Detecting neural replay in sleep with EEG classifiers

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Thesis Summary

A rich literature has shown the importance of sleep for enhancing memories. It has been shown that we can trigger memories by re-presenting sound cues during sleep that were associated with specific memories during wake, this method is called targeted memory reactivation (TMR) and it is used in a lot of recent studies. Recent work showed that it is possible to use machine learning classifiers with TMR and identify memory reactivations during sleep. This inspired me to explore memory reactivations and their characteristics in human slow wave sleep (SWS) and rapid eye movement (REM) sleep.

Chapter 1 is an introduction. In Chapter 2, I used a serial reaction time task (SRTT) and machine learning classifiers and showed that we can identify memory reactivation and its timing after TMR in SWS.

In Chapter 3, new characteristics of reactivations are revealed. I analyse different SWS graphoelements such as slow oscillations (SOs), spindles and show that we can use them to know when to deliver our TMR cues.

In Chapter 4, I use trials of varying lengths to see the impact of this on early reactivations that were detected in Chapter 3 and see the behaviour of reactivation when cues are separated further apart.

In Chapter 5, I take a leap of faith and try classifying memory reactivation in human REM with a new pipeline and that was successful. Detection of memory replay in REM sleep was shown in rodents but not with TMR in humans. I explore the characteristics of the detected reactivations and how they relate to the rodent literature. In Chapter 6, there is a general discussion and conclusion.

The findings of this work pave the way for understanding sleep reactivation better and improving TMR delivery in SWS. REM sleep findings offer a starting point for more investigations to come.

Acknowledgements

My PhD has been a wonderful journey. I would like to thank my supervisor Prof. Penny Lewis from the bottom of my heart for always being supportive, available, providing expert insight and for making my PhD a great experience. My gratitude and love go to all members of my group (NaPs), I have enjoyed being a group member and a friend of yours and have learned a lot from you. Thank you, Matthias Treder, for the support and precious advice I have learned a lot from you. I would like to thank my family for always being supportive and understanding. I would like to thank the European Commission (ERC) for supporting my PhD. I would like to thank my parents, Prof. Eid and Eng. Huda for supporting and helping me to chase my dreams, you were always there for me. Thank you, Prof. Reda, for the support and encouragement. Thank you, my lovely wife Youmn, for all the love, support and encouraging words you gave me.

Codes are available at:

https://github.com/MahmoudAbdellahi

CHAPTER 1

Introduction

"If the human brain were so simple that we could understand it, we would be so simple
that we couldn't."

Emerson M. Pugh

1.1 Sleep physiology

We spend around one-third of our lives asleep. Sleep has been shown to benefit our memories (Squire & Zola, 1996; Stickgold, 2005). It is characterised by unconsciousness and reduced responsiveness to external stimuli. Sleep is essential for us to think clearly and process memories and the more we remain in waking state and deprive ourselves from sleep the longer the duration of sleep we get afterwards, this is known as sleep homeostasis. But why do we enter this state and lose our consciousness and what is the brain doing during that time? Is the brain just taking rest or is the story more fascinating than that?

Luckily, nowadays we can monitor sleep via Polysomnography (PSG) and observe different activities that happen during sleep. With PSG we can monitor different functions such as the activity of the brain with Electroencephalography (EEG), the movements of the eyes with Electrooculography (EOG), muscles activity with Electromyography (EMG), and heart rhythm with Electrocardiography (ECG). EEG is offers an easy way to record the activity of the brain using different electrodes that cover the scalp. It is cheap and non-invasive, so it does not require a surgery. EEGs have low signal-to-noise ratio but offer a high temporal resolution. By analysing the brain activity using EEG during sleep we are certain that the brain is not merely resting. Interestingly, the activity pattern during sleep does not only differ from the activity in wake but also varies from time to time within sleep itself. These different patterns can be organised into different sleep stages, (Patel et al., 2020; Silber et al., 2007), Figure 1.1.

Stage 1 / N1

In this sleep stage, the brain activity gets slower and the brain produces alpha waves of relatively low frequency (~8 to 13 HZ) and the body starts to relax with occasional muscle twitches. It is characterised by rolling of eye movements, it is easy to awake the sleeper from N1. The sleeper can quickly enter the second stage, N2, and usually does not spend a lot of time in N1 if not disturbed. N1, N2, and N3 sleep stages are called non-rapid eye movement (NREM) stages.

Stage 2 / N2

Now the sleeper enters N2, and the muscles are more relaxed and brain activity looks different from N1. A rhythmic brain wave activity is produced which is called a sleep spindle (~10 to 16 HZ). Sleep spindles are shown to be related to learning (Fernandez & Lüthi, 2020; Ulrich, 2016). In N2, the movements of the eyes stop. N2 is also characterised by K-complexes which appear as negative voltage peaks, followed by a positive component (Cash et al., 2009). During the first sleep cycle, N2 can last from 10 to 25 minutes, then as the night proceeds the duration of N2 can become longer. A sleeper spends around half of sleep time in N2.

Stage 3 / N3

N3 is known as deep sleep and slow wave sleep (SWS). During this stage, the body relaxes even more, and the brain activity looks different as it produces identifiable delta waves (1 to 4 HZ) and slow oscillations (SOs) (<2 HZ) with large amplitudes (>75 μV). SOs have an up-going phase (depolarisation), and down-going phase which is a period of neuronal silence with respect to firing (hyperpolarisation) (Amzica & Steriade, 1998). Fast spindles (~13 to 16 HZ) typically occur on the up-going phase (Born & Wilhelm, 2012; Siclari et al., 2014) and they are shown to be linked to memory consolidation (Nishida & Walker, 2007). Deep sleep gets shorter as the night proceeds and more time is spent in rapid eye movement (REM) sleep.

Rapid Eye Movement (REM) sleep

In REM sleep, the eyes move rapidly behind the closed eyelids, it is also characterised by brain activity that looks similar to wake and no muscular activity. Theta activity (~ 4 to 8 HZ) is prominent during this sleep stage (Boyce et al., 2016; Hutchison & Rathore, 2015; Nishida et al., 2009). REM sleep is linked to the most vivid dreams (Crick & Mitchison, 1983). It can take up to 25% of sleep time and the duration of REM gets longer as the night proceeds.

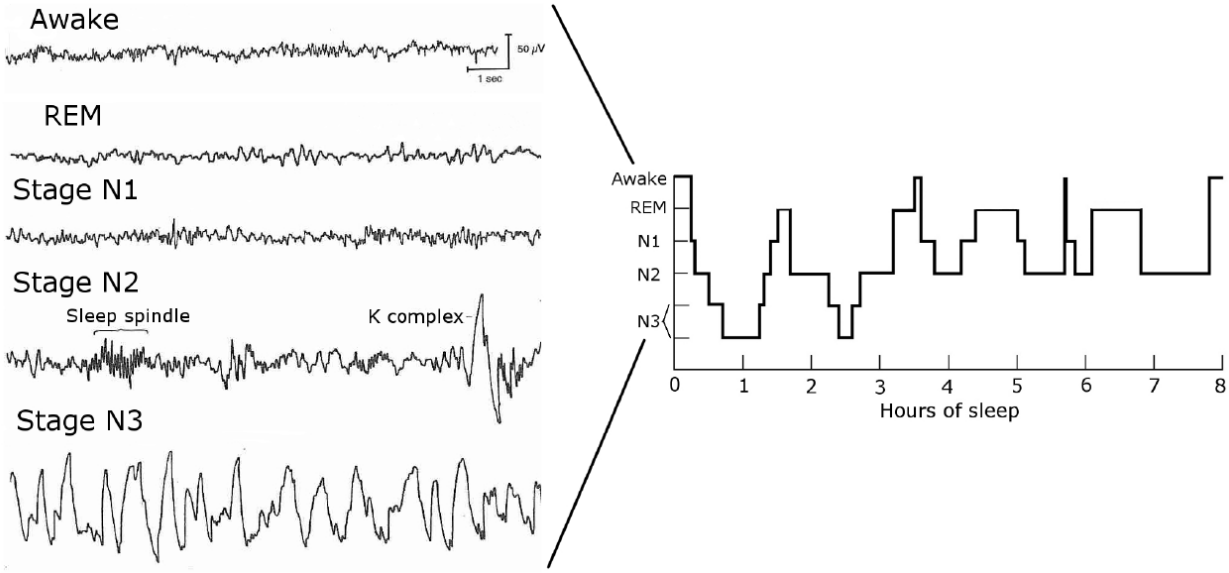


Figure 1.1 A representation of the EEG traces (left side) of different sleep stages and a representation of hypnogram showing the progression of a night of sleep with different stages (right side), (Christian G Fink, 2012).

1.2 Memories: what are they? how are they processed and reactivated? what sleep has to do with memory?

A memory is a piece of information that is encoded, stored, and retrieved by the brain. Memories are crucial for making future actions, without them it would not be possible to

continue developing relationships. Our memories go through three important phases: memory encoding, consolidation, retrieval. During encoding memories are formed and a memory trace is created as a result of perceiving a stimulus. During consolidation, the memory trace is strengthened and integrated into a stable network of memories (Lechner et al., 1999). Memories can then be accessed and retrieved during the retrieval phase. Memories can be divided into different types, some of these are declarative and non-declarative memories (Squire & Zola, 1996). Forms of the declarative explicit memories are like remembering facts and faces which require our awareness. On the other hand, procedural (non-declarative) memories are implicit memories that do not require our awareness, like motor skills.

A small, curved part of the brain called the 'hippocampus' plays a great role in learning, memory, and navigation (Maguire et al., 2000). The hippocampus helps us process and retrieve memories. It was suggested that declarative memories are hippocampus dependant (Squire & Zola, 1996). However, memories could include both explicit and implicit elements at the same time (Peigneux et al., 2001) and the hippocampus was also shown to be involved in the formation of motor memory (Albouy et al., 2008). The hippocampus can be considered as a storage of short-term memories, those that can be later transformed into long-term memories and transferred to another area of the brain: the 'neocortex', sleep plays an important role in this transfer. A cognitive representation of locations is formed inside the hippocampus by 'place cells' that are activated when rodents are in a particular place in the environment, the repetitive replay of the firing pattern is an ongoing process of consolidation. This replay of firing was shown in rodents where place cells that fired when rodents were in specific locations during the task co-fired again during sleep (Pavlides & Winson, 1989). Several studies have now shown that replays of different memories take place during sleep (Lee & Wilson, 2002; Louie & Wilson, 2001; Wilson & McNaughton, 1994). These studies open the door for more investigations on the characteristics of these detectable memory replays and show that sleep is very important to memory.

1.3 Sleep and memory models

One of the models of human memory was firstly introduced in 1971 by Marr, it is called the two-stage memory system (Marr, 1971). It suggests that memories are firstly encoded into the hippocampus and then transferred to a slow learning more stable long-term storage in the neocortex. The fast-learning hippocampus helps ensuring a fast encoding of memories;

however, memories are still unstable. When those memories are repeatedly reactivated the long-term slow learning store is trained on those memories and they transform into long-term memories (Frankland & Bontempi, 2005).

Sleep has different stages. In order to understand the role of sleep stages and memory, a paradigm called the 'night-half paradigm' adopted the idea that different sleep stages benefit different types of memories (Fowler et al., 1973; Yaroush et al., 1971). In the night-half paradigm, the influence of SWS-rich sleep was compared to REM-rich sleep by manipulating the timing of learning and sleep. This idea is now known as the dual-process hypothesis (Plihal & Born, 1997). This hypothesis assumed that declarative memories benefit from SWS, while REM sleep is important for non-declarative memory consolidation. It has been shown that SWS-rich sleep benefited declarative memories and REM-rich sleep benefited procedural memories (Plihal & Born, 1999). Another hypothesis called the sequential hypothesis assumed that memory formation benefits from cycling between SWS and REM sleep (Ambrosini et al., 1988a, 1988b; Rasch & Born, 2013).

A hypothesis that is adopted in many reviews (Ellenbogen et al., 2007; Lewis & Durrant, 2011; Mölle & Born, 2011) and the one we adopt in the current work is the active system consolidation (ASC) hypothesis (introduced by (Diekelmann & Born, 2010)) which is compatible with aspects from both the dual-process hypothesis and the sequential hypothesis. In ASC, sleep is not seen as just a passive shelter. Instead, ASC hypothesis suggests that memory consolidation happens as a result of the newly encoded memories being repeatedly reactivated during SWS and thus transformed from the short-term store (hippocampus) into long-term store (neocortex). The stabilisation is thought to be taking place in REM sleep. In ASC model (Rasch & Born, 2013), a dialogue between neocortex and hippocampus is suggested, where SOs drive reactivation of hippocampal memories with accompanying sharp wave ripples nested into thalamo-cortical spindles. It is suggested that ripples, together with the reactivated memory information, are nested into the troughs of spindles. In this work, we do subscribe to the idea that sleep has an active role and that SO, spindles, and sharp wave ripples play an important role in the reactivation of memories.

1.4 Targeted Memory Reactivation (TMR)

Cues (odour/sound/electrical shock) can be associated with learning material by presenting them at the same time during encoding. We can then redeliver the cues during subsequent sleep and thereby reactivate the cued memory. This method is known as targeted memory reactivation (TMR). In 1987, Hennevin et al. applied TMR in an active avoidance-conditioning (Hennevin et al., 2007; Hennevin & Hars, 1987). They applied it on rats using a mild electrical shock to the ear and found that a redelivery of these shocks during post-learning REM sleep increased both the time spent in REM sleep and the recall of avoidance. A number of studies done in humans have now shown the benefits of using TMR on memory consolidation for declarative (Cairney et al., 2014; Fuentemilla et al., 2013; Rasch et al., 2007; Rudoy et al., 2009) and non-declarative memories (Antony et al., 2012; Monika Schönauer et al., 2014). We use TMR in the current work to associate memories with sound cues and play those sounds in the sleep stage of interest.

1.5 The serial reaction time task (SRTT)

In this work, we use the serial reaction time task (SRTT) and pair it with sounds then use TMR to reactivate this task in sleep. It is important to think about the SRTT and whether we expect this task to reactivate in SWS and REM sleep.

In SRTT, sounds are paired with four different finger presses associated with images that appeared on the four quadrants of the screen, as illustrated in, Figure 2.5. Participants should learn two 12-item sequences, A and B (A: 1 2 1 4 2 3 4 1 3 2 4 3 and B: 2 4 3 2 3 1 4 2 3 1 4 1) and only one of them will be cued during sleep (reactivated sequence). Sequences are matched for learning difficulty such that both contained each item three times. Sequences are presented in blocks and each block contained three repetitions of a sequence. The blocks are interleaved so that a block of the same sequence is presented no more than twice in a row. There are 24 blocks of each sequence (48 blocks in total), and each block is followed by a pause of 15 seconds during which feedback on reaction time (RT) and error-rate are presented. After the 48 blocks of sequences A and B, there are four blocks of random sequences. They contained the same visual stimuli and an ‘R’ displayed centrally on the screen. Two of these blocks are paired with the tone group of one sequence (reactivated in sleep), and the other two are paired with the tone group of the other sequence (non-reactivated). Each sequence is paired

with a group of pure musical tones, either low tones within the 4th octave (C/D/E/F) or high tones within the 5th octave (A/B/C#/D). These tone groups are counterbalanced across sequences. For each trial, a 200 ms tone is played, and at the same time a visual cue appears in one of the corners of the screen. The location indicates which key on the keyboard should be pressed as quickly and accurately as possible: 1 – top left corner = left shift; 2 – bottom left corner = left Ctrl; 3 – top right corner = up arrow; 4 – bottom right corner = down arrow. Visual cues are neutral objects or faces, used in previous studies (Cousins et al., 2014), which appear in the same position for each sequence (1 = male face, 2 = lamp, 3 = female face, 4 = water tap). Visual cues stay on the screen until the correct key is pressed, after which 880 ms inter-trial interval followed.

We included a motor imagery (IMG) version of the SRTT. IMG task consisted of 30 interleaved blocks (15 of each sequence), presented in the same order as during the SRTT. Again, each trial consisted of a 200 ms tone and a visual stimulus, the latter being shown for 270 ms and followed by an 880 ms inter-trial interval. There are no random blocks during the imagery task and no performance feedback during the pause between blocks. An explicit recall test is done after sleep, in which participants are asked if they remember the images' locations of the two sequences to see if one sequence is recalled better than the other one (reactivated vs. non-reactivated).

TMR in SWS shows benefits when the cued sequence is compared to un-cued sequence of the SRTT (Cousins et al., 2014, 2016). Thus, we aim to replicate this. Additionally, classification results from our group showed that it is possible to detect reactivation of the SRTT by above chance accuracy (Belal et al., 2018). Thus, we aim to build classification pipelines that explore the characteristics of reactivation and whether reactivations are similar to wake activation and also to know when to apply TMR. In REM, we would expect REM sleep to relate to the SRTT given its procedural nature (Plihal & Born, 1999). The SRTT shows post-learning changes related to REM sleep suggesting reprocessing of the task in REM. Whether reactivation is detectable after TMR in human REM sleep is still unexplored. However, a study showed that several brain areas activated during the execution of a serial reaction time task during wakefulness were significantly more active during REM sleep for trained vs non-trained participants (Maquet et al., 2000). In that task, markers were displayed on the screen facing the participants and they reacted to a stimulus by pressing the spatially corresponding response key. Similarly, results from another serial reaction time study suggest that regional cerebral

reactivation in REM sleep reflects the reprocessing of learned material (Peigneux et al., 2003). Another serial reaction time study examined experience-dependent functional connectivity of the left premotor area and bilateral cuneus. They showed that the left premotor cortex is correlated with the left posterior parietal cortex and bilateral pre-supplementary motor area during REM sleep of participants trained on the task vs. non-trained (Laureys et al., 2001). This collective evidence suggests that reactivation of the SRTT takes place in SWS and REM sleep. We aim to take this further and relate wake and sleep category specific patterns with high temporal accuracy using EEGs with TMR in pipelines employing multivariate pattern classifiers. Subsequently, we aim to characterise the detected reactivations and explore when to apply our TMR cues.

1.6 What are EEG classifiers? What could they say about memory reactivation during sleep?

We will start by taking a closer look at what classification means in a general machine learning context. Afterwards, we will see how classifiers are useful with EEGs and detecting memory reactivation.

Machine learning is the study of the set of algorithms that enable the computer to make decisions based on a set of observations, known as the 'training data'. A computer can make decisions by following automated algorithms that can iteratively update parameters of the classifier to build a 'model'. In the case of supervised learning, this is done using the training observations and their respective categories. Supervised learning is the approach that we use in the current work, and it means that we need our computer to distinguish between categories ('classes') based on observations from different classes. Simply, assuming that we have 1000 observations from two classes (a 'binary classification problem') and we have two measurements (the 'features') in every observation, we can scatter every observation in a 2d space such that each observation is represented by a point. We can then look at the points in this mapped space (the 'feature space'), as shown in Figure 1.2. In this example, a classifier then tries to draw a separating line between the observations of the two classes, which is the case in linear classification. When the classifier follows the learning algorithm and draws the separating line, it can assign a prediction to the new observations according to which side of the line they are on. We can then calculate the confidence/certainty of the classifier prediction,

which is high for the samples falling further away from the separating line (‘decision boundary’). This can be explained by the maximum posterior probability from the two classes (ranges from 0 to 1), as shown in Figure 1.2. We can then check the category prediction of the classifier (‘predicted label’) and compare it to the actual label of the observation (‘true label’ or ‘ground truth’). In this way, we are able to evaluate the performance of the classifier. Machine learning classifiers have a variety of applications, including speech recognition, natural language processing (NLP), computer vision, brain computer interface (BCI), bioinformatics etc.

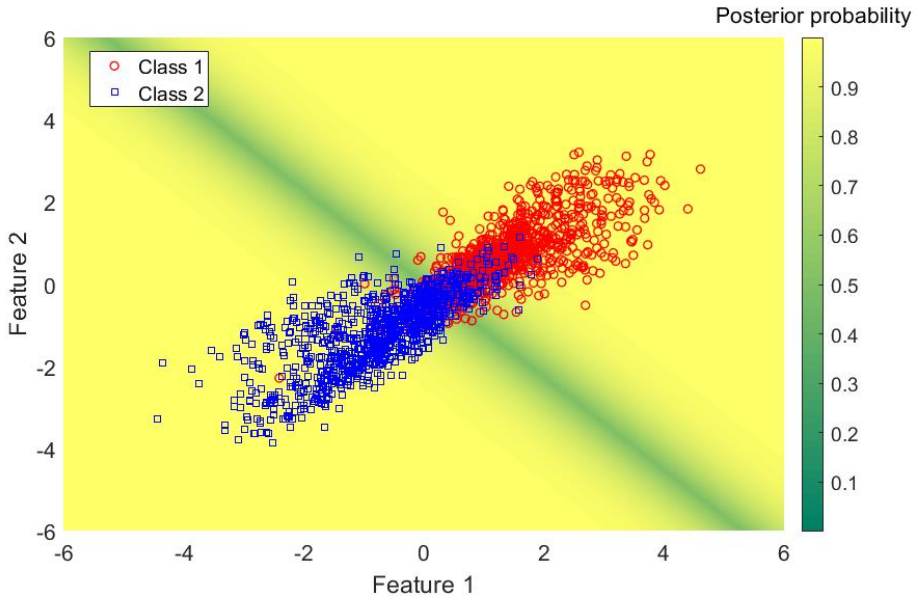


Figure 1.2: Binary classification problem. A linear classifier calculates a separating decision boundary between the two classes and can provide a certainty for every observation.

Now, let us demonstrate how supervised learning can be used to develop EEG classifiers that can differentiate the brain activity states and categorise them. With EEGs, we are able to record the activity of the brain with a high temporal accuracy. This activity is recorded using different electrodes (‘channels’) covering the different areas of the scalp. Let us try to visualise the data and see how a classifier can work with them and classify EEG patterns. At every time instance we get a value from all channels. Thus, assuming we have a sampling rate of 100 samples/second, if we record the activity of the brain for one second we will then have 100 values from every channel. Now, we assume that we have two activities that we want to distinguish: right and left-hand movements. We collected 1000 observations (‘trials’) for the classifier to train on (500 left hand and 500 right hand movements) with a

sound cue instructing participants/subjects with which hand to move. A trial here lasts one second. For simplicity, let us now assume that we expect the difference between left- and right-hand trials to be maximum in two channels, one on each side of the brain. We can organise our EEG data (1000 trials x 2 channels x 100 timepoints) in a 3d shape (3d-tensor), as shown in Figure 1.3. Assuming no need for pre-processing at the moment, we can train our classifier on these raw EEG recordings directly after aggregating channels and timepoints dimensions together by reshaping them into one vector, thereby making our data 2d (1000 trials x 200 channel-time). Now, our classifier can take every trial and learn how to differentiate between left- and right-hand activity. If we want to test our model later, we can test it with trials from a 'testing set' or some left out trials from the training set then see what class they will be assigned to, then compare the predicted class labels to the true labels of those trials. Classification can further be manipulated slightly to reveal new information. For instance, we can do a classification across time and evaluate the discriminability between classes at every time point. This can be illustrated by the same example as in Figure 1.3, except now we build a classifier model at every time point. This means that we build 100 classifier models, one at every time point, such that each classifier will have (1000 trials x 2 channels) to train on. If we take one time slice from our EEG data (Figure 1.3, green area) we can see how the classifier model is built on every timepoint. The posterior probabilities and feature space can be visualised, similar to the one in Figure 1.2, since now the classifier has two features at any given time point. This temporal classification can be beneficial when we are not sure about the exact timing of the effect (not sure when the difference between the classes occurs) or when the effect could only be happening for a small duration, and thus extracting the features from the whole trial could distort the effect. Now, every classifier at every time point can be used to predict the class of some left-out trials (not used in training) from the same dataset 'validation set', or perhaps be used to evaluate all time points from a different dataset, thereby generating a temporal generalisation plot (King & Dehaene, 2014). These time x time temporal generalisation plots are discussed in different chapters of the current work (e.g., Figure 2.1). We can now imagine that if we want to detect memory reactivation, we could train a classifier model on activation of the memory during the encoding phase in wake and then apply the model on sleep EEG, after TMR cues. We can then evaluate the classification performance.

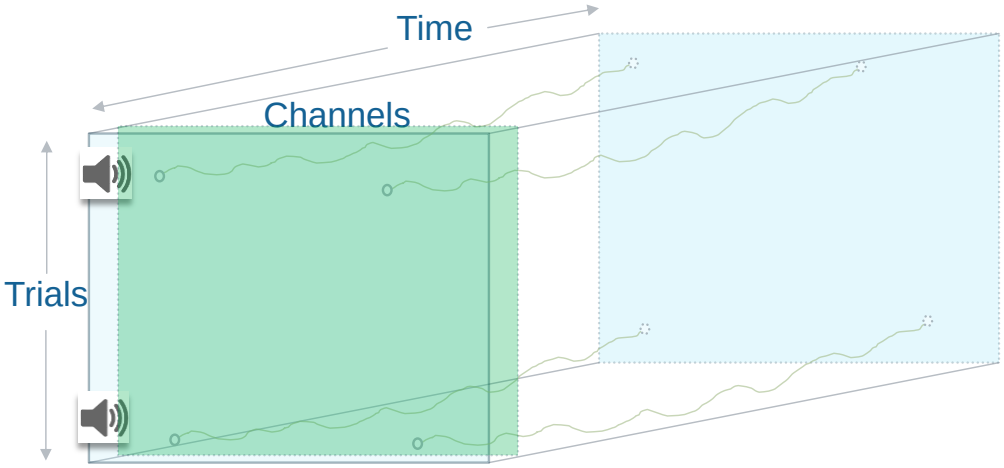


Figure 1.3 A representation of EEG data in 3d space (trials x channels x timepoints). A time slice is shown in green.

Thus, we can use machine learning to build EEG classifiers that can enable us to: (i) detect reactivation during sleep to examine relationships between memory reactivation and consolidation; (ii) reveal interesting characteristics about reactivations, and even (iii) guide us on when to apply the TMR cues, as we will see in later chapters and discussions.

Some studies developed machine learning classifiers for the sake of detecting and discriminating memory reactivations. A study by (Deuker et al., 2013) showed that it is possible to build a machine learning paradigm in which a classifier was trained on wake data during encoding and then applied on subsequent wake and sleep to classify reactivations. Classification in that study was applied on the continuous EEG to detect spontaneous memory reactivation. Another study used machine learning to detect spontaneous reactivation in REM sleep and showed success (M. Schönauer et al., 2017). The classification of spontaneous reactivation is challenging and will be difficult if we want to detect reactivations of multiple memories. Thus, if we know the exact label of the reactivation and roughly the timing of reactivation, we can evaluate our classification results with respect to that. TMR offers exactly that. TMR offers a unique association between sounds and categories, it gives a class label for every trial during wake and sleep. A study adopting TMR showed that it is possible to discriminate reactivation of different memories of a finger tapping task during Non-REM (NREM) sleep (Belal et al., 2018). Several following studies showed that it is possible to detect

memory reactivation in SWS with TMR (Cairney et al., 2018; Schreiner et al., 2018; Wang et al., 2019).

1.7 The relationship between different sleep stage graphoelements and detectable reactivation and consolidation

Different sleep graphoelements in SWS and REM sleep are shown to be related to consolidation and reactivation. In SWS, studies explored interesting relationships between sleep spindles and the detected reactivation. It has been shown that sleep spindles play an active role in reactivation (Antony et al., 2018; Cairney et al., 2018). A study by (Zhang et al., 2018) adopted intracranial EEG (iEEG) and representational similarity analysis (RSA) to show that spontaneous replays are locked to ripples happening in high gamma frequency (Xue et al., 2010; Yaffe et al., 2014; Zhang et al., 2015). SOs are also shown to be important in tuning the timing of TMR sounds. Neurons oscillate between hyperpolarization and depolarisation with sustained firing. Depolarised SO state drives memory reactivation in the hippocampus via interactions with thalamic sleep spindles and hippocampal sharp wave ripples. Additionally, fast spindles that typically occur on the up-going phase (Born & Wilhelm, 2012; Siclari et al., 2014) are shown to be linked to both memory consolidation (Nishida & Walker, 2007) and reactivation (Cairney et al., 2018). In REM sleep, theta activity is prominent (Boyce et al., 2016; Hutchison & Rathore, 2015; Nishida et al., 2009). In wake, theta activity was shown to be linked to memory processing and encoding of new information (Battaglia et al., 2011; Juergen Fell et al., 2011; Kahana et al., 1999; Vertes, 2005). Also, a link between detected replay in rodents and theta activity was suggested (Louie & Wilson, 2001). A study in humans showed increased theta power during post-learning REM sleep indicating a link between theta activity and memory reprocessing (Fogel et al., 2007).

