

Miriam Nokia

The Role of the Hippocampal
Theta Activity in Classical
Eyeblink Conditioning in Rabbits



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UNIVERSITY OF JYVÄSKYLÄ

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ABSTRACT

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Finnish summary

Diss.

Classical eyeblink conditioning is a model of associative learning in which a tone is repeatedly paired with an airpuff towards the eye to produce a learned response, i.e. a blink in response to the tone alone. The neural basis of eyeblink conditioning is well-known: While the cerebellum is crucial at all times, the hippocampus is thought to participate mostly when the task is complex. However, hippocampal activity shows learning-related changes, and the spontaneous level of theta activity, a ~6 Hz oscillation dominating the hippocampal electrophysiological activity, predicts subsequent learning rate also when the task is simple. Then, how exactly, and why, does hippocampal theta activity relate to classical eyeblink conditioning? Three variants of the conditioning paradigm (delay, trace and discrimination-reversal) differing in complexity and hippocampal contribution were applied in rabbits, and extracellular electrical activity was recorded simultaneously. The neural state reflected by the level of hippocampal theta activity was found to affect specifically the rate and strength of association formation between the tone and the airpuff. In addition, the state reflected by the level of hippocampal theta activity changed according to the demands of the external environment mirroring the subjects' persistent yet adjustable tendency to respond to external stimuli. It was also shown that theta oscillations in the hippocampus and the cerebellum synchronize when the relative power of the hippocampal theta activity is high thus possibly mediating communication between these structures both involved in learning during eyeblink conditioning. In sum, the thesis shows how and why hippocampal theta activity relates to classical eyeblink conditioning.

Keywords: hippocampus, classical conditioning, theta, phase synchrony, local-field potential, learning, memory

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ABSTRACT

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1 INTRODUCTION

Classical conditioning of the eyeblink response (Gormezano, Schneiderman, Deaux & Fuentes, 1962) is a task used widely in the study of the neural basis of learning and memory both in humans and in animals. Eyeblink conditioning is mediated by the cerebellum which is thought to store the memory trace of the conditioned response (McCormick, Clark, Lavond & Thompson, 1982; for a review see Thompson, 2005). However, if the conditioning paradigm is complex, also the hippocampus becomes necessary for normal learning (Berger & Orr, 1983; Beylin, Gandhi, Wood, Talk, Matzel & Shors, 2001; Moyer, Deyo, & Disterhoft, 1990; Solomon, Vander Schaaf, Thompson, & Weisz, 1986; Weikart & Berger, 1986). Interestingly, it has been shown that disrupting the functioning of the hippocampus is more detrimental to learning during eyeblink conditioning than removing the structure altogether (Allen, Padilla, & Gluck, 2002; Asaka, Griffin, & Berry, 2002; Berry & Thompson, 1979; Salvatierra & Berry, 1989; Solomon, Solomon, Schaaf, & Perry, 1983). This curious result regarding the role of the hippocampus is likely related to a ~6-Hz oscillation called the theta oscillation which dominates the hippocampal electrophysiological activity (for a review see Bland, 1986 and Buzsáki, 2002).

Over time, the hippocampal theta activity has been connected to a wide variety of cognitive functions, like spatial navigation and associative learning (see for example Buzsáki, 2005; Vertes, 2005). Common to the behaviours associated with hippocampal theta is that they all reflect active processing and orientation toward the stimuli of the environment, the "on-line state" (Buzsáki, 2002). Convincing evidence for the role of the hippocampal theta activity in learning and memory was provided in 1978 by Stephen Berry and Richard Thompson who showed a predictive correlation between the amount of spontaneous hippocampal theta activity and learning rate during subsequent eyeblink conditioning in rabbits. The higher the proportion of time characterized by a dominant theta oscillation in the hippocampus, the faster the animal acquired the conditioned response. This finding was replicated in later studies by Berry and colleagues, also showing that the level of the hippocampal theta activity can be elevated, and learning thus

accelerated, by keeping the rabbits thirsty during the training procedure (Berry & Swain, 1989). Recent studies conducted by Berry's group have shown that by administering the conditioning stimuli during periods of spontaneous activity characterized by strong/weak relative power of hippocampal theta activity, the learning rate can be affected accordingly (Asaka, Mauldin, Griffin, Seager, Shurell, & Berry, 2005; Griffin, Asaka, Darling, & Berry, 2004; Seager, Johnson, Chabot, Asaka, & Berry, 2002). Based on the results reviewed above, the level of the hippocampal theta activity has been suggested to reflect the state of the subject, which in part affects the conditions for learning during a simple motor conditioning task.

Taking into account the lack of direct connecting pathways, it seems intriguing that the hippocampus and the cerebellum are both essential for successful learning during certain forms of eyeblink conditioning. Obviously, some form of co-operation between these two brain structures is required. One possible means of mediating communication between functionally related brain structures has been proposed to be synchronous oscillatory activity (for reviews see Fries, 2005; Klimesch, 1996; Klimesch, Freunberger, Sauseng & Gruber, 2008; Sauseng & Klimesch, 2008). By synchronizing and desynchronizing in time their activity in relation to each other, brain structures can "select" which inputs arrive and are sent during a time window of maximal effect, i.e. cause either excitation or inhibition in the receiving group of neurons (Fries, 2005). Interestingly, the phase of the ongoing hippocampal theta oscillation has been shown to modulate the excitability of neurons (Huerta & Lisman, 1995; Hyman, Wyble, Goyal, Rossi & Hasselmo, 2003; Hölscher, Anwyl & Rowan, 1997), thus affecting synaptic modification, and learning at the cellular level. Also, it has been suggested that the hippocampal theta activity might control the timing of gamma-band oscillatory activity in the neocortex and thus the information flow from the neocortex to the hippocampus (Sirota, Montgomery, Fujisawa, Isomura, Zugaro & Buzsáki, 2008). From this, a question arises, whether the theta oscillation might also play a part in forming a functional connection between the hippocampus and the cerebellum.

The connection between the hippocampal theta activity and learning rate has been studied earlier by applying correlation analyses to assess the effects of spontaneous differences in the level of theta activity (Berry & Thompson, 1978; Berry & Swain, 1989) as well as with the help of group comparisons following direct manipulations of the training paradigm according to the animal's state (Asaka et al., 2005; Griffin et al., 2004; Seager et al., 2002). However, some important basics still remain to be clarified. The aim of this dissertation was to thoroughly explore the connection between the hippocampal theta activity and eyeblink conditioning. Three different conditioning paradigms, delay, trace and discrimination-reversal, were used. During each paradigm the hippocampus is thought to affect learning to a different degree. In addition to exploring the effects of the relative power of the hippocampal theta activity on learning rate at different stages of the learning process (see Prokasy, 1984; 1987), the studies also focused on the effects of training on the level of hippocampal theta activity, an aspect not addressed before. In addition, a possible explanation for the role of the theta

activity as a facilitator of communication between two brain structures involved in eyeblink conditioning, the hippocampus and the cerebellum, was examined. Essentially, this thesis aimed to find out how and why hippocampal theta activity relates to classical eyeblink conditioning.

1.1 Eyeblink conditioning as a model of simple motor learning

Classical conditioning of the eyeblink response is a model of simple motor learning that has been used in the study of the neural basis of learning and memory since the 1960's (Gormezano et al., 1962). During eyeblink conditioning a neutral conditioned stimulus (CS), usually a tone or a flash of light, is presented preceding an unpleasant unconditioned stimulus (US), most commonly an airpuff or a mild electric shock targeted towards the eye. The US always elicits an unconditioned response (UR), in this case the closure of the eyelids, whereas the CS initially does not produce any observable behaviour. As a result of repeated pairings of the CS with the US, the subject learns to associate the two, and starts to close the eyelid already as a response to the CS alone. This learned response is called the conditioned response (CR).

