The Role of Theta Oscillations in Human Associative Memory

by

Andrew Jason Clouter

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School of Psychology

College of Life and Environmental Sciences

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Abstract

Human episodic memory consists of bound, highly rich, multisensory experiences. The sensory cortices that process the sensory information that form part of these memories are distributed across the brain in specialised areas. The binding of the information processed by these sensory regions is likely facilitated by long-term potentiation. This mechanism relies on the precise timing of neural activity. Theta oscillations, a dominant, low-frequency neural oscillation in the hippocampus, is a candidate mechanism involved in facilitating the precise timing required for LTP to take place. Indeed, studies in animals have suggested as much. However, evidence for a causal role of theta oscillations in human associative memory formation is lacking. By modulating the luminance of movies and the amplitude of sounds in an associative memory task, we manipulated the degree of phase synchrony between the respective sensory cortices. We show that associative memory was improved if the stimuli were presented in-phase. Through the experiments presented in this thesis, we provide direct evidence of the causal role of theta oscillations in human memory: Human associative memory relies on the phase of a theta-frequency mechanism.

Dedication

For everyone who has first struggled to accept things, who then laboured to appreciate things, and who then brought themselves to love things. Amor Fati. Let everyone see as beautiful what is.

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Table of Contents

Author Notes

While the design, analysis, conduction, and interpretation of the work described herein was done by myself, it was with the advice and guidance of my advisors. Thus, while the manuscript is written in the first-person plural, in order to fully acknowledge the guidance provided by my advisors, the analyses and opinions presented throughout are my own, and may not always reflect the opinions of my advisors.

Much of the work presented in Chapters 3-5 has been published by myself with my supervisors (Clouter, Shapiro, & Hanslmayr, 2017), principally authored by myself. The work in Chapter 6 was undertaken with the collaboration of Danying Wang and Qiaoyu Chen, principally authored by Danying Wang, and published (Wang, Clouter, Chen, Shapiro, & Hanslmayr, 2018). Every effort has been made throughout this manuscript to ensure that it remains an original piece of work, despite the previous publication of the work presented herein.

Author Contributions

Publications and Presentations

From my doctoral study at the University of Birmingham, the following articles and conference proceedings are in preparation, submitted, or accepted for publication and/or presentation at conferences. For the publications and presentations, Kimron Shapiro and Simon Hanslmayr advised on study design, data analysis and editorial guidance; however, this thesis was prepared by myself alone, without the guidance of my supervisors.

Papers published from this doctoral research

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Abstracts from this doctoral research

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Chapter 1: Introduction

The sights, sounds, smells, and other elements of our perception are bound together in the brain, such that we enjoy a rich, multisensory conscious experience. When we mentally revisit these experiences using the facilities of our memory, we can again experience a richness that approximates the original event, as Proust's narrator experienced when the taste of a madeleine dipped in tea brought forth tastes and smells long past, and the comfortable surroundings in which they were enjoyed. The question of how the memory system in the brain binds together the elements of our senses so that our remembrances of things past are as rich as our personal experiences remains unanswered.

It has been suggested that oscillatory neural activity in the hippocampal region of the brain is the machinery that enables the binding of different sensory elements into memories. However, concrete evidence for such a mechanism remains elusive. Evidence for such a system would allow a better understanding of human memory, and may prove critical in informing future discoveries on the nature of memory, as well as informing potential treatments for disorders that affect memory.

Different Memory Systems

In the study of memory, it is often fruitful to distinguish different forms of memory; these forms are typically delineated by neural or cognitive components that subserve one form of memory, but not others. These different forms of memory are further characterised by a number of other properties, such as their duration (short- or long-term), whether they can be consciously declared, or whether they are of personal experience, general knowledge, and more. The processes of the memory system

are also broken down into phases such as encoding, storage, and retrieval. Different neural populations and mechanisms subserve these different memory processes (Moscovitch, 2004).

Memories that persist beyond the immediate need for the information is called long-term memory. Two different types of long-term memory are conscious declarative memory (that can be only true or false) and procedural memory, that is unconscious and non-declarative (Schacter & Tulving, 1994; Tulving & Schacter, 1990). Declarative memory is again broken down into subtypes: episodic memory and semantic memory (Tulving, 1972). Episodic memory is the memory of personal events, including the sensory experiences, the times, and the places, in our lives—what people usually mean when they speak of memory.

Episodic memory is therefore inherently associative, since our personal lives are inherently multisensory, and thus it requires the internal linking, or binding, of the information received and processed by the different sensory cortical areas of the brain. These sensory areas, which are only connected to each other weakly, project to the medial temporal lobes (MTL) of the brain, where the processed sensory information is bound together to create our rich episodic memories.

The Neural Correlates of Memory 1: The Medial Temporal Lobe

In the early 1950s, Brenda Milner and Wilder Penfield had encounters with two patients, both of whom had persistent and very severe impairments of memory for recent events. Both patients experienced these symptoms after the removal of their medial temporal lobes (Penfield & Milner, 1958). Milner and Penfield reported the two cases to the American Neurological Association meeting in 1955. William Scoville read the abstract of their presentation, and later contacted Penfield to say that he had a patient (patient “H. M.”) with similar memory deficits, also following

the bilateral removal of the MTL. Milner studied the patient with Scoville, describing the patient in 1957 (Scoville & Milner, 1957). Like the patients observed by Penfield and Milner, patient H. M. had what seemed to be a complete inability to form new memories, with no accompanying loss of other cognitive abilities (Scoville, 1954). Subsequent research demonstrated that H. M. was able to form new short-term memories (Milner & Taylor, 1972), but was unable to transfer these into long-term storage (Milner, 1972). H. M. lived the rest of his life with all of the experiences following his surgery failing to make a contribution to his existing store of knowledge.

The MTL, shown to be critical for the formation of episodic memory, is also critical for associative memory (Eichenbaum, Yonelinas, & Ranganath, 2007; Aggleton & Brown, 1999). Damage to the MTL leads to significant impairments in learning new associations (Brasted et al., 2003; Brasted et al., 2002; Murray et al., 2000; Wise & Murray 1999; Rupniak & Gaffan, 1987). It is also clear from studies of patients like H. M., who suffered little impairment of memory for events that occurred before the resection of the MTL structures, that the MTL is required for the formation of new memories, but only until those memories are transferred to the neocortex for permanent storage (Moscovitch et al., 2005; Nadel et al., 2000; Nadel & Moscovitch, 1997). Once the information is permanently stored, it can still be accessed without the requirement for MTL involvement.

The major MTL structures critical for memory include the hippocampus proper, the dentate gyrus, the subicular complex, and the entorhinal cortex. Together these form the hippocampal formation. In addition, the adjacent parahippocampal and perirhinal cortices form critical parts of the MTL memory system (Milner, Squire, and Kandel, 1998). The association cortices of the brain, which process perceptual information, project to the parahippocampal and perirhinal regions. These regions, in turn, project to the entorhinal cortex. The entorhinal cortex projects to the dentate gyrus and to the hippocampus (regions *Cornu Ammonis* (CA) 1 and CA3) via the perforant pathway. The

dentate gyrus projects to a subregion of the hippocampus, CA3, via the mossy fiber pathway. Region CA3 projects to region CA1 through the Schaeffer collateral pathway. Region CA1 projects to the subiculum, which then projects back to the entorhinal cortex, which itself projects back to the association cortices and the neocortex itself. Figure 1 depicts the connectivity within the MTL and between the MTL structures and the major sensory and association areas of the brain.

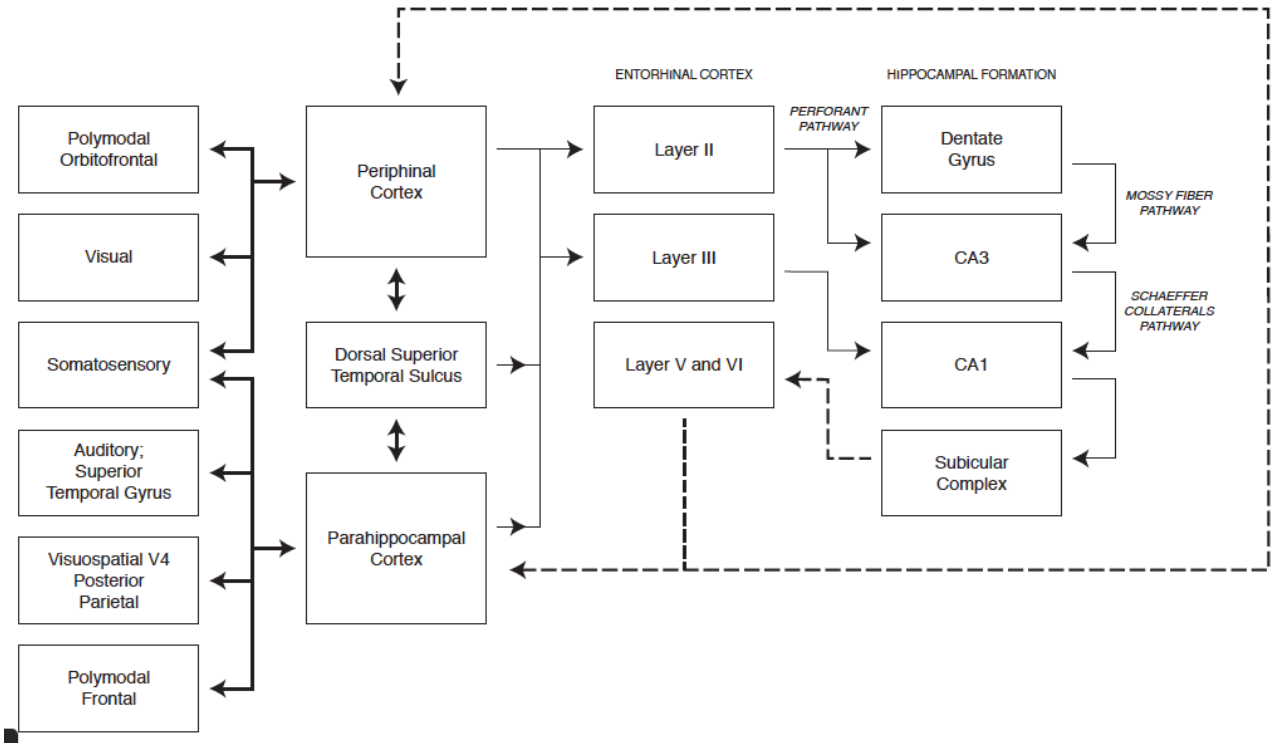

Figure 1. ***Hippocampal connectivity with the cortex, and between the entorhinal cortex and the hippocampal formation.*** The hippocampal formation and the cortex are largely reciprocally connected; the connectivity is via the parahippocampal cortex, the perirhinal cortex, and the entorhinal cortex. Entorhinal cortex layers II and III receive inputs from the rest of the cortex. Outputs from the entorhinal cortex to the rest of the cortex are via layers V and VI. The entorhinal cortex projects to CA3 and the dentate gyrus (the perforant pathway) and to CA1; the dentate gyrus projects to CA3 (the mossy fiber pathway), and CA3 projects to CA1 (the Shaeffer collateral pathway).

The specialised sensory cortices are also connected to the perirhinal and parahippocampal cortices. Thus, the hippocampus can be seen as a convergence zone for sensory information via connections with the unimodal sensory cortices and the polymodal association cortices (Suzuki, 1996).

In the CA1 region of the hippocampus, the pyramidal neurons do not target other pyramidal neurons in the same area. These pyramidal neurons are the only neurons in the hippocampus that target the neocortex either directly or via the subiculum. However, in area CA3, the pyramidal neurons target large numbers of other pyramidal neurons in the same area (Insausti & Amaral, 2004). The different inputs into area CA3 target different regions of the neurons in region CA3. The proximal parts of the apical dendrites of CA3 pyramidal neurons are targeted by the dentate gyrus, the distal parts by the entorhinal cortex; nearby CA3 pyramidal neurons tend to target the central or proximal parts, and more distant CA3 pyramidal neurons tend to target the basal dendrites (Amaral & Lavenex, 2006). The pyramidal neurons in the CA1 region receive inputs from the CA3 pyramidal neurons on the proximal parts of the apical dendrites and on the basal dendrites, and receive inputs from the entorhinal cortex on the distal parts of their apical dendrites (Amaral & Witter, 1989). Thus, with this pattern of connections, the integration of information within the hippocampus proper can occur separately prior to the overall integration of the information.

The Neural Correlates of Memory 2: Molecular Biology

In the late 19th century, Santiago Ramón y Cajal suggested that learning new information does not lead to the growth of new nerve cells. Rather, existing nerve cells grow more branches and strengthen their connections, so as to be able to communicate more effectively (Cajal, 1894). This idea—that the strength of the connections of the synapses between cells change with learning and memory—was refined by Donald Hebb in 1949, who proposed that the strength of the synaptic

connections that is the basis of learning and memory results from presynaptic and postsynaptic neural activity (Hebb, 1949). In the 1970s a variety of studies on non-declarative memory (mostly reflex actions) in invertebrates with relatively simple nervous systems provided the first direct evidence of Cajal's suggestions: the synaptic connections between the neurons involved in a behaviour are not fixed—the connections become modified by learning. These modifications can persist, and thus may serve as the components for memory storage (e.g., Castellucci et al., 1970, 1978; Zucker, 1971; Castellucci & Kandel, 1974). Another major discovery in the 1970s provided a mechanism by which synapses increase the strength of their connections. Bliss and Lømo discovered that synaptic connections within the hippocampus could undergo what is now called long-term potentiation (LTP). Long-term potentiation is a kind of increase in the strength of synaptic connections that can last for days or weeks—the very kind that would be required for memory storage (Bliss & Lømo, 1973). Thus, in the very region of the brain that had been shown to be crucial for the formation of declarative memories, neurons show the degree of plasticity required for learning and memory processes. Learning and the storage of information induces LTP in hippocampal neurons (Pastalkova, Serrano, Pinkhasova, Wallace, Fenton, & Sacktor, 2006; Whitlock, Heynen, Shuler, & Bear, 2006), indicating that these long-term changes in the strength of synapses are indeed the basis for memory.

There are different types of LTP. One major type of LTP is α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-receptor LTP, in which the AMPA receptors in the postsynaptic neuron are modulated. AMPA receptors respond to glutamate as the primary neurotransmitter. In AMPA-receptor LTP, the ion flow through the receptor can be modified, the number of AMPA receptors present can change, and the synapse can be physically enlarged (Bi & Poo, 1998). The change in the number of AMPA receptors can be via the movement of nearby AMPA receptors to the area, or the release of new AMPA receptors from inside the dendrite to the target area (Patterson,

Szatmari, & Yasuda, 2010). The creation of new AMPA receptors in the postsynaptic neuron means that there will be a greater depolarisation in response to future incoming action potentials, and thus a higher probability of producing an action potential in the postsynaptic neuron; that is, an increase in the strength of the synaptic connection between the neurons. The creation of new AMPA receptors requires the influx of calcium ions through N-methyl-D-aspartate (NMDA) receptors (Ascher & Nowak, 1988; MacDermott et al., 1986), discussed below.

There are also different timescales associated with AMPA-receptor LTP. The initial changes in the strength of synapses is called LTP1. LTP1 is replaced by LTP2, which is in turn replaced by LTP3. Without all three of these LTP types occurring, the synapse will revert back to its previous state, prior to the initial increase in synaptic strength (Raymond, 2007; Reymann & Frey, 2007).

In the perforant pathway and the Schaeffer collateral pathway, LTP is NMDA-receptor dependant, and inherently associative in the Hebbian sense, requiring the coincident activity of both presynaptic and postsynaptic neurons. NMDA receptors, like AMPA receptors, are glutamate receptors. NMDA receptors are blocked by a bound magnesium ion at the resting potential; the magnesium is released and the receptors unblocked when the neuronal membrane is depolarised in the area near the receptor. This property makes NMDA receptors coincidence-detecting in the Hebbian sense: the presynaptic firing of a neuron and the subsequent release of glutamate, binding to the receptors of the postsynaptic cell, and the depolarisation of the postsynaptic neuron, must occur almost simultaneously (Bliss & Collingridge, 1993). Thus, NMDA-receptor LTP is the mechanism for LTP in Hebb's model. For NMDA-receptor LTP, there are only two requirements: synaptic stimulation and postsynaptic depolarisation… exactly as postulated by Hebb in 1949.

Since the NMDA receptors of the postsynaptic neuron are located in the dendritic tree, the action potential generated by the postsynaptic neuron must be backpropagated. The backpropagation of action potentials is a way of communicating the information that an action potential has been generated. The magnesium ion blocking the NMDA receptor will only be released if the nearby membrane area is depolarised from a backpropagating action potential. This backpropagation is most likely to arrive at dendrites where there is already some depolarisation, as a result of recent synaptic activity from presynaptic neurons targeting that area (Stuart & Hausser, 2001). The release of the magnesium ion blocking the NMDA receptor, and the subsequent influx of calcium ions, can lead to the increase in the number of AMPA receptors, and thus a change in the strength of the synaptic connection.

Thus, the strength of the synaptic connection between neurons will increase if an incoming action potential is followed by an action potential in the postsynaptic neuron, if that action potential is backpropagated back to the synapse (Bi & Poo, 1998). The relative timing of the action potentials generated by the presynaptic and postsynaptic neurons also influences whether, and by what degree, the synaptic strength is changed: in most pyramidal neurons, LTP only occurs if a postsynaptic action potential is generated within 20ms of the incoming presynaptic action potential (Bi & Poo, 1998).

As mentioned earlier, in area CA3 of the hippocampus, the pyramidal cells receive input from the dentate gyrus, the entorhinal cortex, and from other CA3 pyramidal neurons. The number of connections between CA3 pyramidal neurons greatly exceeds the other types of connections (Ishizuka, Weber, & Amaral, 1990), which had led researchers to believe that these connections between CA3 pyramidal neurons serve as the basis of associative memory. The connections between CA3 pyramidal neurons can also be strengthened via NMDA-receptor-dependant LTP

(Berger & Yeckel, 1991; Zalutsky & Nicoll, 1990; Williams & Johnston, 1988; Harris & Cotman, 1986).

As a mechanism for the storage of learning and memory, LTP has several features: it has been demonstrated in the perforant pathway, the mossy fiber pathway, and the Schaeffer collateral pathway; it has stability over periods of hours to weeks; and it can be induced quickly.

In addition to these changes seen in synaptic neurotransmission in response to learning, the second of Cajal's suppositions was that learning causes neurons to increase the number of branches, and thus connections, with other neurons, which would also yield an increase in the ability to communicate with each other (Cajal, 1894). The increase in the number of connections, or the reorganisation of existing connections—a type of structural plasticity—has also been found to be linked with learning and memory (Moser, 1999; Bailey & Kandel, 1993). Given that an increase in the number of excitatory neural connections in the hippocampus leads to an increase in the amount of excitatory activity (Andersen et al., 1966; Harris & Kater, 1994), it is thought that these types of structural plasticity are also important for the formation of memories. Studies have demonstrated that the density of dendritic spines—small protrusions of the dendrites which contain the majority of excitatory synapses—increases during associative memory, and that the process is NDMA-receptor dependant (Leuner, Falduto, & Shors, 2003). Other findings (e.g., Wenzel et al., 1980; Van Reempts et al., 1992; Stewart & Rusakov, 1995; Ramirez-Amaya et al., 1999; Klein et al., 2002; Desmond & Levy, 1986; Geinisman, 2000; Yuste & Bonhoeffer, 2001) also indicate that dendritic spine density accompanies associative memory formation. Moreover, the influx of calcium through the NMDA receptors of the postsynaptic cells activates calcium-calmodulin-dependant-kinase II (CaMKII). This can increase the size of the dendritic spine by enlarging the cytoskeleton (Bosch et

al., 2014; Herring & Nicall, 2016b; Patterson & Yasude, 2011), again yielding improved communication with the presynaptic neurons.

These principles of neural plasticity within the MTL provide the molecular mechanism for learning and the formation of new memories. However, the system would be faulty if it was not regulated such that unrelated inputs were not erroneously bound together, while ensuring that related inputs were bound together. The oscillating activity of neurons is a candidate mechanism to ensure that the system operates as required.

Basic Principles of Brain Oscillations

The generation of action potentials is the principal means by which neurons communicate with each other. The resting membrane potential of a neuron is -70 mV. If the membrane is depolarised to around -55 mV, then voltage-gated sodium ion channels open on the membrane in the immediate area. The influx of these positively charged ions further change the polarisation of the neuron. Once the membrane area is depolarised to around +30 mV, potassium channels open. This is the trigger to close the sodium channels. The positively charged sodium ions diffuse into the interior of the neuron, restoring the negative -70 mV resting membrane potential. This collapse in the resting membrane potential is very brief, lasting only around a single millisecond. The restoration of the resting membrane potential takes only a couple of milliseconds. The diffusion of the positively charged sodium ions away from the site of the collapse means that the resting membrane potential in adjacent areas of the axon will also depolarise, triggering the opening of more sodium channels, and thus propagating the collapse along the length of the axon, and to all targets of that axon (Kole, Ilschner, Kampa, Williams, Ruben, & Stuart, 2008).

