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REVIEW ARTICLE

Disrupting ripples: Methods, results, and caveats in closed-loop approaches in rodents

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Summary

Hippocampal ripple oscillations have been associated with memory reactivations during wake and sleep. These reactivations should contribute to working memory and memory consolidation respectively. In the past decade studies have moved from being observational to actively disrupting ripple-related activity in closed-loop approaches to enable causal investigations into their function. All together these studies have been able to provide evidence that wake, task-related ripple activity is important for working memory and planning but less important for stabilisation of spatial representations. Rest and sleep-related ripple activity, in contrast, is important for long-term memory performance and thus memory consolidation. In this review, we summarise results from different closed-loop approaches in rodents. Further, we highlight differences in detection and stimulation methods as well as controls and discuss how these differences could influence outcomes.

KEYWORDS
memory, memory consolidation, reactivation, replay, sharp-wave ripples, sleep

1 | INTRODUCTION

The hippocampus has long been known to play an important part in a variety of cognitive functions. Over recent years many research groups have advanced in identifying the neural activity patterns that are instrumental for maintaining these functions. The hippocampal sharp-wave ripple (SWR; 100–200 Hz), an oscillatory event connected to both highly synchronous neural firing within the hippocampus and modulation of neural activity in various brain regions, is one of those patterns (Buzsáki, 2015).

Of note, the sharp-wave and the ripple are two separate components of the SWR, that occur in different layers of the hippocampus (Figure 1). Most studies discussed in this review identify and manipulate the ripple component of the SWR (for overview of studies see Tables 1 and 2). For more details on online and offline detection of ripples see Box 1.

Hippocampal ripples are thought to play a role in both the consolidation and retrieval of memories but have also been proposed to contribute to planning of future behaviour (Buzsáki, 2015). Moreover, hippocampal neuronal firing during ripples has been shown to contain elements from both past, i.e. memory reactivations, and future experiences (Buzsáki, 2015; Genzel et al., 2020; Girardeau & Zugaro, 2011; de la Prida, 2020). While early studies provided correlative links between hippocampal ripples, memory reactivations and memory performance (Dupret et al., 2010; Mölle et al., 2006), it is only in the last decade that a select number of studies started to demonstrate the causal relationship. Among others, the use of closed-loop ripple disruption, by which ripples during either rest/sleep (i.e. targeting memory

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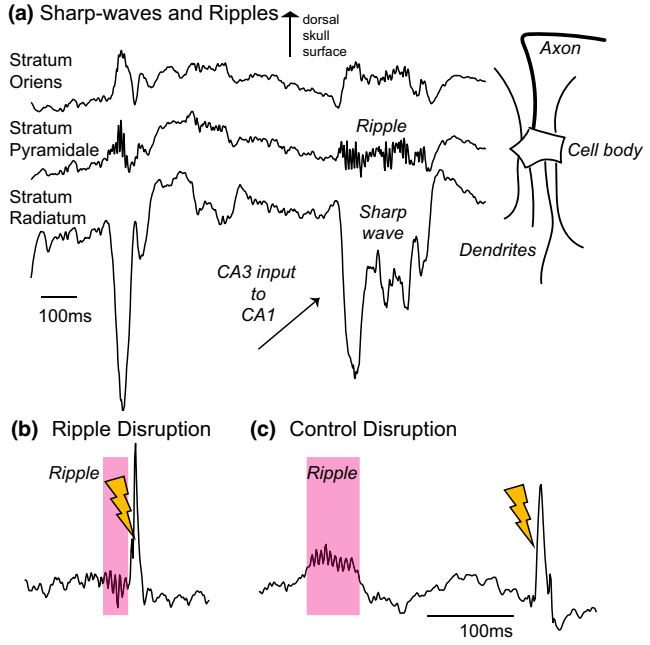

FIGURE 1 Ripples and sharp waves: (a) shown are local field potential recordings from different layers in the hippocampus. The stratum oriens shows an upward deflection during a ripple, the stratum pyramidale the actual ripple oscillations (100–200 Hz) and below the stratum radiatum shows the simultaneously occurring sharp wave. The sharp wave is due to the input coming from CA3 exciting the dendrites of the CA1 pyramidal cells. On the right a cartoon pyramidal cell with the cell body at stratum pyramidale, dendrites both up and down in stratum oriens and radiatum, and the axon leaving upwards. (b/c) Shown are examples for electrical ripple disruption (left) and control disruption with the electrical pulse send with a delay after the ripple (right; from (Aleman-Zapata et al., 2020))

consolidation) or wakefulness (e.g. targeting memory retrieval, working memory, planning or consolidation) are online detected and subsequently disrupted, can alter subsequent memory performance. Both electrical stimulation to the ventral hippocampal commissure as well as direct optogenetic inactivation of selected cells in the hippocampus have been used as disruption methods.

In the present review, our aim is to provide an overview of the different ripple-related, closed-loop intervention methods used in rodent research. We will summarise findings these studies have produced and how they have contributed to our understanding of the function of this oscillation. We will further discuss closed-loop applications used in rodents targeting either other oscillations or more specific cell activity. Finally, we will highlight how detection methods (Box 1), controls (Box 2) and other methods differ across studies (Tables 1 and 2), and discuss the implications of these differences.

2 | RIPPLE DISRUPTION AFTER TASK LEARNING

Ripple events associated with short bouts of neural firing activity in the hippocampus during post-learning rest or sleep have been linked to the reactivation of neural activity of preceding learning experiences and these reactivations have been proposed to be needed for the memory consolidation process (Buzsáki, 2015). While various studies had shown convincing evidence for a link between hippocampal ripples and offline memory consolidation (e.g. Dupret et al., 2010), it was not until the study of Girardeau et al. (2009) that a causal relationship was demonstrated.

Rats were trained in an eight-armed radial maze (Figure 2) daily over 15 days and the authors used closed-loop electrical stimulation upon online detection of hippocampal ripples during the first hour of rest/sleep after learning. This ripple disruption affected performance in the task, while global sleep architecture remained unchanged. In contrast, control rats that either received a delayed stimulation (i.e. stimulation was triggered by the detection of a ripple but delivered with an 80–120 ms delay) or no stimulation, did not show any performance deficits.

Following similar closed-loop electrical stimulation Ego-Stengel and Wilson (2010) disrupted all ripples occurring in the hour after the rats had learned to navigate one of two four-armed radial mazes arranged on a single, large wagon-wheel structure (Figure 2) daily over 8–10 days. Like Girardeau et al. (2009), they found that although stimulation disrupted the ongoing ripples and suppressed further ripples within 1 s of stimulation, no change in sleep/wake structure was observed. Furthermore, rats learned the control maze (not followed by ripple disruption) significantly faster than the test maze (followed by ripple disruption), indicating that ripple disruption during the post-training rest/sleep period impaired memory consolidation and thus slowed down learning the memory task.

Both these studies used a hippocampal-dependent spatial memory task and thus showed that elimination of ripples during the 1-hr post-training consolidation period results in a performance impairment in rats. However, Ego-Stengel and Wilson (2010) also showed that differences in performance by the rats in the test and control maze disappeared little over a week into training. This "delayed" learning could be due to the fact that ripple disruption only took place in the first hour post-training and that for learning to be completely eliminated, longer periods of ripple elimination are needed. Likewise, in the study by Girardeau et al. (2009) rats with disrupted ripples did perform above chance at the end of training. Indeed, Aleman-Zapata et al. (2020) using a one-session learning paradigm in contrast to the previous multi-day learning paradigms showed that a 4-hr post-learning closed-loop electrical stimulation protocol does eliminate learning in the plusmaze (Figure 2) similarly to the performance of rats that received sleep deprivation post-learning. In contrast, the rats performed above chance in the 24-hr test when delayed or no stimulation was applied during this 4-hr period, which is in line with the earlier mentioned studies.

