TRACE EYEBLINK CONDITIONING AND EXTINCTION CONTINGENT TO HIPPOCAMPAL RIPPLES IN RABBITS

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Two distinct hippocampal oscillatory states have been associated to functions in learning and memory. Theta oscillations (3 – 12 Hz) occurring during attentive exploratory behaviors are suggested to reflect the formation of new memory traces to the hippocampus. In a complementary function, sharp-wave ripples (~200 Hz) occurring during slow wave sleep and quiet restfulness, are essential for memory consolidation. There is experimental evidence that presenting learning trials contingent to theta oscillation enhances learning. The effects of ripple contingent training are unknown, but should be expected to result in slower learning. Rabbits were trained with trace eyeblink conditioning and extinction contingent to hippocampal ripple states, while a yoked control group was trained simultaneously independent of their neural state. Contrary to what was expected ripple contingent training accelerated acquisition of the conditioned response, but slowed down extinction. The results are suggested to be explained by hippocampal oscillatory state dependent properties of neuromodulator systems that have different effects on conditioning and extinction.

Keywords: trace eyeblink conditioning, extinction, sharp-wave ripple, hippocampus

INTRODUCTION

Classical conditioning of the rabbit's eyeblink / nictitating membrane response (Gormezano, Schneiderman, Deaux, & Fuentes, 1962) is a behaviorally well-defined form of associative learning that has become a widely used model for studying the neural substrates of learning and memory. In the classical eyeblink conditioning procedure, an initially neutral conditioned stimulus (CS, commonly a tone or a light) is presented before an unconditioned stimulus (US, an air puff to the eye), that elicits an unconditioned response (UR, an eyeblink). After repeated pairings, the CS comes to elicit a conditioned response (CR), an eyeblink resembling the UR. The procedure is defined as delay conditioning when the CS ends during the US or coterminates with it, and trace conditioning when a stimulus free interval separates the CS from the US.

Neural substrates of classical eyeblink conditioning are one of the most widely studied and best understood mammalian learning and memory circuits (for reviews on animals, see (Christian & Thompson, 2003; Thompson & Steinmetz, 2009); for humans, (Cheng, Disterhoft, Power, Ellis, & Desmond, 2008; Gerwig, Kolb, & Timmann, 2007)). In simple delay conditioning, the cerebellum and its associated circuitry are generally considered to be the necessary brain substrates required for acquiring the conditioned response ((Christian & Thompson, 2005; Krupa & Thompson, 1997; McCormick, et al., 1981) for a review, see (Thompson, 2005)). Trace conditioning depends in addition on intact hippocampal functioning for CR acquisition (Moyer, Deyo, & Disterhoft, 1990; Solomon, Vander Schaaf, Thompson, & Weisz, 1986), but not expression after a sufficiently long period from learning (Kim, Clark, & Thompson, 1995), whereas the hippocampus may not be required for simple delay conditioning (Schmaltz & Theios, 1972). Extinction, the reduction in the frequency of the conditioned response, is generally thought to involve new learning that inhibits CR performance. Extinction can be achieved with CS alone presentation trials or by exposure to the conditioning context alone (Kehoe, Weidemann, & Dartnall, 2004; Poulos, Pakaprot, Mahdi, Kehoe, & Thompson, 2006). The neural substrates of extinction are not as well studied as those of acquisition and retention (for a review, see (Robleto, Poulos, & Thompson, 2004)), however CS alone extinction has been shown to be impaired by hippocampal lesions (Akase, Alkon, & Disterhoft, 1989; Moyer et al., 1990).

Two distinct hippocampal electrophysiological oscillatory states have been suggested to represent complementary processes in learning and memory. The hippocampal state defined by slow theta (3-12 Hz) oscillation (for a review, see (Buzsaki, 2002)) concurrent with attentive exploratory behaviors (Vanderwolf, 1969) is suggested to reflect the ongoing transfer of newly obtained information about

the external world into the hippocampus (Buzsaki, 1989; Buzsaki, 1996). In contrast, high-frequency (~200 Hz) network oscillations (ripples) in CA1 of the hippocampus occurring in conjunction with large-amplitude CA1 sharp waves (SPWs) are suggested to be essential in the consolidation of recently formed memory traces (Buzsaki, 1986; Buzsaki, 1989; Buzsaki, 1996; Chrobak & Buzsaki, 1996; Ylinen, et al., 1995). The SPW ripple state is concurrent with behaviors where there is little interaction with the environment, such as eating, grooming, immobility and sleep (Buzsaki, Leung, & Vanderwolf, 1983; Buzsaki, 1986).

There is experimental evidence to support a causal relationship with learning for both the hippocampal ripple and theta states. Girardeau et al. (2009) found, that suppressing ripples during a period of post-training sleep after a hippocampus-dependent spatial learning task impairs learning. Also, in trace eyeblink conditioning, presenting training trials contingent to theta oscillations results in faster learning, whereas presenting trials in the absence of theta impairs learning (Asaka et al., 2005; Griffin, Asaka, Darling, & Berry, 2004).

Considering that ripples occur exclusively in the absence of theta oscillations (Buzsaki, 2002) and are suggested to represent a specific state of memory consolidation (Buzsaki, 1986; Buzsaki, 1989; Buzsaki, 1996; Chrobak & Buzsaki, 1996; Ylinen, et al., 1995), together with the evidence that trace conditioning contingent to the absence of theta impairs learning (Griffin et al., 2004), invites the question, whether ripple-contingent training would be particularly detrimental to learning. Two groups of animals were trained in trace eyeblink conditioning (Gormezano et al., 1962). One group (R+) received trials contingent to hippocampal ripples, while the yoked control (YC) group received trails coincidently with the R+ group regardless of their hippocampal oscillatory state. It was expected that the R+ group would show impaired learning compared to the YC group.

METHODS

Subjects

Subject were 28 adult male New Zealand White rabbits provided by Lidköpings Kaninfarm (Harlan Netherlands/HB) that at the time of surgery were ~4 months of age and weighted ~2.9 kg. The rabbits were housed at the facilities of the University of Jyväskylä animal research unit in individual metal cages placed in a room where the temperature, humidity, and light-dark cycle (12 h light / 12 h dark, lights on at 6.00 A.M.) were controlled. The rabbits had free access to food and water. All

experimental procedures were carried out during the light portion of the light-dark cycle in accordance with the European Communities Council Directive (86/609/EEC) on the care and use of animals for research purposes.

Surgery

For each rabbit, preceding the onset of surgery (~30 min) subcutaneous injections of an analgesic solution [2 ml; 0.1 ml of buprenorphine 0.3 mg/ml (Temgesic, Schering-Plough Europe), diluted in 1 ml of 0.9% NaCl] and of an anti-inflammatory drug [0.1 ml/kg; carprofen 50 mg/ml (Rimadyl Vet, Pfizer Animal Health)] were given. Anesthesia was achieved before surgery with a 0.8 ml/kg i.m. injection of ketamine-xylazine cocktail, and maintained during surgery with subcutaneous 0.8 ml injections of either the cocktail or ketamine every ~20 minutes. Eyes were prevented from drying during surgery with eyedrops (Oftan, Santen Oy). After the surgery an analgesic (as above) was administered in 8 h intervals for 24 – 48 h, depending on the animals recovery rate. To facilitate recovery, a 0.1 ml/kg subcutaneous injection of metoclopramide [5 mg/ml; Primperan (Sanofi Winthrop Industrie)] was administered 8 h after surgery. The animals were allowed at least 1 week for postsurgical recovery.

