Gating Neuronal Activity in the Brain – Cellular and Network Processing of Activity in the Perirhinal-entorhinal Cortex

J.G.P. Willems

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The brain is a unique organ which allows us to receive information and store memories about every day events. Information from the outside world is processed and integrated by several cortical areas. This information can be modulated by other areas such as the amygdala, a brain area involved in emotion, before the memory is stored in the cortex.

The parahippocampal cortex is an integration area which receives information from various brain areas and is involved in memory processes. This information is combined and sent to the hippocampus for further processing and processed information is then projected back to the cortex for memory storage. The parahippocampal cortex consists of various regions which together form the input and output structure of the hippocampus. The lateral (LEC) and medial (MEC) entorhinal cortex are positioned adjacent to the hippocampus and these two entorhinal regions are bordered by the perirhinal (PER) and postrhinal cortex, respectively. The PER and LEC form a gate between the (sub)cortex and the hippocampus and are involved in memory, object information, attention, and motivation processing. These structures are damaged early in the development of for example Alzheimer's disease or epilepsy, which manifests as impaired performance on these functions in early stages of the disease.

Much information about the anatomy of the PER-LEC network is revealed by anatomical studies in the last century. Neurons in the cortex preferentially project to the superficial layers of the PER-LEC. PER-LEC superficial layer neurons project to the hippocampus, implying that the PER-LEC superficial layers form the input network for the hippocampus. Neuronal projections from the hippocampus target neurons in the deep layers of the PER-LEC network, from where PER-LEC neurons project back to the cortex, proposing the PER-LEC deep layers as the output structure of the hippocampus. Despite the large amount of anatomical knowledge about the PER-LEC gate, it is not yet completely understood how information, in the form of neuronal activity, from various sources is received by the PER-

LEC and how this information interacts and is processed before it is transmitted towards the hippocampus.

An important feature of our memory system is that emotion can influence the strength of memory storage. By emotional enhancement, the memory trace for information which involves an emotional event is better stored and more easily recalled. The amygdala is the brain structure involved in emotional processing and projects to the PER-LEC network. How amygdala activity modulates information from the cortex however, is not yet understood.

The research described in this dissertation offers insight in how transmission of neuronal activity is regulated in the PER-LEC network. We investigated how activity induced in the neocortical agranular insular cortex (AiP) by electrical stimulation is processed in the PER-LEC network and which role excitation and inhibition play in processing this cortical activity in the superficial and deep PER-LEC layers. Additionally, we describe how input from the amygdala interacts with the AiP evoked activity. To answer these questions, we performed experiments in acute horizontal mouse brain slices consisting of an important part of the intact connectivity between the AiP, lateral amygdala (LA), PER, LEC, MEC and the hippocampus. We electrically stimulated the AiP and/or LA and recorded the evoked network activity in the PER-LEC network using voltage sensitive dye imaging. Additionally, we recorded how the evoked activity is received and processed in PER-LEC neurons using the whole-cell patch clamp technique. This *in vitro* approach enabled us to investigate the interaction between AiP and LA input in a controlled setting, to address how interaction of a two single synaptic responses from 2 brain areas is processed in the PER-LEC network. Furthermore, by performing paired recordings of excitatory principal neurons and inhibitory parvalbumin (PV) positive interneurons, we could draw conclusions on how excitation and inhibition regulate activity transfer in the PER-LEC network and how the interaction between these cell types is important for this regulation. Hence we explored the processing of propagating neuronal activity on the cellular and network level.

In chapter 2 we show that stimulation of the AiP as well as the LA can activate the PER-LEC network. However, the spatio-temporal recruitment pattern differs: the AiP activates the PER-LEC superficial layers first, after which the deep layers are activated, whereas the LA mainly evokes activity in the deep layers. When inhibition is reduced, using a low concentration of the competitive $GABA_A$ antagonist bicuculline, AiP-evoked activity spreads

further through the PER-LEC and LA stimulation can, in addition to the deep layers, also recruit the superficial layers. This leads to the conclusion that both the AiP and LA synaptic input can activate the PER-LEC network, but the initial activation site is different. Additionally we conclude that inhibition plays an important role in the transmission of neuronal activity evoked by input from both the AiP and the LA through the PER-LEC network.