While action potentials are similar along the axons of the different types of neurons, the frequency with which they can occur can differ. The opening of the potassium channel, which triggers the closing of the sodium channels and leads to the restoration of the -70 mV resting membrane potential, can last between 1-10 ms. During this time, the hyperpolarisation of the membrane makes it much more difficult for the neuron to generate another action potential. The hyperpolarisation of the membrane can also be reinforced if there are positively charged calcium channels in the neuron (Sah, 1996), and can last as long as a few hundred milliseconds. Also, after the generation of several action potentials over a short period of time, different calcium-gated potassium channels can open, leading to a longer-lasting hyperpolarisation (Faber & Sah, 2003). These mechanisms regulate the frequency with which a neuron can generate action potentials.

Interneurons can also inhibit the activity of the pyramidal neurons to which they connect, and can modulate the frequency of the activity of their target pyramidal neurons. The oscillatory patterns of pyramidal neural activity is imposed by the interneurons that activate at the appropriate phase of local neural activity (e.g., Somogyi & Klausberger, 2005). In the hippocampus, for example, axoaxonic interneurons and basket cell interneurons are active at the gamma frequency, with axoaxonic interneurons preferentially firing at the peak of theta frequency oscillations, and basket cell interneurons firing in the descending phase of the theta oscillations (Klausberger, Magill, Marton, Roberts, Cobden, Buzsaki, & Somogyi, 2003).

Populations of pyramidal neurons show temporal ordering in their activity, which can be observed using, for example, local field potential (LFP) or electroencephalogram (EEG) recordings. The summed action potentials of large numbers of individual neurons—themselves the fastest oscillation in the brain—shows that the activity of neurons synchronise at a variety of frequencies. Without such synchronisation, random neural firing would not show the periodic and relatively

large amplitude voltage changes that has been observed. Examples of frequencies of neural oscillations that are important for memory processes in the hippocampus are gamma (30-100 Hz) and theta (4-10 Hz) frequencies (Bragin, Jando, Nadasdy, Hetke, Wise, & Buzsaki, 1995). The activity of pyramidal neurons preferentially occurs at the peaks of these frequencies during learning and memory processes, with activity at its maximum when the peaks of both of the frequencies co-occur (Soltesz & Deschenes, 1993).

Neurons that are close together have relatively shorter conduction times and stronger connections than more distant neurons. Thus, fast oscillations (e.g., in the gamma range) are often found in relatively small neural networks. Slower oscillations, such as those in the theta range, tend to occur between more distal brain regions, synchronising neural activity across longer distances. Thus, there is an inverse relationship between the frequency of the oscillations and its spatial scale. Also, given that the coincident activity of populations of neurons, leading to the oscillations observable using LFP and EEG recordings, necessarily happens less frequently than the firing of neurons in general, the frequencies of neural oscillations follow a characteristic 1/f pattern, which is often seen in nature. In the 1/f distribution, events that occur more frequently have less magnitude (or power) than events that occur less frequently. In EEG recordings, we see that lower frequency oscillations have a much higher amplitude than high frequency, fast oscillations, and the power of the observed oscillations with respect to their frequency follows the 1/f distribution.

The phase of the local oscillations influence the excitability of neurons in the local area, which increases the likelihood of firing of neurons in the area. Thus, the timing of the firing of neurons is influenced by the phase of local oscillations (Elbert et al., 1987; Fröhlich et al., 2010). In particular, output action potentials tend to occur in the depolarised state of the local field potential (Fries, 2005). Also, as mentioned earlier, the likelihood of postsynaptic firing in increased if there is

temporal coincidence in presynaptic inputs to the neuron (Salinas & Sejnowski, 2001; Köing et al., 1996; Singer 1999; Fries 2005). So, oscillations influence both the tendency of neurons to generate an output action potential and increase the sensitivity of neurons in the area to inputs from neurons that target them. Therefore, mechanisms that serve to synchronise neural activity will amplify the processing of information (Nyhus & Curran, 2010). Moreover, given the effect of oscillations on neural firing, optimal communication between different brain areas can be achieved by aligning their oscillatory phases. Such phase alignment would promote information transmission between these regions. During the phase of local depolarisation, neurons in the target area will be more likely to generate an action potential if an input is received. In the area that provides the input to the target area, the depolarised phase is a period where firing to communicate with the target area is more likely. Thus, the aligned phases of different regions leads to oscillating periods of optimal communication between those regions. The alignment of phases across regions of communication means that, as neurons in the input area fire, neurons in the target area are more likely to fire as well (Buzsaki & Draguhn, 2004; Salinas & Sejnowski, 2001; Köing et al., 1996; Singer 1999; Fries 2005; Womelsdorf et al., 2007). The role of phase synchronisation, then, is not limited to facilitating information transfer within an area of the brain, but also across more distant regions (Engel et al., 1992; Singer & Gray, 1995; Engel et al., 2001; Hermann et al., 2004; Fries, 2005; Jensen et al., 2007), such as for the integration of sensory information from different sensory cortices (Engel et al., 2012; Salinas & Sejnowski, 2001; Womerldorf et al., 2007).

As mentioned earlier, the coincident firing of presynaptic and postsynaptic neurons is critical for the induction of LTP. If phase alignment is achieved between the areas of the presynaptic and postsynaptic neurons, the timing of the receipt of the input and the generation of the postsynaptic action potential will facilitate the induction of LTP (Axmacher et al. 2006; Jutras & Buffalo, 2010; Holscher et al., 1997; Huerta & Lisman, 1995; Pavlides et al., 1988). From the perspective of

associative memory formation, with information arriving to hippocampal targets from multiple sensory modalities, if the inputs from the different modalities are received at the same time, they would have a greater impact on target neurons than desynchronised inputs, or indeed than the sum of inputs from the individual sensory modalities separately, and would thus be more likely to induce LTP, facilitating the generation of an association in memory.

Theta Oscillations in the Human Medial Temporal Lobe

Walter and Dovey first proposed that the term 'theta' be used to describe oscillations in the 4-7 Hz range in 1944 (Walter & Dovey, 1944), since others (e.g., Kennard & Nims, 1942; Kennard, 1943) found that lesions of the thalamus in monkeys changed the predominant rhythms of post-central areas to theta-range rhythms, and that "lesions interrupting transmission of centripetal impulses through the thalamus would tend to augment the 4-6 [cycles per second] activity, much as closing the eyes augments the occipital alpha rhythm." (Walter & Dovey, 1944).

There are a number of different interneurons in the hippocampus proper, such as OLM interneurons, which support theta oscillations, and basket cell interneurons, which support gamma oscillations (Kullmann, 2011). The gamma band activity is, in turn, modulated by the theta oscillations (Fischer, Wittner, Freund, & Gähwiler, 2002). Other subcortical structures exhibit strong connectivity with the hippocampus, including the basal forebrain septal nuclei. These nuclei project to hippocampal areas CA1 and CA3 and to the dentate gyrus (Colom, 2006). Hippocampal areas CA1 and CA3 in turn project to the septal nuclei (Siegel, Edinger, & Ohgami, 1974). The septal areas influence theta oscillations in the hippocampus in three different ways: glutamatergic connections exert influence on the frequency of activity; cholinergic connections on the magnitude of the oscillations, and GABAergic connections inhibit interneurons, thus influencing the excitability of hippocampal

pyramidal neurons and promoting theta-frequency activity (Niewiadomska, Baksalerska-Pazera, & Riedel, 2009). While the septal nuclei may generate theta oscillatory activity in the hippocampus, it has been shown that, once generated, the theta oscillations are maintained by interneurons in the hippocampus itself (Fischer, Wittner, Freund, & Gähwiler, 2002). However, since interrupting the inputs from the medial septum to the hippocampus drastically disrupts theta-frequency generation in the hippocampus itself, and the coherence between areas on the hippocampus, it is clear that the septum has a strong influence as an external pacemaker of the hippocampal theta rhythm (Yoder & Pang, 2005).

Evidence from rodent studies demonstrates that within the MTL there are several theta rhythms (Mizuseki, Sirota, Pastalkova, & Buzsaki, 2009; Montgomery, Sirota, & Buzsaki, 2008; Montgomery, Betancur, & Buzsaki, 2009). Theta oscillations recorded in the layer I of the entorhinal cortex has an inverse phase to the oscillation recorded in layers II and III. It is layers II and III that project to the hippocampus (as shown in Figure 1); in area CA1 of the hippocampus, there is inverse polarity of the theta oscillation between the stratum lacunosum-moleculare layer and the stratum pyramidale and stratum radiatum layers. (Hafting, Fyhn, Bonnevie, Moser, & Moser, 2008; Quilichini, Sirota, & Buzsaki, 2010; Deshmukh, Yoganarasimah, Voicu, & Knierim, 2010). To complicate matters further, neurons in different areas tend to fire preferentially at different phases of a reference theta cycle (Mizuseki, Sirota, Pastalkova, & Buzsaki, 2009), and different types of neurons in the same area (e.g., pyramidal and interneurons) tend to fire preferentially at different phases. Even among interneurons, different classes tend to fire preferentially at different phases (Klausberger & Somogyi, 2008; Klausberger, Magill, Marton, et al., 2003; Somogyi & Klausberger, 2005; Klausberger, Marton, Baude, Roberts, Magill, & Somogyi, 2004; Halasy, Buhl, Lorinczi, Tamas, & Somogyi, 1996; Czurko, Huxter, Li, Hangya, & Muller, 2011).

Various models have been proposed that attempt to handle the dual role of the hippocampus in encoding and retrieving memories, while also accounting for the different oscillations (and phases) of the theta rhythm found within the MTL, but each has several serious drawbacks (e.g., the Hasselmo model (Hasselmo, Bodelon, & Wyble, 2002; Hasselmo, 2005; Hasselmo & Eichenbaum, 2005); the acetylcholine hypothesis (Hasselmo, Schnell, & Barkai, 1995; Hasselmo, Wyble, & Wallenstein, 1996; Hasselmo & Schnell, 1994); the "detonator" model (Marr, 1971; McNaughton & Morris, 1987; Treves & Rolls, 1992; Cerasti & Treves, 2010; Leutgeb, Leutgeb, Moser, & Moser, 2007; Henze, Wittner, & Buzsaki, 2002)). A detailed treatment of such models is well beyond the scope of the present manuscript.

Despite the variety of models that try to provide an account for the role of theta oscillations in the MTL, the functions that the models try to account for remain the same. Theta oscillations play a variety of roles in the hippocampus: they have been related to modulating synaptic plasticity in the hippocampus (Buzsaki 2002; Buzsaki 2005; Dragoi & Buzsaki 2006; Dragoi et al., 2003; Sitota et al., 2008; Montgomery et al., 2008; Tort et al., 2008; Mizuseki et al., 2009); the induction of LTP in the hippocampus (Greenstein et a, 1998; Larson & Lynch, 1986; Larson et al., 1986; Rose & Dunwiddle, 1986); memory formation in animals (e.g., Lega et al., 2010); and opening the door for the binding of information from different regions of the brain during memory formation and retrieval (Vertes, 2005; Vertes et al., 2001; Hasselmo 2007; Hyman et al., 2005). Studies have shown that electrical stimulation at the theta frequency is optimal for the induction of LTP in the dentate gyrus and CA1 neurons in the hippocampus (Greenstein et al, 1998; Larson & Lynch, 1986; Larson et al., 1986; Rose & Dunwiddle, 1986). Moreover, this LTP is limited to the positive peak of the theta phase (the depolarised state; Pavlides et al., 1988; Holscher et al., 1997; Huerta & Lisman, 1995; Hyman et al., 2003). Theta plays a variety of other roles in animals, potentially including

movement (Bland and Oddie, 2001; Kelemen et al., 2005; Lenck-Santini et al., 2008; Shin, 2011; Vanderwolf, 1969; Whishaw and Vanderwolf, 1973), velocity (Hinman et al., 2011, 2013; Jeewajee et al., 2008; Maurer et al., 2005; Rivas et al., 1996; Whishaw and Vanderwolf, 1973), attention (Green and Arduini, 1954; Sainsbury et al., 1987), and location (O'Keefe, 1976; O'Keefe and Dostrovsky, 1971; Skaggs et al., 1996), in addition to learning and memory. Again, a detailed treatment of these many roles of theta oscillations in animals is beyond the scope of this manuscript.

Despite arguments for and against the various models that attempt to account for theta oscillations in the MTL, it remains clear that theta oscillations in the medial temporal lobes are optimal for the induction of synaptic plasticity, and thus learning and memory, and for the provision of windows of communication between different brain areas, defining times when long-range information transfer is likely to succeed. Theta oscillations in the 3-4 Hz range appear to mirror the memory-formation-relevant theta oscillations widely studied in animals (Lega et al., 2012; Jacobs 2013).

The Entrainment of Brain Oscillations

Entraining brain oscillations requires that the neural oscillations in the brain align themselves to an external input. That is, the phase of the oscillations in the brain synchronise with the oscillations of the external driver. Both visual and auditory inputs can be used to entrain the visual and auditory sensory cortices, respectively. Photoic driving of the visual system by the presentation of rhythmically flickering visual stimuli can entrain neural activity in the visual cortex at frequencies up to 100 Hz (Adrian & Matthews, 1934; Regan, 1966; Herrmann, 2001). Entraining visual responses is typically done using luminance modulations of visual stimuli or high-contrast reversals of visual stimuli, such as visual checkerboards. Driving of the auditory system can be achieved in a similar way: frequency modulation or amplitude modulation of a presented auditory stimulus can

drive neural oscillations in the auditory cortex at the modulation frequency (Geisler, 1960; Picton, John, Dimitrijevic, & Purcell, 2003).

The entrainment of brain oscillations can be used to take advantage of endogenous oscillations of particular interest. That is, if oscillatory activity at a frequency potentially relevant for cognitive phenomena, such as perception or memory, can be entrained, then that entrainment may impact the cognitive phenomenon in question. Alternatively, the presentation of stimuli can be matched to the phase and frequency of the endogenous oscillation of the relevant behaviour (Schroeder & Lakatos, 2009; Schroeder et al., 2008; Luo et al., 2010; Luo & Poeppel, 2007). Moreover, stimuli from different sensory modalities can be used to entrain the respective cortical sensory areas simultaneously (e.g., de Jong et al., 2010; Saupe et al., 2009; Nozaradan, Peretz, & Mouraux, 2012; Jenkens et al., 2014).

EEG can be used to determine the degree of success in entraining populations of neurons. Successful entrainment is characterised by a number of factors, such as the alignment of the phase of the endogenous oscillation to that of the entraining stimuli, along with an increase in the amplitude of the endogenous oscillation, indicating that an increased number of neurons in the population have been entrained to the phase of the external stimuli (Thut, Schyns, & Gross, 2011).

Overview of the Present Work

Episodic memories are associative: the events in our lives are made up of information from a variety of senses. Theta oscillations play a critical role in facilitating the transfer of information across regions in the brain, as well as influencing the formation of memories in the medial temporal lobes. While theta oscillations in the hippocampus have been shown to be critical for associative

memory formation in animals, there is a notable lack of evidence for the role of theta oscillations in human memory formation.

In the present work, we directly address this issue. By entraining oscillations in the brain, we can generate evidence regarding the behavioural role those oscillations play, and thus improve our understanding of the effect those oscillations have on the behaviour in question. Chapter 2 provides the general methods used throughout our series of experiments. Chapter 3 addresses the question of whether the synchronisation of sensory inputs across different sensory modalities impacts the ability to form associative memories. In Chapter 4, we investigate whether these effects are specific to the theta frequency, or more general effects of the synchronisation of sensory inputs *per se*. In Chapter 5, we use EEG to determine whether the sensory cortices targeted by our to-be-remembered stimuli do indeed entrain to the external rhythm in which the stimuli were presented. Chapter 6 goes a step further to investigate the effects of our manipulations on a single-trial basis. We conclude that memories for associations between visual and auditory stimuli are improved if the stimuli are presented in a fashion such that the visual and auditory cortices are entrained in-phase with respect to each other, that this improvement is specific to the theta frequency, and that this improvement can be shown at the level of a single memory formation trial.

Chapter 2: General Methods

Apparatus

MATLAB (R2013a; The Mathworks, Inc., Natick, MA, USA) and the Psychophysics Toolbox extensions (Brainard, 1997; Pelli, 1997; Kleiner, Brainard, Pelli, Ingling, Murray, & Broussard, 2007) were used to program the tasks. Visual stimuli were displayed using a 21-inch CRT computer display with a 75 Hz screen refresh rate, using a nVidia Quadro K600 graphics card (875 MHz graphics clock, 1024 MB dedicated graphics memory; Nvidia, Santa Clara, CA, USA). Sounds were presented using Sennheiser HD 201 headphones (Sennheiser Electronic GmbH & Co. KG, Wedemark, Germany), through a Sound Blaster Audigy 5/Rx audio card (Creative Technology Ltd., Singapore). Additional software (ASIO4All; 2.12; Steinberg Media Technologies GmbH) was used to further control the presentation timing of the auditory stimuli. The phase offsets between the presentation of the visual and auditory stimuli were verified using a Pico ADC-212 oscilloscope (picotech.com) with a ThorLabs DET36A photodetector (thorlabs.de), using PicoScope for Windows (5.08.6) software. A Windows 7-based PC (3.40 GHz processor, 16 Gb RAM) with a standard QWERTY keyboard and a solid-state drive was used to run the tasks.

Visual Stimuli

The visual stimuli were 96 movie clips, each containing 76 frames displayed at 25 frames per second; thus the moves were of a 3-second duration. The movie clips contained emotionally neutral

human activity or activity in nature, such as vehicles on a road, a turtle moving on a beach, or an animal foraging for food.

In all experiments, the luminance of the movies were modulated using a constructed sine wave, such that the movies flickered from no luminance to full luminance. The details of the luminance modulation differs between the experiments, and will be described in the following chapters. In all experiments, the movies began their presentation at 50% of full luminance, and initially increased in luminance to 100% of full luminance.

Auditory Stimuli

The auditory stimuli were 96 sound clips. Sounds from four different instrument categories were used, including acoustic guitar, electric guitar, synthesiser, and an orchestra. The sound clips were obtained from the Apple Loops for Garage Band (6.0.5) and iLife Sound Effects sound libraries, which are freely available on Apple computers. Additional sound clips were obtained from movie soundtracks. In all cases, the sound clips chosen contained a minimal amount of rhythmic beats and amplitude modulations.

The sound clips were trimmed such that they were all of a 3-second duration. Trimming and preprocessing was completed using Audacity software (2.1.1; audacityteam.org). Preprocessing included applying a 100ms linear amplitude fade-in, a 100ms amplitude fade-out, and normalisation to 0.0 dB maximum amplitude.

In all experiments, the amplitude of the sound clips were modulated using a constructed sine wave, such that the amplitude flickered from no amplitude to full amplitude. The details of the amplitude

modulation differs between the experiments, and will be described in the following chapters. The initial phase of the presentation of the sound clip, with respect to the movie, differed within and between each experiment, and will be described in the following chapters.

Procedure

In all experiments, the completion of a safety screening questionnaire and the provision of informed consent preceded the completion of the tasks. Participants were seated 60 cm from the centre of the computer screen. The experimenter familiarised each participant with the task by walking them through a block of practice trials. Once familiar with the experiment, participants were separated from the experimenter for the completion of the tasks.

The task for associative episodic memory contained six blocks. Each block consisted of three phases: an encoding phase, a distracting phase, and the associative memory test phase, as depicted in Figure 2. The order in which the six blocks were presented was balanced across participants, so that each block was presented first, second, third, etc., an equal number of times across participants in the experiments.

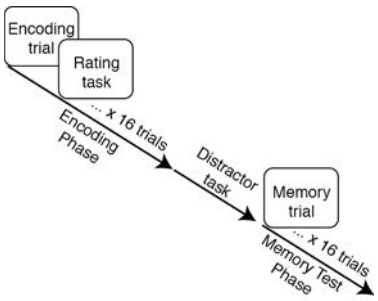

Figure 2. ***Depiction of the experimental procedure.*** After each trial in the encoding phase participants rated how well the sound suited the content of the movie. After a block of 16 trials,

participants completed a distractor task lasting 30 s, followed by an associative memory test for the 16 sound movie associations presented in the encoding phase.

Encoding

The encoding phase of each block contained sixteen movies and sounds. On each trial, participants watched one of the sixteen movies. One of sixteen sound clips was played along with each of the movies. The sound clips were likened to the soundtrack to a movie. Following the presentation of each movie/sound clip pairing, participants made a subjective judgement as to how well the sound clip suited the content of the movie using the number keys 1 (the sound does not suit the content of the movie at all) through 5 (the sound suits the content of the movie very well) on the keyboard. The following guide was used in the task instructions to assist participants with the task:

> "Suppose the movie is of a gorilla sitting in some grass. The sun is up, and the gorilla is scratching itself and looking very content. Now suppose that the sound is some nice, soft, orchestral music… you can almost picture David Attenborough narrating. In that case, you might say that the sound suits the content of the movie. On the other hand, if the sound was a distorted electric guitar, playing a wild solo, using a whammy bar and sounding crazy, then you might say that the sound does not suit the content of the movie very well."

Within each block, participants were instructed to remember which sound was presented with each movie—that is, to remember the association between the sounds and movies, as memory for those associations was to be tested later.