In the beginning of surgery, the rabbit was first placed in a stereotaxic instrument (Kopf Instruments) with the head oriented so, that bregma was 1.5 mm higher to lambda. The scalp was incised longitudinally and four stainless-steel anchoring screws were attached to the skull (5 mm anterior and 5 mm lateral to bregma; 13/10 mm posterior and 5 mm lateral to bregma). Conductive wire was used to attach two of the screws together, and these were used as a reference for the electrophysiological recordings. Three monopolar recording electrodes were chronically implanted into the CA1 of the right hippocampus from holes (5 mm posterior and 4, 5, and 6 mm lateral to the bregma) drilled to the skull (for details, see (Korhonen, 1991)). The exact electrode placement in the dorsal-ventral axis (bregma—6.5–7.2 mm) was determined from a brain atlas (Bures, Petran, & Zachar, 1967) and guided by LFPs and multiunit activity during implantation. After the electrodes were in place and attached to a pin connector, the construction was cemented in place with dental acrylic, leaving only the connector visible.

Conditioning procedure

Two groups of randomly assigned rabbits were first given trace conditioning (500 ms trace period) training for 10 sessions (paired trials). One group (R+, n = 14) received trials contingent to exhibiting hippocampal ripples, while the other group (YC, n = 14) received trials parallel to the R+ group. From

the rabbits that met a learning criterion of having exhibited a CR in 8 of 9 consecutive trials or >50% CRs during a single session, new R+ and YC groups were randomized with an additional rule of having half the animals in the new R+ group selected from the previous YC group. These new groups then received 8 sessions of CS-alone extinction training. All the experimental sessions were conducted once per day on consecutive days, with the length of each daily session limited to 50 min or 60 trials. The minimum intertrial interval for all sessions was 15 s.

These experiments were conducted in a ventilated, electrically insulated, and sound-attenuated conditioning chamber, with the rabbits in Plexiglas restraining boxes. Before the first sessions each rabbit spend 20 minutes in the experimental situation to allow for familiarization for the rabbit and testing of the implanted electrodes.

LabView (National Instruments) was used to control the presentation of the CS (a 2 kHz, 85 dB, 200 ms tone) and the US (a 100 ms, 64 dB corneal airpuff with 0.35 bar source pressure), that was delivered through a nozzle with an inner diameter of 2 mm placed 1 cm away from the eye. A steady background noise (65 dB) created by a fan located behind the rabbit was present in the conditioning chamber.

The online detection of ripples in the R+ group was accomplished by comparing a bandbass filtered (80–250 Hz) signal from the hippocampal layer CA1/CA3 to a variable amplitude threshold (120 – 400 μV) during 100 ms time windows. The amplitude threshold varied for each individual electrode depending on signal quality, but was constant for each electrode over sessions. A trial was triggered by a brain-computer interface after a 200 ms delay when the signal exceeded the threshold, if there had been at least 15 seconds from the last trial (Figure 1).

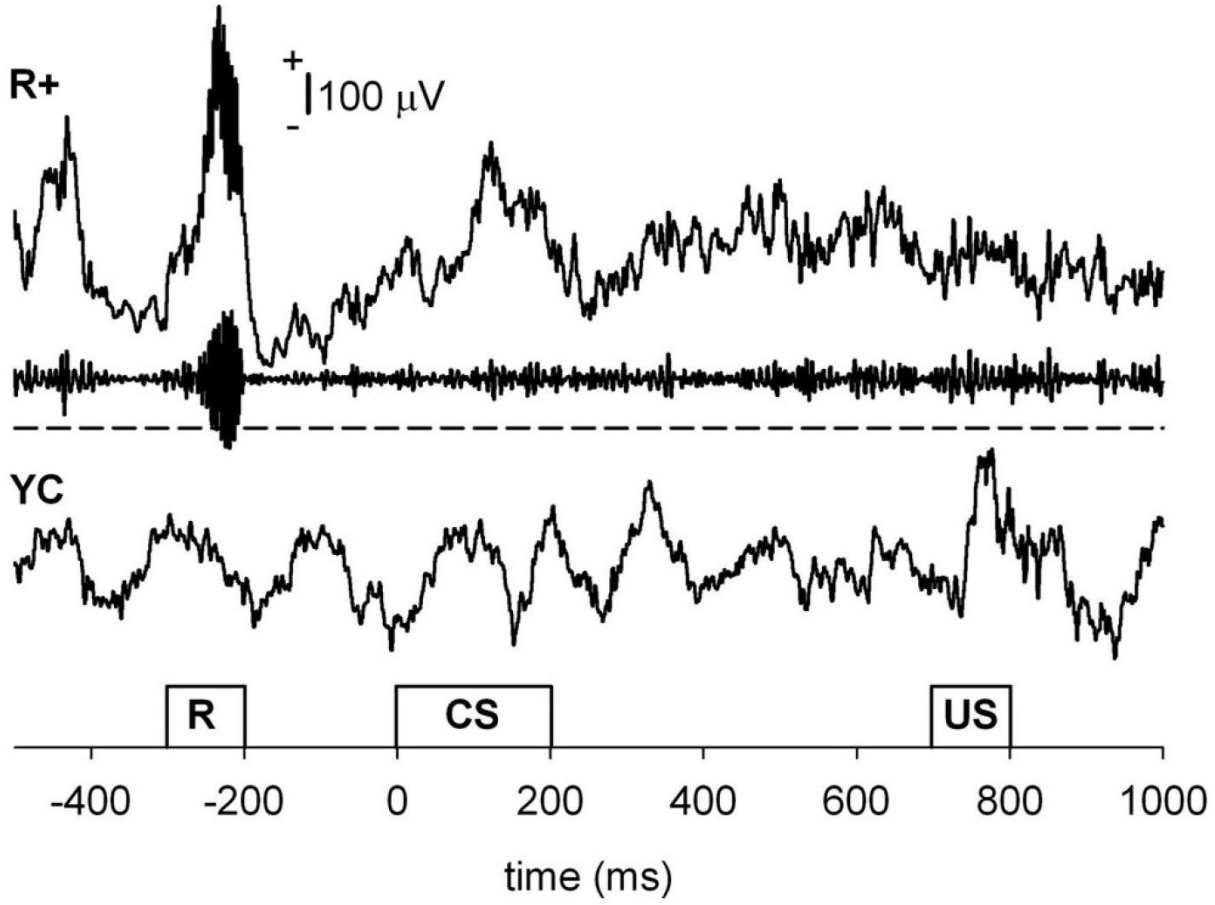

FIGURE 1. Trial presentation example. Trials (CS + US) were triggered simultaneously in both the R+, and YC groups upon the detection of a ripple (R) in the R+ group. The detection of ripples was accomplished by bandpass filtering (80 – 250 Hz) the raw hippocampal LFP signal and comparing it to an amplitude threshold (dashed line).

