**Introduction**

The hippocampus has long been recognized as a key region for the formation and long-term storage of memories. The hippocampus can be grossly divided into three main sub regions: the dentate gyrus (DG) and Cornu Amonis 1 and 3 (CA1 and CA3 respectively). The general information-flow through the hippocampus according to the simplified ‘trisynaptic model’ is the following: The entorhinal cortex (EC) relays to the hippocampus converging inputs from all the cortical association areas via the fiber-tract known as the perforant-path. The perforant path mainly innervates the first station of the hippocampus-proper –the granular cells of the DG sub region. The DG granular cells then send their main output to the CA3 sub region via unmyelinated fibers, known as the mossy-fibers (MF), which terminate with large and complex synapses onto CA3 pyramidal-cells (Hereafter MF-synapses).Next, The CA3 sub region relays the information to the CA1 via the Schaffer collateral, and finally the CA1 projects back onto the EC, and this way closes the loop between the hippocampus and the neocortex.

The MF-synapse is unique among hippocampal synapses for its very low basal release probability (Pr) which provides it with a very dominant short-term plasticity (STP) that manifests as a robust frequency-dependent facilitation ([Salin et al., 1996](#_ENREF_33)). Due to its low basal Pr, the MF-synapse responds with the release of very few synaptic vesicles, if any, to the presentation of single action-potentials (APs) (Mori et al., 2007). However, when presented with high-frequency trains of APs, the MF-synapse reveal its full potential as it undergoes dramatic facilitation that can cause the firing of action-potentials in the target CA3 neuron (Nicoll & Schmitz, 2005). Because of this unique ability of a single MF-synapse to induce spiking in its post-synaptic target, the MF-synapse is sometimes considered to be a ‘detonator synapse’ (Brown &Zador, 1986; McNaughton & Morris, 1987).

The high facilitatory nature of the MF-synapse makes it a very efficient high-pass filter of information transfer, which allows it to propagate its incoming inputs only if they are compressed in bursts of high-frequency activity (Henze et al., 2002). The DG granular cells are also known for their low activity and their sparse connectivity to the CA3 pyramidal cells. The low activity is reflected in the facts that the DG granular cells have low endogenous firing rates (approx. 0.1 Hz; Ylinen et al., 1995; Jung &McNaughton, 1993), and less than 3% of all granule cells are active in a given testing arena (Chawla et al., 2005). The sparse connectivity is expressed in the fact that a single granule cell forms only one synapse with each CA3 pyramidal cells and it innervates relatively low number of CA3 neurons (up to 14 CA3 neurons; Amaral et al., 1990; Rolls, 2013). Such a combination of traits; low activity, sparse connectivity and dominant frequency-dependent facilitation, led computational theorists to hypothesize an important role for the MF pathway in ‘pattern separation’ - a mnemonic process that allows to discern between similar, yet distinct environments (Yassa & Stark, 2011). Indeed, some studies provided support for this theory by showing that lesions and perturbations of the DG and its efferents led to reduced ability of the inflicted animals to distinguish between similar contexts (Gilbert et al., 2001; Morris et al., 2012).

In addition to these unique properties, the MF-synapse also exhibits a special form of pre-synaptic long-term potentiation (LTP) which forms in response to prolonged activity of the synapse at high frequencies and manifests as a sustained increase in Pr. In a seminal study that involved both modeling and experimental approaches, it was shown that synaptic increase in Pr (possibly like the one apparent after MF-LTP) is not translated into a uniform amplification of post-synaptic responses to a given pre-synaptic train of stimuli. Rather it causes a redistribution of post-synaptic responses relative to one another, which actually reflects the change in neurotransmitter-release pattern during a train without changing the overall neurotransmitter release (Tsodyks & Markram, 1997). In the case of the MF-synapse, the redistribution of neurotransmitter release, as a result of LTP, is manifested as a decrease in its synaptic facilitation (Gundlfinger et al., 2007). The implications of such decrease in synaptic facilitation to the high-pass filtering properties of the MF-synapse and on its ability to support pattern-separation are currently unknown. Therefore, one of our aims will be to determine the effects of LTP on information transfer in this synapse.