While these studies point towards an important role for hippocampal ripples during the post-learning sleep period, it remained unclear how learning and subsequent consolidation requirements might influence and regulate ripples during sleep. Hence, Girardeau et al. (2014) trained rats on a spatial memory task and showed that when ripples were disrupted by closed-loop electrical stimulation

TABLE 1 Overview of experimental details across different ripple disruption and extension studies

Authors	Model	*N*	Behaviour	Ripple-d timepoint (sleep/wake)	All ripples or selection	Stim. type
Girardeau et al. (2009)	Rat (male)	17 (test method) 26 (task)	Spatial memory task (8-armed radial maze)	Post training; rest/sleep (1 hr)	All ripples	Single pulse (0.5 ms; 5~30 V) stim. through bipolar electrodes in vHC.
Ego-Stengel and Wilson (2010)	Rat (male)	6 (only 5 in results; 1 of those is stopped after 3 days)	Two identical spatial navigation tasks (4-arm radial maze in one single, large wagon-wheel structure)	Post training after one of test maze tasks; rest/sleep (1 hr)	All ripples during NREM and wake (though ripples could occur in the 2-s recovery interval)	Electrical stim. (two biphasic pulses (20–60 μA) with 10 ms interval) of vHC via 3–6 tetrodes.
Jadhav et al. (2012)	Rat (male)	6 ripple-d 6 control stim 4 un-implanted	Spatial alternation task (W-track task)	During the task; wake	All ripples	Single pulse stim. by a bipolar electrode in vHC.
Girardeau et al. (2014)	Rat (male)	6 (within animal control; though not all rats were tested in each condition)	Spatial memory task (8-armed radial maze) and exploratory locomotor task (circular arena)	Post training in 8-arm radial maze, locomotion in familiar circular arena or home cage; rest/sleep (<1 hr)	All ripples	Stim. electrodes in vHC + recording electrodes in CA1 pyramidal layer. Single pulse stim. (0.5 ms).
de Lavilleon et al. (2015)	Mouse (male)	10 (of 40 implanted animals)	Open field	During task or post exploration; wake and sleep (1 hr)	*Wake*: during spikes of place cell *Sleep*: reactivation-related spiking activity of the selected place cell	Polytrodes in CA1, stim. electrode in MFB. Train lasting 100 ms and composed of fourteen 1 ms negative square-wave pulses (140 Hz).
Kovács et al. (2016)	Mouse (female)	3 (within animal control)	(initial) Novel environment, passive exploratory behaviour	Post training, between 1st and 2nd exposure to same enclosure; rest/sleep (3 hr)	All ripples	Microdrive with 15 tetrodes and bilateral 2 × conical optic fibre (stable, placed in stratum oriens) covering dorsal CA1. Closed-loop optogenetic disruption (laser light pulses, green light 200 ms) only affecting Arch-ECFP pyramidal cells in CA1.

(Continues)

TABLE 1 (Continued)

Authors	Model	*N*	Behaviour	Ripple-d timepoint (sleep/wake)	All ripples or selection	Stim. type
Maingret et al. (2016)	Rat (male)	15 out of 23 (12 implanted and 3 un-implanted)	Spatial object recognition task + flower pot for sleep	Post training; sleep (lasted until 1,000 stims had been delivered [~4,000 s of SWS])	All ripples in HPC followed stimulation of motor cortex	Microdrive with six or 16 tetrodes in right mPFC and CA1, incl. bipolar electrode in left motor cortex. Monophasic single-pulse (0.1 ms) of deep layers of motor cortex, delivered by a constant current stimulator. Optimal stim. voltage (17.5–22.5 V) was the minimum necessary to reliably induce propagating delta waves.
Novitskaya et al. (2016)	Rat (male)	22 implanted (divided over three diff. stim. groups), unknown number un-implanted (total reported: 48)	Radial maze	Post training; rest/sleep (1 hr)	All ripples in HPC followed stim. of LC	Monopolar electrical stim. applied unilaterally to LC using trains of bi-phasic square pulses (0.4 ms, 0.05 mA) at 20-100 Hz for 100–200 ms. Low freq. group: five pulses at 20 Hz, 200 ms. High freq. group: 50/100 Hz, 100/200 ms.
Papale et al. (2016)	Rat (male)		Spatial adjusting delay discounting task	During the task; wake		
van de Ven et al. (2016)	Mouse (male)	8 (within animal control; only 7 for ripple-d)	Novel (irregular shaped) and familiar (round) box	Post training after novel or familiar environment; rest/sleep (1 hr)	Selected ripples during sleep (1: identification of assembly patterns during first exploration, 2: track expression of these during subsequent sleep and re-exposure, 3: ripple-d)	Bilateral: 5 tetrodes, 1 optic fibre in CA1. Closed-loop optogenetic disruption (50 or 80 ms light on). Ensemble recordings of CA1 principal neurons.
Roux et al. (2017)	Mouse (male)	5 (within animal control)	Cheeseboard maze (water reward); new set of 3 goal locations daily. Extra test: cue-guided version of the task.	During the task, at goal location; wake	All ripples in a subset of pyramidal neurons in CA1	Drive with silicone probe (4 or 8 shanks, 32 or 64 sites); one or more shanks equipped with etched optical fibres coupled to head-mounted laser diodes for focal stim. Implanted unilateral (2 mice), bilateral (3 mice). Closed-loop optogenetic disruption (60 ms square pulses, approx. 200 μW, 1 per detection).

TABLE 1 (Continued)

Authors	Model	N	Behaviour	Ripple-d timepoint (sleep/wake)	All ripples or selection	Stim. type
Rangel Guerrero et al. (2018)	Mouse (female)	3 (pilot study)	Cheeseboard maze	In-between training sessions; sleep (3 hr)	All ripples	Microdrive array (15 tetrodes) combined with 4 optic fibres, targeting dorsal CA1. Closed-loop optogenetic disruption via 200 ms long green laser pulses (561 nm).
Fernandez-Ruiz et al. (2019)	Rat (male)	5 out of 20 (15 control rats)	W-maze	During the task; wake	All ripples	Microdrive with silicone probes and optic fibres. Closed-loop optogenetic stim. For prolongation: tapered-onset 100 ms-long light stimulus. For truncation: 10 ms pulses.
Michon et al. (2019)	Rat (male)	8 out of 23 used for ripple-d	Dual-environment reward-place association task	In-between training in the two environments; rest/sleep (2 hr)	All ripples	Microdrive array with 24 tetrodes and 3 stim. electrodes in vHC. Biphasic electrical pulses (0.2 ms) varied from 100–500 mA.
Aleman-Zapata et al. (2020)	Rat (male)	6 (within animal control)	(baseline, track, novelty) plusmaze for ripple-d	Post training; sleep (4 hr)	All ripples	Stimulation electrode in vHC. 1/3 of detected ripples were followed by two stim. pulses at 200 and 400 ms.
Gridchyn et al. (2020)	Rat (male)	4 (within animal control)	(double) cheeseboard maze	Post training; rest/sleep (4 hr)	HSEs	128-channel, independently movable electrode arrays + 4 optic fibres in CA1. Assembly detection performed during initial phase of HSEs, and a laser pulse triggered disruption of HSE firing pattern if HSE encoded for target maze.
Oliva et al. (2020)	Mouse (male)	17 (7 ripple-d, 10 control)	Social-recognition task	Post training, between learning and test; sleep (1 hr)	All ripples	Microdrive with unilateral silicon probe (4 or 5 shanks, 64 or 60 sites) targeting HPC and bilateral optic fibre in CA2. Closed-loop optogenetic disruption via 10 ms high-intensity (5–10 mW) light pulses.
Igata et al. (2021)	Rat (male)	17 (5 rats recording electrodes only, 12 recording and stim. electrodes)	Open field, spatial learning task (start, checkpoint 1, goal; update checkpoint - orig. goal) within 1 hr	During the task, after reward relocation; wake	All ripples	Drive with 16 tetrodes (incl., in some, bipolar electrodes in vHC); right dorsal CA1. Closed-loop electrical stim. (single pulse of 100 µs, 140 to 180 µA, stim. rate max. 4 Hz) applied to vHC.

Abbreviations: freq., frequency; HPC, hippocampus; HSE, high synchrony event; LC, locus coeruleus; max., maximum; MFB, medial forebrain bundle; mPFC, medial prefrontal cortex; NREM, non-rapid eye movement; stim., stimulation; SWS, slow-wave sleep; vHC, ventral hippocampal commissure.

