

FAN

Head: Prof. Dr. Klaus G. Reymann

Research Report 2004-2005

Introduction

The main topics of the FAN gGmbH in close collaboration with the IfN-Project Group Neuropharmacology are "cerebral ischemia" and "phenomens of brain post-lesional plasticity" investigated both under in vitro and in vivo conditions. With different approaches we investigate the underlying mechanisms of neuronal death after stroke and new neuroprotective strategies targeting at ion transporters and proteases. Immunohistochemical methods and confocal microscopy allow us also to study possible repair processes after ischemic injury based on proliferation of endogenous stem cells. Adult mammalian brains have been shown to retain an ability to generate neurones from endogenous stem cells throughout life. These cells could play a central role in regeneration after neuronal loss. Currently we try to "boost" postischemic adult neurogenesis by using growth factors. As an alternative we test the possibility to replace the nerve cells lost after stroke by transplantation of embryonic stem cells. Last but not least, we take advantage of electrophysiological methods to explore the mechanisms underlying various forms of long-term potentiation (LTP) and long-term depression (LTD) and their change after lesion.

1. Ion exchangers and ion channels as novel targets for neuroprotective drugs in cerebral ischemia

U.H. Schröder, S. Busse, M. Straßburger, M. Martinez, K.G. Reymann

Cerebral ischemia and, under in vitro conditions, oxygen/glucose deprivation (OGD) lead to a massive influx of Ca^{2+} and Na^+ into neurons, resulting in damage and subsequent death. There is still some controversy regarding the ion channels involved and a possible contribution of ion exchangers has received scarce attention so far. We studied the short-term survival of hippocampal neurons in organotypic hippocampal slice cultures (OSC) and freshly isolated slices from adult rats by means of electrophysiology, the long-term survival in OSCs by propidium iodide (PI) staining 24 h after the insult as well as the ischemia-induced neuronal Ca^{2+} -uptake with Ca^{2+} -sensitive dyes. We could show that the blockade of the NMDA-receptor by MK-801 and of Na^+ channels by tetrodotoxin and lidocaine is neuroprotective and also strongly reduces Ca^{2+} -influx (Martinez et al., 2004). The inhibition of voltage gated Ca^{2+} -channels and intracellular Ca^{2+} -stores had no effect. The blockade of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger by KB-R7943 also reduced neuronal damage and the ischemia-induced Ca^{2+} rise (Martinez et al., 2004).

The electroneutral cation–chloride cotransporters ($\text{Na}^+\text{-K}^+\text{-2Cl}^-$ and $\text{K}^+\text{-Cl}^-$ -cotransporters) are abundantly expressed in the brain and are involved in the regulation of the intracellular Cl^- concentration and thus γ -aminobutyric acid (GABA)-dependent inhibition of neuronal excitability. In our studies the $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ -cotransport inhibitor bumetanide (1-10 μM) reduced neuronal damage in the OSCs, but not the functional recovery in acutely isolated slices, whereas the $\text{K}^+\text{-Cl}^-$ cotransport inhibitor [(dihydroindenyl)oxy] alkanic acid (DIOA, 50-100 μM) exacerbate damage in both models (Busse et al., 2005). Thus our data suggest that the $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ -cotransporter contributes to neuronal injury at least in juvenile tissue and that the activity of the $\text{K}^+\text{-Cl}^-$ cotransporters is an intrinsic protective mechanism of neurons against ischemic damage.

