Inhibitory Control of Brain Plasticity
Bio-imaging Methods Reveal Brain Physiology

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List of abstracts

1. Homeostatic regulation of GABAergic synaptic transmission following focal cortical lesions

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Focal lesions in the cerebral cortex lead to profound alterations in the functional properties of the surviving cortical networks. In particular, a reduced strength of GABAergic transmission is classically known as one major cellular mechanism underlying neuronal hyperexcitability often developing following these pathological events. In the present study, by using an ex vivo-in vitro model of laser-lesions in the rodent visual cortex, we provide several lines of evidence indicating that this conclusion may be an oversimplification. Instead, we suggest that lesion-induced alterations in inhibition are rather homeostatic in nature. Changes in the inhibitory system were studied in the first week post-injury, in a cortical area located at around 1 mm distance from the lesion. Sham-operated animals were used as controls. Bio- and immunohistochemical analyses revealed neither changes in the expression level of the two GABA-synthesizing enzymes -GAD65 and GAD67- nor any sign of degeneration of GABAergic synaptic terminals. Patch-clamp recordings from layer 2/3 pyramidal neurons, in acute brain preparation, disclosed a reduced frequency of miniature and spontaneous inhibitory postsynaptic currents (mIPSCs, sIPSCs) post-lesion. These changes, accompanied by an increased PPR of evoked postsynaptic inhibitory currents (eIPSCs), suggest an impairment in phasic GABA release. Counteracting the reduced phasic GABA release we observed a prolongation in the kinetics of mIPSCs and eIPSCs as well as an enhancement of $GABA_A$-receptor-mediated tonic inhibition. Interestingly, we found a significant negative correlation between the frequency and the decay-time of mIPSCs. This may suggest that alterations at pre and postsynaptic site are homeostatically regulated to maintain constant the overall strength of inhibition. To further explore the effect of the lesion on inhibition we also performed recordings from layer 2/3 GABAergic interneurons in GAD67-GFP knock-in mice. Interneurons could be classified as fast-spiking or non fast-spiking depending on their firing properties and the expression of the calcium-binding protein, parvalbumin. Surprisingly, the excitability of these two subpopulations of interneurons was shifted in opposite directions by the lesion. Based on the outlined observations we propose a revision to the generally accepted notion that a cortical lesion simply leads to a reduction in the intracortical inhibitory strength. In fact, our new findings describe a series of homeostatic-like mechanisms attempting to attenuate perturbations in neuronal network excitability at expenses of the accuracy of the GABAergic synaptic signaling.

2. Gap junctions and rhythmic oscillations in the limbic cortex

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In the second half of the nineteenth century a discussion took place between proponents of the cell theory, who considered neurons to be independent units, and those who believed that cells were interconnected by protoplasmic bridges. It remained for light microscopy to show that each neuronal cell was surrounded by its own plasma membrane. However, electron microscopy provided further evidence that continuity between certain cells occurs, but in the form of tenuous connections of molecular dimensions, which were further

labelled as gap junctions (GJs). Gap junctions were discovered more than five decades ago, and since that time enormous strides have been made in understanding their structure and function. Despite the voluminous literature concerning the function of GJs, the involvement of these membrane structures in central mechanisms underlying oscillations and synchrony in the neuronal network is still a matter of intensive debate. The presentation has reviewed data concerning the involvement of electrical coupling in the production of theta in the rat hippocampal formation. It has provided evidence that *in vitro* and *in vivo* recorded hippocampal theta field potentials are a very useful model to study the electrical coupling underling the limbic mechanisms of oscillations and synchrony. The amplitude and power of theta rhythm seem to reflect the efficiency of electrical coupling of the hippocampal neuronal network.

3. Glutamic acid decarboxylase 65 – a link between GABAergic plasticity in the lateral amygdala and conditional fear

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GABA, the major inhibitory transmitter of the brain, is critically involved in the control of fear and anxiety through the amygdala. In fact, deficits in GABAergic metabolism are closely related to pathological conditions. Two isoforms of the glutamic acid decarboxylase enzyme, GAD67 and GAD65, provide GABA in the mammalian brain. Previous studies show that a genetically determined deficiency in GAD65 results in generalization of conditioned fear and impairment of fear extinction. Therefore the present study was undertaken to characterize the functional role of GAD65 in synaptic transmission in the lateral amygdala (LA). Whole-cell patch-clamp recordings were performed in acute brain slices containing the LA of GAD65 deficient mice ($GAD65^{-/-}$) and wild-type littermates ($GAD65^{+/+}$) prepared *in vitro*, and after Pavlovian fear conditioning *ex vivo*. Obtained results indicate that GAD65 deficiency is associated with (*i*) a significant decrease in efficacy of evoked $GABA_A$ receptor-mediated synaptic responses, whereas glutamatergic responses were unaffected, (*ii*) an impairment of long term plasticity at monosynaptic GABAergic inputs, and (*iii*) a shift from a heterosynaptic associative form of long-term potentiation (LTP) at cortico-thalamic inputs to non-associative forms, which could be mimicked in $GAD65^{+/+}$ by application of CGP55845 blocking presynaptic $GABA_B$ receptors. Importantly, generalized fear obtained upon over-training in $GAD65^{+/+}$ resulted in a shift from associative to non-associative forms of LTP similar to that observed in $GAD65^{-/-}$. These data support the notion that GAD65 is a critical element for providing sufficient amounts of GABA during periods of increased demands such as high synaptic activity. Since lowered GAD plasma activity and polymorphisms in the GAD2 gene-region have been associated with risk factors for anxiety disorders, these findings raise the possibility that GAD65 enzyme dysfunction could be a pathogenic factor in panic disorder.

4. Thalamic KCNQ channels: mechanism for modulation of neuronal excitability and somatosensory perception

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KCNQ channels are the molecular substrate of the M current (I_M), which operates below action potential threshold and limits neuronal excitability. We detected mRNA and protein expression of these channels in the ventrobasal thalamic complex (VB). To determine the contribution of KCNQ channels to thalamic activity modes and to analyse their possible role in somatosensory and noxious stimulus processing, VB neurons were characterized in vitro, in silico and in vivo. Whole-cell recordings were performed in mouse VB slices. Channel properties were modulated by using the specific KCNQ channel opener retigabine and inhibitor XE991. The consequences of I_M activation were investigated in a TC neuron computer model and in mice

using hot plate tests. In voltage-clamp, KCNQ channels generated a slow K^+outward current which was sensitive to retigabine and XE991. In current-clamp, retigabine reduced tonic firing and promoted the burst-like firing mode. The same effect was produced by adding an I_M component to the TC neuron model. During hot plate testing, intrathalamic injection of retigabine and XE991 significantly increased and decreased the latency to the occurrence of pain behaviour, respectively. These findings indicate that I_M limits TC neurons' excitability. Moreover, membrane hyperpolarization induced by KCNQ channel activation represents a new mechanism for the facilitation of LTS-mediated burst firing. Given the analgesic effect induced by retigabine injection and the known anti-nociceptive effect of thalamic burst firing during noxious stimulation, KCNQ channels may represent a novel target to control pain sensation on thalamic level.

