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CELLULAR NEUROSCIENCE

Diffusion Versus Extrusion: Mechanisms for Recovery of Neurons from Intracellular Sodium Loads

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The balance of extra- and intracellular ion concentrations is of upmost functional importance for the brain and requires constant cellular transport activity and energy supply. Most energy is used for the export of sodium by the Na+/K+-ATPase (NKA), which is constitutively active even at rest. Additional sodium loads are imposed onto neurons by excitatory synaptic transmission; these can be local or global depending on the strength of activity. Since NKA is virtually the only relevant mechanism for export of sodium under physiological conditions, it was hypothesized that recovery from activity-related sodium transients was mainly mediated by the pump's activity. Sodium, however, is an essentially non-buffered, highly mobile ion. Thus, efficient recovery from local sodium increases could additionally be provided by fast diffusion of sodium ions into non-stimulated regions. We addressed this question studying the properties of recovery from glutamate-induced sodium transients in cell bodies and dendrites of hippocampal neurons using ratiometric sodium imaging. In addition, numerical simulations were performed to explore the parameter space of sodium diffusion and sodium pumping in soma and dendrites. Our data show that sodium export upon global sodium increases is largely mediated by NKA and strongly depends on an intact energy metabolism. For recovery from local sodium increases, in contrast, diffusion dominates over extrusion, operating efficiently even during short periods of energy deprivation. While sodium will eventually be pumped out by the NKA, the fast dissemination of dendritic sodium loads to non-stimulated regions by diffusion might reduce local energy requirements.

Sodium Compartmentalization in Spines and Dendrites of CA1 Pyramidal Neurons

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Influx of sodium ions through voltage- and ligand gated channels is a major mechanism for the generation of depolarization and excitation of neurons. While sodium ions are major charge carriers, influencing biophysical properties of dendrites and spines, the characteristics of sodium signals remain largely unexplored. Here, we analyzed diffusion of sodium in spiny dendrites of mouse CA1 pyramidal neurons in situ using whole-cell patch-clamp combined with multi-photon sodium imaging. Sodium transients were induced by extracellular glutamate uncaging, local electroporation or synaptic stimulation. Our data show that sodium ions diffuse with an apparent diffusion coefficient of 160 $\mu m^2/s$ along dendrites. Sodium signals are similar in amplitude and decay time course in dendrites and short-neck spines, indicating fast diffusional equilibration of sodium between these two compartments. In long-neck spines, in contrast, sodium transients generated in the dendrite appear only delayed and dampened, while synaptically-induced sodium signals are faster, larger and display different decay kinetics as compared to parent dendrites. These results are replicated by a numerical simulation of diffusion between dendritic segments and spines of different neck lengths. Taken together, our results provide the first direct experimental evidence that long-spine necks serve as significant diffusional barriers for sodium ions, resulting in a substantial retention of sodium in those spines that experienced direct sodium influx. The demonstrated compartmentalization of sodium supports the proposed electrical compartmentalization of long-neck spines, and may thus be an important mediator of synaptic weight.

Structural Plasticity of Synaptic Environment: a Quest into the Machinery

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Memory formation in the human brain is thought to rely on the structural remodelling of synaptic connections which ultimately leads to the 'rewiring' of neural circuitry. This process is likely to involve thin astroglial pro¬trusions occurring in the immediate vicinity of excitatory synapses. Indeed, recent evidence has associated astroglial Ca^{2+} activity with diverse molecular signals that can affect the efficacy or functional modality of synaptic connections. The phenomenology, cellular mechanisms and the causal relationships of usedependent astroglial restructuring remain however poorly understood. To monitor rapid rearrangement of astroglia on the nanoscale upon induction of long-term potentiation (LTP, an established experimental model of synaptic memory), we combined electrophysiology with two-photon excitation microscopy and photolytic uncaging.

We document NMDA receptor dependent-withdrawal of astroglial processes from the immediate proximity of synapses following LTP induction, both at the level of synaptic populations and at the level of individual potentiated synapses. This reduction in synaptic astroglial coverage facilitates escape of glutamate discharged into the synaptic cleft, thus boosting cross-talk among extrasynaptic NMDA receptors that might well represent neighboring cells. The molecular mechanisms behind astroglial restructuring are accompanied by local Ca^{2+} elevations but do not appear to involve mGluRs or IP3-receptor dependent Ca^{2+} signalling. They however depend on the availability of aquaporin AQP4. Experiments are underway to build a conceptual understanding of the molecular interactions that act within the microscopic vicinity of synapses in the course of synaptic plasticity.

Effects of Thyroid Hormones in Neuron-Glia Interaction

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Aims

L-tri-iodothyronine (3, 3', 5–triiodothyronine; T3) is an active form of the thyroid hormone (TH) essential for the development and function of the central nervous system (CNS). Circulating thyroxine (T4) crosses blood-brain barrier via specific transporters. T4 in the brain is taken up by astrocytes, de-iodinated to produce T3, and then taken by other cells (Fig. 1). In adult CNS, both hypo- and hyper-thyroidism may affect psychological condition and potentially increase the risk of cognitive impairment and neurodegeneration including Alzheimer's disease (AD). We have reported non-genomic effects of T3 on microglial functions and its signaling in vitro (Mori et al., GLIA, 2015). To investigate whether or not different THs level affects glial cells and neurons in vivo, the effects of hyper- and hypothyroidism on microglia, astrocytes and dendritic spines were investigated.

Methods

Hyper- or hypothyroidism was induced by intraperitoneally injecting T4 (0.3 mg/kg) 4 times during 2 weeks or propylthiouracyl (60 mg/kg/day) for 21 days. The morphological changes in microglia and astrocytes in the cerebral cortex and hippocampus in C57/BL6 mice were investigated by immunohistochemical analysis. The dendritic spines were analyzed by electron microscopy. Behavioral changes in young male mice under hyperthyroidism were investigated using open field test and tail suspension test.

Results

Both microglia and astrocytes were morphologically activated with abnormal level of THs. Interestingly, effects of hyper- and hypothyroidism on glial cells were sex- and age-dependent; Only in young male mice, morphologically activated glial cells were observed. With hypothyroidism, activation of glial cells was observed in young female mice, while in old

female mice glial cells showed rather inhibited morphology. In young male mice with hyperthyroidism, increase in general locomotor activity and willingness to explore was observed, while there was no significant change in tail suspension test, one of the most widely used models for assessing antidepressant-like activity in mice.

