

Transcutaneous electrical stimulation enhances episodic memory encoding via a noradrenaline-attention network, with associated neuroinflammatory changes

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ABSTRACT

Background: Attention plays a central role in learning and memory processes. Prior research has demonstrated how goal-directed attention influences successful performance on both attention and working memory tasks. However, an important question remains about whether long-term memory outcomes can be reliably enhanced by targeting attention processes.

Objective: To test the hypothesis that 40 Hz Non-invasive Transcutaneous Electrical Stimulation of the Greater Occipital Nerve (NITESGON) would enhance long-term memory encoding by upregulating theta activity in the dorsal attention network. We also hypothesised that this would be in association with upregulated noradrenaline activity and downregulated cytokine activity.

Methods: In two double-blinded experiments, learning and memory were tested via a Swahili-English word-association task completed on 2 visits (separated by 1 week). 60 individuals were randomized to assess 40 Hz NITESGON's effect compared to active-control (1 Hz) or sham conditions. Before and after stimulation, rs-EEG assessed theta activity in the dorsal attention network, and saliva measures were collected incl. salivary alpha amylase (sAA; a proxy for noradrenaline activity) and cytokines (IL-6, IL-1 β and TNF- α).

Results: Participants receiving 40 Hz NITESGON learned and remembered more words than control or sham groups. There were no significant differences in consolidation between the groups. 40 Hz NITESGON was associated with increased theta activity in the dorsal attention network, and this activation was associated with enhanced learning but not memory performance. The 40 Hz NITESGON group had significantly upregulated sAA post-stimulation, with this associated with learning and memory (supporting a LC-NA mechanism). Modulation of IL-1 β and TNF- α were not frequency specific. However, modulation of IL-6 was specific to 40 Hz and was associated with memory outcomes.

Conclusion: 40 Hz NITESGON can activate a noradrenaline – dorsal attention network, to facilitate goal-directed attention during encoding stages of a long-term memory task, in association with neuroinflammatory changes.

1. Introduction

Having the ability to retain information over time supports our decision-making by enabling us to draw on our past experiences. This ability rests on both memory encoding (how information is attended to and stored temporarily) and consolidation (how information is transferred from short-term to long-term storage) processes. Countless research has investigated memory consolidation and the underlying

brain regions [1–3]. However, an equally important step is the initial stage where information is learnt or encoded, and in which attention plays a central role [4]. Our attention can be driven by stimuli in our environment (i.e. bottom-up factors), or by our goals or expectations in a given moment (i.e. top-down regulation) [5].

A neurobiological model of attention termed the Dual-Attention Theory [5], proposes that attention processes are supported by two neural systems, each relying on separate, but somewhat overlapping

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functional networks connected to the lateral posterior parietal cortex (PPC) [4]. According to this model, activation in a dorsal-PPC network underlies attention that is guided by one's goals [6]. Whereas a ventral-PPC pathway facilitates more bottom-up or stimulus-driven attention processes [4,5]. These networks are often referred to as the dorsal attention network and ventral attention network, respectively. This theory is supported by: functional magnetic resonance imaging (fMRI) during attention-related tasks [6,7]; resting-state functional connectivity magnetic resonance imaging (rs-fcMRI) [6,8]; magnetoencephalography (MEG) [9,10]; and resting-state electroencephalography (rs-EEG) [7]. While the ventral (stimulus-driven) attention network has been shown to be centred on right lateralised temporoparietal cortical and inferior frontal cortical regions [4,5], key nodes of the dorsal (goal-driven) attention network have been identified as the bilateral intraparietal sulci and frontal eye field regions [7,11–13].

In the memory domain, research has demonstrated the role of the dorsal attention network in the encoding stages of a working memory task [14]. In fact, two meta-analyses of neuroimaging data confirm the associations with this dorsal attention network during encoding and subsequent successful memory recall [4,15]. Adding to these findings, theta frequency oscillations in the dorsal attention regions, either at-rest or during a task, have been shown to be both recruited by, and essential for facilitating goal-directed attention in both clinical and healthy samples [7,9].

