Gating neuronal activity in the brain

Cellular and network processing of propagating activity in the perirhinal–entorhinal cortex

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GENERAL INTRODUCTION

1

The brain is a unique organ which allows us to interpret many sources of information and integrates this information to prepare actions in response. The perceived information is received by various primary brain areas and collected in integration areas. In these integration areas, emotion can for example be incorporated in the information to form a weighted representation of our current state of being.

The parahippocampal region is a brain area which receives and processes information from multiple neuronal structures. More than 100 years after the first explorations of the parahippocampal-hippocampal anatomy by Ramón Y Cajal (Ramón y Cajal, 1893), it is still unclear how this neuronal network integrates information coming from these different brain structures. The research presented in this dissertation tries to tackle the fundamental question how this network integrates information that originates from different brain areas and it proposes a mechanism for information processing by the parahippocampal cortex.

The role of the parahippocampal cortex in cognitive function and behavior

The parahippocampal region is a cortical brain area which is involved in cognitive functions like learning and memory, object information, sensory representation, attention, motivation, and spatial orientation (Aminoff, Kveraga, & Bar, 2013; Bos et al., 2017; Eichenbaum, Sauvage, Fortin, Komorowski, & Lipton, 2012; Kealy & Commins, 2011; van Strien, Cappaert, & Witter, 2009). The parahippocampus is a crucial part for cortico-hippocampal crosstalk, since it forms the gateway between the (sub)cortex and the hippocampal formation (Fernández & Tendolkar, 2006; Keene et al., 2016). The parahippocampal cortex comprises a set of anatomically and cytoarchitectonically distinct regions such as the perihinal (PER), postrhinal (POR), and lateral (LEC) and medial (MEC) division of the entorhinal cortex (Figure 1; Burwell, 2000; Burwell & Witter, 2002; Cappaert, Van Strien, & Witter, 2015; van Strien et al., 2009). While the POR-MEC pathway is mainly involved in spatial information processing (Moser et al., 2014), the PER-LEC pathway processes object information (Knierim, Neunuebel, & Deshmukh, 2014). This dissertation focuses on the interconnected PER-LEC network to understand how cortical input is processed before it is transmitted to the hippocampus.

Anatomy of the PER-LEC network

The PER and LEC are situated adjacent to the hippocampus, aligning the rhinal sulcus in the temporal lobe, and form both the input and output structure for hippocampal activity (Figure 1 A-C; for review see van Strien et al., 2009). To gain more knowledge about activity processing by the PER-LEC, this dissertation focuses on the mouse PER-LEC network. In this animal model we were able to visualize a specific interneuron cell type by using genetically modified animals, allowing us to study the interplay between these interneurons and principal neurons. Since the structure and function of the PER and LEC is very comparable between species (Box 1) we ultimately hope to provide advance understanding about the processing of activity in the PER and LEC in higher order animals.

The PER receives input from neocortical areas such as the sensory, temporal and insular cortical areas and subcortical areas like the amygdala, basal ganglia, (hypo)thalamus, raphe nucleus and olfactory bulb (Kealy & Commins, 2011). The LEC afferents originate from piriform, frontal, insular and temporal areas, including the PER (Burwell & Amaral, 1998a). In

Box 1 | Comparison of rodent, monkey and human PER-LEC network

The anatomical localization of the PER and LEC is highly conserved amongst species. In the rodent, monkey and human brain, the temporal lobe contains, amongst others, the hippocampus with adjacent the EC and PER (Burwell, Witter, & Amaral, 1995; Solodkin, Van Hoesen, & Insausti, 2014). The connectivity between these subregions of the temporal lobe is comparable between the various species (Burwell et al., 1995; Insausti & Amaral, 2008; Suzuki & Amaral, 1994; Witter et al., 2017) and the size, relative to the neocortical volume, has been shown to be alike (Burwell et al., 1995).