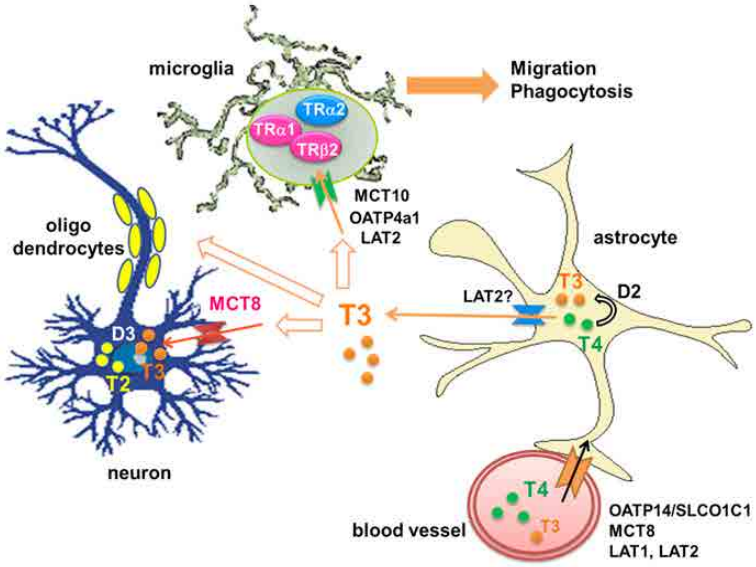

Fig. 1. *Transport of THs to the brain and their metabolism.*

Conclusion

THs are transported into the brain, metabolized in astrocytes and affect microglia and oligodendrocytes, demonstrating an example of glioendocrine system. Dysfunction of THs may impair glial function and thus disturb the brain function. Our results may help to understand physiological and/or pathophysiological functions of THs in the CNS and how hypo- and hyper-thyroidism may cause neurological disorders and their age- and sex-dependence.

Acknowledgements

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Linking AMPA Receptor Nanoscale Organization and Function at Excitatory Synapses

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The spatio-temporal organization of neurotransmitter receptors in the postsynaptic membrane is a fundamental determinant of synaptic transmission and thus information processing by the brain. Ionotropic AMPA glutamate receptors (AMPAR) mediate fast excitatory synaptic transmission in the central nervous system. Using a combination of high resolution single molecule imaging techniques and video-microscopy, we had previously established that AMPARs are not stable in the synapse as thought initially, but undergo continuous entry and exit to and from the post-synaptic density through lateral diffusion.

Using three independent super-resolution imaging methods, on both genetically tagged and endogenous receptors, we have demonstrated that, in live hippocampal neurons, AMPAR are highly concentrated inside synapses into a few clusters of around seventy nanometers. AMPAR are stabilized reversibly in these domains and diffuse freely outside them. Nanodomains are themselves dynamic in their shape and position within synapses as they can form and disappear within minutes, although they are for the most part stable for at least up to an hour. These results open the new possibility that glutamatergic synaptic transmission is controlled by the regulation at the nanometer scale of the position and composition of these highly concentrated nanodomains. In support of this hypothesis, we recently demonstrated that AMPAR conformation strongly impacts their mobility, indicating that desensitized AMPAR can escape synapses. This finding provides a functional support to our hypothesis that fast AMPAR surface diffusion can tune short term plasticity by allowing fast replacement of desensitized AMPAR by naïve ones during high frequency stimulation.

Mechanisms and Consequences of Neuronal Protein SUMOylation in Health and Disease

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Protein SUMOylation is a critically important post-translational protein modification that participates in nearly all aspects of cellular physiology. In neurons, SUMOylation participates in cellular processes, ranging from neuronal differentiation and control of synapse formation to regulation of synaptic transmission and mitochondrial function. SUMOylation is a highly dynamic and usually transient modification that enhances or hinders interactions between proteins and its consequences are extremely diverse. Hundreds of different proteins are SUMO substrates and the mechanisms and protein targets of SUMOylation are activity-dependently controlled and highly sensitive to cell stress. Unsurprisingly, dysfunction of protein SUMOylation is strongly implicated in a many different diseases. I will outline the SUMO system and discuss recent discoveries from our lab that illustrate some of the roles of SUMOylation in healthy and diseased neurons.

Molecular Mechanisms Regulating Synaptic Expression of NMDA Receptors

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NMDA receptors are critical for neuronal development and synaptic plasticity. Functional NMDA receptors are tetramers most often composed of two GluN1 subunits and two GluN2 subunits (GluN2A–D). Although synaptic NMDA receptors are tightly anchored to the postsynaptic membrane via the postsynaptic density (PSD), they are also dynamic at the cell surface. Indeed, we find that phosphorylation and dephosphorylation regulates synaptic expression and endocytosis in a subunit-specific manner. We find that CK2 phosphorylation of GluN2B on S1480 disrupts PSD-95 binding, drives GluN2B endocytosis thus removing GluN2B from synapses resulting in an increase in synaptic GluN2A expression. Furthermore, there is an interplay between S1480 phosphorylation and the nearby GluN2B tyrosine-based endocytic motif (YEKL), providing a molecular mechanism for the observed effects on NMDA receptor trafficking. Namely, there is coordinated phosphorylation and dephosphorylation of two different residues on GluN2B, S1480 within the PDZ domain binding domain and Y1472 within the tyrosine-based YEKL endocytic motif, which play opposing roles in the regulation of NMDA receptor endocytosis and ultimately the surface expression of GluN2B . Both the tyrosine kinase Fyn and the tyrosine phosphatase STEP are known to target GluN2B on Y1472. However, nothing is known about a potential functional interaction between STEP and PSD-95. We find that STEP binds to PSD-95, but not other PSD-95 family members and that PSD-95 drives degradation of STEP. These findings support an unexpected dual role for PSD-95 to stabilize NMDARs by binding directly to GluN2B, but also by promoting degradation of the negative regulator STEP.

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Space and Time Shape The Functions of the Nmda Receptor Coagonists in the Hippocampus

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NMDA receptors (NMDARs) require the coagonists d-serine or glycine for their activation raising controversies "where, when, and how?" glycine and d-serine enter into play to regulate NMDARs at synapses in the brain1,2,3. Whether the identity of the coagonist could be synapse specific and developmentally regulated has remained unexplored. We therefore investigated the contribution of d-serine and glycine by recording NMDAR-mediated responses at hippocampal Schaffer collaterals (SC)-CA1 and medial perforant path-dentate gyrus (mPP-DG) synapses in juvenile and adult rats4. Selective depletion of endogenous coagonists with enzymatic scavengers as well as pharmacological inhibition of endogenous d-amino acid oxidase activity revealed that d-serine is the preferred coagonist at SC-CA1 mature synapses, whereas, unexpectedly, glycine is mainly involved at mPP-DG synapses. Nevertheless, both coagonist functions are driven by the levels of synaptic activity as inferred by recording long-term potentiation generated at both connections. This regional compartmentalization in the coagonist identity is associated to different GluN1/GluN2A to GluN1/GluN2B subunit composition of synaptic NMDARs. During postnatal development, the replacement of GluN2B- by GluN2A-containing NMDARs at SC-CA1 synapses parallels a change in the identity of the coagonist from glycine to d-serine. In contrast, NMDARs subunit composition at mPP-DG synapses is not altered and glycine remains the main coagonist throughout postnatal development. Altogether, our observations disclose an unprecedented relationship in the identity of the coagonist not only with the GluN2 subunit composition at synaptic NMDARs but also with astrocyte activity5 in the developing and mature hippocampus that reconciles the complementary functions of d-serine and glycine in modulating NMDARs during the maturation of tripartite glutamatergic synapses. Our results point out the importance of the spatial and temporal switch in coagonist identity for therapeutic interventions aimed at treating deficits in NMDAR activity in brain disease such as schizophrenia.