Funding: Land Sachsen-Anhalt, Grant LSA 3521/0703A

2. Role of thrombin in transient focal cerebral ischemia

P. Henrich-Noack, K.G. Reymann

Thrombin is well-known as a key-factor involved in the blood-coagulation cascade and by this also as a mediator of induction of stroke (focal cerebral ischemia). However, we and other international laboratories have shown, that thrombin receptors are also expressed in brain tissue and that thrombin has direct effects on the brain parenchyma during and after an ischemic insult. Our recent studies have focussed on the role of protease-activated receptors (PARs) which are activated by thrombin, i.e. PAR1, 3 and 4. Literature data from in vitro studies have provided evidence, that PARs are expressed on all main cell-types of the CNS. However, immunohistochemical studies using ex vivo slices have only confirmed purely neuronal expression so far. It was our hypothesis, that only under certain conditions, such as ischemia, PARs can be also found on other cell-types. Our experiments confirmed this hypothesis. Interestingly, we could detect selective de novo staining on microglia – but not astrocytes – at early post-ischemic time-points. In addition to PAR1, surprisingly also PAR3 appeared on microglia/macrophages in the penumbra zone. This is interesting as PAR3 has not been known to be involved in thrombin-mediated inflammatory processes so far. PAR4 was found in naïve as well as in post-ischemic animals exclusively on neurons. However, the staining was significantly enhanced near and in the infarct area and especially neurons with a damaged/disintegrated morphology expressed this receptor strongly, suggesting that PAR4 may be involved in degenerative neuronal processes after an ischemic insult (Henrich-Noack et al., 2005).

Funding: DFG Re 847/3-1 for FAN/INBC – Otto-von-Guericke Universität

3. Electrophysiological evaluation of neuronal function after global cerebral ischemia

P. Henrich-Noack, C. Pforte, T. Hecht, K.G. Reymann

Neuroprotective interventions are up to now mainly evaluated by quantification of histologically defined structural damage. The main decisive clinical parameter after brain injury however, is a functional one, which is not in full correlation with morphological criteria. We therefore analysed electrophysiologically the neuronal function after transient global ischemia in rats (2-vessel occlusion plus hypotension). In this model we induce selective, delayed neuronal death in the CA1-region of the rat hippocampus. Electrophysiological recordings, however, were performed in the adjacent dentate gyrus, which morphologically looks intact by Nissl-staining. For future regenerative strategies, it is important to know whether the dentate gyrus as an important input structure to CA1 and a neurogenic zone in adult mammals is functionally intact.

Our data demonstrate a selective loss of the population spike in the dentate gyrus already one day after ischemia. In contrast, the synaptic transmission (excitatory postsynaptic field potential) shows no major change. Despite reduced output function LTP can be induced 10 days after ischemia. However, this functional recovery seems to be partial and transient: population spike-values do not reach pre-ischemic values and cease back to the low pre-tetanic baseline values the next day. Electrophysiological measurements *ex vivo* after ischemia indicate that the neuronal dysfunction in the dentate gyrus is not due to locally destroyed structures but that the activity of granular cells is merely suppressed. In summary, global ischemia leaves the morphologically intact area dentata, a major neurogenic zone, at least temporally functionally impaired.

Funding: Land Sachsen-Anhalt, Grant LSA 3480A/1202M assoziiert zu SFB 426

4. Increase in proliferation and gliogenesis but decrease of early neurogenesis in the rat forebrain shortly after transient global ischemia

C. Pforte, P. Henrich-Noack, K. Baldauf, K. Reymann

Ischemic brain injury leads to a massive loss of neurones in the CNS. Over the last decade studies convincingly showed that neurogenesis persists in mammals throughout life in at least two regions of the brain - the subventricular zone (SVZ) and the hippocampal dentate gyrus (DG). In the current study our main interest focussed on the influence of cerebral ischemia on endogenous stem cells and, moreover, the influence of ischemia on activation and proliferation of microglia in the rat forebrain shortly (3 days) after global ischemia (transient two-vessel occlusion combined with hypotension). This interruption of blood flow causes neurodegeneration selectively in CA1 pyramidal neurones. Immunfluorescence together with confocal laser microscopy of BrdU antibodies after incorporation of BrdU into