The PER-LEC network plays an important role in memory formation and object recognition. Studies have shown that lesions in the PER-EC network resulted in impaired object recognition in rats and monkeys (Mumby & Pinel, 1994) and PER and EC lesions are associated with memory impairment in rats, monkeys and humans (Eichenbaum et al., 2012; Kealy & Commins, 2011; Robin, Rai, Valli, & Olsen, 2018; Solodkin et al., 2014; Suzuki, Zola-Morgan, Squire, & Amaral, 1993; Suzuki & Naya, 2014). Considering these anatomical and functional similarities, the studies performed to elucidate the mechanism for activity transmission in the rodent PER-LEC will provide educated hypotheses for further studies in evolutionary higher species.

turn, PER and LEC axons project to the hippocampal formation (Figure 1; for review see Witter, 1993). This parahippocampal pathway, from the PER to the LEC to the hippocampus and vice versa (Figure 1C), is considered an important gateway for the cortical infromation to flow into the hippocampus and reciprocally back to the cortex (Burwell, 2000; Burwell & Amaral, 1998a; Burwell & Witter, 2002; Cappaert et al., 2015; Ohara et al., 2018).

This PER-LEC gateway is divided into a path in the superficial layers, transmitting activity towards the hippocampus, and one in the deep layers which transfers activity from the hippocampus back to the cortex (Ohara et al., 2018; Witter, Doan, Jacobsen, Nilssen, & Ohara, 2017). Anatomical studies have shown that longitudinal connections from the cortex, via the PER and LEC towards the hippocampus mainly originate in the superficial layers (for review see Witter et al., 2017), whereas the deep layers form a return network of connections (Figure 1 C) (Burwell & Amaral, 1998b; Suzuki & Amaral, 1994; Tamamaki & Nojyo, 1993; Witter, Groenewegen, Lopes da Silva, & Lohman, 1989; Witter, Room, Groenewegen, & Lohman, 1986).

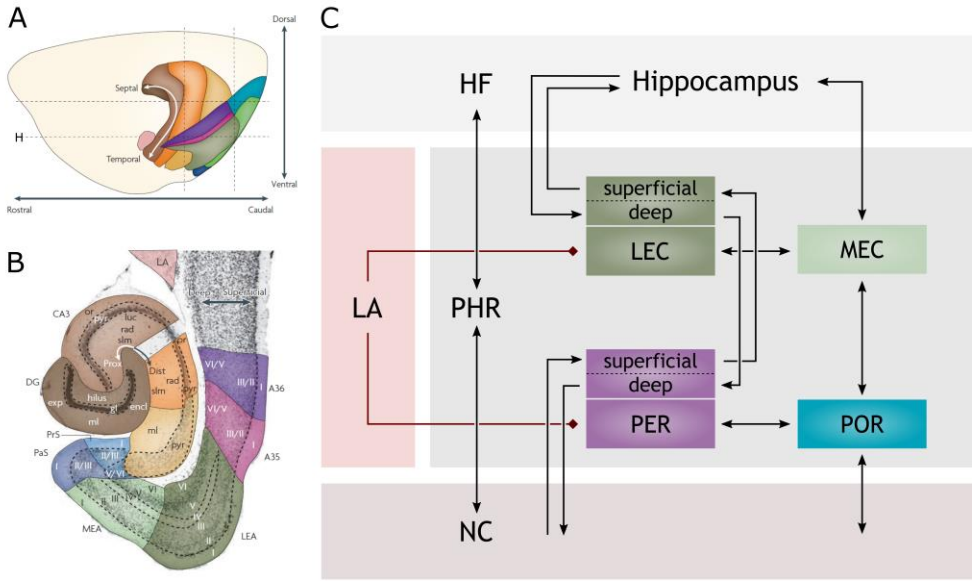

Figure 1. *Anatomy of the parahippocampal network.* **A.** Lateral view of the rodent brain with in red the lateral amygdala, in brown the regions of the hippocampus, in blue the subicular area, in green the entorhinal cortex, in purple the perirhinal cortex and in cyan the postrhinal cortex. **B.** A Nissl stained horizontal section cut at line H indicated in A, with the colors corresponding to the areas in A. **C.** Schematic over view of the connectivity between the neocortex, parahippocampal region and the hippocampus. Arrows indicate the hypothesized connectivity between the different areas. The LA had modulatory projections to the PER and LEC.
Abbreviations: NC, neocortex; LA, lateral amygdala; PHR, parahippocampal region; HF, hippocampal formation; PER, perirhinal cortex; POR, postrhinal cortex; LEC, lateral entorhinal cortex; MEC, medial entorhinal cortex. Adapted from van Strien et al. (2009).