Recordings and data analysis

During the training sessions both eyeblink and neural signals were measured. The Eyeblink signal was acquired by recording EMG (bipolar electrode) with stainless steel wire hooks placed around the upper and lower eyelids. Neural signals (LFPs) from the recording electrodes implanted in surgery were first passed through a low-noise preamplifier (MPA8I, Multi Channel Systems MCS) directly connected to the electrode coupler in the animals head. The neural signals were then led to an amplifier (Axon Cyberamp 380, Molecular Devices) through a flexible, insulated cable. All the signals were recorded with Axo-Scope (Molecular Devices) software, bandpass filtered (0.1 - 400 Hz for the LFPs and 30 - 300 Hz for the EMG), and then digitized (Digidata 1322A, Molecular Devices) using a 2.02 kHz sampling rate. Clampfit (Molecular Devices), MATLAB (The MathWorks), and SPSS were used for the off-line data analyses.

Behavioral data

Eyeblinks were defined from an envelope curve calculated (using the real and imagery parts of the Hilbert transformation) from a high-pass filtered (over 100 Hz) and Hilbert transformed version of the previously digitized EMG signal, as EMG activity exceeding a threshold (MEANpre + 4 x SDpre). From a 500 ms pre-CS period, baseline EMG activity (MEANpre) was calculated as the mean (*M*) of the EMG amplitude, and the mean of the SD of the EMG activity during the same 500 ms pre-CS period (SDpre) was determined. Trials that showed eyeblinks during a 100 ms period immediately preceding CS onset were rejected from data analysis. Conditioned responses (CRs) were considered to be eyeblinks performed during the 500 ms trace period. Subject that performed a CR on 8 of 9 consecutive paired or CS-alone trials or showed >50% CRs during a single session were considered to have met the learning criterion for asymptotic performance. Learning rate was defined as the number of conditioning trials needed to perform the fifth CR (phase 1 of learning) (Prokasy, 1984). Extinction rate was defined as a performance of <30% CRs during one session.

Statistical analyses

Statistical testing of the behavioral data from the 10 conditioning and 8 extinction sessions was performed on compressed blocks of 2 sessions. Changes across training were analyzed with repeated-measures ANOVA with block as a within-subjects factor, and group as a between subjects factor. Greenhouse–Geisser corrected degrees of freedom were used whenever the sphericity assumption was violated. Block by block comparison of the groups was performed with a one-way ANOVA.

Histology

When the experiments were completed each rabbit was anesthetized with an intramuscular injection of ketamine-xylazine cocktail and then killed with an overdosed of pentobarbital (Mebunat Vet, Orion-Yhtymä Oyj) given by intravenous injection. The brain was perfused with physiological saline and formalin (10 %) solutions delivered through the ascending aorta. To assist in later determining the electrode-tip locations a DC current (200 µA, 20 s) was passed through the electrodes to leave a small mark in the tissue. After the brain was removed it was stored in a solution (10 % formalin + 10 % sucrose) for approximately one week. It was then coronally sectioned into 100-µm slices while frozen. The slices, attached to gelatinized slides, were dyed with Prussian blue and cresyl violet to make the electrode-tip locations visible to inspection with a microscope. A stereotaxic atlas (Bures et al., 1967) was used as a reference in determining the exact electrode-tip locations.

RESULTS

Histology

All 28 subjects had at least one recording electrode correctly placed in the right dorsal hippocampus showing ripple activity (Figure 2).

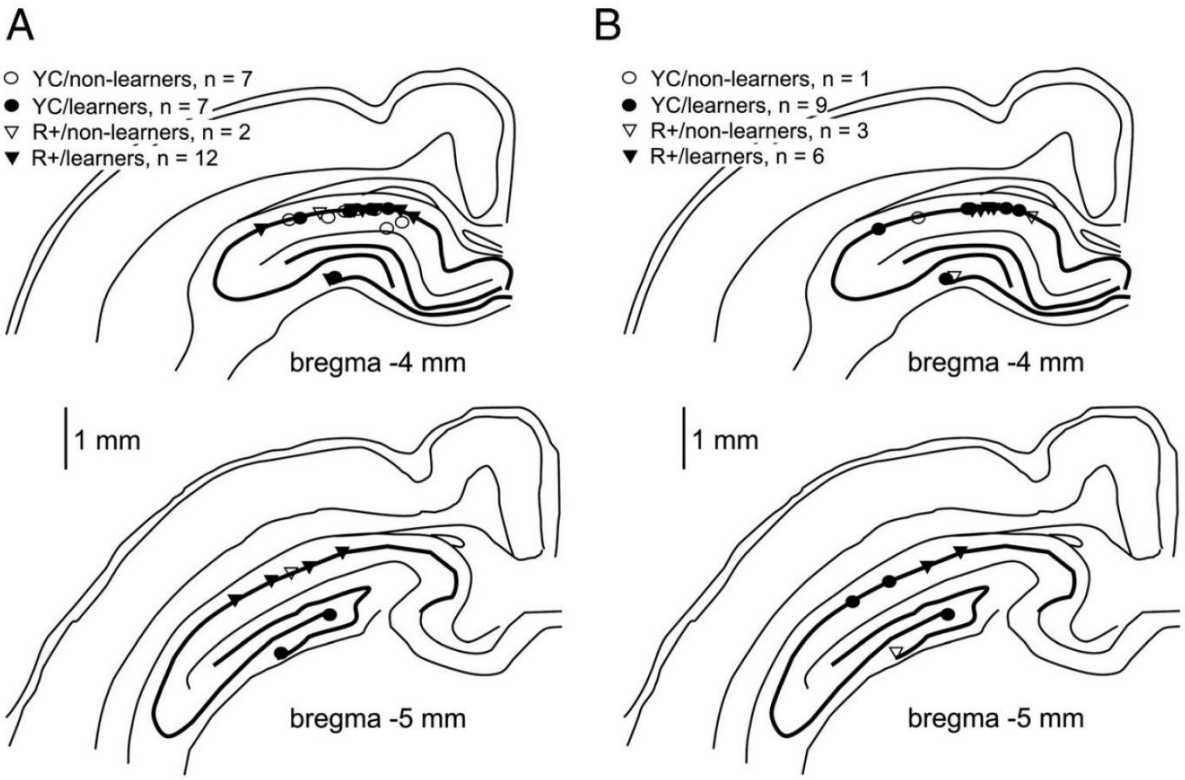

FIGURE 2. Recording electrode locations by group (YC and R+) and learning outcome during conditioning (**A**) and extinction (**B**).