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Optical Quantal Analysis of Glutamate Release

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Information transmission at chemical synapses is a quantal process based on the release of transmitter-filled vesicles from the presynaptic terminal. In the CNS, where each neuron receives thousands of synapses at different electrotonic distances from the soma, classical quantal analysis of postsynaptic potentials is difficult. Optical methods based on fluorescent calcium indicators have been used to isolate the response of individual synapses on dendritic spines in intact brain tissue. The non-linear relations between glutamate concentration, receptor activation and spine calcium concentration, however, complicate a quantal analysis of calcium signals. Here we use direct optical measurements of glutamate concentration in the synaptic cleft based on the genetically encoded glutamate sensor iGluSnFR. We show that the amplitude distributions from individual synapses are well fit by a quantal model if photon shot noise is taken into account. Furthermore, the new method allows localizing the fusion site on the surface of the presynaptic bouton with an accuracy exceeding

the resolution limit of the two-photon microscope. Biophysical modeling of the release process allows us to extract the number of readily releasable vesicles, the occupancy of postsynaptic AMPA receptors and other functional parameters of Schaffer collateral synapses.

Experimental and Modelling Approaches to Studying Ion Channel Function in Small Presynaptic Terminals in Health and Disease

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Some inherited cases of migraine, ataxia and epilepsy are due to mutations in neuronal Ca^{2+}, K^+ and Na^+ ion channels and also in several other presynaptic proteins. We investigate how these mutations affect Ca^{2+} signals in nerve terminals, and how they affect neurotransmitter release. Our aim is to establish how the disease-linked mutations affect neurotransmission at the level of individual synapses which is prerequisite for understanding of the abnormal neuronal network function in paroxysmal neurological disorders.

We have recently developed a set of new methods which, for the first time, allow us to study the relationship between Ca^{2+} entry and vesicular exocytosis, and to probe presynaptic ion channel function in individual small presynaptic terminals. This is based (i) on measuring, with fluorescence microscopy, rapid changes in the concentration of Ca^{2+} ions, as well as the rate at which small vesicles containing chemical neurotransmitters are discharged, and (ii) on using super resolution scanning ion conductance microscopy for nanoscale-targeted patch-clamp recordings in small presynaptic boutons. Using these methods we investigate how different channels that mediate Ca^{2+} influx into the presynaptic terminal control the release of vesicles, how they influence synaptic plasticity, and how synapses are influenced by other modulatory neurotransmitters acting upon presynaptic terminals both in health and disease.

In this talk I will present the data from two ongoing projects that are focused on understanding of the cellular mechanisms of Familial Hemiplegic Migraine Type 1 and of Episodic Ataxia Type 1.

Microglial Cells Promote Glioma Growth

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We have studied the interactions between microglial cells and glioma cells. Based on our first finding that microglia promotes glioma growth (Markovic et al., 2005, J Neuropathol Exp Neurol. 64:754-762), we have identified mechanisms of interaction between these cells. We found that glioma cells induce microglia to express a metalloprotease, MT1-MMP which is necessary for the activation of MMP-2. Glioma cells release MMP-2 in an inactive form which is then activated by the microglial MT1-MMP (Markovic et al., 2009, Proc Natl Acad Sci U S A. 106:12530-12535). The metalloproteases degrade extracellular matrix and thereby promote glioma invasion and expansion. We found that this signalling between microglia and glioma cells is mediated by Toll -like receptor 2 (Vinnakota et al., 2013, Neuro Oncology 15:1457-1468). As ligand for Toll -like receptor 2 we recently identified versican which is released from mouse and human glioma cells (Hu et al., 2015, Neuro Oncol. 17:200-210). Microglial cells also upregulate and release MMP-9 triggered by the same pathway (Hu et al., 2014, Int J Cancer. 135:2569-2578). With a microarray screen on mouse microglia, we also found that glioma cells induce a specific phenotype in microglia which does neither fit into the M1 nor in the M2 classification (Szulzewsky et al. 2015, PLOSone, e0116644. doi: 10.1371/journal.pone.0116644). This we could recently confirm with microglia isolated from human glioma resection material (Szulzewsky et al., submitted).

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VMAT2 in Astrocytes Regulates Morphology of Pyramidal Neurons in Developing Pfc by Modulating Extracellular Levels of Dopamine

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Neuromodulation of neuronal circuits of the prefrontal cortex (PFC) by monoamines influences synaptic plasticity and executive functions and may play critical roles in psychiatric disorders (Arnsten et al., Neuron., 2012). The cellular mechanisms governing the homeostasis of monoamines in the developing PFC are not completely defined. Evidence that astrocytes express the whole enzymatic apparatus for the metabolism of monoamines (Youdim et al., Nature Rev Neurosci., 2006) strongly suggests a possible involvement of astroglial cells in mechanisms governing monoaminergic homeostasis.

Here we report that astrocytes located in PFC express a plasma membrane organic cation transporters 3 (OCT-3) and contain dopamine (DA). Moreover we found that astrocytes express vesicular monoamine transporter 2 (VMAT2) and that the signal of this transporter was readily recognizable on different subset of intracellular organelles such as endosomes and lysosomes. The physiological relevance of VMAT2 in astrocytes is investigated by generating conditional transgenic mice in which VMAT2 is specifically deleted in GFAP expressing cells (aVMAT2cKO). Interestingly, VMAT2 deletion in astrocytes leads to a specific deficits in astrocytes storing DA and a decrease in the extracellular levels of DA in the PFC. Moreover, we found out that the decreased levels of DA in the developing PFC of aVMAT2cKO mice alters the spine formation of pyramidal neurons that can be rescued by chronic administration of L-DOPA or VMAT2 re-expression in astrocytes by lentivirus during postnatal development. These results highlight a critical role for VMAT2 in astrocytes in the regulation of DA levels and the normal development of pyramidal neurons in the PFC.