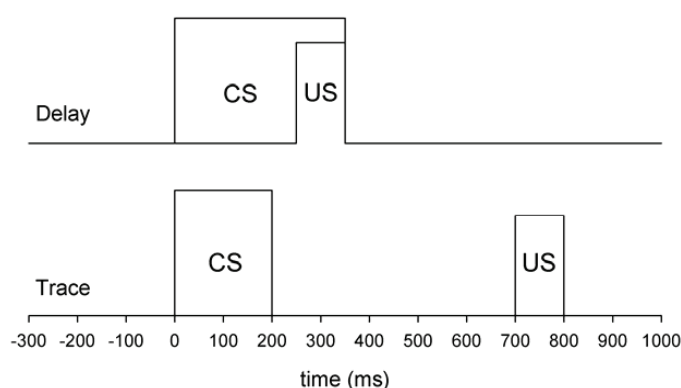


FIGURE 1 The two basic eyeblink conditioning paradigms. CS = conditioned stimulus, usually a tone or a flash of light. US = unconditioned stimulus, usually an airpuff directed towards the eye or a mild electric shock targeted to the muscles surrounding the eye.

There are several paradigms of eyeblink conditioning, of which the delay and the trace paradigms are most commonly used (see Fig. 1 above). During standard delay eyeblink conditioning the CS onset precedes the US onset, and the two stimuli overlap and co-terminate. During trace conditioning the CS and the US are separated by a stimulus-free gap, the trace period, of around 500 ms. Modifications of these two basic paradigms include, for example, the long-delay paradigm, in which the difference between the CS and US onsets is stretched to ~1

s. In addition, all of the above mentioned paradigms can be used in discrimination training, where one CS is consistently followed by a US and another CS is not. The subject must learn to respond to the CS that is followed by the US and suppress responses to the other CS. The task can be made even harder by switching the roles of the two CSs after the initial discrimination training and again training the subject to respond only to the CS followed by the US (reversal).

1.1.1 The roles of the cerebellum and the hippocampus in eyeblink conditioning

The memory trace of the CR acquired during classical eyeblink conditioning is thought to reside in the cerebellum (in humans see, Cheng, Disterhoft, Power, Ellis & Desmond, 2008; Gerwig, Kolb & Timmann, 2007; in animals see, Christian & Thompson, 2005; McCormick et al., 1982; Pakaprot, Kim & Thompson, 2009; for reviews see Attwell, Ivarsson, Millar & Yeo, 2002; Lavond, 2002; Thompson, 2005). Specifically, it has been shown that lesioning the cerebellum ipsilateral to the eye to be trained prevents learning the CR (McCormick et al., 1982) and that lesioning the cerebellum after training abolishes the already acquired CR (Clark, McCormick, Lavond & Thompson, 1984). The cerebellum seems to be the long-term storage of the memory trace since cerebellar interpositus nucleus (IPn) lesions and inactivations carried out as late as 30 days after learning still abolish the CR (Christian & Thompson, 2005). Related to the behavioural CR, an increase in multiple-unit activity (MUA), representing the number of action potentials of a large group of neurons, is seen in the cerebellum (Choi & Moore, 2003; McCormick & Thompson, 1984). Depending on the formation of this cerebellar neural model of the CR (Clark et al., 1984; Sears & Steinmetz, 1990), similar neural models of the CR emerge also in other brain structures, for example in the hippocampus (Berger, Alger & Thompson, 1976). Thus, the behavioural as well as the neural CR both seem to depend on an intact cerebellum.

The second main structure involved in eyeblink conditioning is the hippocampus, which is not crucial for learning when the simplest form of eyeblink conditioning, the delay paradigm, is applied (Schmaltz & Theios, 1972). However, the hippocampus becomes increasingly important if the relations between the stimuli are more complex, like during trace (Moyer et al., 1990; Solomon et al., 1986), long-delay (Beylin et al., 2001) and discrimination-reversal conditioning (Berger & Orr, 1983; Weikart & Berger, 1986). However, the amount of hippocampal MUA in the pyramidal layer of the CA1 elicited by the stimuli used in conditioning increases rapidly early during training, and temporally precedes and models the behavioural CR even when the simple delay paradigm is used (Berger et al., 1976; Berger & Thompson, 1978; for review see Berger, Berry, & Thompson, 1986). The effects of hippocampal lesions have been shown to be most detrimental when they are made in the early phase of learning, before the memory trace of the CS-US association and the of the CR have been consolidated into long-term memory (Kim, Clark & Thompson., 1995; Takehara, Kawahara & Kirino, 2003; Takehara, Kawahara, Takatsuki & Kirino, 2002). Moreover, disrupting the

functioning of the hippocampus by severing or disrupting the functioning of the septum (Allen, Padilla, & Gluck, 2002; Asaka, Griffin, & Berry, 2002; Berry & Thompson, 1979; Salvatierra & Berry, 1989), or systemically administering a substance (scopolamine) interfering with hippocampal electrophysiological activity (Solomon, Solomon, Schaaf, & Perry, 1983), is more detrimental to eyeblink conditioning than lesioning the hippocampus.

In sum, whereas the cerebellum most likely stores the memory trace of the CR (for a review see Thompson, 2005), the hippocampus is thought to participate in the regulation of the adaptive amplitude-time course of the behavioural CR (Berger et al., 1976; 1986), and to contribute to the consolidation of the memory trace especially in the early phases of learning (Kim et al., 1995; Takehara et al., 2002; 2003). In addition, whereas lesioning or inactivating the cerebellum consistently prevents learning and abolishes the CR acquired during eyeblink conditioning (for a review see Thompson, 2005), a badly functioning hippocampus seems to cause more disturbances for learning compared to no hippocampal function at all (Allen et al., 2002; Asaka et al., 2002; Berry & Thompson, 1979; Salvatierra & Berry, 1989). This suggests that appropriate activity in the hippocampus, while not being crucial for learning in eyeblink conditioning, is still involved in it.

1.1.2 The learning process according to Prokasy

As stated above, the role of the hippocampus along with other brain structures involved in eyeblink conditioning varies according to the conditioning paradigm used (see for example Moyer et al., 1990; Schmaltz & Theios, 1972; Solomon et al., 1986) and the phase of the learning process (Kim et al., 1995; Takehara et al., 2002; 2003). In addition, substantial differences in learning rate exist between individual subjects. To be able to study the exact relations between hippocampal theta activity and learning during eyeblink conditioning, a theory of the learning process taking into account the above mentioned facts is in order.

Several theories regarding the nature of the learning process during classical conditioning were proposed already during the 1960's. For example Theios and Brelford (1966) and Prokasy (1965; 1972; 1984; 1987) both presented a model taking into account the sequential nature of learning, the individual differences in the duration of each phase, and the effects of previous performance on the probability of making a CR (for a comparison of the two models see Prokasy, 1972). Both models contain an initial state during which the response probability is assumed to remain at a low and stable level. The finite integer model of Theios and Brelford (1966) comprising of three states further assumes that the transfer between states occurs on a single trial and that the response probability remains stable also within stages two and three. Contrary to this, Prokasy's 2-phase theory does not assume a stationary response probability after the first learning phase but instead suggest a linear increase in it. In addition to being simpler in terms of the number of learning phases, predictions made according to Prokasy's theory seem to fit experimental eyeblink conditioning data better (Prokasy, 1972).

According to the latest version of Prokasy's (1984; 1987) theory, Phase 1 consists of i) the formation of the association between the CS and the US (contingency detection) and ii) the selection of the appropriate CR. Phase 2 consists of iii) response acquisition, during which the frequency of responding changes depending on the reinforcement schedule, and iv) response shaping, during which the CR latency and topography are modified to maximize the adaptivity of the response in regard to the attributes of the CS and the US. Although the four different stages (contingency detection, response selection, response acquisition and response shaping) do not occur strictly in sequence, the previous stage must always be initiated before the next one can begin. In addition, Prokasy (1987) suggests that Phase 1 and Phase 2 are carried out essentially independent of each other, although overlapping, and that the performance profile (i.e. the change in response rate) during conditioning does not straightforwardly reflect the formation or strengthening of associations. Specifically, associations start to form well before any behavioural responses occur (see for example Berger, et al., 1976), and on the other hand the associations acquired early in training continue to strengthen even after behavioural responding has reached asymptote.

Prokasy's theory (1987) has been used in forming the criteria for learning and in interpreting the results of earlier eyeblink conditioning studies (see for example Griffin et al., 2004; Salvatierra & Berry, 1989). Especially, it is thought that the emergence of the 5th CR during eyeblink conditioning indicates the timing of the formation of the CS-US association in Phase 1 (Prokasy, 1987; Thompson, Berry, Rinaldi, & Berger, 1979). In addition, the subject is assumed to have reached asymptotic performance during Phase 2 (Prokasy, 1987) once a CR is present on eight out of nine consecutive trials. These measures are also used in this thesis, and the neural substrates as well as the mutual timing of Phase 1 and Phase 2 addressed.