1.8 Sleep reactivation differs temporally from wake activation

In an interesting study by Schreiner and colleagues, the reactivation classification strength was shown across time and enabled us to see when reactivation is happening after TMR (Schreiner et al., 2018). They showed that the reactivation is occurring more than one time in a word-sound pairing task. Evidence of reactivation reoccurrence was also found in a category specific activity after TMR (Cairney et al., 2018). Those studies offer interesting

findings on SWS reactivation and show that the reactivation is reoccurring. A mechanism for how TMR biases NREM reactivation and causes the recurring reactivation in a hippocampus-cortex dialogue is proposed by Lewis and Bendor (2019). It is suggested that during the up-going state of a SO, a presentation of TMR increases the activity of cortical neurons that are associated with the cued memory and this selects which context will be reactivated by the hippocampus. This leads to a hippocampal bias to spontaneously replay a sequence related to the selected context. Subsequently, hippocampal replay leads to a cortical replay of the same memory, and while the cortical neurons are in the up-going state this hippocampal-cortical replay could repeatedly occur (Lewis & Bendor, 2019). In rats, replay during NREM sleep has different temporal characteristics compared to wake and it has been shown to occur from 10 to 20 times faster (Ji & Wilson, 2007; Lee & Wilson, 2002; Nádasdy et al., 1999). Sleep reactivation in rats was also shown to be compressed 6 to 7 times in comparison to wake suggesting that processing could be faster with the absence of behavioural constraints (Euston et al., 2007). We are interested in exploring the temporal characteristics of detectable reactivation in our data and investigate them further.

1.9 Coming up next

In the upcoming chapters, we will use TMR with a serial reaction time task (SRTT), wherein we will see how we can train a classification model on the activation pattern of a motor imagery memory and classify sleep reactivation. We delve into the relationship between sleep stage graphoelements and their active role in reactivation. We also investigate when to apply TMR and explore the temporal properties of reactivation pattern, we will also study the recurrence of reactivation after TMR and temporal compression.

CHAPTER 2

Initial attempts to classify memory reactivation across time with EEG classifiers

In this study, Anne C. M. Koopman collected data from participants and Monika Śledziowska helped with data collection, Suliman Belal, Martyna Rakowska, and Monika Śledziowska helped with scripts of the study design and behavioural analysis. All the EEG and classification analyses were done by me and developed in Matlab. Penelope A. Lewis supervised and advised on the study and throughout the work and writing. Matthias S. Treder supervised and advised on EEG and classifiers.

2.1 Abstract

Recent studies have adopted different methods to reveal that it is possible to classify memory reactivations in humans during slow wave sleep (SWS). Here, we use targeted memory reactivation (TMR) in which memories are associated with sounds and different classes can be selectively triggered during sleep. Recent efforts show that reactivation could be reoccurring after a sound cue. Here, we use machine learning and build EEG classifiers that can classify memory reactivation during SWS of a motor memory and show that reactivation is delayed in comparison to wake activation. We also show that there is a behavioural improvement as a result of TMR. Furthermore, we tested the same classification pipeline with REM sleep to find that REM reactivation could be classifiable but not yet strong enough.

2.2 Introduction

It is now broadly accepted that sleep can enhance our memories. During SWS memories are spontaneously replayed which helps in strengthening these memories (Rasch & Born, 2013). Sounds and odours that have been associated with a memory can be used to trigger reactivation when presented during specific sleep stages and this has been employed in a number of studies (Cairney et al., 2014; Fuentemilla et al., 2013; Rasch et al., 2007; Rudoy et al., 2009). EEG is an electrophysiological monitoring method used to record electrical activity of the brain and is widely used in Brain Computer Interface (BCI) systems. EEGs are also widely used in sleep studies to define sleep stages. Thus, developing an EEG-based tool can be extremely useful. EEG can be easily employed and is cheaper than other techniques. Yet, EEG signals are accompanied with noise. Thus, a reliable machine learning system and discriminative features should be used to try to detect reactivation in sleep.

Participants first spent an adaptation night in the lab. Next, they performed the SRTT by learning two sequences (A and B) of button presses (Cousins et al., 2014), which differed in order of buttons pressed and the four tones associated. Afterwards, participants heard the tones and imagined pressing the appropriate buttons without movement. Tones associated with one of the sequences were reactivated during either REM or SWS. Importantly, those tones were also played to participants during the adaptation night prior to learning the task, as a

control. Subsequently, we developed a classification pipeline that shows classification performance across time to see when reactivation happens in our SRTT.

We trained a classifier to identify left and right-hand presses using the imagination condition. The trained classifier was then applied to the data after each TMR tone in SWS and REM. We used time domain amplitudes as features and a linear discriminant analysis (LDA) for classification (Blankertz et al., 2011; Mika et al., 1999). We find overnight improvement for the reactivated sequence compared to the non-reactivated sequence which implies TMR cuing benefit. Furthermore, we found that we can classify reactivation of motor imagery (left hand vs. right hand) and we can see that the classification pattern is delayed (initiated around 1 sec. after the onset of the cue) in comparison to wake activation (initiated from 0.7 sec. after the onset of the cue). This suggests interesting temporal property of the detected reactivation and needs further investigation. Classification of REM sleep, however, did not yield significant difference between the experimental and adaptation nights.

2.3 **Results**

2.3.1 Detection of TMR cued reactivation in sleep with classifiers

We were interested to detect neural replays triggered by TMR using EEG classification, determine how these related to behavioural consolidation and how they differed between sleep stages. To achieve this, we first produced a time x time classification in wake, Figure 2.1. Here, the accuracy for classifying left- vs. right-handed trials using a classifier trained at the specified 'training time' and tested at the specified 'testing time' was built one row at a time. We used time domain features of 80 ms averages (40 ms before and after each time point). Using a threshold of 75% correct classification rate that we defined in our method (see classification methods), we identified a time of interest (TOI) in which classification accuracy peaked from 0.7 to 1.1 seconds after cue onset for wake data of the SWS group (Figure 2.1a) and 0.64 to 0.97 seconds after cue onset for wake data of the REM group (Figure 2.1b).

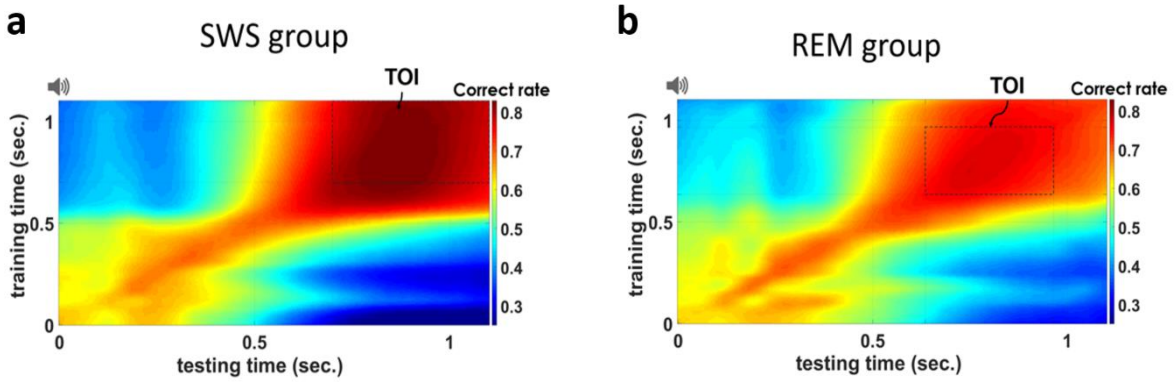


Figure 2.1 Classification in wake: Grand average classification correct rate of wake motor imagery of right hand vs. left hand (wake imagery training and testing) using time domain features with an 80 ms smoothing window and LDA classifier. For **a)** SWS group and **b)** REM group.

Turning to sleep data, we next examined classification in sleep with a second time x time classification procedure, but this time training with wake (y-axis) and testing with sleep (x-axis), Figure 2.2. To avoid double-dipping, we used a leave-one-subject-out approach wherein, for each subject, data from the other subjects were used to select a sleep TOI for that subject. An example of the sleep classification result and the TOI of sleep for one participant from the SWS group is shown in Figure 2.2. Note that the TOI in sleep was defined as the window with the highest average accuracy, where window length was determined by the window length obtained during wake classification. The TOI during sleep varied slightly between participants but interestingly, SWS TOI occurred later for the experimental night than it did during wake, from 0.88 ± 0.04 to 1.28 ± 0.04 seconds after cue onset. For completeness, we also extracted the TOI based on the adaptation night, which should be a time period that does not relate to the encoded memory of the hands, because the task had not yet been completed in this night. Thus, the exact same analysis was performed on the adaptation night to get its TOI which was: 0.55 ± 0.036 to 0.95 ± 0.036 seconds after cue onset. For the REM group the TOI for the experimental night was 0.74 ± 0.004 to 1.07 ± 0.004 seconds after cue onset, and the TOI of the adaptation night was 1.1 ± 0.21 to 1.4 ± 0.21 seconds after cue onset.

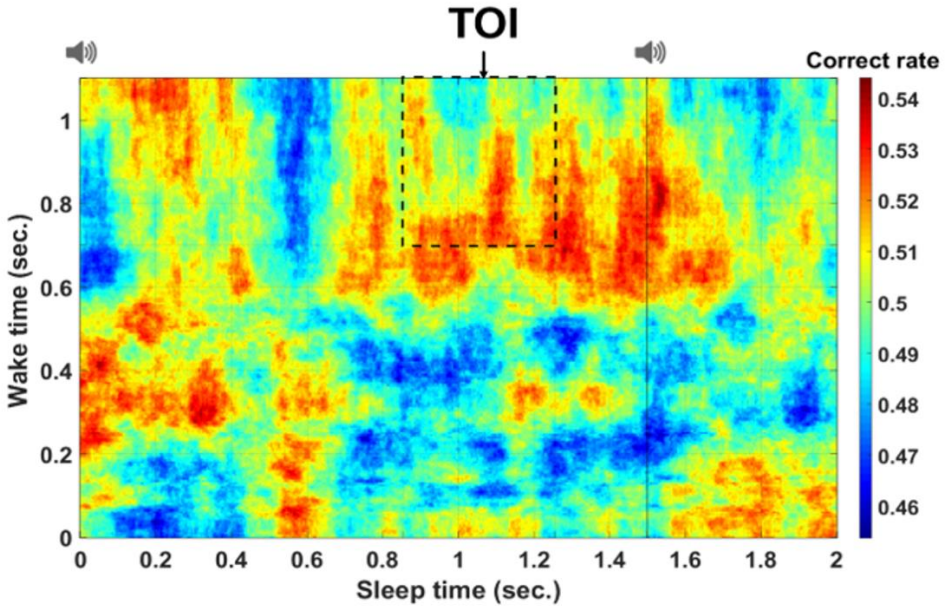


Figure 2.2 Classification of right- versus left-handed trials during the experimental (EXP) night. Note that the classifier was trained during wake (time of training is shown on the y-axis) and applied during sleep (time of testing is shown on the x-axis). The TOI for one participant from the SWS group is shown here. TOI is marked with a dashed square and is calculated by leaving the data of that participant out and getting the maximum window from average classification of all other participants of the same group.

We applied our classifier to SWS and assessed classification performance with accuracy using 300 trials, as this was the maximum number we could use consistently across all participants. Figure 2.3a shows the accuracies for the experimental vs. the adaptation night for the SWS group using the TOI defined by the experimental night. Classification accuracies was significantly higher for the experimental night than the adaptation night (paired t-test, $t(9) = 4.1$; $p = 0.003$). When we consider the TOI of the adaptation night, we would expect no difference between the classification accuracies of the two nights. Indeed, this was the case, a paired t-test showed no difference between the nights in this analysis, (paired t-test, $t(9) = -1.6$; $p = 0.14$). This suggests that only the experimental night contained memory related reactivation that is similar to the encoded memory.

We repeated the process for the REM group for both experimental and adaptation nights. For this group, we included 366 trials, as this was the maximum available for all participants. The

classification performance did not exceed chance level and showed no difference between experimental and adaptation nights (paired t-test, t(13) = 1.57; p = 0.14), as shown in Figure 2.3b using the TOI defined by the experimental night. Interestingly, however, if the outlier participant that obtained 40% correct classification in the experimental night is rejected, there is a difference between experimental and adaptation nights: mean accuracy for the experimental night becomes: 51.6%, and for the adaptation night: 49.4%, (paired t-test, t(12) = 2.93; p = 0.013). Furthermore, with the outlier rejected, the experimental night is significantly higher than chance level 50% (t(12) = 2.93; p = 0.013). As expected, the classification using the TOI defined using the adaptation night did not show a significant difference between the nights (paired t-test, t(13) = 1.01; p = 0.332).

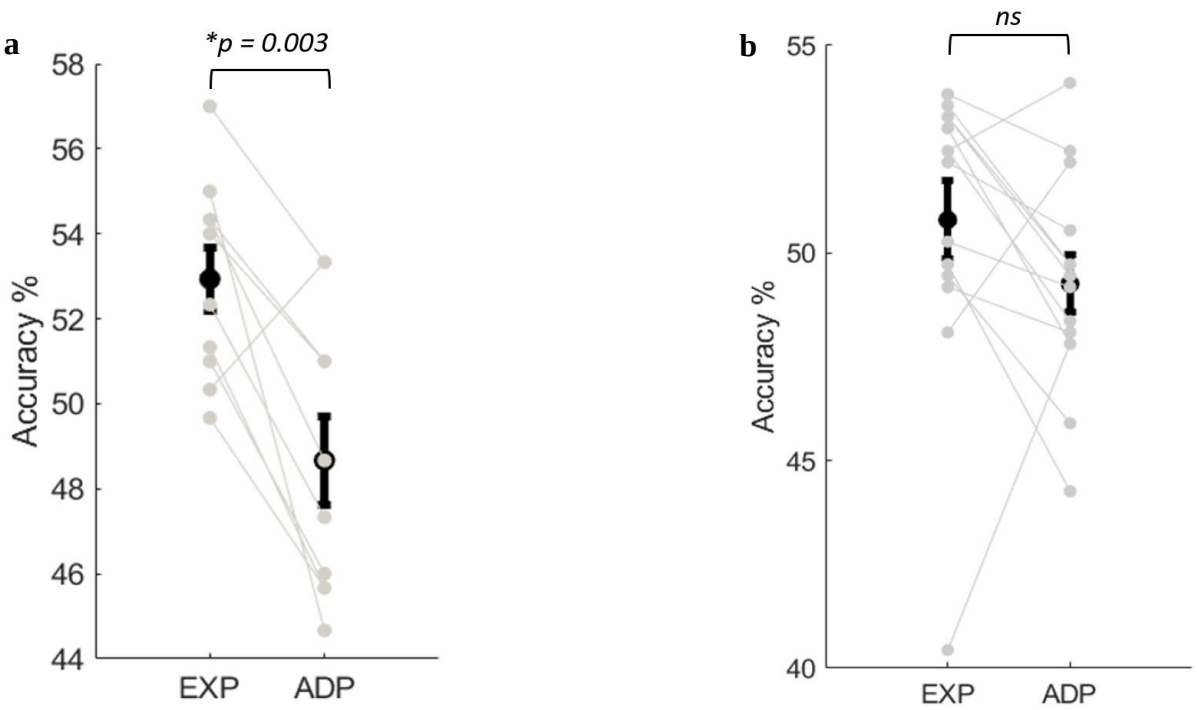


Figure 2.3: Classification accuracy in the experimental and control (adaptation) night, for both the **a)** SWS group, (paired t-test, t(9) = 4.1; p = 0.003) and **b)** REM group, (paired t-test, t(13) = 1.57; p = 0.14), this difference is significant if the outlier point at ~40% is rejected (paired t-test, t(12) = 2.93; p = 0.013).

2.3.2 Correlating classification performance with behaviour

We tested if there is a correlation between the classification performance and behavioural improvement, as summarised in Table 2.1. There was no significant relationship with either early (random blocks after sleep - first four blocks after sleep) or late (random blocks after sleep - last four blocks after sleep) behavioural improvements for any of the groups.

Table 2.1 Correlations between classification performance and either early or late behaviour improvement for both groups.

	REM sleep group n = 14		**SWS group n = 10**	
Early improvement	r = -0.15	p = 0.63	r = -0.16	p = 0.66
Late improvement	r = 0.18	p = 0.55	r = 0.24	p = 0.51

2.3.3 Behavioural performance

We defined a measure called the sequence specific improvement (SSI) to measure how much participants improved after sleep compared to the last blocks before sleep, SSI was calculated as follows:

SSI = (random blocks after sleep - first four blocks after sleep) - (random blocks before sleep - last four blocks before sleep)

We found a significant difference in SSI for the reactivated sequence compared to the non-reactivated sequence in SWS group, Figure 2.4a. (t-test P = 0.042, n=15). Interestingly, when we separated the trials of left and right hand to see the improvement for each hand we found that the significant improvement was derived from the non-dominant hand (left hand) as shown in Figure 2.4b (Koopman et al., 2020).

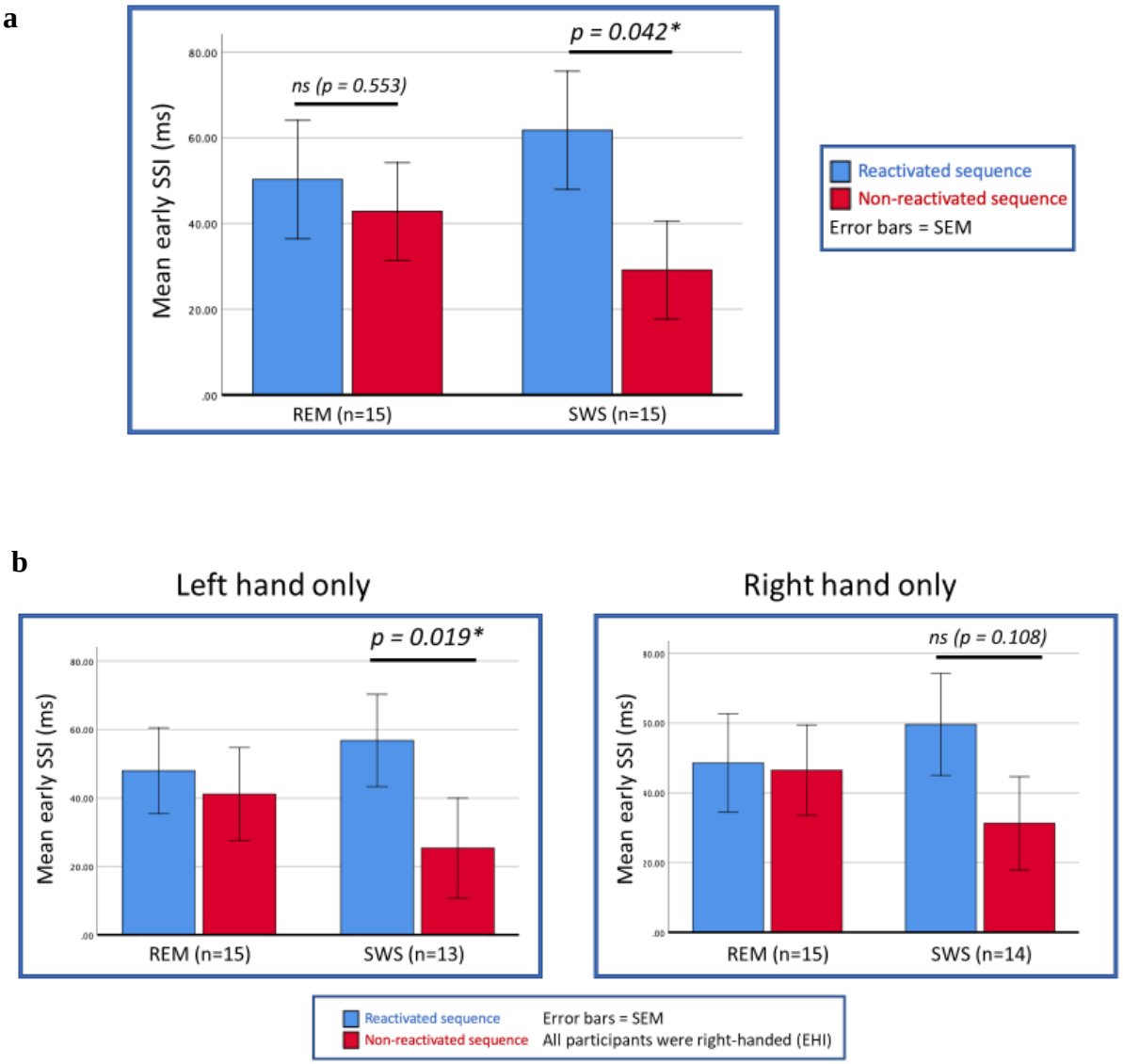


Figure 2.4: Behavioural improvement. **a)** using the sequence specific improvement (SSI) measure, a significant overnight improvement is found for the reactivated sequence compared to the non-reactivated, only in SWS group. **b)** dominant hand is less sensitive to TMR during SWS.

2.4 Discussion

2.4.1 Linear classifiers with time domain features detect reactivation in SWS

While it is well-established that TMR can facilitate consolidation, the question of whether this intervention truly triggers memory reactivation has attracted much attention in the last couple of years (see (Lewis & Bendor, 2019; Schreiner & Staudigl, 2020)). A number of studies have now succeeded in demonstrating neural reactivation after TMR (Belal et al., 2018; Cairney et al., 2018; Murphy et al., 2018; Schreiner et al., 2018; Shanahan et al., 2018), using a variety of methods and measures. In this experiment, we developed a novel pipeline for classification of memory reactivation after TMR using EEG amplitude alone. Although we were able to detect reactivation at above chance levels in SWS, there was no association between the level of detection and measures of behavioural consolidation. This corresponds to the findings of Belal and colleagues, who applied a different classification pipeline on the same task, but found no significant correlation with behaviour (Belal et al., 2018). Interestingly, however, some reports have identified correlations between detected reactivation and subsequent behavioural performance (Cairney et al., 2018; Schreiner et al., 2018; Shanahan et al., 2018; Sterpenich et al., 2021). It is unclear whether this difference relates to the task in question or the specific classification pipeline.

In REM, our classification results were much more marginal. It is true that the removal of an obvious outlier led to above-chance classification in the experimental night, and this was also significantly stronger than classification in the adaption night. However, the actual level of classification accuracy was still very low (averaged 51.6%). While this finding is encouraging, suggesting that TMR in this stage is eliciting some kind of response, it is not sufficient evidence to state that we can definitely detect reactivation in REM. The EEG in this sleep stage is extremely noisy, partially due to the many eye movements. We speculate that removing the eye movements noise from EEG and perhaps changing the pipeline or features of the brain response may be needed to convincingly classify memory reactivation during REM.

2.4.2 Conclusion

In this chapter, we demonstrated that machine learning methods can be used to classify memory reactivation in human SWS. We also showed that we can analyse the temporal characteristics of the reactivation after the TMR sound and we are able to identify the timing of the reactivation

which is delayed in comparison to wake activation. In the next chapter, we will use the SWS data and analyse reactivations to find more about their characteristics. Results in REM sleep showed a slight hint of classification, however, a different pipeline could be more suitable to detect reactivation in REM sleep.

2.5 Methods

2.5.1 Experimental Design

The current study uses EEG from human participants (n=15 for SWS, n=15 for REM). Participants completed a SRTT before and after sleep (SRTT; adapted from (Cousins et al., 2014). As illustrated in Figure 2.5, sounds cued four different finger presses. EEG signals are used in a machine learning pipeline to identify the timing of sleep reactivations and classify them. We used data from 30 participants, they were divided into two groups, the SWS group and the REM group. Participants learned two 12-item sequences, A and B (A: 1 2 1 4 2 3 4 1 3 2 4 3 and B: 2 4 3 2 3 1 4 2 3 1 4 1). Sequences had been matched for learning difficulty; both contained each item three times. Sequences were presented in blocks and each block contained three repetitions of a sequence. The blocks were interleaved so that a block of the same sequence was presented no more than twice in a row. There were 24 blocks of each sequence (48 blocks in total), and each block was followed by a pause of 15 seconds during which a feedback on reaction time (RT) and error-rate were presented. The pause could be extended by the participants if they wanted. After the 48 blocks of sequences A and B, participants performed four blocks of random sequences. They contained the same visual stimuli and an 'R' displayed centrally on the screen. Two of these blocks were paired with the tone group of one sequence (reactivated in sleep), and the other two were paired with the tone group of the other sequence (not reactivated).

Participants were aware that there were two twelve-item sequences, and each sequence was indicated with 'A' or 'B' appearing centrally on the screen, but participants were not asked to learn the sequences explicitly. Counterbalancing across participants determined whether sequence A or B was the first block, and which of the sequences was reactivated during sleep.

Each sequence was paired with a group of pure musical tones, either low tones within the 4th octave (C/D/E/F) or high tones within the 5th octave (A/B/C#/D). These tone groups were counterbalanced across sequences. For each trial, a 200 ms tone was played, and at the same

time a visual cue appeared in one of the corners of the screen. The location indicated which key on the keyboard needed to be pressed as quickly and accurately as possible: 1 – top left corner = left shift; 2 – bottom left corner = left Ctrl; 3 – top right corner = up arrow; 4 – bottom right corner = down arrow. Participants were instructed to keep individual fingers of their left and right hand on the left and right response keys, respectively. Visual cues were neutral objects or faces, used in previous studies (Cousins et al., 2014), which appeared in the same position for each sequence (1 = male face, 2 = lamp, 3 = female face, 4 = water tap). The nature of the cues (objects/faces), participants were told, was irrelevant. Visual cues stayed on the screen until the correct key was pressed, after which an 880 ms inter-trial interval followed.

After completion of the SRTT, participants were asked to do the same task again, but were instructed to only imagine pressing the buttons. Motor imagery (IMG) consisted of 30 interleaved blocks (15 of each sequence), presented in the same order as during the SRTT. Again, each trial consisted of a 200 ms tone and a visual stimulus, the latter being shown for 270 ms and followed by an 880 ms inter-trial interval. There were no random blocks during the imagery task and no performance feedback was presented during the pause between blocks. As a control, participants were asked to sleep in the lab before doing the SRTT training. During control night, sounds were played with the same criteria as the actual experiment.

After the experimental night participants were asked to perform the tasks again, first the motor imagery, then the SRTT. Eventually, they were asked if they remember the images' locations of the two sequences to see if one sequence is recalled better than the other one. Motor imagery data set of each participant was used for classification. The adaptation/control night is useful for eliminating the possibility that a classifier could merely classify sound induced effects on the EEG. Thus, if the classifier can classify the experimental night but not the adaptation night this suggests the classifier is classifying memory reactivations, rather than a simple response to a sound.

None of the participants reported prior knowledge of performing the SRTT. All participants had normal or corrected-to-normal vision, normal hearing, and no history of physical, psychological, neurological, or sleep disorders. Participants did not consume alcohol and caffeine in the 24 hours prior to the study or perform any extreme physical exercise or nap. This study was approved by the School of Psychology, Cardiff University Research Ethics Committee, and all participants gave written informed consents.

Data acquisition. EEG was used in this study from human participants. EEG was collected using 21 electrodes (according to the 10-20 system) on the scalp, they consisted of 13 standard locations: Fz, Cz, Pz, F3, F4, C5, CP3, C6, CP4, P7, P8, O1, and O2, and were referenced to the mean of left and right mastoid channels. Three EMG channels were used on the chin and two on the left and right sides above and below the eyes for collecting EOG, and one ground on the forehead. The impedance values were below 5kΩ for scalp electrodes and below 10kΩ for face electrodes. PSG were scored by two trained sleep scorers and only the parts of the correct sleep stage were kept for further analyses. Data were collected at 200 HZ sampling rate. Sound cues were delivered either during SWS and REM sleep stages according to the group.