5. Synaptic machinery alterations in the hippocampus of dystrophin-deficient mice

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Duchenne muscular dystrophy (DMD) is a lethal neuromuscular disease caused by mutation of the dystrophin encoding gene. Although muscular disorder is the major life-threatening consequence of DMD, it is also one of a few well-known single gene defects, which result in cognitive impairment. In this study putative alterations of the P2X-dependent Ca^{2+} signaling in hippocampal neurons of dystrophic mice are the main focus. Knowing the cellular distribution of P2X7 receptors in the brain would be important not only for our understanding of this receptor function but may explain its involvement in DMD-associated mental retardation. P2X7 receptor level has been found to be inversely dependent on the expression of $GABA_AR$ gene. As such "inhibitory" interactions between $GABA_A$ and P2X native receptors has previously been described we have examined $GABA_AR$ subunits distribution in hippocampi. The only change we have observed concerned decreased synaptic clustering of alpha1 and less inhibitory receptors within the synapse in dystrophic mutants, what may have functional implications. Differences in the density of various inhibitory synapses within stratum pyramidale and stratum radiatum have also been observed. Moreover, immunostaining for proteins relevant to GABAergic synapses showed decreased VGAT, CB1 and NL2 in stratum pyramidale of *mdx* with more than expected puncta in stratum radiatum. This infers aberrant synapse formation and, collectively, may imply the cognitive impairment.

6. Genetically encoded FRET-based biosensor for MMP-9 activity

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Here we developed a genetically encoded FRET-based biosensor to monitor the activity of matrix metalloproteinase 9 (MMP-9). MMP-9 is an extracellular acting endopeptidase implicated in both physiological and pathological processes. A genetically encoded FRET biosensor anchored in the cellular membrane provides an important advantage over currently employed probes. The sensor allows studying the proteolytic activity of MMP-9 with high spatiotemporal resolution at the exact region of MMP-9 action on the cell. Applicability of the sensor, both *in vitro* and *in vivo* in living cells, was demonstrated by ratiometric analysis of cleavage of the sensor by a purified auto-activating mutant of MMP-9.

7. Role of GABAA receptors in learning induced plasticity of the barrel cortex in mice

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We investigated plasticity of facial vibrissae representation in the barrel cortex of adult mice induced with classical conditioning protocol, in which tactile stimulation of one row of vibrissae - conditional stimulus (CS) was paired with a tail shock - unconditional stimulus (UCS). Previously, we showed that this conditioning resulted in: 1. increased GABA-ergic transmission in the area of cortical representation of the "trained" row of vibrissae, 2. expansion of functional activation of the cortical representation of "trained" row of vibrissae revealed by brain mapping with [14]C-2-deoxyglucose autoradiography, 3. progressive reduction of head movements in response to CS, a conditioned reaction, during conditioning. The aim here was to examine the effect of temporary blocking of GABAA receptors (GABAARs) upon development of plastic changes after conditioning and of conditioned reaction. Mice received unilateral injection of GABAzine (100nl of 1mM solution) or saline into the cortex medially and posteriorly to the barrel field. 45 min after injection mice were subjected to the conditioning procedure. This was repeated for 3 days. 24 hrs after the last conditioning session [14]C-2-deoxyglucose brain mapping was performed, during which the "trained" row of vibrissae and corresponding row on the opposite side of the snout were stimulated. Autoradiograms revealed that injections of GABAzine immediately before conditioning resulted in a significant decrease (of 30%) of the area activated by the "trained" vibrissae within barrel cortex in comparison to the control, uninjected hemisphere and to the control (saline) group. The development of the behavioral reaction was also affected. Results indicate that repeated inhibition of GABAARs in the barrel cortex caused a strong reduction of the ability to drive cortical activation by whisker stimulation 24 hours after conditioning and negatively impacted the formation of CS-UCS association.

8. GABAergic neurotransmission involvement in post-traumatic epileptogenesis in APP/PS1 mouse model of Alzheimer`s disease

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To elaborate the role of increased amyloid-β load on post-TBI epileptogenesis we investigated whether traumatic brain injury (TBI) facilitates epileptogenesis in APP/PS1 mouse model, and whether it associates with impaired GABAergic neurotransmission. TBI was triggered in 13-15wk old APP/PS1 mice and wild type (Wt) littermates. Mice were followed-up for 2wk with video-EEG monitoring starting at 6wk and 14wk post-TBI. Gene expression profiling of perilesional cortex, ipsilateral thalamus and hippocampus was performed using Affymetrix microarrays. Video-EEG revealed increased probability of developing epilepsy in APP/PS1 injured mice compared to Wt injured mice ($p<0.01$) and APP/PS1 controls ($p<0.05$). Perilesional cortex showed GABA-Aα2 and α4 downregulation in Wt injured mice compared to Wt controls ($p<0.01$). GAT3 expression in APP/PS1 controls decreased compared to Wt controls ($p<0.01$). APP/PS1 injured mice displayed upregulation of GABA-Aβ1, downregulation of GAT3 compared to Wt injured mice and upregulation of GAT2 compared to APP/PS1 controls ($p<0.01$). In hippocampus of Wt injured mice GABA-Aα2 and β3 were downregulated, whereas GABA-Aα3 and GAT2 showed upregulation compared to Wt controls ($p<0.01$). APP/PS1 injured mice showed decreased expression of GABA-Aρ3 compared to APP/PS1 controls ($p<0.01$). APP/PS1 controls displayed downregulation of GABA-Aα3 compared to Wt controls ($p<0.01$). Thalamus analysis revealed that Wt injured mice increased the fraction of GABA-Aγ2 and decreased the fraction of GABA-Aδ compared to Wt controls ($p<0.01$). Enhanced amyloidogenesis in APP/PS1 injured mice results in more pronounced epileptogenesis, changes in GABA-A receptor subunits composition and GATs expression.

9. mTOR kinase role in dendritic arbor formation of neonatal born neurons

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Protein kinases, one of which is mammalian Target of Rapamycin (mTOR), play an important role in transmitting signals to cellular effectors during dendritogenesis. Dysregulation of mTOR activity is often connected to neurological disorders. Therefore, precise mTOR role in neuron development need to be established. For that we decided to standardize technique which allows *in vivo* visualization of neurons in olfactory bulb (OB). The olfactory bulb is one of two regions in the adult brain where new functional neurons are continuously incorporated into pre existing neuronal circuits. The OB is a destination for neuronal progenitors born in subventricular zone (SVZ), which migrate through the rostral migratory stream (RMS). Therefore, SVZ-RMS-OB is a unique system to study molecular mechanisms of neurogenesis, neuronal development and neuronal network reconstruction *in vivo*. While importance of mTOR has been previously demonstrated for dendritic arbor development of embryonic neurons, it remains unknown if exact same molecular mechanisms drive dendritic arbor development of neonatal born neurons. We have shown a high activity of mTOR kinase in OB, an area of adult-born neurons differentiation. On the other hand, its activity is relatively low in SVZ and RMS. Consequently, we have showed using *in vitro* cultured SVZ-derived, postnatally-born neuroprogenitors, forced to differentiate into neurons that mTOR activity is needed for proper development of their dendritic arbors. Moreover with use of mTOR inhibitor (Rapamycin) and *in vivo* electroporation we showed that decrease level of active mTOR leads to reduction in dendritic branching in olfactory bulb neurons. Both *in vitro* and *in vivo* studies suggest that mTOR is crucial for neuron development of neonatal born neurons. Currently, we are performing experiments, with use of *in vivo* electroporation to provide more direct evidence for a key role of mTOR for incorporation of neonatal born neurons into already functional circuits of OB. This work has been supported and ERA-NET-NEURON/03/2010 (co-financed by the National Centre for Research and Development).