TABLE 2 Overview of online and offline detection techniques, detection assessment and controls across studies

Authors	Online detection	Offline detection	Detection assessment	Controls
Girardeau et al. (2009)	Filtering in the ripple band and thresholding	• Band-pass filtering (100–200 Hz), squaring and normalising, then thresholding the field potential recorded in CA1 pyramidal layer. • Ripples were defined as events peaking at >5 *SD* and lasting <100 ms.	Average online detection rate was 86.0 ± 1.3% (*SEM*) of post hoc detected ripple	Delayed trigger (random 80–120 ms)
Ego-Stengel and Wilson (2010)	• Ripple events detected through hardware; filter 100–400 Hz • Causal online filtering delay was determined and corrected by shifting all signals −7 ms. • No use of zero-phase FIR filter to avoid premature stimulation.	• Double-threshold crossing method on absolute value of LFP (mean ripple power + 3 *SD* and + 10 *SD*). • Minimum duration of 30 ms. Gaps smaller than 50 ms were discarded.		No stimulation
Jadhav et al. (2012)	• Monitored power (100–400 Hz) simultaneously across multiple tetrodes (5–6) in CA1. • Threshold had to exceed on at least 2 tetrodes. • Speed filter with threshold of 5 cm/s to 10 cm/s to prevent false positives.	• LFPs filtered between 150–250 Hz. • Ripples detected when a smoothed ripple envelope was above 3 *SD* of the mean for at least 15 ms on at least one tetrode.		Delayed trigger (random 150–200 ms)
Girardeau et al. (2014)	Filtering in the ripple band and thresholding	See Girardeau et al., 2009	Detection rate >83 ± 2%. False detection rate <19 ± 2%	Delayed trigger (random 80–120 ms)
de Lavilleon et al. (2015)	• Spike detection based on voltage threshold on a polytrode channel with Spike2 software. • Threshold manually adjusted to detect highest action potentials using hexa/octrodes.	Semi-automatic cluster cutting using KlustaKwik and Klusters.	Sensitivity of 43.2% (true positive rate). High specificity (false negative rate)	• Non-rewarded stimulation • Control wake-pairing protocol
Kovács et al. (2016)	• Use of an analogue ripple detector circuit as Nokia et al., 2012. • Band-passed filtered differential signal (subtracted stratum radiatum channel from stratum pyramidale channel).			Delayed trigger (1.32 s)
Maingret et al. (2016)	Threshold crossing on the ripple band (100–250 Hz)	• LFP recording in CA1 pyramidal layer was band-pass filtered (150–250 Hz), squared, low-pass filtered and normalised. • Ripple detected when signal was above 2 *SD* for 30 ms to 100 ms and peaked >5 *SD*.		Delayed trigger (random 160–240 ms)

TABLE 2 (Continued)

Authors	Online detection	Offline detection	Detection assessment	Controls
Novitskaya et al. (2016)	Threshold crossing on band-passed (140–240 Hz) CA1 LFP	• LFP band-passed (120–240 Hz). • Ripple detected when ripple-RMS exceeded 4 *SD* threshold of the mean. • Start and end 2 *SD* with min. duration of 0.02 s.		• Random high freq. stim. with 2–11 s interstimulus intervals. • Ripple-triggered low freq. stim. • No stimulation.
Papale et al. (2016)	See Jadhav et al., 2012	See Jadhav et al., 2012		See Jadhav et al., 2012
van de Ven et al. (2016)	• Every 5 ms a copy of the last 20 ms was filtered (125–250 Hz) and another copy was convolved with a Morlet wavelet (160 Hz central freq.). • Detection of average power in the last 10 ms was 4 *SD* above baseline and the maximum of the wavelet-convolved signal was 3 *SD* above baseline.	• Same as McNamara et al. 2014. Signals were band-pass filtered (135–250 Hz) and a signal from a reference electrode was subtracted. • The power (RMS) was calculated per electrode and summed across all CA1 pyramidal cell layer electrodes.	• In rest sessions without light delivery 80.1% ± 1.0% of the ripples were detected. • Average latency of 7.68 ± 0.30 ms before peak power.	Random silencing independent of ripples. Total number of random pulses equal or higher than the average number of pulses delivered in the ripple disruption condition.
Roux et al. (2017)	• The root mean square of a CA1 pyramidal layer signal (80–250 Hz) was computed in two running windows (RMS1 = 2 s and RMS2 = 8 ms). • Ripples detected when RMS2 exceeded 3 × RMS1 for at least 8 ms.	• Filtered signal (80–250 Hz) and instantaneous power was computed. • Events exceeding 2.5 *SD* from the mean were selected. Events shorter than 15 ms were discarded and those closer to 15 ms were merged.	• 83 ± 4% of visually identified ripples were targeted. • 63 ± 4% of all online detected ripples were considered false positives by visual scoring.	Ripple-delayed place cells (random 100–300 ms) and non-silenced place cells
Rangel Guerrero et al. (2018)	See Kovács et al., 2016			Delayed trigger (1.32 s)
Fernandez-Ruiz et al. (2019)	• Detection of sharp wave (8–40 Hz), ripple (80–300 Hz) and neocortex noise (80–300 Hz). • Ripple detected when both events co-occurred in the absence of a noise signal in the neocortex.	• *Ripple detection*: LFP filtered (80–250 Hz) and instantaneous power (clipped at 4 *SD*) was rectified, and low pass filtered (55 Hz). Power of non-clipped signal exceeded 4 *SD* with a min. duration of 15 ms. • *Sharp waves detection:* LFP from str. radiatum filtered (5–40 Hz) with a duration of 20–400 ms. Events exceeded 2.5 *SD*. • *SWR*: Simultaneous sharp waves and ripples.		• Delayed trigger (random 400–1,000 ms). • No stimulation.

(Continues)

TABLE 2 (Continued)

Authors	Online detection	Offline detection	Detection assessment	Controls
Michon et al. (2019)	• Ripple power was summed across electrodes and ripples were detected when it exceeded a linear combination of mean and mean absolute deviation estimates of the ripple power. • Detection of noise in the cortex to discard spurious ripples.	• The ripple envelope (140–225 Hz) was averaged across recording sites, smoothed and detrended. • A ripple was detected when crossing 8 *SD* above the mean.	• Detection rates above 1 Hz led to increased false positives. • A maximum detection rate of 1 Hz was set in the online detection.	Delayed trigger (random 100–250 ms)
Aleman-Zapata et al. (2020)	• Bandpass-filtered the LFP signal (100–300 Hz). • A user-defined threshold was applied to the rectified band-passed signal.	• LFP band-pass filtered on the ripple spectrum (100–300 Hz). • Thresholding of voltage peaks with a minimum duration of 30 ms. Two detected ripple peaks closer than 50 ms were merged.		• Delayed trigger (200 and 400 ms) • No stimulation.
Gridchyn et al. (2020)	• High synchrony events were detected when, in a 20 ms window, the number of detected spikes exceeded a threshold of 3.5-times the mean spike numbers in a 20-ms windows during the pre-rest session. • Threshold adjusted to achieve a 1 Hz detection rate. • Real-time decoding determined which environment was being encoded.	• Spike sorting and pyramidal unit discrimination performed similarly to Csicsvari et al. 1999. • Environment preference was determined based on unit firing rate increase, coherence, and sparsity of place fields.	• Most events occurred earlier than ripples and ~50% of events were followed by a ripple within 50 ms. • The time interval between the threshold crossing and the deflection in the LFP caused by the light pulse was 1.04 ± 0.09 ms	No stimulation if high synchrony event encoded the control maze.
Oliva et al. (2020)	• Bandpass-filtered the LFP signal between 100 and 300 Hz. • Detection of noise in the neocortex.	• LFP filtered (100–300 Hz) and instantaneous power (clipped at 4 *SD*) was rectified, and low pass filtered (55 Hz). Power of non-clipped signal exceeded 4 *SD* with a minimum duration of 15 ms.		• Delayed tigger (random 500–1,000 ms). • Cre (negative) animal.
Igata et al. (2021)	• A smoothed envelope of a tetrode band passed signal (100–400 Hz) was computed. • Ripples detected when the animal's running speed was <5 cm/s and the envelope exceeded the detection threshold of 3–4 *SD* above the mean computed during periods in the rest box.	• LFP filtered at 150–250 Hz. Smoothed envelope. • Ripple power during periods with a running speed of <5 cm/s in the task periods were computed per tetrode. • Only considered detections with a duration of 50–500 ms.		• Delayed trigger (250 ms). • No stimulation.

Abbreviations: FIR, finite impulse response; LFP, local field potential; min., minimum; RMS, root mean square.

during the subsequent sleep period, ripple occurrence was upregulated compared with control sleep periods where stimulations were delayed in time and thus did not interfere with ripples. Moreover, upregulation of ripples did not occur when ripple disruption was applied during sleep following random foraging in a familiar environment. These results indicate that ripple occurrence during post-training sleep is triggered by learning during the task and not just by a spatial experience.

All studies discussed so far used electrical stimulation to disrupt hippocampal ripples during the post-training period, whether this encompassed quiet rest and/or sleep. However, with the technological advances of recent years, another closed-loop disruption method became available, namely optogenetics. Optogenetics is a neuromodulation method that utilises light of a specific wavelength to control neurones that have been genetically modified to express light-sensitive ion channels. This means that, instead of targeting larger areas of the brain as is done with electrical stimulation, optogenetics can be used to control the activity of single neurones or specific neurone types.