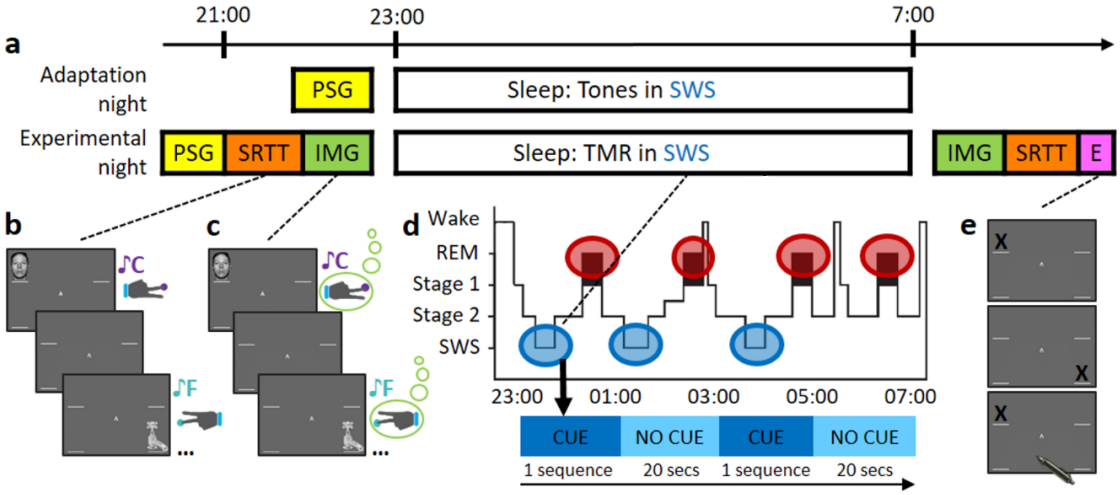


Figure 2.5: The experiment. **a)** The experiment consisted of an adaptation and an experimental night. During the adaptation night, participants were wired-up for EEG and tones were played while they slept as shown in d. During the experimental night, participants were wired-up, then they completed the serial reaction time task (SRTT) and motor imagery task (IMG) as outlined in b and c, respectively. Then, participants went to sleep and TMR was carried out in SWS or REM sleep, depending on the group, as shown in d. After waking up, participants completed IMG then the SRTT, and finally the explicit recall task shown in e. **b)** In the SRTT, four images are presented in two different sequences. Each image is accompanied by a specific pure tone (different for each sequence) and requires a specific button press. **c)** In IMG, Participants view the same sequences of images (paired with the same tones), but this time are instructed to only imagine pressing the buttons. **d)** One sequence was played as long as participants were in the relevant sleep stage, with a 20 second

pause between repetitions. **e)** In the explicit recall 'E', participants marked the order of each sequence on paper. Motor imagery trial duration was 1.1 sec (duration between onsets of cues). Sleep trial duration (between cues duration) was 1.5 sec.

2.5.2 Classification

We trained an EEG classifier to classify right- versus left-handed trials. This classifier was trained and tested using data from the motor imagery task performed just before and after sleep to assess the classifier performance during wake. We band-pass filtered the EEG signal from 0.1 to 50 Hz, and performed smoothing with 80 ms moving average (40 ms before and 40 ms after each individual time point). This 80 ms moving window was applied to the whole trial. The resulting time domain features were submitted to a linear discriminant analysis (LDA) classifier. LDA implementation from Matlab was used. A time x time classification was then performed using features from one time point to train a classifier, and that classifier was then tested on all time points.

We reasoned that if the classifier did not perform well in wake (either because the memory is weakly encoded or because it can somehow not classify the encoded memory), then it would not work during sleep, where noise is much higher and signal is much lower. We therefore used classifier performance during wake as a filter and excluded participants in whom wakeful reactivation could not be classified above 0.7 correct rate from further classification. During memory reactivation after a cue, there may be a time where activation reaches a peak, and other time points may not be very relevant for classification. We therefore used wakeful classification to extract the time period when classification accuracy was highest. This 'peak activation period' is very important for classification. We defined this time period as the time of interest (TOI). Using our wake-to-wake classifier, we identified a TOI based on the time of the highest classification rates. This is the window when we can best discriminate between the two classes, defined using a threshold of 0.75 correct rate on the grand average accuracies of all participants.

Subsequently, we developed an EEG classifier using wake samples and applied it on sleep. This was trained using every time point of wake and applied on sleep after each TMR cue. If reactivation really occurs during sleep and is detectable with the current pipeline, then we would expect the classification to peak around the TOI that we identified during wake motor

imagery. We applied the classifier to data from both adaptation and experimental nights for REM and SWS groups, as the comparison between these two nights allows us to separate the brain response to sounds (adaptation night) from the brain response to memory-related cues (experimental night). If TMR is associated with genuine memory reactivation, classification should be stronger during the experimental night, when participants have associated tones with the task, than during the adaptation night when tones have no memory associations.

We devised a method for removing noisy trials. In this method, trials which had low posterior probability (i.e., those which fell near the decision boundary) were considered noise and eliminated from the analysis. Rather than defining a set cut-off value, we used the maximum number of trials that was available for all participants consistently to determine which trials would be kept. In the SWS group this meant 300 trials, and 366 in the REM group. Importantly, this process does not consider the actual class label – it only considers the distance from the decision boundary. The exact same process was employed for classification of both the experimental and adaptation nights. After we had removed these noisy trials, classification accuracy on experimental and adaptation nights was compared to determine whether the classifier was detecting memory reactivation.

Given that the SRTT is a motor task, and we are classifying right- and left-hand presses, we expected to obtain meaningful results by focusing on the motor area when obtaining features. Thus, we repeated the classification analysis using only the four channels around the motor area: CP3, C5, CP4, and C6, instead of using all channels as in the previous analysis. This final classification pipeline is shown in Figure 2.6. It uses the TOI as identified with the classification using all channels. However, whereas previously each time point had a classification output, here we aggregated the time points inside the TOI together on motor channels to form feature vectors. This allows the classifier to consider more information, which should enable it to learn better. Put differently, this analysis only provides one overall classification for a trial, rather than one for each time point, but it has more information compared to individual time points. Signals were band-pass filtered from 0.1 to 50 Hz, and smoothed and the time domain features were extracted and aggregated from the sleep TOI and the chosen channels and then fed to the LDA classifier.

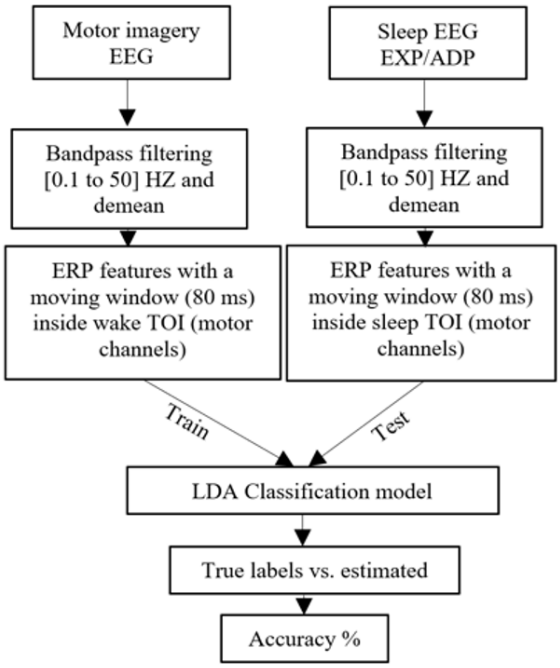


Figure 2.6 Block diagram of the final classification pipeline. Signals were band-pass filtered, and smoothed. Time points inside the TOI were then aggregated using motor channels to form feature vectors that were subsequently given to the classifier for classification.

CHAPTER 3

Targeting targeted memory reactivation: characteristics of cued reactivation in sleep

For this chapter, Anne C. M. Koopman collected data from participants, Suliman Belal and Monika Śledziowska contributed into the scripts of the experiment design. All the EEG, classification and post-classification analyses were done by me and developed in Matlab. Penelope A. Lewis supervised and advised on the study and throughout the work and writing. Matthias S. Treder supervised and advised on EEG analyses and classifiers.

3.1 **Abstract**

Targeted memory reactivation (TMR) is a technique in which sensory cues associated with memories during wake are used to trigger memory reactivation during subsequent sleep. The characteristics of such cued reactivation, as well as the optimal placement of cues to elicit it, remain to be determined. To examine this, we built an EEG classifier that can discriminate between the TMR elicited reactivation of right- and left-handed movements in a finger tapping task. We found that cues which fall on the up-going transition of the slow oscillation (SO) are more likely to elicit a classifiable reactivation related to these movements. Furthermore, we were able to predict the likelihood of eliciting a classifiable reactivation after each cue using pre-cue features of the ongoing SO such as the rising slope and half wave durations. We also found that classifiable reactivations occurred either immediately after the auditory cue or one second later. These findings greatly extend our understanding of memory reactivation in sleep and pave the way for the development of wearable technologies to efficiently enhance memory through cueing in sleep.

3.2 Introduction

Memories are neurally replayed during sleep, and this process is associated with consolidation (Ólafsdóttir et al., 2018; Rasch & Born, 2013; Squire et al., 2015). Targeted memory reactivation (TMR) is a technique in which sensory cues are paired with learned material during wake, then re-presented during subsequent sleep in order to trigger reactivation of the associated material (Cellini & Cappuzo, 2018; Hu et al., 2019). This procedure leads to memory benefits for reactivated material (see (Hu et al., 2019) for a recent meta-analysis). Importantly, several studies have confirmed the reinstatement of learning related brain activity after TMR cues in non-rapid eye movement (NREM) sleep (see (Lewis & Bendor, 2019) for a review). Studies have looked at the neural structures involved in reactivation (Shanahan et al., 2018; van Dongen et al., 2011), and found both positive (Cairney et al., 2018; Schreiner et al., 2018; Shanahan et al., 2018; Wang et al., 2019), and negative (Murphy et al., 2018) relationships between the extent of reactivation and subsequent memory benefits.

Cortical activity during slow wave sleep (SWS) is characterised by high amplitude slow oscillations (SOs) in which neurones oscillate between hyperpolarization and neuronal silence ("down-state") and depolarisation with sustained firing ("up-state"). Depolarised SO up-states drive memory reactivation in the hippocampus via interactions with thalamic sleep spindles (SS) and hippocampal sharp wave ripples (SWRs). One study showed that TMR during the up-going phase was associated with memory benefit, while TMR of the down-going phase was not (Göldi et al., 2017). Another study showed that stimulating the up-going phase of the SO produces a higher ERP response compared to down-going phase (Schabus et al., 2012). This could be due to the fact that neurones are in the process of depolarising and are thus moving closer to the threshold for firing during the up-going phase. Furthermore, fast spindles, which have been linked both to memory consolidation (Nishida & Walker, 2007) and to reactivation (Cairney et al., 2018), typically occur on the up-going phase (Born & Wilhelm, 2012; Siclari et al., 2014).

TMR is thought to prime a memory trace for reactivation (Lewis & Bendor, 2019), and has been shown to trigger SO-spindle complexes (Cairney et al., 2018; Oyarzún et al., 2017; Schreiner et al., 2015). Based on the above observations, we predict that application of such priming during the up-going phase of the slow oscillation, just prior to a spindle event may be more likely to lead to reactivation than application of the same stimulation during the down-

going phase of the oscillation when fast spindles rarely occur and excitability is reduced. SOs vary in terms of generation locus as well as shape, for instance having different periods, trough depths, and peak to trough slopes. These varied morphologies are thought to relate to the degree of synchronisation across neural populations in the cortex (Bernardi et al., 2018; Siclari et al., 2014). Given these differences, some SOs are likely to facilitate reactivation more efficiently than others. We hypothesise that it may be possible to predict this efficiency based on features of the ongoing oscillatory structure of sleep, with specific reference to SOs and spindles, in the time period directly before stimulation. This would not only optimise stimulation, but would also allow selective targeted stimulation, minimising the number of sound cues needed to influence consolidation, and thus minimising the risk of disturbing sleep through provision of excessive cues.

In the current chapter, we set out to characterise memory reactivation after TMR in NREM sleep and to determine whether applying TMR on the up-going phase is more likely to elicit reactivation, and also whether it is possible to predict the optimal time for TMR stimulation using the ongoing morphology of SOs and spindles. Following our prior work on classification of memory reactivation after SWS TMR (Belal et al., 2018), we used a serial reaction time task (SRTT) (Koopman et al., 2020), (Chapter 2), in which participants respond to audio-visual cues by pressing 4 buttons using two fingers on each hand. Each finger press was cued by a picture-sound pair, and the tones associated with the task were replayed during SWS on the night after training to elicit memory reactivation (Figure 2.5). Importantly, we also played the relevant tones during an adaptation night when the participant slept in the lab prior to training each participant on the SRTT task. This provided a night of control data during which tones could not have evoked memory reactivation, as they were not yet associated with any memories. We then trained a classifier to identify neural responses associated with left and right-handed presses in wake and applied it on the data after each TMR tone in SWS on both adaptation and experimental nights. Finally, we used the features of the ongoing oscillation to train another classifier to determine whether TMR applied at a given time in the oscillatory sequence would elicit detectable reactivation.

3.3 Results

3.3.1 TMR improved sequence memory

The SRTT task is reliably facilitated by TMR in SWS (Cousins et al., 2014, 2016; Monika Schönauer et al., 2014). Our data are in line with this, since improvement on sequence memory cueing was associated with a significant advantage in overnight improvement (paired-samples cued vs. uncued t-test, n = 13, p = 0.049) (Koopman et al., 2020)**.**

3.3.2 Multiple reactivations detected after TMR

Prior work (Cairney et al., 2018; Schreiner et al., 2018) has suggested a recurrent pattern of reactivation after a TMR cue, with a reinstatement of the target memory immediately after the cued memory followed by a later reinstatement, see (Lewis & Bendor, 2019) for a discussion. Building on this work, we examined the time course of classification after TMR for evidence of a similar pattern. Our results revealed significantly higher classification performance in the experimental night than the adaptation night with two different effects described by two clusters after TMR onset (Figure 3.1a). An early cluster (p = 0.02) that occurred immediately after TMR onset and a late cluster (p = 0.01) that occurred ~1sec later. Results are corrected for multiple comparisons with cluster-based permutation (see methods for details), trial duration in sleep was 1500 ms.

To test whether this was due to recurrent reactivation of the same response, we examined each trial to see whether it included an early reactivation, a late reactivation, or both. We then looked at whether the same trials were classified correctly at both early and late peaks (Figure 3.1b). This revealed that the majority of trials contain one peak, either early or late, and only 8.7% of trials showed reoccurring reactivation by classifying correctly during both early and late peaks. Comparison of the prevalence of reoccurring reactivation to chance level showed it was below chance (Wilcoxon signed rank test, n = 12, p = 0.002, z = -3.0594) (see methods for details); (Figure 3.1b). Overall, these results suggest that the reactivations we are detecting in this paradigm are not recurrent, but instead normally occur just once after each cue: either early or late within our trial duration.

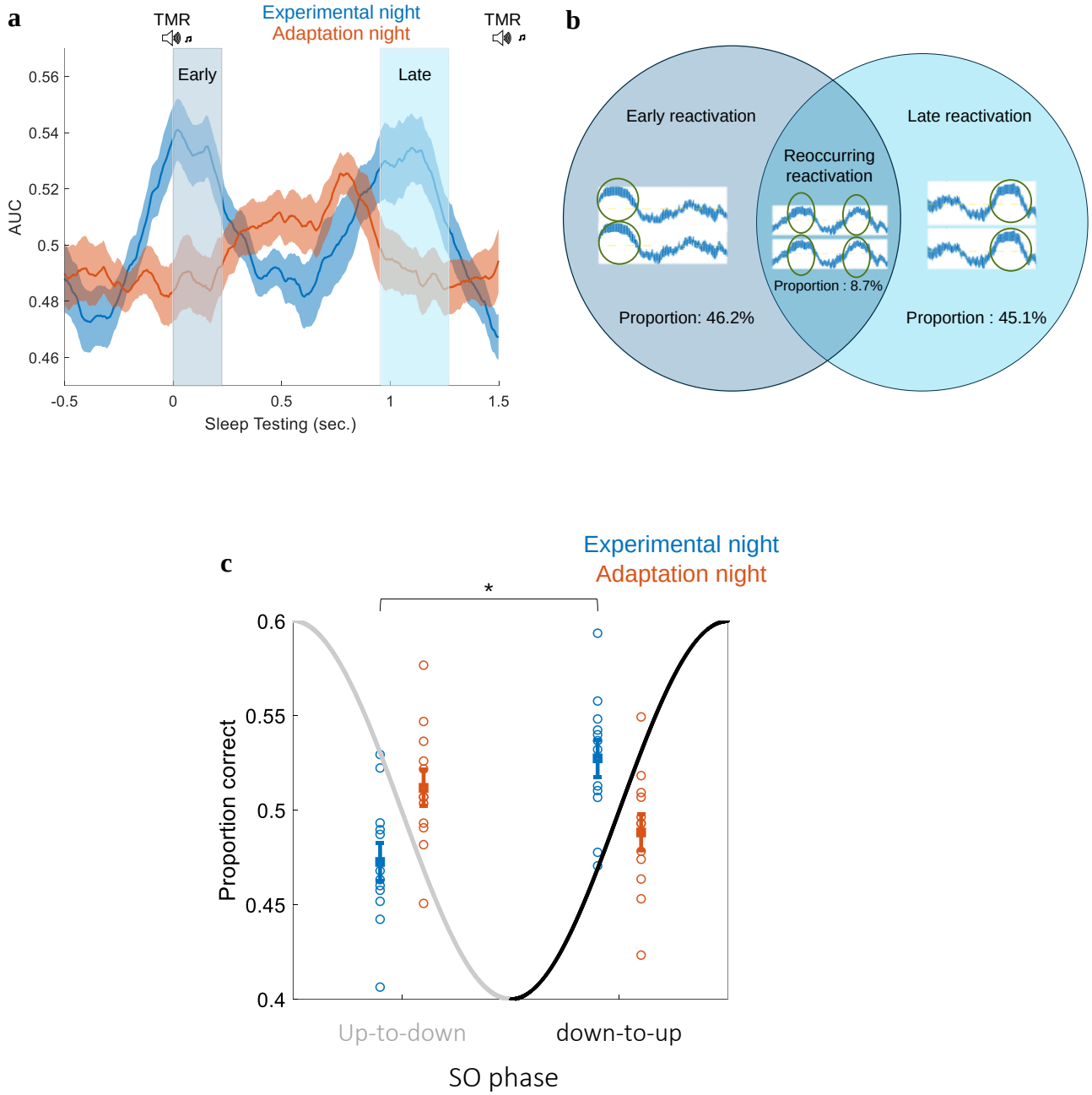


Figure 3.1 Classification results from training on wake and testing on sleep. **a** Classification results for both nights. The blue curve represents the area under the ROC curve (AUC) across time for the experimental night (with standard error shaded around the mean), red curve represents the adaptation night, TMR sounds are presented at the beginning of sleep trials, 'early' and 'late' are used to mark early and late reactivations. Classification results have two significant effects expressed by two clusters, (early cluster, p = 0.02, and late cluster, p = 0.01). **b** Proportions of correct trials with only early reactivation (46.2%), only late reactivation (45.1%), and reoccurring reactivations (8.7%). **c** proportion of correct trials with

the TMR cue falling on different SO phase transitions for the two nights, circles represent participants and the grey curve is a simplified cartoon representation of the phase of a slow oscillation (SO), two phases are marked on the x-axis (Up-to-Down and Down-to-Up) and the y-axis represents the proportion of correct trials. The preferred phase for early reactivation is when the sound falls on the up-going transition of the SO (Wilcoxon signed rank test, n = 12, p = 0.019, z = 2.4) compared to down-going.

3.3.3 Preferred TMR phase for reactivation

There is evidence that TMR may be more effective when applied to the up-going phase of the SO (Göldi et al., 2017). Moreover, fast rhythms, such as spindle, and gamma activity are more prominent in the SO up-going state than in the SO down-going state (Mölle et al., 2002; Piantoni et al., 2013; Valderrama et al., 2012), also there are changes to the ERP when the auditory stimulation is applied during the up-going phase of the SO (Schabus et al., 2012). Building on the extensive literature relating to reactivation during rodent sharp-wave ripples (Kudrimoti et al., 1999; Nakashiba et al., 2009; O'Neill et al., 2008), data from human epilepsy patients has shown that the SO up-going state shows higher gamma oscillations (Van Quyen et al., 2010), and sharp-wave ripples, which have been shown to carry reactivation (Zhang et al., 2018), on the other hand, ripples are supressed during the SO down-going state (Clemens et al., 2007). Thus, up-going state appears to be the preferred time for reactivation (Göldi et al., 2019).

Given this background, we predicted that TMR would more effectively trigger reactivations if applied to the up-going phase of the oscillation. We tested this hypothesis by dividing our correctly classified sleep trials post-hoc based upon the phase at which TMR was initiated, see methods 3.5 for details. In the experimental night, this showed a significantly higher proportion of correct trials for early reactivation when TMR was applied on the up-going compared to the down-going SO transition and the chance level of 0.5 (Wilcoxon signed rank test, n=12, p = 0.019, z = 2.4), Figure 3.1c. As a control, we compared the proportion of correct trials of the adaptation night between these two transitions and also against chance and found no difference (Wilcoxon signed rank test, n = 12, p = 0.24, z = -1.18). We repeated this analysis for the incorrectly classified trials for early reactivation and found no significant difference between

transitions and chance level for the experimental night (Wilcoxon signed rank test, n = 12, p = 0.16, z = 1.4) nor for the adaptation night (Wilcoxon signed rank test, n = 12, p = 0.31, z = -1.02). We also did the same for late reactivation but found no difference between up-going and down-going phase transitions. We then tested whether applying TMR on either the positive or negative half wave leads to detectable early reactivation, by following the same approach. Analysis of the phase values of the correct and incorrect trials did not show a preferred phase (positive or negative half waves) compared to chance for both experimental and adaptation nights.

This analysis shows that TMR cues which fall on the up-going transition of the SO are more likely to lead to a classifiable early reactivation than TMR cues that fall on the down-going phase, supporting the idea that slow oscillations interact with reactivation in some functional way. This could also be important for optimisation of TMR cueing in order to successfully trigger reactivation.

3.3.4 Predicting reactivation using pre-cue Slow Oscillation features

While the literature suggest that reactivation is modulated by SOs, (Inostroza & Born, 2013; Ngo et al., 2018; Rasch & Born, 2013), the mechanism for this modulation remains to be understood. We were interested to determine whether the features of the ongoing SO prior to stimulation could predict whether a given TMR cue would produce a classifiable reactivation. In other words, we wanted to know whether some points in the oscillatory pattern are more optimal than others for delivering TMR, and if so, which features of the ongoing oscillatory structure determine this. To examine this, we performed a second classification analysis, this time training our classifier on SO features. We wanted to see if we could discriminate between trials in which the hand for which movement was being cued was classified correctly vs. incorrectly (the results of main reactivation classifier, Figure 3.1a). To this end, we extracted SO features from the Fz electrode during the two seconds of data before the onset of TMR.

The extracted features are described in Extended Data Table 1. These features were fed to decision tree classifiers (Gordon et al., 1984) which were trained on two classes: correctly classified, and incorrectly classified from the main classifier, see methods for more details. As a control, we compared the results obtained from the experimental night SO-based classifier to a SO-based classifier trained and tested using the adaptation night, Figure 3.2a. The performance of the experimental night classifier was significantly higher than that of the

adaptation night for predicting the early reactivation (Wilcoxon signed rank test, n=12, p = 0.015, z = 2.43) but not late reactivation (p > 0.2). This indicates that it was possible to predict classifiable early reactivation in the experimental night when learned information could actually be reactivated compared to the control condition when nothing had been learned yet. This result shows that we can use SO features to predict when to optimally deliver TMR in order to maximise the probability of producing a classifiable early reactivation.

In addition to the ongoing pattern of SO oscillations, we were interested in how the ongoing pattern of spindles might impact upon the ability of TMR to elicit classifiable reactivations. We therefore repeated the above analysis, now using spindle features. We thus trained a spindle-based classifier to predict whether we could use these higher frequency oscillations to determine whether TMR would produce a correct classification. We used features from channels around the motor area (C5, CP3, C6, and CP4). We thus extracted a binary value representing whether there was a spindle in the 1.5 seconds duration pre-cue (0: no spindle, 1: has spindle) and used this in a decision trees classifier, see methods. This showed that we can discriminate between correctly classified and incorrectly classified trials only in the experimental night and not the adaptation night (Wilcoxon signed rank test for experimental vs. adaptation, n = 12, p = 0.04, z = 2.04), Figure 3.2b. Subsequently, we analysed the trials of each participant to get an idea whether it was the presence or absence of spindles that might predict which trials had been correctly classified by the reactivation classifier. This showed that trials with fewer pre-cue spindles are more likely to have late reactivation (Extended Data Figure 4). This is in keeping with the study by (Wang et al., 2019) in which significant post-cue reactivation was observed in trials with low pre-cue sigma power. It was argued that spindles have a periodicity of about 4 seconds, thus, it is possible that the occurrence of pre-cue spindles which prevented post cue spindles and reactivation in the (Antony et al., 2018) study also prevented late reactivation in our study. However, it is notable that there was no such relationship with early reactivation. Overall, these results suggest that we can use spindle features to predict when to deliver TMR in order to trigger a classifiable late reactivation.

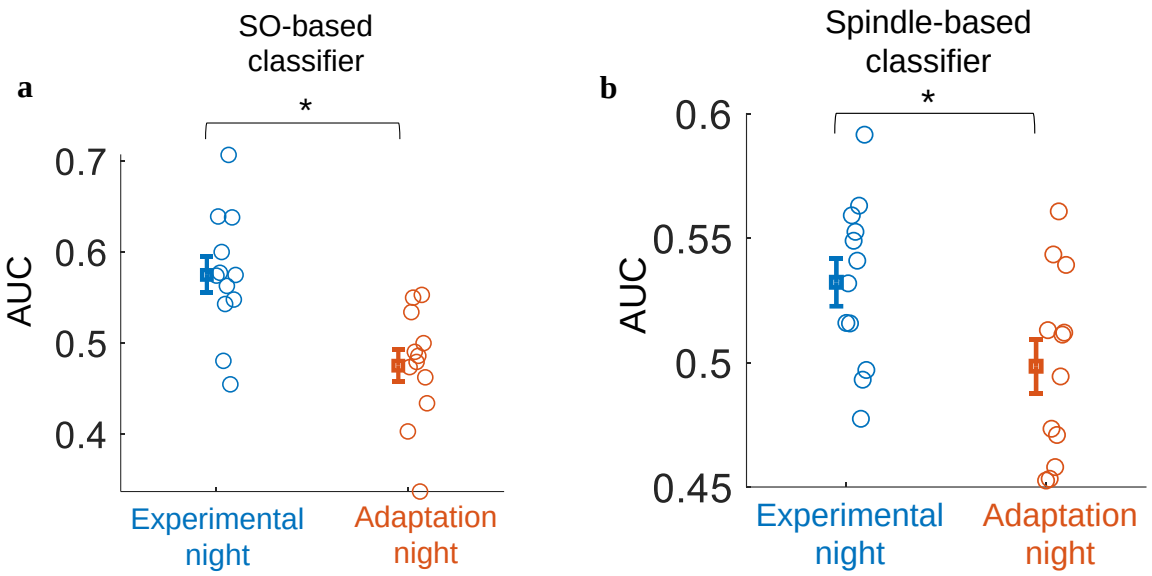


Figure 3.2: Predicting reactivation using pre-cue features. **a)** Classification results of the SO based classifier for the experimental vs. the adaptation night for early reactivation (Wilcoxon signed rank test, n=12, p = 0.015, z = 2.43). **b)** Classification results of the spindle-based classifier for the experimental vs. the adaptation night for late reactivation (Wilcoxon signed rank test, n=12, p = 0.04, z = 2.04).

3.3.5 Characteristics of detected reactivations

Because this is a motor task, we wanted to know whether classification of reactivation was derived from the channels over the motor area. We therefore analysed the selected features that were included for classification after the feature selection step. This showed that the selected features always came from the motor area channels (C5, CP3, C6, CP4), with 66.7% of features being chosen from the right motor channels and 33.3% from left. This shows that the activity patterns in wake and sleep arise from the motor area and are related to the motor task.