10. Why do rats differ in the strength of anxiety reactions?

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In recent years, our studies concentrated on the mechanisms underlying behavioral differences between high- (HR) and low-anxiety (LR) rats selected for their behavior in the contextual fear test (i.e., the duration of the freezing response was used as a discriminating variable). The aim was to model individual susceptibility to acute and chronic stress, depending upon ability to adapt or habituate to stress to an extent related to individual genetic predisposition. We demonstrated that HR rats had more passive behavior (characterized by longer freezing time) and were more susceptible to stressful environmental influences. The more anxiogenic- and depression-like phenotype of the HR rats behavior was accompanied by higher activity of the basolateral amygdala (BLA) and lower activity of the prefrontal cortex (c-Fos studies) when they were exposed to aversive stimuli. Decreased activity of the prefrontal cortex could reflect cognitive impairments and weaker control over subcortical structures such as the amygdala and hypothalamus, which, in turn, control behavioral and hormonal responses and explain their higher vulnerability to stressors. We also showed that the less anxious behaviour of LR animals was accompanied by elevated basal levels of glutamate in the BLA and a stronger elevation of GABA in response to contextual fear when compared to HR rats. Pretreatment of rats with d-cycloserine and midazolam, drugs used in the treatment of post-traumatic stress disorder, significantly increased the concentration of GABA in the BLA and inhibited the expression of contextual fear in the HR group. Moreover, these drugs caused an increase in GABA-A receptor alpha-2 subunits in limbic and cortical areas. Chronic corticosterone administration increased anxiety-like behavior in the HR group, and the behavioral effects in HR rats were accompanied by a decrease in alpha-2 subunit

density in the medial prefrontal cortex (prelimbic cortex and frontal association cortex), and by increased expression of alpha-2 subunits in the basolateral amygdala. The present research may help to better understand the neurobiological mechanisms of individual differences that are responsible for predisposition to mood and emotional disorders accompanied by elevated levels of glucocorticoids.

11. Early depolarizing GABA controls critical period plasticity in the rat visual cortex

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GABA, the main inhibitory neurotransmitter in the adult mammalian brain, plays a fundamental role for development and plasticity of the visual system. Conversely, GABA exerts depolarizing action during early development due to high expression of the $Na^+/K^+/Cl^-$ cotransporter NKCC1. Interestingly, nothing is known about depolarizing-GABA control of plasticity mechanisms in the visual system. Here, we interfered with depolarizing-GABA action by treating rat pups with NKCC1 inhibitor (and FDA-approved diuretic) bumetanide or vehicle from P3 to P8 and examined the time course of plasticity by means of *in vivo* (ocular dominance plasticity) and *in vitro* (long term potentiation, LTP, in acute slices) paradigms. We found that plasticity was high in developing control animals (P20-26) and virtually negligible in young adults (P35). In striking contrast, significant levels of plasticity were still detectable in bumetanide-treated rats at P35, although negligible plasticity was observed in fully adult animals (P75). The effect on plasticity was not due to defects in structural or functional development of the visual system of bumetanide-treated rats. Indeed, migration and morphology of layer II-III pyramidal neurons in the visual cortex and basic physiological parameters developed normally in these animals. Moreover, treatment with the osmotic diuretic mannitol did not mimic bumetanide effects on plasticity, indicating that the latter were specifically due to chloride regulation. To explain the higher level of plasticity in bumetanide-treated rats at P35, we analyzed basal GABAergic transmission and the extracellular matrix, which normally act as brakes for plasticity in the adult. We found a reduction of both GABAergic inhibitory tone and density of perineuronal nets. Finally, as Brain Derived Neurotrophic Factor (BDNF) regulates the time course of critical period plasticity, we analyzed BDNF levels in the visual cortex at P35. We found a significantly lower expression of BDNF in bumetanide-treated animals. In seeking for a possible direct mechanism, we increased BDNF signaling by BDNF-mimetic DHF during bumetanide treatment, as depolarizing GABA has been described to modulate BDNF levels during development. We found that plasticity at P35 was negligible in animals co-treated with bumetanide and DHF, and this was accompanied by a restoration of a normal expression of plasticity brakes. These results demonstrate that depolarizing GABA exerts a long lasting modulation of plasticity of cortical circuits by a strong crosstalk with BDNF, without affecting general development of the visual system.

12. Changes in expression of alpha-2 subunits of GABA-A, NR2B subunits of NMDA receptors and glucocorticoid receptors after chronic restraint stress in low and high anxiety rats

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The aim of the study was to assess the mechanisms underlying behavioural differences between high (HR) and low- anxiety (LR) rats selected according to their behaviour in the contextual fear test (i.e., duration of

freezing response was used as a discriminating variable) after chronic restraint procedure (21 days, 3 h every day). We found that in HR group chronic restraint stress decreased rats activity in the Porsolt test and decreased the levels of corticosterone in their prefrontal cortex. We also analysed the expression of alpha-2 GABA-A receptors and NR2B NMDA receptors subunits as well as glucocorticoid receptors (GR) in selected brain structures (immunocytochemistry). In dentate gyrus of the hippocampus of the HR group we also found a decreased expression of GR and NR2B NMDA subunits in the prelimbic cortex and parvicellular part of the hypothalamus compared to HR control and LR restraint groups. Moreover, in the secondary motor cortex HR restraint group had a lower expression of alpha-2 GABA-A subunits compared to LR restraint group. The present results suggest that in HR rats exposed to chronic restraint stress the hippocampal and cortical GABAergic and glutamatergic neurotransmissions were attenuated. These effects could have negative influence on the negative feedback processes in the HPA axis as well as on hippocampal-dependent behaviours, including impairment of cognitive functions and on depressive symptoms.

