Summary

CA1 pyramidal neurons have tens of thousands of excitatory and inhibitory synaptic connection with other neurons and the integration of these thousands of inputs determinate the electrical activity of the certain neuron. Through the synaptic connections the information transfer are mainly mediated by two neurotransmitters: glutamate and γ-amino-butyric acid (GABA). The excitatory (glutamatergic) synaptic inputs are widely spread out on several hundreds of micrometers on the dendritic arbor, meanwhile the action potential output localized at the proximal site of the axon. These excitatory synaptic transmissions are conveyed by glutamate activated ionotropic channels (iGluR) α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate (AMPA), *N*-methyl-D-aspartate (NMDA) and kainite (KA), which are named for the agonists that selectively stimulate them, located at the postsynaptic site and activated by the presynaptically released glutamate. AMPA receptor mediated glutamate currents are responsible for the rapid information transfer between neurons, whereas the NMDA receptors have a detector function for specific patterns of activity which can induce long-term changes in the synaptic strength by modulating the AMPA responses.

AMPA receptors are tetrameric or pentameric ionotropic channels, built from subunits GluR1-4. These subunits can form certain type of heteromeric or homomeric channels. Recently, several details of transport and insertion of different types of heteromeric AMPA receptor have become evident including the different physiological roles of different subunits determined mostly by their C-termini, where different phosphorylation and transport protein binding sites are located. Recent studies together strongly suggest that different subunit combinations of AMPA receptors are differentially involved in the three types of plasticity (I. Homeostatic, II. Hebbian-type and III. Distance-Dependent-Synaptic-Scaling) and offer a great opportunity to investigate them by manipulation of the receptor subunits.

I. Homeostatic plasticity provides network and cellular stability, globally optimizes synaptic connections and regulates excitability, adjusting gain of the neurons in dynamically changing environment

II. Hebbian type plasticity /LTP, LTD, etc/ produces associative changes of individual synaptic strength, progressively modifies network properties

increasing synaptic weight differences between synapse (depending on synaptic event history). It is believed that it is crucial in computational tasks, learning and memory.

III. Distance dependent synaptic scaling (DDSS) adjusts the weight of every synapse to make them equally capable to have same impact on output regardless of their distance. Distance-dependent synaptic scaling (DDSS) shares many similar features with the previously distinguished, two major forms of synaptic plasticity: homeostatic and Hebbian type. Like homeostatic plasticity, DDSS globally optimizes synaptic connections. The major target of all three forms of plasticity is the alteration of the level of synaptic transmission by changing the number and/or properties of postsynaptic glutamate receptors Homeostatic plasticity globally alters the number of synaptic AMPA receptors by changing their half-life over minutes or hours/days. Hebbian plasticity can change AMPA receptor number within minute. The DDSS also works through increasing the number of AMPA receptors but proportional to the distance from the soma.

Previous works suggested a strong relationship between different forms of synaptic plasticity and the density/properties of synaptically active glutamatergic, GABAergic receptors.

The last few years, we have focused on the DDSS and the Hebbian-type plasticity, to determine the underlying mechanism of these two plasticity forms. We have directly examined single synaptic transmission before and after different electrical/molecular manipulations in control and genetically modified mice avoiding the spatial and temporal influence of other synapses and/or anatomically uneven dendritic conductance differences that distort conventional somatic recordings. To characterize biophysical properties such as single-channel conductance, open probability, kinetic features and agonist affinity of glutamate-activated channels we used outside-out patches excised from dendrites. This provided insight into specific alterations of excitation in chemically and genetically manipulated rats and mice.

At first, in our investigation I focused on the apical dendrites of CA1 pyramidal neurons, innervated by the Schaffer collaterals coming from the CA3 neurons. The Schaffer collateral pathway provides hippocampal CA1 pyramidal cells with a fairly homogeneous excitatory synaptic input that is spread out across several hundred microns of their apical dendritic arborizations. A progressive increase in synaptic conductance with distance from the soma has been reported to reduce the location-dependence that should result from this arrangement. By excising outside-out patches from apical dendrite and rapidly applying glutamate to activate AMPA and NMDA channels, I have characterized the amplitude, agonist affinity, kinetics and single channel properties of glutamate receptor-mediated currents, and compared these properties on patches excising from different locations from the apical dendrites of CA1 pyramidal neurons. The main finding is that the mean amplitude of the AMPA current at least doubles with distance from the soma. This distance-dependent increase in AMPA receptor current could be the result of an increased receptor number, receptor density or some modification of receptor properties. Modified subunit composition or different phosphorylation states can change the kinetic properties, ionic permeability, agonist affinity, current-voltage relationship, single-channel conductance and maximum open-probability of AMPA channels. We examined all of these receptor properties and compared them with distance from the soma, and found no significant differences in any of them, while on the other hand, channel number increased approximately two-fold. These data then strongly suggest that the distance dependent increase in AMPA current is due to a progressive increase in number of AMPAR in the patches and not to alterations in the basic properties of the receptor/channels.