Distractor

Following the presentation of the final sound/movie pairing in the encoding phase, the distractor phase began. In this phase, participants were presented with a random number on the computer screen, and asked to count backwards, out loud, first by subtracting three from the number presented, and then subtracting three from the resultant number. This task lasted 30 seconds.

Recall

Following the distractor phase, memory for the associations between the sounds and videos presented during the encoding phase was tested. Each trial began with the presentation of four still images from four of the movies that were shown during the encoding phase of the same block. One sound that was presented with one of the four movies represented by the still images was played. Participants were instructed to select the movie that was playing while that sound was played in the encoding phase, using the number keys 1-4 on the keyboard. The presentation on the computer screen is depicted in Figure 3.

Figure 3. ***Depiction of the associative rating task.*** A sound is played from one of four instrument categories (represented by the waveforms in blue near the bottom of the image), and still images

from four of the movies that were seen while a sound in the same instrument category was playing during the encoding phase are shown. Participants chose which movie was paired with the particular sound in the encoding phase.

Chapter 3: Associative Memory for Synchronously and Asynchronously Presented Multimodal Stimuli

Chapter Note: The experiment presented in this chapter has previously been published (Clouter, Shapiro, & Hanslmayr, 2017). While I was also the primary author on the previously published manuscript, I have endeavoured to ensure throughout this chapter a minimum of self-plagiarism. However, some of the figures and figure legends demonstrating the results are exactly as those that appear in Clouter, Shapiro, & Hanslmayr, 2017.

Introduction

Oscillations in the brain can be likened to the conductor of an orchestra: the conductor can exert control over the timing and magnitude of activity in the orchestra. Oscillations in the brain can play the same role, influencing the timing of activity of neurons in the region. We have described how this temporal orchestration can facilitate communication between distant regions of the brain. We have also described the molecular mechanisms for memory, and the reliance of such mechanisms on the precise timing of the activity of neurons in the relevant areas. Thus, the role of oscillations in influencing the timing of neural activity is also critical in facilitating the precisely timed neural activity required for LTP—a molecular mechanism for learning and memory.

Theta oscillations are prominent in the hippocampus, a region critical for the successful formation of episodic memories (Aggleton & Brown, 1999). Given the requirement of a hippocampus for successful associative memory formation, and the prominence of theta oscillations in the hippocampus, it has been thought that theta oscillations in the hippocampus play a causal role in the formation of associative memories (Pavlides, Greenstein, Grudman, & Wilson, 1988; Winson,

1978). In animals, it has been shown that disrupting the theta oscillations in the hippocampus leads to deficits in the formation of memory, and that the magnitude of the oscillations in the hippocampus is correlated with associative memory (McNaughton, Ruan, & Woodworth, 2006; Berry & Thompson, 1978; Griffin, Asaka, Darling, & Berry, 2004). In humans, studies have shown a correlation between theta power and the formation of associative memories (Klimesch, Doppelmayr, Russegger, & Pachinger, 1996; Staudigl & Hanslmayr, 2013). However, evidence for a causal link between theta oscillations and the successful formation of multimodal associative memories is lacking.

We sought to provide this missing evidence by demonstrating that associative memory formation in humans is dependent on the degree of phase synchrony between the different sensory cortices involved, and that the dependence is specific to the theta frequency. We did so by modulating the luminance of visual stimuli (movies) and the amplitude of sounds using a 4 Hz (theta-frequency) sine wave. Our critical experimental manipulation was the timing of the presentation of these stimuli: on some trials, the sounds and movies were presented such that entrained neural activity in the auditory and visual sensory cortices were in-phase with respect to each other (synchronous), while on other trials they were out-of-phase (asynchronous)[1]. Given that the information processing pathways from the different sensory cortices converge in the hippocampus, our hypothesis was that the timing of activity in the sensory cortices would influence the activity of the neurons in the hippocampus (see Figure 4). Performance on a task of associative memory showed that memories were significantly better if the stimuli were presented such that neural activity was synchronous between the relevant sensory regions.

1 Phototransduction is, relatively, a much slower process than auditory transduction, taking approximately 50 ms as compared to 10 ms, respectively (Bolz, Rosner, & Wässle, 1982; Lamb & Pugh, 1992; Lennie, 1981; Rodieck & Rodieck, 1998; Schnapf, Kraft, & Baylor, 1987; Corey, & Hudspeth, 1979; King & Palmer, 1985). Thus, in order to ensure that our flickering stimuli entrained the sensory cortices in-phase or out-of-phase, according to our design, the physical onset of the movies preceded the onset of the sounds by approximately 40 ms throughout all experiments presented in this manuscript. Throughout this manuscript, when we refer to in-phase or synchronous, or out-of-phase or asynchronous, we mean the synchrony or asynchrony we aimed to achieve in the sensory cortices, and not between the physical stimuli presented to the participants.

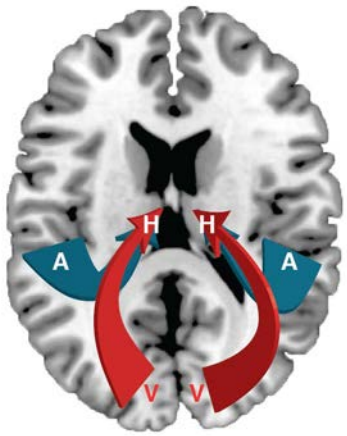

Figure 4. ***Depiction of auditory and visual cortices projecting to the hippocampus.*** Information from the auditory and visual cortices could arrive at the hippocampus at the same time, if the information is processed by the auditory and visual cortices at the same time.

Methods

The design and procedure follow the General Methods outlined in Chapter 2. Twenty-four healthy English-speaking young adults (mean age = 19.96 years, SD = 1.52; 15 female) participated in this experiment. Twenty-three participants were right-handed; one participant was left-handed. Five participants received a small stipend (£10.00), while 19 participants were granted experimental participation credit for their participation in the experiment. All data from the participants were retained for analysis.

The movies and sound clips used in the experiment were modulated using a 4 Hz (theta-frequency) sine wave, such that the luminance of the movies, and the amplitudes of the sounds, flickered from 0% to 100% luminance or amplitude, respectively, at a rate of 4 Hz. The sounds were modulated using sine waves at four different phase offsets from the sine wave used to modulate the movies: 0° phase offset (synchrony), 90°, 180°, and 270° phase offset. Of the four sounds within each

instrument category in each block, one was modulated at each of the four phase offsets with respect to the modulation of the movie. Therefore, within each block of 16 trials, there were four sounds from each of four different instrument categories, each of which contained four sounds at each phase offset. The phase offsets of the flicker of the movies with respect to the sound clips is depicted in Figure 5. During the presentation of the memory test, on all trials, each of the four movies represented by still images on the computer screen was presented with a sound from the same instrument category in the encoding phase, so that the associative memory task could not be made easier by merely remembering the *type* of sound that was associated with the movies, rather the exact sound, given that each of the four movies being represented by the still images was associated with a sound of the same type.

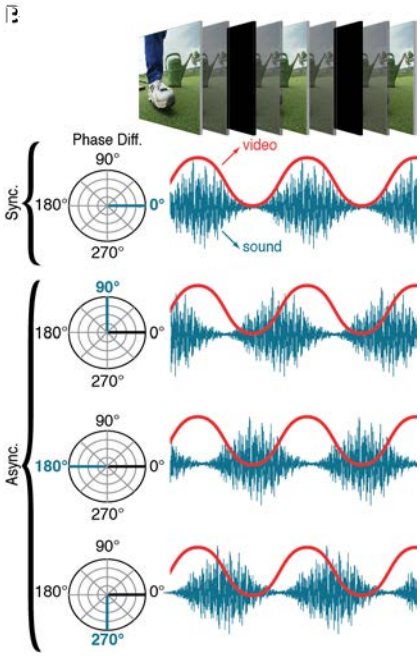

Figure 5. ***Depiction of the experimental paradigm***. Sinusoidally flickering visual (red) and auditory (blue) stimuli are presented either in synchrony or at 90-degree, 180-degree, or 270-degree phase offset.

Following completion of all six blocks of the associative memory task, participants completed an additional block of trials designed to test their ability to detect whether the flicker of the movies and sounds was synchronous or not. The instructions for this task were presented after participants had completed the memory task. This synchrony judgement task consisted of a subset of 24 of the 96 total movie-sound pairings that had been presented already in the experiment. Following the presentation of each sound-movie pair, participants made a two-alternative forced choice judgement as to whether the participant felt that the flicker of the sound and the flicker of the movie were synchronous or asynchronous, using the number keys 1 and 2.

Eighteen participants completed the task as above. For the final six participants, four additional synchrony judgement blocks were introduced to the experiment. In these four additional blocks, the sounds and movies were modulated with sine waves at different frequencies from the 4 Hz theta used in the first synchrony judgement task. 1.652 Hz, 2.4721 Hz, 6.4721 Hz, and 10.4721 Hz sine waves were used to modulate the sounds and videos in each of these four additional synchrony judgement blocks. The data collected from these blocks served as pilot data in designing the experiment described in the following chapter.

The duration of the experiment was approximately 40 minutes for the first 18 participants, and approximately 55 minutes for the final six participants.

Analysis

Effects of phase offset were tested using repeated measures analysis of variance (ANOVA) on the proportion of correct responses in the memory test trials, and followed up with pairwise *t*-tests. We recognise that our data are binomially distributed (that is, categorised by 1s and 0s, indicating that

the response on a particular trial was correct or incorrect, respectively), and that such data can violate critical assumptions of ANOVAs. The use of ANOVAs on binomially distributed data, while common, can yield difficult-to-interpret results (such as when a confidence interval extends beyond 0% or 100%), and spurious results. Therefore, in addition to ANOVA, we used generalised linear mixed models (GLMMs; Pinheiro & Bates, 2006).

Metrics of the strength of the evidence for the effects were generated using comparisons of GLMMs. These GLMMs allow the specification of the distribution of the data; thus, our binomial data could be treated properly as such using a logit regression. The approach compares two models: one that includes the variable of interest and one that does not. The results are presented as a likelihood ratio (corrected for model complexity using Akaike's information criterion; Akaike, 1974). The value of the likelihood ratio, presented in binary digits ("bits" of evidence on a log-base-2 scale), is used to determine whether the evidence favours one model over the other, or whether neither model can be preferred to the other. Strong evidence in favour of one model is evidenced by values greater than 5 (Royall, 1997).

T-tests were used with sensitivity analysis (d-prime) measures to test for a difference between the ability to detect synchronous versus asynchronous presentation of stimuli in the synchrony detection task, and for response bias (beta).

After each trial in the associative memory encoding phase, participants rated how well they thought the sound suited the content of the movie. To rule out the potential confound that, by chance, some sounds were judged to suit the content of the movies more than others, and that those pairings that were judged to be more suited to each other would be more likely to be remembered, we performed additional control analyses. Repeated measures ANOVA were performed on the suitability data to

test whether the sounds in the four different phase offset conditions were judged to suit the movies differently. We then equalised the number of trials in each phase offset condition at each of the five rating categories and performed the same repeated measures ANOVA on the memory performance data as before, to check to see whether the results would remain the same.

Results

Our repeated measures ANOVA revealed that the effect of phase offset on memory performance was significant: ANOVA: $F(3, 69) = 6.74$, $P < 0.01$. Pairwise *t*-tests showed that memory performance was better in the 0° phase offset condition than in the 90° phase offset condition: $T(1, 23) = 3.456$, $P = 0.01$, that memory performance was better in the 0° phase offset condition than in the 180° phase offset condition: $T(1, 23) = 3.915$, $P < 0.01$, and that memory performance was better in the 0° phase offset condition than in the 270° phase offset condition: $T(1, 23) = 4.281$, $P < 0.01$. Further pairwise *t*-tests showed that memory performance in the three asynchronous conditions (90°, 180°, and 270° phase offsets) were not statistically different from each other (all $P > 0.05$). Memory performance in each of the four phase offset conditions is shown in Figure 6.

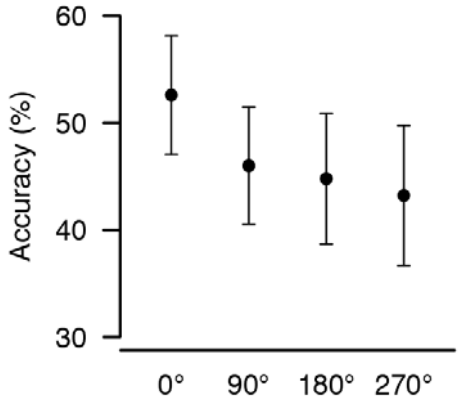

Figure 6. ***Results.*** Accuracy (%) of selecting the correct movies that were presented with particular sounds, when the movies and sounds were presented at each phase offset. Error bars are 95% confidence intervals.

Our GLMMs support the results of the ANOVA: the corrected log-likelihood ratio (9.223) suggests that there was strong evidence in our data in favour of the model that included phase offset as a variable, compared to the model that did not include phase offset. The values of the logit regression coefficients support the results of the pairwise *t*-tests: memory performance was better in the 0° phase offset condition than in the 90° phase offset condition: $Z = -2.238$, $P = 0.025$; memory performance was better in the 0° phase offset condition than in the 180° phase offset condition: $Z = -2.650$, $P < 0.01$; and memory performance was better in the 0° phase offset condition than in the 270° phase offset condition: $Z = -3.180$, $P < 0.01$.

For the analysis of d-prime and beta, one participant had a 100% hit rate. For this participant, we manually substituted one hit for a miss for the purposes of the calculations. Our analysis of discriminability (d-prime) revealed no significant effect: Null hypothesis: Mean = 0; $T(1, 23) = 1.224$, $P = 0.233$. Analysis of response bias revealed no significant effect: Null hypothesis: Mean = 1; $T(1, 23) = -0.174$, $P = 0.864$. Thus, participants could not discriminate between synchronously presented sound-movie pairs and those presented out-of-phase, so perceptual judgements of synchrony were not responsible for performance on the episodic memory task.

Repeated measures ANOVA on responses to how well the sounds suited the content of the video revealed no effect of phase offset on the ratings: $F(3, 69) = 1.970$, $P = 0.125$. Suitability ratings, then, were not influenced by the phase offset between the flicker of sound and that of the video, and

thus the suitability ratings did not contribute to the differences observed in memory performance between the phase offsets.

In order to further check whether the suitability ratings had any effect on memory performance, we equalised the numbers of trials that were rated 1, 2, 3, 4, and 5 amongst the four phase offsets. For this analysis, we removed 256 trials, leaving 2048 trials for the analysis. The repeated measures ANOVA revealed a main effect of phase offset on memory performance: $F(3, 69) = 5.155$, $P < 0.01$, which confirms our results. That is, we arrive at the same result—an improvement in memory for the sound-movie associations in the 0° phase offset condition relative to the other phase offsets, even when we equalise the number of trials at each value of the rating scale in the suitability judgements, indicating again that the suitability judgements did not contribute to the observed differences in memory performance.

Discussion

The critical role that the relative timing of events in learning and memory has been known since Pavlov demonstrated that the degree of conditioning was related to the timing of the conditioned and the to-be-conditioned stimuli (Pavlov, 1927). Our results provide direct evidence that the formation of episodic memories in humans might rely on the precise timing of inputs from the sensory cortices. Our unique behavioural manipulation—to entrain the sensory cortices that process the to-be-associated-stimuli either in-phase with each other or out-of-phase with respect to each other—allowed us to examine the role that the entrained oscillations play in associative memory. We showed that when stimuli are presented such that the sensory cortices were entrained in phase at a 4 Hz theta frequency, memory for associations is improved relative to when the stimuli were presented out-of-phase. Given that the information processing pathways from the different sensory

cortices converge in the hippocampus, our hypothesis was that the timing of activity in the sensory cortices would influence the activity of the neurons in the hippocampus. We have already discussed the critical role that the precise timing of inputs has on the activation of target neurons, and the likelihood of inducing LTP: the coincident timing of inputs to a neural target is more likely to generate a response in the target neuron, and more likely to induce LTP (Fries, 2005; Buzsaki, 2010). While the previous research has been carried out in animals, our findings provide direct evidence that the binding of multisensory information in the hippocampus in humans is facilitated by the synchronous activity of the respective sensory cortices providing the input to the hippocampus, even when the participants were unable to determine whether the stimuli were presented synchronously or asynchronously. Thus, we demonstrate a direct link between the phase of oscillatory activity and the formation of associative memories in humans.

Chapter 4: Testing the Boundary Conditions

Chapter Note: Experiments 1 and 2 presented in this chapter have previously been published (Clouter, Shapiro, & Hanslmayr, 2017). While I was also the primary author on the previously published manuscript, I have endeavoured to ensure throughout this chapter a minimum of self-plagiarism. However, some of the figures and figure legends demonstrating the results are exactly as those that appear in Clouter, Shapiro, & Hanslmayr, 2017.

Introduction

In Chapter 3 we provided evidence suggesting that the precise timing of inputs from the sensory cortices entrained at a 4 Hz theta frequency facilitates the creation of associative memories in humans. Given the hypothesised roles of theta oscillations in the hippocampus—specifically in the binding of information and the management of windows of optimal communication—entraining the sensory cortices at this frequency seemed the ideal manipulation in order to test whether the theta frequency is causally involved in these processes in humans. However, while we were able to demonstrate improved performance on our associative memory task when the flickering multisensory stimuli were flickering in-phase at 4 Hz compared to out-of-phase, we cannot claim that our effect is specific to the 4 Hz theta frequency. In order to demonstrate that the theta oscillation plays a special role in associative memory formation, we would have to demonstrate that our effects are specific to that frequency alone; i.e., that we would not find a similar effect if our stimuli were modulated with sine waves at other, non-theta, frequencies. In addition, in order to make the claim that the theta frequency is important for the formation of associative memories, it would be useful to compare our effect to a case wherein the stimuli were not flickering at all, since non-flickering stimuli would be the best situation in which to perceive and bind the stimuli

(VanRullen, Zoefel, & Ilhan, 2014). Moreover, if binding was taking place at the perceptual level, rather than at the associative-memory-formation level, than one could expect that stimuli flickering in-phase could be better bound than stimuli flickering out-of-phase, which could be argued as an alternate explanation of our results. However, if performance on the memory task was better in the theta case compared to a non-flickering baseline case, than it would be clear that theta oscillations play an important role in associative memory formation.

Anticipating the former, the final six participants in the experiment presented in Chapter 3 were presented with additional synchrony judgement tasks, as detailed in the previous chapter. We created sine waves at four frequencies in addition to the 4 Hz wave used to modulate our stimuli in Chapter 3: specifically, 1.652 Hz, 2.4721 Hz, 6.4721 Hz, and 10.4721 Hz. These frequencies were chosen such that they were one or two "golden means" or "golden ratio" (the irrational number 1.618...) above and below 4 Hz. Such frequencies do not share harmonics with 4 Hz, and thus the choice of such frequencies is critical in determining the potential frequency specificity of 4 Hz (Pletzer, Kerschbaum, & Klimesch, 2010). Given that one golden mean above and below 4 Hz would still yield a frequency within the theta band (6.4721 Hz), we opted to use frequencies two golden means above and below 4 Hz (1.652 Hz and 10.4721 Hz) to test for the frequency specificity of our effect. In order to further determine whether the theta frequency is especially important, and to rule out accounts of our results at the perceptual level, we also compared performance in the theta frequency task to a non-flickering associative memory task.

Experiment 1

Methods

The design and procedure follow the General Methods outlined in Chapter 2. An error in the program code was discovered after ten participants were tested. The data from these participants were discarded. Twenty-four new healthy English-speaking young adults (mean age = 22.92 years, SD = 4.26; 19 female) completed this experiment. All of the participants were right-handed, and all participants received a received a small stipend (£10.00) for their participation. The data from these 24 participants were were retained for analysis.

One-third of the 96 movies (i.e., 32) and sounds were modulated using a 1.652 Hz sine wave, such that the luminance of the movies, and the amplitudes of the sounds, flickered from 0% to 100% luminance or amplitude, respectively, at a rate of 1.652 Hz (in the delta range). One-third (32) movies and sounds were modulated using a 10.4721 Hz sine wave, such that the luminance of the movies, and the amplitudes of the sounds, flickered from 0% to 100% luminance or amplitude, respectively, at a rate of 10.4721 Hz (in the alpha range). The remaining 32 movie and sounds were modulated using a 4 Hz sine wave, such that the luminance of the movies, and the amplitudes of the sounds, flickered from 0% to 100% luminance or amplitude, respectively, at a rate of 4 Hz (the theta range, as in the experiment in Chapter 3).

In Chapter 3, we found that there were no significant differences in memory performance for the associations when the sound-movie pairs were presented at 90°, 180°, and 270° phase offsets. Therefore, in the present experiment, we modulated the amplitudes of the sounds using sine waves at the same four phase offsets with respect to the sine wave used to modulate the luminance of the movie: 0° phase offset (synchrony), 90°, 180°, and 270°; however, we grouped the three out-of-phase modulations together, and compared memory performance on the synchronous trials to performance on the asynchronous trials. That is, within each block of 16 trials, 8 of the sound-movie pairs were presented in-phase, while 8 were presented out-of-phase. Across all blocks, the

number of trials presented at each asynchronous phase offset (90°, 180°, and 270°) was equal (16 at each phase offset: 48 total asynchronous trials; 16 synchronous and 16 asynchronous trials per frequency; 8 of each per block). Two blocks of of trials were presented at each frequency. The order of block presentation was arranged such that blocks at the same frequency modulation were not presented twice in a row, and the order of block presentation was balanced such that each block of trials was presented in each serial position an equal number of times.