1.2 Hippocampal electrophysiological oscillatory activity

Neuronal oscillations, the rhythmic changes in electrophysiological activity of the brain at different frequency bands, have evoked more and more interest among scientist during the past few years (for reviews see Buzsaki & Draguhn, 2004; Steriade, 2006). The oscillatory activity of the brain, and especially synchrony between oscillations of distinct brain regions (see for example Fries, 2005), is thought to form the link between single-neuron activity and behaviour (Buzsaki & Draguhn, 2004), and to reflect the neural state of the animal. The interplay between large-scale neuronal oscillations and the firing of single neurons was recently demonstrated by Li, Poo and Dan (2009) who were able to show that the stimulation of a single cortical cell in the rat was able to induce a change in the oscillatory pattern of the cortex as far as 6 mm away from the initial site of the stimulation.

Three main types of hippocampal electrophysiological oscillatory activity, all implicated in learning and memory, have been identified: Firstly, a dominant 3-12 Hz theta oscillation (for a review see Buzsáki, 2002), secondly a slow oscillatory state characterized by ~1 Hz large-amplitude fluctuations in extracellular voltage (Wolansky, Clement, Peters, Palczak & Dickson, 2006), and thirdly a high-frequency ripple activity (~140-200 Hz; for a review see Buzsáki, 2006) riding on slower sharp-waves.

1.2.1 Theta

The dominant hippocampal electrophysiological oscillation in the awake animal is the ~6-Hz theta oscillation. Hippocampal theta activity originates in the medial septum-diagonal band of Broca (MS-DBB), the entorhinal cortex and the hippocampus itself (Vertes & Kocsis, 1997; for a review see Bland, 1986 and Buzsáki, 2002). The *oscillatory pattern and frequency* of the hippocampal theta activity is thought to be governed by the MS-DBB and the supramammillary region reciprocally connected to it. In addition, also the hippocampal region CA3, forming an auto-associative network, is thought to be capable of acting as a rhythm generator. The *magnitude* of the hippocampal theta activity is regulated mostly by entorhinal input, as well as by the activity of the hippocampus itself, especially within the CA1 and dentate gyrus regions. The hippocampal theta oscillation is weakest in the pyramidal layer of the CA1 (Fig. 2, pyr), and most powerful, and its frequency most regular, near the hippocampal fissure (Fig. 2, fissure), in the stratum lacunosum-moleculare layer of the hippocampal region CA1 from which point onward to the hilus of the dentate gyrus (Fig. 2, DG) it retains roughly the same order of magnitude (Bragin, Jandó, Nádasdy, Hetke, Wise, & Buzsáki, 1995; Buzsáki, 2002). The slower portion of the theta-band activity (3-8 Hz) has been specifically implicated in alert immobility and is eliminated by anticholinergic drugs such as atropine (see Bland, 1986 and Buzsáki, 2002) as opposed to the faster theta (8-12 Hz) usually associated with exploratory behaviours, and unaffected by anticholinergic drugs (see Bland, 1986; Berry & Seager, 2001; O'Keefe & Nadel, 1978).

As already mentioned, theta-band oscillations dominate the hippocampal electrophysiological activity during a multitude of behaviours, like exploratory motor behaviour, and awake immobility, and have been suggested to play a role in a multitude of cognitive processes, like spatial navigation, learning and memory just to name a few (for reviews see Buzsáki, 2005; Hasselmo, 2005; Lisman, 2005; Vertes, 2005). Common to the behaviours associated with theta is that they all reflect active processing and orientation toward the stimuli of the external world, the "on-line state" (Buzsáki, 2002). Very well documented is the relationship of the phase of the hippocampal theta oscillation and cell firing in the hippocampus modified by learning when rats are presented with a task requiring spatial navigation (theta phase precession, for a review see Lisman, 2005). In fact, the hippocampal theta oscillation has been referred to as "the temporal means of navigation in both neuronal space during episodic memory and real space during

selfmotion" (Buzsáki, 2005). What comes to the interplay between oscillations of different frequencies, the recall of associated items from long-term memory in the same serial order they appeared during encoding is thought to be coded in nested hippocampal theta and gamma (~20-80 Hz) oscillations (see Lisman, 2005). Recently it was suggested that the hippocampal theta activity coordinates the timing of neocortical bursts of gamma oscillations and thus the information flow from spatially distributed neuronal assemblies in the neocortex to the hippocampus (Sirota et al., 2008).

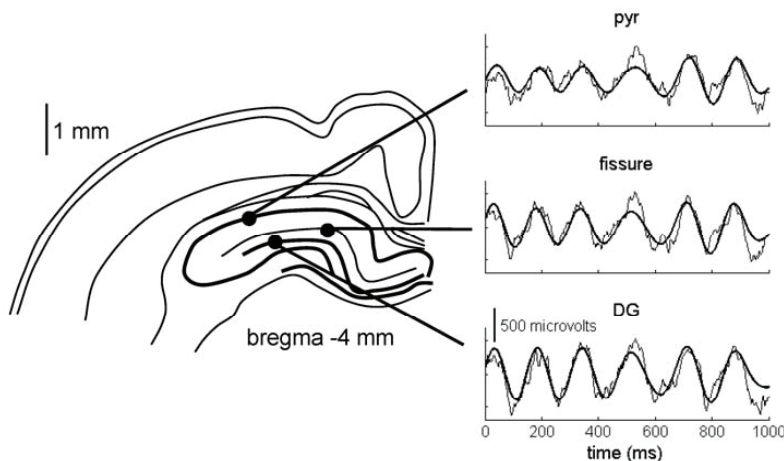


FIGURE 2 Theta oscillation recorded from the rabbit hippocampus. Abbreviations: pyr = pyramidal layer of the CA1, fissure = hippocampal fissure, DG = dentate gyrus. The thin lines depict the raw signal (0.1-200 Hz) and the thick lines show the same signal band-pass filtered at the theta-band (5-8 Hz).

The hippocampal theta oscillation has also been implied to have a role in regulating synaptic modification and plasticity at the cellular level. Namely, it has been shown that hippocampal stimulation arriving at the peak of the CA1 theta oscillation produces long-term potentiation (LTP), whereas stimulation arriving at the trough produces long-term depression (LTD) (Hyman et al., 2003; Hölscher et al., 1997). Recently, Caporale and Dan (2008) reviewed a phenomenon termed spike timing-dependent plasticity, meaning that either LTP or LTD is produced in the hippocampus depending on the timing of sequential weak and strong inputs coming from the entorhinal cortex relative to one another. If a weak input precedes a strong input, LTP is produced and if the order is reversed, LTD is produced. Related to these findings, Vertes (2005) has proposed that stimuli relayed to the hippocampus from the neocortex and thalamus, arriving at the peak of the CA1 theta oscillation, and thus co-occurring with the input coming from the MS-DBB and the entorhinal cortex, would be processed in the hippocampus, i.e. in the short-term memory, more efficiently.

1.2.2 Slow oscillations and ripples

Two other oscillatory phenomena characteristic to the hippocampal electrophysiological activity and briefly introduced next, are the large-amplitude slow oscillations and the high-frequency ripples. The slow oscillatory state (SO) refers to a rhythm earlier found to be widely present in the neocortex during anesthesia (rats: Steriade, Nunez, Amzica, 1993), and discovered only recently to exist also in the hippocampus both during spontaneous periods of sleep as well as during urethane anesthesia (Wolansky et al., 2006). The SO reflects a repeated sequence of so-called UP- and DOWN-states during which neurons are either depolarized, and bound to fire (UP), or hyperpolarized and not likely to fire (DOWN) (Steriade et al., 1993). Related to memory consolidation taking place during sleep, Sirota and Buzsáki (2005) recently suggested that the SO of the neocortex might control the timing and the content of the information transfer from the hippocampus to the entorhinal cortex and onward to the neocortex.