Ripple-contingent training resulted in faster conditioning and slower extinction

Over all sessions conditioning resulted in the acquisition of a CR in both the R+ (n = 14) and the YC (n = 14) groups ($F = 24.46$, d.f. = 4, $P < 0.001$), with no statically significant differences in performance or learning rate between the R+ and YC groups (group: ns; block x group: ns) (Figure 3). Contrary to what was expected, rabbits in the R+ group showed more CRs in the early phase of conditioning (block 1: $F = 5.40$, d.f. = 1, $P < 0.05$) (Figure 3).

The mean number of trials to the fifth CR was 119 (SEM = ± 13.12, min = 13, and max = 297). Rabbits in the R+ group acquired the fifth CR in 94 ± 14.51 trials and those in the YC group in 144 ± 20.24 trials ($F = 3.97$, d.f. = 1, $P < 0.057$). 19 of the 28 animals in trace eyeblink conditioning

acquired a robust CR during 10 sessions. Most of the animals that failed to acquire a robust CR were in the YC group (7 of 9). There was no initial difference in responding between the R+ and YC groups (Figure 4).

The nine animals that failed to meet the learning criterion in the conditioning phase were excluded from the extinction phase. For the extinction phase the animals were regrouped in to R+ (n = 9) and YC (n = 10) groups by counterbalancing their participation to either group during the conditioning phase.

Over all sessions extinction decreased CRs in both the R+ and the YC groups ($F = 15.03$, d.f. = 4, $P < 0.001$), with a larger decrease in the YC group (group: $F = 4.96$, d.f. = 1, $P < 0.05$; block x group: ns) (Figure 5.). In the early phases of learning rabbits in the R+ group showed more CRs than rabbits in the YC group (block 1: $F = 9.32$, d.f. = 1, $P < 0.01$; block 2: $F = 5.20$, d.f. = 1, $P < 0.05$) (Figure 5).

For conditioning the average ITI was 40.89 s (SEM = 0.44 s, min = 17.30 s, and max = 267.02 s), for extinction 39.10s (SEM = 0.40 s, min = 17.00 s, and max = 221.95 s).

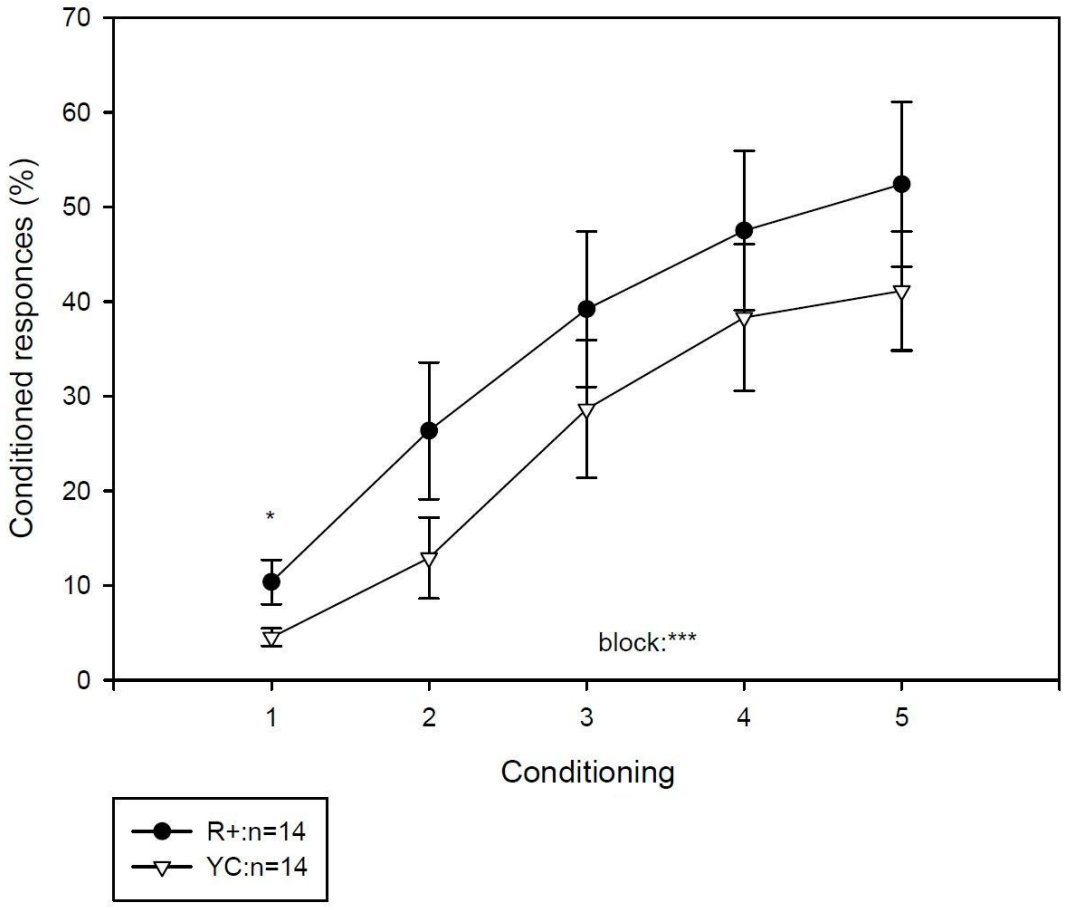

FIGURE 3. CRs as a function of conditioning block. Ripple-contingent training led to faster learning. Asterisks refer to statistical significance: *p < 0.05, ***p < 0.001.