13. Modulation of inhibition in cortical processing of sensory activity by repetitive transcranial magnetic stimulation (rTMS)

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Recently, we could show that theta-burst stimulation (TBS) applied via rTMS affects the activity of inhibitory cortical interneurons indicative by a fast reduction in the calcium-binding proteins parvalbumin (PV) and calbindin (CB), with an intermittent type TBS (iTBS) primarily reducing the number of PV+ cells, while a continuous TBS pattern (cTBS) more likely reduced the CB expression. Using this tool, we now investigated how sensory processing in rat barrel cortex is altered when activity of either PV+ or CB+ neurons is acutely affected. With each of the five iTBS blocks applied a late sensory response of units in upper layer IV to principal whisker (PW) stimulation increased (>19ms) while the early transient response (8-18ms) was not affected. This was accompanied by an increase in negativity of LFPs within the same late time window. The strong suppression of the second response when stimulating PW twice at 20ms interval or when pairing it with an adjacent whisker (AW-PW) at 20ms was strongly weakened with iTBS but not with cTBS. The first cTBS block had almost opposite effects but when cTBS was repeated 4-5 times the effect became similar to iTBS although weaker. Based on these findings we conclude that PV+ neurons are involved in recurrent cortical inhibition, controlling the strength of late response components and the temporal integration of repetitive responses. On the other hand, neither response gain of the early response, nor responses evoked by simultaneous AW-PW stimulation were affected, indicating that feed-forward inhibition described for thalamocortical responses in layer IV had not been affected. One reason may be that due to the stimulation of callosal axons by our rTMS method primarily inhibition driven by cortical inputs had been changed without affecting the thalamic input.

14. The effect of 5-HT_7 receptor activation on GABAergic transmission in the dorsal raphe nuclei

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It has been speculated that an increase in the release of serotonin in the brain occurring after the administration of 5-HT_7 receptor antagonists may result from GABA-glutamatergic–serotonergic interaction in the raphe nuclei. It has also been suggested that 5-HT_7 receptors in the raphe are not localized directly on 5-HT cells but rather on local GABAergic and/or glutamatergic neurons. However, the effects of the activation and functions performed by 5-HT_7 receptors in this brain structure are still not fully understood. In the present study we aimed at establishing whether the mechanism of the modulation of 5-HT neurons in the dorsal raphe nuclei area involves local inhibitory circuitry through the activation of 5-HT_7 receptors.

Whole-cell recordings were obtained from 5-HT neurons which were identified on the basis of a characteristic electrophysiological response. Spontaneous IPSCs were recorded at the holding potential of 0 mV as outward currents. To activate 5-HT_7 receptors, 5-CT (a nonselective 5-HT_7 receptor agonist) was applied in the presence of WAY 100635 (a selective 5-HT_{1A} receptor antagonist). Application of 5-CT (50–500 nM) resulted in a dose-dependent increase in the mean frequency of sIPSCs while the mean amplitude and other parameters characterizing sIPSCs were not altered. The data suggest that the activation of 5-HT_7 receptors results in an enhancement of the GABAergic input to 5-HT raphe nuclei neurons. Thus serotonin, acting through 5-HT_7 receptors, can exerts a complex modulatory influence over 5-HT - and GABA-mediated synaptic transmission. This study provides new information regarding the role of 5-HT_7 receptors located on raphe GABAergic interneurons in the mechanisms which allow serotonin to simultaneously remodel neuronal activity in a functionally appropriate manner.

15. Fluorescence in situ hybridization (FISH) in rat hippocampal slices - imaging the inhibition of gene expression

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FISH is the suitable method to localize and semi-quantitatively analyze specific gene expression level. Combining the FISH staining with immunofluorescent labeling (IF) enable indication of mRNA in a very precisely defined cell type or cell compartments. On the other hand, brain tissue is very susceptible to damage thus the proper tissue preparation is needed to preserve the intact mRNA and proteins. The idea of the study was to find method of hippocampus slices preparation to effectively proceed with FISH plus IF. The genes expression levels of metabolic enzymes in astrocytes and neurons at CA1 region were analyzed. During the study several chemical methods for tissue fixation (4% PFA, Carnoy's, Bouin) and relatively new methanol/ethanol technique were tested. Additionally, different temperatures or times periods of slices preservation were tried. Finally, two types of waxes where tissue was embedded in were applied. We worked with rat hippocampus slices. In experiments we compared the untreated sections (CTR) to slices after basal synaptic stimulation (CTRs) and with electrophysiologically induced long term potentiation (LTP). We counted the relative fluorescence for astrocytes and neurons. The results have proven that the rapid EtOH/MetOH technique combined with low melting polyester wax embedding medium were the best composition to achieve positive results in FISH. We observed the inhibition of gene expression for fructose-bis-phosphatase and pyruvate kinase after LTP induction, comparing to control slices. mRNA analysis CTRs vs. LTP indicates rapid, statistically significant decrease of gene expression (with $p<0,05$). We are able to observe that LTP induction is associated with decrease of metabolic enzymes gene expression in neurons.

16. Potential targets of insulin in rat neocortex

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Concentration of insulin in the central nervous system is 10 to 100 times higher than blood plasma levels. Insulin controls biological metabolism especially the uptake of glucose into cells, feeding, reproduction and cognition. Alterations of insulin levels cause several diseases including diabetes, aging, obesity and Alzheimer's disease. The existence of insulin in the brain is well established but the source of insulin is still a controversial question and a lot to do with its process of degradation also. In spite of its well-known presence the cell-type dependent localization of its target molecules including insulin-sensitive glucose transporter GLUT4 and insulin receptors still remain unclear. We were on the point of determining its distribution between different types of interneurons by immunohistochemical method and single cell digital polymerase chain reaction.

17. Expression of GABA-A receptor alpha-2 subunits in limbic brain structures after chronic corticosterone administration in low- and high-anxiety rats

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The aim of the study was to determine the anxiogenic- and depression-like effects of chronic corticosterone administration (CORT) in low (LR) and high-anxiety (HR) rats selected according to their behavior in the contextual fear test (i.e. duration of a freezing response). We examined the expression of GABA-A receptor alpha-2 subunits in brain structures and concentration of corticosterone in the prefrontal cortex. Chronic CORT (20 mg/kg for 21 days) increased anxiety in an elevated plus maze, decreased activity in a forced swim test, reduced body weight and decreased prefrontal cortex corticosterone concentration in LR and HR rats (these effects were stronger in the HR group). Moreover, repeated CORT increased anxiety-like behavior in the open field test in the HR group. The behavioral effects in HR rats were accompanied by a decrease in alpha-2 subunit density in the medial prefrontal cortex and by an increase in the expression of alpha-2 subunits in the basolateral amygdala. These studies have shown that HR rats are more susceptible to anxiogenic and depressive-like effects of CORT, which are associated with modification of GABA-A receptor function in the medial prefrontal cortex and basolateral amygdala. It appears that innate, individual differences in local GABAergic activity may be causally related to the observed behavioral effects.

18. CB1 and PV immunopositive puncta in the mouse barrel field after classical conditioning training involving facial vibrissae.