Also related to sleep and concomitant memory consolidation, high-frequency ripple activity (Chrobak & Buzsáki, 1996; Chrobak, Lörincz, & Buzsáki, 2000) is present in the hippocampus primarily when the animal is not engaged in active interaction with the environment. The small-amplitude ripple oscillation travels at the peak of a around 100-ms large-amplitude sharp-wave, and engages tens of thousands of neurons in the hippocampal-entorhinal axis to fire synchronously, the bursts occurring at the negative peaks of the ripple (Chrobak & Buzsáki, 1996). In addition to the hippocampus, ripples are also seen in the entorhinal cortex (Chrobak & Buzsáki, 1996). Hippocampal ripple activity has been connected to “off-line” memory consolidation and the transfer of memories from the hippocampus to the neocortex by means of modifying synaptic connections (Axmacher, Elger, & Fell, 2008; Eschenko, Ramadan, Mölle, Born, & Sara, 2008; Ponomarenko, Li, Korotkova, Huston, & Haas, 2008), as well as to the replay of recent experiences (Diba & Buzsáki, 2007).

1.3 Hippocampal theta activity and eyeblink conditioning in the rabbit

As noted above, the hippocampus seems to play at least a modulatory role in eyeblink conditioning and most importantly it seems that a badly functioning hippocampus disrupts learning more than the removal of the hippocampus altogether (see for example Solomon et al., 1983). This effect is likely to be due to the disruption in the hippocampal theta rhythm (for a review see Buzsáki, 2002) described in detail above. In deed, compelling evidence for the association of hippocampal theta oscillation with learning was provided by Berry and Thompson (1978; see also Berry & Swain, 1989), who first showed that the amount of spontaneous hippocampal theta activity before training predicts learning rate, as well as the magnitude of the US-induced change in hippocampal MUA, during

subsequent delay eyeblink conditioning in rabbits (for reviews see Berry & Seager, 2001; Berry, Seager, Asaka, & Borgnis, 2000). Berry and Thompson (1978) recorded hippocampal LFPs from the CA1 region of rabbits for a 2-minute period prior to delay eyeblink conditioning, and, using a zero-crossing technique, found that the amount of time characterized by a high proportion of 2-8 Hz compared to 8-22 Hz activity correlated negatively with the number of trials needed to reach the learning criterion. In addition to the correlation between the pre-training *amount* of theta activity and learning rate, Berry and Thompson (1978) also found a correlation between the *change* in the amount of hippocampal theta activity across training and learning rate, fast learners moving towards a less synchronized state and slow learners towards a more synchronized theta state (Berry, 1982) resulting in more similar amounts of theta activity in all subjects after conditioning.

In order to study the effects of the general motivational state on the hippocampal electrophysiological oscillatory activity and eyeblink conditioning, Berry and Swain (1989) subjected rabbits to water deprivation. As a result, the water-deprived animals i) showed more hippocampal theta activity, ii) learned faster and iii) showed greater hippocampal neural responding to the conditioning stimuli especially early in training compared to the control animals (Berry and Swain, 1989). Since no prior drive induction is necessary for normal eyeblink conditioning, Berry and Swain (1989) suggested that the facilitative effect on conditioning brought about by increasing the general motivational state via water deprivation was related to increased attentiveness towards all external stimulation.

More recent studies by Berry's group have shown that the administration of conditioning trials (using a modern brain-computer interface) in the presence of high hippocampal theta ratio facilitates, and administering trials during low hippocampal theta ratio hinders learning especially in the early phase of training (Asaka et al., 2005; Griffin et al., 2004; Seager et al., 2002). Seager et al. (2002) showed that when delay eyeblink conditioning trials were administered during periods of spontaneous activity in the hippocampus dominated by theta oscillations, acquisition of the CR took only half as many trials compared to when the conditioning trials were administered in the absence of dominating theta activity in the hippocampus. Using the trace paradigm, Griffin et al. (2004) obtained similar results showing that conditioning administered exclusively during periods of hippocampal activity dominated by theta oscillations accelerated the acquisition of both the initial CS-US association measured as the number of trials needed to reach the 5th CR, as well as reduced the number of trials needed to reach asymptotic conditioned responding. However, presenting trials during periods of hippocampal activity characterized by the absence of a dominant theta oscillation only retarded learning in the early phase of training (Griffin et al., 2004), when the CS-US association is formed (see Prokasy, 1987). In addition, the study of Griffin et al. (2004) showed a greater increase in hippocampal firing rate in response to the CS early in training, if the training trials were presented contingent upon dominant theta activity in the hippocampus. Later, Asaka et al. (2005) showed that training contingent upon dominant hippocampal theta activity facilitated learning only in the early phase of trace

eyeblink conditioning in young rabbits, but accelerated learning both early and late in conditioning in old rabbits, thus eliminating the age-related deficit in learning during a hippocampally-dependent form of eyeblink conditioning.

1.3.1 Evaluation

In their 1978 study Berry and Thompson recorded hippocampal activity once before and once after eyeblink conditioning. Thus, although they were able to show the overall direction of change in the level of the hippocampal theta activity, detailed analysis of, for example, the timing of these changes in regard to the learning process was not possible. In their study Berry and Thompson (1978) used a zero-crossing method to detect the presence, or the amount of time dominated by the theta oscillations, whereas today the Fast Fourier Transform can be used to analyze the power of the signal at different frequency bands directly and effortlessly. All in all, although Berry and Thompson's study (1978) showed clear and groundbreaking results, it also left some major questions unanswered, a portion of which were addressed in the first study included in this thesis (see Overview of the original studies, Study I).

The recent studies by Berry's group utilizing hippocampal theta -contingent trial presentation under both trace and delay eyeblink conditioning paradigms show the effects of manipulating the training paradigm according to the state of the animal on learning (Asaka et al., 2005; Griffin et al., 2004; Seager et al., 2002). However, direct evidence of the connection between two continuous variables, the spontaneous level of the hippocampal theta activity and learning rate, has only been obtained using the simplest form of eyeblink conditioning, the delay paradigm (Berry & Thompson, 1978). In addition, the question of how the level of the hippocampal theta activity changes across training still remains unanswered. Moreover, none of the experiments described above included control groups in which animals would have undergone an equal number of stimulus presentations but in an unpaired manner. The above mentioned aspects were addressed in the first three studies included in this thesis (see Overview of the original studies, Study I-III).

1.4 Oscillatory synchrony as a means of communication

The role of the hippocampus in eyeblink conditioning along with the cerebellum seems indisputable in light of lesion studies (see Thompson, 2005). Also, a clear connection exists between the neural state of the subject, reflected by the electrophysiological oscillatory activity in the hippocampus, and learning (Berry & Thompson, 1978). However, the mechanism by which the hippocampus interacts with the cerebellum remains unknown. Few if any direct anatomical connections exist between the hippocampus and the cerebellum: Controversial evidence for a projection from the cerebellar fastigial nucleus to the hippocampus has been

presented in the 1970's (see Heath & Harper, 1974; Crow, Finch & Babb, 1977). Thus, the co-operation of these two brain structures must rely on more indirect means of communication via other brain regions, like the neocortex.

One possible mechanism mediating communication between functionally related brain structures could be synchronous oscillatory activity (for reviews see Fries, 2005; Klimesch, 1996; Klimesch, et al., 2008; Sauseng & Klimesch, 2008). It has been suggested that *phase synchrony* between oscillating signals stemming from various brain structures provides the flexible and effective means of communication, working on top of fixed anatomical connections, and making it possible for the brain to perform complex cognitive functions (Fries, 2005, see also Palva, Palva & Kaila, 2005). In essence, phase synchrony between signals means that the *difference* between the phases of the signals is fixed, that is, the phase of one signal can be derived from the phase of the other signal. By synchronizing and desynchronizing in time their activity in relation to each other, brain structures can "select" which inputs arrive and are sent during a time window of maximal effect, i.e. cause either excitation or inhibition in the activity of the receiving group of neurons. On one hand, (de)synchronization can be viewed over relatively long time periods encompassing several oscillatory cycles in duration. On the other hand, oscillatory synchronization can act as a means for precise timing of excitatory and inhibitory influences when the phases of two oscillations are locked at the level of single oscillatory cycles (for example, ~170 ms for theta, 20 ms for gamma).

