Chapter 8

SUMMARY

The main objective of our research, using anatomical, electrophysiological, and behavioral approaches, was to study the functional organization of the ventral thalamic midline nucleus reuniens (RE), particularly regarding its connectivity with the hippocampus, and proposed importance for cognitive functions.

In chapter 2, using the multiple retrograde tracing technique, we showed that the projections from RE to the hippocampus (i.e., CA1, and subiculum), as well as those to the perirhinal and entorhinal cortices (EC) are non-collateralized. These predominant unilateral projections, displaying a topographical organization, arise from distinct although intermingled population of neurons located mainly in the rostral and mid rostral-to-caudal part of RE. Moreover, within each target area the terminal collateralization is locally restricted instead of distributing axon collaterals throughout the entire target structure. Since RE afferents are also topographically organized, it is conceivable that distinct clusters of RE projection cells may receive separate inputs, which in turn will be relayed to specific targets in the hippocampal-entorhinal complex.

In chapter 3, the RE-CA1 and direct EC-CA1 projections, both known to terminate in stratum lacunosum moleculare, were used as a model to explore and compare the sensitivity, specific properties, and the detectability of three different anterograde tracers, namely Phaseolus-vulgaris leucoagglutinin (PHA-L), rhodamine- (RDA) and biotin-conjugated dextran amine (BDA). We describe a newly developed method that can be used to examine the distribution of axonal terminations of three different afferent systems in a particular brain area, in one and the same animal. Using a simple application procedure, we injected the first tracer bilaterally in RE, a second tracer in the left EC, and a third tracer in the right EC. Subsequently, a newly developed triple staining procedure allowed for the simultaneous and permanent visualization of these three tracers, i.e., detectable as black, brown, and blue-green coloured labelled fibres, respectively. Not only offers this method an attractive approach for anatomical research in general, it also provided the necessary information to be used in our following electrophysiological experiments, i.e., choosing the optimal site for electrical stimulation in RE and EC, as well as the appropriate recording site in CA1 showing substantial overlap of RE and EC terminals in lacunosum moleculare (see chapters 4 and 7).
In chapter 4, we describe our electrophysiological in vivo experiments in which we stimulated the RE-CA1 projection at its origin, and studied the influence of RE input on the neuronal activity in hippocampal field CA1. Anatomically, RE axons in stratum lacunosum moleculare of CA1 are known to form asymmetrical (i.e., excitatory) synaptic contacts with spines and dendrites, suggesting that RE innervates the spinous apical dendrites of pyramidal cells, and presumably also the largely aspinous dendrites of interneurons with a dendritic tree in lacunosum moleculare. Paired pulse stimulation of RE elicited a clear dipole field in CA1, i.e., relatively large negative (subthreshold) deflections in lacunosum moleculare, reversing at the lacunosum moleculare-radiatum border to positive-going ones in strata radiatum and pyramidale, and steadily declining towards the alveus. A current source density (CSD) analysis revealed a clear lacunosum moleculare sink-radiatum source configuration, which is in agreement with an excitatory synaptic RE input onto the apical dendrites of CA1 pyramidal cells. Stimulation of RE at low frequencies (0.13-2 Hz) elicited the largest amplitude field excitatory postsynaptic potentials (fEPSPs). In contrast, stimulation of RE in the theta frequency range (4-10 Hz) evoked only small amplitude fEPSPs. Furthermore, low frequency paired pulse stimulation of the RE-CA1 input resulted in a robust form of short term plasticity, termed paired pulse facilitation (PPF). This appeared largely independent on stimulation (low-to-high) intensity or inter-pulse-interval duration (20-200 ms), indicating that RE can exert a persistent influence on the level of pyramidal cell excitability.

In contrast to subthreshold CA1 cell responses (i.e., we never observed action potential generation in pyramidal cells) we did notice the occurrence of RE-elicited spiking in two types of putative inhibitory CA1 interneurons, both known to mediate feedforward and feedback inhibition of pyramidal cells. Thus, RE-CA1 input partially influences hippocampal activity through activation of (at least two classes of) local inhibitory interneurons.

Next to a clear monosynaptic RE input, we found indications for complex and presumably di-synaptic elicited responses. Using additional anatomical tracing methods we showed that the basis for the observed di-synaptic input in CA1 was a projection from caudal RE-to-rostral RE.