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We studied the cellular localization and the contribution of type 1 cannabinoid receptor (CB1) and parvalbumin (PV) puncta immunoreactivity to the previously reported plastic changes induced in the layer IV of the mouse primary somatosensory cortex (S1) by 3 days aversive classical conditioning paradigm. The aversive trained group (CS+UCS) received conditioned stimulus (CS) consisting of three strokes to the whiskers of row B of the left snout lasting 9s, and at the last second of the last stroke a single tail shock (unconditioned stimulus, UCS) was applied. The paired stimuli were applied 4 time per min for 10 min a day for 3 days. The left hemisphere of these mice was an untrained control. In addition a second control group of mice NAÏVE had no stimulation. Mice were perfused with 4% paraformaldehyde in 0.1M phosphate-buffer saline. We used a rabbit polyclonal CB1 antibody, raised against the synthetic peptide MSVSTDTSAEAL corresponding to C-terminal amino acids 461-472 of human CB1 (1:500; Abcam) and monoclonal mouse anti-parvalbumin (1:12,000; Sigma) to detect immunoreactive axon terminals in the barrel cortex of conditioned (CS+UCS) and NAIVE animals. We demonstrated, by quantitative confocal microscopic analysis, that the numerical density of CB1 immunolabeled puncta in the hollows of the B3 barrel of the S1 increases in experimental (i.e.“trained”) brain hemisphere but not in the control brain hemisphere. The NAIVE control animals did not show any increase in CB1 immunolabeled puncta. When the ratio of CB1 puncta between right and left hemispheres of the CS+UCS mice was compared with that from NAIVE mice it was observed that there was a twofold an increase in CB1 immunopositive puncta numerical density in the hollows of the CS+UCS mice. In tangential sections the pattern of barrels was also present following PV immunoreactivity. The most notable observation was of a lowered numerical density of PV immunopositive puncta concentrated in the hollow of the CS+UCS mice compared to the NAIVE mice. The findings suggest that both, CB1 receptors present on interneuron axons and PV immunopositive axon terminals may play a regulatory role in experience dependent plasticity of the S1. Founded by project 6420/B/PO1/2011/40 to ES.

19. The dynamics of interneuronal spines are regulated by NMDA receptors

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Dendritic spines are specialized membranous protrusions specialized in receiving neuronal input. Although they have been typically studied on excitatory pyramidal neurons, they are also present in some populations of inhibitory interneurons, playing the same role. These neurons express plenty of neurotransmitter receptors, but glutamate NMDA receptors (NMDAR) are especially important. Despite extensively studied in order to understand learning and memory, little is known about their role on the structural plasticity of interneurons. In this study we try to unravel how NMDAR influence the spine dynamics of a subpopulation of interneuronal dendritic spines of hippocampus. In order to do so, we use the highly selective NMDAR antagonist MK-801 on organotypical entorhino-hippocampal cultures, performed on GAD-GFP mice. This technique allows us to study either longitudinally or tangentially spine dynamics and the relative spine density. In the present study, we describe how the apparition turnover rate of interneuronal spines is rapidly decreased 4 hours after the antagonist treatment, remaining low 24 hours later. In addition, we report a decreased relative spine density 24 hours after the treatment. Since NMDAR hypofunction on inhibitory networks is the most accepted hypothesis explaining the molecular basis of schizophrenia, this study can also help to shed light on this important subject.

20. Alterations in the structure, plasticity and connectivity of inhibitory networks in a double hit model of schizophrenia

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Schizophrenia is a complex psychiatric disorder, which results in dramatic changes in behavior, perception and cognition, as well as alterations in the structure and function of cortico-limbic regions, including the prefrontal cortex, the amygdala and the hippocampus. Current pathophysiological theories of schizophrenia point to abnormalities in the development, organization and physiology of inhibitory circuits as responsible for some of these alterations. However, the cellular and molecular bases of these alterations are still unclear. In this line, the generation of animal models reproducing some of the core features of schizophrenia, constitute valuable tools to investigate these alterations. By generating a double developmental/environmental mice model of schizophrenia in a transgenic strain displaying fluorescent interneurons, we sought to mimic a wide range of features of this disorder and find alterations in the structural plasticity of inhibitory circuits. Mice were subjected to a perinatal injection of an NMDA receptor antagonist and were socially isolated from postweaning to adulthood. We found that these mice reproduce some of the behavioral and structural alterations previously seen in other models; such as increased thigmotaxis and locomotor activity and volume reductions in cortico-limbic regions. Interestingly, interneurons in these areas showed increased dendritic arborization and spine density, while the number of perineuronal nets and the expression other plasticity-related molecules (i.e. PSA-NCAM) were reduced. Consequently, abnormalities in the structural plasticity of cortico-limbic interneurons may have a key role in the pathophysiology of schizophrenia.

21. Fluoxetine and serotonin act on excitatory synaptic transmission to suppress pyramidal cell-triggered cell assemblies in the human preftontal cortex

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Selective serotonin reuptake inhibitors are the most widely prescribed drugs targeting the CNS with acute and chronic effects in cognitive, emotional and behavioral processes. This suggests that microcircuits of the human cerebral cortex are powerfully modulated by selective serotonin reuptake inhibitors, however, direct measurements of serotonergic regulation on human synaptic interactions are missing. Using multiple whole-cell patch-clamp recordings from neurons in acute cortical slices derived from nonpathological human samples of the prefrontal cortex, we show that neuronal assemblies triggered by single action potentials of individual neurons in the human cortex are suppressed by therapeutic doses of fluoxetine (Prozac). This effect is boosted and can be mimicked by physiological concentrations of serotonin through 5HT-2A and 5HT-1A receptors. Monosynaptic excitatory connections from pyramidal cells to interneurons were suppressed by application of serotonin leaving the monosynaptic output of GABAergic cells unaffected. Changes in failure rate, in paired-pulse ratio, and in the coefficient of variation of the amplitude of EPSPs suggest a presynaptic action of serotonin. In conclusion, activation of neuronal assemblies are effectively downregulated by the acute action of selective serotonin reuptake inhibitors or serotonin at the site of pyramidal output in human microcircuits.

22. Plasticity of the inhibitory synapses in the mouse barrel cortex under constant darkness

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Mice show a circadian rhythm of activity associated with rhythmic changes in the sensory input which modulates the whisker representations in the somatosensory (barrel) cortex. The previous study under LD 12:12 conditions showed an up-regulation of the density of excitatory synapses located on single-synapse spines during the rest period (light) and an increase in the density of inhibitory synapses located on double-synapse spines during the active period (darkness). The aim of this study was to investigate circadian synaptic plasticity in the barrel cortex associated with locomotor activity level of the C57/BL mice in constant darkness (DD 24h). In such conditions, the circadian activity/rest rhythm is retained. Using stereological analysis of serial TEM sections, we observed a significant increase in the density of inhibitory synapses located on double-synapse spines during the active period. This study demonstrates that the mouse barrel cortex shows cyclic **changes** in the density of synapses synchronized in phase with the locomotor activity rhythm of the animals. Moreover, changes of inhibitory synapse density during the 24 cycle are circadian and light-independent. Hence, the observed daily plasticity seems to be generated by an internal circadian clock.
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23. Early onset binocular pattern deprivation regulates the expression of proteins involved in GABA release and neurite outgrowth