Unraveling the Role of Astroglial Networks in Neuronal Coordination

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Astrocytes interact with neurons to regulate network activity. Although the gap junction subunits connexin 30 and connexin 43 mediate the formation of extensive astroglial networks that cover large functional neuronal territories, their role in neuronal synchronization remains unknown. Using connexin 30– and connexin 43–deficient mice, we showed that astroglial networks promoted sustained population bursts in hippocampal slices by setting the basal active state of neurons. Astroglial networks limited excessive neuronal depolarization induced by spontaneous synaptic activity, increased neuronal release probability, and favored the recruitment of neurons during bursting, thus promoting the coordinated activation of neuronal networks. In vivo, this sustained neuronal coordination translated into increased severity of acutely evoked epileptiform events and convulsive behavior. These results revealed that connexin-mediated astroglial networks synchronize bursting of neuronal assemblies, which can exacerbate pathological network activity and associated behavior.

Role of Astroglial Calcium/Calcineurin-Mediated Signaling in Alzheimer's Disease: in Search of Potential Mechanisms and Mediators

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Alzheimer's disease (AD) is the most common age-related neurological disorder with an enormous social and economical impact. AD is characterized by progressive loss of memory, social deficit and dementia. Currently, there is no cure or preventive therapy for AD and therefore novel approaches for understanding the AD pathogenesis are desperately needed. Deregulation of calcium homeostasis has been proposed to have a crucial role in pathogenic cascade which leads to neurodegeneration. Yet, the possibility that the familial AD (FAD)-associated mutations alongside with the soluble beta-amyloid (Aβ) oligomers affect calcium-signaling in astroglial cells, leading thus to neuronal dysfunctions at the early stages of disease, has been largely over-looked. We have dissected a cascade of events by which Aβ deregulates calcium homeostasis in hippocampal astrocytes. In details: (i) 100 nM Aβ42 leads to an increase in cytosolic calcium; (ii) increased Ca^{2+} leads to activation of calcineurin (CaN), which in turn (iii) directly activates NFAT to up-regulate IP3R1, and (iv) via interaction with Bcl10 and degradation of IkBα activates NF-kB to up-regulate mGluR5 and IP3R2. Furthermore, ATP-induced IP3R1-mediated Ca^{2+} release and IP3R1 protein were augmented in the hippocampal astrocytes form 3xTg-AD mice, a well characterized AD mouse model in which persenilin 1 (PS1)-M146V FAD-related mutation is expressed in astrocytes. Finally, we show that mGluR5 staining is augmented in hippocampal astrocytes of AD patients in proximity of Aβ plaques and co-localized with accumulation of the p65 NF-kB subunit and increased staining of CaNAα. These data indicate that calcium-dependent activation of CaN and NF-kB mediates the remodeling of astroglial calcium signaling toolkit in AD.

Next, using astrocyte-neuronal co-cultures and astrocyte-conditioned medium (ACM) transfer, we found that Aβ42-exposed astrocytes, as well as astrocytes form 3xTg-AD mice produce alterations of dendritic spines and reduce MAP2, PSD95 and Syn38 proteins in cultured neurons in a CaN-dependent manner. Searching for a soluble factor(s) we performed multiplex cytokine assay of ACM, but among 12 cytokines assayed only TGF-β was released at a detectable level. Using qPCR we found that TGF-β2 and TGF-β3 are predominantly expressed at mRNA level. Furthermore, Aβ42-induced TGF-β3 mRNA up-regulation as well as release of TGF-β3 from 3xTg-AD astrocytes were blocked by a CaN inhibitor. In cultured hippocampal neurons, treatment with TGF-β2 and TGF-β3, but not TGF-β1, produced reduction of neuronal proteins, suggesting that the β2 and β3 isoforms may play a role in the early AD-related neuronal pathology.

Finally, in order to characterize transcriptional alterations that astroglial cells undergo in the early AD we performed a whole-genome microarray study on cultured hippocampal astrocytes from 3xTg-AD mice using Non-Tg astrocytes as a control. A set of 963 genes was differentially expressed in 3xTg-AD with respect to Non-Tg astrocytes. Gene ontology (GO) analysis revealed that among the up-regulated, the genes involved in nucleotide binding, regulation of transcription and mitochondrial function were significantly overrepresented. Instead, among the down-regulated, the overrepresented genes are involved in cell-cell communication and regulation of synaptic transmission.

Taken together, our data indicate that transcriptional Ca^{2+}/CaN-dependent remodeling of astroglial cells, taking place in the early AD stages, may participate to the development of synaptic and neuronal dysfunction.

Role of Synaptic Plasticity in AMPA Receptor Intracellular Trafficking

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AMPA receptors (AMPARs) mediate fast excitatory synaptic transmission in the central nervous system. Their abundance at the synapse is essential for the establishment and maintenance of synaptic function. Many studies characterized trafficking of AMPARs in spines at basal state or after induced plasticity. Their synaptic localization is dependent on a highly dynamic exocytosis, endocytosis and plasma membrane trafficking events. Our hypothesis is that synaptic localization of AMPARs is also regulated by their earlier intracellular trafficking. However, AMPARs post-ER traffick-

ing toward the plasma membrane still remains poorly understood.

Using a new biochemical tool combined with photonic live imaging, we controlled and followed the dynamic secretion of tagged AMPAR subunits in cultured rat hippocampal neurons. We characterize AMPAR trafficking from the ER to the Golgi apparatus and from Golgi to the plasma membrane.

We analyze the characteristics of basal AMPAR transport. We studied the influence of synaptic plasticity in the intracellular transport of GluA1 containing AMPAR. Since the scaffold protein SAP97 has been shown to be involved in the intracellular AMPARs traffic via its PDZ interaction with GluA1, we have studied its role in the GluA1 vesicular trafficking. We show that an abolishment of the PDZ interaction between GluA1 and SAP97 alters the GluA1 vesicular trafficking. We are characterizing how interaction of GluA1 with SAP97 can regulate the intracellular transport of AMPARs.

Extrasynaptic Protease, MMP-9 in Healthy and Diseased Mind

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Matrix metalloproteinase 9, MMP-9 is an extracellularly operating enzyme that has been demonstrated as important regulatory molecule in control of synaptic plasticity, learning and memory. Either genetic or pharmacological inhibition of MMP-9 impairs late phase of long-term potentiation at various pathways, as well as appetitive and spatial memory formation, although aversive learning remains apparently intact in MMP-9 KO mice. MMP-9 is locally translated and released from the excitatory synapses in response to neuronal activity. Extrasynaptic MMP-9 is required for growth and maturation of the dendritic spines to accumulate and immobilize AMPA receptors, making the excitatory synapses more efficacious. Animal studies have implicated MMP-9 in such neuropsychiatric conditions, as e.g., epileptogenesis, autism spectrum disorders, development of addiction, and depression. In humans, MMP-9 appears to contribute to epilepsy, alcohol addiction, Fragile X Syndrome, schizophrenia and bipolar disorder. In aggregate, all those conditions may be considered as relying on alterations of dendritic spines/excitatory synapses and thus understanding the role played by MMP-9 in the synaptic plasticity may allow to elucidate the underpinnings of major neuropsychiatric disorders.