DNA during cell replication was used to demonstrate the proliferation and migration of cells. In the global ischemia model three days after ischemia, proliferation (BrdU⁺) significantly increased throughout the forebrain (hippocampus, striatum, SVZ). The vast majority of newly generated cells were microglia/macrophages, which were activated, proliferated and invaded morphologically damaged as well as undamaged regions. Early neurogenesis, detected by doublecortin (DCX) labelling, on the other hand, was restricted to the neurogenic zones of the DG and the SVZ. In the DG global ischemia reduced the overall number of DCX⁺ cells, particularly in the upper blade of the DG. However, proliferation of newly derived progenitors (DCX⁺/BrdU⁺) was nearly unchanged at this early time. Additionally we used nestin as a marker which is broadly recognized as a marker for pluripotent neural stem cells. Under control conditions nestin was expressed in the DG and in the SVZ. After ischemia, however, an intensive staining of this marker appeared all over the hippocampus and in striatal areas. Nestin was co-localized almost completely with the astrocytic marker GFAP (Pforte et al., in press). The expression of the neural precursor marker nestin on astrocytes is in line with the assumption that activated astrocytes might be a possible source of nascent neurons.

Funding: Land Sachsen-Anhalt, Grant LSA 3480A/1202M associated to SFB 426 and Land Sachsen-Anhalt, Grant LSA 3521A/0703

5. EGF and bFGF stimulate or suppress region specific endogenous neurogenesis in the endothelin-1 model of focal ischemia

K. Baldauf, K.G. Reymann

Ischemia leads to a dramatic loss of neurones in the CNS, followed by functional impairment. The persistence of neurogenesis in the adult mammalian forebrain suggests that endogenous precursors may be a potential source for neuronal replacement after injury or neurodegeneration. But only a minor part of proliferated cells could be detected 4-6 weeks after ischemia. Growth factors such as basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF) can facilitate neural precursor proliferation in the adult rodent subventricular zone (SVZ) and dentate gyrus. As the application of EGF and bFGF was found to boost neurogenesis after global ischemia, in this study we investigated whether a combined intracerebroventricular (i.c.v.) EGF/bFGF treatment over a period of 2 weeks affects the proliferation of newly generated cells in the endothelin-1 model of transient focal ischemia in adult male Sprague Dawley rats as well.

As assessed by toluidine blue staining, EGF/bFGF substantially increased the infarct volume in ischemic animals. Chronic 5'-bromodeoxyuridine (BrdU) i.c.v. application revealed an EGF/bFGF induced increase in cell proliferation in the lateral ventricle 14 days after surgery. Proliferation in the striatum increased after ischemia, whereas in the dentate gyrus and in the dorsal 3rd ventricle the number of cells decreased. Analysis of the neuronal fate of these

cells by co-staining with a doublecortin (DCX) antibody showed that the growth factors concomitantly nearly doubled early neurogenesis in the ipsilateral striatum in ischemic animals, but diminished it in the dentate gyrus (Baldauf and Reymann, 2005). Because of the increased infarct volume and unclear long-term outcome further modifications of a chronic treatment schedule with growth factors are needed before final conclusions concerning the perspectives of such an approach can be made.