Because sleep is characterised by relatively low frequencies such as SOs (0.5 – 1.5 Hz), delta waves (1.5 – 4 Hz), and theta (4 – 8 Hz), we hypothesised that these would be the most important for our classification. To investigate this, we applied a low pass filter with cut-off frequency of 10 Hz without smoothing the signals. The resulting classification pattern was similar to the result without this filter in Fig. 2a (early cluster, p = 0.01, and late cluster, p = 0.03), suggesting that feature of the low frequency range is deriving classification.

As shown in Figure 3.1a, we found that reactivation could occur at either of the two different timepoints - either early after the onset of the cue or approximately one second later. This shows the temporal characteristic of reactivations within trial duration. We also wanted to examine the characteristics of reactivations occurring at these two different times across the time course of stimulation. Our prior work on this task suggested that classification performance decreases as the number of stimulations in a night increases (Belal et al., 2018). We were therefore interested to know if this finding would hold with our new classification pipeline and data. We tested whether more correct classifications occur before or after the middle of the stimulation time by indexing trials that were classified as correct for early/late reactivation to range from 0 (first trial in stimulation) to 1 (last trial) for every subject, then we compared the indices of all trials to 0.5 (middle of stimulation) across subjects. This revealed that reactivations could be detected to a similar extent at any time during stimulation and was not more prevalent at the beginning or end. Neither reactivations which occurred right after the TMR tone, nor reactivations which occurred ~1 second after the TMR tone differed significantly from the middle of the stimulation time (Wilcoxon signed rank test, $n = 12$, $p = 0.39$, $z = 0.86$, and $p = 0.58$, $z = 0.55$ for early and late reactivations, respectively).

Finally, we wanted to examine how the performance of early and late reactivations varied across the night of stimulation. Thus, we obtained a performance curve across stimulation time for each peak by observing the changes of classification performance during the time of that peak throughout trials of stimulation (Extended Data Figure 1). We used a 50-trial block to calculate classification performance and slid this by one trial at a time to progress across the stimulation time. We then normalised the stimulation time to have the range [0 to 1], with 0 being the first stimulation in the night and 1 the last stimulation. Interestingly, classification performance differed between the two peaks around approximately 0.6, that is, 60% of the way through stimulation time, with early reactivation more likely to occur at this time (Extended Data Figure 1).

3.3.6 The relationship between behaviour and classification performance

Some prior reports have shown a positive relationship between detectable reactivation after TMR tones and the extent of TMR related benefit (Bendor & Wilson, 2012; Cairney et al., 2018; Schreiner et al., 2018). We searched for this relationship by testing for correlations between classification and behavioural performance. Because different trials classified

correctly at early and late timepoints after the cue, and because such temporally distinct reactivation may potentially also have distinct functional characteristics, we performed all correlations twice, using the classification rate at first the early peak and then the late peak. This revealed a negative correlation between pre-sleep reaction time for the reactivated sequence and classification AUC in the early peak (Spearman r = -0.60, uncorrected p = 0.04), Figure 3.3a. In other words, faster pre-sleep performance was associated with a more classifiable reactivation immediately after the TMR cue. This could mean that a stronger representation had formed, and this could reactivate more easily.

Interestingly, the late peak showed quite different associations from the early peak. Here, classification AUC negatively predicted the extent to which responses on the cued sequence sped up across the night of sleep (performance just before sleep – performance early post-sleep), (Spearman r = -0.72, uncorrected p = 0.01), Figure 3.3b. We refer to it as overnight improvement, however, this improvement is the improvement of reaction time for the task and not the improvement in learning the sequence. The late peak AUC also predicted slower reaction times for the non-cued sequence after sleep (Spearman r = 0.68, uncorrected p = 0.02), Figure 3.3c. Thus, the stronger the late peak the slower the non-reactivated sequence was performed immediately after sleep. These results could suggest that when reactivation occurs ~1 second after the TMR cue it somehow disrupts both the spontaneous consolidation of the non-reactivated sequence and the cued consolidation of the reactivated sequence. Late reactivation could have this property which is in-line with the study that showed a negative correlation between reactivation and improvement (Murphy et al., 2018).

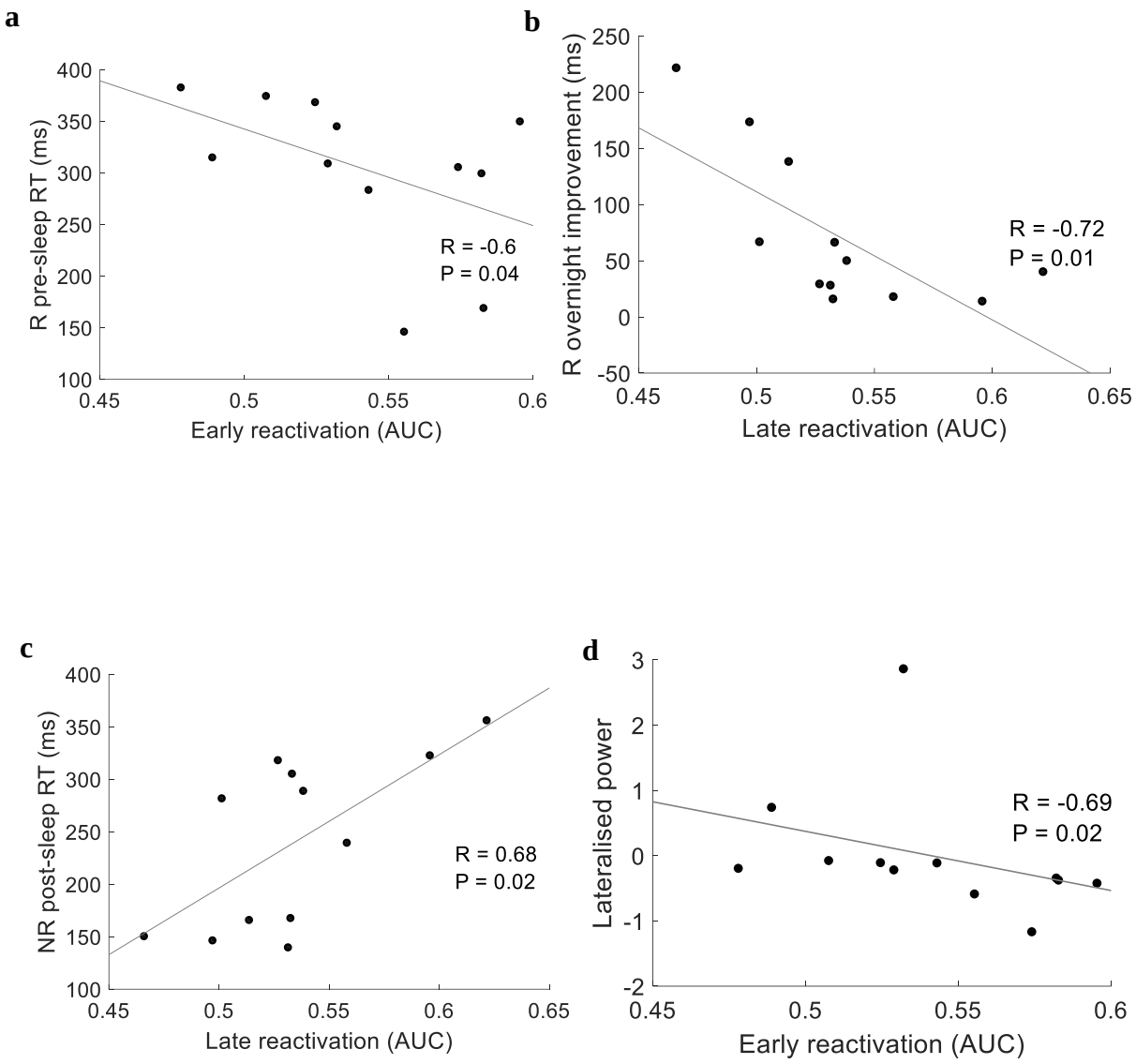


Figure 3.3: Correlation with behavioural results. **a)** negative correlation between the classification performance (AUC) of the first peak and the average reaction time of the last four blocks before sleep for the reactivated (R) sequence (spearman correlation = -0.60, uncorrected p = 0.04). **b)** Late peak correlated negatively with the overnight improvement of the reactivated sequence (spearman correlation = -0.72, uncorrected p = 0.01). **c)** Late peak predicted slower reaction times after sleep for the non-reactivated (NR) sequence (spearman correlation = 0.68, uncorrected p = 0.019). **d)** Correlation of lateralized sigma power (z-transformed) with classification performance for the early peak (Spearman correlation = -0.69, p = 0.016).

3.3.7 The relationship between sleep spindles and classification performance

Sleep spindles have been strongly linked with memory reactivation (Antony et al., 2019; Klinzing et al., 2019; Rasch & Born, 2013). Work in rodents shows that replays correlate with spindles (Peyrache et al., 2012). Lateralised spindle density during cue periods has been shown to predict TMR-related benefit (Cousins et al., 2014), and lateralised spindle power over motor cortex is strongly associated with overnight improvements in finger tapping tasks (Nishida & Walker, 2007). We tested for a relationship between sigma power at (11 to 16 HZ) and classification performance. We found that, even though participants used both hands in this task, the lateralized sigma power was negatively associated with the early classification peak, (Spearman $r = -0.69$, $p = 0.016$) as shown in Figure 3.3d. Thus, the more the lateralised spindles right before the stimulus compared to after, the more likely we were to classify reactivation immediately after the TMR cue (more details about power calculation in methods). This is interesting in light of a prior analysis of our behavioural data showing TMR-related improvement in the weaker left, but not the stronger right hand over sleep (Koopman et al., 2020). This correlation suggests that a lateralised spindle response may provide a marker for more classifiable early reactivation.

3.4 Discussion

This study examined memory reactivations after auditory TMR of a finger tapping task in SWS using EEG classifiers. We found evidence of reactivation both immediately after TMR cues and about one second later. Importantly however, most correctly classified trials contained a reactivation at just one of these time points. We also found that TMR cues applied during the up-going state of the SO were more likely to result in a classifiable reactivation than those applied during the down-going transition. Furthermore, we showed that the pattern of ongoing slow oscillations and spindles before a TMR cue can be used to predict whether that cue will produce a classifiable reactivation. These findings markedly deepen our understanding of neural reactivations after TMR cues in sleep and may lead to improved methods for efficient boosting of memory via the TMR manipulation.

3.4.1 Timing of reactivation after the cue

The delay between TMR onset and triggered reactivation is a matter of current investigation. Thus, rodent work showing a reverberation of reactivation between cortex and hippocampus

(Rothschild et al., 2017) has led to the suggestion that replays may 'echo back' again and again after TMR. In fact, work in rodents (Bendor & Wilson, 2012) suggests that TMR cued replay can continue to repeat for up to 10 seconds after the offset of the auditory cue and a second cue can interrupt this replay. Turning to humans, one study showed strong reactivation about two seconds after the cue, with a trend towards an earlier reactivation immediately after the cue (Cairney et al., 2018). Another study showed recurrent reactivation after a TMR cue, one immediately after the cue and a second one about two seconds after the cue (Schreiner et al., 2018). Our findings are in keeping with this work since they suggest that reactivation can occur either immediately after the cue or around one second later. Because our inter-trial interval was only 1500 ms, it is possible that the start of the next trial, marked by a TMR cue, may have disrupted the reactivation pattern such that we were unable to identify reactivations after this time. Importantly however, our data shows that within a single trial, reactivation does not occur at both early and late timepoints, but only at one or the other. It is possible that this may also have been the case in the prior human studies (Cairney et al., 2018; Schreiner et al., 2018), as they looked at the average across trials instead of examining individual trials.

Since our data show that reactivations occur at different delays (immediately after the cue and one second later) on different trials, we must ask whether such differences in timing are important. Interestingly, we found that the early peak in reactivation was predicted by pre-sleep behavioural performance, while the late peak showed no such correlation and was instead negatively correlated with overnight improvement in reaction time on the cued sequence. It is difficult to interpret these findings, but one possibility is that a strongly encoded memory of the task leads to more immediate reactivation after a TMR cue. On the other hand, late reactivation may result from a weaker memory trace, and might actually disrupt consolidation of the task. It is also possible that weaker memories that do not reactivate immediately after the TMR cue could become distorted during the delay, such that late reactivation is counterproductive to consolidation instead of beneficial. After all, reactivation is associated with spindles (Antony et al., 2018) and TMR is thought to trigger SO-spindle complexes (Cairney et al., 2018; Oyarzún et al., 2017). Spindles have been shown to gate Ca2+ influx into dendrites, thereby facilitating synaptic plasticity (Rosanova & Ulrich, 2005; Seibt et al., 2017) and this Ca2+ influx is strongly amplified when spindles coincide with SO up-states (Niethard et al., 2018). One recent study even showed that the more closely spindles coincided with SOs after a cue, the higher the fidelity of the associated reactivation signal (Schreiner et al., 2020).

Thus, delayed reactivation that does not correspond to a SO coupled spindle may be counterproductive.

We also aim at analysing whether the early reactivation was caused by the TMR cue at time 0 or whether the brain was able to predict the upcoming cue and thus reactivated its contents before it was presented. This possibility seems likely given that the task is a sequence that gets repeated many times and the intertrial delay is fixed. This could enable a temporal predictability after representing the sequence many times such that the brain adapts to the intertrial delay and knows the upcoming cue because it follows the sequence order that was encoded in wake.

3.4.2 Optimal timing of TMR cues

The exact mechanisms by which TMR triggers reactivation are unknown, but the up-going phase is clearly more reactive to stimulation than the down-going phase, since neurones are preparing to fire as the slow oscillation approaches its peak and beginning a silent period as it enters the trough. Stimulation after the negative peak of the SO, during the up-going phase, was shown to produce a higher amplitude than stimulating during the down-going phase, this finding shows a phase dependent ERP response and may suggest a different relationship between reactivation and up- vs. down- going phase of the SO (Schabus et al., 2012). SOs are highly heterogeneous, differing both in locus of generation and in terms of shape. For instance, SOs differ in period, trough depth, and peak to trough slope (Bernardi et al., 2018; Siclari et al., 2014). Importantly, the SO down-state is thought to be required for the generation of a thalamic down-state which triggers a spindle (Mak-McCully et al., 2017). On the other hand, the SO up-going state is thought to initiate memory reactivation with sharp wave ripples and thalamo-cortical spindles (Sirota & Buzsáki, 2005). Given the established association between memory reactivation and spindles, and given that spindle initiation apparently requires a sharp SO trough, it is reasonable to suppose that TMR stimulation of some SOs may be more likely to trigger reactivation than TMR stimulation of others. For instance, SOs with a deeper trough or steeper slope, or some combination of these might be more likely to carry reactivation-bearing spindles. Such differences could explain why we were able to predict which stimulations would be successful based on the features of the ongoing SO before the TMR cue, although, notably, the combination of features was necessary and no single SO feature was sufficient for this prediction. Related to this, we also found that trials with fewer pre-cue spindles are more likely to have late reactivation (Extended Data Figure 4). This is in good

keeping with work from Wang and colleagues, (Wang et al., 2019), showing that less pre-cue spindles predicted more post-cue reactivation, and that such reactivation begins around one second after the onset of the cue.

Importantly, such predictive analysis could potentially be used to boost the efficacy of TMR by ensuring that stimulation occurs only at the times when it is most likely to be effective. This could minimise any potential disturbance from TMR, which does often lead to arousals when delivered indiscriminately. Such increased precision of cue delivery could be important for translation of the TMR technique from lab to the home environment.

3.4.3 Conclusion

This study elucidates several interesting characteristics of TMR cued reactivation, how this relates to the ongoing oscillatory pattern in slow wave sleep, and how best to elicit it. For a start, we show that reactivation can occur at different times after the cue and these times are different from wake. Detected reactivations are not recurring after one sound cue. Early and late reactivations also appear to have different functional significance. Furthermore, we show that the SO up-going transition is a preferred window for TMR delivery, probably because it heralds the spindle-bearing upstate. Finally, we show that both pre-cue SO morphology and spindle incidence can be used to predict TMR cued reactivation, providing a clear mechanism for more efficient stimulation in future studies as well as delivery of TMR by wearable devices for at-home manipulation of reactivation to facilitate memory. In our next study, we would like to explore whether the triggered early reactivation is caused by the current cue at time 0 or whether it could be a result of the brain expecting the upcoming cue and thus reactivating its information. We would test this by jittering the onset of TMR such that, if the early reactivation is absent this could mean that the brain was reactivating the information of the upcoming cue.

3.5 **Methods**

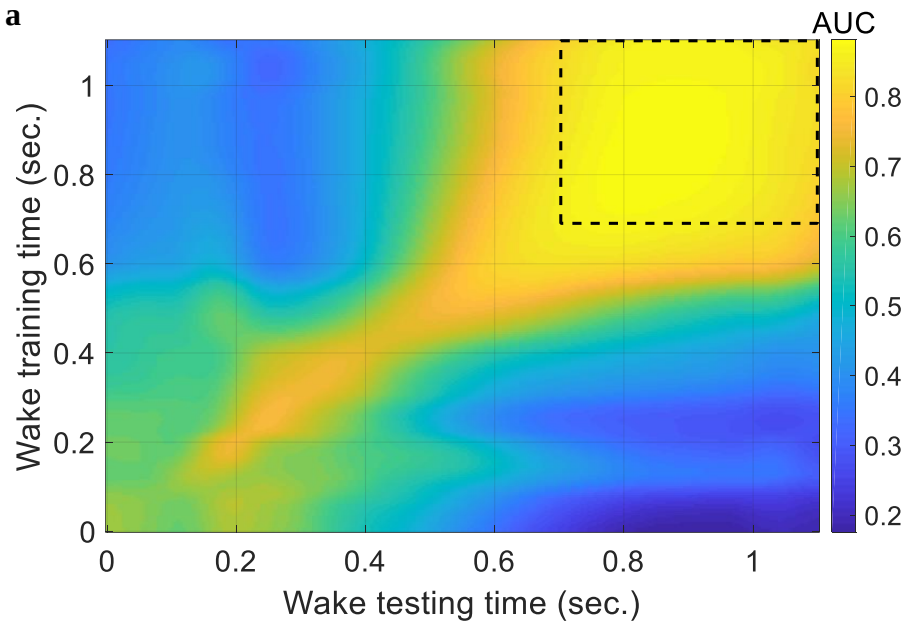
3.5.1 Wake-to-wake classification to locate a time of highest classification rate

We started the analysis by performing a wake-to-wake motor imagery classification. This was performed for each subject separately, with trials serving as observations and are being labelled according to the hand they belong to. EEG signals were band-pass filtered (0.1 to 50 Hz) and the mean was subtracted. Features were extracted by calculating time-domain amplitude

averages of 80 ms (40 ms before and 40 ms after every time point). Subsequently, features were fed to a linear discriminant analysis (LDA) classifier (Blankertz et al., 2011). Each classifier was trained and tested on data during the wake experiment before sleep, in a time x time fashion (King & Dehaene, 2014). The classifier was trained on a specific time point and tested with all time points to build one row in the time x time classification, illustrated in Figure 3.4a. We assumed that if a classifier is not classifying at a considerably high rate during wake, then this would decrease the possibility of that classifier to classify sleep reactivation where noise is higher. Consequently, we chose the subjects with wake-wake classification with Area Under the ROC Curve (AUC) >= 0.7, (n = 13). One subject was neglected because of a technical problem during the collection of sleep data. The rest of data was used for classification (SWS: n = 12). We also do realise the rich literature of motor imagery classification with common spatial patterns (CSP) and other methods (Blankertz et al., 2008; Lemm et al., 2005; Pfurtscheller et al., 1997, 2006; Ramoser et al., 2000). However, given the differences between wake and sleep data sets and their different nature of noise and oscillations we decided to use time domain features with our classifiers.

Initial investigations revealed a higher classification performance for left- vs. right-hand (where both fingers were aggregated into one class) than for faces vs. objects. Therefore, we conducted the analysis on left- vs. right-hand imagery. The trial length was defined as the duration between cue onsets (1.1 sec. in wake). Sound cues had a duration of 200 ms and were played from time 0 of the trial. During sleep, trial length was 1.5 sec.

Motor imagery classification during wake shows a time period with maximum classification performance (marked with dashed box in Figure 3.4a). This time region should be useful for discriminating left hand and right hand. We defined this time period as the time of interest (TOI). A TOI is a time window that has high classification rate, indicating its ability to discriminate the classes. It acts as a temporal marker of expected discrimination. To locate this window, we used a threshold of 0.85 on the average classification AUC from all subjects.



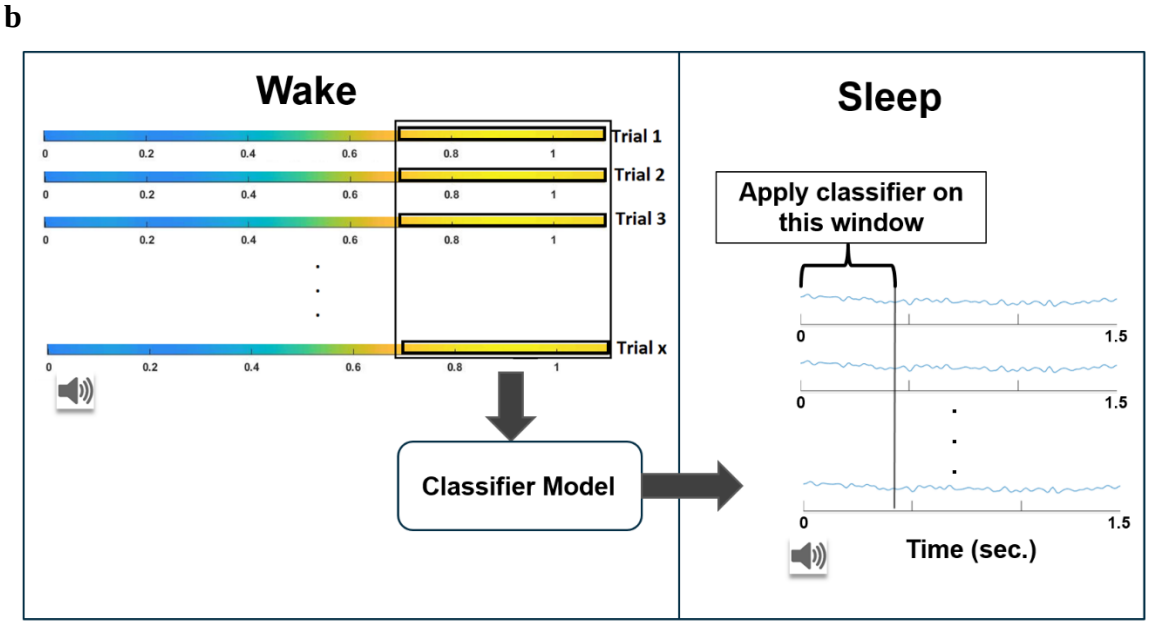


Figure 3.4: Classification with a classifier trained using wake motor imagery. **a)** Grand average classification AUC for left- vs. right-hand motor imagery using a sliding 80ms smoothing window and LDA classifiers, dashed box represents the time of interest (TOI). **b)** Illustration of classification procedure of left- vs. right-hand (training: wake and testing: sleep) which is applied for both the experimental night and adaptation (control) night. A sliding window approach is performed, wherein a classifier is tested on a window from sleep

and the classification result replaces the centre of that window then the window is slid by one time point to construct a performance curve across time (blue curve).

3.5.2 Wake-sleep Classification

Once we had built a classifier on wake data, we tested it on sleep data. We applied it to sleep using a sliding window approach, as shown in Figure 3.4b. Using the sliding window approach the classification was applied on the first testing window in sleep, for example: [0 to 0.38] second, which matches the length of the TOI. Then, the classification performance is placed at the centre time of this window, i.e., at 0.190 second. Subsequently, the sliding sleep window is shifted by one time point and the process is repeated. Thus, the results of classification are AUC values across time.

The wake-to-sleep classifier used the concatenated averages inside the TOI as features. These concatenated time points were reduced to the most informative contiguous time points using mutual information on wake data for each participant. The reason for that is to reduce the features to the most informative time points since the reactivation might be temporally short compared to wake activation. Consequently, we slide a shorter window that contains the most informative features which enables the classifier to detect the reactivation if it was temporally short or long. The most informative time points were chosen such that the time points are contiguous and contain the highest 10% of the mutual information values.

We devised a method for removing noisy trials with no TMR effect. Let us imagine that the noisy trials belong to a new ‘no effect’ class which is different from right- and left-hand. The features of those trials in the feature space should fall near the decision boundary, in a region of uncertainty of the classifier; Extended Data Figure 2a. Thus, we define trials as ‘no effect’ if they fall in that area. We rejected noisy trials falling close to the boundary and used 300 clean trials from every participant, as there were 300 clean trials in the participant with the lowest number of such trials, and we wanted to be consistent among participants. Those 300 trials correspond to a certainty average of 0.86, with 0.1 standard deviation. Importantly, to avoid any bias, this cleaning process was unsupervised, meaning that the information of the ground truth class labels of sleep data was not used. Moreover, we verified that this cleaning process

would not be useful if the data we were trying to clean was random and contained no useful information, as illustrated in (Extended Data Figure 2b). This was the case with the control night. It would also not be useful if sleep data was not scattered in a similar way to wake training samples because the decision boundary position and orientation which are determined using wake will then be meaningless for sleep samples. Thus, this cleaning process only works if the data is not random. Importantly, the exact same cleaning procedure was performed for both the experimental and adaptation night for completeness.

3.5.3 Preferred TMR phase analysis

Phase information was extracted using Hilbert transform on the band pass filtered signal (0.5 to 2 HZ) using electrode FZ. We divided phase values into two ranges: [0 to π] and (π to 2π], indicating the two transitions: down-going and up-going, respectively. For each participant, we determined the number of correctly classified trials in which TMR fell on either phase range in each night, then normalised by the total number of correct trials. We compared the proportion of correct trials where TMR occurred in the down-going and up-going transitions of the SO. The same process was repeated for the incorrect trials of the experimental night also, the correct and incorrect trials of the adaptation night.

3.5.4 Lateralised sleep sigma power analysis

The lateralized sigma power [11 16] HZ was calculated using short time Fourier transform during the duration: [0 to 0.5] sec. relative to cue onset which is around the early reactivation. Lateralised power was calculated as the difference between left and right motor channels (C6, CP4, C5, CP3) and was baseline corrected ([-0.2 0] sec. relative to cue onset). Consequently, percentage change from baseline was calculated.

3.5.5 Reoccurrence of reactivation

We statistically tested if one reactivation (early or late) is more likely to happen or whether reactivation is reoccurring after a sound cue. Thus, we took the accuracy for recurring reactivation (i.e., the ratio of correct trials during the time of both early and late reactivation simultaneously) and compared it to the probability of both reactivations happening simultaneously after a sound cue (the accuracy for early reactivation multiplied by the accuracy

for late reactivation) as a chance level. We performed this analysis for every subject and compared the accuracy of reoccurring reactivation to chance level.

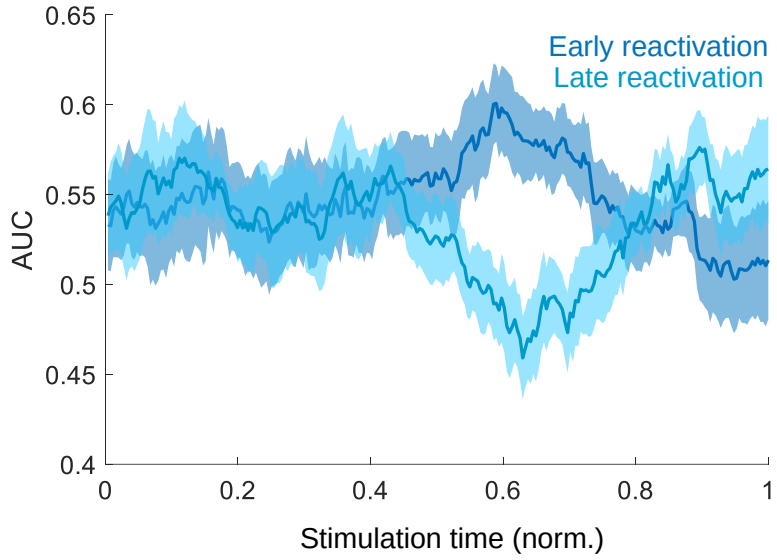
3.5.6 SO based classification

The SO based classification consisted of 200 decision trees ensemble. Leave one out classification is used wherein the data of all participants except one is used to train the classifier and the left-out participant is used for testing the classifier. This gives a classification result for the left-out participant and the process is then repeated until the classification performance is calculated for all participants. Every decision tree is trained on a random subset of trials from the training set and tested on the testing set and the final result is the aggregated votes from all decision trees.