Spontaneous Neurotransmitter Release and Synaptic Plasticity

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Spontaneous neurotransmitter release is a salient feature of all presynaptic nerve terminals. Recent studies have shown that these action potential independent release events are essential regulators of synaptic homeostasis; in particular, they are involved in the maintenance of synaptic strength in terms of both presynaptic release rate and postsynaptic sensitivity. Moreover, there is growing evidence that postsynaptic receptors and signaling elements that respond to spontaneous release events diverge from those that respond to evoked release, suggesting a spatial segregation of these two forms of neurotransmission. We have previously shown that application of NMDA receptor antagonists – ketamine (20 μ) and MK801 (10 μM) at rest potentiates synaptic responses in the CA1 regions of rat and mouse hippocampus. This potentiation requires protein synthesis, brain-derived neurotrophic factor expression, eukaryotic elongation factor-2 kinase function, and increased surface expression of AMPA receptors. The same synaptic potentiation could be elicited by deplete neurotransmitter selectively from spontaneously recycling vesicles. In recent experiments, we found that this form of synaptic potentiation does not fully occlude subsequent long-term potentiation elicited by theta burst stimulation (100 Hz theta-burst protocol: 100 Hz, four pulses per burst; 15 bursts at 200 ms intervals). In these experiments, we could detect an additional ~20% increase in synaptic efficacy following ketamine mediated potentiation of responses (~30% above baseline). In this setting, theta burst stimulation alone could elicit up to ~50% potentiation above baseline. Taken together, these findings demonstrate that selective presynaptic impairment of spontaneous release, without alterations in evoked neurotransmission, is sufficient to elicit synaptic potentiation, which shows limited overlap with canonical long term potentiation elicited by repetitive activity.

A New Pathway for Presynapse to Nucleus Communication: Potential Implications for Information Storage in the Brain

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Formation of long-term memories requires synapto-nuclear communication that in turn regulates expression of relevant genes in neurons. While various signaling pathways and protein mediators for communication between the postsynaptic and dendritic compartments with the nucleus have been identified our knowledge about presynapse to nucleus signaling is rather limited (1). Recent studies in our laboratory have revealed that the multifunctional protein CtBP1 (C-terminal binding protein-1) may fulfill such a task. CtBP1 has been discovered originally as a transcriptional co-repressor. CtBPs including CtBP1 and CtBP2 are widely expressed proteins involved in developmental gene regulation and chromatin modification. In addition CtBP1 (also named BARS-50) has been implicated in regulating intracellular membrane trafficking processes and, in neurons, it has been localized to presynaptic boutons.

Synaptic localization of CtBP1 depends on its interaction with the presynaptic cytomatrix proteins Bassoon and Piccolo – giant proteins involved in the organization of the apparatus for neurotransmitter release at the active zone (2). Presynaptic and nuclear pools of CtBP1 can communicate in an activity-dependent manner and the absence of the two large presynaptic anchor proteins causes increased levels of nuclear CtBP1, what in turn affects the expression of activity-regulated genes (3). The interaction of CtBP1 with Bassoon and Piccolo is regulated by cellular NAD/NADH levels and thus may act as a sensing system for presynaptic activity and metabolic state. Accordingly, CtBP1 is a prime candidate for a protein mediator that couples activity-driven changes in presynaptic performance with plasticity-related alterations of neuronal gene expression.

Potential implications for the interaction of the CtBP signaling pathway with postsynapse to nucleus communication pathways and for setting of the gene expression pattern in a given neuron will be discussed.

Acknowledgements

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Synaptic and Extrasynaptic Neuron-Glia Interactions

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Brain is often viewed as large neuronal connectome where the information is encoded in the patterns of action potentials and stored in the changes of synaptic strength or appearance of new wiring routes. However, recent studies have demonstrated that astrocytes also possess complex patterns of calcium signals influenced by neuronal activity. Astrocytic calcium signals regulate various functions of these cells including release of gliotransmitters and morphological changes in the astrocytic processes (Tanaka et al., 2013). It has been tempting to suggest that information in astrocytes is encoded in the frequency of calcium events, similar to patters of neuronal action potentials. Synaptically released neurotransmitters thought to trigger new calcium events in perisynaptic astrocytic processes (PAPs) though activation of metabotropic glutamate receptors (mGluRs). In contrast, our recent findings suggest that PAPs are devoid of calcium stores that are required for mGluR-mediated calcium signaling (Patrushev et al., 2013). This makes unlikely any significant role of mGluRs in triggering calcium events in PAPs. Instead, we show that activation of 'extrasynaptic' astrocytic mGluRs increases proportion of spatially extended calcium events in the power-law based distribution of calcium event sizes (Wu et al., 2014). This effect takes place without any significant increase in the frequency of calcium events. These findings suggest that astrocytic response to surrounding neuronal activity is rather encoded in spatial

characteristics of their calcium events and fundamentally different from temporal information coding in neurons (e.g. coincidence detection, action potentials sequences etc). Nevertheless, we cannot exclude local ionic changes in PAPs in response to synaptic activity. For example, potassium ions accumulate in the synaptic cleft of glutamatergic synapses during repetitive activity. We have demonstrated that the bulk of these ions is contributed by potassium efflux through postsynaptic NMDA receptors (Shih et al., 2013). Potassium mediated depolarization of presynaptic terminal increases glutamate release probability. Now we have found that accumulation of intracleft potassium during repetitive synaptic activity could also inhibit astrocytic glutamate uptake by depolarizing PAPs. This extends glutamate dwell-time in the synaptic cleft and boosts glutamate spillover effects.

Acknowledgements

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Cell Protective and Trophic Properties of GDNF and its Derivatives