In chapter 3 we gain more detail about how synaptic input from the AiP is processed in the PER-LEC deep layers. We recorded how principal neurons and PV interneurons are activated after stimulation of the AiP superficial layers. We show that synaptic input onto principal neurons consists of a small excitatory and large inhibitory component. AiP stimulation strongly excites PV interneurons, which subsequently send a strong inhibition to the principal neurons in the deeper layers. The activation of PV interneurons occurs directly and probably monosynaptically after stimulation, evoking action potentials in the PV interneurons which results in fast inhibition of the postsynaptic principal neurons. We conclude that the excitatory network in the deep PER-LEC layer neurons is actively suppressed by inhibition originating from PV interneurons when the neocortical activity arrives in the PER-LEC network.

In chapter 4 is described how cortical activity from the AiP is transmitted through the PER-LEC network. To compare how synaptic input is processed in different parts of the network, we performed paired recordings in superficial and deep layer principal neurons. AiP stimulation evokes action potential firing in a larger group of principal neurons in the superficial layers, compared to the deep layers. This supports the hypothesis that the activity from the cortex is mainly transmitted towards the hippocampus via the superficial layers of the PER-LEC network. The difference in neuronal activity between the superficial and deep layers can be explained by the different strength of the synaptic input and the timing of the evoked excitation and inhibition, resulting in a larger net excitation in the superficial layers. Paired recordings of superficial principal neurons and PV interneurons show that the timing of the inhibition is very consistent and originates from local network PV interneurons. This solid inhibitory input is overruled when the excitation is large and is received early enough, which is the case in superficial but not deep layer principal neurons.

In chapter 5 we elucidate how LA activity interacts with the synaptic input from the neocortical AiP. Principal neurons as well as PV interneurons receive synaptic input from the AiP and LA, suggesting that these projections share a network in which AiP and LA activity is processed and integrated. How these two inputs interact in principal neurons and PV interneurons is examined by simultaneously stimulating the AiP and the LA. In principal neurons, this interaction mainly takes place in the inhibitory component of the synaptic input. A larger, summated excitatory input in the PV interneurons will make them reach firing threshold at an earlier moment in time, resulting in the earlier arrival of the inhibition in principal neurons. This temporal shift narrows the time window for principal neurons to fire their action potentials which is hypothesized to enhance the precision and synchrony of the network activity.

In this dissertation we increase the knowledge about how the PER-LEC gates neuronal activity. The previously reported anatomical separation of the superficial and deep layers also functionally promotes that cortical activity is transmitted through the PER-LEC superficial layers towards the hippocampus for further processing. It is expected that synaptic input from many other cortical areas is also transmitted via the superficial layers while the deep layers are strongly inhibited, since cortical projections mainly target the superficial layers. These projections target superficial layer neurons on their proximal dendrites and deep layer neuron on their distant apical dendrites. The cortical input onto the deep layer neurons is hypothesized to provide the deep layers with information about the activity travelling towards the hippocampus. Via the activation of deep layer PV interneurons, the output pathway of the hippocampus is blocked when activity is transmitted towards the hippocampus. How the output from the hippocampus is transmitted back towards the cortex via the deep layers remains to be revealed. Studies in related brain areas have shown that for the activity which returns towards the cortex, the reversed phenomenon takes place: the superficial layers are inhibited while the deep layers are active.

It is known that emotion can modulate memory storage and retrieval, which increases the chances for survival. The amygdala, a structure involved in emotion, can reinforce the transmission of activity through the PER-LEC network and herewith facilitate memory formation. In this dissertation, we add a mechanism for modulation of activity by the

amygdala to this conclusion. Although it has long been thought that synaptic input from the LA and cortex mainly interacted on the excitatory synaptic responses, we showed that modulation of inhibition probably plays a crucial role in the regulation of transmission by the LA. The prompter action potential firing of the inhibitory neurons will synchronize the firing of principal neurons. This synchronization, caused emotional modulation, is suggested to ensure information transmission to the projection areas, causing fast, strong and efficient memory formation.