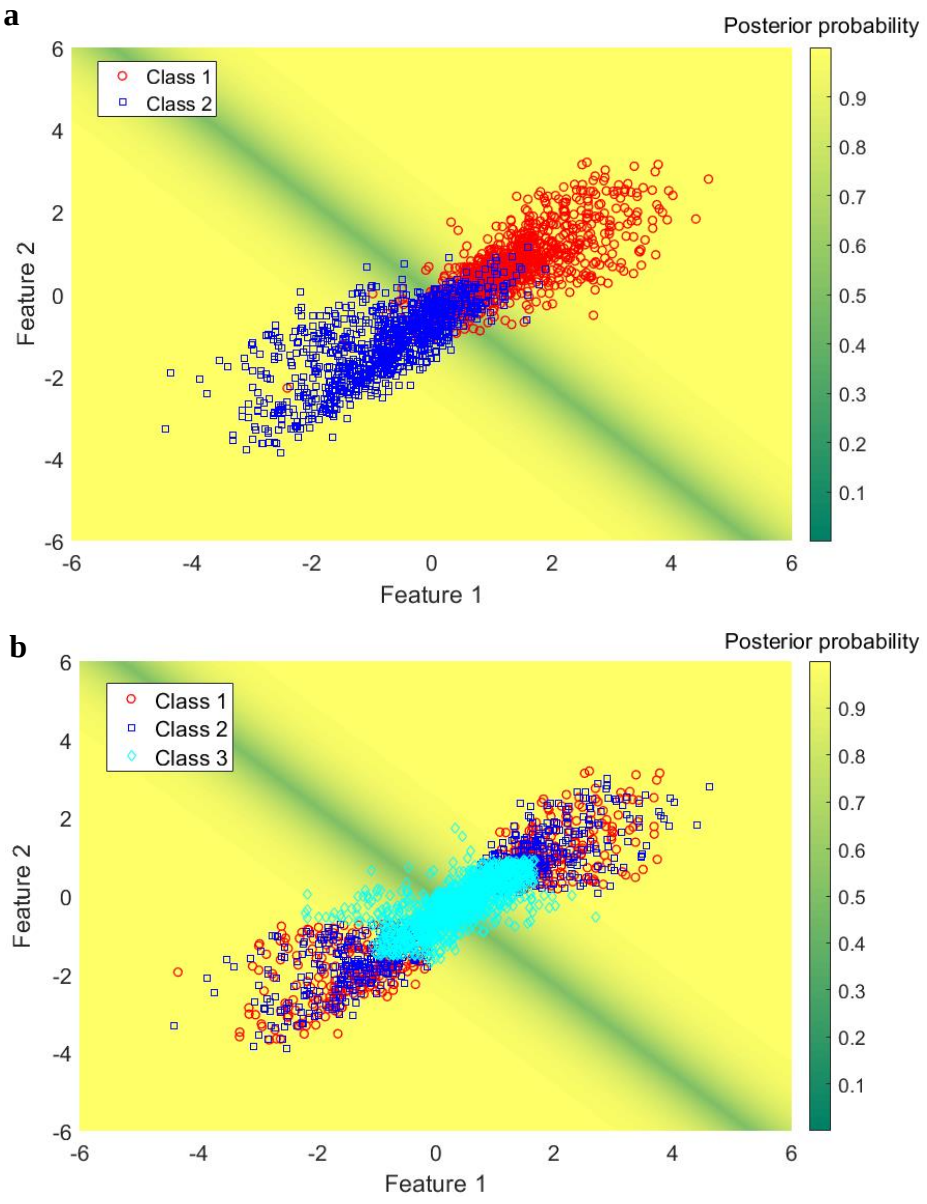
3.5.7 Statistical testing

To assess the statistical significance of the classification results, we compared the classification performance of the experimental night against the adaptation/control night. Sounds played during the adaptation night were the same sounds used in the experimental night but because the adaptation night was before participants had been trained on the experimental task, these sounds were not yet associated with any memories. This control was used to make sure that classification is not derived due to some sound induced features/noise in EEG.

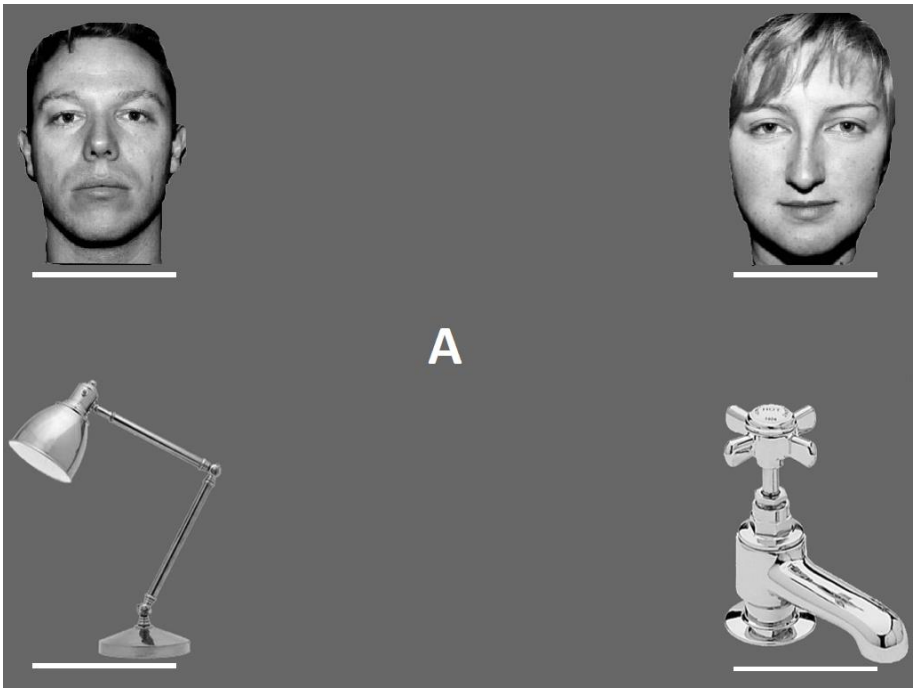
Statistical analysis was performed using the classification results of the two nights with cluster-based permutation using Fieldtrip (Oostenveld et al., 2011). Monte Carlo was used with a sample-specific test statistic threshold of 0.05, a permutation test threshold for clusters of 0.05, and 10,000 permutations. The correction window used in the test was the whole length of sleep trial, i.e., [0 to 1.5] sec.



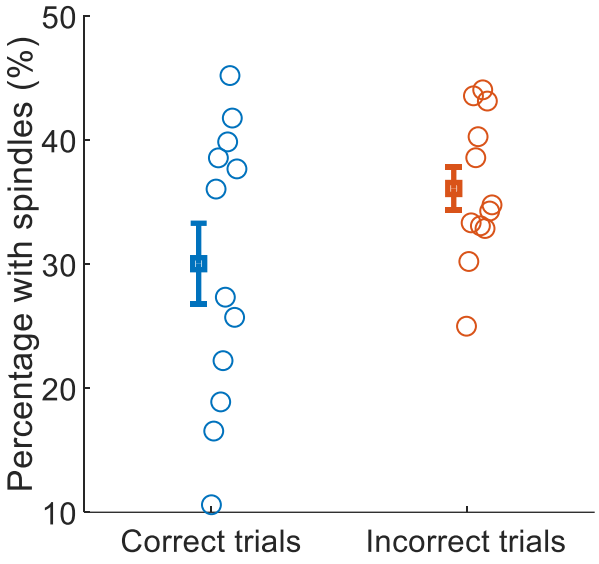
Extended Data Figure 1: performance of classification peaks throughout stimulation time (stimulation time is normalised to have the range [0 to 1]) the performance was calculated for each 50-trial block, the shaded area represents the standard error (SE) and the solid line represents the mean of different participants. After the middle of the stimulation time, early and late reactivations show different behaviour.



Extended Data Figure 2: Illustrating classification feature space using one subject. **a)** Classification of left hand (class 1) vs. right hand (class 2), posterior probability is illustrated. Wake data from one subject is used and the area of 'no effect' is near the decision boundary corresponding to low posterior probability (green). **b)** If trials were random and did not contain discriminative information, then rejecting some trials that fell near the decision boundary (cyan) will not lead to improved classification performance. Thus, cleaning random data will not be useful and only the data with actual classifiable effect would benefit from this cleaning.



Extended Data Figure 3: The four images that appeared to participants in the task: two faces and two objects. One image appeared at the beginning of every trial, all images are shown together for illustration.



Extended Data Figure 4: likelihood of pre-cue spindles [-1.5 to 0] sec. for correct and incorrect trials. Percentages of trials with spindles are shown for correct and incorrect trials of reactivation classifier which shows that the lack of pre-cue spindles accompanies classifiable late reactivation. Each point represents one participant.

Feature	Description	Variable
cosPhase	Cosine of the phase of auditory stimulation	Continuous
sinPhase	Sine of the phase of auditory stimulation	Continuous
vSOTrough	Voltage of SO trough before the click	Continuous
vSOPeak	Voltage of SO peak in the click wave	Continuous

vSOPeakTrough	Voltage of SO peak-trough before the click	Continuous
tSONegWave	Time of duration for the negative wave before click	Continuous
tSOPosWave	Time of duration for the positive wave before click	Continuous
tSORising	Time of duration from the trough to zero crossing before click	Continuous
tSOPeakTrough	Time of duration for the peak to trough before click	Continuous
tStimSOCrossing	Time between zero-crossing to click time	Continuous
tStimSOTrough	Time between trough before click to click time	Continuous
tStimEstimPeak	Time between click time to wave peak	Continuous
rmsSONegWave	Area under curve for trough section before click (troughArea)	Continuous
rmsSOPosWave	Area under curve for peak section before the click	Continuous
rmsSOWave	Area under curve for all wave previous to stimulation	Continuous
numSOTroughs	Number of troughs in the negative wave before click	Ordinal
numSOPeaks	Number of peaks in the positive wave before click	Ordinal

risingSOSlope	Slope from the trough to zero crossing before click	Continuous
SOwaveRatio	Duration ratio for the wave before click (Slope)	Continuous
SOhalfWaveRatio	Duration ratio for the negative wave before click	Continuous
FSonStim	Presence of fast spindle on stimulation	Binary
SSonStim	Presence of slow spindle on stimulation	Binary
existFSonSO	Presence of fast spindle on the wave before stimulation	Binary
existSSonSO	Presence of slow spindle on the wave before stimulation	Binary

Extended Data Table 1: Description SO features used for predicting reactivation.

CHAPTER 4

The effect of temporal jittering of cues on TMR reactivation in SWS sleep

In this study, me, Martyna Rakowska, and Penelope A. Lewis designed the experiment. Me, Martyna Rakowska, and Paulina Bagrowska collected data from participants. All the EEG, classification and post-classification analyses were done by me and developed in Matlab. Penelope A. Lewis supervised and advised on the study and throughout the work and writing. Matthias S. Treder supervised and advised on EEG and classifiers.

4.1 Abstract

In this chapter, we delve deeper into the temporal characteristics of the detected reactivations. Following our previous study (Chapter 3), we repeated the experiment but jittered the timing of the TMR cues during sleep to study the impact of such jittering on reactivations. As in chapter 3, we identified multiple reactivations above chance level. However, we found a markedly different timing of reactivations after jittering. Specifically, reactivation occurred for extended periods after a jittered cue and continued until the presentation of a second cue we also do not get the early reactivation that we found in chapter 3. More precisely, reactivations occurred at 1 second after the onset of the cue and again at 2 seconds. These results suggest that reactivation can occur for extended periods and there is an element of predictability of TMR cues which influences the timing during which we can detect reactivation. We also found that the lack of pre-cue spindles predicted both reactivations.

4.2 Introduction

Sleep is essential for both declarative and non-declarative memory consolidation (Diekelmann & Born, 2010; Rasch & Born, 2013; Squire et al., 2015), during sleep, memories are reactivated such that brain activity is reinstated during offline periods which facilitates the consolidation process (Ólafsdóttir et al., 2018; Wilson & McNaughton, 1994). These memories can be selectively triggered by representing odours or sounds that were originally present during the encoding of those memories. This method is called targeted memory reactivation (TMR) and it has shown great success in biasing reactivation and affecting the behavioural improvement as a result of cuing (Belal et al., 2018; Cellini & Cappuzo, 2018; Hu et al., 2019; Schreiner et al., 2018; Shanahan et al., 2018; Wang et al., 2019). TMR has been shown to have positive (Cairney et al., 2018; Schreiner et al., 2018; Wang et al., 2019) as well as negative effects (Murphy et al., 2018) on memory. Several studies have confirmed the reinstatement of learning related brain activity after TMR cues in Non-REM (NREM) sleep (see (Lewis & Bendor, 2019)). We also showed in (Chapter 3), that we can detect TMR elicited reactivation in SWS.

In this study, we use a highly sleep dependent serial reaction time task (SRTT) which was shown to be affected by TMR in SWS in human participants (Cousins et al., 2014). We updated the study design by jittering the delivery of TMR and including three follow up sessions. In the follow up sessions, participants performed the SRTT again. In the final follow up session, participants performed a sequence recall task where they marked the order of each sequence, Figure 4.1. It has been shown that memory reactivation during SWS can be detected using EEG (Belal et al., 2018; Cairney et al., 2018; Schreiner et al., 2018; Wang et al., 2019), chapter 2 and 3. fMRI (Deuker et al., 2013; Shanahan et al., 2018), and also intracranial EEG (iEEG) (Zhang et al., 2018). In some studies, reactivation was detectable with above chance accuracy with information about the exact timing of these reactivations. Various studies found the reactivation to be locked to the onset of the TMR sound (Wang et al., 2019), locked to TMR onset and mediated by spindle activity (Cairney et al., 2018), locked to onset and mediated by phase of theta activity (Schreiner et al., 2018), locked to ripples (Zhang et al., 2018), or locked to slow oscillation (SO)-spindle complexes (Schreiner et al., 2020). In our prior work (Chapter 3), we found a TMR-locked evidence of reactivation and by applying TMR on the up-going phase of the SO we can trigger an early reactivation. Additionally, the lack of pre-cue spindles can predict occurrence of detectable reactivation after the cue.

It has been shown that the delay between the onset of reactivation and the TMR cue onset is different from the delay in wake, chapter 2 and 3, also the reactivation in sleep can echo repeatedly after TMR (Bendor & Wilson, 2012; Rothschild et al., 2017). In rodents, it was shown that TMR replay can repeat several times after the offset of a cue (Bendor & Wilson, 2012). In humans, it has been shown that reactivation could be reoccurring (Schreiner et al., 2018). In the current study, we explored the timing of activation during wake and when the activation pattern is strongest and compared this to when the reactivation pattern during sleep peaked. We found that indeed the reactivation pattern is delayed (after 1 second and at 2 seconds) compared to activation in wake (around 0.7 seconds).

In the current study, we sought to detect reactivation of our SRTT and determine the temporal characteristics of detectable SWS reactivation using EEG signals and linear machine learning models. Following our prior work on this problem (Chapter 3), we wanted to understand the effect of extending the trial durations after a TMR cue and making these durations unpredictable. We therefore jittered the onset of cue timings to be able to see how this would affect the detected reactivations.

In Chapter 3, we showed that reactivation can be detected with EEG classifiers and the detected reactivations happen at two different timings. That study used a trial duration of 1.5 second and we were able detect an early reactivation which happened immediately after a TMR onset and another reactivation which happened ~1 second after the cue onset. In the current study, we jitter the timing of TMR onsets from 2.5 to 3.5 seconds in hopes of determining whether reactivations also occur later than 1 second after the TMR cue. The jittering should also enable us to determine if the early reactivation at TMR onset is caused by the current cue onset at time 0 or whether a previous TMR cue is enabling the brain to predict the upcoming cue. If this is the case, then the brain reactivates the upcoming cue information without waiting for the cue to be actually presented which is a possibility given that the task is a sequence.

Brain activity during SWS is characterised by SO. SO have an up-going phase which is related with sustained firing depolarisation state and during which faster phenomena like sleep spindles (Born & Wilhelm, 2012; Siclari et al., 2014) which are linked to memory consolidation (Nishida & Walker, 2007) and to reactivation (Cairney et al., 2018). On the other hand, a hyperpolarisation state of neuronal silence is linked to the down-going phase of the SO. Additionally, memory benefit was shown to be linked to the up-going phase of the SO (Göldi

et al., 2017). It has also been shown that we could produce higher ERP responses by stimulating the up-going phase of the SO vs. the down-going. Here, we use jittered cues and aim to investigate whether the delivery of TMR during the up-going phase of the SO can produce better classifiable responses compared to down-going phase. We also aim to explore the relationship between sleep spindles and reactivation following our work in Chapter 3 and (Wang et al., 2019).

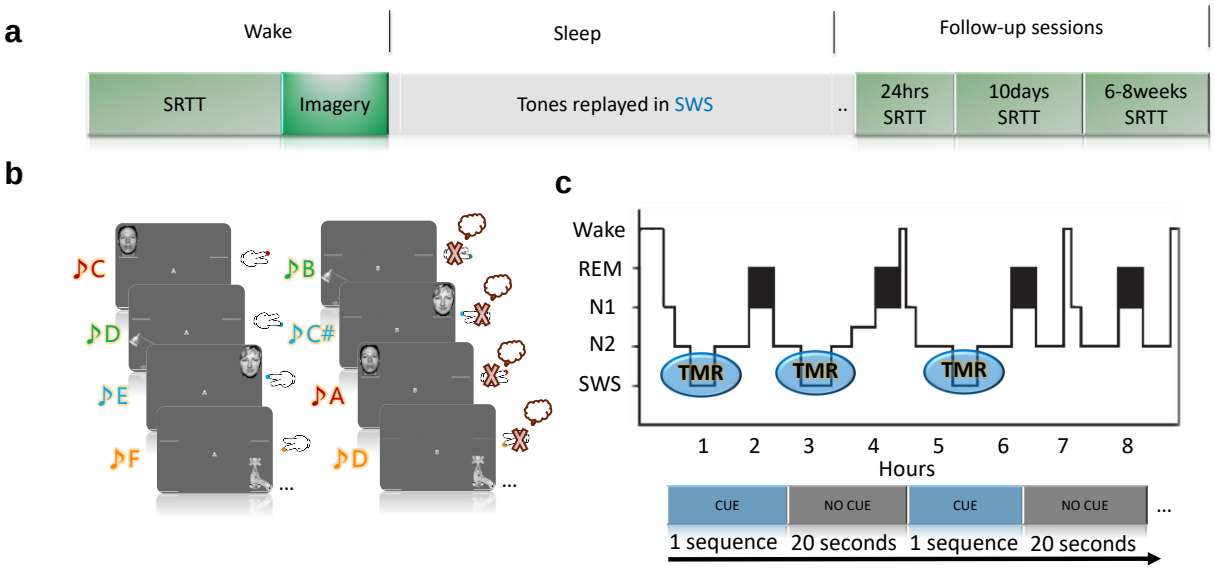


Figure 4.1: Study design. **a)** Participants were wired-up, afterwards they completed the serial reaction time task (SRTT) and motor imagery task (IMG), respectively. Then, participants went to sleep and TMR was carried out in SWS, as shown in c. After that, participants had three follow up sessions of SRTT. During the final follow up session, participants performed an explicit recall task where the order of each sequence had to be marked. **b)** In SRTT, four images are presented in two different sequences. Each image is accompanied by a specific tone (different for each sequence) and requires a specific button press. In IMG, Participants view the same sequences of images and imagine that they are pressing the buttons. **c)** Schematic representation of the TMR protocol. Reactivation took place in SWS. Sequences were followed by a 20-second pause.

4.3 **Results**

4.3.1 TMR elicits multiple reactivations

We sought to analyse the temporal characteristics of detected motor imagery reactivation. The detection of reactivation was shown to reoccur (Cairney et al., 2018; Schreiner et al., 2018), with the reinstatement of the target memory immediately after the cued memory, followed by a later reinstatement (Lewis & Bendor, 2019). Our own work (Chapter 3) also shows two reactivations, one immediately after the TMR cue and another after ~1 second. Thus, in the present study, we wanted to examine the time course of classification performance between the reactivation of left- and right-hand activity given that we here jitter the timing of cues. Thus, we build classification models using EEG activity of the motor imagery during wake. These models discriminate the activity of different hands at every time point after the onset on the cue. Once trained, we tested the models on the activity that arises after the onset of TMR in SWS. Our results show late reactivations, but not the early reactivations that we found in (Chapter 3). This suggests that the early reactivation may have resulted from the brain predicting the upcoming cue due to the fact that our task is sequence based and that the intertrial delay was fixed. Interestingly, in the jittered design, we see a reactivation after around 1 second after the cue onset and also see reactivation at around 2 seconds from the onset ($n = 12$, $p = 0.01$, $p = 0.0045$ for both clusters after 1 second and at 2 seconds, respectively) (Figure 4.2). Results were corrected for multiple comparisons with cluster-based permutation (see methods for details). Trial duration in sleep was jittered between 2500 ms and 3500 ms.

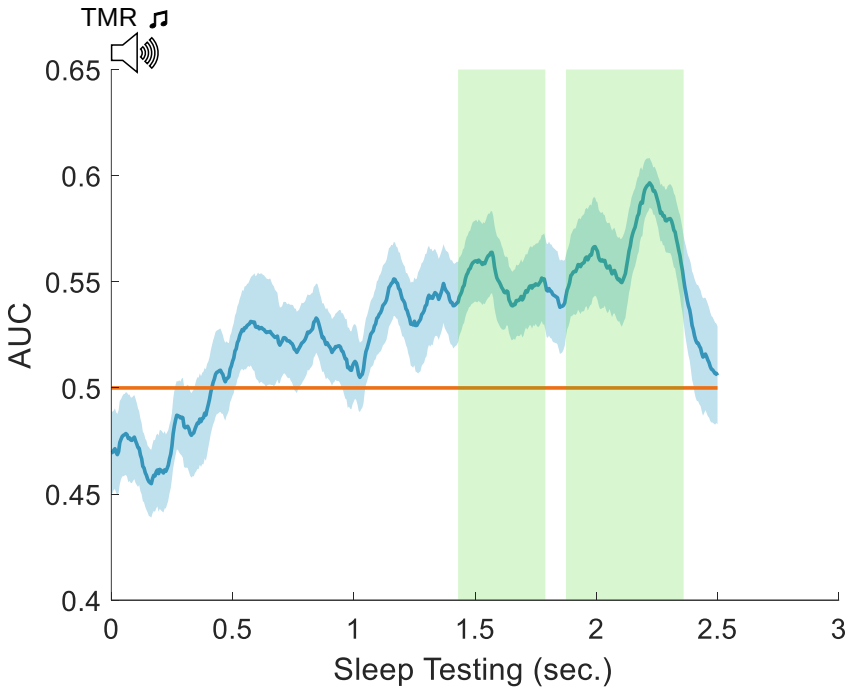


Figure 4.2: Classification results after training the classification model on wake and testing with sleep. The blue curve represents the area under the ROC curve (AUC) across time (with standard error shaded around the mean). TMR sounds are presented at the beginning of sleep trials (time 0). Green shaded areas mark the timing of the two clusters indicating reactivations. Classification results have two significant effects expressed by two clusters (1-second cluster, p = 0.01, and 2-second cluster, p = 0.0045).

4.3.2 Reactivation is reinstated on the motor area

To be sure that the detected reactivation is related to our motor task we used the channels on the motor area for classification. We chose 12 electrodes on the motor area (C1, C3, C5, CP1, CP3, CP5, C2, C4, C6, CP2, CP4, CP6). The classification performance shown in Figure 4.2, is using these motor channels which ensures that the detected pattern is a reactivation of the task. Classification with motor channels followed our previous work in chapter 3, where we showed that classification was derived from the motor area.

4.3.3 Reactivations are not reoccurring

As in our previous analyses with this task (Chapter 3), detected reactivations appear to reoccur. However, this pattern arises as a result of combining different trials and different participants. Thus, reactivation could actually happen at just one of the two detected time durations of reactivations in each trial but appears to reoccur as a result of looking at the overall effect across many trials. To examine this, we compared the probability of having a correct trial at the timing of both peaks with the probability of finding both peaks simultaneously (multiplication of both probabilities as chance level) after a TMR. We found that the probability of having trials classified correctly at the timing of both peaks simultaneously is significantly lower than chance level (Wilcoxon signed rank test, $n = 12$, $p = 0.004$, $z = -2.90$). This suggests that, as in Chapter 3, it is unlikely that reactivations are reoccurring in the same trial after a sound cue.

4.3.4 Lack of pre-cue spindles facilitates post-cue detectable reactivation

We analysed the duration before the onset of the stimulus and extracted the information of whether there was a spindle happening before the stimulus or not (see methods for more details). We found that when we analyse the pre-cue periods of the correctly classified trials, the occurrence of spindles is less likely compared to incorrect trials. This suggests that the lack of pre-cue spindles facilitates reactivation. We performed this analysis for both peaks, at 1 second and 2 seconds, to find that this was true for both of them: first peak (1 second) (Wilcoxon signed rank test for percentage of trials with pre-cue spindles for correct vs. incorrect trials, $n = 12$, $p = 0.023$, $z = -2.28$), and second peak (2 seconds) (Wilcoxon signed rank test, $n = 12$, $p = 0.002$, $z = -3.061$), Figure 4.3.

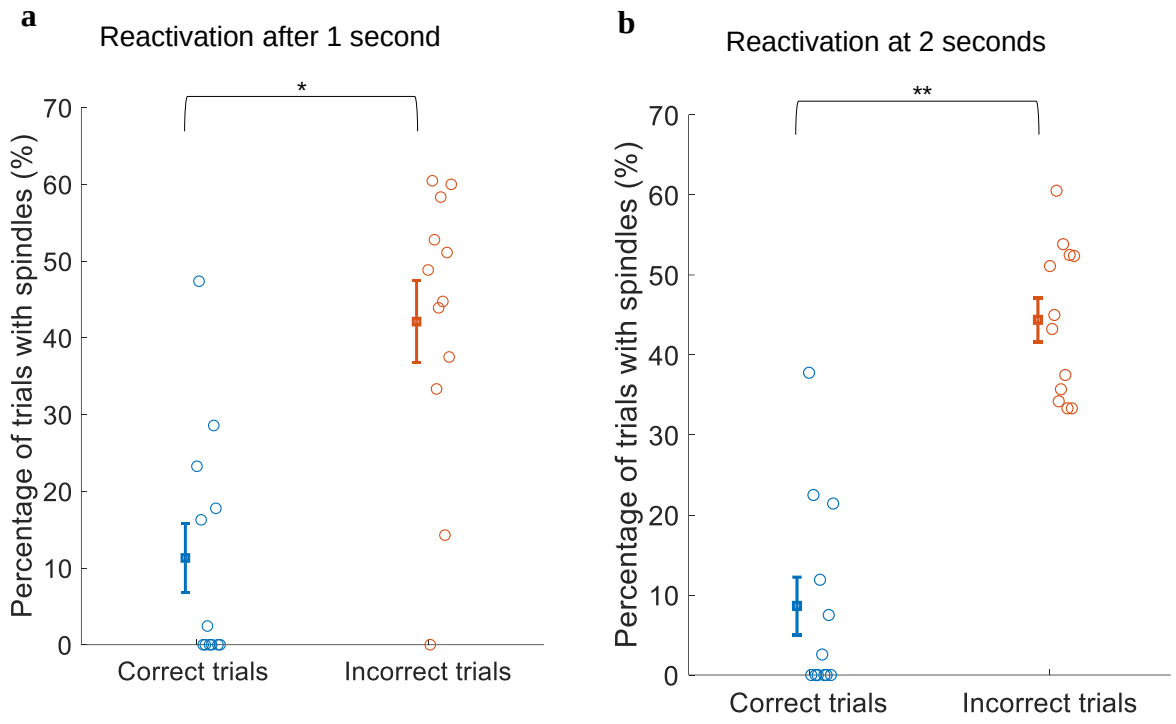


Figure 4.3: Lack of pre-cue spindles predicted correctly classified reactivations. Percentages of trials containing spindles are shown for correct and incorrect trials of reactivation classifier for both 1-second reactivation (a) and 2-second reactivation (b) for each participant. This shows that the lack of pre-cue spindles is related to both detectable reactivations after TMR. First peak (1 second) (Wilcoxon signed rank test for percentage of trials with pre-cue spindles for correct vs. incorrect trials, n=12, p = 0.023, z = -2.28), and second peak (2 seconds) (Wilcoxon signed rank test, n=12, p = 0.002, z = -3.061).