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During early postnatal brain development, visual input modifications lead to specific alterations of functional connectivity in the mammalian visual cortex (Wiesel and Hubel, 1963, 1965). We applied a gel-based screening approach to compare the protein expression patterns of normally stimulated and binocularly deprived cat primary visual cortex (area 17) of 2 and 4 month old kittens (2BD and 4BD). CRMP4 (Collapsin response mediator protein 4) levels increased in 2BD group as compared to age-matched controls, whereas CRMP2 levels remained unaffected. As CRMP4 is expressed in a subset of inhibitory interneurons and CRMP2 in excitatory pyramidal cells of adult cat visual cortex (Cnops et al., 2006), we chose to focus on possible changes in the expression of proteins involved in GABAergic transmission due to BD. By means of Western analysis we confirmed the upregulation of CRMP4 in 2BD kittens. Also the level of Septin 5, which inhibits exocytosis of GABA vesicles in synapses (Beites et al., 1999; Kinoshita et al., 2000), was increased in 2BD animals. GAD65, the GABA synthesizing enzyme at the synapse, was downregulated in 4BD kittens, while GAD67, the enzyme producing the main cellular GABA pool, was not modified under BD. Altogether, these protein expression changes indicate that BD results in a negative regulation of inhibitory transmission during area 17 development and in specific structural remodeling of inhibitory interneurons as CRMPs regulate neurite outgrowth (Goshima et al., 1995). Project co-financed by the European Union from the European Regional Development Fund within the frame of International PhD Projects Programme (MPD4-504).

24. Influence of extracellular matrix modifier on dendritic spine turnover and morphology

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MMP-9 is a member of matrix metalloproteinases (MMPs) family, secreted and activated in the extracellular space in response to enhanced neuronal activity. MMP-9 can cleave and release signaling molecules from extracellular matrix (ECM) which affects morphology and turnover of dendritic spines. Recently, we have shown that enzymatic activity of recombinant autoactivating MMP-9 influences spine morphology. To investigate the impact of endogenous MMP-9 on dendritic spines morphology and turnover we employed a chemically induced long-term potentiation (cLTP) model. We have demonstrated that cLTP increases activity of endogenous MMP-9 localized on the dendritic spines and causes changes in their morphology. Furthermore, the quantitative analysis of dendritic spine turnover shows that MMP-9 is engaged in spine elimination mechanism.

25. Excitatory activity-dependent regulation of synaptic $GABA_A$ receptor lateral mobility probed with optogenetic tools

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The lateral mobility of neurotransmitter receptors is an important determinant for the short- and long-term modulation of synaptic responses. Although a considerable effort has been made to characterize receptor diffusion at both excitatory and inhibitory synapses in response to neuronal activity, the influence of the activation of individual glutamatergic synapses on the mobility of both AMPA and $GABA_A$ synaptic receptors is poorly understood. To address this issue, we exploited light-sensitive glutamate receptors (LiGluK2) in combination with the single particle tracking technique: this optogenetic tool allows the control of the receptor activation with high spatial and temporal precision. Using the single particle tracking technique, we investigated the lateral mobility of both LiGluK2 and $GABA_A$ receptors ($GABA_A$Rs) in response to different durations of LiGluK2 activation.We report that short LiGluK2 light-induced openings differentially and reversibly affect the lateral diffusion of surface LiGluK2 and $GABA_A$Rs at excitatory and inhibitory synapses, respectively. Moreover, we aimed at assessing whether the modulation of synaptic $GABA_A$Rs lateral diffusion might be regulated by changes in intracellular Ca2+ induced by LiGluK2 activation. To this purpose we studied the Ca2+ permeability of LiGluK2 and we observed that receptor activation elicited a transient intracellular Ca2+ rise. This effect was dependent on the Q/R editing of LiGluK2 in a site known to control the Ca2+ permeability in endogenous AMPA/Kainate receptors. Interestingly, suppression of the Ca2+ permeability of LiGluK2 abolished the effect of LiGluK2 activation on synaptic $GABA_A$Rs lateral mobility. This study demonstrates a functional crosstalk between excitatory and inhibitory synaptic receptor activation and mobility. Moreover this work presents LiGluK2 as a novel optogenetic tool for a fine spatial and temporal control of intracellular Ca2+ for the regulation of $GABA_A$Rs lateral mobility at synapses.

26. Hydrophobic residue in GABA binding site

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$GABA_A$ receptors are crucial for neuroinhibition in adult mammalian brain. These channels are pentamers and show anionic selectivity. GABA binding site is located at β(+)/α(-) interface and contains different hydrophobic aminoacids. In the present study we checked the impact of α_{1F64} mutation on binding and gating properties of $\alpha_1\beta_1\gamma_2$ recombinant $GABA_A$ receptors. As expected, mutations in this position affected apparent affinity of the receptor and this effect was confirmed by the use of competitive antagonist in so called race experiments. However, analysis of currents evoked by ultrafast applications of GABA revealed that α_{1F64} mutations impair macroscopic desensitization and slow down current onset suggesting that α_{1F64} is additionally involved in channel gating. Non-stationary variance analysis showed that α_{1F64C} mutation decreases maximum open probability without altering channel conductance. Surprisingly, kinetics of currents evoked by partial agonist (P4S) in WT receptors closely resembled that for response evoked by saturating GABA in the case of $\alpha_{1F64C}\beta_1\gamma_2$ mutant indicating that the mutation interferes with receptor's efficacy. In the case of $\alpha_{1F64L}\beta_1\gamma_2$ receptors, application of saturating muscimol elicited currents larger than GABA and partially rescued fast desensitization suggesting that examined mutations do not eliminate receptor's ability to desensitize. We conclude that α_{1F64} residue plays a key role both in binging and gating properties of $GABA_A$ receptors. Our analysis points to a notion that the major impact of α_{1F64} residue mutation concerns the preopening (flipping) transition which precedes opening or desensitization.

27. Synaptic agonist transient and binding-gating cross-talk – powerful determinants of GABAergic synapse function

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Time course of synaptic inhibitory currents (IPSCs) depends on two major factors: duration of synaptic GABA transient and kinetic properties of GABAA receptors. There is no tool to directly measure synaptic GABA but two indirect methods can be applied. The first is based on the use of rapidly dissociating competitive antagonists and agonist time course is inferred from receptor unblocking upon synaptic transmission. The second one, developed by our group, relies on the use of modifiers of binding and gating. The mechanism of a modifier action is described by analyzing current responses and its effect on IPSCs is numerically reproduced by optimizing the time course of synaptic agonist. Since synaptic transient is short this method is optimal for drugs affecting the binding rate (for chlorpromazine and benzodiazepines we assessed the time constant of agonist transient as 0.1 – 0.3 ms). Intriguingly, GABA binding sites on GABAA receptor are remarkably distant (ca. 50 angstoms) from the channel gate. This structural feature raises the question about molecular mechanisms underlying the energy transfer from binding process to conformational transitions. Recently, we have found that mutation of binding site residue α_{1F64} affects not only binding but has also a strong effect on conformational transitions. Extensive experimental data and model simulations indicated that the major mechanism of α_{1F64} mutation impact on receptor gating is an interference with a conformational transition called preopening or flipping linking binding process with opening/closing and desensitization. We propose that α_{1F64} residue may participate in the process of energy transfer from agonist binding to conformational transitions.

