SCIENTIFIC REPORT

Project title: Sensory Entrainment for Improving Spatial Navigation

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ABSTRACT

Spatial Navigation (SN) is a complex skill essential for many animal species, including animals that requires coordingation of serveral cognitive processes, for instance episodic and working memory. At present, both invasive and non-invasive neuroimaging studies in animals (specially rodents) and humans have revealed the relevance of the hippocampus and a wide-spread brain network in SN. Theta brain oscillatory have been proposed to be the responsible for the organization of SN information into cognitive maps and to be crucial both for encoding and retrieval of SN information. Recent studies suggest that Theta oscillatory activity can be entrained by means of rhythmic sensory stimulation and result in improved associative memory.

The aim of current study was to improve SN in humans in a realistic SN task by means of rhythmc sensory stimulation in the Theta range. To do that, we developed a VR tool for generatin realistic scenarios while sensory (visual or auditory) stimulation was provided. In addition, electroencephalographic activity of subjects was recored during the SN task in order to verify successful entrainment of brain oscillations.

We performed 3 experiments and we concluded that, although we found evidence of entrainment of brain oscillatory activity in the EEG recordings, this entrainment did not result in a behavioral benefit in any of the experiments. Therefore, we conclude that sensory (audio or visual) entrainment cannot be used in VR applications in humans to improve SN skills either at short or long term.

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KEYWORDS

Spatial Navigation, Electroencephalography, Brain oscillations, Sensory Entrainment, Virtual Realtiy

BACKGROUND

Spatial Navigation (SN) is a complex skill, relevant for the survival of many animal species, including humans. Successful SN requires the coordination of several cognitive processes such as working and episodic memory, spatial positioning, sensorimotor coordination decision making, among others (Ekstrom et al., 2017). Age-related decline of SN abilities results in a loss of autonomy and life quality (Burns, 1999) that has a very negative impact on the life of elderly population. At present, Due to its relevance, SN has been thoroughly investigated by Psychologists and Neuroscientists both in non-human (Buzsáki, 2005; O'Keefe & Conway, 1978) and human populations (Epstein et al., 2017). Impaired SN skills have been related correlated with midlife risk of suffereing Alzheimer (Newton et al., 2024) and have been suggested as a reliable biomarker of cognitive decline (Plácido et al., 2022).

Because of their good SN skills, rodents have been thoroughly used in SN studies. Electrophysiological studies in rodents have revealed the importance of the hippocampal Theta oscillatory activity (3 to 8 Hz) in SN (O'Keefe & Nadel, 1978) to organize spatial information into cognitive maps that allow to integrate external information (like sensory cues, for instance) for flexible behavior. At present, although recent theories claim that the hippocampus is not restricted to the organization of SN, but all mneomnic information (Buzsáki, 2005; Eichenbaum, 2017; Epstein et al., 2017), there is no doubt about the relevance of hippocampus and theta oscillatory activity in SN.

In the last decades, studies in human population using both invasive and non-invasive neuroimaging thechniques (Baumann & Mattingley, 2021; Caplan et al., 2001; Ekstrom et al., 2017; Epstein et al., 2017; Hyman et al., 2005) have shown that SN is coordinated by means of a wide-spread brain network including (but not restricted to) hippocampus, parietal cortex and prefrontal cortex. Several studies indicate that it is possible to extract SN-related information from non-invasive, scalp, electroencephalography (EEG). Non-invasive electrophysiological recordings have also observed correlates of brain oscillatory activity (mostly in the Theta range, 3-5 Hz) to SN (Chrastil et al., 2022; Do et al., 2021; Liang et al., 2018; White et al., 2012). Scalp-recorded EEG offers the advantage of having increased temporal resolution and portability compared to fMRI studies and, contrary to invasive electrophysiological studies, allows the access to both clinical and non-clinical populations.

Although all the abovementioned studies are correlational, there is some evidence supporting a causal connection between Theta oscillatory activity and SN. In rodents, disrupting hippoampal theta oscillations has been shown to result in impaired SN (Winson, 1978). In humans, episodic and working memory (two key processes in SN) have been modulated by means of non-invasive entrainment of Theta oscillatory activity. For instance, modulating Theta peak frequency by means of parietal Transcraneal Alternate Current stimulation (TACs) changes working memory capacity (Wolinski et al., 2018). Also, using Transcranial Magnetic stimulation (TMS) disruption of frontal and parietal Theta activity results in impaired working memory (Morgan et al., 2013) whereas rhythmic Theta frontal TMS stimulation improved working memory capacity (Riddle et al., 2020). Finally, studies using sensory (audio and visual) entrainment have reported behavioral benefits in associative (Wang et al., 2018) and episodic (Roberts et al., 2018) memory, although other authors fail modulate working memory capacity by means of sensory entrainment (Pileckyte, 2024).

In the last decades, the field of SN has relied more and more in the use of realistic tasks, in particular Virtual Reality (VR) environments for the study of SN. VR environments canproduce close-to-realistic immersive experiences whilst allowing for experimental control (Diersch & Wolbers, 2019). Furthermore, VR has been proposed as a valuable tool for the assessment of navigational disorders, as well as a potential tool for interventions (Cogné et al., 2017), as the correlation between SN performance in VR environments and real life is high (Cushman et al., 2008; Diersch & Wolbers, 2019). In particular, some studies indicate age-related decline in SN skills is comparable between real life and VR situations (Cushman et al., 2008).

HYPOTHESIS AND PREDICTIONS

The aim of the present project is to modulate SN skills in healthy humans performing a realistic SN task in a VR environment by means of sensory (audio and visual stimulation). In particular, we aim at improving SN navigation skills by using sensory Theta (4 Hz) stimulation in different sensory modalities (visual, auditory and audiovisual) while participants navigate through T-Junction mazes. Electroencelographic activity will be recorded in order to assess entrainment of brain oscillatory activity.

The hypothesis and predictions of current project are the following:

1. Entrainment effect: The general hypothesis is that, if Theta band fluctuations favour encoding and maintenance of sequences for solving spatial navigation tasks, we expect that entrainment of functionally-relevant Theta rhythm will have a positive impact in spatial navigation performance (lower error rate, shorter solving time).

2. Spectral specificity: If the abovementioned improvement is related to the rhythmic character of the fluctuations, and not an epiphenomenon related to increased arousal due to exogenous stimulation per se, then we expect to observe larger behavioural effects (improvement) upon rhythmic compared to non-rhythmic entrainment conditions.

3. Cross-modal generalization: If entrainment is general across modalities, not exclusive of the visual modality (required to solve the maze visual), then it will be possible to resonate the task-relevant neural network with a non-visual modality: audio entrainers.

4. Multisensory benefit: If Theta multisensory entrainment facilitates binding of information across modalities by synchronizing different sensory brain areas in memory formation, we expect that this multisensory entrainment will result in steeper improvements than single-modality entrainment in spatial navigation tasks.