Of the four sounds within each instrument category within each block of 16 trials, one was modulated at each of the four phase offsets with respect to the modulation of the movie. Therefore, within each block of 16 trials, there were four sounds from each of four different instrument categories, one of which was modulated at each of the four phase offsets. During the presentation of the memory test, on all trials, each of the four movies represented by still images on the computer screen was presented with a sound from the same instrument category, so that the associative memory task could not be made easier by merely remembering the type of sound that was associated with the movies, rather the exact sound, given that each of the four movies being represented by the still images was associated with a sound of the same type.

Following completion of all six blocks of the associative memory task, participants completed three additional blocks of trials designed to test their ability to detect whether the flicker of the movies and sounds was synchronous or not, at each frequency. The instructions for this task were presented after participants had completed the memory task. Each of the three blocks of this synchrony judgement task consisted of a subset of 24 of the 96 total movie-sound pairings that had been presented already in the experiment. Following the presentation of each sound-movie pair, participants made a two-alternative forced choice judgement as to whether the participant felt that

the flicker of the sound and the flicker of the movie were synchronous or asynchronous, using the number keys 1 and 2.

The duration of the experiment was approximately 60 minutes for all participants.

Analysis

Effects of synchrony at each frequency were tested using *t*-tests, and supported by GLMMs, as in Chapter 3.

T-tests were used with sensitivity analysis (d-prime) measures to test for a difference between the ability to detect synchronous versus asynchronous presentation of stimuli in the synchrony detection task, and for response bias (beta), in each of the frequency conditions.

Additional analyses grouped participants using a median split (i.e., participants were split into two groups, depending on whether their performance was better or worse than the median) on the synchrony judgement each, at each frequency. Three 2 (group) by 2 (synchrony) repeated measures ANOVAs were run to check for a significant effect of group or an interaction between group and synchrony, on performance in the associative memory task. These analyses would reveal whether participants better able to judge whether the sound-movie stimuli were flickering in synchrony, versus asynchronously, influenced memory for the pairs.

As in the experiment presented in the previous chapter, in order to rule out the potential confound that the ratings of how well the sounds suited the contents of the movie might influence the ability

to form the associative memories for those pairs, we ran an additional 3 (frequency) by 2 (synchrony) repeated measures ANOVA, with follow-up *t*-tests at each frequency.

Additional analyses (*t*-tests) were performed to determine whether the suitability judgements influenced the results by equalising the number of trials at each point on the rating scale (1-5) within the two synchrony conditions at each frequency conditions.

Results

Performance on the associative memory task for synchronously-presented and asynchronously-presented trails, in each of the three frequencies, is shown in Figure 7, along with the results from the Chapter 3 for comparison.

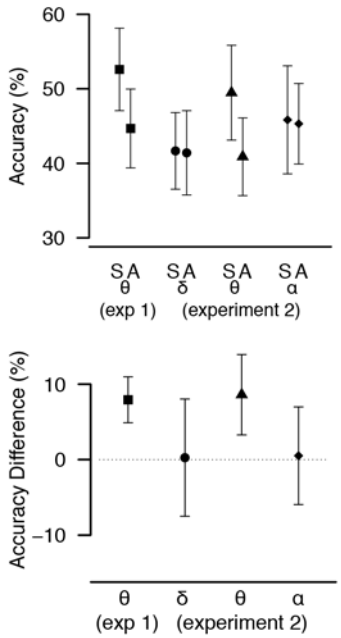

Figure 7. ***Results.*** Top: Accuracy (%) of selecting the correct movies that were presented with particular sounds when the movies and sounds were flickering in synchrony (S) or out of synchrony (A; 90-degree, 180-degree, and 270-degree phase offsets combined) at theta in experiment 1

(previous chapter) and at delta, theta, and alpha frequencies. Bottom: The difference in accuracy between the synchronous and asynchronous conditions at theta in experiment 1 and at delta, theta, and alpha frequencies in experiment 2. Error bars represent 95% confidence intervals.

At the theta frequency, performance was better for synchronous trials, compared to asynchronous trials: $T(1, 23) = 3.34$, $P < 0.01$. The GLMM supported the result of the *t*-test, with the corrected likelihood ratio of 5.62 suggesting strong evidence in favour of the model that included synchrony as a variable. This replicates the results of Chapter 3. At the delta frequency, there was no significant difference in memory performance between the two synchrony conditions: $T(1, 23) = 0.07$, $P = 0.95$; the GLMM supported the result, showing no evidence (corrected likelihood ratio = -2.88) in favour of one model over the other (i.e., there was no evidence to suggest that the synchrony manipulation had any effect on memory performance). At the alpha frequency, there was also no significant difference in memory performance between the two synchrony conditions: $T(1, 23) = 0.17$, $P = 0.87$. The GLM supported this result: the corrected likelihood ratio (-2.85) suggests that there was no evidence that the synchrony manipulation had an effect on memory performance.

The difference in performance between synchronous and asynchronous trials at the theta frequency was compared to the difference at the control frequencies: the difference was significantly larger at the theta frequency than at the control frequencies: $T(1, 23) = 2.46$, $P = 0.01$ (one-sided). Also, performance on synchronously-presented trials only was compared between the theta frequency and the control frequencies: $T(1, 23) = 1.93$, $P = 0.03$ (one-sided), suggesting that the effect of improved performance at the theta frequency is due to an improvement in memory on trails that were presented synchronously at the theta frequency.

The discriminability analysis in the synchrony judgement task showed that there was no effect of discriminability (d-prime) for trails in the theta frequency condition: Null hypothesis: Mean = 0; $T(1, 23) = 1.269$, $P = 0.217$. There was also no effect of response bias (beta): Null hypothesis: Mean = 1; $T(1, 23) = 0.143$, $P = 0.888$. In the alpha frequency condition, there was no effect of discriminability: Null hypothesis: Mean = 0; $T(1, 23) = 0.002$, $P = 0.999$, or response bias (Null hypothesis: Mean = 1: $T(1, 23) = -0.3062$, $P = 0.762$. In the delta frequency condition, we found a significant effect of discriminability: Null hypothesis: Mean = 0; $T(1, 23) = 3.059$, $P = 0.006$, and a significant effect of response bias: Null hypothesis: Mean = 1; $T(1, 23) = -4.642$, $P < 0.001$. Thus, synchrony was more easily detected in this condition. Figure 8 shows the discriminability index in each condition, along with the discriminability index from the experiment in the previous chapter for comparison.

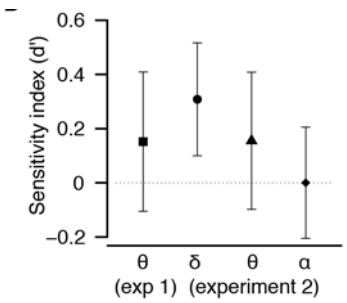

Figure 8. ***Sensitivity index.*** Sensitivity index (d-prime) on the synchrony judgement task, measuring the ability to discriminate between synchronous and asynchronous stimuli at theta in experiment 1 and at delta, theta, and alpha frequencies in experiment 2.

In each frequency condition, participants were split into two groups, dependant on their performance on the synchrony judgement task. Participants with performance greater than the median performance were assigned to one group, and participants with performance worse then the median to another group. The 2 (group) by 2 (synchrony) repeated measures ANOVA on memory

performance revealed no effect of group in the theta condition: $F(1, 22) = 0.226$, $P = 0.639$. There was no interaction effect between group and synchrony: $F(1, 22) = 0.003$, $P = 0.960$. The main effect of synchrony remained significant: $F(3, 22) = 10.663$, $P = 0.004$. The same analysis in the delta condition also revealed no main effect of group: $F(1, 22) = 0.186$, $P = 0.671$, no interaction between group and synchrony: $F(1, 22) = 0.217$, $P = 0.646$, and no main effect of synchrony: $F(1, 22) = 0.027$, $P = 0.871$. The same analysis in the alpha condition also revealed no main effect of group: $F(1, 22) = 2.406$, $P = 0.135$, no interaction between group and synchrony: $F(1, 22) = 0.220$, $P = 0.644$, and no main effect of synchrony: $F(1, 22) = 0.005$, $P = 0.946$. These analyses suggest that judgements of synchrony were not responsible for performance on the associative memory task.

Additional analyses were used to confirm that the suitability judgements made by participants did not influence performance on the associative memory task. First, a 3 (frequency) by 2 (synchrony) repeated measures ANOVA was performed on the suitability judgements, and revealed an interaction between frequency and synchrony: $F(2, 46) = 15.396$, $P < 0.001$. Follow-up pairwise *t*-tests were used in each frequency condition. In the theta condition, the sounds were judged to have suited the movies better in the asynchronous condition than in the synchronous condition: $T(1, 23) = -4.668$, $P < 0.001$. This was unexpected, but clearly did not influence our results: if the suitability judgements had a positive influence on memory for those items, one would have expected better memory performance in the asynchronous condition. Thus, our primary result persisted despite this unexpected finding in this experiment. In the alpha frequency condition, the was no difference in the judgement as to how well the sounds suited the content of the videos between the synchrony conditions: $T(1, 23) = 0.418$, $P = 0.680$. In the delta condition, the sounds were judged as suiting the content of the movies more in the synchronous condition than the asynchronous condition: $T(1, 23) = 3.075$, $P = 0.005$. However, memory performance was no different between these two conditions

at the delta frequency, again suggesting that the subjective ratings of suitability did not influence memory performance.

Our final analyses involved equalising the number of trials that were assigned each of the five suitability ratings (1-5) between the two synchrony conditions, across the three frequency conditions. We removed 208 trials, leaving 2096 trials for the analysis. Analysis confirmed the memory performance effect in the theta frequency condition: $T(1, 23) = 2.484$, $P = 0.021$. We did not see an effect of synchrony on memory performance in the delta condition: $T(1, 23) = -0.162$, $P = 0.873$, or in the alpha condition: $T(1, 23) = -0.162$, $P = 0.873$. These analysis confirm our primary results.

Experiment 2

Methods

The design and procedure follow the General Methods outlined in Chapter 2. Twenty-four new healthy English-speaking young adults (mean age = 20.1 years, SD = 1.21; 19 female) completed this experiment. All of the participants were right-handed, and all participants received a received a small stipend (£10.00) for their participation. The data from these 24 participants were were retained for analysis.

The experiment contained four blocks of 16 trials. In two of the blocks, the movies and sounds were modulated using a 4 Hz sine wave, as in Chapter 3, with the luminance of the movies, and the amplitudes of the sounds, flickered from 0% to 100% luminance or amplitude, respectively, at a rate of 4 Hz. Given that there were no significant differences in memory performance for the

associations when the sound-movie pairs were presented at 90°, 180°, and 270° phase offsets in previous experiments, we presented our stimuli in the present experiment at only two phase offsets: 0° phase offset (synchrony), and 180° (asynchrony), and compared memory performance on the synchronous trials to performance on the asynchronous trials. Thus, within each block of 16 trials, half of the sound-movie pairs were presented in-phase, and half were presented at 180° (out-of-phase). In the other two blocks of trials, the stimuli were not modulated with a sine wave, and were instead presented, unmodulated, at the same times that the sounds and videos were presented in the flickering blocks. However, given that unmodulated sound-movie pairs, lasting 3 seconds, would contain twice the amount of information as sound-movie pairs that were flickering from 0% to 100% amplitude and luminance, we trimmed the length of the sounds and videos in the two unmodulated blocks of trials to half their length (i.e., to 1.5 seconds each). The order of block presentation was balanced such that each block of trials was presented in each serial position an equal number of times.

In the flickering blocks of trials, of the four sounds within each instrument category within each block of 16 trials, two were modulated at each of the two phase offsets with respect to the modulation of the movie. Therefore, within each block of 16 trials, there were four sounds from each of four different instrument categories, two of which were modulated at each of the two phase offsets. During the presentation of the memory test, on all trials, each of the four videos represented by still images on the computer screen was presented with a sound from the same instrument category, so that the associative memory task could not be made easier by merely remembering the type of sound that was associated with the movies, rather the exact sound, given that each of the four movies being represented by the still images was associated with a sound of the same type. Similarly, in the non-flickering blocks of trials, during the presentation of the memory test, the four

videos represented by the still images on the screen were presented, during encoding, with sounds from the same instrument category.

The duration of the experiment was approximately 30 minutes for all participants.

Analysis

Repeated-measures analysis of variance (ANOVA) was used to determine the effect of condition. Follow-up pairwise *t*-tests were used to reveal the direction of the effects for each comparison. Given that there were no synchrony-judgement blocks in this experiment, there were no additional control analysis as in previous experiments. Indeed, the control in this experiment is a the non-flickering baseline condition.

Results

Performance on the associative memory task for synchronously-presented and asynchronously-presented trails at 4 Hz, and for non-flickering trials, is shown in Figure 9.

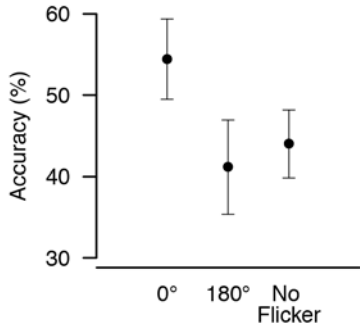

Figure 9. ***Results.*** Accuracy (%) of selecting the correct movies that were presented with particular sounds, when the movies and sounds were presented at 0-degrees phase offset, 180-degrees phase

offset, or were unmodulated, in the control experiment. Error bars represent 95% confidence intervals.

Analysis of variance revealed a main effect of condition: $F(2, 46) = 9.48$, $P < 0.01$. Pairwise *t*-tests showed that memory performance was better in the synchronous condition compared to the asynchronous condition: $T(1, 23) = 4.01$, $P < 0.01$, replicating our previous results. Pairwise *t*-tests also showed that memory performance was not different between the asynchronous condition compared to the non-flickering condition: $T(1, 23) = -0.92$, $P = 0.37$, but was significantly better in the synchronous condition than in the non-flickering condition: $T(1, 23) = 3.24$, $P < 0.01$.

Discussion: Experiments 1 and 2

In Chapter 3 we showed that memory performance was greater when multisensory stimuli flickering at 4 Hz were presented in-phase with respect to each other, compared to out-of-phase. However, such an effect could be explained in a number of ways: it could be that the effect is merely a result of the phase of the flicker, and not specific to 4 Hz, and it could be that the effect is a result of improved binding at the perceptual level, rather than the binding of highly processed sensory information at the memory-creation level. We aimed to address these potential explanations by comparing memory performance for synchronously and asynchronously flickering stimuli at three different frequencies: delta, theta, and alpha, and by comparing memory performance for synchronously and asynchronously flickering stimuli to that for stimuli that were not flickering at all.

In addition to providing replications of the effect discovered in Chapter 3, we showed that the improvement in memory performance for the synchronously flickering stimuli was specific to the

theta frequency. That is, we did not find the same improvement in memory for stimuli flickering in-phase relative to out-of-phase when the stimuli were flickering at the delta or alpha frequencies. Moreover, performance for stimuli flickering in-phase was better if the stimuli were flickering at the theta frequency than at the delta and alpha frequencies. Thus, entrainment at the theta phase specifically led to the memory enhancement effect, suggesting a causal role of theta oscillations in the successful formation of associative memories. Surprisingly, memory performance was also better when the stimuli were flickering in-phase at a theta frequency than for sound-movie pairs that were not flickering at all. This was an unexpected result: the flickering sound-movie pairs were perceptually distracting, compared to non-flickering pairs. It seems fair to expect that, at best, there would be no difference between theta-flickering pairs compared to non-flickering pairs. Such a result would still suggest an important role of theta oscillations in the formation of associative memories, as a lack of difference would at least suggest that entrainment at that oscillatory frequency boosts memory enough to offset the distracting effects of the flicker. It is worth noting that in our regular flickering conditions, the average luminance of the videos and amplitude of the sounds is 50% over the course of their presentation, and we thus chose to equate the information content of the non-flickering stimuli by adjusting their presentation time to 50% of the length of the time that the flickering stimuli were presented (1.5 seconds vs. 3 seconds, respectively). However, another way to control the information content between the two conditions would have been to keep the duration constant, but adjust the luminance of the videos and the amplitudes of the sounds in the non-flickering trials to 50% of maximum throughout the 3-second presentation. While both methods control the information content between the two conditions, it could be that memory performance would differ between the two methods. Regardless, that the in-phase flickering stimuli were remembered better than their non-flickering counterparts suggests the causal role of theta oscillations even more strongly, while also suggesting that memory for in-phase flickering pairs was improved, rather than memory for out-of-phase pairs impaired. Together, the results from these

experiments suggest that the effect is specific to theta, and cannot easily be explained by accounts of early perceptual binding. Therefore, we have termed the effect the "Theta-Induced Memory Effect" (TIME).

Given the hypothesised roles of theta oscillations, entrainment at 4 Hz (the hypothesised frequency in humans equivalent to the observed theta oscillations in the rodent hippocampus) yielded results supporting the causal role of oscillations at 4 Hz in associative memory formation. However, thus far, the success of our entrainment have been assumed. That is, in order to ensure that our effect is theta-frequency driven, we would need to demonstrate that we did successfully entrain the auditory and visual sensory cortices with our flickering stimuli, that our manipulations of phase offset were successful at the sensory cortex level, and that our effect in fact relies on such entrainment.

Experiment 3

Methods

The design and procedure follow the General Methods outlined in Chapter 2. Twenty-four healthy English-speaking young adults (mean age = 20.17 years, SD = 2.30; 19 female) participated in this experiment. Twenty-one participants were right-handed; three participants were left-handed. All participants received a small stipend (£10.00) for their participation in the experiment. All data from the participants were retained for analysis.

Half of the 92 movies and sounds (48) were modulated using a 4 Hz (theta-frequency) sine wave, such that the luminance of the movies, and the amplitudes of the sounds, flickered from 0% to 100% luminance or amplitude, respectively, at a rate of 4 Hz. The sounds were modulated using

sine waves at four different phase offsets from the sine wave used to modulate the movies: 0° phase offset (synchrony), 90°, 180°, and 270° phase offset. Of the four sounds within each instrument category, one was modulated at each of the four phase offsets with respect to the modulation of the movie. Within each block of 16 trials, there were four sounds from each of four different instrument categories, however 8 of the sounds were modulated at a 0° phase offset with respect to the movie, and 8 were modulated at the 90°, 180°, and 270° phase offsets, such that there were equal numbers of trials at each asynchronous phase offset across blocks. During the presentation of the memory test, on all trials, each of the four movies represented by still images on the computer screen was presented with a sound from the same instrument category, so that the associative memory task could not be made easier by merely remembering the type of sound that was associated with the movies, rather the exact sound, given that each of the four movies being represented by the still images was associated with a sound of the same type.

The remaining 48 sounds and movies were modulated using a constructed sine wave. This sine wave was constructed from one full cycle from eight different waves: 1.135 Hz, 1.536 Hz, 2.135 Hz, 2.635 Hz, 5.365 Hz, 5.835 Hz, 6.365 Hz, and 6.865 Hz. The average of these frequencies is 4 Hz, and none of the frequencies fall between 3 Hz and 5 Hz. Sine waves were constructed by randomly shuffling the above vector of frequencies to create 48 waves; an example is depicted in Figure 10. These 48 waves were used to modulate the luminance of the movies and the amplitude of the sounds. As with the theta-modulated sounds and movies, the sounds were modulated using sine waves at four different phase offsets from the sine wave used to modulate the movies: 0° phase offset (synchrony), 90°, 180°, and 270° phase offset. Of the four sounds within each instrument category, one was modulated at each of the four phase offsets with respect to the modulation of the movie. Within each block of 16 trials, there were four sounds from each of four different instrument categories, however 8 of the sounds were modulated at a 0° phase offset with respect to the movie,

and 8 were modulated at the 90°, 180°, and 270° phase offsets, such that there were equal numbers of trials at each asynchronous phase offset across blocks. During the presentation of the memory test, on all trials, each of the four movies represented by still images on the computer screen was presented with a sound from the same instrument category, so that the associative memory task could not be made easier by merely remembering the type of sound that was associated with the movies, rather the exact sound, given that each of the four movies being represented by the still images was associated with a sound of the same type.

Figure 10. ***Depiction of a randomly-constructed wave used to modulate stimuli***

The task consisted of six blocks of trials, three of which contained trails with sound-movie pairs modulated at 4 Hz, and three blocks with the sound-movie pairs modulated with the random waves. The order of block presentation was balanced such that each block appeared in each serial position an equal number of times across participants.

Following completion of all six blocks of the associative memory task, participants completed two additional blocks of trials designed to test their ability to detect whether the flicker of the movies and sounds was synchronous or not. The instructions for this task were presented after participants had completed the memory task. Each block of the synchrony judgement task consisted of a subset of 24 of the 48 total movie-sound pairings that had been presented already in the experiment; one block was presented for each wave type. Following the presentation of each sound-movie pair, participants made a two-alternative forced choice judgement as to whether the participant felt that

the flicker of the sound and the flicker of the movie were synchronous or asynchronous, using the number keys 1 and 2.