28. Molecular mechanisms of postsynaptic potentiation of GABAergic synapses: a single particle approach

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Postsynaptic long-term potentiation of inhibition (iLTP) can rely on increased $GABA_A$ receptors ($GABA_ARs$) at synapses by promoted exocytosis. However, the molecular mechanisms that enhance the clustering of postsynaptic $GABA_ARs$ during iLTP remain obscure. Here we demonstrate that, during iLTP, $GABA_ARs$ are immobilized and confined at synapses, as revealed by single particle tracking of individual $GABA_ARs$. iLTP expression requires the synaptic recruitment of the scaffold protein gephyrin from extrasynaptic areas, which in turn is promoted by CaMKII-dependent phosphorylation of $GABA_AR$-β3-Ser^{383}. We also report that, while gephyrin moderately contributes to the maintenance of $GABA_AR$ synaptic clustering in basal conditions, this protein is essential for the synaptic rearrangements underlying receptor accumulation during iLTP. Indeed, impairment of gephyrin assembly prevents iLTP and, in parallel, blocks the accumulation and immobilization of $GABA_ARs$ at synapses. Importantly, an increase of gephyrin similar to that observed during iLTP in cultures is found in the rat visual cortex following an experience-dependent plasticity protocol that potentiates inhibitory transmission in vivo. Thus, phosphorylation-dependent accumulation of gephyrin at synapses and receptor immobilization are crucial for iLTP and are likely to modulate network excitability.

29. Lynx1 closes a critical period for auditory thalamocortical plasticity

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Neural circuits undergo dynamic readjustments in response to the environment during developmental critical periods. However, molecular 'brakes' may limit such plasticity in adulthood, thus restricting therapeutic approaches to improve adult brain function. The cholinergic neuromodulatory system has long been a target to induce plasticity in the adult cortex. In fact, recent work elucidates a role of nicotinic acetylcholine receptors (nAChRs) in engaging a disinhibitory microcircuit in the auditory cortex that is required for fear learning. Here, we propose that auditory cortical plasticity is regulated by developmental changes in nAChR signaling by Lynx1, a membrane-anchored protein that inhibits nAChR function. We examined plasticity of auditory thalamocortical circuits in Lynx1 knockout (KO) mice. These mice exhibit enhanced nAChR signaling and heightened plasticity in the adult visual cortex. Our results show that Lynx1 KO mice continue to show robust sound-induced modification of thalamocortical connectivity beyond the critical period. Whole-cell recordings suggest that Lynx1 deletion promotes plasticity by enhancing the activity of cortical layer (L) 1 inhibitory cells. L1 cells may reduce parvalbumin (PV) inhibitory cell function through a disinhibitory circuit, thereby adjusting the excitatory/inhibitory network balance. This study identifies Lynx1 as a molecular 'brake' for auditory plasticity and as a potential therapeutic target to reinstate plasticity beyond early life.

30. Cooperative involvement of serotonergic signaling and extracellular matrix in synaptic plasticity

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The brain plasticity is a re-organization of the neuronal and synaptic networks that allows for changes in response to incoming environmental stimuli. Pathological forms of neuronal plasticity underlie the multiple neuropsychiatric disorders like depression. Clinical observations on the efficacy of antidepressants targeting serotonergic system strongly suggest that serotonin and its receptors play a pivotal role in modulation of pathological plasticity. It is known that matrix metalloproteinase-9 (MMP-9) is one of the most important biomarker in depression and polymorphism in this protein affect bipolar disorder. However, underlying molecular mechanisms, and in particular possible interplay between serotonergic system and ECM, remain poorly understood. We have recently shown that MMP-9, having an established role in synaptic plasticity, influences dendritic morphology in a similar way to that obtained after the 5-HT7 receptor stimulation, e.g. it induces formation of long, thin dendritic spines. It is also known that stimulation of 5-HT7 receptor leads to activation of small Rho GTPase - Cdc42 in fibroblast cell line and in neurons. In this work we investigate whether MMP-9 substrate represents a novel downstream effector of 5-HT7 receptor. Our results indicate that stimulation of the 5-HT7 receptor increases MMP-9 activity toward its synaptic substrates and results in activation of small Rho GTPases.

31. Long-term plasticity in excitatory synapses onto hippocampal inhibitory interneurons in anaesthetised rat

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Synaptic plasticity is considered the neuronal substrate for memory formation. Learning-associated neuronal activity patterns can elicit long-term potentiation (LTP) or depression (LTD) in synaptic inputs onto principal cells and interneurons of hippocampus. Studies in acute brain slice preparation have discovered various types of LTP and LTD in hippocampal interneurons, but it still remains unknown whether any of these take place in the intact brain *in vivo*. We have addressed this question by studying plasticity of excitatory synaptic pathways onto hippocampal CA1 interneurons in urethane-katemine/xylazine anesthetised rats. We electrically stimulated contralateral excitatory afferents (every 5 s) to evoke an action potential in post-synaptic interneuron in dorsal hippocampus. Recordings were performed extracellularly in a juxta-position. Following a stable baseline (for at least 10 min), plasticity-inducing high-frequency stimulation pattern (20 trains of 100 Hz 5 pulses, delivered at 5 Hz for 4 s) was applied to the pathway. Alterations in the excitatory transmission strength were measured (at least 20 min but up to 3 h) through changes in stimulus-evoked postsynaptic action potential probability and delay. Finally, the recorded neurons were juxtacellularly labelled with neurobiotin to reveal their anatomical structure and identify a cell type. Our results show both LTP and LTD can be elicited in hippocampal interneurons *in vivo.* At least in some interneuron types plasticity is akin to what has been reported in a slice preparation.

32. GABAergic contribution to learning-dependent brain plasticity

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Learning brings about reorganization of brain connectivity, creating changes of synaptic strength in specific neuronal circuits. It is well known that these modifications require alterations of excitatory neurotransmission. Our data show that many aspects of GABA-ergic transmission also are significantly affected. Our learning model is a simple classical conditioning in which stimulation of a row of vibrissae (CS) is paired with tail shock (UCS). Conditioning results in a plastic change of functional cortical representation of the “trained” row of whiskers, revealed by 2DG mapping. Following the conditioning, several changes were registered in the barrels of the “trained” row. The density of GABA and GAD IR neurons increases. The increases is due to upregulation of GAD in somatostatin containing interneurons. The frequency of IPSC in excitatory neurons in layer IV increases, suggesting increased GABA release. Tonic inhibition is affected in both inhibitory and excitatory neurons. New inhibitory synapses are formed on spines and more GABA-IR is seen in the synapses. Increased number of polysomes, which can be considered a marker of activation, is observed near these synapses. HPLC measurements show that conditioning causes an increase of GABA level in the barrel cortex. Blocking GABAa receptors for the duration of the training interferes with the formation of plastic change of vibrissal representation. We propose that the GABA-ergic upregulation is a necessary component of learning-induced cortical plasticity.