Funding: Land Sachsen-Anhalt, Projekt LSA 3521 E/0703 M

6. Transplantation of exogenous stem cells after eMCAO-induced stroke in rats

C. Bühnemann, H. Braun and K.G. Reymann

In parallel to the investigations regarding the endogenous stem cells we were interested in the feasibility of transplantation of embryonic stem cells (ESC) after stroke in rats. Thus, ES-cells were pre-differentiated into nestin expressing neural precursor cells and transplanted into the brain of rats after transient focal ischemia. Ischemia was induced by stereotaxical application of the vasoconstrictor endothelin-1 near to the middle cerebral artery (eMCAO). Transplantation was performed 1 week after stroke. We tested various transplantation sites, i.e. directly into the damage, outside of the necrotic area and contralateral. Transplanted rats survived 1, 4 and 12 weeks after transplantation. By means of fluorescence microscopy and confocal analysis we investigated survival, migration and differentiation of transplanted cells. Grafted cells survived up to 12 weeks, but no differences in differentiation and migration was observed independent of the transplantation site and the different survival times. Already after 1 week almost no Nestin expression was detectable on transplanted cells. Also no neuronal or astrocytic marker was expressed at this time. Four and 12 weeks after transplantation about 30% of transplanted had differentiated into neurons detected by their NeuN-expression. Only 7-8% had differentiated into astrocytes (expression of GFAP) and not more than 1% into oligodendrocytes (expression of CNPase). Furthermore, immunostaining for SV2, a typical marker for presynaptic vesicles, showed a possible interaction of grafted cells and host cells. In summary, these data show a survival and differentiation of exogenous precursor cells, even after transplantation into the ischemic damage (Bühnemann et al, 2005).

Funding: ESF-Project to FAN

7. Early neurogenesis in organotypic hippocampal slice cultures

O. Chechneva, K. Dinkel, K.G. Reymann

For the investigation of postnatal stem cell proliferation and neurogenesis an appropriate tissue-based in vitro model would be helpful. We characterized early neurogenesis in organotypic hippocampal slice cultures under serum free conditions. Proliferation was

assessed by bromodeoxyuridine incorporation, neurogenesis by bromodeoxyuridine- double labeling with doublecortin or β -III tubulin. We showed for the first time that in addition to the dentate gyrus the organotypic hippocampal slice cultures include a second neurogenic zone: the posterior periventricle, which is a part of the lateral ventricle wall. This structure lining the stratum oriens contained Nestin-positive precursors. Morphological and functional differences between dentate gyrus and posterior periventricle precursor populations were identified. Our data demonstrate that basic fibroblast growth factor treatment induced a fast but short-lasting neurogenic response in the dentate gyrus while the posterior periventricle showed a more pronounced and long lasting neurogenic effect of basic fibroblast growth factor. Thus two neurogenic zones with different neurogenic properties were identified in organotypic hippocampal slice cultures (Chechneva et al., in press).

Drittmittel: EU Key Action ORCA QLRT-2001-00407

8. Repair mechanisms in organotypic hippocampal slice cultures after traumatic injuries

K. Dinkel, O. Chechneva, A.Laskowski, K. Reymann

We studied cell proliferation, glio- and neurogenesis respectively after brain injury in organotypic hippocampal slice cultures using a focal trauma by transecting Schaffer collaterals in the CA2 region. After determination of cell death using propidium iodide, neurogenesis was analysed by immunohistochemical doublelabeling (BrdU/Marker) at different time points post injury. Exogenous bFGF enhanced neurogenesis significantly in the Gyrus dentatus, CA3 and CA1 region. Neutralising anti-bFGF against endogenous bFGF revealed a significant decrease of basal and trauma- induced proliferation. RT-PCR studies exhibited a downregulation of FGF RNA transcription after the antibody treatment. In contrast, EGF increased proliferation, but not neurogenesis. A combination of bFGF and EGF displayed an EGF-dominant effect on both- proliferation and neurogenesis. These results demonstrate, that in our model bFGF but not EGF sustain neurogenesis, whereas together the two growth factors permit an increased proliferation but not neurogenesis in OHCs (Laskowski et al., 2004).