The duration of the experiment was approximately 50 minutes for each participant.

Analysis

The effects of wave type and synchrony were rested using a 2 (wave type) by 2 (synchrony) repeated measures analysis of variance (ANOVA) on the proportion of correct responses in the memory test trials, and followed up with pairwise *t*-tests.

T-tests were used with sensitivity analysis (d-prime) measures to test for a difference between the ability to detect synchronous versus asynchronous presentation of stimuli in the synchrony detection task, and for response bias (beta) in both wave type conditions.

After each trial in the associative memory encoding phase, participants rated how well they thought the sound suited the content of the movie. To rule out the potential confound that, by chance, some sounds were judged to suit the content of the movies more than others, and that those pairings that were judged to be more suited to each other would be more likely to be remembered, we performed additional control analyses. Repeated measures ANOVA were performed on the suitability data to test whether the sounds in the four different phase offset conditions were judged to suit the movies differently, in each wave type condition. We then equalised the number of trials in each phase offset condition for each of the five rating categories and performed the same repeated measures ANOVA and follow-up *t*-tests on the memory performance data as before, to check to see whether the results would remain the same.

Results

The repeated measures ANOVA revealed no main effect of wave type: $F(1, 23) = 0.32$, $P = 0.58$, and no interaction between wave type and synchrony: $F(1, 23) = 0.54$, $P = 0.47$. The main effect of synchrony was significant: $F(1, 23) = 12.93$, $P < 0.01$. Pairwise *t*-tests showed that memory performance was significantly better in the synchronous condition compared to the asynchronous condition for the theta wave type: $T(1, 23) = 3.36$, $P < 0.01$, but not for the random wave type: $T(1, 23) = 1.89$, $P = 0.07$. Thus, despite the lack of interaction between wave type and synchrony, only the theta condition showed a significant difference between the synchrony conditions. A one-sided *t*-test on the difference between synchrony and asynchrony, between the two wave types, did not show that the differences were significantly different: $T(1, 23) = 0.74$, $P = 0.23$.

Interestingly, performance in the synchrony condition was exactly the same between the wave types (50.17%). However, performance in the asynchrony condition was slightly (and non-significantly) higher in the random wave type condition (45.14%) than in the theta wave type condition (42.19%). These data show that the lack of a significant difference between the synchronous and asynchronous conditions in the random wave type condition was a result of higher performance on asynchronous trials, rather than lower performance on synchronous trials. Memory performance is shown in Figure 11.

The discriminability analysis showed no significant effect of discriminability in the theta wave type condition: Null hypothesis: Mean = 0; $T(1, 23) = -0.29$, $P = 0.77$, and no response bias effect: Null hypothesis: Mean = 1; $T(1, 23) = 0.44$, $P = 0.67$. In the random wave type condition there was no significant effect of discriminability: Null hypothesis: Mean = 0; $T(1, 23) = 0.73$, $P = 0.47$, and no

response bias effect: Null hypothesis: Mean = 1; $T(1, 23) = 0.45$, $P = 0.66$. In the random wave type condition, one participants had 100% hits, and another participant had 100% false alarms. For the purposes of the above analysis, one miss was substituted in for a hit, and one correct rejection was substituted in for a false alarm. In both wave type conditions, participants could not discriminate between synchronously presented sound-movie pairs and those presented out-of-phase, so perceptual judgements of synchrony were not responsible for performance on the episodic memory task.

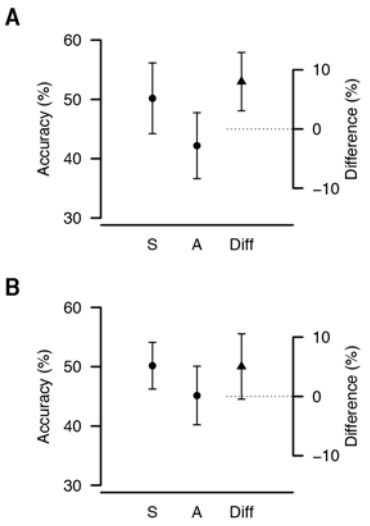

Figure 11. ***Results.*** A: Accuracy (%) when the stimuli were modulated with a 4 Hz theta wave. B: Accuracy (%) when the stimuli were modulated with the randomly-constructed wave. S = synchrony, A = asynchrony. The right y-axis corresponds to the difference between synchrony and asynchrony. Error bars represent 95% confidence intervals.

The 2 (wave type) by 2 (synchrony) repeated measures ANOVA on the ratings of how well the sound suited the content of the movies showed a main effect of wave type: $F(1, 23) = 24.96$, $P < 0.01$, and a wave type by synchrony interaction: $F(1, 23) = 24.09$, $P < 0.01$. The main effect of

synchrony was not significant. The data show that, in the theta wave type condition, the sounds were judged as suiting the movies better when the sound and movie were presented out-of-phase. In the random wave type condition, the sounds were judged to have suited the movies better when the sound and movie were presented in-phase. These results are not surprising, and, in the theta wave condition, are no different from the results presented in Chapter 4. Despite the fact that the sounds were judged to have suited the movies better in the asynchronous condition, memory performance was better in the synchronous condition. Thus, the memory performance in the synchronous condition could not partially be explained by higher judgements of suitability—such pairs might more easily be remembered. However, in this experiment, by chance (since the movies and sounds were randomly paired between experiments), the sounds were judged as better suiting the movies in the synchronous condition of the random wave type condition. This result might partially explain the improved memory performance for synchronously-presented pairs in the random wave type condition.

In order to further check whether the suitability ratings had any effect on memory performance, we equalised the numbers of trials that were rated 1, 2, 3, 4, and 5 amongst the four phase offsets, across both wave type conditions. For this analysis, we removed 142 trials, leaving 2162 trials for the analysis. The repeated measures ANOVA revealed a main effect of synchrony on memory performance: $F(1, 23) = 13.29$, $P < 0.01$, in-line with our earlier results. The main effect of wave type remained statistically non-significant: $F(1, 23) = 0.28$, $P = 0.60$, and the interaction between wave type and synchrony remained non-significant: $F(1, 23) = 0.28$, $P = 0.61$. Pairwise *t*-tests showed that memory performance was significantly better in the synchronous condition compared to the asynchronous condition for the theta wave type: $T(1, 23) = 2.87$, $P < 0.01$, but not for the random wave type: $T(1, 23) = 1.93$, $P = 0.07$. Again, despite the lack of interaction between wave

type and synchrony, only the theta condition showed a significant difference between the synchrony conditions.

Discussion: Experiment 3

While we successfully replicated our earlier results by showing improved memory performance when to-be-remembered sound-movie pairs were flickering in-phase at 4 Hz, our comparison condition gave surprising and difficult to interpret results. Memory performance in the random wave condition was similar to the theta condition when the waves were flickering in synchrony, but better than the theta condition (albeit non-significantly) when the waves were flickering out-of-synchrony. While equating the suitability ratings did little to change the results, there could be an explanation for these difficult-to-interpret results. Specifically, there may have been issues with how we constructed our random wave.

In designing the random wave, we were faced with the decision to select a set of frequencies that would average to 4 Hz, or a set of frequencies such that the cycle length of the frequencies would average to 250 ms. With no previous literature as a guide, we opted to select eight frequencies that arithmetically averaged to 4 Hz, such that frequencies between 3 Hz and 5 Hz were avoided: 1.135 Hz, 1.536 Hz, 2.135 Hz, 2.635 Hz, 5.365 Hz, 5.835 Hz, 6.365 Hz, and 6.865 Hz. However, the respective cycle lengths of the frequencies chosen were (rounded to 3 decimal places and in seconds): 0.881, 0.612, 0.468, 0.380, 0.186, 0.171, 0.157, and 0.146. The average cycle length was 0.375, which corresponds to 2.67 Hz. Had we chosen to develop our random waves from single cycles of frequencies that averaged 250 ms in the length of the cycles, our results may have been more clear and easier to interpret.

Whatever the explanation, our next steps were to ensure that the entrainment that we were, as yet, hypothesising, was in fact taking place at the level of the sensory cortices. In order to make such a determination, we turned to EEG.

Chapter 5: The Role of Entrainment in the Theta-Induced Memory Effect

Chapter Note: The experiment in this chapter has previously been published (Clouter, Shapiro, & Hanslmayr, 2017). While I was also the primary author on the previously published manuscript, I have endeavoured to ensure throughout this chapter a minimum of self-plagiarism. However, some of the figures and figure legends demonstrating the results are exactly as those that appear in Clouter, Shapiro, & Hanslmayr, 2017. Hector Cervantes provided valuable assistance in collecting the data for this experiment, and assisted with the programming of the scripts used for analysis.

Introduction

One way to study the role of oscillations in the human brain is using entrainment; the best evidence would come from studies that show a behavioural effect of entrainment in addition to demonstrating, for example using EEG, that the entrainment succeeded as intended (Thut et al., 2011). While the TIME is a behavioural effect, we have not shown that the effect results from entrainment of the visual and auditory sensory cortices, and we have not shown that rhythmic entrainment is necessary to produce the effect.

Given that the entrainment of brain oscillations requires that the activity of a population of neurons aligns in-phase with the external input, analysis of EEG measurements of the activity of neurons in the visual and auditory cortical areas would be sufficient to demonstrate successful entrainment. In order to demonstrate entrainment, we aimed to provide evidence that oscillations, measured at the scalp level using EEG, aligned with the frequency and phase of our sine-wave modulated stimuli. Using source localisation techniques and EEG waveform reconstruction, we aimed to determine

whether entrainment was successful in both the visual and auditory cortices. Photoic driving (entrainment) of the visual system is well established (e.g., Adrian & Matthews, 1934; Regan, 1966; Herrmann, 2001) using luminance modulations of visual stimuli. Similarly, entrainment of the auditory system is well established, and is achieved using either amplitude or frequency modulation of presented auditory stimuli (Geisler, 1960; Picton, John, Dimitrijevic, & Purcell, 2003).

In this experiment, and in the following chapter, we demonstrate that we achieved our aim of entraining the visual and auditory cortices at the hypothesised phase offsets with respect to each other.

Methods

The design and procedure follow the General Methods outlined in Chapter 2, with the exceptions outlined below. Ten healthy english-speaking young adults completed the experiment (including the author; mean age = 26.55 years; SD = 4.21, 3 female). One participant was left-handed. The data from one participant was excluded from the final analysis as a result of technical problems with the EEG recording.

The number of movies and sound clips was doubled to 192 movies and sound clips. As in the experiment in Chapter 3, all movies were luminance modulated with a 4 Hz sine wave, such that the movies visually flickered from no luminance to full luminance at 4 Hz, and all sounds flickered from no amplitude to full amplitude at 4 Hz. Eight, rather than four, instrument categories were used, each of which contained 16 sounds. The sounds were presented through insert earphones (ER-3C; Etymotic Research, Elk Grove Village, IL) via a Sound Blaster Audigy 5/Rx audio card (Creative Technology, Singapore).

During the preparation of the participant for EEG data collection, and during the practice trials, participants sat in a well-lit testing room. The testing room was darkened when the experiment proper began, and the experimenter monitored the participant from an adjoining room via a webcam. The experiment consisted of 14 blocks of trials; the first 12 blocks were the same as the experiment presented in Chapter 3, with the exception that within each block of 16 trials, there were four sounds from each of four different instrument categories, and eight of the sounds were modulated at a 0° phase offset with respect to the video, and eight were modulated at the 90°, 180°, and 270° phase offsets, such that there were equal numbers of trials at each asynchronous phase offset across blocks, and 96 total trials in-phase and 96 total trials out-of-phase (with 32 at each of the 90°, 180°, and 270° phase offsets). The 13th block consisted of sound clips only, with no corresponding movie, and contained 50 trials; the 14th block contained movies only, with no corresponding sound clip, and also contained 50 trials. In both blocks, participants were instructed to rate how pleasant they thought the sound or movie was using the number keys 1-5, with 1 corresponding to "very unpleasant" and 5 corresponding to "very pleasant". The ratings data from these blocks were not used in any analysis; these blocks were used for EEG source localisation.

EEG Methods[2]

Continuous EEG data was recorded using a 128 channel BioSemi ActiveTwo system (BioSemi, Amsterdam, the Netherlands). Electrode positions were the 128 standard equidistant BioSemi sites; EOG generated from eye movements and blinks were recorded from three additional electrodes placed approximately 1 cm to the left of the left eye, 1 cm to the right of the right eye, and 1 cm below the left eye. Data were digitized using the BioSemi ActiView software, with a sampling rate

2 Given the standard nature of presenting these methods, what is presented here is nearly an exact copy of what appears in our published manuscripts.

of 2048 Hz. Offline analysis were performed with SPM12 (Wellcome Trust Centre for Neuroimaging) and FieldTrip (version 2016031538; Oostenveld, Fries, Maris, & Schoffelen, 2011).

EEG data were re-referenced to the average reference, and highpass filtered with a cutoff of 1 Hz (butterworth filter, zero phase, order 5), prior to resampling at 512 Hz. The data were then lowpass filtered with a cutoff of 20 Hz (butterworth filter, zero phase, order 5), and epoched (1000 ms before to 4000 ms after stimulus onset) and baseline corrected. Trials and channels with obvious artefacts (other than ocular artefacts that occurred between 3000 and 4000ms after stimulus onset) were removed by visual inspection, and by using Fieldtrip's visual artefact rejection procedure. Independent components analysis (ICA) was then applied to the data, and components related to ocular artefacts were removed. Any remaining artefacts were removed during a final visual inspection of the data. On average, 22.72% of trails were removed from the data, and 5.4% of channels were removed. Sensor data were then interpolated (via triangulation of nearest neighbours) and re-referenced to the average reference, if any channels had been removed during the artefact rejection procedure.

EEG electrode positions were measured on the first participant's head using a Fastrak electromagnetic digitiser (Polhemus, Colchester, VT, USA), and warped to match the orientation of the standard 10-5 electrode template provided in FieldTrip. Using the geometric locations of the electrodes and the template FieldTrip volume conduction and source models, sources were reconstructed over time and space using a linear constrained minimum variance (LCMV) beamformer. Given that activity in the auditory cortices have highly correlated time courses with binaural stimulation, which poses a problem for accurate source localization using a LCMV beamformer, we followed an established method that allows detection of correlated sources by

calculating the surface Laplacian of the data and the leadfields to improve the spatial resolution of the data in the unimodal auditory and multimodal conditions (Murzin, Fuchs, & Kelso, 2013).

In the unimodal conditions, time-frequency analysis was applied to each source ERP using Morlet wavelet (width = 7) at our frequency of interest (4 Hz). Evoked power at each dipole was determined by averaging the evoked power between 0.75 and 2.75 s post stimulus onset. The evoked power at each dipole was contrasted with evoked power at each dipole in a randomized condition. For the randomized conditions, individual trials for each participant were randomly assigned a 0, 90, 180, or 120 phase offset by shifting the signal forward in time by 0, 32, 64, or 96 samples (0, 62.5, 125, or 187.5 ms). The expected value of the evoked power at relevant auditory and visual sources would thus be zero in the randomized conditions. For each participant, the evoked power difference at each source in each of the unimodal conditions was interpolated to the MNI 305 MRI template. The grand average of these power differences was interpolated onto the MNI 305 MRI template and displayed using MNI-space templates included with CARET (version 5.65; Washington University School of Medicine) for visualization of sources of power greater than 90% of the maximum. Coordinates for auditory and visual sources were determined by finding the maximum power difference in regions corresponding to auditory and visual sensory areas.

In the multimodal conditions, we obtained common source filters separately. Source grand average EEG signals were calculated at the determined coordinates for the auditory and visual sources, and interpolated to the template structural MRI. Individual trial data were lowpass filtered with a cutoff of 15 Hz and grand averaged in each multimodal condition (0°, 90°, 180°, and 270°) at each determined source. The Hilbert transformation was applied to the grand averaged signals obtained at each determined source for each phase offset condition. The instantaneous phases were derived from the Hilbert transformed data, and unwrapped. The unwrapped instantaneous phase differences

(modulus 2 p) between the auditory source and the visual source was calculated between 0.75 s and 2.75 s in order to avoid effects of stimulus onset and offset, for each phase offset condition.

Analysis

Reconstructed EEG waveforms at the identified auditory and visual sources were analysed using Rayleigh's uniformity test, which tests for a uniform distribution in the phase difference between the two signals, and the V test (Zar, 2010), in which the alternative hypothesis is that the phase difference between two signals is a particular phase angle. The test parameter reflects the probability that the phase difference is distributed uniformly. The analyses were conducted on a segment of the signals of a 2 second duration, between 0.75 s and 2.75 s, which would exclude neural activity related to stimuli onset and offset (thus, 1026 samples in each phase offset condition).

Results

The reconstructed EEG waveforms at the auditory and visual sources in the right hemisphere is shown in Figure 12, and the left hemisphere in Figure 13. In the right hemisphere, the EEG waveform was reconstructed at the coordinates determined by source localisation: MNI coordinates: 28, -96, -6 for the visual cortex and MNI coordinates: 64, -24, 6 for the auditory cortex. In the left hemisphere, the EEG waveform was reconstructed at the coordinates determined by source localisation: MNI coordinates: 16, -88, -2 for the visual cortex and MNI coordinates: -46, -18, 14 for the auditory cortex.

The results of Rayleigh's uniformity test and the V test are shown in Table 1.

	Left Hemisphere				Right Hemisphere			
	0°	90°	180°	270°	0°	90°	180°	270°
n	1026	1026	1026	1026	1026	1026	1026	1026
μ (rad)	-0.728	1.442	2.805	-1.916	-0.582	1.900	-3.118	-2.041
(deg)	318.29°	82.62°	160.71°	250.22°	326.65°	108.86°	181.35°	243.06°
r	0.661	0.497	0.607	0.712	0.492	0.745	0.653	0.655
p(R)	0	0	0	0	0	0	0	0
p(V)	0	0	0	0	0	0	0	0

Table 1. Parameters of the circular analyses of the difference in the phase offsets between the auditory and visual sources in the left and right hemispheres, at each phase offset. Symbols: n = number of data; μ = mean circular vector; r = length of μ; *p*(R) = Rayleigh's test parameter; *p*(V) = V test parameter.

As shown in Table 1, in each phase offset condition, in both hemispheres, the Rayleigh test rejected the null hypothesis of a uniform distribution of the phase differences between the signals at the visual and auditory sources. Moreover, the V test rejected the null hypothesis of a uniform distribution of the phase differences between the signals at the visual and auditory sources, in favour of the alternative hypotheses: That the phase differences were at each of the hypothesised angles: 0°, 90°, 180°, and 270°. Thus, we can conclude that our visual and auditory entrained the visual and auditory cortices, and that the phase difference between the entrained activity was as hypothesised.

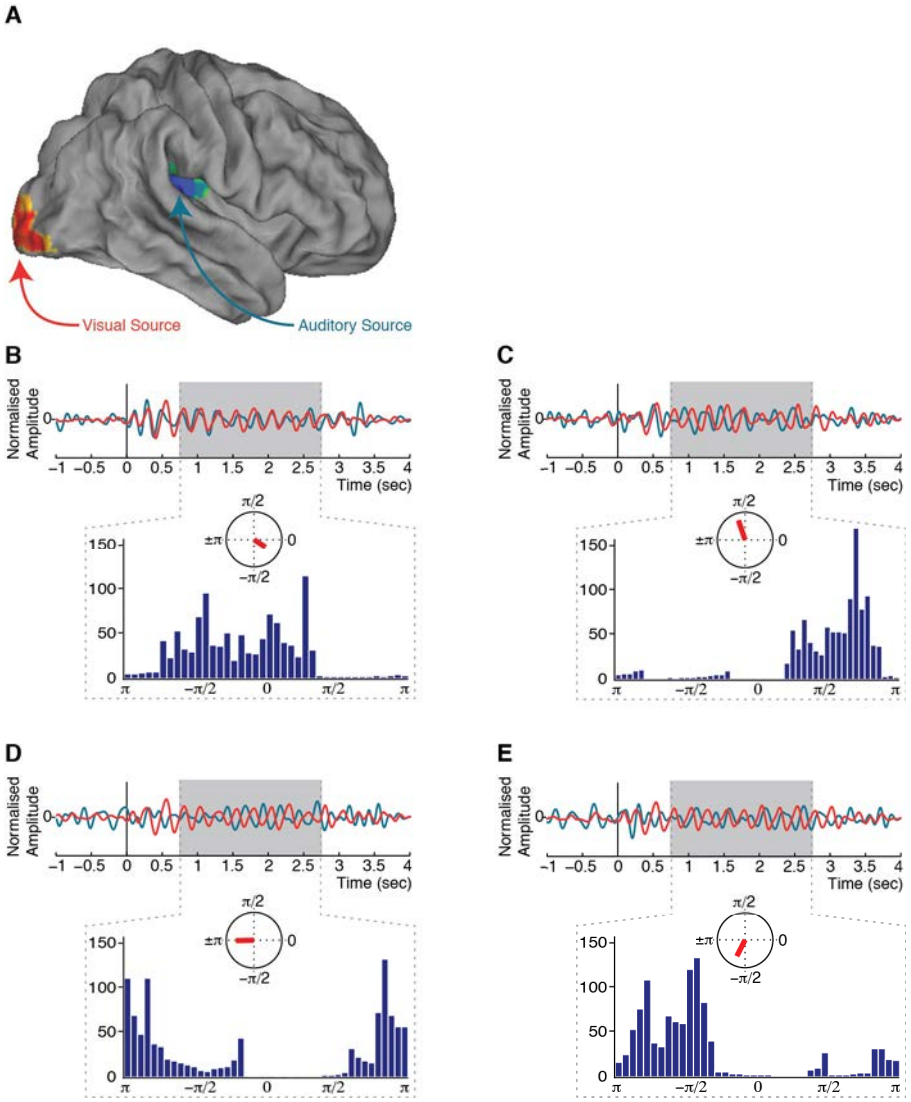

Figure 12. ***Results of the analysis of the phase differences between visual and auditory sources at each phase offset*.** Visual (red) and auditory (blue) sources are shown. (A) Maps of evoked power obtained from the visual only and auditory only unimodal conditions projected onto visual and auditory cortices, showing visual (red) and auditory (blue) sources. Evoked power was calculated as the difference between average evoked power between 0.75 and 2.75 s at each dipole in the auditory and visual unimodal conditions, contrasted with the average evoked power in the same condition when trials were randomly assigned to each phase offset condition (wherein the expected evoked power would be zero). (B) Top: amplitude-normalized grand-averaged signals from the visual and auditory cortices (low-pass filtered at 15 Hz) at 0-degree phase offset. The stimuli onset at time = 0. Bottom: wrapped count histogram of the instantaneous phase difference between the visual and

auditory signals for the shaded time region (0.75–2.75 s). Inset: the direction and length of the mean resultant vector of the phase differences. (C–E) Same as (B), but for 90-degree (C), 180-degree (D), and 270-degree (E) phase offsets.