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GDNF is a major factor for a survival of the dopamine neurons of the midbrain. It supports the axon growth as well as survival of the neurons. For different models of the Parkinson disease GDNF could prevent the neurotoxically provoked death of the dopamine neurons, and supports recuperation of its functional activity. Though some by-side effects are also known, like loosing weight and chance of neoplastic transformation. We prepared a genetic construct caring human GDNF gene, introduced it into HEK293 cells, and then transplanted the cells into parenchyma of the mouse brain. Transgenic cells, which express GDNF, essentially reduce the glial scar formation. Therefore GDNF could be applied during transplantation into the brain to improve the transplant survival. In humans GDNF gene supplies two versions of mRNA for: pre-(α)pro-GDNF and truncated pre-(β) pro-GDNF (1). Pre-(α)pro-GDNF is secreted through Golgi apparatus and pre-(β) pro-GDNF is located in the secretory vesicles and moves by fast secretion pathway. Probably, pre-(α)pro-GDNF is needed for conventional neuron survival, and pre-(β) pro-GDNF serves as SOS system during traumatic injury of neurons or neurodegenerative diseases. To study 'pro' region function during fast transport and factor induction properties several derivatives of GDNF were made. A secretion of the factor into medium has been shown by western blot analysis. All modified GDNF were introduced into HEK293 cells, and transgenic cell lines were maintained (2). After culturing the cells with modified GDNF, the condition media was added into culture medium of rat embrional spinal ganglion explant, and growth of neural sprouts were analyzed. Deletion of 'pro' region essentially increases GDNF effects as neural inductor. A study of culture of dissociated spinal ganglion and calculation of neural sprouts yielded the same results. HEK293 cells were transfected with a vector encoding an isoform of the human. GDNF gene with deleted pre- and pro-regions (mGDNF) in the medium conditioned by the transfected cells was shown to induce axonal growth in PC12 cells. Then the early Parkinson's disease model was established by injection of the dopaminergic proneurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) into C57Bl/6 mice. Transgenic HEK293/mGDNF/GFP cells were transplanted into the striatum (caudateputamen) of experimental mice. The motor activity was monitored 1 and 2 weeks after MPTP injection. After the experiment, the motor coordination of experimental animals was evaluated in the rotarod test, and dopaminergic neurons in the substantia nigra pars compacta were counted in cross-sections of the midbrain. MPTP administration lowered the number of tyrosine hydroxylase immunopositive cells in the substantia nigra pars compacta, decreased motor coordination. The transplantation of HEK293/mGDNF cells into the caudate-putamen smoothed the effects after MPTP, while the control transplantation of HEK293 cells showed no notable impact.

Conclusions

Transplantation of transgenic cells with GDNF gene lacking the pre- and pro-sequences can protect dopaminergic neurons in the mouse midbrain from the subsequent administration of the pro-neurotoxin MPTP, which is confirmed by polysomnographic, behavioral and histochemical data. Hence, GDNF is released from transfected cells, and provides the differentiation activity and neuroprotective properties.

Acknowledgements

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Excitation-Energy Coupling and Vesicle-Based Signaling in Astrocytes

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Astrocytes, a heterogeneous glial cell type, get excited when neurotransmitters, such as noradrenaline (NA) and ATP bind to their membrane receptors and respond back by releasing their own signals. This involves vesicles, which store chemicals termed gliotransmitters or more generally gliosignaling molecules. In the former case chemical messengers get released from astrocytic sites proximal to the synapse, which defines communication to occur in the micro-space of contact between the synapse and the astrocyte. In contrast gliosignaling molecules may also be released into the extracellular space and get transported to locations far away from the active astrocyte. This mode of release resembles the endocrine system. Hence astrocytes are considered to be part of the gliocrine system in the brain, where the glymphatic system mediates the convection of released molecules. This complex system not only plays a role in cell-to-cell communication but also synchronizes the provision of energy for neural networks. Astrocytes contain glycogen, a form of energy store. Excitation of astrocytes by volume transmitters, such as NA, released by locus coeruleus neurons, activates adrenergic receptors and stimulates glycogenolysis, providing lactate. This lecture will discuss how astrocytes operate to synchronize excitation and energy provision. Moreover, Ca^{2+}-dependent fusion of the vesicle membrane with the plasma membrane in astrocytes will be presented.

Using an approach to study single astrocytes by quantitative imaging confocal microscopy, we studied how stimuli like noradrenaline or ATP activate cytosolic calcium signals and how the mobility of fluorescently labelled secretory vesicles is affected by physiological states of astrocytes. By fluorescence resonance energy transfer (FRET) nanosensors we also measured second messenger cAMP and metabolites, such as D-glucose and L-lactate. Stimulation of astrocytes by noradrenaline increases cytosolic calcium and cAMP in distinct time-domains. Vesicle mobility was differentially modulated, depending of the vesicle cargo, by elevations in cytosolic calcium levels. NA also stimulated glycolysis monitored as an increase in FRET-based cAMP and cytosolic L-lactate increase, while cytosolic D-glucose levels were decreased due to facilitated consumption in glycolysis.It is proposed that excited astrocytes liberate energy by enhanced glycolysis, while a complex vesicle -ased signalling response is taking place in the same time domain. Hence, excitation-energy coupling is time-associated with alterations in astrocytic vesicle-based communication capacity.

Offline Effects of Single and Paired Pulse TMS

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All effects produced by TMS are mainly two types: online or offline. Online TMS effects (behavior and electrophysiological) described as lasting less than 1 second after stimulation. Offline TMS on the other hand means that the stimulation effects lasting seconds and minutes. Single and paired plus (ppTMS) stimulations are considered as online (Terao et.al.,

2006). We checked EEG state before and after blocks of random single (110% MT) and paired (90% - 110% MT, 2 or 10 ms IPI (intracortical facilitation (ICF) and short-interval intracortical inhibition (SICI)); 4 – 8 sec between single or paired pulses) pulse stimulation (about 300 total stimuli) by calculation of Long-Range Temporal Correlations (LRTC, Linkenkaer-Hansen et al., 2001; Nikulin et al., 2012) in EEG . LRTC in the alpha frequency range were calculated with Detrended Fluctuation Analysis applied to multichannel EEG recordings.

LRTC in the alpha frequency range showed moderate intra-class correlation between the two rest sessions thus indicating a relative stability of the temporal dynamics between the sessions. Topographically, intra-class correlation was least pronounced over the stimulated areas thus demonstrating a potential long-lasting offline effect of TMS on neuronal activity. This was further confirmed by showing that there was a positive correlation between the magnitude of ICF and the magnitude of LRTC in 8–13 Hz range. Importantly, such correlation was regionally specific demonstrating strongest values over sensorimotor areas where TMS was applied. Our data suggests single and paired pulses TMS can have a previously unobserved long lasting effect on the temporal dynamics of neuronal oscillations and can be considered as offline.

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Role of the Oligodendrocyte Lineage in Acute CNS Trauma

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Acute brain injuries activate signaling cascades essential for scar formation. Here, we report that acute lesions associated with a disruption of the blood-brain barrier (BBB) trigger re-programming of the oligodendrocyte lineage. Differentiated oligodendrocytes and their precursor cells can generate another neuroglial cell type: astrocytes. By in vivo 2P-LSM analysis we followed oligodendrocytes after injury in PLP-DsRed/GFAP-EGFP transgenic mice. Adjacent to the lesion site, oligodendrocytes first turned into an intermediate cell stage with astro- and oligodendroglial gene expression properties (AO cells). Subsequently, portions of AO cells differentiated into astrocytes, while others stayed in the oligodendrocyte lineage. In split-Cre mice, AO cells showed a clear glia-restricted differentiation potential that also depended on local cues. At the lesion higher expression levels of glial differentiation factors were detected. And indeed, local injection of IL-6 promoted the formation of AO cells. In summary, our findings highlight the plastic potential of oligodendrocytes in acute brain trauma.