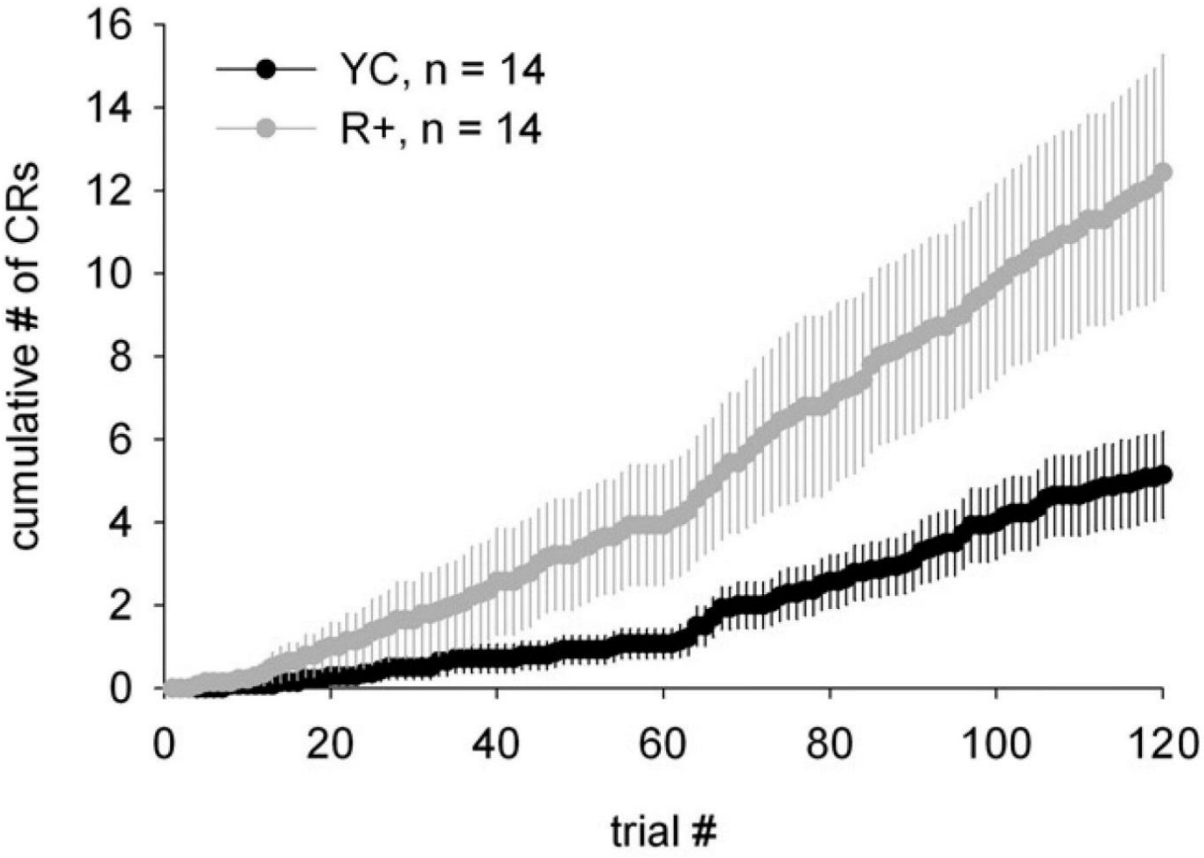

FIGURE 4. Cumulative number of CRs during conditioning plotted as a function of trail number for the R+ and YC groups. Vertical lines depict the SEM.

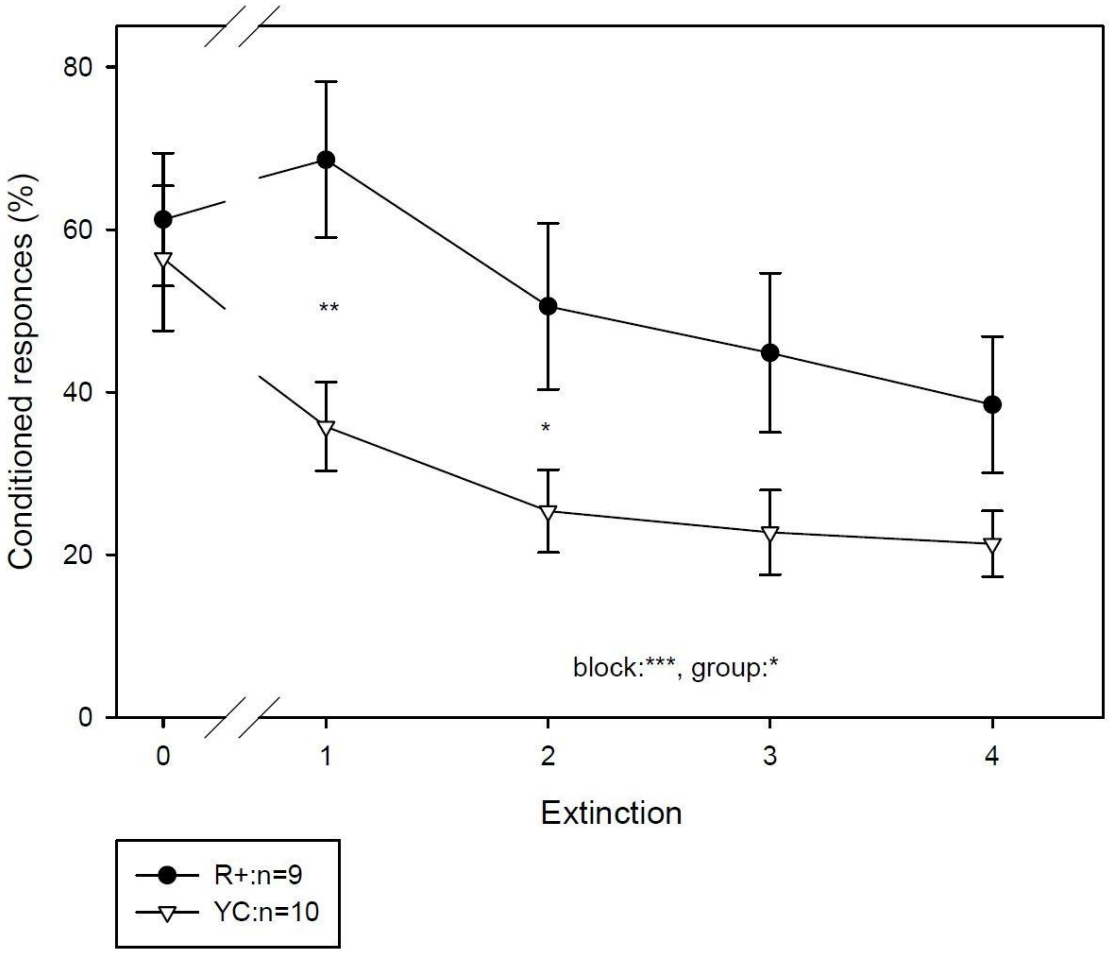

FIGURE 5. CRs as a function of extinction block. Ripple-contingent training led to slower extinction. Asterisks refer to statistical significance: *p < 0.05, p** < 0.01, ***p < 0.001.

DISCUSSION

The above results show that presenting training trials contingent to hippocampal ripple states can affect learning rate in trace eyeblink conditioning and CS alone extinction. Contrary to what was expected, ripple contingent training led to faster acquisition in the initial phase of learning, yet retarded extinction.

Acquisition

The two hippocampal states differentiated as the theta stage by the presence of theta band oscillations and as the ripple state by the presence of sharp wave ripple complexes (SPWRs) and absence of theta band oscillations, have been suggested as complementary stages in memory trace formation (Buzsaki, 1989). Since presenting training trials systematically when the hippocampus is in a particular oscillatory state leads to faster acquisition contingent to the theta stage (Asaka et al., 2005; Griffin, Asaka, Darling, & Berry, 2004; Seager, Johnson, Chabot, Asaka, & Berry, 2002) and also contingent to the ripple state, there are learning enhancing effects from hippocampal state contingent training in relation to at least two separate hippocampal states.