5. Memory consolidation: If the (expected) improvement in spatial navigation (encoding and maintenance) during sensory entrainment facilitates the consolidation in episodic memory (a part of long term memory), then the resulting behavioural benefit of entrainment for immediate path learning will persist in subsequent repetitions of the way finding task using the same paths, at later times.

EXPERIMENTAL PROTOCOL

We collected behavioral data from 78 subjects (30 in Visual Experiment 1, 24 in Auditory Experiment and 24 in Visual Experiment 2), and EEG data from 30 subject (Visual Experiment 1). Participant exclusion criteria were: history of epilepsy, being under medication for neurological or psychiatric disorders, and susceptibility of suffering motion sickness, screened online using the Cybersickness Susceptibility Questionnaire .

The experiment took part in a laboratory room (CBC labs, Universitat Pompeu Fabra, Barcelona, Spain). On arrival, subjects were screened for the potential to suffer cybersickness adverse symptoms (for instance headache, blurred vision, eye strain) using the Simulator Sickness Questionnaire (Kennedy et al., 1993). None of the subjects reported severe symptoms and, therefore, all proceeded to the experiment. Once the subjects were screened, they sat up on a chair, fitted the VR (HTC Vive Pro) goggles and the SN paradigm started.

The SN task consisted on learning sequential paths in T-junction virtual mazes (Figure 1). Each of the mazes had 8 junctions and were generated using the custom-made maze generation tool based on Unity developed during this project. The tool allows to generate mazes with varying lengths, trajectories and designs as well as to include backgroun sensory stimulation.

We designed a total of 25 different mazes: 9 were used in Visual Experiment 1, 8 in Auditory Experiment and 8 in Visual Experiment 2. Each of the mazes used in the series of experiments was unique in the following features:

- Each of the mazes had an unique sequence of left/right turns.
- Each maze had a different and disctintive wall pavement and colour material.

Additionally, within one experiment, each of the maze was unique in the following features:

- Each maze had walls decorated with windows and doors of different style and in different dispositions, and was populated with benches of a distinctive style.
- Each of the mazes contained one other distinctive street element (hydrant; streetlight; rubbish bin; plant; mailbox; phone; fountain; container; low wall).
- Skybox rotation. Although all the mazes were positioned in the center of the same Skybox (simulating a city center), each of them was rotated a different angle, in steps of 40 degrees.

In all experiments, subjects view all the mazes generated for the experiment. However, the condition assigned to each of the mazes was randomized across subjects. In each experiment, all mazes were presented in all experimental conditions.

For each maze, subjects first navigated the entire maze with direction cues indicating the correct turns (Guided mazes). Subjects had up to 3 attempts to correctly follow the path, but most participants completed the maze at the first attempt in this stage. Immediately after, they had 3 attempts to solve the same maze without direction cues (Immediate recall mazes). Each attempt ended upon an error or after successful completion. After studying the all mazes, participants were allowed to exit the VR environment to take a resting break. After the break, Subjects entered again the VR environment and they had to solve again the mazes learned in the first part of the experiment now presented in random order and without visual cues (Delayed recall mazes).

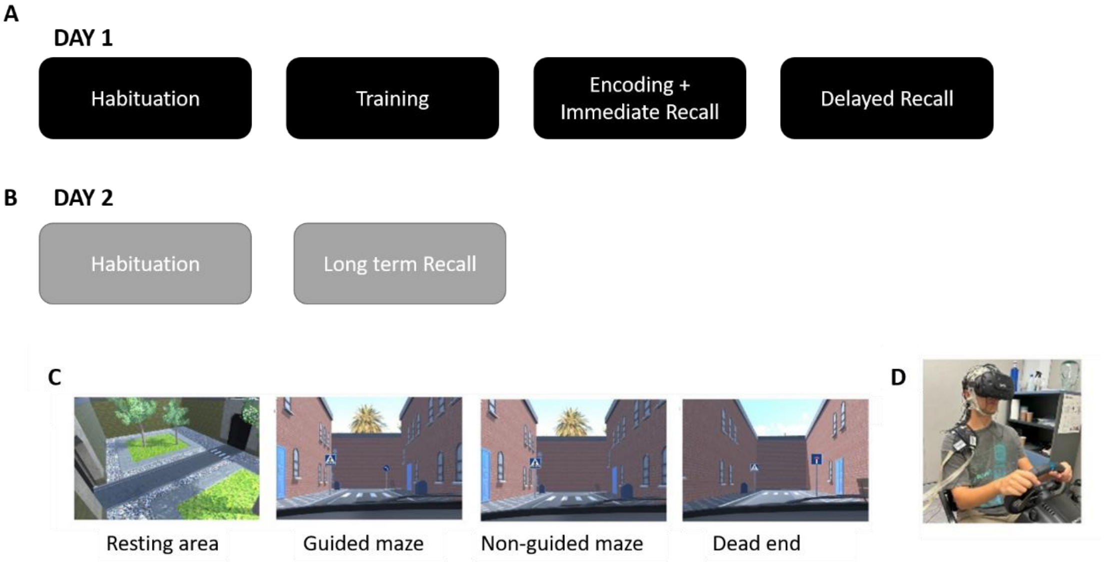

Figure 1: A: Schema of the paradigm for Session 1. B: Schema of the paradigm for Session2. C: Snapshots of the VR environment. D: Subject during the experiment.

Visual Experiment 1

In Visual Experiment 1, subjects performed 2 sessions: In Session 1, both EEG and behavioural data were collected, whereas in session 2 only behavioural data was collected. Sample size (30 subjects) was suited to detect effect sizes of Cohen's d=0.46 with 80% statistical power, and d=0.52 with 95% statistical power (at an alpha level of 0.05 in both cases).

In Session 1, subjects were presented with 6 mazes, in randomized order, 2 in each of the experimental conditions:

1. Baseline: no sensory modulation.
2. Theta: visual entrainment by means of rhythmic modulation (4 Hz) of the luminance of the scenario: display luminance was increased during 22 ms at intervals of 244 ms (square wave).
3. Control: visual modulation was performed by 22 ms duration luminance increases (square shape) presented at random latencies with the total number of increases presented equated to the rhytmic condition.

In Session 2, subjects were presented with 9 mazes, in randomized order: 6 of the mazes were the ones learned in Session 1, whereas 3 new mazes (fillers) were presented (1 per experimental condition).

Auditory Experiment

The results obtained in Visual Experiment 1 casted doubts on the possibility of modulating SN skills by means of sensory entrainment. However, exploratory analyses (Results: Visual Experiment 1) suggested that using the individual endogenous values of Theta peak frequency during task resulted in improvement of SN skills. In addition, the order of learning of the maze seem to have and impact on the subsequent recall.

Therefore, we pre-registered a modified version of the paradigm in which entraining frequency was tailored to the individual Theta frequency of each of the subjects. Sample size was selected based on two factors: Subjects available from previous experiment and control for serial position effect. To control for serial position effect, we needed to collect sample sizes multiple of 8. Given that the total number of subjects availables from previous experiments was 35, we decided to collect 24 subjects, which allowed us to capture an effect with Cohen's d=0.52 with 80% statistical power and d=0.69 with 95% statistical power.