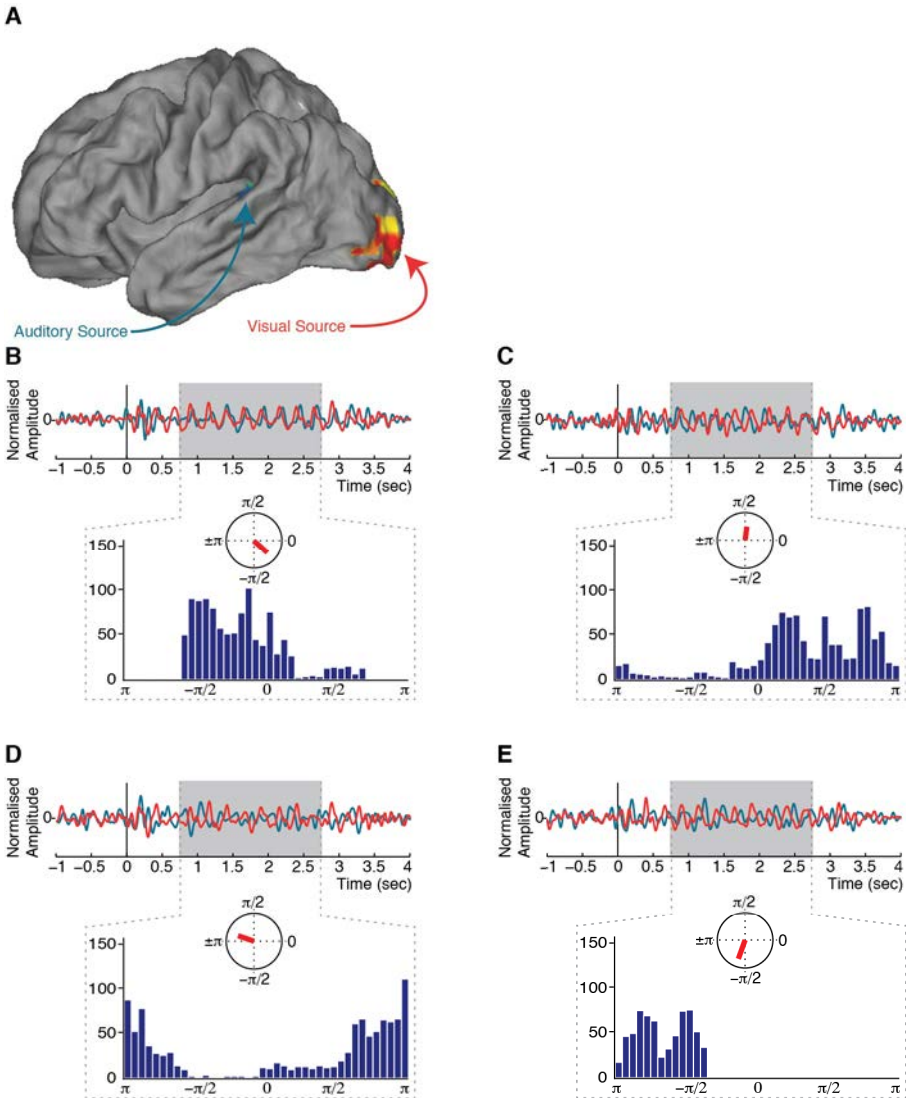

Figure 13. ***Results of the analysis of the phase differences between visual and auditory sources at each phase offset*****.** Visual (red) and auditory (blue) sources are shown. (A) Maps of evoked power obtained from the visual only and auditory only unimodal conditions projected onto visual and auditory cortices, showing visual (red) and auditory (blue) sources. Evoked power was calculated as the difference between average evoked power between 0.75 and 2.75 s at each dipole in the auditory and visual unimodal conditions, contrasted with the average evoked power in the same condition

when trials were randomly assigned to each phase offset condition (wherein the expected evoked power would be zero). (B) Top: amplitude-normalized grand-averaged signals from the visual and auditory cortices (low-pass filtered at 15 Hz) at 0-degree phase offset. The stimuli onset at time = 0. Bottom: wrapped count histogram of the instantaneous phase difference between the visual and auditory signals for the shaded time region (0.75–2.75 s). Inset: the direction and length of the mean resultant vector of the phase differences. (C–E) Same as (B), but for 90-degree (C), 180-degree (D), and 270-degree (E) phase offsets.

Discussion

By presenting our participants with two blocks of stimuli in which only the movies, and only the sounds, were presented, we were able to use EEG source localisation techniques to determine the appropriate coordinates in the visual and auditory cortices in each hemisphere. Then, we were able to reconstruct the EEG waveforms at those two sources, in each hemisphere, in the trials in which the movie and sound were played together. The reconstructed signals were analysed to determine whether the phase difference between the signals at the visual and auditory cortices were as expected based on our manipulations of the phase differences in the physical stimuli presented to the participants. Our results demonstrated that were were successful in our entrainment of the sensory regions, and that the phase differences between the signals at the sources in the two sensory cortices were as expected.

Without corresponding EEG evidence, we could not be certain that our modulations of the visual and auditory stimuli did in fact entrain the visual and auditory cortices, respectively, despite the results of our behavioural experiments. Converging evidence from both EEG and behavioural studies would provide strong evidence for the role of the theta oscillation in the formation of

associative memory in humans. That presenting rhythmically flickering stimuli modulates neural activity in the sensory cortices is not a new finding (e.g., Pantev, Roberts, Elbert, Ross, & Wienbruch, 1996; Picton, John, Dimitrijevic, & Purcell, 2003). However, to aid the interpretation of the results of our series of experiments, it was necessary to ensure that entrainment had taken place, and that activity in the sensory cortices were modulated with the phase differences that we had selected.

Chapter 6: The Theta-Induced Memory Effect at the Single-Trial Level

Chapter Note: The experiment presented in this chapter has been previously published (Wang, Clouter, Chen, Shapiro, & Hanslmayr, 2018). I was not the first author of the published manuscript, and so, while the material presented here follows what is presented in the published manuscript, it has been substantially re-written. I was involved in designing and programming the experiment, and in training Danying Wang and Qiaoyu Chen to collect the data and perform the analysis. Danying Wang and Qiaoyu Chen collected the data, performed the analysis, and Danying Wang was the primary author on the published manuscript. Some of the figures and figure legends demonstrating the results are exactly as those that appear in Wang, Clouter, Chen, Shapiro, & Hanslmayr, 2018, as this can scarcely be avoided.

Introduction

We have shown that precisely controlling the relative timing of sensory inputs, with the concurrent entrainment of the sensory cortices at a 4 Hz theta frequency, dramatically improves memory relative to non-flickering stimuli, stimuli flickering out-of-phase, and stimuli flickering in-phase, but at a non-theta frequency. Our results provide strong evidence for a causal role of the theta frequency in the formation of associative episodic memories. However, the strength of the evidence could be increased if we were also able to demonstrate that, at the level of a single memory-formation trial, the degree of synchrony between the auditory and visual cortices predicts the successful formation of the associative memory.

In our previous experiments, we have shown that, *on average* (i.e., when we collapsed our trials across the in-phase and out-of-phase conditions), memory performance was better when the stimuli were modulated with a theta wave, and flickering in-phase (at the level of the sensory cortices). However, we have not been able to show that, on any given memory formation trial, the degree of the synchrony actually observed between the sensory cortices predicts the ability to successfully encode the association. We can achieve this goal by sorting all trials into those trials that were successfully remembered and those that were not remembered, and then analysing the degree of phase synchrony between the auditory and visual cortices as we did in Chapter 5 during the encoding phase. Increased strong evidence for a causal role of theta oscillations would result if we could show that, within the different synchrony conditions, subsequent memory was better when, at the level of a single trial, memories were more likely to be formed when the measured phase difference between the sensory cortices were close to 0°, which is what we show.

Methods

The design and procedure follow the General Methods outlined in Chapter 2, with the exceptions outlined below. Thirty-one healthy english-speaking young adults completed the experiment (mean age = 21.48 years; range: 18-29 years, 16 female). One participant was ambidextrous, and all other participants were right-handed. Twenty-seven participants received a small stipend for their participants; the remaining four participants received experimental credits. The data from one participant was excluded from the final analysis as a result of technical problems with the EEG recording. The data from three additional participants were removed as a result of memory performance at chance (25%) levels. The data from three additional participants were removed as a result of poor EEG quality. All data from the 24 remaining participants were retained for the final analyses.

The number of movies and sound clips was doubled to 192 movies and sound clips. As in the experiment in Chapter 3, all movies were luminance modulated with a 4 Hz sine wave, such that the movies visually flickered from no luminance to full luminance at 4 Hz, and all sounds flickered from no amplitude to full amplitude at 4 Hz. Eight, rather than four, instrument categories were used, each of which contained 16 sounds. The sounds were presented through insert earphones (ER-3C; Etymotic Research, Elk Grove Village, IL) via a Sound Blaster Audigy 5/Rx audio card (Creative Technology, Singapore).

During the preparation of the participant for EEG data collection, and during the practice trials, participants sat in a well-lit testing room. The testing room was darkened when the experiment proper began, and the experimenter monitored the participant from an adjoining room via a webcam.

The experiment consisted of 15 blocks of trials; the first 12 blocks were the same as the experiment presented in Chapter 3, with the exception that within each block of 16 trials, there were four sounds from each of four different instrument categories, and eight of the sounds were modulated at a 0° phase offset with respect to the video, and eight were modulated at 180° phase offset. The 13th block of trials consisted of a synchrony judgement task, as used in the experiment in Chapter 3. This block contained 24 trials, using a subset of sounds and movies drawn from the 192 used throughout the experiment. On half of the trials, the sounds and movies were presented at a 0° phase offset, and half were presented at 180° phase offset. Following the presentation of each sound-movie pair, participants rated whether they thought that the flicker of the sound was in-synchrony with the flicker of the video, using the number keys 1 and 2 on the keyboard, where 1 corresponded to asynchrony, and 2 corresponded to synchrony. The 14th block consisted of sound clips only, with

no corresponding movie, and contained 50 trials; the 15th block contained movies only, with no corresponding sound clip, and also contained 50 trials. In both blocks, participants were instructed to rate how pleasant they thought the sound or movie was using the number keys 1-5, with 1 corresponding to "very unpleasant" and 5 corresponding to "very pleasant". The ratings data from these blocks were not used in any analysis; these blocks were used for EEG source localisation.

EEG Methods[3]

Continuous EEG data was recorded using a 128 channel BioSemi ActiveTwo system (BioSemi, Amsterdam, the Netherlands). Electrode positions were the 128 standard equidistant BioSemi sites; EOG generated from eye movements and blinks were recorded from three additional electrodes placed approximately 1 cm to the left of the left eye, 1 cm to the right of the right eye, and 1 cm below the left eye. Data were digitized using the BioSemi ActiView software, with a sampling rate of 2048 Hz. Offline analysis were performed with SPM12 (Wellcome Trust Centre for Neuroimaging) and FieldTrip (version 2016031538; Oostenveld, Fries, Maris, & Schoffelen, 2011).

EEG data were re-referenced to the average reference, and highpass filtered with a cutoff of 1 Hz (butterworth filter, zero phase, order 5), prior to resampling at 512 Hz. The data were then lowpass filtered with a cutoff of 20 Hz (butterworth filter, zero phase, order 5), and epoched (2000 ms before to 5000 ms after stimulus onset) and baseline corrected. Trials and channels with obvious artefacts (other than ocular artefacts that occurred between 3000 and 4000ms after stimulus onset) were removed by visual inspection, and by using Fieldtrip's visual artefact rejection procedure. Independent components analysis (ICA) was then applied to the data, and components related to ocular artefacts were removed. Any remaining artefacts were removed during a final visual

3 Given the standard nature of presenting these methods, what is presented here is nearly an exact copy of what appears in our published manuscripts.

inspection of the data. Sensor data were then interpolated (via triangulation of nearest neighbours) and re-referenced to the average reference, if any channels had been removed during the artefact rejection procedure. Data from participants with less than 16 trials remaining in any of the conditions listed below were excluded from the final analyses. The average number of trials per participant remaining in each condition were: sound-only: 42; movie-only: 42; 0° phase offset, subsequently remembered: 34; 0° phase offset, subsequently forgotten: 43; 180° phase offset, subsequently remembered: 31; 180° phase offset, subsequently forgotten: 46.

EEG electrode positions were measured on the first participant's head using a Fastrak electromagnetic digitiser (Polhemus, Colchester, VT, USA), and warped to match the orientation of the standard 10-5 electrode template provided in FieldTrip. Using the geometric locations of the electrodes and the template FieldTrip volume conduction and source models, sources were reconstructed over time and space using a linear constrained minimum variance (LCMV) beamformer. Three participants had structural MRI head scans, and so head models were created with those scans, using the Statistical Parameter Mapping (SPM, version 8, http://fil.ion.ucl.ac.uk/spm) toolbox. Their structural MRI scans were segmented into the brain, cerebrospinal fluid, skull, and scalp layers, and their electrode positions were aligned to their head models.

Given that activity in the auditory cortices have highly correlated time courses with binaural stimulation, which poses a problem for accurate source localization using a LCMV beamformer, we followed an established method that allows detection of correlated sources by calculating the surface Laplacian of the data and the leadfields to improve the spatial resolution of the data in the unimodal auditory and multimodal conditions (Murzin, Fuchs, & Kelso, 2013).

In the unimodal conditions, time-frequency analysis was applied to each source ERP using Morlet wavelet (width = 7) at our frequency range of interest (3.5 – 4.5 Hz). Evoked power at each dipole was determined by averaging the evoked power between 0.75 and 2.75 s post stimulus onset. The evoked power at each dipole was contrasted with evoked power at each dipole in a randomized condition. For the randomized conditions, individual trials for each participant were randomly assigned a 0, 90, 180, or 120 phase offset by shifting the signal forward in time by 0, 32, 64, or 96 samples (0, 62.5, 125, or 187.5 ms). The expected value of the evoked power at relevant auditory and visual sources would thus be zero in the randomized conditions. For each participant, the evoked power difference at each source in each of the unimodal conditions was interpolated to the MNI 305 MRI template.

In the multimodal conditions, we obtained common source filters separately. Source grand average EEG signals were calculated at the determined coordinates for the auditory and visual sources, and interpolated to the template structural MRI. Individual trial data were lowpass filtered with a cutoff of 15 Hz and grand averaged in each multimodal condition (0° and 180°) at each determined source. The Hilbert transformation was applied to the grand averaged signals obtained at each determined source for each phase offset condition. The instantaneous phases were derived from the Hilbert transformed data, and unwrapped. The unwrapped instantaneous phase differences (modulus 2 p) between the auditory source and the visual source was calculated between 0.75 s and 2.75 s in order to avoid effects of stimulus onset and offset, for each phase offset condition.

LCMV beamforming can assign a random sign to the signals at each dipole, corresponding to the direction of the dipole. To correct for this random assignment, we plotted the signals reconstructed from the left and right hemisphere auditory sources and the visual sources for each participant on each trial. If the signal was flipped, we multiplied the timeseries data by -1 to flip the data, such that

the early auditory and visual components (P1, N1, P2) were in the correct direction. This data flipping procedure was carried out across all trials in all conditions.

Analyses

Effects of phase offset were tested using a paired-samples *t*-test on the proportion of correct responses in the memory test trials. *T*-tests were used with sensitivity analysis (d-prime) measures to test for a difference between the ability to detect synchronous versus asynchronous presentation of stimuli in the synchrony detection task.

After each trial in the associative memory encoding phase, participants rated how well they thought the sound suited the content of the movie. To rule out the potential confound that, by chance, some sounds were judged to suit the content of the movies more than others, and that those pairings that were judged to be more suited to each other would be more likely to be remembered, we performed additional control analyses. Repeated measures ANOVA were performed on the suitability data to test whether the sounds in the two different phase offset conditions were judged to suit the movies differently. We then equalised the number of trials in each phase offset condition for each of the five rating categories and performed the paired-samples *t*-test on the memory performance data as before, to check to see whether the results would remain the same.

Reconstructed EEG waveforms at the identified auditory and visual sources were analysed using Rayleigh's uniformity test, which tests for a uniform distribution in the phase difference between the two signals, and the V test (Zar, 1979), in which the alternative hypothesis is that the phase difference between two signals is a particular phase angle. The test parameter reflects the probability that the phase difference is distributed uniformly. The analyses were conducted on a

segment of the signals of a 1 second duration, between 1 s and 2 s, which would exclude neural activity related to stimuli onset and offset (thus, 513 samples in each phase offset condition).

In order to quantify the strength of entrainment for each trial, we calculated the phase difference between the reconstructed signals at the auditory and visual sources, and compared that difference to the phase difference between the auditory and visual stimuli. The resultant vector length was determined for the 1 s period for each trial. A 2 (synchrony) by 2 (subsequent memory: remembered or forgotten) repeated-measures ANOVA was run on this phase entrainment measure, and followed up with pairwise *t*-tests in each phase offset. Also, given that our memory performance data are binomially distributed, we fit a logistic regression model to the data, using entrainment strength as a predictor of subsequent memory performance.

Finally, we created four bins into which to randomly assign 12 trials each for each participant. These bins were centred at 0°, 90°, 180°, and 270°, with each bin width +/- 45° from the centre. We calculated the proportion of remembered trials in each bin, and repeated the procedure 10 times for each participant. We then ran a repeated-measures ANOVA on memory performance across the four bins.

Results

A paired-samples *t*-test revealed that memory performance was better in the 0° phase offset condition than in the 180° phase offset condition: $T(1, 23) = 2.069$, $P = 0.025$ (one-sided), which replicates our previous results. The discriminability analysis of performance in the synchrony judgement blocks of trials showed no significant effect of discriminability: Null hypothesis: Mean = 0; $T(1, 23) = 1.317$, $P = 0.201$, so perceptual judgements of synchrony were not likely responsible

for performance on the episodic memory task. Memory performance in the two synchrony conditions is shown in Figure 14.

