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.

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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.

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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.,