The prevailing theory of memory consolidation proposes that labile memory traces initially formed into the hippocampus during exploratory behavior, coincident with hippocampal theta oscillations, are gradually, by the process of memory consolidation, transferred to the neocortex and strengthened to from long term memories (Buzsaki, 1989). Hippocampal sharp wave-ripple (SPWR) complexes, occurring during slow-wave sleep and quiet wakefulness, are essential for this memory consolidation (Girardeau & Zugaro, 2011). SPWRs are high frequency field oscillations measured from the CA1 pyramidal cell layer, consisting of the activity of CA1 pyramidal cells and interneurons. They are initiated via Shaffer collaterals by bursts of activity in subsets of CA3 pyramidal cells, weakly potentiated during exploratory behavior by fast-firing granule cells. This CA3 activity reflect a 'replay' of previous sensory experience in a temporally compressed manner and has the potential to induce long term synaptic changes inside the hippocampal network and cortical structures ((Buzsaki, 1989) (for a review; Girardeau, 2011)). Since presenting training trials (Nokia, Penttonen, & Wikgren, 2010), or a light stimulus (Nokia, Mikkonen, Penttonen, & Wikgren, 2012) contingently to ripples leaves the ripple oscillation intact, and suppressing ripples impairs learning (Ego-Stengel & Wilson, 2010; Girardeau, Benchenane, Wiener, Buzsaki, & Zugaro, 2009; Jadhav, Kemere, German, & Frank, 2012), the ripples in the R+ group were most likely not suppressed or interrupted, although

the hippocampal oscillatory state after the triggering of a CS from the ripple was likely affected (Nokia et al., 2012). Thus, the result of faster acquisition in the R+ group is not in contradiction with the other evidence cited, as the result are likely not caused by changes to the SPWRs themselves.

In comparison to results obtained from theta contingent trace eyeblink conditioning (Griffin et al., 2004; Hoffmann & Berry, 2009), where the benefits to learning are more evident through the whole learning process until asymptotic learning has been reached, ripple contingent trace eyeblink conditioning accelerated acquisition of the CS – US association only in the initial phase 1 of learning, consisting of contingency detection and response selection (Prokasy, 1984).

Involvement of the hippocampus is crucial in forming associations between temporally separated events, like the CS and the US in trace eyeblink conditioning (Shors, 2004). Generally it has been accepted that trace eyeblink conditioning is hippocampus dependent and delay eyeblink conditioning does not require the hippocampus, although it has been argued that this view is too simplified and does not fit the full range of experimental data (Moustafa et al., 2013). Since hippocampal processing is crucial for the initial phase of learning in eyeblink conditioning, then underlying the different results for theta contingent and ripple contingent training, there are likely to be differences in hippocampal processing during and/or immediately after trial presentation.

There are implications that the hippocampus is more responsive to incoming sensory stimulation when the stimulation is initiated in the ripple state, compared the theta state (Buzsaki, 1986). Thus the training trials could have different effects on the hippocampus depending on the instantaneous hippocampal oscillatory state during which their presentation is initiated. It was reported by Nokia et al. (Nokia et al., 2010) that ripple contingent trace eyeblink conditioning elicits phase locking of hippocampal theta oscillations to the CS early in training. Phase locking to an external stimulus is indicative of stimulus related neuronal processing (Sauseng & Klimesch, 2008) and also, theta band phase locking/reset, in response to relevant sensory stimuli, has been associated to long-term-potentiation (LTP) (Givens, 1996; McCartney, Johnson, Weil, & Givens, 2004; Williams & Givens, 2003), a mechanism of plastic changes in synaptic efficacy directly linked to learning and memory (Shors & Matzel, 1997). Since there was faster learning in the R+ group during acquisition and CS-evoked phase locking has been reported for ripple contingent training (Nokia et al., 2010), it is implied that for the R+ group, the CS was better able to cause a phase locked theta band oscillatory response in the hippocampus early in learning. Thus, it is possible that, in the R+ group, a greater initial CS-evoked phase locking in the theta band (Nokia et al., 2010), enabled by the neural state coincident with hippocampal sharp wave ripples (Buzsaki, 1986), induced stronger plastic changes into the hippocampus, facilitating faster acquisition of the CS-US contingency association in the first phase of learning.

However, in accordance with encoding versus retrieval scheduling models of hippocampal theta phase (Hasselmo & Stern, 2014), theta phase-locking in response to stimuli could reflect memory retrieval, as has recently been suggested by Nokia and Wikgren (Nokia & Wikgren, 2013). Considering this, it is more likely that the theta band phase-locking in ripple contingent training reflects the re-activation of neural ensembles (Fries, 2005) previously associated to the CS. Considered together with the fact that a stimulus (light) unrelated to learning a CS-US association, evokes a similar theta band response, whether presented contingent to ripples or not (Nokia et al., 2012), it seems unlikely that a stronger initial theta band phase locking induced greater plastic changes in the R+ group, but rather a stronger phase locking to the CS would have emerged earlier in training, reflecting faster formation of a memory trace in the hippocampus and it's activation in response to the CS. Thus, a more plausible explanation would seem to be, that an initially similar theta response to the CS (Nokia et al., 2012) in both groups (R+ and Yoked control) was, due to a greater capacity for plastic change (Buzsaki, 1986), able to induce stronger plastic changes when driven from a ripple state by the CS in the R+ group, facilitating faster acquisition of the CS-US contingency association in the first phase of learning (Prokasy, 1984). Stronger phase locking to the CS would emerge in the R+ group earlier in training (Nokia et al., 2010), reflecting the faster acquisition of a memory trace and it's activation in response to the CS (Nokia & Wikgren, 2013).

Extinction

In line with expectations, ripple contingent training lead to slower extinction. However, because CS-alone extinction is a hippocampus-dependent (Akase, Alkon, & Disterhoft, 1989; Moyer, Deyo, & Disterhoft, 1990) learning process, where the original memory trace formed during acquisition is preserved and new learning, that is at least behaviorally inhibitory to the previously learned response, is acquired (Robleto, Poulos, & Thompson, 2004), the results would seem to be in conflict with faster acquisition during conditioning. However, there is conclusive evidence that extinction of the conditioned eyeblink response is dependent on plastic changes distinct from acquisition, at least in part of the cerebellar leaning circuitry (Robleto et al., 2004), that is critical for all eyeblink conditioning (Thompson & Steinmetz, 2009). Thus, the different results for acquisition and extinction might be explained by differences in the neural bases of these two forms of learning. A comparison to theta contingent extinction would be informative, however this is not possible, since no such results have been published.

Neurotransmitters have been implicated as essential in hippocampal-dependent memory (Easton, Douchamps, Eacott, & Lever, 2012). For example, modulation of cholinergic transmission affects

learning rate of aging rabbits in trace eyeblink conditioning (Disterhoft et al., 1996; Disterhoft & Matthew, 2003). Neurotransmitter antagonists and agonists most likely affecting on the hippocampus have been shown to have different effects on acquisition and extinction (Scavio & Wills, 1992; Simon, Knuckley, & Powell, 2004). Interestingly, somewhat similar effects on acquisition and extinction, as the results obtained in this study, have been received with posttraining intravenous injections of the NMDA (N-methyl-D-aspartate) receptor antagonist ketamine, where ketamine accelerated acquisition of trace eyeblink conditioning, but retarded CS-alone extinction (Scavio & Wills, 1992).