33. GABA-dependent plasticity in the aging brain

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Neuronal circuits are vulnerable to synaptic alterations that contribute to age-related cognitive impairments causing an age-related plasticity decline. In our studies different forms of cortical plasticity (deprivation-induced and learning-induced) turn out to have different susceptibility for detrimental effects of aging, with learning-induced plasticity being more vulnerable. We have shown an age-related decline of cortical plasticity measured as an expansion of functional cortical representation of a row of vibrissae stimulated in a classical conditioning paradigm, where whisker stimulation was paired with tail shock. Plastic change induction was possible after extending the conditioning from 3 to 7 days. It suggests that aged cortical connections do not support quick learning-induced reorganisation which may be linked to altered excitatory-inhibitory balance that controls cortical excitability. Using molecular and biochemical analyses we found an age-related alteration in discrete balance of excitatory (glutamatergic) and inhibitory (GABAergic) neurotransmission systems in barrel cortex as well in the presynaptic machinery fundamental for neurotransmission. Glutamate/GABA ratio, measured with HPLC, was decreased in aged animals and GABA increase normally observed after training was absent. Western blot and RT-PCR analysis of presynaptic markers in barrel cortex revealed an age-related decreased expression of synaptophysin, vglut2, GAD67 and VGAT protein, as well as mRNA for VGAT. Our data support the notion of an imbalance towards inhibition in the aging somatosensory cortex and point to its role in the impairment of learning-dependent cortical plasticity. Aging affects neurotransmission as early as at the level of neurotransmitter synthesis and transport to the synaptic vesicles.

34. Homeostatic regulation of GABAergic synaptic transmission following focal cortical lesions

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Focal lesions in the cerebral cortex lead to profound alterations in the functional properties of the surviving cortical networks. In particular, a reduced strength of GABAergic transmission is classically known as one major cellular mechanism underlying neuronal hyperexcitability post-injury. In the present study we used an *ex vivo-in vitro* model of focal laser-lesions in the rodent visual cortex to provide evidence that this conclusion may be an oversimplification. Instead, we suggest that lesion-induced alterations in inhibition are rather homeostatic in nature. Changes in the inhibitory system were studied in the first week post-injury, in a cortical area located at around 1 mm distance from the lesion. Sham-operated animals served as controls. Biochemical and immunohistochemical analyses revealed neither changes in the expression level of the two GABA-synthesizing enzymes, GAD65 and GAD67, nor any sign of degeneration of GABAergic synaptic terminals. Patch-clamp recordings from layer 2/3 pyramidal neurons in acute brain preparation disclosed a reduced frequency of miniature and spontaneous inhibitory postsynaptic currents (mIPSCs, sIPSCs) post-lesion. These changes were accompanied by an increased PPR of evoked postsynaptic inhibitory currents (eIPSCs) suggesting an impaired phasic GABA release. Counteracting this reduced phasic GABA release we observed a prolongation in the kinetics of mIPSCs and eIPSCs as well as an enhancement of $GABA_A$-receptor-mediated tonic inhibition. Interestingly, we found a significant negative correlation between the frequency and decay-time constant of mIPSCs. This may suggest that alterations at the pre- and postsynaptic site are homeostatically regulated to maintain a constant overall strength of inhibition. Additionally, we performed recordings from layer 2/3 GABAergic interneurons in GAD67-GFP mice. Interneurons could be classified as fast-spiking or non fast-spiking based on their firing properties and the expression of the calcium-binding protein, parvalbumin. Surprisingly, the excitability of these two subpopulations of interneurons was shifted in opposite directions by the lesion. Based on the outlined observations we propose a revision to the generally accepted notion that a cortical lesion simply leads to a reduction in the intracortical inhibitory strength. In fact, our new findings describe a series of homeostatic-like mechanisms attempting to attenuate perturbations in neuronal network excitability at expenses of the accuracy of the GABAergic synaptic signaling.

35. Changes in GABA(A) receptor subunits and Gephyrin expression caused by BDNF overproduction after complete transection of adult rat spinal cord

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Our recent study showed that BDNF overproduction in the lumbar (L) spinal cord, isolated from supraspinal inputs by its complete transection at low thoracic (Th11-12) level, led to improvement of locomotor functions. However, fast recovery was followed by symptoms of hyperactivation: myoclonus and hindlimb jerks. Searching for its mechanisms we showed that 7 weeks post-lesion, BDNF overproduction significantly increased GAD67 and GABA which were reduced by the lesion, which in conjunction with reduced expression of KCC2 cotransporter, responsible for Cl^- extrusion, could reverse the inhibition by GABA and explain increased excitability. To address contribution of GABA(A) receptors in altered GABAergic transmission, in the current study we examined expression (with qPCR) of GABA(A) receptor subunits α1, α2, γ2 and its key postsynaptic organizer, gephyrin, in the L1-2 spinal segments of spinalized rats, injected with either AAV-BDNF (SP-BDNF; n=4) or PBS (SP-PBS; n=3). Intact rats served as Controls (n=5). Spinalization down-regulated α1 and γ2 subunit (both by 58%), and α2 subunit (by 37%). Surprisingly, it strongly up-regulated gephyrin (by 93%). BDNF overproduction led to (a) an increase of α2 (b) a further decrease of γ2; α1 subunit and gephyrin levels were not changed by BDNF. Similarly increased gephyrin in both spinalized groups suggests that lesion-evoked facilitation of accumulation of the remaining GABA(A) receptors and their dwell-time in the synapse are not modulated by BDNF, contrary to the subunit composition of GABA(A) receptors. Support: PL-German S007/P-N/2007/01, NN401324739 grants.

36. GABAergic contribution to inhibitory deficits after spinal cord injury: the effects of BDNF treatment on GABAergic signaling

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Spinal interneurons are organized into networks that control activity and output of the motor system. After a complete spinal cord transection at low-thoracic level, isolated lumbo-sacral spinal circuitries adapt to the loss of supraspinal inputs reorganizing their connections. Since a view prevails that a loss of stepping ability after spinal cord injury is caused by impaired balance between excitatory and inhibitory systems, we studied to which extent molecules related to GABA signaling contribute to biochemical adaptation in adult spinal rats 7 weeks after spinalization. A greater capacity to induce locomotor pattern which is attributed to the rostral (L1-2) than to the caudal lumbar segments (L3-6), where the motoneurons innervating the majority of hindlimb muscles are located, accounted for segmental analysis. In L1-2, GABA and mRNA coding for GAD65 (presynaptic inhibition) and GAD67 (postsynaptic inhibition) were significantly decreased, accompanied by a decrease in KCC2 cotransporter, responsible for Cl^- extrusion, and in GABA(A) receptor (R)α1, α2, γ2 subunits. BDNF, which contributes to locomotor recovery after lesions, delivered via AAV to L1-2 segments, led to increased GABA, GAD65, GAD67 and α2 GABA(A)R expression in L1-2, elevated GABA above control in L3-6, but did not increase reduced KCC2 levels. We conclude that spinalization impairs GABAergic transmission, which can be improved by BDNF treatment. However, a persisting reduction of KCC2 in conjunction with elevated GABA may reduce the generation of Cl^--dependent hyperpolarizing GABA(A)R currents, increase the probability of reversal of inhibitory signaling and cause hyperactivity of motoneurons ,translating into episodes of clonic movements observed late after lesion. Support: PL-German S007/P-N/2007/01, NN401324739 grants.