The paradigm was the same as Session 1 in Visual Experiment 1 with two main differences. On one side, we used auditory stimulation instead of visual stimulation. Auditory entrainment consisted on tones (440 Hz, 40 ms duration, 20 ms ramp up and down) presented at the frequency of the entrainment overimposed on white noise. On the other, 8 mazes were presented, 2 for each of the experimental conditions:

4. Baseline: no sensory modulation was performed during this condition.
5. Theta: auditory entrainment at the endogenous frequency.
6. Upregulation: auditory entrainment at the endogenous frequency + 2Hz.
7. Downregulation: auditory entrainment at the endogenous frequency – 2Hz.

Visual Experiment 2

Visual Experiment 2 was identical to Auditory Experiment, save for the fact that sensory modulation was performed by display luminance increases, 22 ms duration, at intervals of 244 ms (square wave), similar to Visual Experiment 1.

RESULTS

Visual Experiment 1

Next, we briefly summarize the results reported in previous progress report, plus additional exploratory analyses that we used to improve the design of experiment.

We successfully verified the presence of entrainment in our data, according to the standard measures used in the field (Steady State Visual Evoked Potential, SSVEP, and cross-coherence, XCOH). As expected, we observed significantly higher values of SSVEP and XCOH at the entraining frequency in sensory areas (Figure 2) and better recall in Session 2 for learned compared to filler mazes.

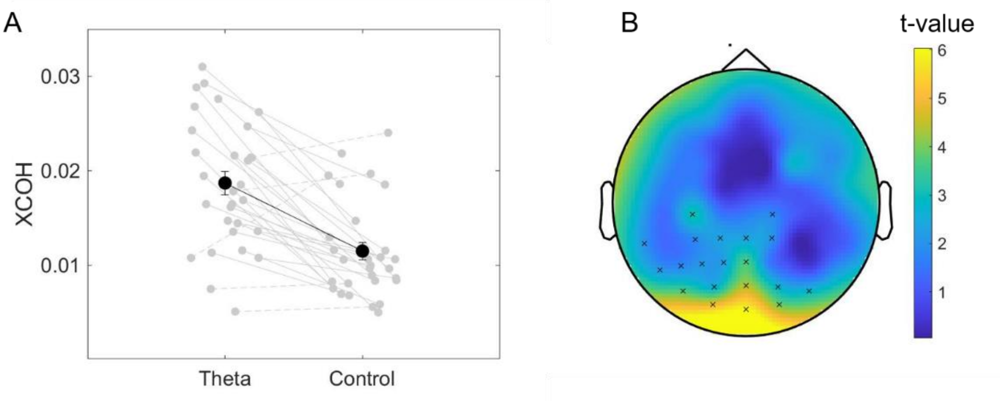

Figure 2: (A) Amplitude of the 4.1 Hz cross-coherence for Theta and Control conditions. Grey dots indicate individual values and black dots average across subjects, error bars correspond to standard error of the mean. (B) Cluster of significantly larger coherence at 4.1 Hz for Theta compared to Control condition.

However, we failed to observe a significant modulation of SN skills related to entrainment condition, either in Session 1 or Session 2 (Figures 3 and 4)

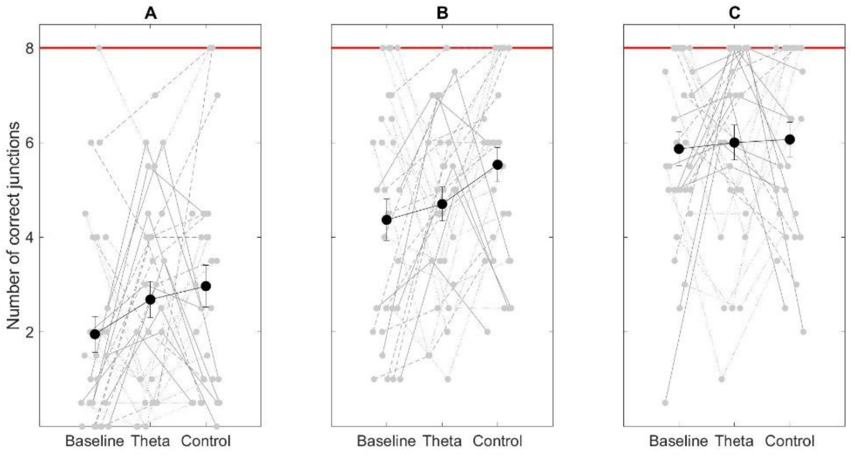

Figure 3: Performance in Session 1: Number of correct junctions for the three experimental conditions for repetition 1 (A), 2 (B) and 3 (C). Grey dots correspond to individual data, solid lines indicate subjects that have a performance in line with the hypothesis, dashed lines subjects that have a performance in line with the hypothesis in at least one of the contrasts and dotted lines indicate subjects that do not have a performance in line with the hypothesis for any of the contrasts. Black dots correspond to the average across subjects and red

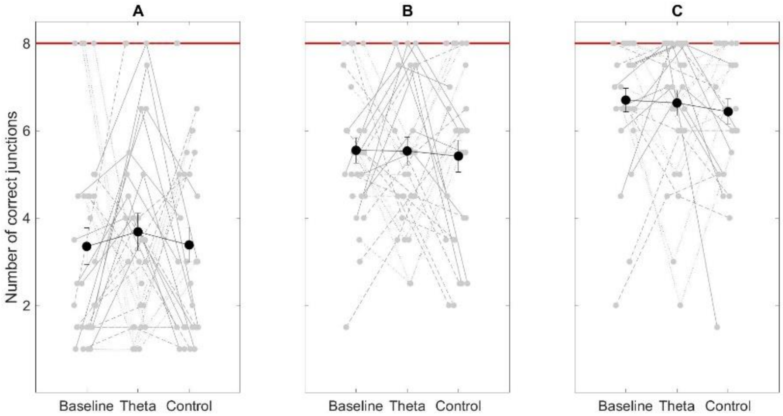

Figure 4: Performance in Session 2: Number of correct junctions for the three experimental conditions for repetition 1 (A), 2 (B) and 3 (C). Grey dots correspond to individual data, solid lines indicate subjects that have a performance in line with the hypothesis, dashed lines subjects that have a performance in line with the hypothesis in at least one of the contrasts and dotted lines indicate subjects that do not have a performance in line with the hypothesis for any of the contrasts. Black dots correspond to the average across subjects and red horizontal line indicates the maximum possible number of junctions (8).