There are known differences in neurotransmitter dynamics during different hippocampal oscillatory states that can have influences on learning induced plasticity. Acetylcholine (Ach), a neuromodulator that has long been implicated in learning and memory, is present in high levels in the hippocampus during novelty and exploration correlated with hippocampal theta (Douchamps, Jeewajee, Blundell, Burgess, & Lever, 2013; Easton, Douchamps, Eacott, & Lever, 2012). In contrast, Ach levels are low in slow wave sleep and quiet restfulness when hippocampal SPWRs are prominent. Encoding versus retrieval scheduling (ERS) models (Hasselmo, Bodelón, & Wyble, 2002; Hasselmo & Stern, 2014) posit that the hippocampus performs the encoding of novel information, and the retrieval of stored representations on different phases of the hippocampal CA1 pyramidal layer theta oscillation. Encoding occurs at the peak of theta, and retrieval occurs at the trough. Ach in the hippocampus can affect this encoding-vs-retrieval dynamic by shifting it towards encoding when Ach levels are high, and towards retrieval when Ach levels are low (Douchamps et al., 2013; Easton et al., 2012).

Presuming lower levels of Ach in the hippocampus immediately after ripples, the ERS framework would predict, for the R+ group, better retrieval during acquisition, and interference from the previously learned associations during extinction, as it would have received trials systematically in a state of low hippocampal Ach (Easton et al., 2012). Ach is suggested to work in the hippocampal network by suppressing recurrent inputs, thus reducing interference from stored associations during encoding (Easton et al., 2012). Better performance, in the R+ group, in the initial phase of acquisition could thus have resulted from enhanced retrieval/consolidation in the hippocampal network. Significantly reduced encoding in the acquisition phase due to low Ach levels would not be predicted, as there were no competing associations for the CS (Easton et al., 2012). In the extinction phase low Ach levels would predict poor inhibition of the previously learned association (Easton et al., 2012), and could explain the results for the extinction phase. It is thus a possibility that the results of faster acquisition and slower extinction in the R+ group could be explained by hippocampal oscillatory state

dependent properties of neurotransmitter systems (Ach levels) affecting differently for acquisition and extinction due to their different neural bases.

Conclusions

Although hippocampal ripples are unquestionably important to memory processing (Girardeau, 2011), their exact role in the hippocampal network is not yet clear (Carr, Jadhav, & Frank, 2011). The current study showed ripple contingent trial presentation to have different effect for two different kinds of learning, accelerating acquisition and retarding extinction. There are solid theoretical reasons to suppose these effects are due to hippocampal oscillatory state dependent properties of neurotransmitter systems in the hippocampal learning circuitry, having different effects in the ripple state for acquisition and extinction due to differences in the neural basis of these two form of learning.

REFERENCES

Akase, E., Alkon, D. L., & Disterhoft, J. F. (1989). Hippocampal lesions impair memory of short delay conditioned eye blink in rabbits. *Behavioral Neuroscience, 103*, 935-943.

Asaka, Y., Mauldin, K. N., Griffin, A. L., Seager, M. A., Shurell, E., & Berry, S. D. (2005). Nonpharmacological amelioration of age-related learning deficits: the impact of hippocampal theta-triggered training. *Proc Natl Acad Sci U S A, 102*, 13284-13288.

Bures, J., Petran, M., & Zachar, J. (1967). *Electrophysiological methods in biological research.* New York: Academic.

Buzsaki, G. (1986). Hippocampal sharp waves: their origin and significance. *Brain Research, 398*, 242-252.

Buzsaki, G. (1989). Two-stage model of memory trace formation: a role for "noisy" brain states. *Neuroscience, 31*, 551-570.

Buzsaki, G. (1996). The Hippocampo-Neocortical Dialogue. *Cerebral Cortex, 6*, 81-92.

Buzsaki, G. (2002). Theta oscillations in the hippocampus. *Neuron, 33*, 325-340.

Buzsaki, G., Leung, L. W., & Vanderwolf, C. H. (1983). Cellular bases of hippocampal EEG in the behaving rat. *Brain Research, 287*, 139-171.

Carr, M. F., Jadhav, S. P., & Frank, L. M. (2011). Hippocampal replay in the awake state: a potential substrate for memory consolidation and retrieval. *Nature Neuroscience, 14*, 147-153.

Cheng, D. T., Disterhoft, J. F., Power, J. M., Ellis, D. A., & Desmond, J. E. (2008). Neural substates underlying human delay and trace eyeblink conditioning. *Proc Natl Acad SciU S A, 105*, 8108-8113.

Christian, K. M., & Thompson, R. F. (2003). Neural Substrates of Eyeblink Conditioning: Acquisition and Retention. *Learning & Memory, 10*, 427-455.

Christian, K. M., & Thompson, R. F. (2005). Long-term storage of an associative memory trace in the cerebellum. *Behavioral Neuroscience, 119*, 526-537.

Chrobak, J. J., & Buzsaki, G. (1996). High-frequency oscillations in the output networks of the hippocampal-entorhinal axis of the freely behaving rat. *Journal of Neuroscience, 16*, 3056-3066.

Disterhoft, J. F., & Matthew, O. M. (2003). Modulation of cholinergic transmission enhances excitability of hippocampal pyramidal neurons and ameliorates learning impairments in aging animals. *Neurobiology of learning and memory, 80*, 223-233.

Disterhoft, J. F., Kronforst-Collins, M., Matthew, O. M., Power, J. M., Preston, A. R., & Weiss, C. (1996). Cholinergic facilitation of trace eyeblink conditioning in aging rabbits. *Life sciences, 64*, 541-548.

Douchamps, V., Jeewajee, A., Blundell, P., Burgess, N., & Lever, C. (2013). Evidence for encoding versus retrieval scheduling in the hippocampus by theta phase and acetylcholine. *Journal of Neuroscience, 33*, 8689-8704.

Easton, A., Douchamps, V., Eacott, M., & Lever, C. (2012). A specific role for septohippocamapal acetylcholine in memory? *Neuropsychologia, 50*, 3156-3168.

Ego-Stengel, V., & Wilson, M. A. (2010). Disruption of ripple-associated hippocampal activity during rest impairs learning in the rat. *Hippocampus, 20*, 1-10.

Fries, P. (2005). A mechanism for cognitive dynamics: neuronal communication through neuronal coherence. *TRENDS in Cognitive Sciences, 9*, 474-480.

Gerwig, M., Kolb, F. P., & Timmann, D. (2007). The involvement of the human cerebellum in eyeblink conditioning. *Cerebellum, 6*, 38-57.

Girardeau, G., & Zugaro, M. (2011). Hippocampal ripples and memory consolidation. *Current Opinion in Neurobiology, 21*, 452-459.

Girardeau, G., Benchenane, K., Wiener, S. I., Buzsaki, G., & Zugaro, M. B. (2009). Selective suppression of hippocampal ripples impairs spatial memory. *Nature Neuroscience, 12*, 1222-1223.

Givens, B. (1996). Stimulus-evoked resetting of the dentate theta rhythm: relation to working memory. *NeuroReport, 8*, 159-163.

Gormezano, I., Schneiderman, N., Deaux, E., & Fuentes, I. (1962). Nictitating membrane: Classical conditioning and extinction in the albino rabbit. *Science, 138*, 33-34.