As opposed to the associative NMDA-dependent LTP, which is the most-studied form of LTP and is generally considered to be expressed post-synaptically, MF-LTP does not require any coordinated post-synaptic activity for its induction (Zalutsky & Nicoll, 1990; Tong, Malenka & Nicoll, 1996; Langdon, Johnson & Barrionuevo, 1995; but see Jaffe & Johnston, 1990). The fact that the MF-LTP is non-associative might suggest that it has alternative physiological relevance to memory storage processes than those related to classical NMDA-dependent LTP. In support for alternative role of MF-LTP are the findings that knock-out mice in which MF-LTP and LTD are impaired show no deficits in learning tasks (Huang et al., 1995; Yokoi et al., 1996). Another support for this idea comes from a recent study in which the researchers provided evidence that homeostatic plasticity processes are prevalent and centered in the MF-synapse in response to pharmacological perturbations to network activity. In-light of these observations, the authors suggested a new role for the MF-synapse as a gain-control device that helps keeping excitation levels in the hippocampal circuitry in a certain physiological range (Lee et al., 2013).

The aforementioned unique features of the MF-synapse, together with its special form of presynaptic LTP, raise some fascinating questions regarding the complex relationship between short-term and long-term plasticity in the MF-synapse. On the one hand, LTP raises the Pr of MF-synapses and thus makes them more responsive to single APs and to low frequency inputs in general. On the other hand, according to the notion that MF-LTP is pre-synaptic in origin and thus leads to redistribution of neurotransmitter release pattern without changing the total neurotransmitter release (Tsodyks & Markram, 1997), it is predicted that LTP, by means of reducing the facilitation, will also reduce the late responses to a train of stimuli (e.g. the fourth and fifth responses to a burst of 5 APs). Together these two modifications imply that following induction of LTP, STP is affected in a way that somewhat counteracts its ability to act as a high-pass filter, a feature that is considered to be important for its ability to support pattern-separation. Such dual effects involved in presynaptic forms of LTP are interesting because they suggest that in-order to understand these kind of plasticity processes it is necessary to depart from the perspectives usually used to understand the implications of more classical forms of LTP (i.e. NMDA-dependent, associative LTP that increase information transfer at a given synapse). Therefore, we suggest that full understanding of the implications of plasticity changes of the MF-synapse could only be achieved when these processes are studied in the context of the synapse’s hypothesized specialization as a high-pass filter synapse. As a first step to resolve this discrepancy, we propose here to characterize the changes in STP of the MF-synapses as a result of MF-LTP, and their effects on the network information transfer through the DG-CA3 network.

**Major aims**

**Aim #1**

What are the effects of MF-LTP on information processing performed by the DG-CA3 subnetwork?

Several synapses are involved in the computations performed by the DG-CA3 subnetwork. Among them are the MF-CA3 synapse and the MF-IN synapse, both of them exhibit dominant STP properties (marked facilitation and depression, respectively; Ref).In-addition, both synapse types were shown to undergo pre-synaptic LTP when presented with high-frequency stimulations. Under the assumption that the STP properties of these synapses are important for the computations performed in the DG-CA3 network, it will be of high relevance to examine how LTP induction affects the STP of these synapses, and how in turn these effects might alter information processing in the network.

Specifically we will examine the effects of LTP induction on action-potential propagation of CA3 pyramidal cells following MF activation using burst of various stimulation frequencies. This we believe will provide indication of how the output of the DG-CA3 subnetwork changes in response to MF-LTP. Next, we will dissect the contribution of the DG-CA3 subnetwork components and examine how STP changes in both the MF-CA3 synapse and in the MF-IN synapse as a result of LTP, and how these changes jointly affect the excitation/inhibition balance on the CA3 neuron.

We plan to achieve these goals by combining electrophysiological measurements together with pharmacological and genetic manipulations of defined components of the DG-CA3 subnetwork.