Information processing in the PER-LEC-hippocampal network

The profound connectivity of the cortico-hippocampal-cortical circuit, with the PER-LEC network as an intermediate structure, forms a pathway for information to flow through the network. This information is shaped by a combination of ongoing neuronal activity and neuronal activity evoked by a stimulus, for example a sensory input (Engel, Fries, & Singer, 2001). The information is coded in patterns of action potential firing. Two hypotheses are proposed to explain how neurons encode information: the rate-coding and time-coding hypotheses (Quian Quiroga & Panzeri, 2009). The rate-coding hypothesis states that information is coded in the mean firing rate of the neuron (Rieke & Warland, 1999) and the time-coding hypothesis states that the precise timing of spikes defines the information content of the message (Optican & Richmond, 1987). Both hypotheses are shown to be present in information processing: neuronal activity will consist of a rate-coded component

and a time-coded component. The distinction, however, is relevant for how information is processed and transmitted.

The information, coded in a neuronal spike pattern, is transferred from neuron to neuron via synaptic activation and postsynaptic action potential firing in the involved areas. The action potential firing patterns in the EC are shaped by the input from the (sub)cortical areas and in turn seed hippocampal neuronal activity (Battaglia, Benchenane, Sirota, Pennartz, & Wiener, 2011). Furthermore, temporal synchronization of neuronal firing patterns between brain areas in the form of oscillations will result in binding of information between areas like the PER-LEC network and the hippocampus (Buzsáki & Draguhn, 2004).

Physiology of the PER-LEC gate

Although there are anatomical projections present from the (sub)cortex, through the PER and LEC, to the hippocampal formation (Cappaert, Van Strien, & Witter, 2014), the synaptically evoked neuronal activity in the PER-LEC network does not under all circumstances induce neuronal activity in the hippocampus (Biella, Uva, & de Curtis, 2002; Koganezawa et al., 2008; Pelletier, Apergis-Schoute, & Paré, 2005; Willems, Wadman, & Cappaert, 2016). This suggests that the PER-LEC network, instead of simply acting as a relay station, actively processes and selects information before transmission to the hippocampus (de Curtis & Paré, 2004). The neuronal mechanism behind this gating capability is not understood. It is shown though, that the firing rate of principal neurons in both the PER and LEC decreases when a cortical input is received (Pelletier, Apergis, & Paré, 2004). This decrease is likely due to the activation of inhibitory interneurons in the local network. Reducing the inhibition resulted in a consistent activation of the PER-LEC network by neocortical synaptic input, implying a role for γ-aminobutyric acid (GABA)ergic interneurons in the gating of activity transmission in the PER-LEC network (Koganezawa et al., 2008; Willems et al., 2016).

It is hypothesized that activity travelling towards the hippocampus does so via the superficial layers of the PER-LEC and is transferred back to the cortex by the deep layer network. This hypothesis is supported by physiological data showing that stimulation of the PER superficial layers results in activation of the LEC superficial layers specifically (de Villers-Sidani, Tahvildari, & Alonso, 2004) and superficial EC neuronal firing seeds hippocampal

neuron activity (Battaglia et al., 2011). Additionally, the PER-LEC deep layers are actively inhibited to block the output network of the hippocampus when neocortical information has to be transmitted towards the hippocampus (Biella et al., 2002; Willems, Wadman, & Cappaert, 2018). Hippocampal CA1 and subiculum neurons project back to the LEC deep layers, forming an output structure of the hippocampus (Witter et al., 2017). When the LEC deep layers are stimulated, mainly the PER deep layers show synaptic excitation, allowing hippocampal activity to be transferred via the PER-LEC deep layers back to the cortex (Gnatkovsky & de Curtis, 2006).

Inhibitory control of neuronal activity transmission

The functional separation of the input and output layers of the PER-LEC network comes about by inhibitory control of the firing of excitatory principal neurons. Previous studies showed that when neuronal fibers distal to the neurons in the PER-LEC network were stimulated, the responses were only excitatory, likely evoked by direct excitatory synaptic inputs (Biella, Uva, & Curtis, 2001; Martina, Royer, & Paré, 2001). When a stimulus was applied in the more proximal, local PER-LEC network however, neurons received excitatory as well as di-synaptic inhibitory synaptic input. This inhibition evoked in the PER and LEC, after application of cortical stimuli, originates from the synaptically activated firing of the inhibitory interneurons (Martina et al., 2001; Willems et al., 2018). Furthermore, an ultrastructural study revealed that the GABAergic neurons are directly targeted by excitatory projections, indicating that they are organized in a feed-forward manner (Pinto, Fuentes, & Paré, 2006).