The first study to report ripple disruption using this method is by Kovács et al. (2016). In this study, mice were trained on a novel maze after which they were allowed to sleep for 3 hr before being re-exposed to the same maze. During half of the experiments, optogenetic disruption in mice expressing archaerhodopsin (Arch-enhanced green fluorescent protein [EGFP]) in the pyramidal cells of the CA1 area was performed upon ripple detection during the first post-exploration sleep period, while during the rest of the experiments, detection-triggered light pulses were delivered with a delay as a control condition. Kovács et al. (2016) found that newly formed representations in CA1 are not affected by optogenetic ripple disruption and they thus conclude that, at least for passive exploratory behaviour, ripple-related plasticity is not needed for the stability of CA1 cell ensembles.

Van de Ven et al. (2016) utilised a similar method to disrupt ripples in mice in the first hour post-training in a novel or familiar environment. In particular, cell assembly patterns formed by repeated neuronal co-firing in the mouse hippocampus were identified during the exploration of one of the two environments, then tracked during subsequent rest/sleep and then tested in the context of re-exposure. During some of the 1-hr rest/sleep periods between the two maze exposures ripple disruption was performed via a single light pulse upon ripple detection, while during other rest/sleep blocks random silencing (i.e. via a matched number of pulses delivered independently of ripples) took place. They show that reinstatement of assembly patterns representing a novel, but not familiar, environment correlated with their offline reactivation and was impaired by the optogenetic ripple disruption. Furthermore, only those assembly patterns that gradually gained strength during initial encoding of the novel environment depended on offline reactivation.

How can these differences in results between two studies that both use a passive exploratory task and similar disruption techniques be explained? First of all, while both use optogenetic disruption techniques, the study by Kovács et al. (2016) disrupted ripples for 3 hr after the task, while Van de Ven et al. (2016) only applied ripple disruption during the first hour after exploration. Furthermore, Kovács et al. (2016) used a delayed, ripple-triggered disruption as a control condition, whereas Van de Ven et al. (2016) randomly delivered a matched number of light pulses independently of ripples as a control. Although these differences in methods could explain the opposing results, there is also another explanation possible. As Van de Ven et al. (2016) show that early stabilised neuronal patterns in a novel environment do not seem to require reactivation during sleep, it is possible that those are the newly formed representations that Kovács et al. (2016) targeted in their disruption study.

So far, the outlined studies examined the effect(s) of ripple disruption in the CA1 region of the hippocampus during the resting period following a spatial task. However, the hippocampus is also proposed to play a role in non-spatial memories in which ripples may as well play a role. To investigate this further, Oliva et al. (2020) used closed-loop optogenetics to examine the role of CA2 ripples during the consolidation of social memory (i.e. the ability to recognise and remember a member of one's own species). They show that disrupting CA2 ripples upon detection, in contrast to random delayed disruption, during the 1-hr post-training session impaired the social-memory recall of mice in the following test period. A previous study on social memory also reported that the genetically targeted inactivation of CA2 pyramidal neurones does not affect spatial memory performance (Hitti & Siegelbaum, 2014), which suggests a functional difference between CA1 and CA2 ripples, with CA1 being more involved with spatial memory and CA2 with social memory.

Combined these studies using ripple disruption during rest/sleep show that ripples occurring during the post-training rest/sleep period are needed for consolidation and thus correct performance in different spatial (and social) memory tasks during re-exposure. Conversely, ripples and thus ripple-related plasticity seems to not be required for the stability of CA1 cell ensembles during the rest period following a passive exploration task. Thus, ripple regulation during post-training rest/sleep seems to be triggered by the learning component of the task and not just by a spatial experience.

3 | RIPPLE DISRUPTION DURING TASK EXECUTION

Besides occurring during NREM sleep, ripple events are also known to occur during the awake state, specifically during moments of inactivity while performing a task. This is in contrast to awake replay during quiet rest after they finished the task (included in the section above) (Buzsáki, 2015; Samanta et al., 2020). Ripples during tasks are linked to awake memory retrieval (*reviewed in* Carr et al., 2011) and are thought to play a role in navigational planning by means of prospective replay (Genzel et al., 2020; Pfeiffer & Foster, 2013). However, there is currently still a debate on their role in prospective replay (Gillespie et al., 2021; Papale et al., 2016).

The first study to investigate the causal relationship between awake ripple activity during task execution and the memory function

BOX 1 Ripple detection techniques offline/online

Developing methods for online detection of hippocampal ripples has been a crucial step in closed-loop studies. In general, most online detection methods mimic the offline detection of ripples, which typically consists of bandpass-filtering CA1 channels in a defined ripple range and thresholding above a selected rectified amplitude or power value. Ripples are detected when this threshold is exceeded for a minimum duration of 15–30 ms.

Online detection methods

Methods used for the online detection of hippocampal ripple can vary widely across studies. One variation is how the ripple frequency range is defined. The ranges used across studies span from 80 Hz up to 400 Hz. In particular, Ego-Stengel and Wilson (2010) and Jadhav et al. (2012) used a wide range of 100–400 Hz, while studies like Novitskaya et al. (2016) and Dutta et al. (2019) use narrower ranges such as 140–240 Hz and 150–250 Hz, respectively. Besides the difference in frequency ranges, the implementation of the signal pre-processing and ripple detection can vary depending on if it is being done digitally or with analogue circuits with reduced latencies (Ego-Stengel & Wilson, 2010; Nokia et al., 2012). The use of a single- or multichannel detection of the hippocampal ripple is another difference across studies. Kovács et al. (2016) and Rangel Guerrero et al. (2018) used a differential signal subtracting a channel in stratum radiatum from a channel in stratum pyramidale or subtracting a cortical reference electrode from a hippocampal channel. Jadhav et al. (2012) monitored the power across five to six tetrodes in CA1 and triggered a stimulation when a threshold was exceeded in at least two of them. Given that this study focussed on the detection of awake ripples it also made use of a speed filter to prevent false positives due to rodents high-speed movements. A particular method of detecting awake hippocampal ripple is the one used by Fernandez-Ruiz et al. (2019). With their method both a ripple and a sharp-wave (8–40 Hz) channels were rectified and a detection was considered when both events co-occurred. The simultaneous detection of the sharp-wave-ripple complex by Fernandez-Ruiz et al. (2019) may be a good way to reduce potential false positives but it relies on the assumption that only those ripples coupled to a sharp-wave constitute the true positives. Further, this method can only be applied when recording with silicone probes or other electrodes that are placed in different layers of the hippocampus. One disadvantage of most methods employed is that while large ripples are easy to identify, it could be more difficult to decide if smaller, high-frequency oscillations are true positive ripples.

Comparison of online and offline ripple detections

To assess the performance of the online detection of ripples it is important to compare its detections with those of an offline ground truth. This ground truth could be an offline detection of hippocampal ripples, visually scored ripples (Roux et al., 2017) or synthetic ripples (Dutta et al., 2019). Few publications report metrics comparing online and offline detections (also see Table 1), but those that do, do generally report detection rate, false positive rate, average latency, sensitivity, and specificity. Girardeau et al. (2009), Van de Ven et al. (2016) and Roux et al. (2017) reported on average a detection rate of 80%.

Besides the detection rate, the detection latency of ripples could be of special interest. Disrupting at different phases of the hippocampal ripple may have implications that are still unknown. For this reason, the latencies of the online ripple detectors may be an important metric to control and to consider when performing closed-loop experiments. The characterisation of the system used by Dutta et al. (2019) allowed the differentiation between hardware and algorithmic delays, showing that algorithmic delays varied significantly depending on parameters used, namely the detection threshold. The authors propose the use of a relative latency metric. This metric determines what fraction of the ripple was transmitted prior to its detection. Relative latencies of 20%–50% were computed for a range of detection thresholds. These values coincide with Ego-Stengel and Wilson (2010) who reported that stimulations occurred about one-third after the ripple onset. As Dutta et al. (2019) suggest, a fractional disruption of the ripple may explain the impairment found in experiments with goal-directed behaviours that require reactivation of sequences, compared to other experiments which found no impairment in non-goal-directed tasks. An environment recognition task is less dependent on sequential memories than a goal-directed task and therefore its consolidation is expected to be less affected by ripple disruption. A fractional disruption after a non-goal directed task may allow rapid consolidation of a subset of memory traces as observed by Van de Ven et al. (2016) who showed that early stabilised cell assembly patterns of novel environments were rapidly consolidated and were not affected by ripple disruption.