In summary, the results indicated high variability in the effect of entrainment: although for a proportion of subjects the performance was improved when sensory theta entrainment was applied, for other subjects it was impaired. One of the potential explanations for this result was that we did not successfully entrain oscillations relevant for SN. In order to avoid adverse syntoms during navigation in the VR environment, the amplitude of the visual entrainment was small, and this could have prevented the entrainment of SN-related activity for subjects with endogenous theta frequencies far from the entraining frequency. It is commonly accepted that the amplitude of the modulation required to successfully entrain brain oscillatory activity depends on the distance between endogenous and entraining frequency, the larger the difference, the larger the amplitude required for the entrainment (Notbohm et al., 2016). Recently, a experimental study using transcranial alternate current stimulation (tACS) in primates (Krause et al., 2022) extended our knowledge of entrainment by showing that entraining at frequencies far of the endogenous frequencies at low amplitudes not only resulted in a failure of entraining brain oscillations, but also on a disruption of endogenous activity. Therefore, according to the results of Krause et al. (2022), a potential explanation of our results would be that we had successfully entrained relevant SN activty for subjects with an endogenous theta frequency close to the entraining frequency (4 Hz) whereas for those subjects with frequencies far from the entraining frequency, we could have even disrupted endogenous activity resulting in an impairment of SN. If this was the case, we would expect a negative correlation between the modulation in performance in the entrainment condition and the distance between entrainment and endogenous frequency. This was what we observed in our results.

In order to verify this, we first extracted endogenous theta peak for each of the subjects during the spatial navigation task. To do that, we used Independent Component Analysis (Artoni et al., 2018) to decompose EEG data of the subjects during each of the conditions of the SN task into pausible brain sources, and we selected for each subject the first component presenting a peak in the Theta range (3 to 8 Hz). Next, we calculated the position of the peak for Baseline, Theta and Control condition separatelly for the selected component of each subject (Figure 5). We observed that the peak of the subjects was highly variable (Figure 6), two of the subjects were discarded from the analysis because they did not present a reliable theta peak.

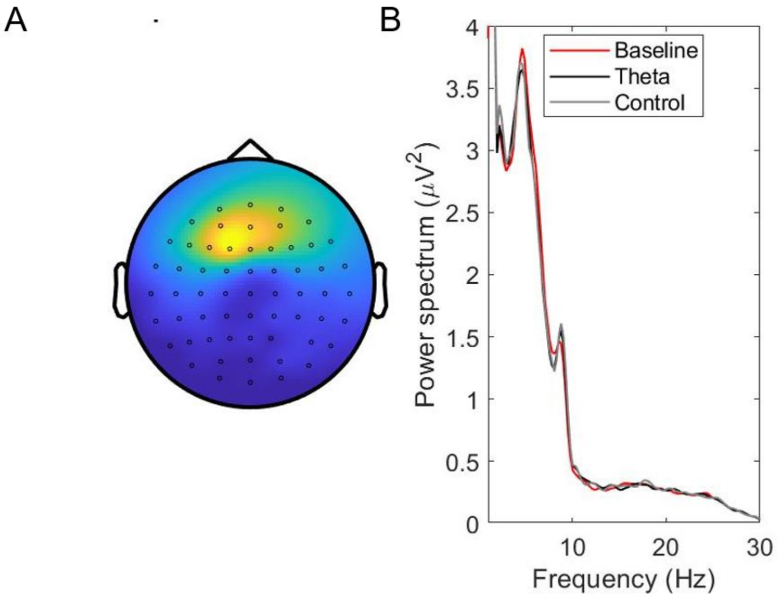

Figure 5: Example of Theta peak detected during SN task for one of the subjects. A: Scalp distribution of the component selected fo extract theta peak. B: Power spectrum of the selected component for the different experimental conditions. The power spectrum presents a clear peak in the Theta range, around 4 Hz, and a secon peak around 10 Hz, in the Alpha range.

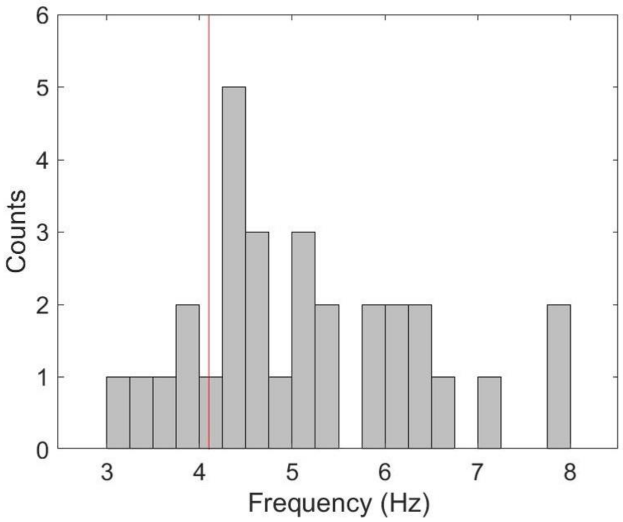

Figure 6: Distribution of the peak of endogenous theta activity for all the subjects included in the analysis. Red vertical line indicates the position of the entraining frequency.

Next, we calculated the behavioral benefit due to entrainment as the improvement in the entrainment condition with respect to the average performance of the subject as follows:

$$Normalized\ NJ = \frac{NJ - \langle NJ \rangle}{\langle NJ \rangle}$$

Where NJ stands for the number of consecutive correct junctions achieved by the subject in one condition, and $\langle NJ \rangle$ is the average number of correct junctions achieved by the subject across all conditions. $Normalized\ NJ$ ranges from -1 (impaired performance) to 1 (improved performance). We expected that subjects with endogenous frequencies close to the entraining frequency (4 Hz) will present a behavioral benefit, whereas subjects with frequencies far from the endogenous frequency will present a behavioral impairment.

Next, we calculated the correlation between endogenous peak position and behavioral benefit in the Theta condition. As expected, we observed a negative correlation between behavioral benefit and distance between endogenous peak position and entraining frequency (Figure 7, Table 1), interestingly, the correlation was still observed when we used the position of the peak calculated during the entrainment condition (Table 1). Finally, as a reality check, we calculated the behavioral benefit during Control condition using the formula previously stated and we calculated the correlation with the endogenous peak measured during Control condition. As expected, no significant correlation was observed (Table 1).

Behavioral index	*Theta peak*	*r*	*p-value*	*CI*
Theta	Baseline	-0.47	0.01	[-0.64 -0.20]
Theta	Theta	-0.37	0.02	[-0.60 -0.12]
Control	Control	0.14	0.77	[-0.15 0.49]

Table 1: Correlation between Behavioral benefit index and Theta peak measured during the different conditions of the SN task. The values presented correspond to Pearson correlation.

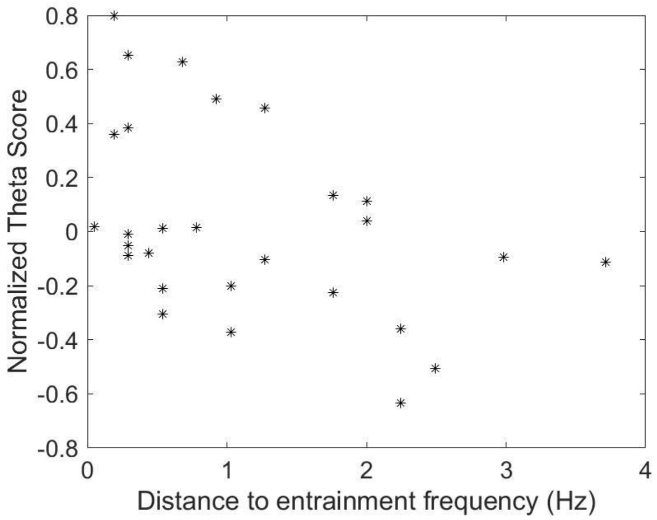

Figure 7: Behavioral benefit during the Theta condition as a function of the distance between endogenous theta peak measured in the Baseline condition and entraining frequency (4 Hz), each dot represents the data of one subject.