Funding: EU Key Action ORCA QLRT-2001-00407

9. P2 receptors and microglial CNS inflammation

F. Cavaliere, K. Dinkel, K.G. Reymann

The discussion on the exact role of microglia and inflammation after a cerebral insult is accompanied by the effect of extracellular ATP in CNS inflammation. Activation of microglia represents the immediate inflammatory response after brain stroke and seems to have a dual regulatory function in facilitating or blocking neurogenesis after the infarct. By acting on P2

receptors, extracellular ATP can regulate microglia activation and, depending on concentration and exposure duration, contribute to the release of toxic cytokines or beneficial factors, e.g plasminogen. In this work, we used a unique cortical/striatal/subventricular zone organotypic model to analyze the presence of several P2 receptors in microglia by immunofluorescence and co-localization with the microglial marker OX42. We also evaluated in the cortex the P2 receptors subset modulation after oxygen glucose deprivation on the protein level. The confocal analysis demonstrated that, among the P2 receptors tested, P2X4, P2X7 and P2Y4 showed a membrane co-localization with OX42, while P2X1 and P2Y1-2-12, although present in the slices, did not co-localize, finally, P2X6 was not detected. Among these microglial P2 receptors, only P2X4 and P2X7 are effectively upregulated after oxygen/glucose deprivation, the upregulation in microglia was also confirmed in the N9 cell line by Western blot analysis (Cavaliere et.al., 2005).

Funding: Marie Curie Host fellowship HPMD-CT-2001-00114 to Reymann/FAN

10. Microglia provides migration-dependent neuroprotection after ischemia

K. Dinkel, J. Neumann, K.G. Reymann

Our aim was to identify the role of microglia in the early phase of the cerebral ischemic insult. We have established a model, where we applied exogenous fluorescence-labelled BV-2 microglia directly onto an organotypic hippocampal slice culture after oxygen glucose deprivation (OGD). With this model we are able to investigate exclusively the role of microglia by excluding the *in vivo* participating infiltrating blood cells (granulocytes, monocytes etc.). Further, with this model we can reach pathophysiological relevant numbers of microglia in our system and we can simulate the migration of microglia from other brain regions towards the site of injury. Neuronal cell death after oxygen-glucose deprivation (OGD) was determined by PI incorporation and Nissl staining. Migration and interaction with neurons was analysed by time resolved three-dimensional 2-photon microscopy. Using a transgenic mice, which expresses eYFP in a subset of neurons and fluorescently labelled microglia, a migration of microglia into the slice and a close cell-cell interaction with the neurons after was observed in the slice. This microglia application protects against OGD-induced neuronal damage even when applied up to 4h after OGD (Neumann et al., 2005). Neuroprotection and migration of microglia was not seen with integrin regulator CD11a deficient microglia. The induction of migration and neuron-microglia interaction deep inside the slice was markedly increased under OGD conditions. In acute injury such as trauma or stroke appropriately activated microglia may primarily have a neuroprotective role. Thus, anti-inflammatory treatment within the protective time window of microglia would therefore be counterintuitive.

11. The role of ATP-regulated K⁺ channels in synaptic plasticity

U.H. Schröder, K.G. Reymann

ATP-regulated K⁺ channels (K_{ATP}⁺-channels) are widely expressed in the brain and are inhibited by intracellular ATP. Recent studies indicate that activators and inhibitors of these channels could affect learning and memory. The role of K_{ATP}⁺-channels in synaptic plasticity is virtually unknown. Since K_{ATP}⁺-channel blockers are developed to treat non-insulin-dependent diabetes mellitus and cardiac arrhythmias, it is important to know if and to what extent they can also alter processes involved in learning and memory formation.

We found that the novel and highly selective K_{ATP}⁺-channel blocker HMR-1372, a novel putative class III antiarrhythmic, did not affect basal synaptic transmission, paired pulse inhibition or the LTD induced by 3,5-dihydroxyphenylglycine in submerged rat hippocampal slices. Both HMR-1372 and glibenclamide, a broad spectrum K_{ATP}⁺-channel inhibitor and antidiabetic, did not alter the LTP induced by weak stimulation, but significantly ameliorated the LTP induced by a strong stimulus (Schröder et al., 2004). The recruitment of additional K_{ATP}⁺-channels by K_{ATP}⁺-channel activators (Diazoxid, Y-26763) had no effect on LTP. Our data suggest that K_{ATP}⁺-channels are stimulus dependently maximally activated during or after LTP induction and play a role in controlling synaptic excitability.

