contribute more to the control of synaptic gain and may not be an event on its own. The potent group III mGlu activation resulting from AP4 application would, under normal conditions, not take place and thus contribute more to a system in maladjustment. The effect on cell viability found in this work contributes to this perspective.

4.4 Answers

4.4.1 What has mGlu5 to do with synaptic plasticity?

The mGlu5 receptor (as demonstrated by MPEP blockade), seems to be responsible for triggering an event resulting in the synthesis of new proteins. This protein synthesis stabilizes plasticity resulting in long-lasting changes (late-LTP maintenance, see Fig. 4.1).
The type of proteins synthesised strongly depend on the activation of additional factors. For example, mRNA translation resulting in PSD-95 proteins needs the co-activation of group I mGlu and the fragile X mental retardation protein (FMRP) [Todd et al., 2003].
The results of this study clearly demonstrate, that the *induction* of LTP and LTD is not dependent on mGlu5 activation. Nevertheless, mGlu5 activation positively supports LTP. In the CA1 region, mGlu5 facilitates LTP by enhancing NMDA receptor currents.
Facilitation of LTD expression due to mGlu5 activation seems to depend tightly on NMDA receptor co-activation. In case of reduced NMDA receptor activation, mGlu5 activation results in LTD expression. These conditions result experimentally in NMDA inactivation due to antagonist application [Kotecha et al., 2003] or by separate mGlu5 activation, as shown in this study with CHPG application.

The fact that mGlu5 antagonism by MPEP did not affect LFS-LTD, whereas mGlu5 receptor activation induces stable LTD might have various explanations.

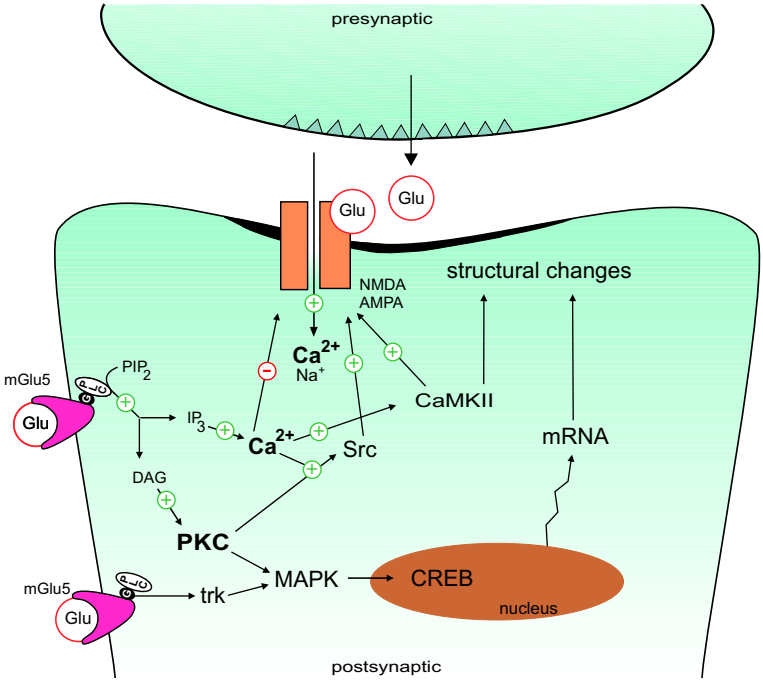

Figure 4.1: Proposed mGlu5 activity involved in the regulation of synaptic plasticity

Schematic illustration representing the main mechanisms induced by mGlu5 activation. Abbreviations: CaMKII: calcium-calmodulin dependent protein kinase II; CREB: cAMP response element binding protein; DAG: diacylglycerol; Glu: glutamate; IP_3: inositol triphosphate; mGlu: metabotropic glutamate receptor; PIP_2: phosphatidyl inositol; PLC: phospholipase C; PKC: protein kinase C; trk: tropomyosin receptor kinase; Src: non-receptor tyrosine kinase.

It cannot be excluded that the antagonist concentration used was adequate for inhibition of LTD. Furthermore, future, more specific

antagonists may reveal that a splice variant (mGlu5a–d)-specific regulation of both plasticity phenomena exists [Romano et al., 2002, Romano et al., 1996, Malherbe et al., 2002, Mion et al., 2001]. Differences in specific G-protein coupling [Kleppisch et al., 2001] may also have resulted in an insufficient antagonism by MPEP.
This study, thus reveals an essential role for mGlu5 in the persistent maintenance of LTP. A role for mGlu5 activation in the induction of chemical LTD in the dentate gyrus is also supported, whereas mGlu5 does not appear to contribute to LTD induced by electrical stimulation.