Therefore, these results suggest that the failure to obtain an average improvement in the SN task could be due to the fact that for subjects with endogenous Theta peak, sensory stimulation may, as a matter of fact, disrupted endogenous activity and therefore result in an impairment in perfromance.

Auditory Experiment

In view of these results, we modified the design of the Auditory Experiment as explained in the Experimental Protocol section. Only behavioural data was collected in this experiment. We used four experimental conditions (Baseline, Theta, Downregulation and Upregulation) and, for each subject, we selected endogenous Theta peak as the entraining frequency.

According to the registered analysis, we did not observe a significant improvement in SN skills due to Theta entrainment (Figure 8). We ran a repeated measures ANOVA with the factors Run and Condition and we observed that only Run had a significant impact on performance (Figure 8, Table 2).

Cases	*Sum of Squares*	*df*	*Mean Square*	*F*	*p*
Run	652.130	2	326.065	153.647	<0.001
Residuals	97.620	46	2.122		
Condition	26.813	3	8.938	1.465	0.232
Residuals	420.0896	69	6.100		
*Run*Condition*	6.141	6	1.023	0.766	0.598
Residuals	184.276	138	1.335		

Table 2: Results for the repeated measures ANOVA in Auditory Experiment.

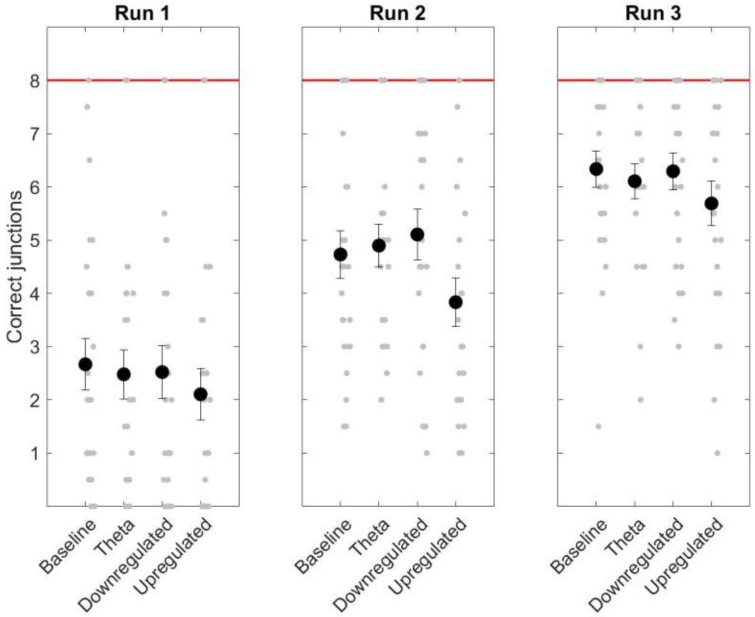

Figure 8: Auditory Experiment: Mean number of correct junctions for each of the experimental conditions and Runs. Grey dots indicate individual data, whereas black dots correspond to average values, error bars indicate the standard error of the mean. Red horizontal line indicates the maximum possible number of consecutive correct junctions.

One of the problems that we encountered in the data, similar to what happened in Experiment 1, was the variability across subjects. Therefore, we ran a complementary analysis using linear mixed models, that are well suited to incorporate individual variability as a random factor, in order to fully investigate the dataset. The number of consecutive junctions was the dependent variable, fixed factors Run (1, 2 or 3) and Condition (Baseline, Theta, Upregulated and Downregulated) and Participant number was included as Random factor. We considered the full model:

$$Proportion\ solved \sim Run + Condition + Run * Condition + \ (1|Participant)$$

And built all possible reduced models. The one with the best performance (measured with Akaike Information Criterion) was a model including both Run and Condition, but not the interaction:

$$Proportion\ solved \sim Run + Condition + \ (1|Participant)$$

A closer inspection of the model, indicated that performance increased significantly across Runs, whereas the Condition was only marginally significant (Table 3). In particular, corrected post-hoc comparisons indicated that the performance in the Upregulation condition was significantly worse than the performance for the Downregulation condition.

	χ^2	*df*	*P*(>χ^2)
Run	167.8312	2	<2-16
Condition	6.8606	3	0.08

Table 3: Type II Wald chi-square test for the mixed model selected for Auditory Experiment.

Visual Experiment 2

In view of this null result, we decided to run a new version of Visual Experiment, using the same design as Auditory Experiment but with visual stimulation instead of Auditory, because it would be possible that, given that auditory modality was completelly uninformative in the experimental design, it would be possible that entrained activity did not reach relevant brain network. Therefore we ran a modified version of Visual Experiment in to determine if Visual entrainment at the endogenous frequency was indeed causally related to SN. For this experiment, we collected 24 subjects for which we had access to Endogenous Theta peak during SN.

Similar to the Auditory Experiment, we did not observe a significant improvement of SN in the Theta condition (Table 4, Figure 9), although, overall, performance was better for all the entrainment conditions (Theta, Downregulated, Upregulated) compared to baseline condition.

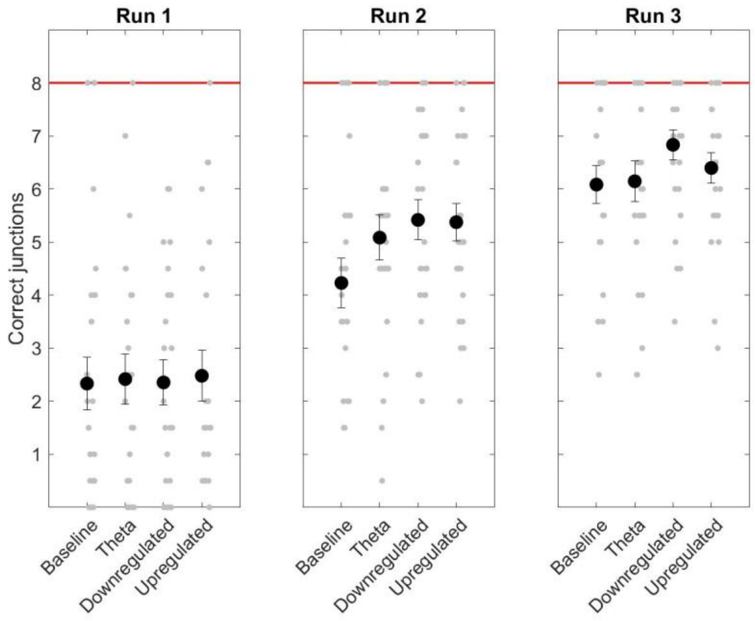

Figure 9: Visual Experiment 2. Mean number of correct junctions for each of the experimental conditions and Runs. Grey dots indicate individual data, whereas black dots correspond to average values, error bars indicate the standard error of the mean. Red horizontal line indicates the maximum possible number of consecutive correct junctions.