12. Enhancement of LTP and learning by Neurogranin/RC3 promoted calcium-mediated signalling

D. Balschun, T. Jäger, K.G. Reymann

Neurogranin/RC3 (Ng) is a 78-amino acid neuronal protein, which binds calmodulin (CaM) at low level of Ca²⁺ and is implicated in the modulation of Ca²⁺- and Ca²⁺/CaM-mediated signaling pathways. This protein is a specific substrate of PKC and is highly concentrated in cytoplasm and dendrites of selective neurons within the forebrain. In collaboration with K.-P. Huang (NIH, Bethesda, USA) we described a central function of the postsynaptic PKC substrate neurogranin (Ng) in the initial steps during the induction of NMDA-dependent LTP. Biochemical and confocal [Ca²⁺]_i measurements demonstrated that Ng and Ca compete for calmodulin binding therefore critically determining the strength of plasticity induced. At the behavioural level, hippocampal concentration of Ng and performances in the Morris water maze were found to be significantly correlated (Huang et al., 2004)

13. Do the proteoglycans brevican and neurocan have a function in long-term depression?

D. Balschun, K.G. Reymann

Chemical synapses are embedded into a dense meshwork of extracellular matrix (ECM) components, which are discussed as mediators in normal brain function and neuronal plasticity. Extracellular proteoglycans are found tightly associated with synaptic membranes and the postsynaptic density (PSD) fraction. Brevican and neurocan are two brain-specific members of the lectican family of proteoglycans that are organized in macromolecular complexes and interact with tenascin isoforms and neural membranes. To study the role of these proteoglycans in synaptic plasticity, double knock-out mice deficient in brevican and neurocan (BC/NC-DKO) were analyzed in tight collaboration with the Department of Neurochemistry (C. Seidenbecher, E. Gundelfinger).

These mice are viable, fertile, and have no obvious deficits in general performance. In the hippocampus of BC/NC-DKO the CA1 pyramidal cell layer exhibits a dysplastic phenotype. Perineuronal nets, a specialized form of brain ECM surrounding neurons and isolating synapses appear disturbed. Strikingly, homosynaptic long-term depression (LTD) in the CA1-region is significantly augmented (Balschun et al. 2005). This is contrasting to earlier findings with these mice that long-term potentiation (LTP) is completely abolished. Our data indicate an involvement of brevican and neurocan in the tuning of hippocampus-dependent synaptic plasticity.

14. The efficacy of mGluR5 modulation in hippocampus-dependend learning

D. Balschun

According to our previous findings, group I mGluRs play a pivotal role in certain types of learning and synaptic plasticity. In collaboration with W. Wetzel (Special lab Behavioural Pharmacology) we investigated whether an allosteric potentiation of mGluR5 has any consequences on hippocampus-dependent learning. Intracerebroventricular application of the allosteric mGluR5 potentiator DFB (3,3'-difluorobenzaldazine) immediately after learning, i.e. during a critical period for memory consolidation, resulted in a marked improvement of spatial alternation retention when tested 24 hours after training. This promnesic effect increased with the difficulty of the task and was apparently due to a substantial strengthening of consolidation (Balschun and Wetzel, submitted). Our results suggest an important function of post-training mGluR5 activation in types of hippocampus-dependent spatial learning.