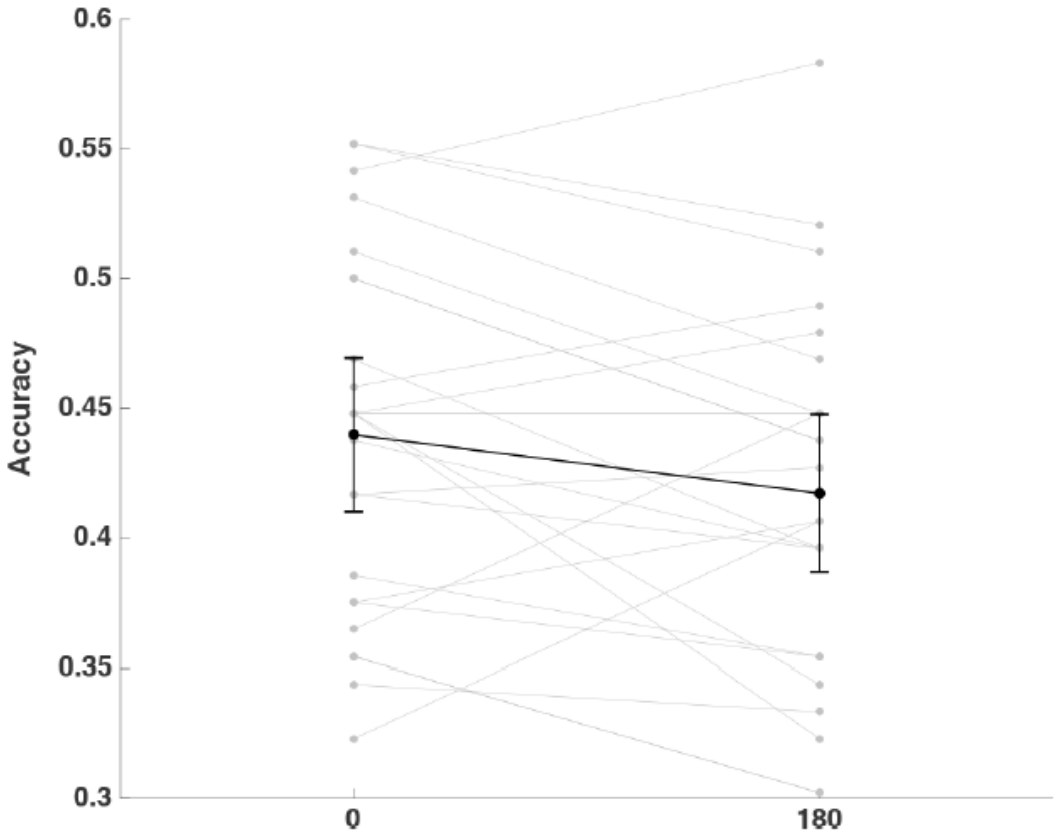

Figure 14. ***Associative memory task performance.*** Proportion of the correctly selected movie scenes that were associated with presented sounds in each phase offset condition. Note that chance level is at 25%. Error bars represent 95% confidence intervals of means in 0 and 180 phase offset conditions. Individual data for correct associative memory performance in 0 and 180 phase offset conditions is shown in grey.

A 2 (subsequent memory) by 2 (synchrony) repeated-measures ANOVA on the subjective rating of how well the sounds suited the contents of the movies revealed a main effect of subsequent memory: $F(1, 23) = 24.244$, $P < 0.01$, and a main effect of synchrony: $F(1, 23) = 7.962$, $P = 0.01$. Taken alone, these results could suggest that memory performance was influenced by how well

participants thought that the sounds suited the contents of the movies. To control for this potential interpretation, we equalised the number of trials that were assigned to each of the 5 choices on the rating scale for each participant by randomly selecting a subset of trials and re-calculating memory performance for those trials. This procedure was repeated 10 times for each participant. We then averaged memory performance in each synchrony condition across the 10 repetitions of the procedure, and re-ran the paired-samples *t*-test. Our results show that, when ratings are equalised, we still see a significant improvement in memory in the synchronous condition compared to the asynchronous condition: $T(1, 23) = 1.903$, $P = 0.035$ (one-sided), which confirms our earlier results.

The locations of the visual and auditory sources resulting from the source localisation steps are shown in Figure 15.

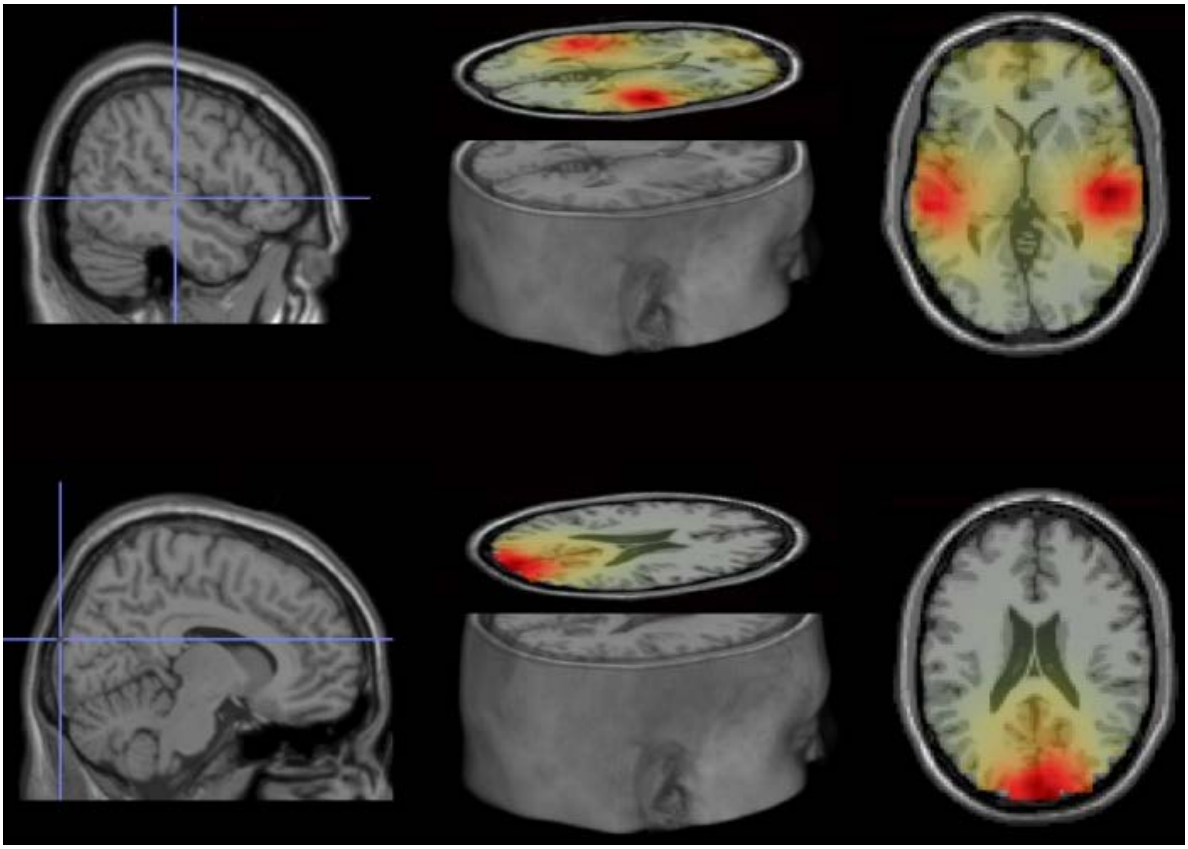

Figure 15. ***Source localisation of theta power in the unimodal conditions.*** Top: Auditory sources, MNI coordinates of ROIs: right, 50, -21, 0; left, -60, -29, 0. Bottom: Visual source, MNI coordinates of ROI: 10, -99, 20. Evoked power was averaged over 3.5 and 4.5 Hz, between 0.75 and 2.75 s at each virtual electrode in the unimodal movie and sound conditions and the baseline conditions (see Materials and Methods). Grand average power differences between unimodal conditions and baseline conditions were interpolated to a MNI MRI template. The source coordinates were determined by where the maximum grand average power differences were.

The grand average reconstructed EEG waveforms at the visual and auditory sources, in the 0° and 180° phase offset conditions, are shown in Figure 16.

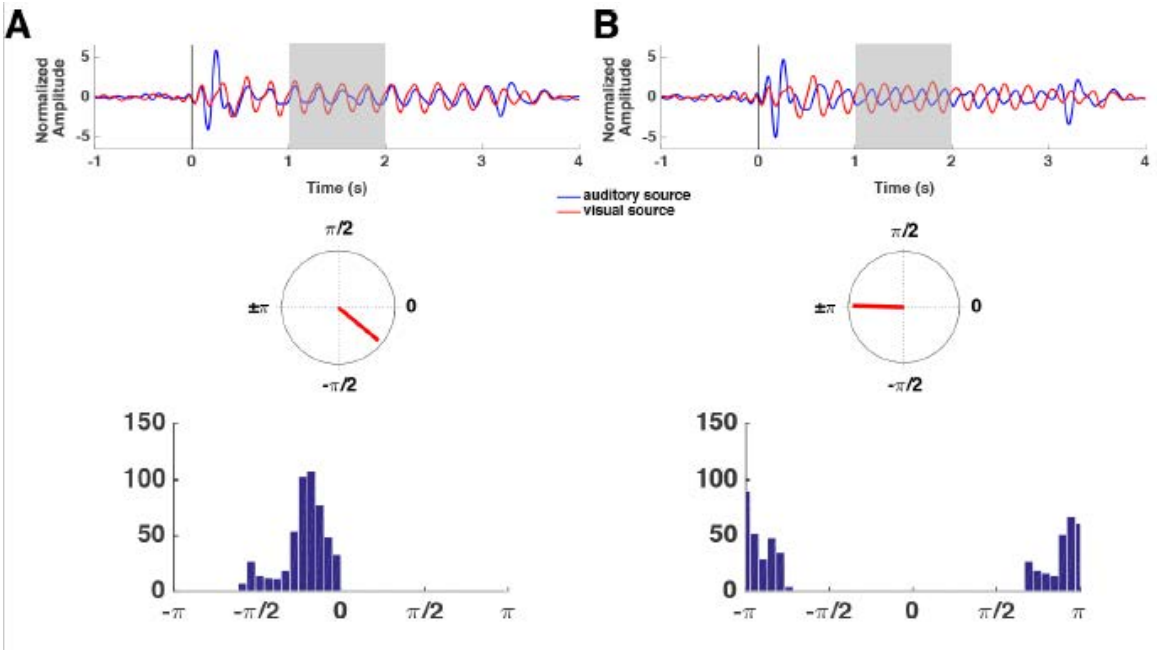

Figure 16. ***Phase differences between auditory and visual sources in each phase offset condition.*** A, Phase differences between auditory and visual sources in 0º offset condition. Top: Amplitude normalized grand average ERP signals at auditory (blue) and visual sources (red). Middle: Mean resultant vector of instantaneous phase differences between auditory and visual sources, between 1

and 2 s (shaded time window on the grand average ERPs) is plotted on a unit circle. Bottom: Histogram of wrapped instantaneous phase differences between auditory and visual sources, between 1 and 2 s, using 40 equally-sized bins. B, As in A, but for the 180º offset condition.

The Rayleigh test of uniformity and the V test were performed for the 0° and 180° phase offset conditions separately, using the phase differences between 1 s and 2 s after stimuli onset. The Rayleigh test showed that the phase differences were not uniformly distributed in the phase offset conditions: 0°: resultant vector length: 0.9197, $P \sim 0$; 180°: resultant vector length: 0.9192, $P \sim 0$. The V test showed that the hypotheses of uniformly distributed phase differences could be rejected in favour of the specified alternative hypotheses: that the phase differences were distributed around 0° and 180°, for each condition: both Ps ~ 0. Thus, our auditory and visual stimuli entrained the auditory and visual sensory cortices, respectively, at the hypothesised phase angles.

For each trial for all participants, we calculated the measure of phase entrainment to the external stimuli as described in the EEG Methods. A resultant vector length close to 1 means that the phase difference between the sensory cortices closely matched the phase difference between the physical stimuli, thus indicating strong entrainment. A resultant vector length close to 0 corresponds to trials in which entrainment was not significant. The results from four single trials is shown in Figure 17.

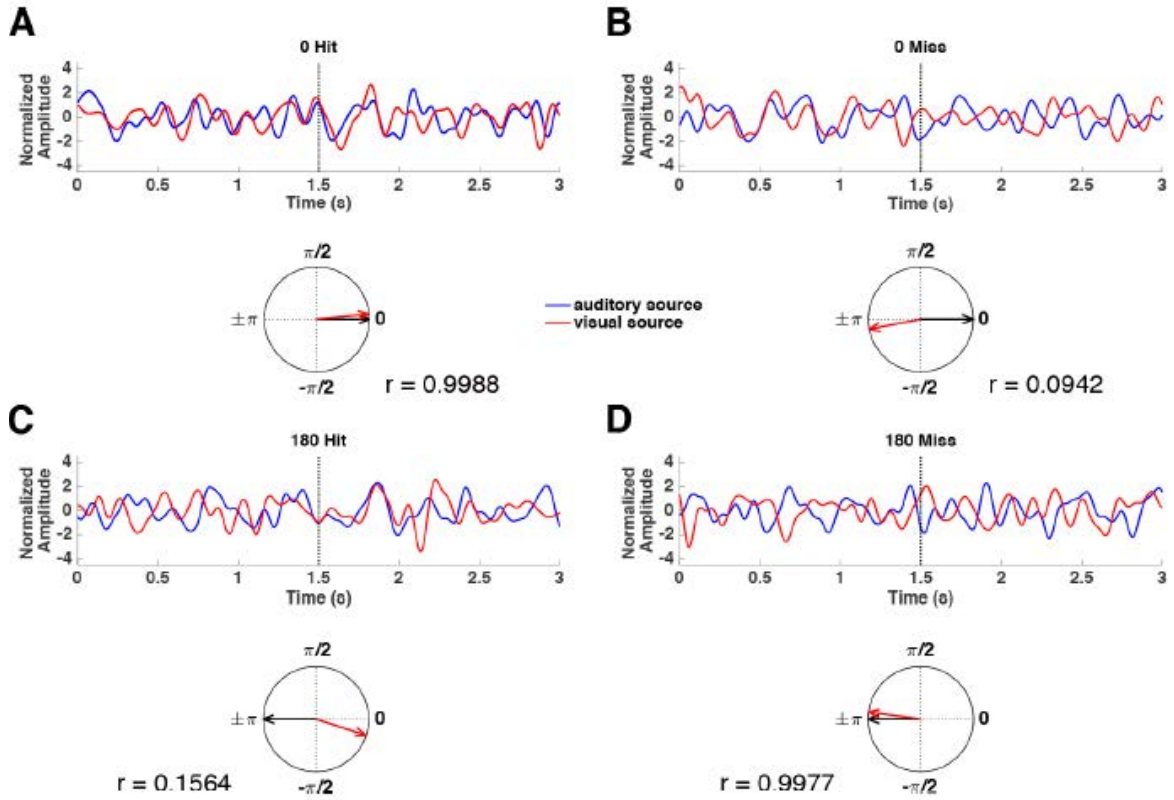

Figure 17. ***An example of four single trials in 0º remembered, forgotten conditions and 180º remembered, forgotten conditions, respectively.*** A, Top: A band-pass filtered (1.5-9 Hz) single trial from auditory (blue) and visual (red) sources in 0° offset remembered condition. Amplitude was normalized. Bottom: instantaneous phase difference between auditory and visual sources at a certain time point 1.5 s of the single trial is plotted on a unit circle (red line). Single trial phase entrainment was calculated as the resultant vector length between the measured phase difference (red arrow) and the entrained phase offset 0º (black arrow), which was 0.9988 in this case. B, same as in A but for 0° offset forgotten; C, same as in A but for an 180° offset remembered trial; D, same as in A but for an 180° offset forgotten trial.

The 2 (synchrony) by 2 (subsequent memory) repeated measures ANOVA on the measure of the strength of entrainment revealed a significant interaction between synchrony and subsequent

memory: $F(1, 23) = 4.627$, $P = 0.042$. Follow-up pairwise *t*-tests showed that there was significantly weaker entrainment for trials that were subsequently remembered than those subsequently forgotten in the 180° phase offset condition: $T(1, 23) = -2.165$, $P = 0.021$ (one-sided), and that in the 0° phase offset condition, there was a trend towards significant for stronger entrainment for subsequently remembered trials versus subsequently forgotten trials: $T(1, 23) = 1.496$, $P = 0.074$ (one-sided). The results show that subsequently remembered trials showed slightly stronger entrainment in the 0° condition, and weaker entrainment in the 180° condition, than forgotten trials, and that the opposite is true for subsequently forgotten trials. Figure 18 shows the results, demonstrating that 0° phase offset is the optimal condition for successful encoding, and that 180° for unsuccessful encoding. In Figure 17, we can see in Panel A that the metric of entrainment is strong for trials subsequently remembered. In Panel C we can see that the entrainment metric is weak for trials that were subsequently remembered in the 180° condition. In Panel B we can see weak entrainment for trials in the 0° condition that were subsequently forgotten and strong in trials in the 180° condition that were subsequently forgotten.

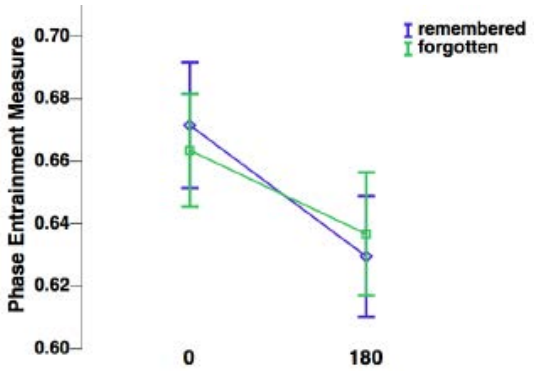

Figure 18. ***Memory performance as a function of phase entrainment.*** The phase entrainment measure was averaged between 1 and 2 s for each trial. These single-trial phase entrainment values are plotted as a function of subsequent memory (remembered: purple; forgotten: green) in each phase offset condition.

An additional analysis step was to estimate the coefficient of the slope parameter in a logistic regression model predicting memory performance. A significantly positive coefficient would indicate that the strength of the entrainment directly predicted memory performance on the trial – that is, a direct relationship between the strength of the entrainment and subsequent memory. A one-sample *t*-test revealed that the slope was significantly positive: $T(1, 23) = 1.958$, $P = 0.031$ (one-sided), indicating such a relationship.

Finally, we created four bins centred at 0°, 90°, 180°, and 270°. Each bin had a width of +/- 45°. For each participant, we assigned their individual trials to each bin, depending on the direction of the mean resultant vector. We then randomly selected 12 trials from each bin and calculated the proportion of correctly remembered trials, and repeated the procedure 10 times for each participant, and calculated the grand average of correctly remembered trials across the 10 iterations of the procedure. A repeated-measures ANOVA of the phase bin on memory performance revealed a significant effect of the phase bin: $F(3, 69) = 6.014$, $P < 0.01$. Follow-up pairwise *t*-tests show that memory performance was better in the 0° bin than the 90° bin: $T(1, 23) = 3.061$, $P < 0.01$, better in the 0° bin than in the 180° bin: $T(1, 23) = 2.739$, $P = 0.01$, and better in the 0° bin than in the 270° bin: $T(1, 23) = 4.293$, $P < 0.01$. Memory performance was no different between the 90°, 180°, and 270° bins: all Ps > 0.8. Results are shown in Figure 19.

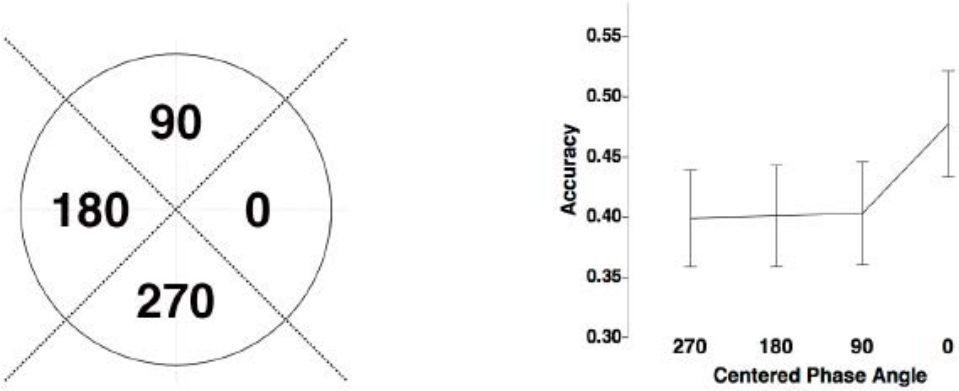

Figure 19. ***Recall accuracy of each phase offset bin that consisted of trials which mean phase direction of each trial was 270°, 180°, 90°, and 0°, ±45° respectively***. Left: An illustration of how the four phase offset bins were defined. Four bins were centered at 0º, 90º, 180º, 270º, with a bin boundary of ±45°. Right: Recall accuracy was calculated in each bin. The x-axis represents the centered phase angle of each bin. For each trial, the mean phase direction between 1 and 2 s was computed. Then, each trial was sorted into one of the four bins depending on the mean direction (e.g. if the mean phase direction of a trial was a value between -45º and 45º, the trial would be sorted into the 0º bin). The proportion of remembered trials was calculated for 12 trials that were randomly selected from each bin for each iteration. The recall accuracy was averaged across 10 iterations.

Discussion

We once again demonstrate that, when collapsed across (averaged) into the synchrony and asynchrony conditions, memory performance was better when the stimuli were modulated with a theta wave, and flickering in-phase (at the level of the sensory cortices). However, we have now provided additional strong evidence that on any given memory formation trial, the degree of the synchrony actually observed between the sensory cortices predicts the ability to successfully encode the association. By sorting trials into those trials that were successfully remembered and those that were not remembered, and then analysing the degree of phase synchrony between the auditory and visual cortices at the level of single trials, we have shown that memory performance is improved if, in the synchronous condition, entrainment strength is strong, and, in the asynchronous condition, entrainment strength is weak. That is, in both the synchronous and asynchronous conditions, the

closer the phase difference between the sensory cortices was to the phase difference between the presented stimuli, the more likely the stimuli would be successfully encoded into an associative memory. Thus, we have shown that the strength of entrainment during an individual encoding trial predicts subsequent memory for the association in that trial. These results strengthen our previous results, revealing that the theta frequency at encoding is part of the mechanisms for associative memory encoding, while providing additional evidence of this causal role of theta oscillations in forming multisensory associative memories. The relative timing of inputs and theta oscillations is thus one of the primary mechanisms underlying the formation of associative memories.