Dutta et al. (2019) also investigated several trade-offs between the metrics that are determined by the parameters used in the design of the online detection of ripples. The accuracy of a ripple detection depends on the selected threshold and the variability of ripple amplitudes. A low threshold would give a high percentage of true positives but also a high number of false positives. In contrast, a higher threshold would reduce false positives but would omit the smaller ripples that are true positives. Furthermore, considering that a ripple has a rising time before reaching its peak, increasing the detection threshold also increases the latency of the detection. Finally, given that larger ripples also tend to be longer, large ripples show lower relative latencies than smaller ripples. In other words, accuracy and latency metrics are not homogeneous for larger and smaller ripples.

Overall, the selection of a threshold depends on the assessment that the investigators make about whether they should prioritise having a higher sensitivity (high true positives and false positives) or a higher selectivity (less false positives and true positives). This principle also applies in the threshold selection used to identify online spiking activity (Ciliberti et al., 2018; de Lavilleon et al., 2015).

BOX 2 Controls

To assess the effects of the ripple-triggered stimulation, several control methods have been applied when investigating closed-loop electrical and optogenetic interventions. One control condition employed is the complete absence of stimulation. In this condition the investigators avoid disrupting neural activity upon ripple detection. This control condition was initially used by Ego-Stengel and Wilson (2010) to investigate the differences in learning in the presence and the absence of hippocampal ripples, and their associated replay events. They justify the sufficiency of this control by showing that the electrical stimulation used did not have an effect on either the sleep architecture of the resting period, the baseline level of neuronal activity or the activity during the ripples. Several studies included a non-stimulation control in addition to other controls (Aleman-Zapata et al., 2020; Fernandez-Ruiz et al., 2019; Roux et al., 2017; Van de Ven et al., 2016). Fernandez-Ruiz et al. (2019) found no differences in rat performance on the outbound component of a W-maze after closed-loop ripple prolongation when using either a non-stimulation or a delayed-stimulation control. On the other hand, Van de Ven et al. (2016) and Roux et al. (2017) found the non-stimulation to be a better control compared to their other respective control methods for optogenetic-ripple disruption.

In order to consider the effect of the stimulation independently of ripple disruption, a method of control is to stimulate after a period of time following ripple detection. The duration of this delay period is often defined as a random value within an interval of time, but it could also be a fixed value. Examples of delay periods employed in ripple-triggered closed-loop experiments are shown in the Figure of this box. Most of the delay periods used with electrical stimulation have a duration of <300 ms. Notably, experiments making use of optogenetic stimulation showed the most diverse delay intervals. Kovács et al. (2016) and Rangel Guerrero et al. (2018) used a fixed delay of 1.32 s, which would allow the occurrence of multiple consecutive ripples compared to shorter delay values. Roux et al. (2017) used a random delay interval similar to those used in electrical stimulation experiments but with a larger variance. Fernandez-Ruiz et al. (2019) used the largest random delay interval found, with a minimum value of 400 ms and maximum value of 1 s. A large interval like this one allows a more random-like stimulation time, as well as keeping the control stimulation farther away from the ripple and potential ripple bursts. Lastly, Aleman-Zapata et al. (2020) made use of a stimulation delay of 200 ms after ripple detection, which was followed by a consecutive stimulation at 400 ms for one-third of the detected ripples. The purpose of this procedure was to control for the homeostatic-like increase of ripple occurrence following ripple disruption during sleep found by Girardeau et al. (2014), and to maintain a similar number of stimulations delivered in both control and disruption conditions.

A delayed-stimulation aims to control the effect of the stimulation independently of the ripple disruption by ensuring that the control stimulation is still in the same brain state e.g. non-rapid eye movement (NREM) sleep. However, the stimulation delay is time-locked to ripple detection, thereby not making it completely random nor independent of ripple occurrence. In addition to a non-stimulation control, Van de Ven et al. (2016) made use of a random stimulation control, which is not dependent on ripple occurrence. In their approach, random intervals between stimulation pulses are sampled from a uniform random distribution. The random durations between pulses could range between 30 ms and X ms, where X is a value determined per animal to ensure that the total number of random pulses is equal or higher to the number of pulses delivered during the ripple disruption condition. The random control used by Van de Ven et al. (2016) has been criticised by Rangel Guerrero et al. (2018) in their discussion. They argue that by not including a ripple-triggered delayed stimulation, the investigators did not control specifically for ripple disruption and may have instead created light-related inhibition and artefacts that may explain the differences between their results and those of Kovács et al. (2016).

Some experiments require stimulation of specific cell types in a region of the brain, which cannot be done using electrical stimulation. Optogenetic stimulation is a method that has been used to express specific hippocampal cells when studying ripple disruption. In particular, Roux et al. (2017) used a light-delivery system designed to stimulate only small circuits of the CA1 region of the hippocampus. By using this selective stimulation, they were able to classify place cells into silenced, delayed, and a non-silenced control group. Roux et al. (2017) found that non-stimulation was a better control compared to a random delay and reported that the delayed control used in their comparisons of place fields destabilisation often did not show consistent effects nor reliable correlations, suggesting that place field stability depends on the timing of neuronal suppression with respect to ripples. To investigate the need of CA2 ripples in the consolidation of social memory, Oliva et al. (2020) used optogenetic stimulation in Amigo2-Cre mice that expressed channelrhodopsin-2 (ChR2) in CA2 after having received Cre-dependent adeno-associated viral (AAV) injections. One control group comprised Cre^{+} animals expressing ChR2 with stimulation after a random delay (500–1,000 ms). A second control group comprised Cre^{-} animals, injected with the same Cre-dependent ChR2 AAV, which received light stimulation upon ripple detection. In summary, this study shows that the non-expression of a protein which leads to light excitability is also a valid control method for ripple-triggered optogenetic disruption.

There are other types of control conditions that are specific to each experiment. Novitskaya et al. (2016) used a ripple-triggered electrical stimulation in the locus coeruleus (LC), which had either low- or high-frequency pulses. This was done to stimulate either

spontaneous or robust burst firing of LC neurones that regulate noradrenaline release. In addition to the ripple-triggered low-frequency stimulation, this study included a random high-frequency stimulation and the absence of stimulation as controls.

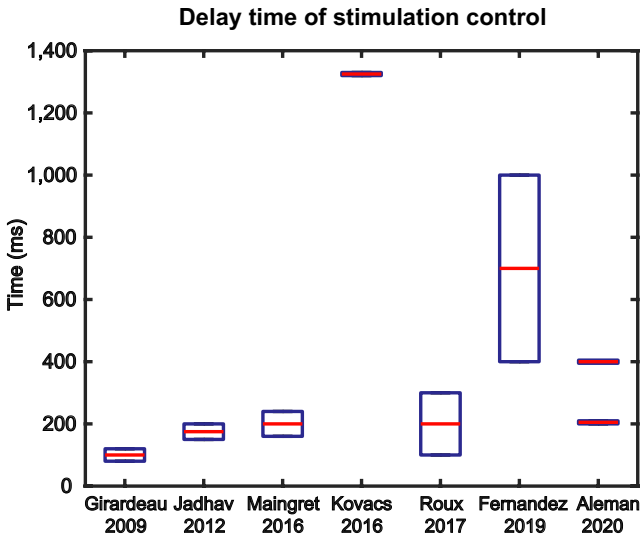

Figure Box 2: Delay intervals used in ripple-triggered stimulation controls. The red line indicates the median value within each interval.

of the hippocampus was by Jadhav et al. (2012). They trained rats in a spatial alternation task, the W-track maze (Figure 2), and disrupted all awake hippocampal ripples upon detection, during the 8 days of the learning the task, using closed-loop electrical stimulation similar to the previously discussed studies disrupting ripples during post-training sleep (Ego-Stengel & Wilson, 2010; Girardeau et al., 2009, 2014). After receiving awake ripple disruption during the task, rats showed a performance deficit for the outbound component of the task but not for the inbound component, while place field activity and ripple reactivation during post-training rest/sleep remained intact. Thus, this study shows that awake ripples support memory-guided decision-making, as the outbound component of the task requires both retrieval of remote memory and working memory. The unaffected inbound performance and the fact that both place field activity and ripple reactivation during the post-training rest period remained unchanged, suggest that the loss of awake ripples does not disrupt subsequent memory consolidation. Nonetheless, how awake ripples, hippocampal maps, and memory consolidation are linked remained to be investigated.