Griffin, A. L., Asaka, Y., Darling, R. D., & Berry, S. D. (2004). Theta-contingent trial presentation accelerates learning rate and enhances hippocampal plasticity during trace eyeblink conditioning. *Behavioral Neuroscience, 118*, 403-411.

Hasselmo, M. E., Bodelón, C., & Wyble, B. P. (2002). A proposed function for hippocampal theta rhythm: separate phases of encoding and retrieval enhance reversal of prior learning. *Neural Computation, 14*, 793-817.

Hasselmo, M. E., & Stern, C. E. (2014). Theta rhythm and the encoding and retrieval of space and time. *Neuroimage, 85*, 656-666.

Hoffmann, L. C., & Berry, S. D. (2009). Cerebellar theta oscillations are synchronized during hippocampal theta-contingent trace conditioning. *Proc Natl Acad SciU S A, 106*, 21371-21376.

Jadhav, S. P., Kemere, C. P., German, W. P., & Frank, L. M. (2012). Awake hippocampal sharp-wave ripples support spatial memory. *Science, 336*, 1454-1458.

Kehoe, E. J., Weidemann, G., & Dartnall, S. (2004). Apparatus exposure produces profound declines in conditioned nictitating-membrane responses to discrete conditioned stimuli by the rabbit (Oryctalagus cuniculus). *Journal of Experimental Psychology: Animal Behavior Processes, 30*, 259-270.

Kim, J. J., Clark, R. E., & Thompson, R. F. (1995). Hippocampectomy impairs the memory of recently, but not remotely, acquired trace eyeblink conditioned responses. *Behavioral Neuroscience, 109*, 195-203.

Korhonen, T. (1991). A method for rapid implantation of multielectrode systems. *Physiol Behav, 49*, 401-403.

Krupa, D. J., & Thompson, R. F. (1997). Reversible inactivation of the cerebellar interpositus nucleus completely prevents acquisition of the classically conditioned eye-blink response. *Learning & Memory, 3*, 545-556.

McCartney, H., Johnson, A. D., Weil, Z. M., & Givens, B. (2004). Theta reset produces optimal conditions for long-term potentiation. *Hippocampus, 14*, 684-687.

McCormick, D. A., Lavond, D. G., Clark, G. A., Kettner, R. E., Rising, C. E., & Thompson, R. F. (1981). The engram found? Role of the cerebellum in classical conditioning of nictitating membrane and eyelid responses. *Bulletin of the Psychonomic Society, 18*, 103-105.

Moustafa, A. A., Wufong, E., Servatius, R. J., Pang, K. C., Gluck, M. A., & Myers, C. E. (2013). Why trace and delay conditioning are sometimes (but not always) hippocampal dependent: a computational model. *Brain Research, 1493*, 48-67.

Moyer, J. R., Deyo, R. A., & Disterhoft, J. F. (1990). Hippocampectomy disrupts trace eye-blink conditioning in rabbits. *Behavioral Neuroscience, 104*, 243-252.

Nokia, M. S., Mikkonen, J. E., Penttonen, M., & Wikgren, J. (2012). Disrupting neural activity related to awake-state sharp wave-ripple complexes prevents hippocampal learning. *Frontiers in Behavioral Neuroscience, 6*, 1-10.

Nokia, M. S., Penttonen, M., & Wikgren, J. (2010). Hippocampal ripple-contingent training accelerates trace eyeblink conditioning and retards extinction in rabbits. *The Journal of Neuroscience, 30*, 11486-11492.

Nokia, M. S., & Wikgren, J. (2013). Phase-locked hippocampal theta-band responces are related to disrciminative eyeblink conditioned responding. *Behavioural Brain Research, 256*, 575-579.

Poulos, A. M., Pakaprot, N., Mahdi, B., Kehoe, E. J., & Thompson, R. F. (2006). Decremental effects of context exposure following delay eyeblink conditioning in rabbits. *Behavioral Neuroscience, 120(3)*, 730-734.

Prokasy, W. F. (1984). Presidential address, 1983. *Psychophysiology, 21*, 1-13.

Robleto, K., Poulos, A. M., & Thompson, R. F. (2004). Brain mechanisms of extinction of the classically conditioned eyeblink response. *Learning & Memory, 11*, 517-524.

Sauseng, P., & Klimesch, W. (2008). What does phase information of oscillatory brain activity tell us about cognitive processes? *Neuroscience and Biobehavioral Reviews, 32*, 1001-1013.

Scavio, M. J., & Wills, J. C. (1992). Posttraining effects of amphetamine, chlorpromazine, ketamine, and scopolamine on the acquisition and extinction of the rabbit's conditioned nictating membrane responce. *Behavioral Neuroscience, 106*, 900-908.

Schmaltz, L. W., & Theios, J. (1972). Acquisition and extinction of a classically conditioned responce in hippocampectomized rabbits (Oryctolagus cuniculus). *Journal of Comparative and Physiological Psychology, 79*, 328-333.

Seager, M. A., Johnson, L. D., Chabot, E. S., Asaka, Y., & Berry, S. D. (2002). Oscillatory brain states and learning: impact of hippocampal theta-contingent training. *Proc Natl Acad Sci U S A, 99*, 1616-1620.

Shors, T. J. (2004). Memory traces for trace memories: neurogenesis, synaptogenesis and awareness. *TRENDS in Neuroscience, 27*, 250-256.

Shors, T. J., & Matzel, L. D. (1997). Long-term potentiation: what's learning got to do with it? *Behavioral and Brain Sciences, 20*, 597-655.

Simon, B. B., Knuckley, B., & Powell, D. A. (2004). Galantamine facilitates acquisition of a trace-conditioned eyeblink response in healthy, young rabbits. *Learning & Memory, 11*, 116-122.

Solomon, P. R., Vander Schaaf, E. R., Thompson, R. F., & Weisz, D. J. (1986). Hippocampus and trace conditioning of the rabbit's classically conditioned nictitating membrane response. *Behavioral Neuroscience, 100*, 729-744.

Thompson, R. F. (2005). In search of memory traces. *Annual Review of Psychology, 56*, 1-23.

Thompson, R. F., & Steinmetz, J. E. (2009). The Role of the Cerebellum in Classical Conditioning of Discrete Behavioral Responses. *Neuroscience, 162*, 732-755.

Vanderwolf, C. H. (1969). Hippocampal electrical activity and voluntary movement in the rat. *Electroencephalogr Clin Neurophysiol, 26*, 407-418.

Williams, J. M., & Givens, B. (2003). Stimulation-induced reset of hippocampal theta in the freely performing rat. *Hippocampus, 13*, 109-116.

Ylinen, A., Bragin, A., Nadasdy, Z., Jando, G., Szabo, I., Sik, A., & Buzsaki, G. (1995). Sharp wave-associated high-frequency oscillation (200 Hz) in the intact hippocampus: network and intracellular mechanisms. *Journal of Neuroscience, 15*, 30-46.