Review of thesis of Dr. Karri Lamsa for the title of the Doctor of the Hungarian Academy of Sciences:

The thesis for the title of Doctor of Sciences of the Hungarian Academy of Sciences submitted by Karri Lamsa is based on five papers published between 2005 and 2016 in most prestigious journals. Papers were thoroughly reviewed by several reviewers before acceptance and publishing. This fact makes easier my role in reviewing the thesis on the one hand, however it lefts little space for the present reviewer on the other hand.
The doctoral thesis of Dr. Karri Lamsa summarizes his research made on the learning-related cortical GABAergic interneuron plasticity specifically in the excitatory synaptic input to these neurons. Experiments were performed on rodent and human brain neurons especially on hippocampal slice preparations (cut made between CA1 and CA3 regions) and neocortical cells. Human neocortex tissue samples resected in deep brain surgery were used to investigate basic function of human interneurons.

In order to perform the experiments sophisticated and complex methodological approach is applied throughout the experiments. Somatic perforated and double or triple whole cell patch clamp is used for current and voltage recordings. Anatomical characterization of the investigated neurons was performed in immunofluorescence, electron microscopic, confocal microscopic experiments. My first question is related to the applied perforated patch: The perforated patch feature is that electrical access to the cell interior is obtained through inclusion of pore-forming antibiotic molecules for example nystatin or amphotericin B in the patch area of membrane in contact with the patch pipette. Gramicidin used by the author shares the same basic principle with previously used methods of perforated patch recording, namely formation of channels selective to small ions and non-electrolytes, in addition; however, gramicidin channels lack of chloride permeability. It is well known however that upon activation, the GABAA receptor selectively conducts Cl^- ions through its pore so my question is that gramicidin would not interfere with GABA activated channels someway?

Normally QX-314 has no effect on Na-current when applied extracellularly but does induce block when applied intracellularly. Earlier it was however observed that intracellularly applied QX-314 decreases also Ca^{2+} currents in acutely isolated CA1 pyramidal cells from rat hippocampus. It was observed that in neurons dialyzed with 10 mM QX-314 (bromide

salt), the amplitude of the high-threshold Ca2+ current decreased by on average 80% and the current-voltage relationship was shifted right on along the voltage axis. Several findings support the possible role of Ca2+ ions in post-tetanic potentiation or depression, as well. It is supposed that the most important for the induction of LTP may be changes induced in the postsynaptic structures by Ca2+ ions entering through ligand-gated channels opened by the action of synaptic transmitters. For example glutamate-activated channels, which dominate in the nervous system, especially channels of an NMDA type, whose Ca2+ conductivity is about 70 times higher than those of other ligand-gated channels. It is also demonstrated that injections of Ca2+ chelators into postsynaptic structures diminish or completely remove LTP. So my question is in relation to the inclusion of QX-314 into the intracellular solution: why was it necessary to include this lidocaine derivate into the intracellular pipette solution both in perforated patch and whole cell experiments since it may interfere with the Ca-signals in the postsynaptic cell?

The synaptic plasticity is one of the important bases of learning and memory. As memories are thought to be encoded by modification of synaptic strength, LTP and LTD are widely considered as major cellular mechanisms that underlies learning and memory. In elegant electrophysiological experiments Dr.Lamsa convincingly demonstrated that GABAergic interneurons exhibit several form of long term synaptic plasticity, which could be specific in anatomically distinct cortical interneuron types, when cytoplasmic integrity is preserved. In stratum radiatum interneurons the pairing-evoked NMDA mediated LTP earlier was not observed probably as a consequence of the run-down effect of the EPSPs when whole cell patch-clamp was used. In general the input-output relationship of neuronal networks depends both on their synaptic connectivity and on the intrinsic properties of their neuronal elements. In addition to altered synaptic properties, profound changes in intrinsic neuronal properties are also observed. These changes reflect alterations in the functional properties of dendritic and somatic voltage- and Ca2+-gated ion channels. The molecular mechanisms underlying this intrinsic plasticity are complex and comprise the highly specific transcriptional or post-transcriptional regulation of ion-channel expression, trafficking and function. Earlier and recent studies suggest that intrinsic plasticity, in conjunction with synaptic plasticity, can fundamentally alter the input-output properties of neuronal networks in CNS. Studies in invertebrate model systems—such as the lobsters and crabs, and neurons of molluscs—have provided insights into how the intrinsic electrical properties of neurons shape network activity

and animal behaviour. It is observed that an intracellular injection of electric current via micropipettes is able to modify the pacemaker activity both in intact and completely isolated neurons emphasising the importance of intrinsic plasticity of a neurons and probably even the ionic channels. The experiments made on the completely isolated neuronal soma demonstrated the processes of habituation and adaptation to the injected anionic and cationic current. In addition some neurons can participate simultaneously in more than a single network, and the properties of a network may be modulated by the actions of neurotransmitters and hormones. Changes in the intrinsic excitability of a single command neuron or command systems of neurons can trigger a complicated and long-lasting behaviour. My question is: is it possible that the intrinsic properties of neurons building up neuronal microcircuits significantly contribute to the observed neuronal activity? Furthermore, non-synaptic plasticity is a form of neuroplasticity that involves modification of ion channel function in the axon, dendrites, and cell body that results in specific changes in the integration of postsynaptic potentials (EPSPs, IPSPs). It interacts with synaptic plasticity, but it is considered a separate entity from synaptic plasticity. Intrinsic modification of the electrical properties of neurons plays a role in many aspects of plasticity from homeostatic plasticity to learning and memory itself. Non-synaptic plasticity affects synaptic integration, subthreshold propagation, spike generation, and other fundamental mechanisms of neurons at the cellular level. These individual neuronal alterations can result changes in higher brain function, especially learning and memory. Could you comment the possible contribution of intrinsic plasticity of individual neurons and the non-synaptic release of neurotransmitters?

It is also described by the author that NMDA receptor mediated EPSPs are recovered when whole-cell patch clamp recording is terminated. What was the time window for recovery? I guess that the recovery process in not unlimited it may depend on the duration of the whole-cell recording.

Neuropeptides are out of scope of this study but still may I ask your opinion or comment about the possible contribution of low molecular neuropeptides to the neuronal plasticity in investigated neuronal circuits?

Finally I would like to summarize original results obtained by Dr.Lamsa:

- The learning-related cortical GABAergic interneuron plasticity is cell specific.

-It is observed that GABAergic interneuron plasticity is able to elicit long lasting changes in local neuronal network.

- Cellular mechanisms that regulate the accuracy of timing of action potentials, both within a network and in different parts of a neuron, are important for the ability of a neuron to modify the strength of the connections it makes with other neurons.

-In experiments using extracellular recording it is demonstrated that PV+ and NOS+ cells can generate either LTP or LTD and the observed effect may be the result of „different activation of receptors and molecular pathways by electrical stimulation".

- It is shown that GABAergic inhibitory circuits in human neocortex express specific functional features typical for the human brain. Such specific feature is the occurrence of very large synaptic potential activated with single action potential and the reorganization capability of a neuronal microcircuit.

The efforts of Dr. Lamsa made in studying neuronal microcircuits are highly appreciated and acknowledged. Conclusions made in the thesis are based on original data and genuine experimental data. The doctoral thesis is proposed for open discussion. Based on previous and recent achievements presented in his thesis the title of doctor of the Hungarian Academy of Sciences for Karri Lamsa is strongly supported.

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