Roux et al. (2017) hypothesised that hippocampal ripples are required for the stabilisation of the spatial map coded by place cells in CA1 during learning. To test this hypothesis, they used focal, closed-loop optogenetic disruption of a subset of pyramidal neurones upon detection of ripples (i.e. at the goal location), while the mouse performed in a hippocampus-dependent spatial memory task named the "cheeseboard" maze (Figure 2). They then compared the place fields of disrupted place cells with those of simultaneously recorded non-disrupted place cells and with those of place cells that were disrupted after a random delay following ripple detection (i.e. control conditions). Place fields of ripple-disrupted place cells remapped, and their spatial information remained unaltered, compared to a gain in the delayed-disrupted or non-silenced cells. Furthermore, although ripple disruption did not impact the firing rates or proportion of place cells, interference of ripples during learning did prevent stabilisation and refinement of the hippocampal map. In contrast to this and similar to the results from Jadhav et al. (2012), ripple disruption of place cells during wake did not impact their activity during NREM sleep ripples (i.e. firing rates, participation, spike count, and gain between pre- and post-learning). While Roux et al. (2017) thus provided evidence that neuronal activity associated with hippocampal ripples is required for both stabilising and refining hippocampal maps, no behavioural effect was shown.

Recently, Igata et al. (2021) used a new spatial task in which rats learned, over the span of 2 hr, new trajectories towards a stable goal location to show that the majority of place fields remained stable irrespective of learning, indicating that a generalised memory map is formed within the hippocampus. Closed-loop electrical ripple disruption during the learning process resulted in unstable trajectories of the rats across trials (in contrast to rats that received the stimulation with a delay or stimulation while not learning a new trajectory) but no change was observed in the number of trials needed to reach the learning point, number of trials until the rat first did a start to check point 2 direct run (meaning a new trajectory) or number of trials needed from when the rat first took this new trajectory until learning point. The authors thus concluded that learning-dependent ripples and replay events support the evaluation and/or reinforcement of specific behavioural patterns during the learning process, which of the two cannot be distinguished from the behavioural readout.

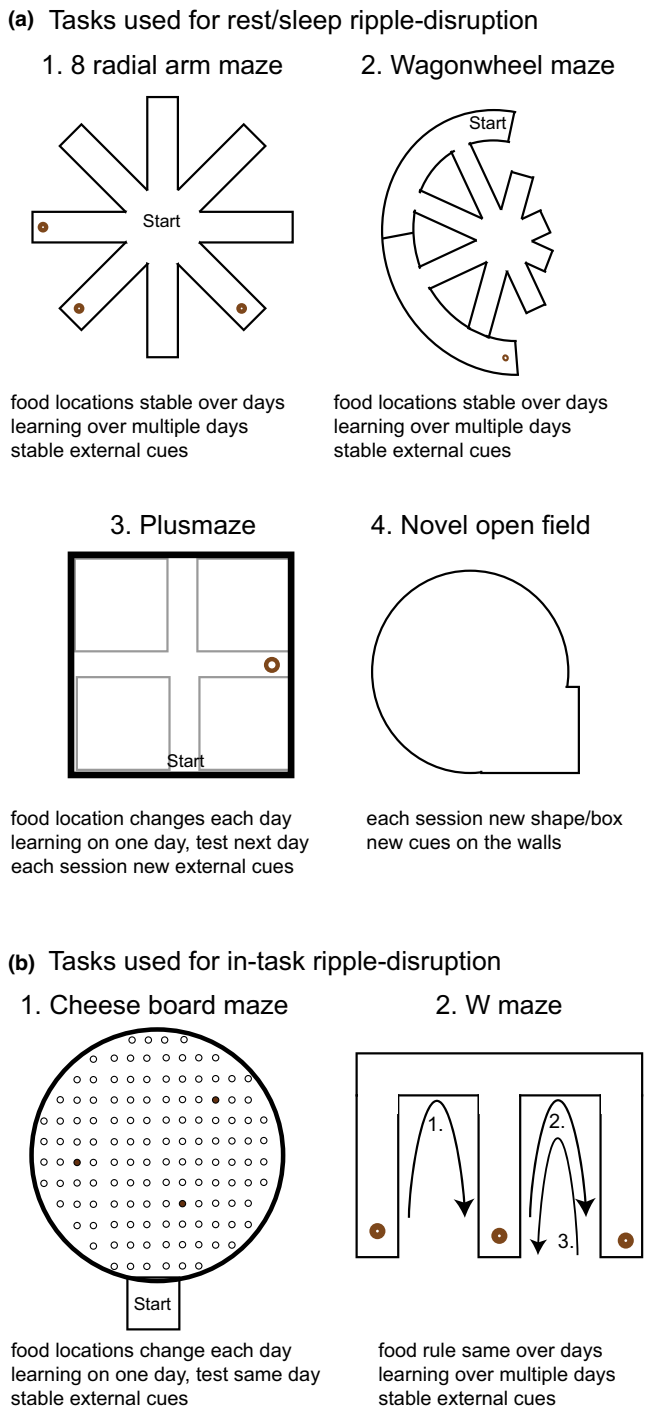

FIGURE 2 Tasks used in ripple disruption experiments targeting rest/sleep (a) and targeting the task period (b). (a.1) In the radial arm maze animals learn which of the eight arms are baited with food (usually three). Animals need to be trained for multiple days before performing above chance. (a.2.) In the wagon wheel maze animals learn to navigate from one fixed start location to one fixed goal location. Animals need to be trained for multiple days to perform well. (a.3) Plusmaze as used by (Aleman-Zapata et al., 2020). Each day (and therefore for each condition) a new arm is baited and each trial in that day has a different start location (different start arm). Each day also will include new external cues for orientation. Animals only need to be trained for 20 min to perform above chance at the 24-hr test. (a.4) For the novel open field environment an animal is placed in a new box, with a new shape and new cues on the wall and is free to explore. (b.1) In the cheese board maze each day (and therefore for each condition) the animal learns three new goal locations (baited ports), which are tested after a short (~1 hr) delay period on the same day. After pre-training the animals perform stable over a long time period; the external cues are stable over time. (b.2) For the w-maze (also known as m-maze) the animal learns the rule to go from outside arm in (1), then from the inside arm to the other outside arm (2) and then back from the outside arm in (3). After pre-training the animals perform stable over a long time period; the external cues are stable over time

Taken together, studies examining ripple disruption during wakefulness indeed indicate a role for these awake ripples in online navigational planning and thus in performance.

4 | CELL ACTIVITY AND ENSEMBLE APPROACHES

Most of the previously discussed closed-loop studies have focussed on the detection of the hippocampal ripple to trigger either an electrical or optogenetic stimulation. However, such an approach remains unspecific and does not allow the detection of specific neural activity, which encodes spatial memories and is reactivated during ripples. With the aim of solving this issue, several investigators have developed new detection methods based on the spiking activity of neural populations.

By using hexatrodes and octrodes, de Lavilleon et al. (2015) measured action potentials in the CA1 pyramidal layer of the hippocampus, which they used to detect spikes after applying an amplitude threshold. The experimenters adjusted the threshold and compared spike waveforms online until identifying a putative pyramidal neurone, which firing maps displayed a place field. Subsequently, this neurone was included in an open-loop brain state-dependent stimulation (terminology as described in Antony et al., this issue) approach during which each time the spike of the place cell was detected, a rewarding stimulation of the medial forebrain bundle was triggered with a delay of <2 ms. This spike-triggered stimulation took place both during a wake-pairing and a sleep-pairing protocol, and as a result induced a preference of the animal towards the corresponding place field of the place cell when returned to the box. Thus, with this approach de Lavilleon et al. (2015) provided the first causal proof that a place cell encodes spatial information (i.e. a specific place in the open field), which can be coupled to a positive valence during wakefulness and sleep.

Despite its improved performance over ripple-detection approaches, the method used by de Lavilleon et al. (2015) did not detect the specific neural pattern associated with the reactivation of cell assemblies instead only single neurone activity was detected. For the online detection of neural sequences, it is necessary to use a neural decoding method that directly relates spikes amplitude features to the location of the rodent during the task (Kloosterman

et al., 2014). To achieve this, Ciliberti and Kloosterman (2017) developed a multithreaded software platform to detect bursts of multiunit activity at low latencies. In a later work, Ciliberti et al. (2018) used this system to identify hippocampal neuronal replay sequences with a 50 ms latency. The performance of this system was further improved by using a graphics processing unit (GPU)-powered implementation, which allowed parallelisation of the neural decoding and its application in high-density silicone probe recordings (Hu et al., 2018).