Chapter 7: General Discussion

Oscillatory neural activity in the theta frequency range can be observed in the human brain, but low-frequency theta oscillations (at around 4 Hz) predominate in the human hippocampus. Given the necessity of the hippocampus in the formation of associative episodic memories, it has been hypothesised that theta oscillations in the hippocampus forms part of the mechanism for associative memory (e.g., Pavlides, Greenstein, Grudman, & Winson, 1988; Winson, 1978).

Research in animals has shown that theta oscillations also play a role in other behaviour, such as spatial movement through locations in an environment (Vanderwolf, 1969), and the coding of specific places in an environment (Burgess, O'Keefe & Reece, 1993; Skaggs, McNaughton, Wilson, & Barnes, 1996; Johnson & Redish, 2007), in addition to memory. Interfering with theta oscillations in the hippocampus in rodents has been found to eradicate learning (McNaughton, Ruan, & Woodnorth, 2006), but that learning can be reinstated by stimulating the hippocampus at the theta frequency. Moreover, correlational evidence suggests that the power of the theta oscillations in the rodent hippocampus is positively related with learning in associative conditioning research (Berry & Thompson, 1978; Griffin, Asaka, & Berry, 2004).

Other animal research has shown that the phase of the theta oscillation plays a critical role in the molecular mechanism of memory encoding: long-term potentiation (LTP). Excitatory input stimulation to the hippocampus at the peak, but not the trough, of the local theta oscillation can induce LTP (Pavlides et al., 1988; Huerta and Lisman, 1995; Hölscher et al., 1997; Hyman et al., 2003). Thus, LTP relies on the precise timing of inputs from pre-synaptic neurons and post-synaptic neural activity. Dendritic processing at synapses is not linear; the coincident activity of numerous inputs will yield a much greater post-synaptic response than non-coincident inputs (Buzsáki, 2010;

Fries, 2015). Theta frequency oscillations can facilitate the precise timing that is required (Huerta & Lisman, 1995; Hanslmayr, Staresina, & Bowman, 2016; Hasselmo, 2005; Hasselmo, Bodelón, & Wyble, 2002). Given the results of these, and many other, experiments, it is easy to assume that hippocampal theta oscillations play a causal role in associative memory processes.

Research in humans has shown that theta oscillations may be related to episodic memory (Hsieh & Ranganath, 2014). During the encoding of episodic memories, the power of theta oscillations has been positively associated with subsequent memory for words (Klimesch, Doppelmayr, Russegger, & Pachinger, 1996), and for associations (Burke, Zaghloul, Jacobs, Williams, Sperling, Sharon, & Kahana, 2013). However, research into the role of theta power in memory formation has shown mixed results: both increases (Staudigl & Hanslmayr, 2013) and decreases (Burke et al., 2013) in the strength of theta oscillations have been positively related to successful encoding. Those studies could, at best, reveal an association between theta power and memory formation—a correlation—and cannot provide evidence for a causal role of theta oscillations for memory formation in humans. Moreover, the studies all focussed on the strength of the theta oscillation, and not the role of the phase of the oscillation.

Since the role of the phase of the theta oscillation plays a causal role in inducing LTP, and given nonlinear dendritic processing, we hypothesised that the precise timing of inputs to the hippocampus, facilitated by sensory entrainment at the theta frequency, would reveal the hitherto supposed causal role of hippocampal theta oscillations in the formation of associative memories in humans—evidence to date has been correlational (see also Fell et al., 2003; Rutishauser et al., 2010; Backus et al., 2016).

In our series of experiments, we have provided the first evidence for a causal role of the phase of theta oscillations in human associative memory. The behavioural manipulation, which involved entraining the visual and auditory sensory cortices at a theta frequency, both in-phase and out-of-phase with each other, gave us the opportunity to test the role of the entrained theta oscillation in the formation of associative memories. Using EEG, we showed that our experimental manipulation successfully entrained the sensory cortices at the theta frequency, and at the intended phase offsets with respect to each other. We demonstrated improved encoding of associative memories when the to-be-remembered sounds and movies were flickering at a theta frequency, such that neural activity in the respective sensory regions would be entrained in-phase with each other. This improvement in memory specific to the theta frequency—flickering the stimuli at slower or faster frequencies did not show the same result. Moreover, and importantly, memory was better in the theta entrainment condition than when the sounds and movies were not flickering at all. Thus, our manipulation improved memory relative to baseline performance. Finally, we investigated whether the strength of entrainment at the level of individual associative memory encoding trials could predict success in encoding the memory. We demonstrated that the strength of entrainment does predict memory performance, which strengthens the evidence of the causal role of theta oscillations in associative memory formation. Given the necessity of the theta frequency in achieving our results, we have called it the "theta-induced memory effect" (TIME). Our results support previous research, which has suggested a role of theta oscillations in the encoding of episodic memories. However, our results go further, by extending the support for a role of theta oscillations by providing the first direct causal evidence in humans.

Limitations and Alternate Accounts

Reliability, Validity, and Generalisability of Results

The results of our series of experiments raises other important questions, and seem to allow for alternate explanations. First, how general are these effects? In our behavioural experiments, we successfully replicated the effect with each new set of results. In these experiments, we used the same set of 96 sounds and videos. Initially, we showed a highly significant effect of phase offset on memory performance when the stimuli were modulated with a theta-frequency sine wave. In testing whether the effect was frequency-specific, we randomly assigned 32 each of the sounds and videos to the three frequencies that we tested. In the behavioural experiment in which the wave used to modulate the sounds and videos was randomly created by combining a single full cycle from each of 8 different frequencies that averaged to 4 Hz, we again randomly assigned half of the sounds and videos to each condition. Throughout all experiments we successfully replicated our initial results. This suggests that our findings are highly replicable.

When we tested whether the strength of entrainment could predict memory performance on a single-trial level, we doubled the number of stimuli used to 192 sounds and 192 movies. While we were still able to replicate our results, our behavioural results were weaker and more variable. One possible reason for this is that, as we increased the size of our stimuli sets, it became increasingly difficult to find movies and sounds that were ideal for our manipulation. In particular, for the sounds, it became increasingly difficult to find sets of sounds that could provide some generalisability to "real-world" situations (that is, refraining from using "laboratory-situation" stimuli such as clicks and beeps). The sounds that we used were chosen, as best as we were able, to have a reasonably constant amplitude envelope throughout the three second duration of the sound clip. Moreover, we attempted to find sounds that had few percussive beats in the sounds. However, fluctuations in amplitude and percussive beats are naturally found in almost every "real-world" musical sound, and as we increased our stimuli set, more of the new sounds that we used contained

more natural amplitude fluctuations and percussive beats. Both fluctuations in amplitude and percussive beats can entrain the auditory system (using the very same mechanism that we were taking advantage of: amplitude-modulated entrainment), thus interfering with our attempts to entrain the auditory system at precisely 4 Hz. The same is true of the videos: the videos themselves contained some natural luminance changes throughout the three second duration. In both cases, these were difficulties that we were prepared to deal with: in an effort to increase the ecological validity of our results, we risked imperfect entrainment. On the other hand, the very variability in our behavioural results, and the strength of entrainment between trails, allowed our investigation of single trial entrainment strength as a predictor of memory formation. Overall, we maintained good ecological validity, while being able to successfully replicate our own results, and all the while allowing sufficient variability to investigate our effects at the single-trial level.

Perceptual Accounts

It could be argued that the sine-wave modulation of the auditory and visual stimuli interfere with perception, and that it is the effects on perception that lead to the memory improvement that we have observed. That is, one could argue that it is possible that the effect might not be related to hippocampal theta oscillations at all. In particular, binding at the perceptual level is improved when stimuli are flickering in synchrony, relative to asynchronously (VanRullen, Zoefel, & Ilhan, 2014).

This possible account of the effect seems very unlikely. First, we have shown that memory performance was improved when the stimuli were modulated in the theta frequency range, and presented in-synchrony, relative to a condition in which the stimuli were not modulated at all. The case in which stimuli are not modulated at all is the best case for optimal perception (VanRullen et al., 2014), given that the stimuli are not perceptually distracting as they are in the flickering

conditions. That memory performance is nonetheless improved in the flickering condition, but only when the stimuli are presented in-phase, is evidence against this perceptual account.

Another argument is that the in-phase entrainment of the auditory and visual sensory cortices that we achieved captures and enhances attention (Senkowski et al., 2008), and that the improvements in memory that we show are a result of this increased attention. However, research has shown that there is a positive relationship between attention and the strength of entrainment (Müller et al., 2006; Lakatos et al., 2008; Saupe et al., 2009; Nozaradan et al., 2012). Thus, if enhanced attention in the synchronous conditions was responsible for our memory effect, we would have seen stronger measurements of entrainment remembered trials relative to forgotten trials, in both the synchronous and asynchronous conditions. However, this was not what we observed: we observed stronger phase entrainment for remembered trials in the synchronous condition, but weaker entrainment for remembered trials in the asynchronous condition. Moreover, this interaction between the synchrony conditions and subsequent memory was demonstrated between 1 and 2 seconds after the onset of the stimuli--not earlier or later. This result suggests that the memory effect was not a result of phase-coupling or early evoked responses in the sensory cortices, as seen in the literature on multisensory integration (e.g., Senkowski et al., 2008). Rather, the memory effect is more likely to have been determined by continuous in-phase theta synchronisation, which has been argued to be a plausible mechanism of associative memory (Summerfield & Mangels, 2005).

The Role of the Hippocampus

In our experiments, we did not measure the activity of neurons in the hippocampus directly. Thus, we cannot be sure that the entrainment of the auditory and visual sensory cortices propagated to the hippocampus. However, given that associative memory is a hippocampal-dependant activity

(Gonzalo et al., 2000; Eichenbaum & Cohen, 2004; Staresina & Davachi, 2009), we can assume that we were able to affect hippocampal activity to some degree.

Success in encoding episodic memories has been related to functional connectivity between the medial temporal lobes and the cortex (Summerfield et al., 2006; Schott et al., 2013). Furthermore, integrating information with existing memory traces, and the binding of to-be-remembered items with their context, have been related to theta-frequency coupling between the MTL and the cortex (Staudigl & Hanslmayr, 2013; Backus et al., 2016). Finally, the in-phase activity of the sensory cortices that we achieved with our entrainment. Given that these sensory areas project to the MTL, the result should be coincident input activity at the target area. As mentioned earlier, coincident presynaptic activity is more likely to induce LTP. That is, whether or not the hippocampal theta oscillations were entrained by propagation of the entrained activity in the sensory regions, the theta-frequency entrainment of the sensory cortices should lead to optimal conditions for LTP induction. However, given that we saw improved memory in the synchronous condition for the theta frequency only, and that memory performance at that frequency was better than the synchronous conditions at lower and higher frequencies, a specific role of theta is suggested.

Ultimately, invasive electrical recording directly from the hippocampus is the ideal way to test whether hippocampal activity was influenced by our entrainment paradigm. Other behavioural experiments could also shed light on the issue, but not address it directly. For example, a similar experiment that tested hippocampal-independent memory, should not show the same results. Similarly, one could use the same paradigm in patient groups without an intact hippocampus, and show no effects of frequency, phase, or even entrainment.

Generating A Theta State

It could be argued that the memory effect that we have demonstrated is a result of the entrainment creating an entrained state, which promotes associative memory formation. That is, the precise timing and the phase relationships of our stimuli may not be necessary to achieve the effect, but rather the memory effect could be a result of a general enhancement in theta power. In our experiments, theta power would be greater in the 0 phase offset condition than in the 90 and 270 phase offset conditions, which in turn would be greater than the 180 phase offset condition, as shown in Figure 20.

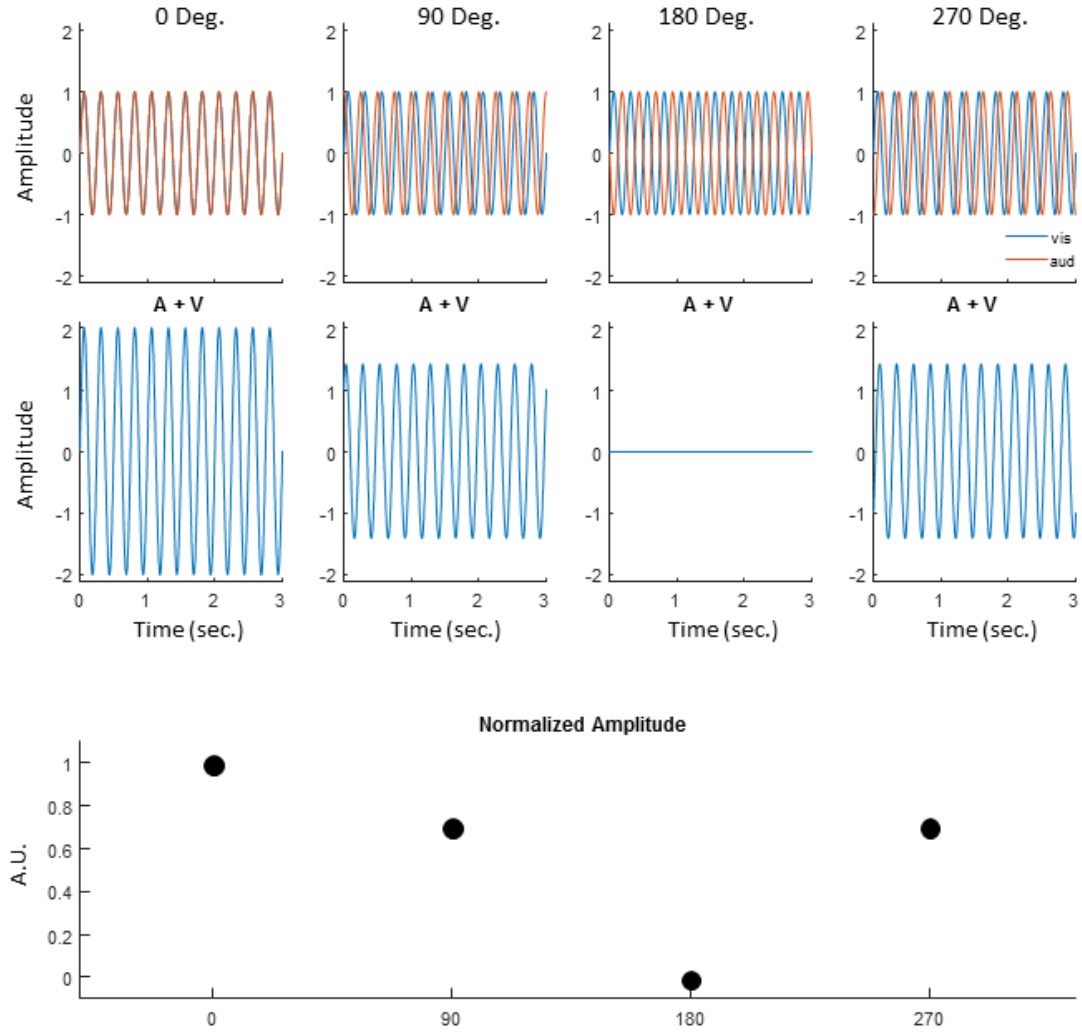

Figure 20. ***Wave summation.*** Two waves (top row) were created with a 0, 90, 180, and 270 phase offset with respect to each other (columns 1-4, respectively), simulating the entrainment between the visual and auditory cortices. The second row depicts the amplitude of a wave created by summing the two individual waves. The third row graphs the amplitude of the waves from the second row. The amplitude of the theta wave created by summing the two individual waves is greater when the individual waves are offset by 90 or 270 degrees, compared to 180 degrees.

However, this possible account for our effect should result in better memory performance in the 90 and 270 phase offset conditions than in the 180 phase offset condition, which we did not find in our results in our behavioural experiments, and we did not find that pattern of results when we sorted trials into the four phase bins in our single-trial experiment. While we cannot state that theta power is not related to successful associative memory formation—indeed it has been suggested by the literature (Klimesch et al., 1996; Staudigl & Hansmayr, 2013; Burke et al., 2013)—we can claim that theta power alone cannot account for our findings. Our effect may be a result of theta phase synchrony, or a combination of theta phase synchrony and power: there is no reason to require that associative memory formation is subserved by only one neural mechanism. However, without further experimentation involving direct recordings from the hippocampus and other MTL regions, we cannot make a firm claim as to the exact nature of the mechanism—only that theta phase, at least, is a constituent component.

Looking to the Future

While our experiments have provided insight into the mechanisms of associative memory in humans, the results suggest that the entrainment paradigm may have value in clinical contexts or in the ageing population. For example, in the elderly, simple multi-tasking becomes more difficult;

simply trying to remember where you put the keys, for example, could impair your balance or gait; Falls in the elderly often have life-changing consequences. In patients with dementia or Alzheimer's disease, the paradigm may also prove to be useful. However, in such circumstances, it would not be feasible to continuously entrain sensory inputs. In fact, the perceptual distraction could make performing other tasks even more difficult. Rather, the paradigm could perhaps be used to assist patients in encoding memories for critical pieces of information.

Moreover, other research has demonstrated that transcranial magnetic stimulation of areas with strong functional connectivity with the hippocampus can facilitate the encoding of new memories (Wang, Rogers, Gross, Ryals, Dokucu, Brandstatt, Hermiller, & Voss, 2014). Our paradigm demonstrates that entrainment of the sensory cortices also facilitates memory formation. However, entrainment need not be via flickering stimuli. While it remains to be seen, it seems plausible that non-invasive transcranial electrical stimulation of the sensory cortices might achieve similar results. In that case, patients or the elderly need not worry about the perceptual distraction of flickering stimuli, and a simple home-based electrical stimulation kit could be designed to improve memory formation in these populations. The potential avenues of further development of entrainment paradigms in order to facilitate successful memory encoding have a bright and exciting future.

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Appendix A: Consent and Safety Screening

Visual Cognition TCS Protocol

Consent form

This information is being collected as part of a research project concerned with the capacity of visual working memory by the School of Psychology in the University of Birmingham. The information which you supply and that which may be collected as part of the research project will be entered into a filing system or database and will only be accessed by authorised personnel involved in the project. The information will be retained by the University of Birmingham and will only be used for the purpose of research and statistical and audit purposes. By supplying this information you are consenting to the University storing your information for the purposes stated above. The information will be processed by the University of Birmingham in accordance with the provisions of the Data Protection Act of 1998. No identifiable personal data will be published.

Please answer the following:

1) Have you filled out the screening questionnaire?
2) Have you read the information sheet provided?
3) Have you had an opportunity to ask questions and had your questions answered is a satisfactory manner?
4) Have you received enough information about the study?

5) During the experiment you will see simple images. If you do not wish to be exposed to these images, you should not participate in this experiment. If you are uncertain, are you willing to view some examples images?

6) You may withdraw from the study at any time and you will be entitled to any agreed reward up to the point where you withdraw. Do you understand this statement?

7) You may ask for your data to be destroyed at any time prior to the publication of the research findings based upon it. The deadline for this request is *6 months from today*. Do you understand this statement?

8) Do you consent to take part in this study?

Please give your full name _____

I confirm that I have read the consent form and have completed the above questionnaire. The nature, purpose and possible consequences of the procedures have been explained. I confirm that I have been through the [EEG/TMS/TCS/ **Behaviour**] screening procedures and that I agree to participate in this study.

Signature (participant) _____Date: _____

Experimenter's name _____

Experiment's Signature_____Date: _____

Safety Screening Questionnaire

Do you have a neuropsychological injury?	Yes	No
Do you have a history of psychiatric disorder?	Yes	No
Do you have a history of epilepsy?	Yes	No
Does anyone in your immediate or distant family suffer from epilepsy?	Yes	No
Did you suffer from febrile seizures as an infant?	Yes	No
Do you have or have you ever had recurrent fainting spells?	Yes	No
Do you have a visual impairment that cannot be corrected with spectacles?	Yes	No
Do you have significant hearing loss?	Yes	No
Have you ever had a neurosurgical procedure (or an eye surgery?)	Yes	No
Are you on any currently not-prescribed or prescribed medications (besides oral contraceptives) ?	Yes	No
Are you currently undergoing anti - malarial treatment?	Yes	No
Have you drunk more than 3 units of alcohol in the last 24 hours?	Yes	No
Have you drunk alcohol already today?	Yes	No
Have you had more than one cup of coffee, or other sources of caffeine, in the last hour?	Yes	No
Have you used recreational drugs in the last 24 hours?	Yes	No
Did you have very little sleep last night?	Yes	No

[For TMS/TCS only]

Have you already participated in a TMS/TCS experiment today?	Yes	No
Have you participated in more that a TMS/TCS experiment in the last 6 months?	Yes	No
Is there any chance that you could be pregnant?	Yes	No
Do you currently have any of the following fitted to your body?	Yes	No
Heart pacemaker		
Cochlear implant		
Medication pump		
Surgical clips		

[For food studies only]

Are you allergic to nuts?	Yes	No
Are you allergic to the food colouring and flavouring used in candy	Yes	No

Note: If you have ticked "yes" in any of the boxes above you should not take part in these experiments.

Sex: Male Female **Age** (in years) _____

Dominant Hand: Left Right English as first language? Yes No

Participant Name in capital letters:	
Participant Signature:	**Date:**

Researcher witness: Print Name: Signature:	**Date:**