4.3.5 Preferred SO TMR phase for detectable reactivation

During the up-going state of the SO, fast rhythms such as spindle, and gamma activity are more prominent than in the SO down-going state (Mölle et al., 2002; Piantoni et al., 2013; Valderrama et al., 2012). In rodents, sharp-wave ripples seem related to reactivation

(Kudrimoti et al., 1999; Nakashiba et al., 2009; O'Neill et al., 2008). Likewise, in humans sharp-wave ripples have been shown to carry reactivation (Zhang et al., 2018). Thus, we could assume that by applying the stimulation on the up-going phase of the SO we would expect reactivation to occur.

We tested this by dividing our sleep trials and analysing the number of correctly classified trials that had the TMR on the up-going phase of the SO and comparing that to the correct trials that had the TMR on the down-going. We performed this analysis for both peaks and revealed that the up-going phase is preferred for TMR to elicit the reactivation at 2 seconds (Wilcoxon signed rank test, $n = 12$, $p = 0.013$, $z = 2.47$). In other words, the majority of correct trials had TMR on the up-going phase of SO. We repeated this for incorrect trials and found no difference between up-going and down-going phase transitions (up-going vs. down-going Wilcoxon signed rank test, $n = 12$, $p = 0.11$, $z = 1.6$). We also repeated this for the peak at 1 second but found no difference between phase transitions (for correct trials Wilcoxon signed rank test, $n = 12$, $p = 0.21$, $z = 1.25$), (for incorrect trials Wilcoxon signed rank test, $n = 12$, $p = 0.64$, $z = -0.47$). This shows that when the sound cues are delivered during the up-going phase of the SO they are more likely to elicit a classifiable reactivation 2-second after the onset of the cue which goes in line with the mentioned studies and emphasises the importance of the SO phase in the delivery of TMR, Figure 4.4.

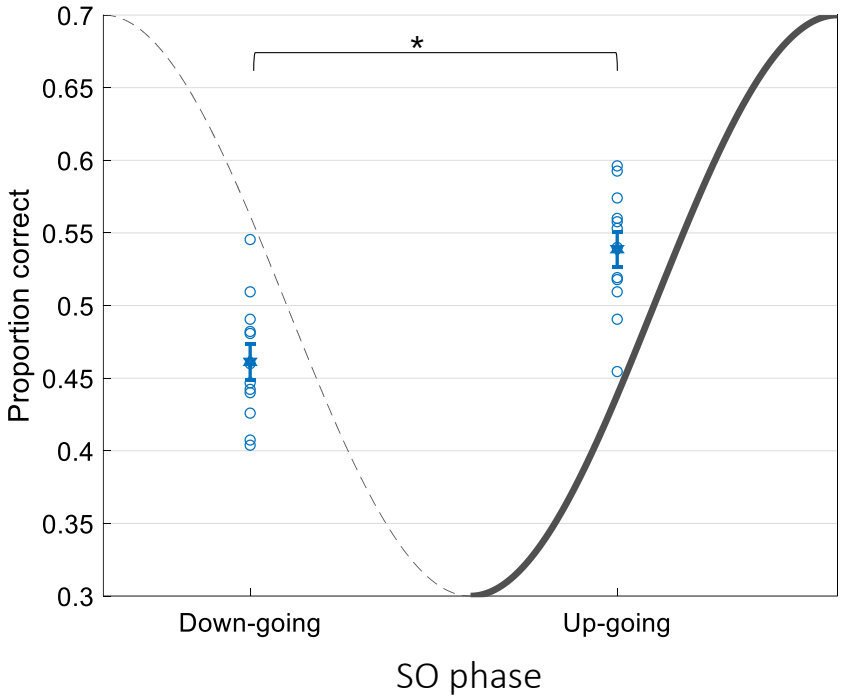


Figure 4.4: Correctly classified trials had TMR on the up-going SO phase. For every participant, the proportion of correct trials with the TMR cue falling on both SO phase transitions down-going and up-going are shown. The shown curve is a simplified representation of the phase of a slow oscillation (SO), two phases are marked on the x-axis and the y-axis represents the proportion of correct trials. The preferred phase for 2-second reactivation is when the sound falls on the up-going transition of the SO (Wilcoxon signed rank test, n = 12, p = 0.013, z = 2.47) compared to down-going.

4.3.6 Employing pre-cue Slow Oscillation features to predict detectable reactivation

Reactivation has been suggested to be modulated by SOs, (Inostroza & Born, 2013; Ngo et al., 2018; Rasch & Born, 2013), however, the mechanism for this modulation remains to be understood. Following our prior work (Chapter 3), we were interested to see if we can use the pre-cue SO features to predict whether each cue will trigger reactivation. In chapter 3, we found that pre-cue SO features can be used to discriminate between the correctly and incorrectly classified trials and that was happening for an early reactivation. Here, we used the same SO

features as in (Chapter 3, Extended data Table 1), to examine this. These features are used with decision tree classifiers to differentiate between the classes (correctly classified reactivation vs. incorrectly classified reactivation), see methods for more details. We found that we were not able to use the SO features to predict those later reactivations (those of the current study: at 1 second and 2 seconds compared to the reactivations in chapter 3). In other words, the classification using SO based features did not yield a discrimination between correct and incorrect trials for the peak at 1 second vs. chance (Wilcoxon signed rank test, $n = 12$, $p = 0.42$, $z = 0.80$) nor for the peak at 2 seconds vs. chance (Wilcoxon signed rank test, $n = 12$, $p = 0.58$, $z = 0.5491$), Figure 4.5a, b. In Chapter 3, the SO based classification was able to discriminate correct from incorrect trials only for the early reactivation. Since the early reactivation is not existing here, we would expect the classifier to not be able to predict the later peaks that we found here.

4.3.7 Classification performance across the time of stimulation

In our prior work (Chapter 3), we showed two reactivations and analysed the classification performance across stimulation time by calculating the performance during the time of the peaks using a 50-trial blocks and sliding this by one trial. The analysis of Chapter 3 showed that both reactivations occur throughout almost the whole stimulation time. Here, we used a similar approach using a 50-trial sliding blocks and observed that we see the two reactivations at 1 second and 2 seconds consistently during stimulation time, Figure 4.5c.

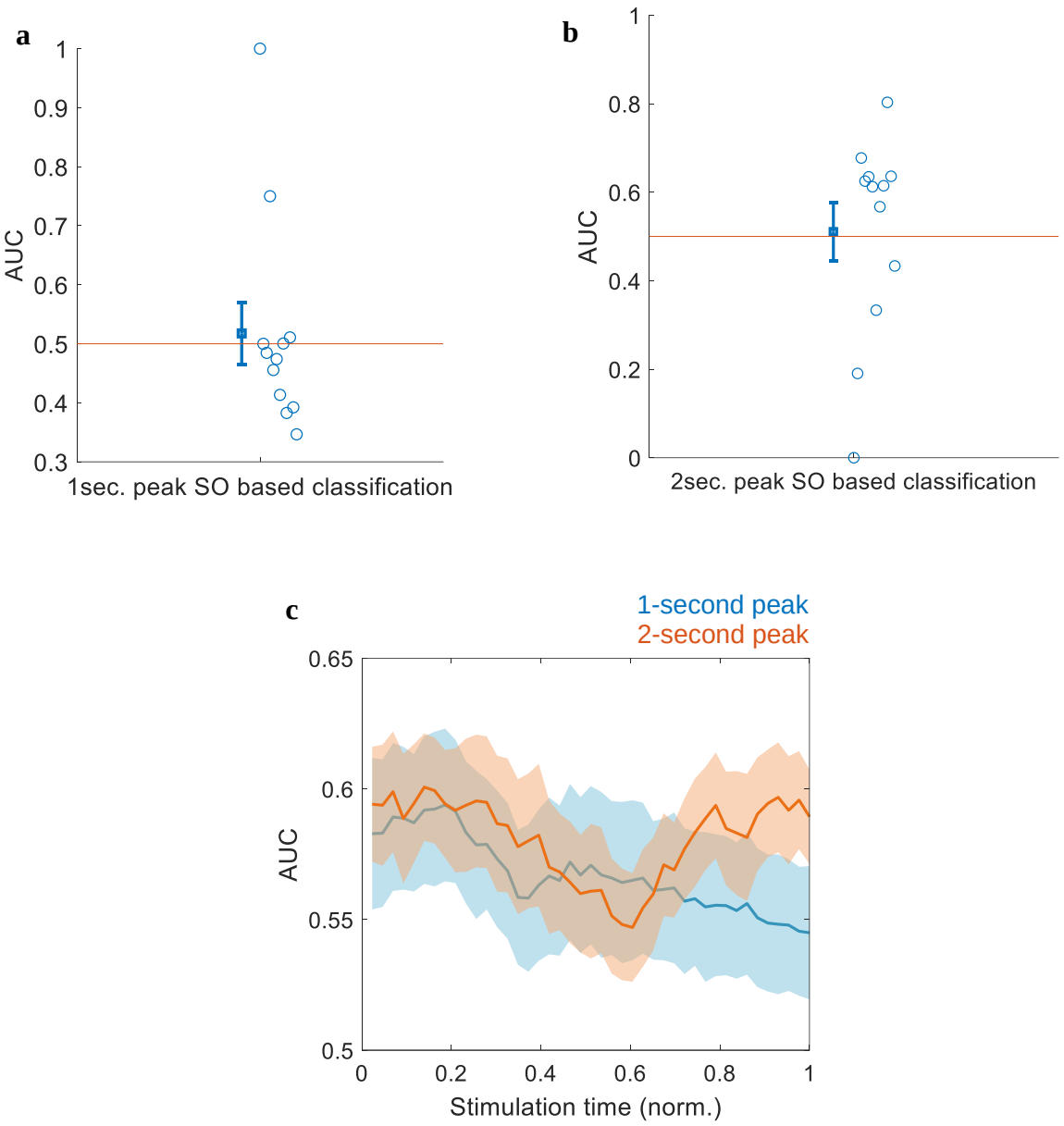


Figure 4.5: Discriminating the correct vs. incorrect trials based on the pre-cue SO features. For every participant, decision trees were used to discriminate between correctly and incorrectly classified trials to test whether it is possible to predict classifiable reactivations using pre-cue SO features. This showed no significant effect $p > 0.4$ for both **a)** 1-second and **b)** 2-second peaks suggesting that we cannot use pre-cue SO features to perform this prediction of late peaks. **c)** Classification performance across stimulation time for both peaks did not show a variation in the patterns of both peaks and suggests that reactivation is happening throughout stimulation time.

4.3.8 Correlation of classification performance with behaviour

We performed a spearman correlation analysis between the classification performance during the timing of the two peaks (the peak performance of the cluster at 1 second and the peak performance of the cluster at 2 seconds) and behavioural measures. Behavioural measures included: the average reaction time of the 4 blocks before sleep and the average of the best 4 blocks before sleep and also a sequence specific skill (SSS) measure. SSS_pre is defined as the average of the last 4 blocks before sleep subtracted from the average of the random blocks before sleep. SSS_best is the average of the best 4 blocks before sleep subtracted from the average of the random blocks before sleep. For the first peak at 1 second and the second peak at 2 seconds, no significant correlation was found between behavioural measures and classification performance. We suspect that this could be a result of jittering the delivery of TMR, as explained in the discussion.

4.4 **Discussion**

In this work, we tested the effect of jittering TMR trial durations during sleep by delivering the sound cues with varying inter-cue durations from 2500 ms to 3500 ms. We found two significant effects indicating two separate reactivations. The first was after 1 second from the onset of the cue and the other was 2 seconds after the onset. We analysed these effects to see if a reactivation is reoccurring after the same sound cue and found that it is not. Interestingly, the lack of pre-cue spindles accompanied correctly classified trials. We also found that the phase of the SO at which TMR is applied plays an important role in predicting the correctness of the reactivation at 2 seconds and suggests that the stimulation of the up-going phase of the SO is more likely to elicit this late reactivation. These results are in line with the results of (Chapter 3) and also emphasise the importance of pre-cue spindle and the importance of stimulating the up-going phase of the SO.

4.4.1 TMR did not improve sequence memory

In our previous investigation with the SRTT, we showed that there was a sequence memory improvement as a result of TMR when we compared the reactivation vs. the non-reactivated sequences (Cousins et al., 2014; Koopman et al., 2020). The improvement was observed when we tested the overnight improvement of sequence memory. In the present study, performance after sleep in the 24-hrs session was better for the reactivated vs. the non-reactivated sequence, however, the difference was not significant (Wilcoxon signed rank test, n = 12, P = 0.14, Z =

1.5). We think that because in the current study we changed the timing of trials by jittering the onset of the cues we disrupted the monotonicity of the sequence and thus it became harder for the brain to process the cues as a sequence. Jittering the time of trials added an element of temporal unpredictability with every sound played and made it harder to process the information as a sequence. A model by Polyn and colleagues explored temporal information and showed that it relies on associations formed during memory encoding (Polyn et al., 2009). We think that the temporal information is a necessary part of the sequence memory and jittering could be interfering with it.

4.4.2 Temporal characteristics of detected reactivation

Studies have shown that reactivation may have different temporal characteristics from that of awake activation. Schreiner and colleagues showed that reactivation reoccurs after TMR cues, with one reactivation immediately after the cue and another after 2 seconds from onset (Schreiner et al., 2018). Our previous work on this task also showed multiple reactivations, however not reoccurring, with one reactivation immediately after TMR onset and another after 1 second from onset (Chapter 3). Additionally, work in rodents showed that memory replay may echo multiple times (Bendor & Wilson, 2012). In our current study, reactivation did not happen immediately after the onset of the jittered cues as in chapter 3, with equally spaced cues. We think that, in chapter 3, this could be because the brain is predicting the next stimuli and starts reactivating and showing the pattern of the next cue before it actually takes place. This could be the reason because the sequences get repeated many times and cues are spaced equally. Interestingly, the reactivations do not occur in the same trials meaning that a TMR cue is likely to trigger one reactivation after 1 second or 2 seconds, but not both. We have already seen this with the early and late reactivations found in (Chapter 3) where the detected reactivations show this pattern.

Because we were curious to look for SWS phenomena that might predict reactivation, we analysed the pre-cue spindles to find that the lack of pre-cue spindles predicts the peaks after both 1 second and 2 seconds. This is in line with our previous findings with no cue jittering, and with other work showing that less pre-cue spindles predicted more post-cue reactivation, and that such reactivation begins around 1 second after the onset of the cue (Wang et al., 2019).

The down-going phase of SOs is related to neuronal silence, on the other hand, the up-going phase is related to a depolarised state of sustained firing during which sleep spindles occur (Born & Wilhelm, 2012; Siclari et al., 2014). In line with our expectations based on the previous findings, we found that we get more classifiable reactivations when TMR is applied on the up-going phase of SOs.

4.5 Conclusions

Overall, our results are broadly in line with our previous findings on the same task. In this work, we explored different characteristics of the detected reactivations. Results show that reactivation can happen at different timings after a TMR cue and are not reoccurring after a sound cue. Also, results emphasise the importance of stimulating the up-going phase of the SOs and stimulating with few pre-cue spindles in order to get classifiable reactivations. Results of jittering TMR delivery showed that the arising EEG pattern will differ from that obtained with no jittering (Chapter 3) and the element of predictability will be eliminated. Meanwhile, jittering the time of TMR could disrupt the temporal consistency of the sequence, thus, preventing improvement of the sequence memory in the follow up sessions.

4.6 Methods

Participants. In the present study, we collected EEG and behavioural data from human participants (n = 23) (13 females, age mean ±SD: 19.5 ±1.1; 10 males, age: 20.9 ±1.9). The number of participants was further reduced because 4 participants were 2 standard deviations away from mean behavioural performance. Also, data had to be rejected to fit with the analysis requirement following the same procedure of classification employed in (Chapter 3)**.** Thus, the final number of participants with higher than classification threshold set for wake (ROC Curve (AUC) > = 0.7) was 12 participants. Participants completed a SRTT before sleep and during three follow up sessions, the first one was after the night of stimulation (24 hours), the second after 10 days later, and eventually the final session after 6 to 8 weeks. None of the participants reported prior knowledge of the SRTT. All participants were right-handed. All participants had normal or corrected-to-normal vision, normal hearing, and no history of physical, psychological, neurological, or sleep disorders. Responses in a pre-screening questionnaire reported no stressful events and no travel before commencing the study. Participants did not consume alcohol or caffeine in the 24 hours prior to the study or perform any extreme physical

exercise or nap. This study was approved by the School of Psychology, Cardiff University Research Ethics Committee, and all participants gave written informed consents.

Study Design. The SRTT that we use in this work was shown to be facilitated by TMR in SWS (Cousins et al., 2014, 2016; Monika Schönauer et al., 2014). We collected the SRTT behavioural data in three sessions after the stimulation night, with one the next day (24 hours) after performing the task and spending the night in the lab, the second one after 10 days and the third after 6 to 8 weeks. The SRTT was similar to that in chapter 2, Figure 2.5.

During the night of stimulation cues were presented in during N2 and N3 of NREM sleep with the continuous supervision of experiments. In this work, N3 data is analysed for memory reactivation. Stimulation was paused with any signs of arousals until the experimenters observe approximately three 30-second epochs with stable N2 or N3. In the follow up sessions (24 hours, 10 days, and 6 to 8 weeks) after the task, participants were asked to perform the SRTT again. Eventually, in the last session, they were asked if they remember the locations of images of the two sequences in order to see if one sequence is recalled better than the other one. Motor imagery data set of each participant was used for classification. As a behavioural measure, we use sequence specific skill (SSS), calculated for pre-sleep as: reaction time of random blocks pre-sleep – reaction time of last 4 blocks pre-sleep.

Data acquisition. The current study uses EEG from human participants. EEG was collected using 64 actiCap active electrodes with 62 channels on the scalp including the reference electrode at CPz and ground electrode at AFz. Two electrodes were used on the left and right sides above and below the eyes for collecting electrooculography (EOG) signals and two electrodes on the right and left sides of chin for collecting the electromyography (EMG). Data were collected at 500 HZ and 250 HZ and subsequently resampled to 200 HZ for all EEG analysis. Sound cues were delivered during N2 and N3 sleep stages.

EEG cleaning. We cleaned the EEG data using a short cleaning pipeline that consisted of band-pass filtering (0.1 to 30 HZ) and centring. For sleep data, sleep was scored manually and only the trials in the epochs scored as N3 were used in this work. Afterwards, we removed outliers based on statistical measures (variance, max, min) and a trial is considered as an outlier if it is higher than the third quartile + (the interquartile range *1.5) or less than the first quartile - (the interquartile range*1.5) in more than 25% of channels. If a trial was bad for <25% of channels

it was interpolated using neighbouring channels with triangulation method in Fieldtrip. Furthermore, because our task is motor-related we defined a number of channels around the motor area (C6, C4, C2, C1, C3, C5, CP5, CP3, CP1, CP2, CP4, and CP6) and a trial was rejected if it is bad on >25% of these channels otherwise bad channels are interpolated and the trial was kept.

4.6.1 Classifying memory reactivation during SWS

Based on our previous findings on this task (Chapter 3), we used a time during which the classification was highest during wake (time of interest (TOI)) to be consistent with our previous study and to ensure that we are training our classifier model with the same ERP component and thus be able to compare results of the current and previous studies to some extent. Time domain features were extracted by extracting the amplitude averages of 80 ms, that is, 40 ms before and 40 ms after every time point. Subsequently, features were fed to a linear discriminant analysis (LDA) classifier (Blankertz et al., 2011). Each participant had their own classifier model that was trained on wake data using the features during the timing of TOI. For each participant, a classifier model was built using wake data from that participant and applied on sleep data from the same participant in a sliding window fashion. We use the same approach we used in (Chapter 3). With the sliding window, the classification was applied on the first window in sleep, for example: [0 to 0.38] second which matches the length of the TOI. Then the classification performance was placed at the centre time of this window: 0.190 second and the sliding window was shifted on sleep data by one time point and the process was repeated. Using the same cleaning approach that we used in the previous study, we cleaned the trials and kept approximately 100 trials from each participant as this was the maximum number we could get consistently from all participants. Thus, the results of classification are AUC values across time, as shown in Figure 4.2.

4.6.2 Preferred TMR phase analysis

We band-pass filtered our signals using channel Fz. After that, we used Hilbert transformation to extract instantaneous phase values as we did in (Chapter 3). We then divided phase values into two ranges: [0 to π] and (π to 2π], indicating the two transitions: down-going and up-going, respectively. For each participant, we determined the number of correctly classified trials in which TMR fell on either phase range, then normalised this value by the total number of correct trials. This yielded a data point for every participant in each phase transition. We

compared the proportion of correct trials where TMR occurred in the down-going vs. up-going transition of the SO. Afterwards, we repeated the same process for the incorrectly classified trials.

4.6.3 Reactivation recurrence

We tested whether the reoccurrence effect that we observe in the classification performance is a genuine recurrence or whether it is caused by the fact that we look at the grand effect of many trials. To do this, we compared the accuracy of classifying a reoccurring reactivation which is the number of trials that are classified correctly at both: the time of reactivation after 1 second and 2 seconds at the same time. We then compared this number to the multiplication of probabilities of correct trials at both peaks simultaneously which can be seen as a chance level. This showed that the probability of reoccurring reactivation is below chance (Wilcoxon signed rank test, $n = 12$, $p = 0.004$, $z = -2.9$). This shows that reactivation happens once after TMR cue at either time point but not both simultaneously.

4.6.4 SO based classification

Similar to Chapter 3, the SO based classification consisted of 200 decision trees ensemble. Leave one out classification is used wherein the data of all participants except one is used to train the classifier and the left-out participant is used for testing the classifier. This gave a classification result for the left-out participant and the process was then repeated until the classification performance was calculated for all participants. Every decision tree is trained on a random subset of trials from the training set and tested on the testing set, and the final result is the aggregated votes from all decision trees.

4.6.5 Statistical testing

Statistical analysis was performed using Fieldtrip (Oostenveld et al., 2011). Monte Carlo was used with a sample-specific test statistic threshold = 0.05, permutation test threshold for clusters = 0.05, and 10,000 permutations. The correction window used on sleep classification data was from 0 to 2.5 seconds.

CHAPTER 5

Identifying memory reactivation in human REM sleep using EEG classifiers

In this study, Anne C. M. Koopman collected data from participants, Suliman Belal and Monika Śledziowska contributed to study design. All the EEG, classification and post-classification analyses were done by me and developed in Matlab. Penelope A. Lewis supervised and advised on the study and throughout the work and writing. Matthias S. Treder supervised and advised on EEG and classifiers.

5.1 Abstract

Memories are reactivated during non-rapid eye movement (NREM) sleep, but the question of whether equivalent reactivation also occurs in rapid eye movement (REM) sleep is hotly debated. To examine this issue, we used a technique called targeted memory reactivation (TMR) in which sounds are paired with learned stimuli in wake, and then re-presented in subsequent sleep, to trigger reactivation. We then used time domain features to train a linear classifier model on discriminating between stimulus classes and found evidence of TMR-induced reactivation in REM. Our analysis revealed that reactivation was temporally compressed by approximately five times in REM compared to wakeful performance of the task, and often occurred twice within a single trial. Interestingly, reactivation was only apparent in trials with a high theta power. Our data provide the first evidence for memory reactivation in human REM sleep after TMR as well as an initial characterisation of this reactivation.

5.2 Introduction

While the reactivation of memories in non-REM sleep is widely supported by evidence from humans, rodents, and other animals (Bendor & Wilson, 2012; Ji & Wilson, 2007; Lee & Wilson, 2002; Rasch & Born, 2013; Wang et al., 2019; Wilson & McNaughton, 1994), it is still unclear whether equivalent reactivation occurs in REM. Reactivation in non-REM has been identified using EEG classifiers (Belal et al., 2018; Cairney et al., 2018; Schreiner et al., 2018; Wang et al., 2019), with fMRI (Rasch et al., 2007; Shanahan et al., 2018), and with intracranial recording (Zhang et al., 2018). Targeted memory reactivation (TMR), a technique which allows the active triggering of memory reactivation, causes both neural and behavioural plasticity when applied in non-REM sleep (Lewis & Bendor, 2019). However, very few studies in rodents show evidence for reactivation in REM, (Hennevin et al., 1995; Hennevin & Hars, 1985; Louie & Wilson, 2001). Furthermore TMR in REM typically fails to produce any measurable behavioural impact (Cordi et al., 2014; Rasch et al., 2007), though work on conditioning seems to be the exception to this rule (Rihm & Rasch, 2015; Sterpenich et al., 2014). A study showed that spontaneous reactivation can be detected in human REM sleep (M. Schönauer et al., 2017), their finding was motivating for us to use TMR with EEG classifier to classify reactivation and see if it is possible to detect reactivation after TMR. We also aimed to explore the temporal characteristics and dynamics of such reactivation.

We were specifically interested in theta activity because this frequency is prominent in REM sleep (Boyce et al., 2016; Hutchison & Rathore, 2015; Nishida et al., 2009), and human studies suggest a possible relationship between memory and theta activity (Jürgen Fell et al., 2002; Klimesch et al., 2001; Sederberg et al., 2003). In wake, theta activity is suggested to be a preferable window for the encoding of new information (Battaglia et al., 2011; Juergen Fell et al., 2011; Kahana et al., 1999; Vertes, 2005). Of the few studies that have provided support for memory reactivation in REM, two showed a link to theta activity (Louie & Wilson, 2001; Poe et al., 2000). We were therefore interested to determine whether theta is associated with TMR cued reactivation in human REM.

Reactivated memories can have different temporal structure compared to their trace at encoding. In rats, replay during non-REM sleep has been shown to occur from 10 to 20 times faster in comparison to wake (Ji & Wilson, 2007; Lee & Wilson, 2002; Nádasdy et al., 1999). Sleep reactivation in rats was also shown to be compressed 6 to 7 times in comparison to wake

suggesting that processing could be faster with the absence of behavioural constraints (Euston et al., 2007). We were interested to know whether we could find evidence of temporal compression during memory reactivation in human REM sleep.

Memory reactivation can reoccur more than once after a TMR cue in non-REM sleep (Schreiner et al., 2018), and this has been argued to be of functional significance for consolidation from the hippocampus to the cortex (Lewis & Bendor, 2019). Building on this we were interested to determine whether a similar pattern is apparent for REM sleep reactivation.

We used a serial reaction time task (SRTT), which is known to be sleep sensitive (Born & Wilhelm, 2012; Spencer et al., 2006) and also sensitive to TMR in non-REM sleep (Cousins et al., 2014, 2016; Monika Schönauer et al., 2014) to examine these questions. In our SRTT, participants were presented with audio-visual cues and responded by pressing 4 buttons (two from each hand). Cues were organised in a 12-item sequence of presses. Sounds were replayed softly during subsequent REM to trigger the associated memories of left- and right-hand presses, Figure 5.1. We used two sequences and replayed only one of them in sleep. For control, we also included an adaptation night in which participants slept in the lab and we played the same tones that would later be played during the experimental night. This provided data in which tones could not have evoked memory reactivation, as participants had not yet learned the behavioural task, so the sounds were meaningless.

Our findings demonstrate that it is possible to use machine learning to build EEG classifiers that can detect reactivation after TMR cues in REM sleep. This classification pipeline uses linear classification of time domain amplitudes to discriminate between reactivation of left- and right-hand button presses. We also reveal that theta activity is associated with detected reactivation. Furthermore, we show that the detected reactivations reoccur within each given trial and are temporally compressed ~5x compared to wake activation.