In fact, coherent theta-band activity between different cortical areas has been observed in healthy humans undergoing tasks related to working memory (Sarnthein, Petsche, Rappelsberger, Shaw & von Stein, 1998; for recent reviews, see Klimesch et al., 2008; Sauseng & Klimesch, 2008). Furthermore, Sirota and Buzsaki (2005) have suggested that through oscillatory synchrony the slow oscillations common to the neocortex and the hippocampus (Steriade et al., 1993; Wolansky et al., 2006) might control the timing and the content of the information transfer from the hippocampus to the entorhinal cortex and further to the neocortex during memory tasks in rats. Furthermore, Sirota and colleagues (2008) recently suggested that the hippocampal theta activity might coordinate the timing of neocortical bursts of gamma oscillations and thus the information flow from the neocortex to the hippocampus. The same idea could also apply to the role of the theta oscillation in the assumed co-operation between the hippocampus and the cerebellum taking place during eyeblink conditioning.

1.4.1 Evaluation

The findings regarding coherent and synchronized oscillatory activity briefly reviewed above support the idea of synchronous activity as a flexible and effective means of communication between functionally related brain structures (Fries, 2005). In the fourth study included in this thesis, the possible role of synchronized theta activity in enabling or facilitating communication between the hippocampus and the cerebellum was explored.

1.5 Summary and aims

In sum, learning during classical eyeblink conditioning depends on the cerebellum (Thompson, 2005), but also on the hippocampus if a complex training paradigm is used (see for example Moyer et al., 1990). In addition, disrupting the functioning of the hippocampus, and thus the theta oscillation predominant for the hippocampal electrophysiological activity (Bland, 1986; Buzsaki, 2002; 2005), is more detrimental to learning than removing the structure altogether (see for example Solomon et al., 1983). In fact, the level of the hippocampal theta activity reflects the neural state of the animal and predicts the learning rate during eyeblink conditioning in rabbits (Berry & Thompson, 1978). However, further studies are still required to elucidate the role of the hippocampal theta activity in different stages of learning (Prokasy, 1987), and to explore if and how the hippocampal theta activity changes as a function of training and learning. In addition, how the hippocampus and the cerebellum interact in the absence of major direct anatomical connections also raises questions.

Based on the above-mentioned, in Study I we aimed to verify the results of Berry and Thompson (1978) and to investigate how the relative power of spontaneous hippocampal theta activity changes during unpaired training followed by delay conditioning. As a logical continuation, we then aimed to examine the relationship between the relative power of the hippocampal theta activity and learning during trace eyeblink conditioning (Study II). In addition to the effects of hippocampal theta activity on learning rate (see Griffin et al., 2004), we also studied the impact of training and learning on the relative power of the of the hippocampal theta activity, a question not approached before. In Study III, we sought to determine if the hippocampal theta ratio is mostly connected to the detection of the contingency between the stimuli used in conditioning (Prokasy, 1987) or also to the learning of more complex inhibitory associations when a highly demanding and hippocampally dependent delay discrimination-reversal eyeblink conditioning paradigm is used. Finally, in Study IV we explored the possibility that theta-band phase synchrony (Fries, 2005; Palva et al., 2005) might mediate communication between the cerebellum and the hippocampus, and thus explain how these structures work together in order to facilitate learning during classical eyeblink conditioning.

2 OVERVIEW OF THE ORIGINAL STUDIES

2.1 Method

A more detailed description of the methods can be found in the original papers.

2.1.1 Subjects

The subjects were adult New Zealand White rabbits aged ~4 months and weighing 2.5–4.5 kg at the time of surgery. Female rabbits were used in studies I and III, and male rabbits in studies II and IV. All the experimental procedures were implemented in accordance with the European Communities Council Directive (86/609/EEC) on the care and use of animals for research purposes.

2.1.2 Surgery

Two to four monopolar recording electrodes made of Teflon-insulated stainless steel wire (bare diameter 125 μm) mounted inside a 27-gauge hypodermic stainless steel tubing were chronically implanted into the right hippocampus (for details, see Korhonen, 1991) 5 mm posterior and 4–7 mm lateral to the bregma. During implantation, MUA and LFPs were monitored to define the preferred depth of the electrode (6.5–7.2 mm below bregma). In Studies I and III the electrodes were aimed at the hippocampal subregion CA1, and in Studies II and IV the electrodes were aimed at the dentate gyrus. In Study IV, according to the same procedure, one electrode was implanted into the right cerebellar cortex lobule HVI 1 mm anterior, 5 mm lateral to and 8–10 mm below the lambda. In addition, two electrodes were implanted into the left medial prefrontal cortex 4–5 mm anterior, 0.8 mm lateral to and 6.5 mm below the bregma.

2.1.3 Conditioning procedure

The delay paradigm with a 250-ms inter-stimulus interval (ISI) was used in Study I in its basic form and in Study III in the context of discrimination-reversal training (see Fig. 1 and Introduction). The trace paradigm with a 500-ms ISI was used in Studies II and IV. The mean inter-trial interval (ITI) during conditioning was 40 s in Studies I, II and IV, and 25 s in study III. During unpaired presentations of the CS and the US the ITI was 20 s (Studies I, II and IV). The training sessions lasted ~1 hour at a time, and were conducted once per day on consecutive days, excluding weekends (Study III). In Study I, all animals went through unpaired treatment followed by delay conditioning. In Studies II and IV two separate groups of animals were subjected to either unpaired training or conditioning.

The stimuli used as CSs were 85-dB tones either 350 (delay) or 200 ms (trace) in length with a frequency of either 1 kHz (Study I), 2 kHz (Studies II-IV) or 8 kHz (Study III). The US in all studies was a 100-ms corneal airpuff (sound pressure level 64 dB) delivered through a nozzle (inner diameter 2 mm) placed approximately 1 cm away from the eye. The source pressure of the airpuff was 0.45 bars in Study I, 0.35 bars in Studies II and IV, and 0.2 bars in Study III.

2.1.4 Recordings

In Study I, eyeblinks were recorded with a nylon loop attached to the rabbit's nictitating membrane, and connected to a minitorque potentiometer with a rigid stainless steel hook. In Studies II and IV eyeblinks were recorded as electromyographic (EMG) activity measured with stainless steel wire hooks placed around the upper and lower eyelids for the duration of the session. In Study III movement of the eyelids and the nictitating membrane was recorded using an infrared recorder (see Ryan, Detweiler, Holland, Hord, and Bracha, 2006) attached to the airpuff nozzle.

All data was recorded using a ~10 kHz sampling rate. The LFPs were band-pass-filtered between 0.1-200 Hz, the MUA between 500-4000 Hz, and the EMG between 100-300 Hz.

2.1.5 Histology

After the experiments, the rabbits were anesthetized with an i.m. injection of ketamine-xylazine cocktail and then overdosed with an i.v. injection of pentobarbital. Next, the brain was perfused by putting physiological saline followed by 10% formalin through the ascending aorta. The locations of the electrode tips were marked by passing a DC current (200 μ A, 20 s) through it. The brains were frozen and coronally sectioned with a microtome into 100- μ m-thick slices which were stained with Prussian blue and cresyl violet. The electrode-tip locations were determined with the help of a microscope and stereotaxic atlases (Bures, Petran, & Zachar, 1967; Lavond & Steinmetz, 2003).

2.1.6 Data analysis

To assess learning rate, two basic measures were taken. Firstly, the number of trials needed to reach the 5th CR was calculated to quantify the learning rate in Phase 1 (Prokasy, 1987). Secondly, the number of trials needed to reach the conditioning criterion (trials-to-criterion, TTC), a robust CR on 8 out of 9 consecutive CS-alone or paired trials, was calculated to determine the learning rate in Phase 2 (Prokasy, 1987). In Study III, the rate of discrimination learning was quantified as the number of sessions needed to reach a 25% and a 50% difference in the response rates to the CS- and the CS+.