**Current-clamp measurements of suprathreshold activity before and after MF-LTP induction:**

For the first set of trials we will record CA3 responses to a train of electrical MF-stimulation using various frequency protocols. After obtaining baseline responses to these trains of stimuli, we will introduce high-frequency stimulation (HFS; two 5 seconds bursts of 25 Hz, with inter-burst interval of 10 seconds) in-order to induce MF-LTP, in the presence of the NMDA-blocker – AP-V, to prevent NMDA-dependent LTP from occurring at other hippocampal synapses. Then we will estimate how the filtering properties of the synapse have changed in terms of the post-synaptic responses to train of stimuli of different frequencies. In-order to measure changes in supra-threshold activity of CA3 neurons in response to MF-LTP, we will use current-clamp measurements will allow us to measure the supra-threshold activity of CA3 pyramidal-cells before and after MF-LTP under different stimulation conditions, and this way to determine the effects of MF-LTP on information transfer through the DG-CA3 subnetwork. In-addition, we will utilize the approach used in a recent study by Milstein et al., (2015), in which the researchers measured EPSPs of different cell-types of the CA1 subnetwork in response to a train of three-pulses at different frequencies, and calculated the extent to which the summation of the EPSPs is supralinear. This way the researchers could assess whether supralinear summation appears when cells are stimulated with high-frequencies or low-frequencies and according to this property they could determine the filter properties of different components of the CA1 subnetwork (Milstein et al., 2015). When experiments are done in the presence of GABA blockers, and.

For this reason taking into account the latencies of the responses into account will be essential in these experiments. Minimal stimulation protocol.

**Outcome**

**Characterization of synaptic summation of inhibitory and excitatory currents before and after MF-LTP induction:**

**Voltage-clamp measurements of inhibitory and excitatory currents before and after MF-LTP induction:**

In-order to more accurately dissect the relative contributions of the different components of the DG-CA3 subnetwork,

Because of its pre-synaptic origin, MF-LTP is expected to cause a redistribution of neurotransmitter release without changing the overall neurotransmitter-release in response to a train of stimuli. To empirically test this hypothesis, we will use voltage-clamp measurements of CA3-pyramidal cells and measure their EPSCs in response to trains of stimuli. If MF-LTP indeed only changes the distribution of EPSCs without changing the total charge transfer, we expect to see that the total charge transfer after LTP is equivalent to the total charge-transfer before LTP.

In-order to further elucidate the contribution of the inhibitory network we will also record Inhibitory post-synaptic currents (IPSCs) clamping the neurons on depolarized voltages that will emphasize the IPSCs on the expense of the EPSCs

**Potential pitfalls:**

**Outcome:**

**Aim #2**

Under what conditions MF-LTP develops?

Conventional LTP protocols involve the simultaneous activation of numerous MF-synapses; some of them converge on the same CA3-PCs. This situation is capable of creating glutamate spillover that can activate glutamate auto-receptors which can be part of the mechanism underlying MF-LTP. Consistently, several studies have provided evidence that MF-LTP is dependent on this kind of glutamate auto-receptors such as metabotropic glutamate receptors (mGluRs; Ref). However, under physiological conditions, the probability of a simultaneous activation of multiple converging synapses is very low when taking into account the low activity of DG granular-cells and their sparse connectivity to CA3-PCs.

This leads us to ask the following questions: first, what role do glutamate spillover play in the induction of MF-LTP? Second, if glutamate spillover contributes to the development of MF-LTP, can it be induced when only a single synapse per CA3 neuron is activated, as it likely occurs under physiological conditions?

**Aim #3**

Does MF-LTP leads to homeostatic adaptation in the DG-CA3 network?

Physiological and morphological homeostatic adaptations were shown to be prevalent in MF-CA3 synapses in response to prolonged pharmacological perturbations(Lee et al., 2013). Will similar adaptations be observed in response to alterations in network-activity that result from more natural neuronal processes, such as MF-LTP?