We modelled the data of Visual Experiment 2 using linear mixed model, similar to what we did in Auditory Experiment. The model that best explained the data was also the one including both Run and condition but not the interaction:

$$Proportion\ solved \sim Run + Condition + \ (1|Participant)$$

In this case, both factors contributed significantly to the model (Table X). Post-hoc tests indicated that the performance in the Downregulation condition was significantly better than in the baseline condition and in the Upregulation condition was marginally better than in the downregulation condition.

	χ^2	*df*	$P(>\chi^2)$
Run	155.375	2	<2-16
Condition	10.282	3	0.02

Table 4: Type II Wald chi-square test for the mixed model selected for Visual Experiment 2.

DISCUSSION

Overall, our findings do not support that sensory entrainment in the Theta range can be used to boost performance in a SN task in humans. Analyses of EEG data in the first experiment indicated successful entrainment, but this entrainment failed to improve SN of subjects.

Next, exploratory analyses, suggested that for subjects with endogenous activity close to the entraining frequency, a boost in performance had indeed happened, whereas for subjects with frequencies much larger than the frequency of entrainment, performance was worse. These results could be explained by a recent study on primates from Krause et al. (2022), in which entrainment at frequencies far from the endogenous frequency of the system resulted in an impairment of oscillatory activity. This could have explained why the impairment in performance we observed was restricted to subjects with endogneous frequencies far from the entraining frequency: disruption of Theta oscillations has been shown to impair performance in a working memory task (Morgan et al., 2013).

Therefore, we ran two behavioral experiments, in which we used entraining frequencies close to the endogenous activity of the subjects, in order to verify this potential explanation. Overall, although we observed better performance for the rhythmic stimulation conditions, endogenous Theta entrainment did not result in a significant improvement in SN performance. In addition, best condition corresponded to Downregulation condition, in which we entrained at frequencies 2 Hz slower than the endogenous frequencies of the subjects. It would be tempting to interpret this result in line with the results of Wolinski et al. (2018), that modulated working memory capactiy of subjects by means of TACs in parietal areas: slower stimulation frequencies (4 Hz) resulted in increased working memory capacity compared to faster stimulation frequencies (7 Hz) but, in that case, we would expect a negative correlation between endogenous activity and performance in the SN task for the no entrainment condition, and this is not supported by our results: there was no significant correlation between the average number of correctly recalled junctions in the baseline condition and endogenous Theta activity for either the Auditory (r=0.37, p-value=0.07) nor the Visual task (r=0.18, p-value=0.4).

Notice that, although it is possible to argue that stronger amplitudes of entrainment could result in significant entrainment, we based our selection in the comfort of the subjects: we tested our stimuli in subjects and selected the stronger values that did not result in discomfort and adverse symptoms (and therefore could not be used for practical applications).

As we anticipated in the project proposal, this was a highly risky project, but the potential benefits of a positive result were so high that it was indeed worth to investigate this path. At present, the evidence supporting the efficacy of sensory entrainment is mixed (Gallina et al., 2023): Although previous studies have successfully modulated behaviour by means of sensory stimulation (Albouy et al., 2017; Hanslmayr et al., 2019; Mathewson et al., 2012; Torralba Cuello et al., 2022; Wang et al., 2018), other studies have failed to improve or impair behavior by means of sensory stimulation (Lin et al., 2022; Pileckyte, 2024; Vilà-Balló et al., 2022).

VR TOOL FOR THE STUDY OF SN IN HUMANS

An unexpected outcome of this project was the VR tool for generating experimental protocols for the study of SN in humans. The VR proved to be very flexible, easy to use and therefore we decided to share it with other researchers, in order to make VR tools to neuroscientists without VR programming skills. The first version of the tool included the following features:

1. Flexible design of T-Junction mazes
    - 1.1. Custom maze definition. Following parameters can be defined:
        - 1.1.1. Number of junctions.
        - 1.1.2. Sequence of turns (for instance: LRRLRLLR).
        - 1.1.3. Number of allowed mistakes.
    - 1.2. Custom definition of wall and floor textures.
    - 1.3. Selection of different decoration patterns.
    - 1.4. Rotation of skybox.
    - 1.5. Choice between different navigation modes:
        - 1.5.1. All turns correct: for familiarizing with VR environment.
        - 1.5.2. Guided mode: visual cues indicate the correct direction to turn.
        - 1.5.3. Non-guided mode: T-junction maze without cues indicating the correct path.
2. Inclusion of sensory stimulation: auditory and/or visual.
    - 2.1.1. Visual stimulation consists on modulations of the overall luminance of the system.
    - 2.1.2. Auditory stimulation consists on an audio track.
3. Syncronization with EEG recording system.
4. Behavioural information at each of the decision points of a maze.

On a second step, we decided to further improve the VR environment generation tool in order to make it even more flexible and accessible to other researchers. In the second version we included the following improvements:

1. Increased flexibility in the desing of the environment: The new version of the tool includes T-Junction mazes and Grid designs, therefore increasing the type of tasks that can be designed with the maze (wandering, looking for a target, following a preset path, recalling a learnt path…).
2. Including Eye-Tracking data: in addition to behavioural and electrophysiological data the tool records time-resolved information of the fixations made for the subject. During fixations, we can access the information about the foveal area of the field of vision of the subject, and as the tool allows to pre-define targets of interest, we can have information about where the gaze of the subject is being directed to during the navigation.
3. Increased flexibility in the synchronization with EEG recording system: in the new version, new events can be included, in case the ones defined a priori do no tinclude key events of the designed experiment.
4. Including the option of controlling the VR environment with generic VR commands, therefore it is not necessary to use a speciffic steering wheel to run the program.
5. Customizable avatar appearance. In the first version, subjects were represented by a white male avatar, and this could decrease the sense of immersion of a large amount of subjects. In the current version of the tool, subjects can select between different options of an avatar in order to maximize the sense of immersion.
6. Improved navigation. Based on the experience of the subjects collected during the pilots and experiments of the project, we improved some details of the navigation in order to reduce cybersickness: inertia at the stop of the car and possibility to decrease movement speed and acceleration during the turns.
7. Code accessibility: Different versions of the VR environment generation tool will be shared and documented, allowing for different levels of personalization:

7.1. Compiled version: this version is directed to researchers that want a ready to use tool, and allows for customization of different items (for intstance maze path, difficulty and appearance, sensory stimulation files, avatar appearance…).
7.2. Semi-compiled version: this version allows for further customization in a Unity environment using tools of the Unity program menu, without hard coding. This will allow, for instance, to include additional events for the EEG, or to add new decorations.
7.3. Open source version: this version allows to access and modify any part of the VR environment generation tool.