To analyze the hippocampal theta activity a Fast Fourier Transform (FFT) was run on the LFPs using a Hamming window of 8192 samples in length with a 50% overlap. Next the power of the theta activity (~ 3.5–8.5 Hz) was divided by that of the combined power of delta (~ 0.5–3.5 Hz) and theta activity, yielding the hippocampal theta ratio. A relative measure [$\text{theta} / (\text{theta} + \text{delta})$] was used to assess the power of the hippocampal theta activity because the absolute power values obtained with FFT vary according to the signal amplitude. The signal amplitude, in turn, varies according to the exact location of the electrode tip, as well as according to the minor differences in the physical properties of the recording electrode. Thus, to gain a measure that is comparable between subjects, and also over time within subjects, the relative power of the hippocampal theta activity was determined. Delta- and theta-band power was used as the sole reference, first, because in absolute power delta and theta frequencies are fairly comparable and, second, because the absolute power of the higher frequencies (8 Hz <) is considerably smaller than that of theta. Figure 1 of Study IV shows how the absolute LFP power in the theta and delta bands differ during periods of high (1A) and low (1B) hippocampal theta ratio: When the hippocampal theta ratio is high, also the absolute power of hippocampal theta-band activity is high and when the hippocampal theta ratio is low, also the absolute power of hippocampal theta-band activity is low. This is to show that changes in the hippocampal theta ratio are only partly due to changes in the absolute power of delta-band activity (see Study IV, Fig. 1).

Phase synchrony (PS) between two signals was calculated as earlier described by Palva et al. (2005). First, the LFP signals were band-pass filtered and transformed into complex form using the Hilbert transform. Next, the amplitudes of the signals were normalized to 1 by dividing each data point by its absolute value. Then, the phase difference of the two signals was calculated by multiplying the first signal with the complex conjugate of the second signal. At last, the phase synchrony was derived by averaging the phase difference matrix over trials and taking the absolute value. Standardization was done by comparing the real PS and PSs calculated for surrogate datasets generated by shuffling real data trials.

2.2 Study I: Hippocampal theta-band activity predicts learning rate during delay eyeblink conditioning in rabbits

In the first study, we aimed to verify the result of Berry and Thompson (1978), who first showed a predictive correlation between the pre-training level of spontaneous hippocampal theta activity and learning rate during delay eyeblink conditioning in rabbits. In addition, we aimed to investigate how the relative power of spontaneous hippocampal theta activity changes during unpaired training followed by conditioning, an aspect not approached in previous studies. Also, we sought to determine how the relative power of hippocampal theta activity during the pre-CS period affects the properties of the following behavioural CR and the hippocampal neural model of the CR (Berger et al., 1976; Griffin et al., 2004).

For the purposes of this study, hippocampal LFPs from a 1-s pre-stimulus period were recorded during each trial and the theta ratios calculated. The relation of the hippocampal theta ratio to CR acquisition rate, CR performance and the hippocampal correlate of the behavioural CR shown as increases in MUA (Berger et al., 1976) were analyzed.

Our results showed that animals with a higher hippocampal theta ratio immediately before conditioning learned faster and also that in these animals the theta ratio was higher throughout both experimental phases. In fact, while the hippocampal theta ratio remained stable in the fast learners as a function of training, it decreased in the slow learners already during unpaired training. In addition, a high hippocampal theta ratio enhanced the hippocampal model of the CR and seemed to be beneficial for CR performance in terms of peak latency during conditioning, but did not have any effect when the animals showed asymptotic learning. Interestingly, whereas slow learners took longer than fast learners to start showing CRs, the following CR acquisition and shaping (Prokasy, 1987) proceeded at a rate compatible with that of the fast learners.

Together with earlier findings, these results implied that the state in which the relative power of the hippocampal theta activity is low is detrimental for learning, and that the state in which hippocampal theta activity dominates is beneficial for learning, at least early in training, before asymptotic performance is achieved.

2.3 Study II: Hippocampal theta-band activity predicts learning rate only early in training during trace eyeblink conditioning in rabbits

As a logical continuation for our first study, we now aimed to examine the relationship between hippocampal theta activity and trace eyeblink conditioning.

As opposed to Griffin et al. (2004) we explored a linear correlation between two continuous data sets (hippocampal theta ratio and learning rate), and not the effect of a manipulation of the training paradigm. In addition to the effects of hippocampal theta activity on learning rate, we also studied the impact of both trace eyeblink conditioning and unpaired training on the magnitude of the hippocampal theta activity at large, a question not approached before.

Hippocampal LFPs were recorded before, during, and after each training session, and the theta ratio was derived. Recordings from multiple time periods were analyzed because the hippocampal theta activity is generally thought to reflect the state of awareness or openness to external stimulation, and we anticipated that this neural state would possibly be altered by the presentation of the conditioning stimuli. Learning rate was quantified both in early (trials to 5th CR) and late (trials to criterion) phases of learning as defined by Prokasy (1987).

As expected, a high theta ratio in the hippocampus predicted faster acquisition of the conditioned response during trace conditioning, but, contrary to previous results obtained using the delay paradigm (Berry & Thompson, 1978; see also Study I), only in the initial stage of learning, when the CS-US association is being acquired (Prokasy, 1987). The presentation of the conditioned stimulus overall elicited an increase in the hippocampal theta ratio. The theta ratio decreased in the unpaired group as a function of training, remained high throughout conditioning in the fast learners and rapidly increased in the slow learners initially showing a low theta ratio.

Our results indicated a reciprocal connection between the hippocampal oscillatory activity and associative learning. The hippocampal theta ratio seems to reflect changes and differences in the subjects' alertness and responsiveness to external stimuli, which affect the rate of learning early in training, and which are in turn affected by both conditioning and unpaired training.

2.4 Study III: Hippocampal theta activity is selectively associated with contingency detection but not discrimination in rabbit discrimination-reversal eyeblink conditioning

Next, we sought to determine if the hippocampal theta ratio is mostly connected to the detection of the contingency between the stimuli used in conditioning or also to the learning of more complex inhibitory associations when a highly demanding and hippocampally dependent (Berger & Orr, 1983; Weikart & Berger, 1986) delay discrimination-reversal eyeblink conditioning paradigm is used.

A high hippocampal theta ratio was not only associated with a fast increase in conditioned responding in general, but also correlated with a slow emergence of discriminative responding due to sustained responding to the non-reinforced conditioned stimulus. No changes in the hippocampal theta ratio across training

were found, probably reflecting the complexity and the high attentional demands set by the task.

The results indicated that the neural state reflected by the hippocampal theta ratio is specifically linked to forming associations between stimuli rather than to the learning of inhibitory associations needed for successful discrimination. This is in line with the view that the hippocampus is responsible for contingency detection in the early phase of learning in eyeblink conditioning (Prokasy, 1987). In addition to previous studies showing a predictive connection between the hippocampal theta ratio and the speed of learning, the present study showed a connection also between the hippocampal theta ratio and the strength of the CS-US association being learned.

2.5 Study IV: Hippocampo-cerebellar theta-band phase synchrony in rabbits

Hippocampal functioning modulates (delay conditioning; Berger et al., 1976; 1986) and, in certain conditions, like early in trace conditioning (Kim, et al., 1995; Takehara et al., 2003; 2002), is crucial in eyeblink conditioning, although it is primarily a cerebellum-dependent form of learning (McCormick et al., 1982; for a review see Thompson, 2005). Thus, the contribution of the hippocampus on eyeblink conditioning is possible only if there is a functional connection between the hippocampus and the cerebellum. A possible means of forming a functional connection is synchronous oscillatory activity, which has been suggested as a mechanism of communication between anatomically distant but functionally related brain structures (Fries, 2005).

We recorded LFPs during trace eyeblink conditioning in rabbits simultaneously from the hippocampus, the cerebellar cortex lobule HVI, and the medial prefrontal cortex, and assessed synchronous activity between these structures by means of calculating the phase synchrony (Palva et al., 2005) at different frequency bands (delta ~2 Hz, theta ~6 Hz, alpha ~12 Hz, beta ~20 Hz and gamma ~60 Hz).

Our results showed that there is a prominent theta oscillation also in the cerebellum and that the cerebellar theta oscillation is synchronized with that of the hippocampus. Furthermore, the degree of phase synchrony in the theta-band increased both as a function of the relative power of the hippocampal theta activity, and as a response to external stimuli. No change across training or correlation with learning rate of this hippocampo-cerebellar theta-band synchrony was found, implying that it is a general and rather stable phenomenon.

We suggest that, while not having causal explanatory power on learning rate, theta-band synchronization reflects communication between crucial brain areas during a conditioning task dependent both on the hippocampus and the cerebellum. This finding helps us understand how hippocampal function can affect eyeblink conditioning.