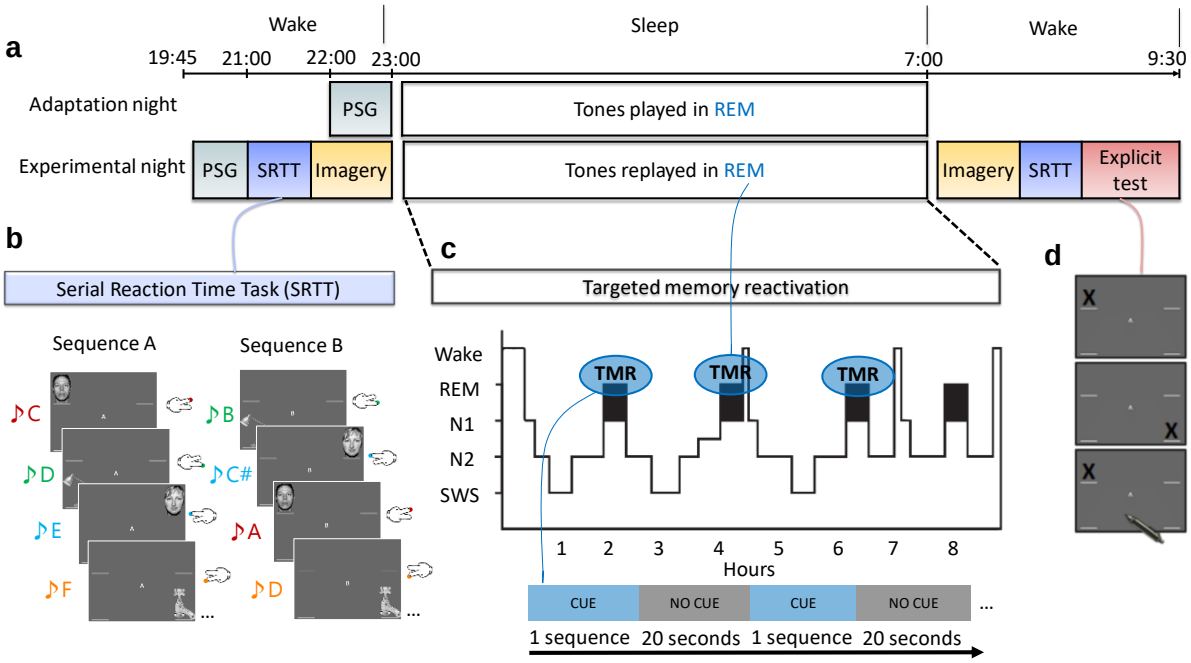


Figure 5.1: Experimental design. **a)** The experiment consisted of two nights: an adaptation and an experimental night. In the adaptation night, tones were presented to the participants during REM sleep and EEG recordings were acquired. In the experimental night, participants were wired-up for EEG, then completed the SRTT and an imagery task. Afterwards participants slept in the lab and TMR cues were presented as shown in c). After waking up, participants completed the motor imagery and SRTT again, and finally they did the explicit recall task as illustrated in d. **b)** In the SRTT, images are presented in two different sequences each with a different set of tones. Each image is associated with a unique tone and requires a specific button press. In the imagery task, participants were cued with pictures and sounds but were told to only imagine performing the finger tapping (without movement). **c)** The sounds of only one learned sequence (cued sequence) were played in the correct order during REM sleep, with a 20 second pause between repetitions. **d)** Explicit recall test. Participants were asked to mark the order of each sequence on paper as accurately as they can remember.

5.3 **Results**

5.3.1 Detection of memory reactivation after TMR cues

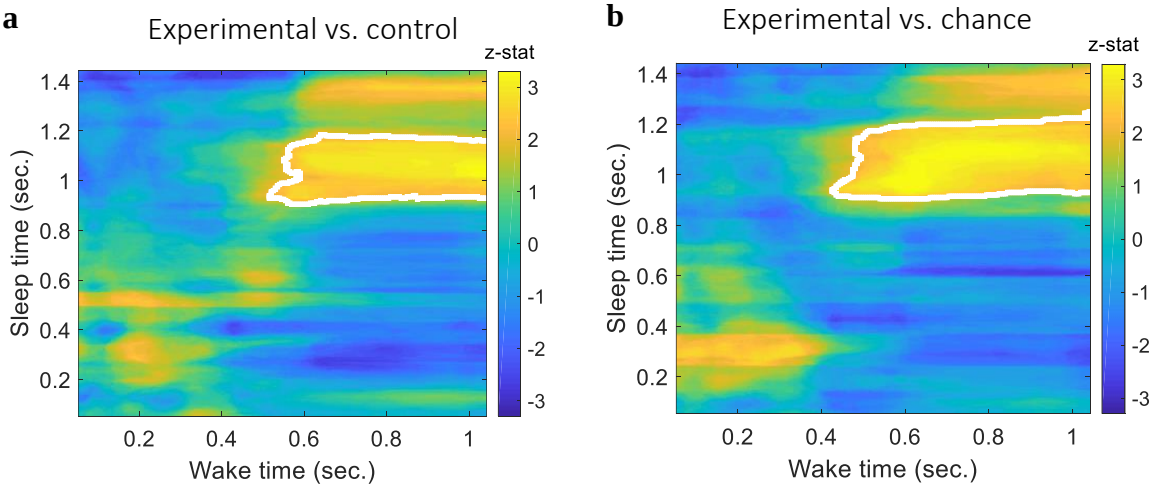
We trained our classification models using sleep data and then tested them on wake data. This was done partly following Loui and Wilson (Louie & Wilson, 2001) who took a template from sleep data and slid it across wake to detect replay. Training a model on wake could have caused it to weigh features which are dominant in wake very highly even if those features were entirely absent from sleep. By training classification models on sleep data, we ensured that the features associated with reactivation were used by the models, and the models were thus able to look for these in the stronger, less noisy, signals recorded during wake.

For classification, we used linear discriminant analysis (LDA) classifier in a time x time classification procedure (King & Dehaene, 2014), see methods for details. We repeated the classification process using the adaptation night to be certain that the classification was not caused by sound induced effect or EEG noise rather than reactivation of the encoded memory. We compared the results from the two nights, both to each other and to chance level. In the adaptation night, no significant clusters were detected vs. chance (area under the receiver operating characteristic curve (AUC) = 0.5), demonstrating that classification of this control condition did not differ from chance level. By contrast, comparison of the experimental night against chance showed a significant effect (Figure 5.2a) which occurred about 1 to 1.2 sec. after the onset of the cue (Figure 5.2b). Comparison of the experimental night to the adaptation night also showed a significant effect, described by a cluster in this timeframe (Figure 5.2a). This means that we can detect memory reactivation and discriminate between right- and left-hand movements during REM sleep and this is evaluated against both control and chance level.

5.3.2 High theta activity mediates reactivation

To test for a relationship between theta power and reactivation, we performed a median split on theta power, creating two groups of trials for each participant: those with high theta power and those with low theta power, see methods. This split was performed for both experimental and adaptation nights. We then compared the classification results of each half of the median split (high and low theta trials) in the experimental night to both chance level and to the equivalent high or low theta power trials in the adaptation night. For high theta trials, this showed a significant effect (Figure 5.2c), explained by a cluster occurring around the same

time as in the classification result using all trials, Figure 5.2a-b, (n = 14, p = 0.028), there was also a significant different against chance level, Figure 5.2d, (n = 14, p = 0.001). The low theta power trials showed no significant effect (Extended Data Figure 4). These findings demonstrate an association between high theta power and reactivation. To determine whether theta activity offered a preferred window for classification or if it was the actual feature causing the discrimination of classes, we band-pass filtered the signal in the theta band and re-ran our classification analysis. Interestingly, classification did not differ from chance in this filtered data ($p > 0.4$) suggesting that while high theta activity offers a preferred window for reactivation, theta activity itself does not discriminate the classes. To be sure that the recordings quality and any other noise were not causing the classification seen with high theta activity, we performed classification in different frequency bands. In this control, we used three different bands lower and higher than theta and a broad range of frequencies: [0.5 3] HZ, [9 16] HZ, and [0.5 30] HZ. We band pass filtered the signals in these bands and performed the same median split we performed on theta band, none of the classification using these bands produced significant cluster(s). This ensures that theta activity mediates reactivation and this is unique to theta band.



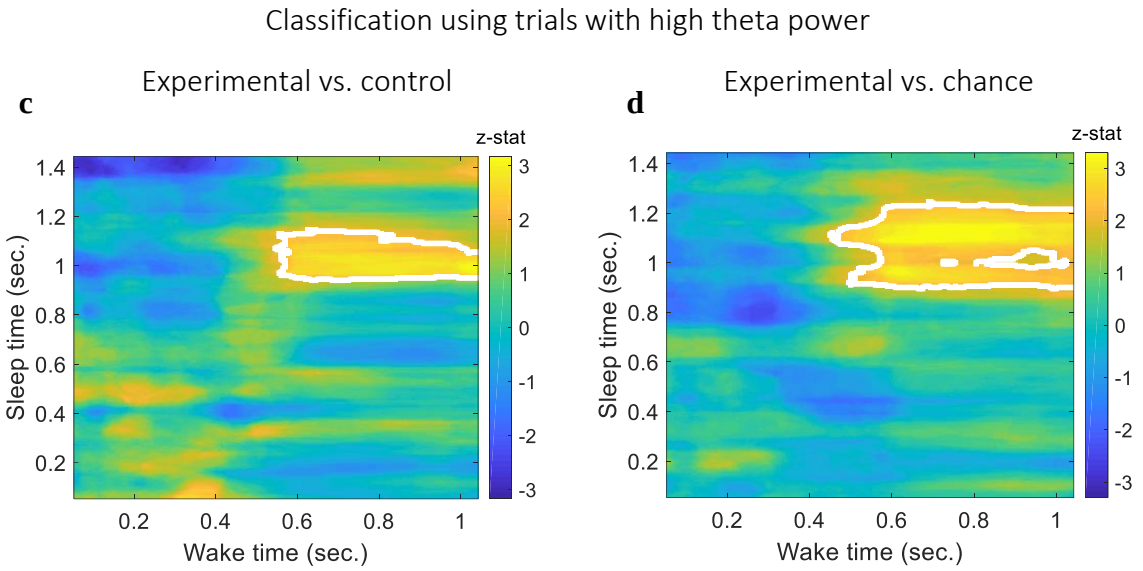


Figure 5.2: Classification of left hand vs. right hand during REM sleep. **a**) A comparison between the classification performance of the experimental vs. adaptation night using all trials reveals a significant effect described by a cluster which shows a higher classification performance for the experimental night compared to the adaptation night (n = 14, p = 0.01), z-statistics values at every point is shown and cluster edges are marked with white after correcting for multiple comparisons with cluster based permutation (see methods for details). **b)** classification performance for the experimental night was also significantly higher compared to chance (AUC: 0.5) as shown by the corrected cluster (n = 14, p< 0.0001). **c**) A comparison between the experimental and adaptation night classification using trials with high theta power reveals a significant effect described by a cluster that shows a higher classification performance for the experimental night compared to the adaptation night (n = 14, p = 0.028), z-statistics values at every point are shown and corrected cluster edges are marked with white. **d**) A comparison between the classification of experimental night using trials with high theta power and chance level shows a significant effect described by the shown corrected cluster (n = 14, p = 0.001).

5.3.3 Correlation of classification performance with behaviour

We next tested for a relationship between classification performance and sequence specific improvement on the behavioural task using a spearman correlation, see methods for details. This revealed a positive correlation (n = 14, r = 0.74, Bonferroni corrected p = 0.01), (Figure 5.3a) indicating that stronger detected reactivation was associated with greater overnight

sequence improvement. Importantly, this correlation only existed for the reactivated sequence (for non-reactivated: n = 14, r = 0.24, uncorrected p = 0.4). This finding suggests that the degree of detecting reactivation in REM positively predicts the extent to which sequence memory is improved over the night. Nevertheless, it is notable that REM TMR did not lead to any overall benefit in performance when considered without the classifier results (Koopman et al., 2020).

5.3.4 Analysis of temporal compression of reactivation

Prior work has shown that reactivation in non-REM sleep is often temporally compressed with respect to wake (Euston et al., 2007; Ji & Wilson, 2007; Lee & Wilson, 2002; Nádasdy et al., 1999). Recent finding showed that wake and N1 reactivation could be sometimes compressed and sometimes dilated within the same data (Eichenlaub et al., 2020). This motivated us to determine whether reactivation in REM lasts for the same amount of time as the original experience in wake, we performed an analysis of temporal compression. First, we applied our time x time classification method on EEG amplitude without any temporal smoothing. Thus, in this analysis we did not perform smoothing to get the precise temporal information. This non-smoothed analysis revealed that reactivation occurs more than once within the timeframe of the cluster we had originally identified (Figure 5.2a). Furthermore, both of the two sub-clusters we identified were temporally compressed during sleep as compared to wake (Figure 5.3b), for more details see methods. Specifically, the activation in wake is ~450 ms long and the two reactivations identified by this non-smoothed analysis are ~80 ms long and therefore last approximately 20% of the duration of activation in wake (Figure 5.3b). This suggests that reactivation of the memory in REM sleep is approximately five times faster than the activation in wake when the memory was encoded.

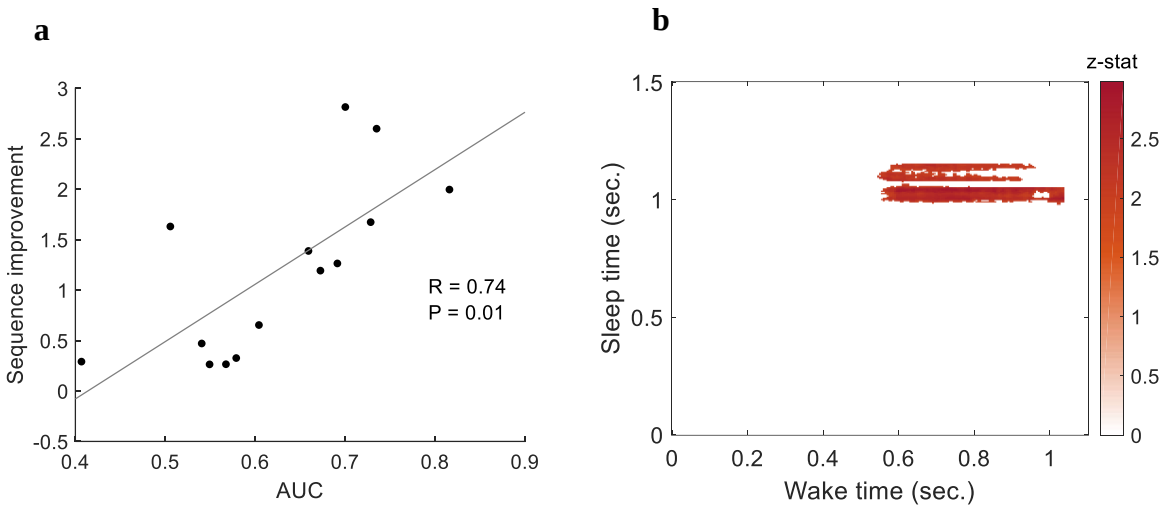


Figure 5.3: Characteristics of detected reactivation in REM sleep. **a**) Classification performance was positively correlated with behavioural improvement for the reactivated sequence (spearman correlation, n = 14, r = 0.74, Bonferroni corrected p = 0.01). **b**) Classification performance using EEG signals without smoothing showed two significant effects described by two clusters, around 1 second after the onset of the cue when compared to the adaptation night (n = 14, p = 0.008 for the earlier and, p = 0.025 for the later cluster). Z-statistic values are shown and cut with respect to clusters. Locations of significant effects and their temporal characteristics reveal that reactivations in REM sleep are approximately five times faster than the activation in wake.

5.3.5 Reoccurrences of reactivation

Finally, we were interested to know if TMR cued reactivation in REM occurs multiple times within each trial, or if the apparent recurrence might instead be due to averaging, with reactivation only happening once during each trial (e.g. at ~1sec. or after 1sec, but not both). To address this, we evaluated the classification correctness of each sleep trial at the times of each of the two clusters (see methods for details). We then determined whether classification was correct for both clusters or just one cluster (Figure 5.3b). We found that a significantly higher proportion of trials show reactivation at both time points (early and late), than at just one time point (Wilcoxon signed-rank test, n = 14, p = 0.001). This shows that reactivation is

recurrent in the majority of trials, and could potentially repeat even more than twice if the duration of trials was longer. This is in keeping with observations from rodent data showing repeated reactivation after a TMR cue in non-REM sleep (Bendor & Wilson, 2012).

5.4 Discussion

We showed that memory reactivation can be detected in human REM sleep using EEG classifiers. Such reactivation appears to be delayed by about one second after the sound cue onset. Reactivation is associated with high theta power, which appears to provide a preferred window for such activity, although it does not carry the discriminative information needed to actually detect reactivation. Interestingly, reactivations reoccur twice within a single trial (1500 ms) and are temporally compressed approximately five times compared to wake. We also demonstrated that stronger detection of REM reactivation predicts greater overnight sequence improvement on our behavioural task, and this is specific to the reactivated sequence.

Because our task was a motor one, we used motor channels for classification. This ensures that reactivation is related to the encoded motor memory and shows that reactivation retains the same features of wake. Moreover, comparison of classification results between experimental and control nights allowed us to confirm that classification is not derived by sound induced EEG noise.

Reactivation after a reminder TMR cue is delayed during REM sleep as compared to wake. During wake, neural activity associated with our motor imagery task starts around 0.6 seconds after cue onset and lasts until the presentation of the next cue at 1.1 seconds. In REM, on the other hand, reactivation of this task starts around 1 second after the cue and ends at around 1.25 seconds from onset. This delayed onset could potentially happen because the brain takes more time to process the information and reactivate the memory during REM sleep than during wake. In keeping with this suggestion, reactivation of a picture memory task in SWS has also been shown to be delayed, appearing about 2 seconds after cue onset (Cairney et al., 2018). This delayed SWS reactivation was also found in chapter 2, 3, and 4.

Our results show that classification performance in REM positively predicts overnight sequence improvement. Similar correlations between classification performance and behaviour were found in non-REM TMR (Shanahan et al., 2018; Wang et al., 2019; Zhang et al., 2018)

and also spontaneous reactivation (M. Schönauer et al., 2017). These findings can lead one to speculate that more reactivation means more consolidation, and therefore better post-sleep performance. However the absence of a group-level REM TMR benefit in our behavioural performance (see (Koopman et al., 2020) for a full analysis of the behavioural data), as well as the fact that other studies have observed negative correlations between reactivation evidence and post-sleep improvement (Murphy et al., 2018) lead us to treat such interpretations with caution.

REM sleep is dominated by theta activity, which is thought to support the consolidation process (Diekelmann & Born, 2010), and has been linked to reactivation (Louie & Wilson, 2001). Theta activity is linked to attention during wake (Biel et al., 2021; Gaillard & Ben Hamed, 2020; Keller et al., 2017) and is more prominent with higher executive control (Magosso et al., 2021). Wakeful theta is also associated with the encoding of new information and memory processing (Buzsáki, 2005; Kahana et al., 2001; Vertes, 2005). Furthermore, neuronal firing relative to theta phase has been shown to impact upon whether synapses are strengthened or weakened, since stimulation of the positive theta phase induces long-term potentiation and stimulation of the negative theta phase induces depotentiation (Hölscher et al., 1997; Huerta & Lisman, 1995). A similar pattern of phase dependent potentiation and depotentiation was shown in REM sleep (Poe et al., 2000), REM sleep reactivation and wakeful reactivation may be structured in a similar way (Battaglia et al., 2011; Jouvet, 1969; Kahana et al., 1999). In SWS, it has been shown that theta phase similarity is high with sleep suggesting the importance of theta phase as reactivation (Schreiner et al., 2018). It has also been shown that the increase of theta power is important for successful cueing during sleep (Schreiner & Rasch, 2015).

Our data demonstrated that trials with higher theta power also show greater evidence of memory reactivation but theta band in isolation does not provide sufficient information to detect reactivation. Theta therefore appears to be a marker for reactivation but does not embody the reactivation in and of itself. This pattern of results requires more investigation but could potentially indicate that theta is providing some kind of timing function which determines the impact of reactivation, while the reactivation itself occurs at other frequencies.

Studies in rats have shown that replay is temporally compressed with respect to run, or actually performing the task in question, and the rate of that compression varies between sleep and wake. For instance, replay in both wake and SWS has been shown to occur at a faster rate than

the original task (Davidson et al., 2009; Diba & Buzsáki, 2007; Lee & Wilson, 2002). However some studies of rodent non-REM sleep showed a compression of 6 to 7 times compared to wake (Euston et al., 2007), while other studies showed compression rates varying between 10 and 20 times faster than wake (Ji & Wilson, 2007; Lee & Wilson, 2002; Nádasdy et al., 1999). Our analysis is in line with this literature, since it suggests that TMR reactivation in human REM sleep is temporally compressed by approximately 5 times with respect to wake.

Schreiner et. al. (2018) showed that the reactivation elicited by TMR in human SWS reoccurs repeatedly within a single trial (Schreiner et al., 2018). A parallel study in rodents (Bendor & Wilson, 2012) also supports this by showing that TMR cued replay can continue to repeat for up to 10 seconds after the offset of the auditory cue, though this process appears to be interrupted by the presentation of a second stimulus. These observations of repeated replay are in keeping with the suggestion of a cortico–hippocampal–cortical loop. Memories are strengthened with a reverberation of replay between the cortex and hippocampus (Rothschild, 2019), although one might potentially expect such reverberation to occur on a shorter timescale. We tackled the question of whether replay occurs repeatedly after a single TMR cue in REM and found two repetitions following a single TMR cue, which is in keeping with the results from previous studies (Bendor & Wilson, 2012; Schreiner et al., 2018). Notably, our trials were just 1.5 seconds long, so it is possible that further reoccurrence would have been observed in our paradigm if the cues were spaced farther apart.

5.4.1 Characteristics of reactivations that happen in SWS, and REM sleep

While there is already a large and growing body of literature about reactivation in non-REM sleep (Bendor & Wilson, 2012; Cairney et al., 2018; Ji & Wilson, 2007; Lee & Wilson, 2002; Rasch & Born, 2013; Schreiner et al., 2021; Wang et al., 2019; Wilson & McNaughton, 1994), our findings provide initial information about human reactivation in REM. As such, they suggest several parallels between reactivation in these two sleep stages. For instance, similar to non-REM (Cairney et al., 2018), reactivation in REM is delayed after cue onset compared to wake. Furthermore, reactivation in REM is somehow related to the oscillatory structure of sleep (e.g. theta activity), which parallels the known relationship between reactivation in non-REM and graphoelements like SOs (Mölle et al., 2002; Valderrama et al., 2012), spindles (Antony et al., 2019; Cairney et al., 2018), and ripples (Zhang et al., 2018). Importantly, detected reactivations are reinstated in the same area of the brain that is related to the task and

the extracted features of reactivation are similar to wake activation which is why it is detectable with machine learning models. Reactivations in REM sleep appear temporally compressed by about five times in comparison to wake, which is close to the 6 to 7 times compression observed in non-REM (Euston et al., 2007).

The question of whether memories reactivate in REM as well as non-REM sleep has been debated for some years now. REM reactivation was suggested by modelling (Hasselmo, 2008) and was shown in the rodent literature (Louie & Wilson, 2001). Some evidence of learning dependent activation in human REM sleep were observed in humans (Maquet et al., 2000; Peigneux et al., 2003), however, null findings from human REM TMR studies (Rasch et al., 2007; Rasch & Born, 2013) lead to scepticism in the community. Our current findings put such scepticism to bed by providing clear evidence of TMR cued reactivation in REM. Furthermore, our analysis of this reactivation uncovers many important properties of this phenomena, showing strong parallels with non-REM reactivation. Further work is needed to explore this topic in detail, for instance determining how such reactivation links to behavioural and neural plasticity, and how this differs across a variety of cognitive tasks.

5.5 Methods

5.5.1 Participants

EEG data and behavioural data were collected from human participants (n = 16) (8 females, 8 males, and age mean: 23.6). One participant was excluded due to a technical problem (n = 15). Participants completed a SRTT before and after sleep, and spent an adaptation night in the lab the night before the task. All participants were right-handed and none of them reported familiarity with SRTT. All participants had normal or corrected-to-normal vision, normal hearing, and no history of physical, psychological, neurological, or sleep disorders. Responses in a pre-screening questionnaire reported no stressful events and no travel before commencing the study. Participants did not consume alcohol in the 12 hours before the study and caffeine in the 24 hours prior to the study or perform any extreme physical exercise or nap. This study was approved by the School of Psychology, Cardiff University Research Ethics Committee, and all participants gave written informed consents. The SRTT is used here, (Cousins et al., 2014) and Chapter 2, with the cues delivered in REM sleep only.

5.5.2 EEG pre-processing

EEG signals were band-pass filtered in the frequency range from (0.1 to 50 HZ). Subsequently, trials were cleaned based on statistical measures consisting of variance and mean. Trials were segmented -0.5 sec. to 3 sec. relative to the onset of the cue. Trials falling two standard deviations higher than the mean were considered outliers and rejected if they show to be outliers for more than 25% of the channels. If trials were bad in less than 25% of the channels, they were interpolated using triangulation of neighbouring channels. Thus, 9.8% and 10.5% of trials were considered outliers and removed from the experimental night data and the adaptation night, respectively.

Data was subsequently analysed with independent component analysis (ICA), to remove eye movement artifacts which can occur during REM. Components identified by the ICA were correlated with the signal from the eye electrodes, and components that were significantly correlated (corrected for multiple comparisons) were removed. In the final artifact rejection step, all channels for each participant were manually inspected. Because TMR will not be effective with all trials, we also rejected trials with low variance that do not differ from their mean across time since they are unlikely to contain a response. The number of clean trials kept after cleaning was consistent among participants such that they contribute equally to the group-level analysis and that number was 366 trials, it was determined according to the participant with the lowest number of such trials. All cleaning was done on all trials irrespective of cue information and stimulation night to avoid bias.

5.5.3 Time x time classification with time domain features

We adopted a time x time classification approach after smoothing the EEG signals using 100 ms window such that every time point is replaced with the average of the 50 ms of both sides around it. Since we know that this task is motor-dependent we focused our classification on the motor area, thus we used four channels around the motor area for classification (C5, CP3, C6, and CP4). In the time x time classification, every time point from sleep was used to train LDA classifier, which was applied to all time points from wake in order to get one row of classification results in the time x time classification plot. The process was repeated until all time points after a sound cue in sleep were finished (trial length in sleep was: 1.5 sec. and 1.1 sec. in wake) (Extended Data Figure 2). We use the area under the receiver operating

characteristic curve (AUC) as the performance measure in our binary classification, which is preferable in assessing the performance of classification over accuracy. Analyses were done using FieldTrip toolbox, MVPA-Light toolbox in Matlab, and customised scripts using Matlab 2018a.

5.5.4 Training classifier models with sleep data

We trained our classifiers on sleep data which allows the models to adapt to sleep data and weigh the features according to their discriminative ability in sleep given that there might be some spatial shifts between the best features of sleep and wake. Thus, by doing this, we ensure that the classification models adapt to the noisier sleep data and get a chance to adapt to the noise of sleep data. In LDA classifiers, between-class covariance is maximised, and within-class is minimised, that within class covariance represents noise and thus the models see the noise of sleep data and are more sensitive to the differences between classes in sleep. We suggest that studies in the future adopt a similar approach and train classification models using sleep data. We should also address that this comes at a cost of making it harder to conduct post-hoc analyses on sleep trials and analyse them (e.g., correct vs. incorrect trials as in the SO-based classifier and spindle-based classifier in Chapter 3, thus training with wake was done in that Chapter) due to the fact that sleep is now the training set.

5.5.5 Theta power calculation

We calculated theta power using band-pass filtering in the range (4 to 8 HZ) and Hilbert transform. The power of a trial is calculated as the average of all power of different time points of that trial and all channels. We then divided the trials based on the median power of all trials. This gave us the trials with high theta power (higher than median) and low theta power (lower than median).

5.5.6 Temporal compression of reactivation

We analysed the temporal compression by applying the time x time classification using the EEG amplitudes without smoothing the EEG signals. We used the cluster we found from the first classification (around 1 sec.) as a clustering window, we used this window as a marker of time of interest because if the reactivation is compressed then it will be temporally short.

Duration of sleep reactivation is calculated as the average durations of the two sub-clusters in sleep time. Duration of wake activation was determined by the average durations of the sub-clusters in wake time, Figure 5.3b. Analysis was done with customised scripts using Matlab 2018a.