4.4.2 What has mGlu1 to do with synaptic plasticity?

The results obtained in this study strongly support that mGlu1 is mainly involved in short-term synaptic processes (see Fig. 4.2). The mGlu1 antagonist LY367385 shows in contrast to the mGlu5 receptor antagonist MPEP a strong influence on paired pulse facilitation supporting the involvement of presynaptic mGlu1 receptors in these phenomena.
The effects on synaptic plasticity seen in this thesis work indicate an involvement of mGlu1 receptor in the induction phase of LTP and LTD: it was demonstrated that the LY367385-mediated blockade of both LTP and LTD was absent when the antagonist was injected after the induction.
In accordance with these results, mGlu1 does not demonstrate a specific role in either LTP or LTD. Rather a general reinforcement of synaptic plasticity, resulting in either LTP or LTD seems to be mediated by mGlu1. This reinforcement might help regulate the signal-to-noise ratio and facilitate the storage of synaptic information which surpasses certain excitability/induction thresholds.

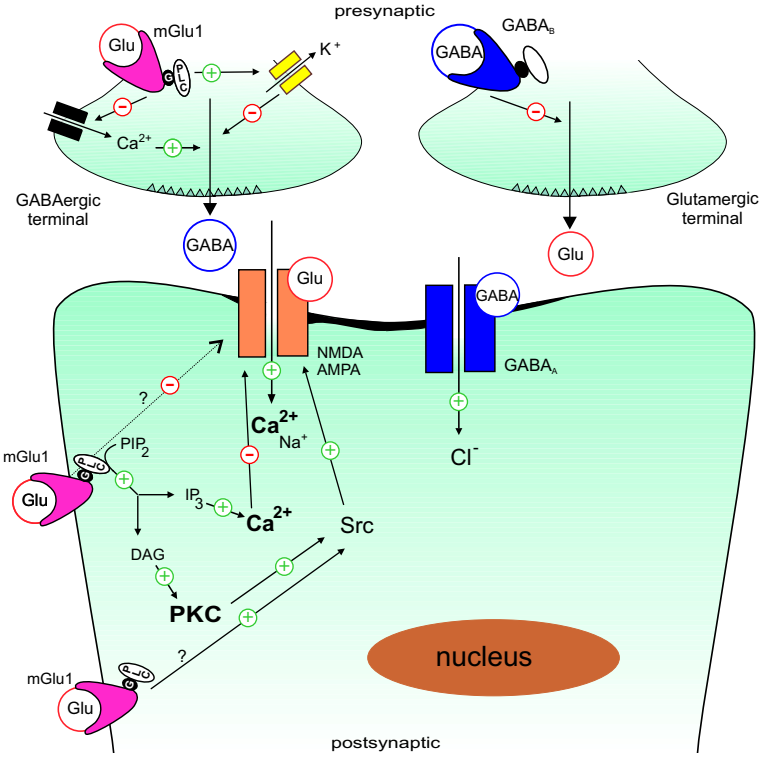

Figure 4.2: Proposed mGlu1 activity involved in the regulation of synaptic plasticity

Schematic illustration representing the main mechanisms induced by mGlu1 activation. Abbreviations: CaMKII: calcium-calmodulin dependent protein kinase II; CREB: cAMP response element binding protein; DAG: diacylglycerol; GABA: Gamma amino butyric acid; $GABA_{A,B}$: GABA receptor A,B; Glu: glutamate; IP_3: inositol triphosphate; mGlu: metabotropic glutamate receptor; PIP_2: phosphatidyl inositol; PLC: phospholipase C; PKC: protein kinase C; Src: non-receptor tyrosine kinase.

4.4.3 How can mGlu modulate both plasticity and pathology?

The data of this thesis support that mGlus may regulate both induction of synaptic plasticity and synaptic pathology. These receptors thus appear to play a very important role in maintaining synaptic function in healthy dynamic ranges, and furthermore, play a critical role in the induction of both stable plasticity and persistent information storage. A malfunction or overactivation of these receptors has catastrophic consequences for the cell viability, on the other hand. Thus group III mGlu activation results in LTD, but this effect is coupled with a slight cell deterioration. Group I receptors regulate multiple forms of synaptic plasticity, but potent agonist activation of group I mGlu results in a form of synaptic plasticity (slow onset potentiation) that results in dramatic cell death [Manahan-Vaughan et al., 1999a]. The results obtained in this and several other studies indicate a strong involvement of mGlu malfunction in processes leading to pathology [Bruno et al., 2001]. In situations of abnormal brain function, like epilepsy or ischemia, an overactivation of mGlus has been demonstrated. It is likely that this causes disruption of homeostatic mechanisms which would normally be activated to counterbalance neuronal excitability, but in the context of mGlu malfunction result in the disruption of calcium homeostasis and induction of pathological processes.

4.5 Conclusion

Taken together, this thesis work has given insight into the subtype specific regulation of synaptic plasticity in the dentate gyrus of freely moving rats. Whereas the group I mGlu subtype 5 seems to be more

related to long-term processes and thus useful in the formation of enduring memory engrams, mGlu1 seems to be more important in mechanisms underlying synaptic gain control (LTD and short-term plasticity) and therefore leading to the facilitation of synaptic events. Group III receptors contribute to synaptic depression which may be independent of memory formation processes. These receptors clearly play a vital role in the regulation and induction of synaptic plasticity, and offer targets for therapeutic approaches which address synaptic pathology related to disruptions in excitability and the regulation of synaptic strength.