15. Kindling-induced long-term changes of hippocampal synaptic plasticity are mediated by group I mGluRs

R. Y. Nagaraja, D. Balschun, K.G. Reymann

In collaboration with A. Becker (University Magdeburg) we examined whether kindling-induced long-term aberrations in hippocampal synaptic plasticity and learning can be

prevented by application of group I mGluR antagonists (Nagaraja et al. 2004; Nagaraja et al., 2005). Rats received an intracerebroventricular application of either the mGluR1-specific antagonist LY 367385 or the mGluR5-specific antagonist MPEP during kindling development 30 min before injecting PTZ. Both substances showed anticonvulsant efficacy against seizures induced by lower doses of PTZ, but they were ineffective in counteracting seizures evoked by higher PTZ doses. While PTZ kindling in itself results in a worsening of shuttle-box learning, application of LY 367385 improved shuttle-box learning when administered in the course of kindling development or when given prior to the learning experiment. This suggests protective and restorative effectiveness. In contrast, MPEP was only effective on the learning performance of kindled rats when given prior to the shuttle-box experiment, which demonstrates restorative effectiveness (Nagaraja et al., 2004).

In electrophysiological experiments, kindling resulted in a higher magnitude of long-term potentiation (LTP) induced by a strong high-frequency stimulation in the hippocampal CA1-region in vitro (Nagaraja et al., 2005). When LY 367385 or MPEP were given 30 min prior to PTZ, this kindling-induced enhancement of LTP was almost completely prevented. In addition, application of MPEP led to an impaired maintenance of population spike LTP in kindled animals. LY 367385 applied to unkindled control animals caused a reduction of the initial magnitude of population spike LTP. MPEP, in contrast, left the initial magnitude untouched but resulted in a faster decay of potentiation. A single administration of LY 367385 and MPEP, respectively, directly into the bath had almost no effect. Our data suggest that the long-lasting aberrations of hippocampal synaptic plasticity induced by the repeated occurrence of generalized epileptic seizures ultimately require a concurrent operation of mGluR1 and mGluR5 (Nagaraja et al., 2005).

Funding: Land Sachsen-Anhalt, Grant LSA 3316/0021B

16. Enhanced hippocampal long-term potentiation in transgenic mice displaying neuropathological features of human alpha-mannosidosis

D. Balschun

Mice with alpha-mannosidase gene inactivation provide an experimental model for alpha-mannosidosis, a lysosomal storage disease with severe neuropsychological and psychopathological complications. Neurohistological alterations in these mice were previously found to be similar to those in patients and included vacuolations and axonal spheroids in the CNS and peripheral nervous system. In collaboration with groups in Leuven (Belgium), Kiel and Göttingen anatomical, electrophysiological and cognitive behavioral alterations in these mice were characterized (D'Hooge et al., 2005).

Vacuolation was most prominent and evenly distributed in neuronal perikarya of the hippocampal CA2 and CA3 regions, whereas CA1 and dentate gyrus were weakly or not

affected. Field potential recordings from CA1 region in hippocampal slices showed enhanced theta burst-induced long-term potentiation (LTP) in alpha-mannosidase-deficient mice. In contrast, learning of an aversively motivated task and acquisition of signal-shock associations were impaired. Likewise, acquisition and reversal learning in the water maze task, passive avoidance learning in the step-through procedure, as well as emotional response conditioning in an operant procedure were deteriorated. Brainstem auditory-evoked potentials and basic neuromotor abilities were not impaired and did not deteriorate with age. We propose that prominent storage and enhanced LTP in hippocampus have contributed to these specific behavioral alterations in alpha-mannosidase-deficient mice (D'Hooge et al., 2005).

17. The function of cytokines in synaptic plasticity and learning

D. Balschun

Together with H. Besedovsky (University Marburg) and W. Wetzel (Special Lab Behavioral Pharmacology) we characterized the role of interleukin-6 (IL-6) in hippocampal LTP in vitro and in vivo as well as in hippocampus-dependent learning. In contrast to interleukin-1 which has a supportive function in synaptic plasticity, IL-6 appears to confine LTP maintenance and long-term memory storage. Our results implicate IL-6 in the mechanisms controlling the kinetics and amount of information storage (Balschun et al., 2004). Together, our data suggest that a specific ratio between the enhanced expression levels of individual cytokines is likely to ensure an adequate fine-tuning of mechanisms controlling the consolidation of potentiation and memory (Balschun et al., 2004).