Key players in altering neuronal excitation in the PER-LEC are the inhibitory interneurons in the local network. The PER contains mainly calbindin, calretinin and parvalbumin (PV) expressing interneurons (Barinka et al., 2012a). The EC comprises three main groups of interneurons: PV, somatostatin and 5HT3r expressing interneurons (Leitner et al., 2016; Rudy, Fishell, Lee, & Hjerling-Leffler, 2011). The exact functional role of different interneuron types however, is not known.

Potential candidates for efficient inhibitory control of principal neurons are PV interneurons (Pfeffer, Xue, He, Huang, & Scanziani, 2013). This interneuron type is present in all layers of the PER and even more abundantly in the LEC (Barinka et al., 2012b; Cappaert

et al., 2014; Wouterlood, Härtig, Brückner, & Witter, 1995). PV interneurons project onto the axo-somatic site of principal neurons, where they can effectively evoke large inhibitory postsynaptic currents upon action potential firing (Pfeffer et al., 2013). Hence, PV interneurons are capable of hyperpolarizing the membrane potential and consequently can regulate at which moments in time the principal neuron is most prone to fire action potentials. These interneurons can therefore determine principal neuron output by shaping action potential firing patterns and herewith oscillatory activity (Cunningham et al., 2006; Sohal, Zhang, Yizhar, & Deisseroth, 2009).

Loss of inhibition in the PER-LEC is associated with pathologies involving hyperexcitability such as temporal lobe epilepsy and schizophrenia (Cunningham et al., 2006; Kumar & Buckmaster, 2006). PV interneuron numbers decrease tremendously in the PER as a consequence of seizures (Biagini et al., 2013) and PV interneuron activation can terminate, or even prevent, epileptic activity in epileptic mice (Assaf & Schiller, 2016).

The inhibitory component of the network likely plays a prominent role in the regulation of activity transfer through the PER-LEC in both healthy and diseased conditions (Cunningham et al., 2006; de Villers-Sidani et al., 2004; Kumar & Buckmaster, 2006; Willems et al., 2016). It is therefore hypothesized that neuronal activity transmission can be altered when the activity of the inhibitory neurons is modulated by the interaction of various cortical and subcortical synaptic inputs.

The insular cortex and lateral amygdala

In this dissertation, we examined two PER-LEC afferents: the neocortical agranular insular cortex (AiP) and the lateral amygdala (LA). The AiP is a neocortical area involved in emotional, interoceptive and exteroceptive signal processing (Nieuwenhuys, 2012). The AiP is located rostrally to the PER and projects to the PER and LEC (Burwell, 2000; Mathiasen, Hansen, & Witter, 2015). In rodents, afferents from the AiP to the PER-LEC network originate in all cortical layers and consist of both glutamatergic and GABAergic projections. These projections from the AiP most densely target the superficial layers of the PER and deep layers of the LEC (Mathiasen et al., 2015; Pinto et al., 2006; Unal, Pare, Smith, & Pare, 2013).

The amygdala plays a pivotal role in emotion processing (McDonald & Mott, 2016; Paz, Pelletier, Bauer, & Paré, 2006). The emotional enhancement of memory is an important

feature of the memory system and plays a crucial role in the survival of species (Christianson, 1992). It has been shown in animal studies as well as in humans that the amygdala can modulate medial temporal lobe activity (including the PER and LEC) and enhances memory performance on emotional versus neutral stimuli (Cahill & McGaugh, 1998; Dolcos, LaBar, & Cabeza, 2004; Kilpatrick & Cahill, 2003).

The amygdala projects to the PER-LEC network (Krettek & Price, 1977; Pikkarainen & Pitkänen, 2001; Pitkänen, Pikkarainen, Nurminen, & Ylinen, 2000). The lateral nucleus of the amygdala contains the heaviest projections to the PER, whereas the accessory and basal nucleus only project moderately to the PER deep layers (Pikkarainen & Pitkänen, 2001). The basolateral complex, the lateral capsular portion of the central nucleus (CLC), and all three subdivisions of the cortical nuclear complex mainly target the LEC (McDonald & Mott, 2016). This dissertation focuses on the input from the lateral amygdala (LA), since the PER and LEC are both synaptic targets of LA projections (Figure 1C), suggesting that the LA can modulate PER-LEC activity. All PER and LEC cortical layers are targeted by LA projections: PER afferents from the LA are mainly glutamatergic while projections to the LEC are both GABA - and glutamatergic (McDonald & Zaric, 2015; McDonald & Mott, 2016; Pitkänen et al., 2000; Smith & Paré, 1994).