It is important to mention that the code will be shared using a Creative Commons Licence: Attribution, Non-Commercial, Share-Alike that prevents the commertial use of the code by third parts.

8. Documentation: The VR programming tool will include a wiki (work in progress), in which all the features of the code will be detailed. The link for the wiki is: LINK.

ACHIEVED OBJECTIVES

Design, implementation and testing of a VR tool for generating the experimental materials required for the project.

The objective was completelly fullfilled: We generated a tool using Unity Software that allowed to generate mazes with tailored difficulty and include visual or/and auditory stimulation selectively and co-register EEG activity of the subjects while they were performing the task. We used approximatelly 8 months for the generation of the tool and 2 months for thecalibration and time-testing (synchronization with EEG data and times of sensory stimulation). The outcome of this part of the project was an undergraduate thesis (Marcos Sánchez Torrent: *Tool for generating and controlling SN experiments in VR*, supervisors: Mireia Torralba Cuello and Josep Blat, Universitat Pompeu Fabra 2022), an oral contribution in Iberian Conference in Perception (Sergi Àvila Sangüesa, Marcos Sánchez Torrent, Salvador Soto-Faraco, Lluis Fuentemilla, Josep Blat and Mireia Torralba Cuello: *Virtual Reality maze generator tool for the study of spatial navigation in humans*, Barcelona 2022), and the VR tool that was optimized and expanded in the last part of the project. The tool at present is stored in a private repository in GitHub: https://github.com/mireiatorralba/T-Lab_Gen, that will contain the three versions of the code (compiled, semi-compiled, raw code), and the detailed instructions for the installation and control of the VR tool. This repository will be made public on the publication of a paper about the VR generation tool, that is under preparation and will be submitted to Journal of Neuroscience Methods or a similar journal.

Development and testing of the sensory stimulation (visual, auditory and audiovisual) for entrainment in the theta range

This objective was completelly fulfilled: We generated rhytmic visual and auditory tracks at the frequencies of interest at different amplitude levels. Next, given that the use of strong visual or auditory stimulation could increase the chances of suffering cybersickness or adverse symptoms (headache, eye strain,dizziness…) in subjects, we performed pilot studies in order to determine the maximal amplitudes of the stimulation that could be used without creating adveser symptoms.

Open science: pre-registration of three experiments

We accomplished the objective. Previous to data analyses we registered the three experiments ran in the project: Visual Experiment 1, Auditory Experiment and Visual Experiment 2. The three registrations can be found in Open Science Foundation, in the link Sensory Entrainment for Improving Spatial Navigation.

Data collection and analysis

As planned, we collected data of 3 different experiments. However, in view of the null results obtained data collection had some modifications. EEG+behavioural data was collected for 30 subjects in Visual Experiment 1, and behavioral data was collected for 48 subjects in Auditory

Experiment and Visual Experiment 2 (24 subjects each). The data of the three experiments has been analyzed according to the pre-registration, and additional exploratory analyses have been applied to the data. Preliminary results were presented in EEG Garden (Mireia Torralba Cuello, Luis Fuentemilla Garriga, Josep Blat, Angela Marti-Marca, Márta Szabina Pápai and Salvador Soto-Faraco: *Using visual sensory entrainment for modulating Spatial Navigation in Virtual Reality environments,* San Sebastián 2023). and, at present, we are preparing a publication of the results obtained in the project.

EXPECTED AND ACHIEVED OUTPUT INDICATORS

Output indicators	Expected (according to application)	Achieved
PhD thesis	0	0
Master's thesis	2	1
Organization of seminar or conference	0	0
Book	0	0
Book chapter	0	0
Conference presentation	2	2
Conference paper	0	0
Journal article	2	0
Software tool for generation of VR environments	0	1

BIBLIOGRAPHY

Albouy, P., Weiss, A., Baillet, S., & Zatorre, R. J. (2017). Selective Entrainment of Theta Oscillations in the Dorsal Stream Causally Enhances Auditory Working Memory Performance. *Neuron*, *94*(1), 193-206.e5. https://doi.org/10.1016/j.neuron.2017.03.015

Artoni, F., Delorme, A., & Makeig, S. (2018). Applying dimension reduction to EEG data by Principal Component Analysis reduces the quality of its subsequent Independent Component decomposition. *NeuroImage*, *175*, 176-187. https://doi.org/10.1016/j.neuroimage.2018.03.016

Baumann, O., & Mattingley, J. B. (2021). Extrahippocampal contributions to spatial navigation in humans: A review of the neuroimaging evidence. *Hippocampus*, *31*(7), 640-657. https://doi.org/10.1002/hipo.23313

Burns, P. C. (1999). Navigation and the mobility of older drivers. *Journals of Gerontology - Series B Psychological Sciences and Social Sciences*, *54*(1), 49-55. https://doi.org/10.1093/geronb/54B.1.S49

Buzsáki, G. (2005). Theta rhythm of navigation: Link between path integration and landmark navigation, episodic and semantic memory. *Hippocampus*, *15*(7), 827-840. https://doi.org/10.1002/hipo.20113

Caplan, J. B., Madsen, J. R., Raghavachari, S., & Kahana, M. J. (2001). Distinct Patterns of Brain Oscillations Underlie Two Basic Parameters of Human Maze Learning. *Journal of Neurophysiology*, *86*(1), 368-380. https://doi.org/10.1152/jn.2001.86.1.368

Chrastil, E. R., Rice, C., Goncalves, M., Moore, K. N., Wynn, S. C., Stern, C. E., & Nyhus, E. (2022). Theta oscillations support active exploration in human spatial navigation. *NeuroImage*, *262*, 119581. https://doi.org/10.1016/j.neuroimage.2022.119581

Cushman, L. A., Stein, K., & Duffy, C. J. (2008). Detecting navigational deficits in cognitive aging and Alzheimer disease using virtual reality. *Neurology*, *71*(12), 888-895. https://doi.org/10.1212/01.wnl.0000326262.67613.fe

Diersch, N., & Wolbers, T. (2019). The potential of virtual reality for spatial navigation research across the adult lifespan. *Journal of Experimental Biology*, *222*(Suppl_1), jeb187252. https://doi.org/10.1242/jeb.187252

Do, T.-T. N., Lin, C.-T., & Gramann, K. (2021). Human brain dynamics in active spatial navigation. *Scientific Reports*, *11*(1), 13036. https://doi.org/10.1038/s41598-021-92246-4

Ekstrom, A. D., Huffman, D. J., & Starrett, M. (2017). Interacting networks of brain regions underlie human spatial navigation: A review and novel synthesis of the literature. *Journal of Neurophysiology*, *118*(6), 3328-3344. https://doi.org/10.1152/jn.00531.2017

Epstein, R. A., Patai, E. Z., Julian, J. B., & Spiers, H. J. (2017). The cognitive map in humans: Spatial navigation and beyond. *Nature Neuroscience*, *20*(11), 1504-1513. https://doi.org/10.1038/nn.4656