With the aim to disentangle the contribution to spatial learning of individual cell assemblies occurring during ripples, Gridchyn et al. (2020) used a real-time decoding system to, during sleep, detect and optogenetically disrupt the reactivation of cell assemblies that encoded a specific goal location in a cheeseboard maze. A second identical maze, but with different extra-maze cues and goal location, was used as a non-disruption control. Disrupting the reactivations of the goal location in the target maze led to a recall impairment, while recall of the undisrupted goal location in the control maze was unaffected. In addition, only the place map of the disrupted environment was not reinstated during memory recall. Finally, the investigators showed that the original place map of the disrupted goal location was reinstated after the animal relearned the goal. Besides being able to decode reactivations of specific place fields in the cheeseboard, this cell assembly detection approach allowed inclusion of reactivations occurring during low amplitude ripples, which are usually missed in ripple-triggered closed-loop experiments.

Overall, with this cell-assembly specific approach, Gridchyn et al. (2020) could show that sleep reactivation of a cell assembly encoding a specific location facilitates the retrieval of such memory without affecting other spatial memories. However, sleep reactivation may not be needed for the stabilisation of place fields themselves given that they re-emerged following retraining of disrupted goal locations.

5 | IN SITU AND IN VIVO RIPPLE CREATION

Although studies using ripple disruption have shown that intact hippocampal ripples are required for both memory consolidation and retrieval, they do not provide an answer to the question of how ripples are generated within the hippocampus. In the following section we will focus on recent studies that made use of stimulation techniques in slice preparations or in vivo to create or positively manipulate ripples either directly within the hippocampus or via another brain region.

5.1 | Hippocampus – in situ

One way to investigate the role of the hippocampus in ripple generation is to use hippocampal slices and electrically stimulate the different hippocampal regions. Behrens et al. (2005) used this technique to stimulate rat hippocampal slices with high frequency and theta burst stimulation. They show that induction of long-term potentiation (LTP), a neurophysiological correlate of learning and memory, in the recurrent CA3 network can facilitate the generation of ripples. Moreover, ripples generated in CA3, propagated to CA1 and the subiculum, and showed similar physiological and pharmacological characteristics of naturally occurring ripples.

Sadowski et al. (2016) build further on this and used a selection of spike trains recorded from CA3 and CA1 place cells of implanted rats on a single day during post-training rest, in order to stimulate acute hippocampal slices prepared from naive, non-implanted rats. In addition, in order to examine if these spike trains were capable of inducing plasticity had they been synaptically coupled, whole-cell patch-clamp recordings from CA1 pyramidal cells were made. They show that reactivated (place cell) firing patterns are indeed able to induce LTP at synapses between CA3 and CA1 cells but only if they are accompanied by ripple-associated synaptic activity and thus resulting dendritic depolarisation. Moreover, the timing of coincident CA3 and CA1 place cell spikes in relation to ripple onset needs to be precise for the induction of LTP.

This study thus confirms an important role for ripples in both triggering and fine tuning the plasticity processes that underlie memory consolidation in the hippocampus during rest or sleep. However, large stimulation of CA3 is not essential to facilitate the emergence of ripples. Jiang et al. (2018) administered weak (~1 Hz) stimulation to CA3 and simultaneously recorded LFP from the CA1 region of mouse hippocampal slices. Not only did this stimulation result in the emergence of ripples in CA1 these ripples had a large variation in both amplitude and ripple pattern, which are similar to spontaneously occurring ripples. An interesting result given that the stimulus parameters were identical and supposedly activated the same CA3 neurones around the stimulation electrode.

5.2 | Hippocampus – in vivo

A logical follow up of the in situ studies was to investigate if ripples could be created or positively manipulated in vivo as well. Fernández-Ruiz et al. (2019) used closed-loop optogenetic stimulation (i.e. 100-ms light pulses) targeting neurones in CA1 of implanted rats through which they were able to double the length of spontaneous occurring ripples. They then tested the memory performance of these rats in a hippocampus-dependent W-maze task (Figure 2) and showed that closed-loop ripple prolongation significantly increased performance on the outbound component of the task compared to no-stimulation or random-stimulation controls but had no effect on the inbound component. The prolongation of CA1 ripples thus improved working memory performance and the experiments were an elegant counterpart of the previous wake-ripple disruption in the same maze by Jadhav et al. (2012). Interestingly, the lengthening of CA1 ripples through optogenetic stimulation did not generate repeated spiking of already active neurones but rather initiated spikes from a low-firing population of pyramidal cells and thus increased

the diversity of the participating neurones. This increase of active neurones may explain why memory performance improved following closed-loop prolongation of spontaneous ripples. Future research should elucidate what the information content of extended ripples is, as one would expect that information has to be memory specific to be able to create a gain-of-function effect. Without this information, it remains speculation how exactly extending ripples lead to better memory consolidation.

5.3 | Via other brain regions

Besides directly stimulating different areas of hippocampus to examine how ripples are regulated, another approach would be to try to manipulate hippocampal ripples via regions known to be connected to the hippocampus. Wang et al. (2015) performed in vivo simultaneous recordings in the mouse median raphe region (MnR), of which the neurones project to the entire hippocampal formation (Acsády et al., 1996; Azmitia & Segal, 1978; Varga et al., 2009; Vertes et al., 1999) and hippocampus. They show that when a group of MnR neurones were active, ripples were absent. Next, applying optogenetic stimulation or inhibition to MnR neurones caused ripple activity to be suppressed or increased, respectively. In addition, Wang et al. used optic-fibre implanted mice in a fear-conditioning test and through this were able to show that photostimulation of MnR neurones interfered with memory consolidation. Taken together the results of the study by Wang et al. (2015) indicate an important role for MnR in regulating hippocampal ripples and memory consolidation.

Another region connected to the hippocampus is the locus coeruleus (LC). The LC is the brain's main source of noradrenaline but can also release dopamine (Duszkiewicz et al., 2019), both which are implicated in synaptic plasticity (*reviewed in* Tully & Bolshakov, 2010). To examine how ripple-associated LC activation affected hippocampal and cortical activity, and spatial memory, Novitskaya et al. (2016) trained implanted rats on a radial maze. Following the first hour after each daily training session, open-loop electrical stimulation was applied to LC upon detections of ripples. Although low (20 Hz) stimulation pulses did not cause changes in neural activity nor had an effect on spatial learning, high frequency (100 Hz) pulses temporarily blocked the generation of ripple-associated spindles in the cortex and caused a memory deficit. The timing of LC stimulation is key in this, as random LC stimulation did not cause any memory impairment. The decoupling of co-ordinated hippocampal-cortical activity due to LC activation and the following negative effect on memory shows that hippocampal–cortical communication has an important role in offline memory consolidation.

These two studies, targeting regions functionally connected to the hippocampus, show that ripples are not only regulated from within the hippocampus but that also the activity or silence of other subcortical regions like the MnR and LC play a vital role in the generation or function of hippocampal ripples.

Taken together, while these in situ and in vivo studies are able to show that the generation of hippocampal ripples is dependent on more than just the CA1 and CA3 region of the hippocampus, the question for future research remains how the large variation in both amplitude and ripple pattern of naturally occurring ripples arises.

6 | OTHER CLOSED-LOOP STUDIES IN RODENTS

Thus far we have discussed closed-loop applications used in rodents targeting ripples directly in the hippocampus or indirectly via connected brain regions, during both sleep and wakefulness. While the results of all previously mentioned studies point towards an important role for hippocampal ripples in memory consolidation and retrieval, they are certainly not the only brain rhythm linked to memory. Especially memory consolidation is thought to involve an interplay of hippocampal and cortical oscillations (Latchoumane et al., 2017). In the following section we will briefly look into closed-loop studies targeting cortical slow oscillations, thalamo-cortical sleep spindles or hippocampal theta.

6.1 | Slow oscillations and spindles

Maingret et al. (2016) examined the role of the hippocampal–cortical dialogue in memory consolidation. They trained rats on a short-exposure object location task, which should cause encoding of information but not result in memory consolidation and thus long-term memory expression. Subsequently, while the rats slept, ripple-triggered electrical stimulation was applied to the neocortex targeting the deep layers of the motor cortex. This open-loop stimulation to the neocortex induced both propagating delta waves and sleep spindles, which resulted not only in a reorganisation of the neuronal activity in the selected medial prefrontal cortex neurones but also increase the prefrontal responsiveness to the task on the next day. Moreover, the rats showed a higher recall performance during the task compared to when the same rats did not receive open-loop stimulation during post-training sleep. Maingret et al. (2016) thus present a causal role for hippocampal–cortical communication during sleep in memory consolidation, by illustrating the involvement of a fine-tuned co-ordination between hippocampal ripples followed by cortical delta waves and sleep spindles as an underlying mechanism.