The amygdala can modulate how activity from the neocortex is transmitted through the PER-LEC circuitry of the rhinal gate (Paz et al., 2006). Previous studies showed that both neocortical and LA stimulation leads to PER-LEC neuronal population activity in brain slices under the condition of a partial $GABA_A$ block (Kajiwara, Takashima, Mimura, Witter, & Iijima, 2003; Koganezawa et al., 2008; Willems et al., 2016). Furthermore, once the inputs from the PER and amygdala coincide in the deep layers of the LEC (Koganezawa et al., 2008), propagation of neuronal activity from the PER through the LEC into the dentate gyrus of the hippocampus is promoted (Kajiwara et al., 2003; Koganezawa et al., 2008). *In vivo* field recordings also showed that amygdala activation increases responsiveness of PER neurons to neocortical stimuli (Pelletier et al., 2005).

McGarry and Carter (2016) studied a possible mechanism for modulation of cortical responses by the amygdala in the prefrontal cortex. They showed that the amygdala can regulate the excitability of principal neurons via recruitment of local PV interneurons, resulting in fast feedforward inhibition that regulates emotional behavior (McGarry & Carter,

2016).

Since the inhibition recruited in the PER-LEC network is likely to regulate the transmission of neuronal activity, it is hypothesized that the PV interneurons in the PER-LEC deep layers are a target for the amygdala driven modulation of PER-LEC activity. To address whether input from the amygdala can alter synaptic activity from the AiP, this dissertation provides data on how synaptic input from the AiP and LA interacts in the PER-LEC network, microcircuit, and in single PER-LEC neurons.

Structure of this dissertation

This dissertation addresses the fundamental hypothesis that the PER-LEC regulates information transmission by gating neuronal activity. By using electrophysiological recording methods (Box 2), we addressed how the PER-LEC network is activated when synaptic input arrives. Especially the temporal aspect of information processing was studied by examining the activity evoked by a single stimulation at two different brain areas. Several questions are posed and answered: how is the PER-LEC network recruited by signals originating in the AiP and in the LA (chapter 2), what is the time pattern in which the excitatory and inhibitory neurons in the deep layers are recruited (chapter 3) and is that distinct from the time-pattern in the superficial layers of the PER-LEC (chapter 4), and how do the synaptic inputs from the AiP interact with those from the LA in single neurons, small circuits and the network in the PER-LEC (chapter 5).

Chapter 2

Stimulation of the PER or LA synaptic input can activate the LEC (Kajiwara et al., 2003; Koganezawa et al., 2008). In this chapter, we compared the spatiotemporal aspect of the activity propagation through the PER/EC network in response to AiP and LA stimulation. We determined the functional organization of the AiP or LA projections to the PER/EC network in horizontal mouse brain slices, at a high spatio-temporal resolution with voltage sensitive dye (VSD) imaging. We compared the recruitment sequence of the AiP or LA evoked activity in the PER-EC network in control condition and after $GABA_A$ dependent inhibition was reduced. We showed that synaptic input from the AiP and LA both activated the PER-LEC

Box 2 | Methods to record neuronal activity

In this dissertation, we aimed to study the processing of synaptic responses in 1) the single neuron, 2) the microcircuit, and 3) the network. Much of what is known about the functional properties of neurons and neuronal networks is discovered by recording neuronal activity *in vitro*, e.g. in the acute brain slice. Neuronal activity in the single neuron can be recorded by single or multiple cellular recordings while network activity can be visualized by voltage sensitive dye (VSD) imaging.

Whole cell patch clamp recordings provide detailed information on the processing of synaptic input in single neurons. Whole cell patch clamp recordings monitor the currents that flow through the cellular membrane as well as the transmembrane voltage, with the choice to control either one. When synapses in the neuronal membrane are activated by neurotransmitters, the resulting postsynaptic current, postsynaptic potential and eventually the generation of an action potential or a more complex firing pattern are revealed. In dual recordings we can study the connectivity between multiple cells in the microcircuit.