3 GENERAL DISCUSSION

In sum, the results of the first three studies showed that the neural state reflected by the strength of the hippocampal theta activity i) predicts overall learning rate during simple associative motor learning when the paradigm used in training supports linear acquisition of the learned response (Studies I and III), ii) only predicts the rate of the initial contingency detection if the paradigm used induces more variability in, and less linear accumulation of, learned responding (Study II), iii) and that the predictive power encompasses also the strength of the associations formed between the stimuli used in conditioning (Study III). The results imply that the state in which the relative power of the hippocampal theta activity is low hinders, and that the state in which hippocampal theta activity dominates is beneficial for the formation of associations between external stimuli, both in terms of speed and strength. The results regarding changes in the level of hippocampal theta activity as a function of training suggest that it reflects changes and differences in the subjects' alertness and responsiveness to external stimuli, which affect the rate of learning, and are in turn affected by the demands of the external conditions. The results of the fourth study further imply that one possible mechanism explaining the connection between the hippocampal theta activity and associative learning might be the increased synchrony between theta oscillations in the hippocampus and the cerebellum especially during periods of dominating hippocampal theta activity (Study IV).

3.1 The relative power of the hippocampal theta activity reflects an adjustable tendency of attending to the external environment

One clear focus of discussion is whether the hippocampal theta ratio primarily reflects a stable feature of the subject or the fluctuation of the state of the subject dependent on, for example, the time of day. The studies included in this thesis provide evidence for both views. Firstly, as shown by repeated measures taken

daily over a period of ~2 months (Study III), the hippocampal theta ratio recorded during the pre-stimulus period (semi-spontaneous activity) showed minimal change. Taken into account that the recordings in all studies were carried out between 8 am and 6 pm, varying the exact timing, the hippocampal theta ratio recorded in the absence of stimulation appears to reflect a rather stable tendency of orienting towards the external environment. However, the demands for cognitive processing set by the external environment, in the form of the experimental stimuli, also caused changes in the hippocampal theta ratio. In the absence of associative stimuli the hippocampal theta ratio decreased and in the presence of demanding associative tasks it stayed high or even increased (Studies I-III). From this point of view, the hippocampal theta activity seems to reflect a constantly altering state of the subject. As a conclusion, the results of this thesis suggest that the hippocampal theta ratio reflects the subject's stable predisposition in attending to external stimuli regulated according to the current demands set by the external environment.

3.2 The tendency of attending to the external environment affects the rate and strength of association formation

As shown by the results from previous studies conducted in Berry's group (Asaka et al., 2005; Berry & Swain, 1989; Berry & Thompson, 1978; Griffin et al., 2004; Seager et al., 2002) and the results presented in this thesis (Study I-III), the tendency/state reflected by the relative power of the hippocampal theta activity recorded prior to training predicts the speed and strength of the association formation during subsequent classical conditioning of the eyeblink response in rabbits. The formation of the association occurs in the first phase of the learning process (Prokasy, 1987), and in some cases requires conscious and explicit recollection of the predictive temporal relationship between the CS and the US (Clark & Squire, 1998; Manns, Clark, & Squire, 2000). Generally, trace eyeblink conditioning is thought to require hippocampus-dependent declarative memory of the CS-US association for successful learning: Studies in humans have shown that awareness of the predictive association between the CS and the US is a prerequisite for learning during differential trace eyeblink conditioning (Clark & Squire, 1998), and facilitates learning during single-cue trace eyeblink conditioning (Manns et al., 2000) – the paradigm also used in Study II and in the study of Griffin et al. (2004). What can be classified as a conscious recollection or awareness in animals, and how, is of course debatable. However, it is likely that the hippocampus through its interaction with the neocortex (for a possible mechanism see Sirota et al., 2008) is somehow involved. Consistently with Prokasy's (1987) theory, with studies regarding the role of awareness in humans (Clark & Squire, 1998; Manns et al., 2000), and with the results of Study II, lesioning the hippocampus impairs conditioned responding after trace eyeblink conditioning only if it is carried out early in the process of memory consolidation (rabbits: Kim

et al., 1995; mice: Takehara et al., 2003; rats: Takehara et al., 2002). Furthermore, the effects of theta-contingent eyeblink conditioning, during which trials are systematically administered when the hippocampal theta ratio is either high (T+) or low (T-), are strongest in the early phases of learning (Asaka et al., 2005; Griffin et al., 2004; Seager et al., 2002), when the CS-US association is being formed. All of these results point toward an understanding of the hippocampal theta activity as an indicator of the tendency/state of the subject related especially to openness to external stimuli, and to the readiness to detect and form associations between stimuli occurring close in time.

3.3 Theta activity binds hippocampal and cerebellar function

According to the results of this thesis, whereas the relative amplitude of the hippocampal theta oscillation during a given time period preceding training predicts the rate and strength of association formation during subsequent conditioning (Studies I-III), the hippocampo-cerebellar theta-band phase synchrony does not (Study IV). It seems that although the phase synchrony is stronger during periods of high hippocampal theta ratio (Study IV), the hippocampo-cerebellar phase synchrony in itself does not add to the effects of the amplitude of the hippocampal theta oscillation. Rather, it seems that the hippocampo-cerebellar theta-band phase synchrony binds hippocampal and cerebellar function at some level at all times, thus forming a “landline” between the two anatomically distant but functionally related brain structures. In addition, this continuous binding is especially strong when the hippocampal theta ratio is high, whether as a consequence of spontaneous activity in the brain or as a result of external stimulation. Consistent with the current findings, a role has been suggested for the hippocampal theta activity in coordinating information flow from the neocortex to the hippocampus (Sirota et al., 2008).

3.4 Contingency detection and response acquisition: overlapping or sequential?

In addition to providing information about the role of the hippocampal theta activity as a precursor of learning and memory in reflecting the ability to detect and form associations between external stimuli, the studies included in this thesis also gave new insight into the structure of the learning process itself. The predictive power of the hippocampal theta ratio encompassed both Phase 1 and Phase 2 (Prokasy, 1987) when the delay paradigm was used in conditioning (Berry & Thompson, 1978; Berry & Swain, 1989; Study I and Study III) and the acquisition of the conditioned response proceeded linearly. However, when a more complex paradigm was used (Study II) the level of the pre-training hippocampal theta ratio

predicted learning rate only early in conditioning (Prokasy, 1987). Since the subjects in Study II undergoing trace conditioning and showing significant differences in the rate at which they reached the 5th CR showed equal learning rates later in conditioning, it seems that the two phases of learning – acquisition of the CS-US association and acquisition of the CR – are at least in part independent from each other, when the training paradigm is temporally more complex. In fact, the number of trials needed to reach the 5th CR correlated strongly with the number of trials to asymptotic learning criterion in delay eyeblink conditioning (Study I), whereas during trace eyeblink conditioning this correlation was absent (Study II). Consistently, Griffin et al. (2004) showed that in rabbits receiving trace eyeblink conditioning trials contingent upon low levels of hippocampal theta activity learning was retarded only in the early phase of training.

In sum, the existing evidence supports Prokasy's (1987) theory of two distinct phases in learning: the formation of the CS-US association seems to be dependent on the state reflected by hippocampal oscillatory activity, while response acquisition seems to be mediated by other brain structures, perhaps the medial prefrontal cortex (Takehara et al., 2003) or the cerebellum (for a review see Thompson, 2005). It could be that the two stages of learning, contingency detection in Phase 1 and response acquisition in Phase 2 (Prokasy, 1987), are temporally overlapping processes during delay conditioning, yet sequential processes during trace conditioning.

3.5 Limitations and future directions

First of all, the results obtained in this thesis about the stability of the feature/state of the subject reflected by the hippocampal theta ratio are ambiguous. A possible solution would be to first classify the subjects according to the hippocampal theta ratio they show spontaneously during the first recording session (shown to predict future performance in eyeblink conditioning) and then condition them according to a T+ -procedure, where the trials are presented during periods of spontaneous activity characterized by a high hippocampal theta ratio (see for example Griffin et al., 2004). If the hippocampal theta ratio reflects a stable feature of the individual, T+ -training should not accelerate learning in subjects initially showing a low hippocampal theta ratio.