Gallina, J., Marsicano, G., Romei, V., & Bertini, C. (2023). Electrophysiological and Behavioral Effects of Alpha-Band Sensory Entrainment: Neural Mechanisms and Clinical Applications. *Biomedicines*, *11*(5), 1399. https://doi.org/10.3390/biomedicines11051399

Hanslmayr, S., Axmacher, N., & Inman, C. S. (2019). Modulating Human Memory via Entrainment of Brain Oscillations. *Trends in Neurosciences*, *42*(7), 485-499. https://doi.org/10.1016/j.tins.2019.04.004

Hyman, J. M., Zilli, E. A., Paley, A. M., & Hasselmo, M. E. (2005). Medial prefrontal cortex cells show dynamic modulation with the hippocampal theta rhythm dependent on behavior. *Hippocampus*, *15*(6), 739-749. https://doi.org/10.1002/hipo.20106

Kennedy, R. S., Lane, N. E., Berbaum, K. S., & Lilienthal, M. G. (1993). Simulator Sickness Questionnaire: An Enhanced Method for Quantifying Simulator Sickness. *The International Journal of Aviation Psychology*, *3*(3), 203-220. https://doi.org/10.1207/s15327108ijap0303_3

Krause, M. R., Vieira, P. G., Thivierge, J.-P., & Pack, C. C. (2022). Brain stimulation competes with ongoing oscillations for control of spike timing in the primate brain. *PLOS Biology*, *20*(5), e3001650. https://doi.org/10.1371/journal.pbio.3001650

Liang, M., Starrett, M. J., & Ekstrom, A. D. (2018). Dissociation of frontal-midline delta-theta and posterior alpha oscillations: A mobile EEG study. *Psychophysiology*, *55*(9), e13090. https://doi.org/10.1111/psyp.13090

Lin, W. M., Oetringer, D. A., Bakker-Marshall, I., Emmerzaal, J., Wilsch, A., ElShafei, H. A., Rassi, E., & Haegens, S. (2022). No behavioural evidence for rhythmic facilitation of perceptual discrimination. *European Journal of Neuroscience*, *55*(11-12), 3352-3364. https://doi.org/10.1111/ejn.15208

Mathewson, K. E., Prudhomme, C., Fabiani, M., Beck, D. M., Lleras, A., & Gratton, G. (2012). Making Waves in the Stream of Consciousness: Entraining Oscillations in EEG Alpha and Fluctuations in Visual Awareness with Rhythmic Visual Stimulation. *Journal of Cognitive Neuroscience*, *24*(12), 2321-2333. https://doi.org/10.1162/jocn_a_00288

Morgan, H. M., Jackson, M. C., Van Koningsbruggen, M. G., Shapiro, K. L., & Linden, D. E. J. (2013). Frontal and parietal theta burst TMS impairs working memory for visual-spatial conjunctions. *Brain Stimulation*, *6*(2), 122-129. https://doi.org/10.1016/j.brs.2012.03.001

Newton, C., Pope, M., Rua, C., Henson, R., Ji, Z., Burgess, N., Rodgers, C. T., Stangl, M., Dounavi, M.-E., Castegnaro, A., Koychev, I., Malhotra, P., Wolbers, T., Ritchie, K., Ritchie, C. W., O'Brien, J., Su, L., Chan, D., & Programme, for the P. D. R. (2024). Entorhinal-based path integration selectively predicts midlife risk of Alzheimer's disease. *Alzheimer's & Dementia*, *20*(4), 2779-2793. https://doi.org/10.1002/alz.13733

Notbohm, A., Kurths, J., & Herrmann, C. S. (2016). Modification of Brain Oscillations via Rhythmic Light Stimulation Provides Evidence for Entrainment but Not for Superposition of Event-Related Responses. *Frontiers in Human Neuroscience*, *10*. https://doi.org/10.3389/fnhum.2016.00010

O'Keefe, J., & Conway, D. H. (1978). Hippocampal place units in the freely moving rat: Why they fire where they fire. *Experimental Brain Research*, *31*(4), 573-590. https://doi.org/10.1007/BF00239813

Pileckyte, I. (2024). *The Functional role of theta oscillations in visual working memory performance* [Universitat Pompeu Fabra]. http://hdl.handle.net/10803/689852

Plácido, J., de Almeida, C. A. B., Ferreira, J. V., de Oliveira Silva, F., Monteiro-Junior, R. S., Tangen, G. G., Laks, J., & Deslandes, A. C. (2022). Spatial navigation in older adults with mild cognitive impairment and dementia: A systematic review and meta-analysis. *Experimental Gerontology*, *165*(April). https://doi.org/10.1016/j.exger.2022.111852

Riddle, J., Scimeca, J. M., Cellier, D., Dhanani, S., & D'Esposito, M. (2020). Causal Evidence for a Role of Theta and Alpha Oscillations in the Control of Working Memory. *Current Biology*, *30*(9), 1748-1754.e4. https://doi.org/10.1016/j.cub.2020.02.065

Roberts, B. M., Clarke, A., Addante, R. J., & Ranganath, C. (2018). Entrainment enhances theta oscillations and improves episodic memory. *Cognitive Neuroscience*, *9*(3-4), 181-193. https://doi.org/10.1080/17588928.2018.1521386

Torralba Cuello, M., Drew, A., Sabaté San José, A., Morís Fernández, L., & Soto-Faraco, S. (2022). Alpha fluctuations regulate the accrual of visual information to awareness. *Cortex*, *147*, 58-71. https://doi.org/10.1016/j.cortex.2021.11.017

Vilà-Balló, A., Marti-Marca, A., Torralba Cuello, M., Soto-Faraco, S., & Pozo-Rosich, P. (2022). The influence of temporal unpredictability on the electrophysiological mechanisms of neural entrainment. *Psychophysiology*, *59*(11), e14108. https://doi.org/10.1111/psyp.14108

Wang, D., Clouter, A., Chen, Q., Shapiro, K. L., & Hanslmayr, S. (2018). Single-Trial Phase Entrainment of Theta Oscillations in Sensory Regions Predicts Human Associative Memory Performance. *The Journal of Neuroscience*, *38*(28), 6299-6309. https://doi.org/10.1523/JNEUROSCI.0349-18.2018

White, D. J., Congedo, M., Ciorciari, J., & Silberstein, R. B. (2012). Brain Oscillatory Activity during Spatial Navigation: Theta and Gamma Activity Link Medial Temporal and Parietal Regions. *Journal of Cognitive Neuroscience*, *24*(3), 686-697. https://doi.org/10.1162/jocn_a_00098

Winson, J. (1978). Loss of Hippocampal Theta Rhythm Results in Spatial Memory Deficit in the Rat. *Science*, *201*(4351), 160-163. https://doi.org/10.1126/science.663646

Wolinski, N., Cooper, N. R., Sauseng, P., & Romei, V. (2018). The speed of parietal theta frequency drives visuospatial working memory capacity. *PLOS Biology*, *16*(3), e2005348. https://doi.org/10.1371/journal.pbio.2005348