That co-ordination of different brain rhythms during sleep is vital for memory consolidation is also shown in the study of Latchoumane et al. (2017). First mice were trained in a combined cued/contextual fear-conditioning task, then during the 6 hr post-learning period the mice were allowed to sleep and received one of three stimulations protocols: in phase, out phase, or no stimulation. Optogenetic open-loop stimulation (i.e. four light pulses) was applied to the thalamic reticular nucleus, the subcortical generator of sleep spindles, as soon as the animal entered NREM sleep in order to induce spindle activity. Spindles induced in-phase with cortical

slow oscillation up-states, but not out-of-phase induced spindles, improved consolidation of hippocampus-dependent memory during sleep. This in contrast to in-phase optogenetic suppression of thalamic spindles, which impaired hippocampus-dependent memory. Furthermore, optogenetically stimulated spindles were as efficient as spontaneously occurring spindles in nesting hippocampal ripples within their excitable troughs, stimulation in-phase with the slow oscillation up-state increased spindle co-occurrence and frontal spindle-ripple co-occurrence, ultimately resulting in increased triple coupling of slow oscillation-spindle-ripple events without affecting overall sleep architecture. Next, they tested the hippocampal dependency of the effect of in-phase spindle stimulation, in the same mice, using an object-location recognition task, which is known to be sensitive to the effects of sleep (Binder et al., 2012). Similarly, the mice showed enhanced place memory after receiving stimulation in-phase, but not out-phase, with the slow-oscillation up-state during post-learning NREM sleep. Taken together, the results of this study suggest that spindle stimulation phase-locked to the slow oscillation up-state specifically enhances consolidation of hippocampus-dependent memory during sleep.

6.2 | Hippocampal theta

Theta oscillations, between 4–12 Hz, occur among other brain regions within the hippocampus during REM sleep and wakefulness (i.e. motor activity), and they have been linked to encoding memory and spatial navigation. Under normal circumstances theta oscillations and hippocampal ripples do not occur simultaneously. Nokia et al. (2012) show what effect it would have on learning if these two rhythms would coincide. They trained adult rabbits on a hippocampus-dependent associative learning task called the "trace eye blinking task". During the inter-trial interval, when the rabbits were awake, they were presented with a bright light either upon ripple detection or at random, or no light was presented as a control treatment. Rabbits that were presented light upon ripple detection showed decreased learning compared to the controls. Although the light stimulation did not disrupt the actual ripple; it did evoke a theta-band oscillation to occur coincidently with the ripple. The results of this study thus suggest that accurate consolidation, at least partly, depends on neuronal activity taking place directly after hippocampal ripples.

Nonetheless, the theta cycle has its own role in learning and may help the brain to keep incoming information separated from information already stored in memory. Using closed-loop optogenetic stimulation, Siegle and Wilson (2014) inhibited the dorsal CA1 region of the hippocampus during specific phases of the endogenous theta rhythm while mice performed in a spatial navigation task. They show that the performance of mice in this task, that required both encoding and retrieval of a reward location on every trial, was enhanced due to this intervention. Stimulation given during encoding enhanced performance when inhibition was triggered by the peak of theta. On the other hand, when inhibition was triggered by the trough of theta an enhancement in performance during retrieval could be observed. Together these results suggest the different phases of the theta cycle play a role in encoding and retrieval of task-relevant information.

Overall, not only the actual occurrence of hippocampal ripples, but a fine-tuned co-ordination between hippocampal ripples and other hippocampal (i.e. theta) and cortical oscillations (i.e. slow oscillations and spindles) is important for the correct encoding, coconsolidation,nd retrieval of memories.

7 | CONCLUSION AND OPEN QUESTIONS

With the start of the closed-loop disruption experiments, research investigating the importance of ripples moved from purely correlative, observational nature to testing necessity. More recently these approaches have become more refined to specifically target cell types with optogenetics or information content with assembly decoding. In the future, the approaches are likely to become even more sophisticated and will target e.g. cells sending specific projections to or receiving from other brain areas. Furthermore, current approaches are still mostly focussed on the hippocampus, therefore in the future we will likely see more interventions targeting ripple-related activity in other brain areas. Finally, the first experiments using approaches with gain-of-function and targeting sufficiency have been provided, opening up many new avenues of research. We are still at the beginning of this type of research, and many new and exciting studies are definitely still to come.

However, with many of these studies we should keep an open and critical mind when it comes to caveats and issues. New technologies often come with new issues and rethinking of necessary controls. An example is optogenetics: The studies by Kovács et al. (2016) and Van de Ven et al. (2016) highlight how the use of different controls and slightly different study designs can result in opposing outcomes and conclusions. The current diversity in the use of e.g. control stimulations (see Box 2) can be seen as a weakness (non-standardisation) but also a strength when outcomes are the same despite the differences.

In the future, there will also be more sufficiency experiments, where experimenters will try to mimic specific oscillations or other neural activity artificially, to test if they are sufficient to induce an effect. When it comes to artificially creating e.g. oscillations, we will have to ask the question what defines such an oscillation. Is it just the frequency band? Is therefore imposing specific frequencies on a brain area sufficient to draw conclusions of the function of an oscillation? Or does the shape and form also play a role? For example, is it sufficient to create the frequency of a spindle or does the waxing and waning spindle shape also have to be present? Spindles originate in the thalamus, can anything created in the cortex then still be termed a spindle? Further, if simply the frequency is enough, we also need to agree on the range of the frequency. For example, currently spindle frequency ranges in different papers widely differ and can be anything from 8 Hz to 20 Hz. How can we then differentiate if

it really is a spindle or else perhaps beta or fast theta? Moreover, as mentioned in Box 1, the frequency range used to detect hippocampal ripples is not standardised across studies and can be very broad, with some studies using a range of 100–400 Hz. It is open to discussion if there are any subtypes of ripples within this frequency band (Ramirez-Villegas et al., 2015), as well as if this range generalises across species. Human studies on ripples usually use a range within 80–120 Hz, which in rodents would be considered as high-gamma.

Another challenge will be the disentanglement of the contribution of different, co-occurring oscillations. Maingret et al. (2016) provided elegant evidence that the triple co-occurrence of ripples, slow oscillations, and spindles is important for long-term memory; however, their experiments highlight the difficulty in isolating specific components of the triple: once they created artificial slow oscillations, they also induced an increase in spindles that occurred after the slow oscillations. Here the triple was the aim, but the difficulty will be in the future to isolate components. If we manipulate one oscillation in the triple, we seem to automatically change the other components as well (Latchoumane et al., 2017; Maingret et al., 2016). Therefore, in the future it will be a great challenge to find ways to selectively manipulate only one component/oscillation at the time to be able to draw conclusions about the specific functions of each.

Recent studies have furthermore identified cortical ripples in humans and rodents, and their co-occurrence with the hippocampal ripple after different behavioural conditions (Aleman Zapata et al., 2020; Khodagholy et al., 2017; for theory on generators please see McKenzie et al., 2020; Vaz et al., 2019). In the future, closed-loop studies will be necessary to elucidate the generation, function, and potential subtypes of such events.

Another interesting challenge for the future will be the targeting of non-hippocampal memories. While memory reactivations are most known to be associated with hippocampus-based memory, Pennartz et al. have shown memory reactivations in the striatum, which were in close temporal association with hippocampal ripples (Lansink et al., 2009; Pennartz et al., 2004, 2011). Tasks are generally thought to depend either on the striatum or the hippocampus, but evidence is increasing that striatum-based memories can still involve hippocampal processing even if it may not be necessary for good performance (Samanta et al., 2021; Sawangjit et al., 2018; Schapiro et al., 2019). Applying ripple-disruption to tasks that are not known to be dependent on intact hippocampal functioning but still show hippocampal memory reactivations, will allow us to gain insight on the importance of such reactivations. But when performing these experiments it will be important to critically consider how memory performance is tested. Perhaps animals will still be able to perform on such tasks after ripple disruption, but their behaviour may be less flexible and e.g. reversal learning or any other form of memory adaptation may be worse (Genzel, 2020).

In conclusion, with closed-loop experiments we are at the cusp of a new research era in sleep and memory, to date these experiments have already provided many exciting and novel findings and confirmed long-standing ideas. In the future, we can look forward to using these techniques to finally tackle the fine-tuned mechanisms during sleep that make memories more long lasting.

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CONFLICT OF INTEREST

None of the authors report a conflict of interest.

AUTHOR CONTRIBUTIONS

Jacqueline van der Meij and Adrian Aleman-Zapata wrote the first draft, Lisa Genzel supervised and refined the article.

DATA AVAILABILITY STATEMENT

Data sharing not applicable – no new data generated.

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