In the second part of our investigation, we have characterized the properties of dendritic AMPA receptors, spontaneous synaptic currents and postsynaptic AMPA receptor responsiveness in hippocampal CA1 pyramidal neurons from both wild type and GluR1 -/- mice. The AMPA receptor currents from outside-out patches pulled from apical dendrites of GluR1 -/- mice are severely reduced in amplitude. The currents from the KO mice also appear to decay faster and have a lower probability of opening than WT currents. Spontaneous synaptic currents are also smaller in amplitude in the KO mice and the degree of this reduction is dependent on the dendritic location of the synapse, with

distal synapses showing the greatest reduction. Statistical analyses of the synaptic currents indicate that the distal SC synapses of KO mice lack a normal increase in postsynaptic responsiveness, and focal application of glutamate onto postsynaptic spines confirms this scenario. We interpret these data to indicate that the extra-synaptic pool of AMPA receptors is almost entirely composed of GluR1-containing AMPA receptors (probably GluR1/2 heteromers) and that distance-dependent scaling of SC synaptic weight is the result of an increased delivery of these receptors to distal synapses. Furthermore, this regulated delivery probably involves receptor cycling of AMPA receptors between the synaptic and extra-synaptic pools.

The third part of our study examined the changes in dendritic AMPA receptors in hippocampal CA1 pyramidal neurons from adult rats that are produced by, or at least coincide with, LTP induction. Tetanus induced potentiation significantly increased the amplitude of AMPA receptor mediated glutamate currents in outside-out patches pulled from the apical dendrites of adult rats. This increase is due to an increase in the number of AMPA receptors in the patches. These currents also appear to decay more slowly and have a slightly higher probability of opening than control or unpotentiated neurons. Other properties of AMPA receptors, such as single-channel conductance, channel rectification and glutamate affinity do not show any alterations following potentiation. Increases in intracellular CaMKII activity mimic the changes in AMPA receptor properties that were observed following tetanus-induced potentiation. The dendritic region affected by the synaptic stimulation is not much greater than the area receiving input and requires the presence of excitatory synapses. We interpret our data to indicate that the stimuli used here produce an increased delivery of AMPA receptors (probably GluR1/2 heteromers) to synaptically-active regions of the apical dendrite without inducing any significant changes in their basic biophysical properties. We suspect that the primarily extra-synaptic receptors sampled by our patches will ultimately arrive at activated PSDs via the regulated process of receptor cycling between the synaptic and extra-synaptic pools of AMPA receptors.

All of our results are compressed into a simplified model, indicating that the changes in number and/or properties of AMPA receptors are the crucial underlying mechanism of different forms of synaptic plasticity, and that certain types of subunit

composed channels are differentially involved. The GluR1 containing AMPA receptors are a basic component of the synaptic pool of glutamate receptors at Schaffer-collateral synapses, particularly at distant synapses. Also GluR1 containing AMPA receptors are the main component of the extra-synaptic AMPA receptor pool. In addition Location-dependent insertion of GluR1 containing AMPA receptors mediates distance-dependent scaling in hippocampal CA1 pyramidal neurons. Together these data suggest that the highly regulated cycling of AMPA receptors between synaptic and extra-synaptic receptor pools is dependent upon the presence of GluR-1 containing receptors, and furthermore that two functionally diverse forms of synaptic plasticity, LTP and distance-dependent scaling, may both use this cycling system to regulate synaptic strength. Also, the lack of GluR1 subunits in KO animals suggests that every synapse is formed by roughly the same number of GluR2/3 subunits regardless of distance from soma. This observation indirectly suggests, that GluR1 containing receptors are added to ˝top-up˝ synapses depending on their distance from the soma. On the top of these distance scaled synapses, more GluR1 containing receptor can be added during long-term potentiation. It is certain that, these plasticity forms have an affect on each other and do not work separately to maintain the homeostasis of the neuronal network and retain the ability to react properly in a dynamically changing environment.