Also the selection of recording sites in the studies included in this thesis merits further discussion. In Studies I and III recordings of hippocampal activity were made from near the pyramidal layer of the CA1, where the neural model of the CR is formed during eyeblink conditioning (Berger et al., 1976; Berger & Thompson, 1978). In Study III, our decision to record from near the hippocampal fissure and the dentate gyrus was based on the fact, that within the hippocampus, the theta oscillation is biggest in amplitude in those regions (Bragin et al., 1995; Buszáki, 2002). This variation between studies poses a limitation since there is a possibility, although unlikely, that the relative power of the theta activity in the

CA1 has a correlation with learning rate different from the correlation of the fissure/dentate gyrus and learning rate. In the ideal case, recordings in all animals and in all studies would have been made from both the CA1 pyramidal layer as well as from the lower subregions of the hippocampus.

The fact that no correlation between the rate of learning during trace eyeblink conditioning and the hippocampo-cerebellar theta-band phase synchrony was found in Study IV also raises questions. Although a linear correlation was not found, it is still possible that a more subtle and/or non-linear connection does exist between these two phenomena. In addition, the exact phase difference between hippocampal and cerebellar theta and its possible connection to learning rate remains to be examined. Also the precise location of the recording electrodes might have affected the outcome. In Study IV the recordings from the hippocampus came from the fissure/dentate gyrus. In addition, cerebellar activity was recorded from the cerebellar cortex, and not from the interpositus nucleus (IPn), the structure often suggested storing the memory trace of the CR (for a review see Thompson, 2005). It might be that the hippocampo-cerebellar phase synchrony is somehow different if theta activity from the CA1 and the IPn is contrasted, and this phase synchrony might also show a correlation with learning rate.

Some technical limitations also existed when this thesis was conducted. Namely, it was not possible to record single-cell activity along with LFPs in our laboratory, and thus it was not possible to determine whether cells in the cerebellar cortex show rhythmic bursting related to the theta oscillation in the cerebellum and/or in the hippocampus. If in forthcoming studies a correlation was found between the cerebellar single-cell activity and the theta oscillation, it would offer further support to the idea of the theta oscillation as the means of mutual communication between the hippocampus and the cerebellum (Fries, 2005).

What comes to future studies regarding the connection between the state reflected by the level of the hippocampal theta activity and learning rate, some basic paradigms, like long-delay conditioning and extinction, still remain to be tested. It has been shown that hippocampal lesions impair CR acquisition during delay conditioning if the ISI is 1400 ms (Beylin et al., 2001), which implies that the hippocampal theta oscillation might have a crucial part in learning during this more difficult version of the delay conditioning paradigm. In addition, the results of Study III regarding the connection between hippocampal oscillatory activity and learning during discrimination-reversal conditioning suggest that the stronger the hippocampal theta ratio the slower the extinction.

4 CONCLUSION

The results of this thesis confirmed and further clarified that the state in which the relative power of the hippocampal theta activity is low hinders, and the state in which the hippocampal theta activity dominates is beneficial for the formation of associations between the conditioning stimuli, not only in terms of speed but also in terms of strength. As to the nature of the neural state reflected by the hippocampal theta ratio, changes in the level of the hippocampal theta activity as a function of training and learning seem to reflect changes and differences in the subjects' tendency to respond to external stimuli, which affect the rate of association formation, and are in turn affected by the demands set by the external environment. In addition, regarding the question of the hippocampo-cerebellar co-operation necessary in order for the hippocampus to affect learning during eyeblink conditioning, it was shown that a phase-synchronized theta oscillation exists in the hippocampus and the cerebellum especially during periods of strong hippocampal theta ratio. In conclusion, not only does the state reflected by the hippocampal theta activity affect the rate and strength of association formation but also the theta oscillation itself seems to facilitate the co-operation between brain structures essential in associative learning.

YHTEENVETO

Hippokampuksen theta-aktiivisuuden rooli klassisessa silmäniskuehdollistamisessa kaneilla

Klassista silmäniskuehdollistamista on käytetty oppimisen ja muistin hermostollisen perustan tutkimiseen ihmisillä ja eläimillä 1960-luvulta lähtien. Oppiminen tämän kokeellisen asetelman aikana riippuu erityisesti pikkuaivojen toiminnasta, mutta mikäli ehdollistamisessa käytetyt ärsykejärjestelyt eivät suosi nopeaa ja suoraviivaista oppimista, myös hippokampuksen rooli korostuu. Yllättävää kyllä, hippokampuksen tuhoaminen häiritsee oppimista vähemmän kuin sen toiminnan häiritseminen. Tämä johtunee siitä, että hippokampukselle ominainen 6 Hz:n taajuinen sähköinen värähtely eli theta-aktiivisuus näyttelee tärkeää osaa assosiativisessa oppimisessa, oli kyseessä sitten ajallisesti lähekkäisten tapahtumien tai avaruudellisesti lähekkäisten sijaintien yhdistäminen toisiinsa. Erityisesti on aiemmin todettu, että hippokampuksen theta-aktiivisuuden suhteellinen voimakkuus mitattuna ärsykeettömässä tilanteessa ennustaa oppimisnopeutta mittausta seuraavan silmäniskuehdollistamisen aikana. Mitä voimakkaampaa on perustilassa mitattu hippokampuksen theta-aktiivisuus, sitä nopeammin oppiminen tapahtuu. Oppimisen eri vaiheiden tarkempi suhde hippokampuksen theta-aktiivisuuden tasoon oli kuitenkin vielä selvittämättä samoin kuin se, miksi ja miten hippokampuksen suhteellinen theta-aktiivisuuden voimakkuus ennustaa oppimisnopeutta tehtävän aikana, joka riippuu lähinnä pikkuaivojen toiminnasta.

Tämän väitöskirjan kolmessa ensimmäisessä tutkimuksessa selvitettiin tarkemmin, miten hippokampuksen theta-aktiivisuuden suhteellinen voimakkuus yhdistyy oppimiseen klassisen silmäniskuehdollistamisen aikana kaneilla. Sen lisäksi, että tutkittiin miten hippokampuksen theta-aktiivisuuden suhteellinen voimakkuus vaikuttaa oppimisnopeuteen, tarkasteltiin myös, miten ehdollistaminen ja oppiminen vaikuttavat hippokampuksen theta-aktiivisuuden suhteelliseen voimakkuuteen. Oppimista mallinnettiin monimutkaisuudeltaan vaihtelevia ehdollistamisasetelmia käyttäen, ja samanaikaisesti mitattiin solun ulkopuolisen sähköisen jännitteen vaihtelua eli kenttäpotentiaaleja hippokampuksesta, pikkuaivoista ja isoaivokuorelta. Hippokampuksen kenttäpotentiaaleista määritettiin Fast Fourier -muunnosta apuna käyttäen theta-aktiivisuuden suhteellinen voimakkuus, jota verrattiin oppimisnopeuteen. Väitöskirjan neljännessä tutkimuksessa selvitettiin toiminnallisesti yhteen liittyvien hippokampuksen ja pikkuaivojen yhteistoimintaa klassisen silmäniskuehdollistamisen aikana käyttäen menetelmänä vaihesynkronian laskemista kahden samantaajuisen signaalin välillä.

Tulokset osoittivat, että tila, jossa hippokampuksen theta-aktiivisuuden suhteellinen voimakkuus on matala vaikeuttaa, ja tila, jossa hippokampuksen theta-aktiivisuuden suhteellinen voimakkuus on korkea edistää nopeaa ja voimakasta assosiaation muodostamista ehdollisen ja ehdottoman ärsykkeen välille.

Oppimisen ja opettamisen tuloksena tapahtuvat muutokset hippokampuksen theta-aktiivisuuden suhteellisessa voimakkuudessa näyttävät heijastavan eläimen taipumusta reagoida ulkopuolisiin ärsykkeisiin, mikä vaikuttaa assosiaation muodostukseen, ja toisaalta muuttuu ympäristön asettamien vaatimusten mukaan. Lisäksi havaittiin, että hippokampuksen ja pikkuaivojen theta-aktiivisuus on synkronoitunutta, erityisesti silloin kun hippokampuksen theta-aktiivisuuden suhteellinen voimakkuus on korkea. Vaikka tämä vaihesynkronia ei ollutkaan yhteydessä opettamiseen tai oppimiseen, tarjoaa se jatkuvan mahdollisuuden hippokampuksen ja pikkuaivojen yhteistoimintaan, ja saattaa näin selittää, miksi hippokampuksen theta-aktiivisuus on tärkeä pikkuaivoista riippuvaisen silmänisku-ehdollistamisen aikana.

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