5.5.7 Correlation of classification performance with behaviour

Classification performance was averaged inside the cluster. AUC values from the high theta power classification were tested for correlation with the behavioural improvement. The behavioural improvement was calculated as: [(random blocks after sleep - the best 4 blocks after sleep) – (random blocks before sleep – the best 4 blocks before sleep)] / (random blocks before sleep – the best 4 blocks before sleep). The result was corrected for other measures using Bonferroni correction. We extracted three behavioural measures: early blocks improvement, late blocks improvement, best blocks improvement (described above). Early blocks improvement was defined as: [(random blocks after sleep - the first 4 blocks after sleep) – (random blocks before sleep – the last 4 blocks before sleep)] / (random blocks before sleep – the last 4 blocks before sleep). Late blocks improvement was defined as: [(random blocks after sleep - the last 4 blocks after sleep) – (random blocks before sleep – the last 4 blocks before sleep)] / (random blocks before sleep – the last 4 blocks before sleep).

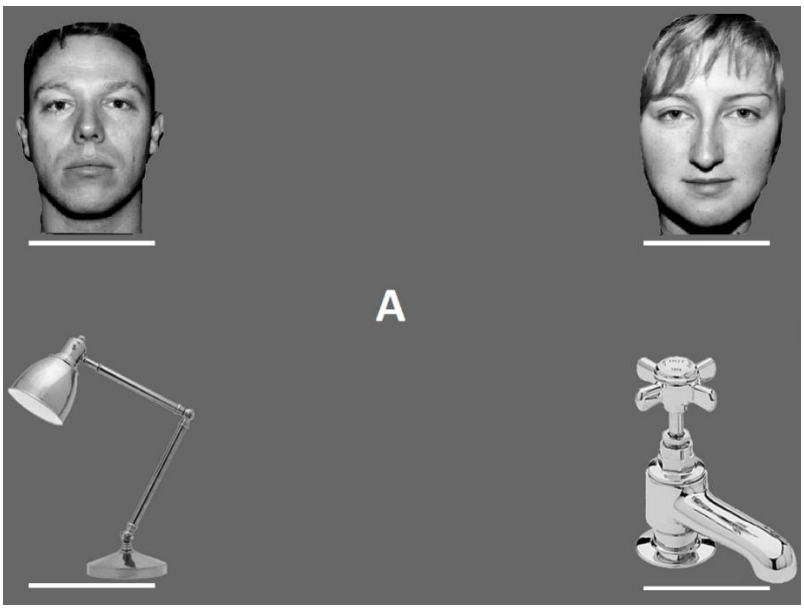
5.5.8 Reoccurrence

We analysed whether the classification is reoccurring within the same trial or whether the reactivation is happening once while it only appears to be reoccurring as a result of averaging. For this, we under-sampled the length of the trial in wake, that is, we used wake time according to the two clusters in Figure 5.3b and under-sampled the wake trial to match the length of sleep cluster. Then, for every trial of sleep, we performed spearman correlation with all trials from wake such that we ended with one vector of correlation coefficients for every trial from sleep. Subsequently, we counted the number of times a trial from sleep positively correlated with wake trials of the same class, and the other class. A trial from sleep is then considered ‘correct’ if the number of positive significant correlations is higher for similar class than different class. Afterwards, we determined if reoccurrence is happening in sleep trial if it is correct during both the earlier and later clusters. Then we counted the number of trials with different types: recurrent, only earlier cluster, and later cluster. Afterwards, we normalised by the total number

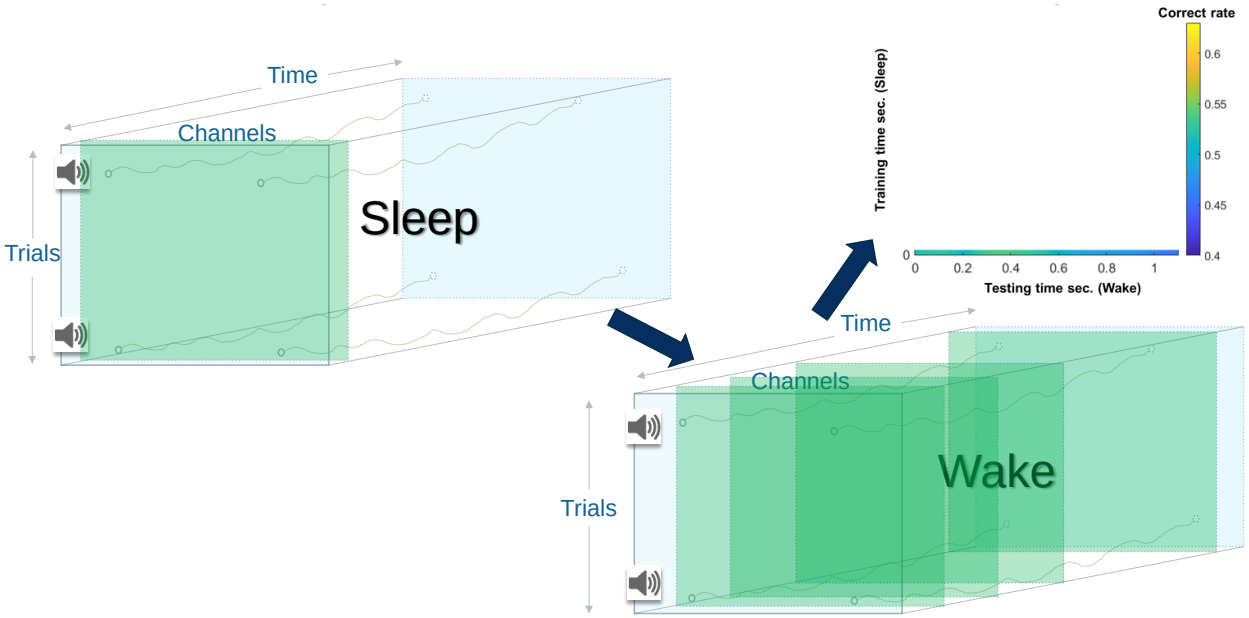
of trials for every subject which gave a datapoint for every subject for every type. Subsequently, the number of trials for every type was compared to chance 0.333 and recurrent reactivation was the only type that showed higher than chance trial count (Wilcoxon signed-rank test, n = 14, p = 0.001). On the other hand, earlier cluster only: (Wilcoxon signed-rank test, n = 14, p = 0.0009) was below chance. Also, later cluster: (Wilcoxon signed-rank test, n = 14, p = 0.0015) was below chance. Analysis was done with customised scripts using Matlab 2018a.

5.5.9 Correcting for multiple comparisons

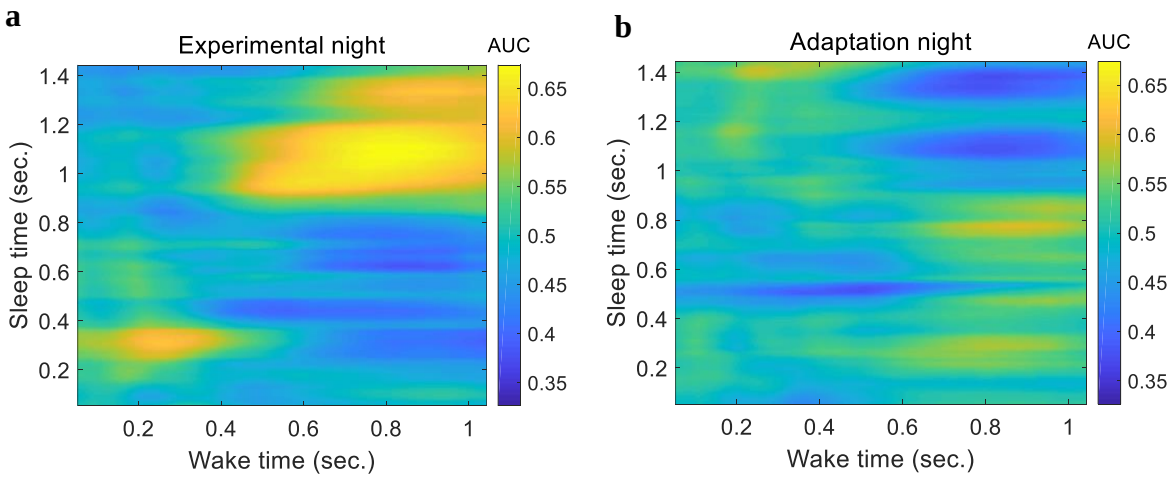
Multiple comparisons correction was done using MVPA-Light toolbox in Matlab (Treder, 2020) and customized scripts. Cluster-based permutation testing was used and a Wilcoxon based sample-specific testing with threshold of 0.05. Permutation test threshold for clusters was 0.05, and 10,000 permutations were calculated.

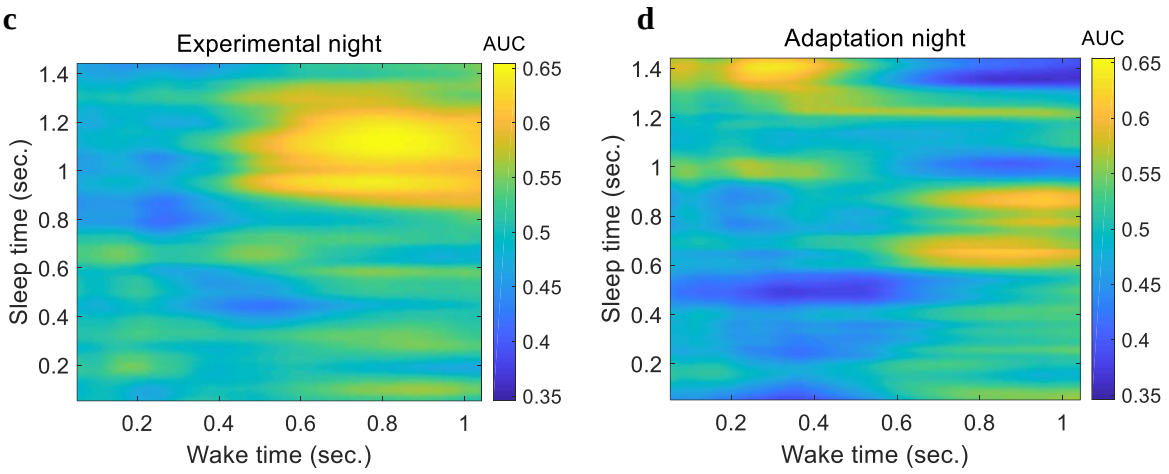


Extended Data Figure 1: Illustration of the four images that appeared in the task: two faces and two objects.

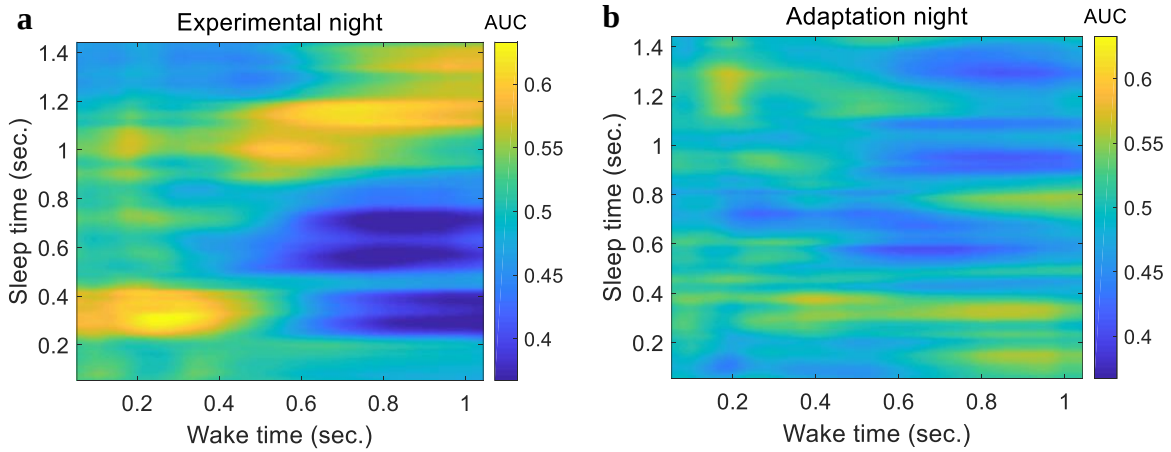


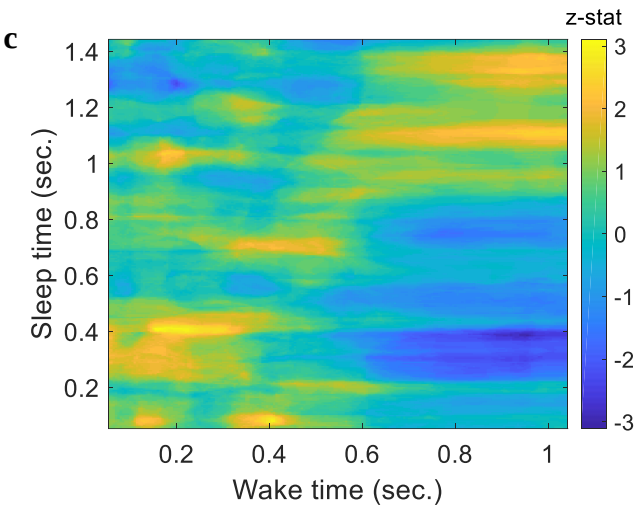
Extended Data Figure 2: Example of time x time classification wherein one time point is used from sleep to build a classifier model and all wake time points were used for testing.





Extended Data Figure 3: Classification of left hand vs. right hand. **a)** Classification performance by training on sleep and testing on wake. **b)** Classification performance when classifiers were trained with the adaptation night sleep and tested on wake. **c)** Classification performance of training on sleep during the experimental night and testing on wake using trials with high theta power. **d)** Classification performance of training on sleep during adaptation night and testing on wake using trials with high theta power.





Extended Data Figure 4: classification with trials with low theta power and the correlation of performance with behaviour. **a)** Classification of left hand vs. right hand using trials with low theta power using the experimental night did not show significant difference against the chance level. **b)** Likewise, the classification using the adaptation night did not show significant difference against the chance level. **c)** Z-values of the comparison between the classification of the experimental and the adaptation nights when the trials with low theta power were used.

General discussion and conclusion

> Everything we do, every thought we've ever had, is produced by the human brain. But exactly how it operates remains one of the biggest unsolved mysteries, and it seems the more we probe its secrets, the more surprises we find.
>
> Neil deGrasse Tyson

6.1 Summary of findings

In this work, we developed different pipelines with the aim of detecting memory reactivations of a motor memory during slow wave sleep (SWS) and rapid eye movement (REM) sleep. For this, we used TMR to associate motor memories with sounds and trigger those memories in sleep. TMR enabled us to feasibly evaluate the classification performance.

In chapter 2, we showed that we can use time domain features and linear classification to detect SWS reactivation which appeared around 1 second from the onset of the cue. In the same chapter, we explored the possibility of detecting reactivation in REM, however, results in REM were more marginal and we could not draw a firm conclusion.

In chapter 3, we further explored SWS reactivation. We found reactivation at two different timings, early after stimulus and around 1 second after. We then took a closer look at the results and found that the two peaks of classification are not reoccurring, and we do not get both reactivations after every TMR cue. Consequently, we started post-classification analyses which showed the active role of slow oscillations (SOs) and sleep spindles in predicting the detectable reactivations. In that chapter, we demonstrated that the up-going phase of the SO is the optimal timing for applying TMR cues in order to get detectable reactivations. Furthermore, we found that both peaks of classification showed different relationships to behaviour: early reactivation showed a negative correlation with pre-sleep reaction time, while late reactivation had a disruptive role for the memory of the task.

In chapter 4, we were curious to investigate whether the early reactivation happened as a result of the stimulus at time 0 or whether the brain could predict the upcoming cues and thus reactivated the information of the upcoming cue before it took place. For this, we collected new data and jittered the timing of cue delivery. We found that reactivation pattern was different, and that the early reactivation did not appear. We think that the brain was predicting the upcoming cues of the reactivated sequence and that is why we detected early reactivation. We also noticed that by extending the duration of trials we could see two detectable reactivations, after 1 second and after around 2 seconds, the observation that we could not make in chapter 3 given the shorter trials (1.5 second). In chapter 4, we think that as a result of jittering, the temporal consistency of the sequence was disrupted and thus we did not find TMR benefit in the first follow up session.

Eventually, in chapter 5, we wanted to see if we can use TMR and EEG classifiers to detect reactivation in REM sleep, which is a debated topic in the memory replay community. We developed a classification pipeline that adapts itself to sleep data and then we applied the trained classifier models on wake and were able to identify memory reactivation in REM sleep. We also found that trials with higher theta activity embody reactivation. Given the rodent literature, we performed post-classification analyses on recurrence and temporal compression of reactivation to find that reactivation in REM sleep is reoccurring more than once after TMR and is temporally compressed in comparison to wake. In the same chapter, we show that there is a positive relationship between the improvement of the memory and the extent we can classify reactivation.

Now, we will discuss some points about classification, memory reactivation and its characteristics as seen in this work and literature.

6.2 A classification perspective on reactivation

6.2.1 Is it a pipeline that fits all?

We would like to borrow a theorem that is famous in optimisation and machine learning, stated by David Wolpert in 1997 (Wolpert & Macready, 1997). We would borrow the gist of it and say that there will be no single pipeline that can be used to detect reactivations of all memories. The discussion on classification pipelines must be candid; we state that the problem in hand

controls the nature of the pipeline and extracted features. In other words, the classification pipeline is developed to tackle the classification problem of motor imagery in the SRTT experiment. This means that for classifying a different activation pattern, one ought to think about the kind of features to be extracted and fed to the classifier, also the channels from which to extract these features or the spatial filtering method to be performed. Consequently, the nature of the encoded memory guides the development of a valid and reliable classification paradigm.

6.2.2 A classifier should not overfit the training set

Machine learning models could overfit the training data and this is a famous problem in classification. Overfitting might occur when developing a classification pipeline as a result of creating a complex classification model that could simply memorise observations and the whole training data rather than learning useful information. Such stringent models are very hard to generalize and test with unseen data and need to be updated or regularised. Given the differences of the oscillatory patterns and noise in wake and sleep, if we build a classification model using e.g., wake EEG pattern, we want that model to learn the EEG pattern that could generalise to sleep as well, without tailoring the model very stringently to wake data. This is the reason why we extract features that we think can generalise to unseen datasets that has different graphoelements. Under the same point, we are aware of some of the famous approaches adopted in the BCI literature for classifying motor imagery. We had a priori hypothesis that methods that rely on the transformation of data and the use of spatial filters in different frequency ranges will not yield optimal results with the task in hand that requires generalisable models across sessions. We think that our a priori hypothesis was correct because we did try some of the other methods in a paradigm trained on wake data from one session and tested on wake data from a different session and the results was not as good as the current pipeline. Adding more features (power, phase, etc.) is clearly possible but it could overcomplicate the model and lead to overfitting. Even if the classification was successful with more features, it would make it difficult to do post-classification analyses and interpret the important features for classification. Although, it is possible to analyse the weights that a classifier model gives to every feature, it would still be a mixture of different features. Additionally, EEG has low signal to noise ratio, thus, a complex nonlinear model could fit the

noise rather than the signal, so, cleaning EEG to isolate signal from noise as much as possible seems reasonable and then a linear classification can be employed.

6.3 The role of stage-specific graphoelements in reactivation

6.3.1 The active role of SWS spindles and SOs in applying TMR during SWS

In this work, our results show that there is indeed an active role of SOs and sleep spindles in memory reactivation. During SWS, neurons enter a duration of silence during the hyperpolarised state during the down-going phase of the SO. On the contrary, depolarisation during the up-going phase of the SO is more reactive to stimulation. Thus, deilvering TMR during the up-going phase can lead to larger ERP responses and possibly different effects than stimulating the down-going phase (Schabus et al., 2012). Related to the same point, fast phenomena such as spindles, and gamma activity are more prominent in the SO up-going state than in the SO down-going state (Mölle et al., 2002; Piantoni et al., 2013; Valderrama et al., 2012). A number of studies showed a relationship between replay in rodents and sharp-wave ripples (Kudrimoti et al., 1999; Nakashiba et al., 2009; O'Neill et al., 2008). Moreover, data from human epilepsy patients has shown that the SO upstate shows higher gamma oscillations (Van Quyen et al., 2010), and sharp-wave ripples, which have been shown to carry reactivation (Zhang et al., 2018). Thus, we think that the up-going state is the preferred time for delivering TMR. The results of chapter 3 and 4 met this expectation, where we analysed the phase of the SO during which the TMR was applied to find that the early reactivation found in chapter 3 was related to the delivery of TMR on the up-going phase of the SO. Meanwhile, in chapter 4, delivering cues on the up-going phase of the SO triggered more classifiable pattern around 2 sec. from TMR onset. This shows the importance of the SO stimulation phase and when we should target our cues. Our analyses in chapter 3, showed that we can use the ongoing pre-cue SO features and predict successful post-cue reactivation. This demonstrates the active role that SOs play in reactivating memories which goes in line with the literature supporting this idea. Since not all SOs are carrying reactivations, we think that by extracting the pre-cue SO features we were able to identify the footprint of some SOs that carry reactivation.

We also see an important role for SWS spindles. In 2018, Cairney and colleagues showed that reactivation is mediated by the increase of spindle power and happens around 2 sec. from the onset of TMR (Cairney et al., 2018). A relationship was shown between spindles and detected

reactivation, where less pre-cue spindles predicted more post-cue reactivation (Wang et al., 2019). Our results in chapter 3 and 4 go in line with what Wang and colleagues have shown. Specifically, we showed that the lack of pre-cue spindles yields a more classifiable post-cue reactivation. This effect happens for the late reactivation in chapter 3 (around 1 sec. from cue onset) and was also true in chapter 4 for both reactivations (after 1 sec. and at 2 sec. from TMR onset). This evidence demonstrates an active role of SWS spindles, it also helps in directing our TMR to successfully trigger detectable reactivation and helps in understanding the relationship between SWS spindles and memory reactivation.

6.3.2 The role of REM sleep theta in reactivation

Now, let us consider REM sleep and its most prominent phenomenon which is theta activity (Boyce et al., 2016; Hutchison & Rathore, 2015; Nishida et al., 2009). Previous studies showed a relationship between theta activity and encoding of new information (Battaglia et al., 2011; Juergen Fell et al., 2011; Kahana et al., 1999; Vertes, 2005). Also, some studies showed a link between replay and theta activity (Louie & Wilson, 2001; Poe et al., 2000) which gave us reason to think that theta activity could be important for reactivation. In chapter 5, we showed that this assumption is correct. When we analysed theta activity, we found that REM sleep reactivation happens during the times of high theta activity. It is notable that we found that theta activity was offering a preferred timing for reactivation and theta activity itself is not the discriminative feature that the classifier uses to classify reactivation. We would be able to say that the classifier uses theta activity to classify reactivation if, for example, we extract theta power, and it was high for one class and low for the other. However, in chapter 5, we see that the oscillatory pattern in a wide frequency band is needed for successful classification. This evidence can help us understand the role of theta in facilitating reactivation and also when to deliver TMR cues to trigger reactivation.

6.4 Temporal characteristics of detected reactivation

6.4.1 The temporal information of the reactivated pattern is different from wake

A number of studies showed that it is possible to detect reactivation after TMR in humans (Belal et al., 2018; Cairney et al., 2018; Murphy et al., 2018; Schreiner et al., 2018; Shanahan et al., 2018). In our first attempts for detecting reactivation in Chapter 2, we showed that we

can use time domain features with linear classification on features from the motor area and successfully detect memory reactivation in human SWS. These results showed that the timing of reactivation is delayed compared to wake encoding. This temporal difference of reactivation goes in line with some studies that showed delayed reactivation after TMR in SWS (Cairney et al., 2018). The temporal difference between wake and sleep is shown in rodents, with replay repeating many times after a reminder auditory cues and up to 10 seconds or until the presentation of another cue (Bendor & Wilson, 2012). We see a difference in timing between SWS reactivation and wake in chapter 3, where we found two classification peaks one immediately after the TMR cue and another around 1 second from the TMR onset. Additionaly, in chapter 4, we see that the reactivation pattern can be altered by jittering the timing of TMR delivery. The point remains that wake activation occurs at different timing from sleep reactivation. This was also the case for REM reactivation: in chapter 5 we show that TMR elicited reactivation around 1 second after TMR onset. All together, we can conclude that, in this work, reactivation was shown to be happening at different timing from wake activation, in accordance with the literature.

6.4.2 Is reactivation recurring after a stimulus?

Recurrence of reactivation is an interesting property and was shown in human SWS sleep (Schreiner et al., 2018) and in rodents (Bendor & Wilson, 2012). Recurrence of replay is supported by a study suggesting a reverberation of replay between cortex and hippocampus (Rothschild, 2019). We wanted to tackle this issue in our data and see if the pattern of reactivation is genuinely recurrent or not. Consequently, we used an approach in which we compared the likelihood of having recurrence in a particular trial to getting only a single reactivation per trial. For SWS reactivation, we found that it is more likely for a trial to have one of the reactivations. Interestingly, for REM reactivation, it was more likely to get multiple reactivations after TMR cue compared to single reactivation. This suggests a difference between SWS reactivation and REM reactivation, even though the classification pattern may seem similar at a glance. It also shows that the reactivation pattern can show hidden features when we look at individual trials instead of the grand effect from many trials/participants.

6.4.3 Temporal compression of reactivation

Looking at another interesting temporal characteristic of reactivation, we see that detected reactivation in chapter 5 appears temporally compressed in comparison to wake activation. Temporal compression of replayed contents is supported by some rodent studies, they show that replay in both wake and SWS occur at a faster rate than the original task, (Davidson et al., 2009; Diba & Buzsáki, 2007; Lee & Wilson, 2002). The rate of compression varied between studies; while some showed a compression in non-REM of 6 to 7 times faster than in wake (Euston et al., 2007), other studies showed it to occur 10 to even 20 times faster (Ji & Wilson, 2007; Lee & Wilson, 2002; Nádasdy et al., 1999). Interestingly, it was also shown that there could be no compression compared to wake experience (Louie & Wilson, 2001). Another recent finding showed that replay in wake and stage 1 of NREM sleep could show the evidence of both temporal compression and temporal dilation simultaneously (Eichenlaub et al., 2020). These findings trigger our curiosity to check for temporal compression of reactivation in our REM sleep data. Thus, when we analysed the detected reactivation, we found evidence of temporal compression and the reactivation was 5 times faster than wake. This suggests a temporal difference between wake activation and REM sleep reactivation. We hope that future studies and investigations will chase this interesting point to know the mechanisms causing the replay to appear compressed and sometimes dilated. Also, whether this relates to the timing of stimulation or paradigm (TMR vs. spontaneous replay) or perhaps the task and how the brain reactivates it.

6.5 Reactivation and behaviour

Let us move on to the relationship between detected reactivation and behaviour. Interestingly, the detected reactivations in SWS correlated differently with behaviour which shows that different reactivations could have different impact on memory improvement. The early reactivation shown in chapter 3 showed higher successful classification when participants were faster with the task before sleep. This early reactivation could have occurred because the memory was formed strongly before sleep so it could reactivate easily in subsequent sleep. On the other hand, the late reactivation which could be initiated by a weaker memory trace has a disruptive characteristic and resulted in a decrease of task memory improvement. Notably, when we jittered the timing of the cues, this could have caused temporal unpredictability and thus a disruption of the sequence memory. The reason is that the temporal information of each sequence presentation is now different, and we think this is why we do not see a TMR benefit in chapter 4 while in chapter 3 there was a TMR benefit. In chapter 5, we showed that the extent we can classify REM sleep memory reactivation predicted memory improvement. These results suggest that there is a relationship between detectable reactivation and behaviour. However, more studies should be done to determine how different reactivations are initiated and how different memory traces are processed to understand why a detectable reactivation could have e.g., a disruptive role.

6.6 Conclusion

In this work, we explored different characteristics of detectable memory reactivation and found interesting properties that can offer a guidance on the timing of TMR delivery to best trigger reactivation. We think that the findings of SWS reactivations deepen our understanding of reactivation and show that even when we have multiple reactivations this does not mean that they are necessarily reoccurring after every cue. Furthermore, each reactivation has its own characteristics and correlation with behaviour. We also demonstrated that we could use pre-cue SO and spindle features to predict correct classification. This emphasises the active role of SWS graphoelements. In addition to understanding reactivation, all these findings offer a mechanism by which we could build a closed loop stimulation paradigm to try and maximise detectable reactivation by delivering TMR in a more precise manner. The impact of this on memory improvement could be explored. We also show for the first time that memory reactivation of human REM sleep is possible and detectable with EEG classifiers. We demonstrated interesting properties of REM reactivation and how it differs temporally from wake and appears to be compressed and recurrent. We showed that theta activity in REM sleep is offering a preferred window for stimulation in order to trigger detectable reactivation.

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