Taken together, we concluded that RE can influence the CA1 pyramidal cell activity through direct excitatory and indirect inhibitory mechanisms. We proposed a closed circuit between rostral RE – CA1 – subiculum – caudal RE – rostral RE, which may allow RE to modulate the activity level in CA1 depending on the hippocampal output.

In chapter 5, we examined the RE axo-dendritic contacts in stratum lacunosum moleculare of hippocampal field CA1 at the ultrastructural level. To label RE axons, the anterograde tracer biotin-conjugated dextran amine (BDA) was injected into RE. Subsequently we
combined the visualization of BDA with staining of GABA, the latter to identify local inhibitory interneurons in CA1. Our results showed that a considerable part of the BDA-labeled RE axons form asymmetrical (i.e., excitatory) synapses on GABA-positive dendrites. These findings confirmed our previous electrophysiological observations, which indicated that RE is able to discharge inhibitory interneurons in CA1 (see chapter 4).

In chapter 6, we compared the effects of neurotoxic lesions of the RE or the mediodorsal (MD) thalamic nuclei on performance in a standard (reference memory) water maze task. Diencephalic or thalamic amnesia is characterized by deficits that resemble those of medial temporal lobe (hippocampal) amnesia or prefrontal dysfunction. Nuclei in the medial thalamus are connected with either the temporal lobe, or prefrontal cortex, or with both, and thus thalamic amnesia may be due to disconnecting the temporal and prefrontal systems at the thalamic level. Alternatively, it may result from the loss of specific thalamic contributions to these systems. In rats, both RE and MD are heavily and reciprocally connected with the medial prefrontal cortex (mPFC), but only RE innervates hippocampal field CA1, a structure of crucial importance for learning and memory. In the standard water maze task, the hippocampal formation is engaged in the spatial aspects of learning and memory, whereas the mPFC is more involved in behavioral flexibility and execution of strategies rather than in encoding or storage of spatial information. Therefore, a RE lesion was expected to cause a mixed deficit in hippocampal related spatial learning/memory as well as in mPFC related flexibility/strategy learning, while a MD lesion was assumed to result predominantly in an acquisition deficit in behavioral flexibility, i.e. a mPFC-related impairment. Unexpectedly, our observations during the acquisition phase (i.e., learning to located an invisible platform, using room cues to guide the search), probe test (i.e., a memory test, with room cues visible but platform removed), and cue test (i.e., a test to examine sensorimotor and/or motivational deficits; visible platform, but room cues no longer visible) revealed that neither RE, nor MD lesions did prevent learning and later memory of the task per se. Instead, RE and MD lesions affected the normal flexible use of search strategies and/or the flexibility with which a change in task conditions can be accommodated. That is, a RE lesion resulted in very flexible/impulsive behavior, whereas a MD lesion caused perseverative behavior, indicating that RE and MD may play opposing roles in non-mnemonic processes like strategy shifting, or in more general aspects of behavioral flexibility.

In chapter 7, using in vivo single and simultaneous low frequency stimulation of the RE-CA1 and lateral EC-CA1 projections, we investigated their combined effects on neuronal activity in hippocampal field CA1. Our results revealed that paired pulse stimulation of either RE or EC evokes subthreshold responses, showing strong homosynaptic paired
pulse facilitation (PPF), whereas combined paired pulse stimulation of RE and EC does not result in heterosynaptic PPF. Coinciding RE and EC inputs, however, resulted in a major enhancement of the fEPSPs in stratum lacunosum moleculare, yet action potential generation in pyramidal cells does not occur. This inability to induce CA1 cell firing is likely due to persistent inhibitory influences mediated by both inputs separately, as well as an additional peri-somatic inhibitory effect mediated by coinciding RE/EC inputs. A CSD analysis revealed complex interactions between RE and EC inputs throughout the depth profile of CA1, indicating an (at least partial) convergence of RE and EC synapses in lacunosum moleculare on the same dendritic branch of CA1 pyramidal cells, as well as on subclasses of inhibitory interneurons with dendrites in lacunosum moleculare. We discussed the functional relevance of coinciding RE and EC inputs in CA1, and proposed that low frequency RE-CA1 input is important for the synchronization of slow oscillations in hippocampus and mPFC. Furthermore, our data strongly suggested that by directly and indirectly facilitating the EC-CA1 input during slow oscillations, RE can contribute to the dialogue between hippocampus and mPFC which is of crucial importance for the consolidation of hippocampal-dependent memories.