Molecular Changes in Penumbra After Focal Photothrombotic Stroke in the Rat Cerebral Cortex

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Aims

Ischemic stroke is a leading cause of human disability and mortality. Vascular occlusion and energy deficit rapidly, for a few minutes induce cerebral infarct. The cell damage propagates from the infarct core to surrounding tissues (penumbra). Acute cell necrosis inside the infarct core cannot be prevented, but tissue damage in penumbra develops slower,

during several hours, and this "therapeutic window" provides time for neuroprotection. However, the efficient neuroprotective drugs for stroke pharmacotherapy are still not found. Therefore, deeper studies of biochemical mechanisms that regulate neurodegeneration and neuroprotection in penumbra are necessary for development of new approaches to stroke treatment.

Methods

We studied morphological and biochemical consequences of the focal photothrombotic infarct (PTI) in the rat cerebral cortex induced by the local laser irradiation (532 nm diode laser, 64 mW/cm2, Ø3 mm, 30 min) after i.v. administration of Bengal Rose (20 mg/kg). This hydrophilic photosensitizer does not cross the blood brain barrier and accumulates in microvessels. The following laser irradiation induces focal occlusion of microvessels. In this stroke model location, size, and degree of the injury are well controlled and reproducible. Preliminary histological and ultrastructural studies showed that PTI core was surrounded by 1.5-2 mm penumbra. To characterize changes in expression of neuronal and signaling proteins in penumbra, we used proteomic microarrays "The Panorama Antibody Array – Neurobiology" (Sigma-Aldrich). Each microarray contains 448 microdroplets with immobilized antibodies against 144 neuronal proteins and their isoforms and epitops (totally 224 spots in duplicate). At 1, 4 or 24 hours after PTI, the rat cortex was extracted, and the penumbra ring was excised. The symmetrical piece from the non-irradiated contralateral cortex was used as a control. After homogenization, the samples were incubated with fluorochromes Cy3 or Cy5. In another set, these samples were stained oppositely, by Cy5 and Cy3, respectively. Such swapped staining provides verification of results and compensation of a bias in binding of Cy3 or Cy5 dyes to protein samples. This provides the double test and full control of the experiment. Two microarrays were incubated with these labeled samples. After washing and drying the microarrays were scanned on the GenePix 4100A Microarray Scanner (Molecular Devices, USA) at 532 and 635 nm (fluorescence maximums of Cy3 and Cy5, respectively). The fluorescence images of the antibody microarrays were normalized (ratio-based normalization). The median fluorescence value determined over all spot pixels was used for estimation of the protein content in each spot, and the ratios of the experimental to control values characterized the difference in the protein levels between photothrombotic and untreated cortical tissue. The standard statistical treatment based on Student's t-test was used. The cut-off level was 30%.

Results

The histological and ultrastructural studies showed edema and degeneration of neurons, glia and capillaries in the PTI core. Morphological changes decreased gradually across the penumbra. The ultrastructure of the penumbra region adjacent to the PTI core was similar to that inside the core, but at a distance of 1.5-2 mm it looked almost normal except edema around some vessels and neurons. The proteomic study showed that the changes in protein expression in penumbra were maximal 4 hours after PTI (upregulation of 22 proteins and downregulation of 21 proteins) as compared with 1 hour (18 and 6 proteins) and 24 hours reperfusion intervals (10 and 9 proteins, respectively).

Diverse cellular subsystems were involved in the complex response of the penumbra tissue 4 hours after PTI: signal transduction pathways including protein kinase Bα/GSK-3, protein kinase C and its β1 and β2 isoforms, Wnt/β-catenin (axin1, GSK-3, FRAT1), Notch/NUMB, DYRK1A, TDP43; axon outgrowth and guidance (NAV-3, CRMP2, PKCβ2); intercellular interactions and tissue integrity (N-cadherin, PMP22); cytoskeleton elements (neurofilament 68, neurofilament-M, doublecortin); vesicular trafficking (syntaxin-8, TMP21, Munc-18-3, synip, ALS2, VILIP1, syntaxin, synaptophysin, synaptotagmin); biosynthesis of neuromediators (tryptophan hydroxylase, monoamine oxidase B, glutamate decarboxylase, tyrosine hydroxylase, DOPA decarboxylase, dopamine transporter); ubiquitin-mediated proteolysis (ubiquilin-1, UCHL1); mitochondria quality control (Pink1, parkin, HtrA2); miscellaneous proteins (LRP1, prion protein, β-amyloid). Additional immunohistochemical and Western blot studies of expression of monoamine oxidase B, UCHL1, DYRK1A, and Munc-18-3 confirmed the proteomic data.

Conclusions

The obtained data on changes in expression of more than 40 proteins that participate in various cellular subsystems provided the integral view on the cellular and tissue responses in the penumbra to photothrombotic infarct. They are involved either in neurodegeneration, or in neuroprotection. These changes were most expressed 4 hours after photothrombotic impact and reduced at the next day. Some of these proteins may serve as potential targets for ischemic stroke therapy.

Acknowledgements

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OM&P

Generation of Multi-Innervated Dendritic Spines in Memory Formation in Ageing

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Aims

Aged-related memory decline is associated with impairment of synaptic strengthening, and memory formation in older animals is slower and less flexible in comparison to younger animals. If strengthening is impaired, hippocampus-dependent memory formation depends on modification of neural circuits and in particular the generation of multi-innervated dendritic spines (MIS- Figure 1) (Radwanska et al., 2011, PNAS, 108 (45) 18471–18475). The objectives of this study were to demonstrate the importance of MIS for the persistence of contextual memory in old, but not young, mice.

