Because only the supra-threshold activity (i.e. the spiking activity) of the CA3 pyramidal cells is eventually transferred to the next stage of the trisynaptic pathway (i.e. the CA1 sub region), it will be of high-relevance to assess how supra-threshold activity of CA3 pyramidal cells is affected by MF-LTP. For this purpose we will use whole-cell recordings in current-clamp configuration of CA3-pyramidal-cells, combined with electrical MF-stimulation of various frequencies and intensities. Control over the intensities and frequencies of the MF-stimulation enables us to induce different patterns of spiking activity in the CA3 pyramidal-cells with great reliability (e.g. it is possible to give a five-pulse train of a certain frequency and intensity that will cause the spiking response only to the fifth pulse; We can attach here a recording that shows that). After obtaining baseline responses to trains of stimuli that induce different spiking patterns, 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. Subsequent comparison between evoked spiking activity before and after MF-LTP will be made.

**Outcome:**

This approach will allow us to characterize the evoked supra-threshold activity of CA3 pyramidal cells, and how MF-LTP, by means of changing the distribution of neurotransmitter-release, changes the pattern of supra-threshold activity, and thus will also lead to a greater understanding of the way MF-LTP alters information-transfer in the hippocampal trisynaptic pathway. However, this set of experiments alone does not provide mechanistic explanations for any changes that will be observed, and therefore the next set of experiments is aimed to elucidate the mechanism behind our observations.

The first obstacle will be overcome by assuring that the latencies of the MF-responses are consistent with mono-synaptic time scales (approx. 5 milli-seconds).

The second obstacle is less due to the stochastic nature of the supra-threshold spontaneous activity, it is anticipated to average out. In-addition, during data analysis we will ignore APs that are not time-locked to the to the stimulations pulses.

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

In-order to better understand how MF-LTP leads to changes in the filtering properties of the MF-synapse, and to gain further insight into the mechanism underlying the changes in information transfer, we intend to conduct a series of experiments inspired by a recent study by Milstein et al., (2015). In their study, Milstein et al. assessed the filtering properties of different components of the CA1 subnetwork by measuring the temporal summation of synaptic inputs in response to a three-pulse stimulation given in various frequencies. In-order to extract the filtering properties from the data, the researchers compared the synaptic summation observed for a given synapse to the expected summation under simple linear summation. Using this method, the researchers found that many synaptic responses showed supra-linear summation under specific conditions which is indicative of their characteristic filtering properties. According to this approach, when supralinear summation is observed for high-frequency stimulations but not for low-frequency stimulations, it is indicative of high-pass filtering properties (and low-pass filtering properties for the opposite situation). In addition, consistent with the notion that STP contributes to the filtering properties of the synapse, and thus any changes in Pr are predicted to alter its filter properties, the researchers have shown that changes in Pr induced by increasing the extracellular Ca2+ levels, converted the filtering properties of the synapse to a much more low-pass filter. In addition, the researchers measured the synaptic summation both in the presence and in the absence of inhibition, in-order to determine the contribution of the inhibitory network to the filtering properties. In the present study we will use similar approach in-order to study the filtering properties of different components of the DG-CA3 subnetwork, and the changes to these properties that arise as a result of LTP induction. Specifically, we will use current-clamp measurements of CA3 pyramidal-cells (and maybe stratum-lucidum interneurons?) while inducing subthreshold EPSPs by MF-stimulation of various frequencies (using an intracellular QX – a blocker of voltage-gated Na currents to prevent spiking of the measured cell). Next, we will produce MF-LTP using electrical HFS, and will measure the changes in the filtering properties of the synapses, and their correlation to alterations of STP. We will then repeat these experiments in the presence of GABA inhibitors which will enable us to measure the filtering properties of the system when ‘stripped’ from any inhibitory influence, and from this to evaluate the contribution of the inhibitory components.

**Outcome:** We anticipate that this set of experiments will provide a more comprehensive description of the filtering properties of DG-CA3 subnetwork and In particular, we expect to pinpoint how the filtering characteristics of the different components of the DG-CA3 subnetwork are translated into their ability the integrate synaptic inputs.

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

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 (Tsodyks & Markram, 1997). To empirically test this hypothesis, we will use voltage-clamp measurements of CA3-pyramidal cells in the presence of QX in the recording pipette to avoid any supra-threshold activity, and measure their EPSCs in response to trains of stimuli. If MF-LTP indeed only changes the distribution of neurotransmitter release without changing the total neurotransmitter released, we expect to see that the total charge transfer after LTP is equivalent to the total charge-transfer before LTP. Another advantage for voltage-clamp measurements is the ability to measure synaptic responses when the voltage is clamped at different values. When neurons are clamped to depolarized potentials around the reversal-potential of the glutamatergic receptors (around 10 mV), mostly inhibitory responses will be induced.

**Outcome:**

**Aim #1 - Potential pitfalls:** whole-cell measurements of mossy-fibers responses of CA3 cells are known to be technically demanding because of several reasons. First, CA3 pyramidal cells give-rise to elaborate recurrent associational connections that make it difficult to isolate synaptic activity that is induced by MF-activity from any synaptic activity that stems from the activity of other hippocampal pathways, and especially the CA3-CA3 associational connections. Second, the spontaneous activity of the CA3-CA3 recurrent collaterals generates some background spiking activity in the CA3 pyramidal-cells that can mask our evoked- supra-threshold responses.