The evoked synaptic current in a neuron contains components that originate from excitatory and inhibitory synapses. Decomposition of the synaptic current into two underlying components, based on their different reversal potential, is a solution to extract the inhibitory and excitatory components of the response without using pharmacologicals, since this will affect the whole network. If the objective of the study is to examine the activation of the neuron by the surrounding neuronal network, it is preferred to leave the integrity of the network intact. To be able to decompose the synaptic current, the post-synaptic cell is clamped at potentials between -90 mV and -50 mV, while the same, voltage-independent, synaptic current is evoked. The recorded currents are the result of the excitatory and the inhibitory synaptic conductances and their respective driving forces: the differences between membrane voltage and the reversal potentials. With the evoked currents and the reversal potentials, the excitatory and inhibitory conductance can be separated based on their unique current/voltage relationship.

In VSD imaging neuronal membranes are loaded with a light sensitive molecule that changes its absorbance depending on the local electrical field strength (Chemla & Chavane, 2010). Bath application will load the neuronal membranes of all neurons present and thus provide information at the network level. Since the largest surface of neuronal membranes is found in the dendrites, VSD imaging mainly shows changes in dendritic voltage as a result of synaptic input. It is excellent in providing temporal information, but does not provide calibrated information on membrane voltage. With a fast camera the technique allows imaging of neuronal activity at a high spatial and temporal resolution and provides information about the propagation of neuronal activity through the slice.

network but the initial activation site was spatially separated, indicating that the functional projection from the AiP and LA is different. Activity transmission only moderately progressed in control conditions. Activity transmission is promoted when inhibition is reduced, indicating a role for the inhibitory interneurons in the regulation of neuronal activity transmission in the PER-LEC network.

Chapter 3

A role for local PER-LEC GABAergic interneurons has been implied in controlling the active selection and processing of activity (de Curtis & Paré, 2004). In chapter 3 we investigated how the excitatory and inhibitory neurons are activated in the PER-LEC deep layers and whether the interplay between principal neurons and PV interneurons plays a role in processing of synaptic input from the AiP in acute mouse brain slices. We examined the stimulus evoked synaptic input and action potential firing patterns in principal neurons and PV interneurons to address the functional output of the PER-LEC deep layer network once synaptic input is processed in the local excitatory and inhibitory components of the network. We showed that the excitatory input from the AiP onto deep layer principal neurons is overruled by strong feedforward inhibition. The PV interneurons, with their fast, extensive stimulus-evoked firing, deliver this fast evoked inhibition onto principal neurons. We concluded that the deep layers principal neurons are not involved, and even actively silenced, when synaptic activity is travelling from the neocortex towards the hippocampus.

Chapter 4

Since deep layer neurons are silenced by inhibition when a synaptic input enters the PER-LEC network, it is expected that the superficial PER-LEC neurons react to neocortical input with action potential firing in order to transmit activity from the PER, via the LEC towards the hippocampus (de Villers-Sidani et al., 2004; Willems et al., 2018). In this chapter we questioned whether differences in synaptic input and action potential firing are present between superficial and deep layer principal neurons and which role of the local superficial inhibitory network plays in the firing of principal neurons in response to input from the AiP.

Paired recordings of superficial and deep layer neurons in acute horizontal mouse brain slices revealed that superficial principal neurons receive stronger synaptic input and respond

with action potential firing after AiP stimulation than deep layer neurons. Furthermore, the timing of the evoked inhibition and excitation was more favorable for action potential firing in superficial layer neurons, likely because the evoked excitatory conductance was faster and larger and the inhibitory conductance arose slightly later. Firing of PV interneurons from the superficial layer local network evoked this inhibitory conductance after AiP stimulation at a very consistent timing, creating a solid inhibitory input, whereas the activated excitation was shifted earlier to ensure action potential firing.

Chapter 5

In chapter 5 we hypothesized that the synaptic input from the AiP and LA interacts when it coincides in the PER-LEC network, since the amygdala modulates PER-LEC neuronal activity transmission (Paz et al., 2006). We stimulated the AiP and LA and recorded principal neurons and PV interneurons in the PER-LEC deep layers to examine the interaction of synaptic input. We showed that both neuron types received synaptic input from the AiP as well as the LA, although the synaptic strength from the AiP input was larger than from the LA input. We found that synaptic inputs originating in the AiP and LA mainly alter the timing and amplitude of the inhibitory input evoked in PER-LEC deep layer principal neurons. Inhibition is evoked earlier in these principal neurons after simultaneous AiP and LA stimulation, because the interaction of AiP and LA inputs in PV interneurons triggers the first PV spike to be evoked earlier, likely causing the temporal window of opportunity for coincidence detection of excitation to be more precise.