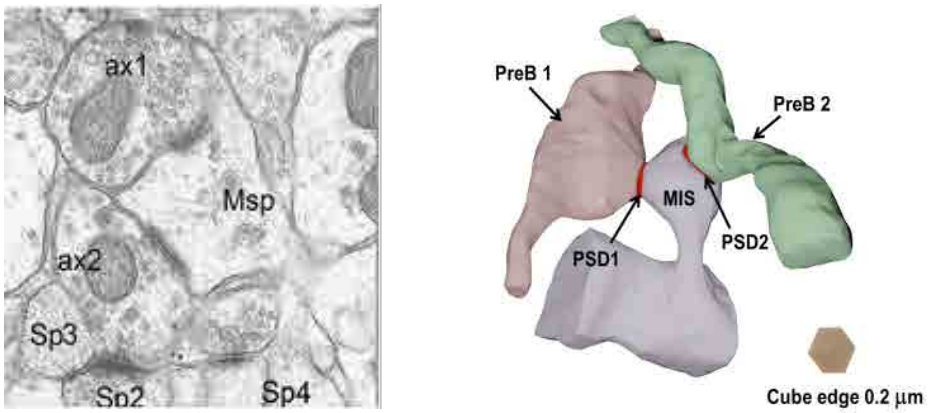

Fig. 1. *MIS – multi-innervated spines Left- Transmission EM with multi-innervated spine (Msp or MIS) contacted by 2 synapses, from axon 1 (ax1) and ax2–. Right- 3D reconstruction from serial EM sections of multi-innervated spine (MIS) contacted by 2 presynaptic boutons (PreB,) from 2 axons; PSD, postsynaptic density.*

Methods

We have analysed modification of neural circuits and in particular the generation of multi-innervated dendritic spines in the hippocampus before and after contextual fear conditioning (CFC) in wild-type C57BL/6J female mice: young adult (3 months): (i) naïve and (ii) 24 hours after CFC; and in aged mice (18 months): (i) naïve, (ii) 2 hours and (iii) 24 hours after CFC. 3-D reconstructions from serial ultrathin sections were performed for quantitative ultrastructural analyses of structural changes in dendritic spines and post-synaptic densities in hippocampal CA1 stratum radiatum. Two MIS types were analysed: excitatory, with more than one excitatory input from different axons; and dual, which have one excitatory and one inhibitory contact.

Results and conclusions

Mushroom spine number increases significantly after training in young but not aged mice. There is a greater overlap between classes of thin and mushroom spines in young mice after training suggesting a maturation process has occurred, with a transition from thin to mushroom spines. In contrast, aged mice show a separation between frequency classes of thin and mushroom spines where there is an increase in the number of large thin spines not yet transformed into mushroom spines. MIS analyses with excitatory synapse contacts shows that the number of MIS is significantly higher in aged naïve mice compared to young naïve mice. Moreover, training induces a significant increase in MIS number in aged but not young mice. Inhibitory synapse analyses show that both aged and young mice have an increased percentage of inhibitory synapses after training, due to an increase in number of dual MIS (excitatory + inhibitory), but not shaft inhibitory synapses.

Morphological Changes in Synapses During Memory Formation in Ageing

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Long-term memory formation is related to functional strengthening of synapses. If the functional strengthening is impaired, hippocampus-dependent memory formation depends on modification of neural circuits and in particular the generation of multi-innervated dendritic spines (MIS) (Radwanska et al., 2011). Aged-related memory decline is associated with synaptic strengthening impairment. Memory formation in older animals is slower and less flexible in comparison to younger animals.

Aims

Our overall aim is to test the hypothesis that in old age hippocampus-dependent memory formation depends on modification of neural circuits in the hippocampus and specifically the generation of multi-innervated dendritic spines (MIS).

Methods

We have analysed MIS generation in the hippocampus before and after contextual fear conditioning (CFC) in the following wild-type groups of C57BL6N female mice:

- young adult (n=6, 3 months): (i) naïve and (ii) 24 hours after CFC;
- aged (n=4, 18 months): (i) naïve, (ii) 2 hours and (iii) 24 hours after CFC.

Mice were perfused intracardially and brain slices taken for electron microscopy, 3D reconstructions from serial ultrathin sections were performed to allow quantitative analyses of structural changes in dendritic spines and post-synaptic densities in hippocampal CA1 stratum radiatum. Two MIS types were analysed: excitatory, which has more than one excitatory input from different axons; and dual, has one excitatory and one inhibitory contact (see Fig.1).

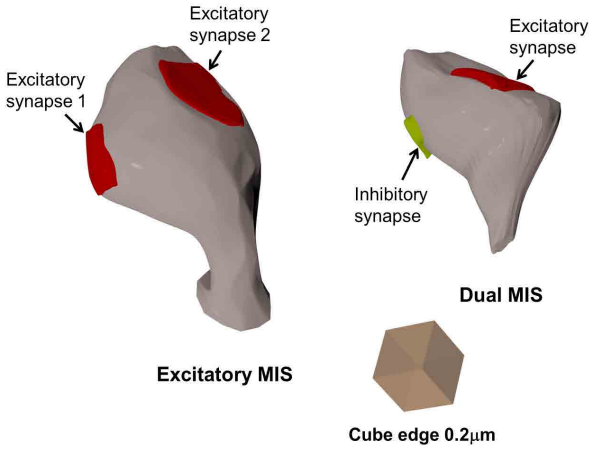

Fig.1. *An example of an excitatory and dual multi-innervated spine reconstructed from serial ultrathin sections*

Results

Our electron microscopy analysis showed no change in total synaptic density between young/aged before/after training. Young Trained 24h group of mice had an increased proportion of mushroom spines compared to the Young Untrained showing synapse maturation after memory formation. However there was no difference in distribution of spines types for Aged Trained 24h compared to Aged Untrained confirming synaptic strengthening impairment with aging. Excitatory MIS analysis showed that the number of MIS was significantly higher in Aged Untrained mice compared to Young Untrained. Moreover, training induced a significant increase in MIS number in aged but not young mice. Analysis of Dual MIS showed its significant up-regulation in Young Trained 24h group of mice but not Aged Trained.

Conclusions

Our results showed that normal synaptic plasticity dependant mechanisms of memory formation are indeed lacking at synaptic level in aged mice. This suggests the ground-breaking hypothesis that different synaptic mechanisms maintain contextual memory in young and old age.

Signal Transduction Underlying Structural Plasticity of Dendritic Spines

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Activity-dependent changes in synaptic strength and structure are believed to be cellular basis of learning and memory. A cascade of biochemical reaction in dendritic spines, tiny postsynaptic compartments emanating from dendritic surface, underlies diverse forms of synaptic plasticity. The reaction in dendritic spines is mediated via signaling networks consist of hundreds of species of proteins. We have developed unique optical techniques to elucidate the operation principles of such signaling networks. First, based on 2-photon fluorescence lifetime imaging and highly sensitive biosensors, we have developed techniques to image signaling activity in single dendritic spines. We have succeeded in monitoring activity of several key signaling proteins in single spines undergoing structural and functional plasticity. This provided new insights into how the spatiotemporal dynamics of signaling are organized during synaptic plasticity. In particular, we have recently developed sensitive and specific protein kinase C isozymes (α, β, γ, δ etc) and a receptor tyrosine kinase TrkB signaling sensors. In addition, we have developed an optogenetic inhibitor for calmodulin-dependent kinase. This inhibitor allows us to manipulate activity of with the temporal resolution of seconds in vitro and in vivo. By monitoring and manipulating signaling components with high spatiotemporal resolution, we expect to reveal the mechanisms underlying the spatiotemporal regulation of signaling dynamics underlying synaptic plasticity and learning and memory.