Interestingly, it has been postulated that shifting of attention between top-down and bottom-up regulation, is in part modulated by noradrenaline (NA) from the locus coeruleus (LC) [5]. Supporting this, a recent rodent study demonstrated how dorsal and ventral attention pathways can be modulated by LC-NA projections to distinct cortical areas, with differential impacts on top-down vs bottom-up attention respectively [16]. These findings and others emphasise how LC-NA activity can modulate attentional processes and play critical roles in learning, memory and plasticity [17–19]. One of the mechanisms through which LC-NA activity can impact synaptic plasticity processes is through mediating communication between the nervous system and immune system, modifying immune system responses and altering microglial morphology [20].

With regards to chemical messengers of the immune system, the role of three cytokines (IL-1 β , IL-6 and TNF- α) has been emphasized [21], especially for their ability to synergistically modulate neuronal activation, and plasticity processes, consequently influencing larger neural networks, and learning and memory processes [22,23]. Moreover, chemical ablation of LC neurons is accompanied by altered cytokine levels [24] and increased neuroinflammation and neurodegeneration [25]. Additionally, NA specifically can act on microglia via their α and β adrenergic receptors, mediate their activity [26], and influence pro-inflammatory cytokine activity [27], with reduced cytokine levels associated with improved neuroinflammation [28] and neuroprotection at multiple levels [29]. Consequently, NA has been suggested as the most likely candidate to facilitate communication between the nervous system and immune system [20]. Thus, techniques that can modulate LC-NA activity, will likely have a multitude of positive interactions on attention, synaptic plasticity, learning and memory pathways.

In recent years, peripheral nerve stimulation has emerged as a viable mechanism behind traditional non-invasive electrical brain stimulation approaches [30–32]. Empirical studies have demonstrated that behavioural effects elicited from stimulation of peripheral nerves critically rests upon activation of LC-NA activity [33–35]. Specifically, stimulation of the vagus nerve, trigeminal nerve or greater occipital nerve have each been shown to influence activation of the LC [36], and NA release, with LC-NA activation being essential for successful learning [37], motor [32] and memory processes [34].

For example, in a prior double-blinded study, our lab applied non-invasive transcutaneous electrical stimulation of the greater occipital nerve (NITESGON) using direct current while participants completed a word association task. Memory recall was measured, and several

markers of LC-NA activity were measured (namely salivary alpha-amylase (sAA), pupil diameter and the P3b ERP). Our findings demonstrated that NITESGON using direct current resulted in increased sAA levels post-stimulation in comparison to pre [34]. This change in sAA levels was correlated with both pupil diameter and the P3b ERP. Moreover, this sAA change was correlated with memory outcomes on day 7, but not initial encoding [34].

Adding to this, a more recent study by our lab reported how using NITESGON to apply either 40 Hz alternating current or direct current, impacted long term memory outcomes differentially [38]. In that study both direct current and 40 Hz NITESGON led to enhanced memory recall 7 days later. The 40 Hz group's enhanced memory recall stemmed from greater learning on the first day. However, while the direct current group also demonstrated significantly enhanced memory recall, this stemmed from offline consolidation benefits, and not enhanced initial learning. Moreover, only the direct current NITESGON group demonstrated significantly reduced rates of forgetting over the 7 days [38]. Thus taken together, NITESGON using direct current can be seen to enhance memory via consolidation [38], with long-term memory performance associated with increased sAA [34]. Whereas NITESGON using 40 Hz alternating current enhanced memory via upregulated encoding [38].

Further preliminary studies indicate that peripheral nerve stimulation of the vagus nerve downregulates inflammatory cytokines including IL-1 β , IL-6 and TNF- α [39,40] that goes together with improved cognitive responses [41]. In parallel, recent research demonstrates that entraining oscillations at 40 Hz, using a non-invasive light flicker treatment, reduces inflammatory responses [42], improves memory and is protective against neurodegeneration in animals [43]. However, there is a dearth of research looking at the combined CNS and immune system effects of peripheral nerve stimulation on learning and memory processes, especially in humans. This is particularly surprising given the significant crosstalk that exists between the CNS and immune system [44], and their overlapping roles in neuroplasticity and learning and memory processes.

Here we conducted 2 double-blinded experiments to test the hypothesis that 40 Hz NITESGON (known for its peripheral LC-NA mechanism) would enhance long-term memory by upregulating encoding-stage processes. Based on the literature [39,40] and prior work by our lab [38,45,46], it was hypothesised that 40 Hz NITESGON's impact on long-term memory performance would specifically stem from an online encoding benefit, in association with theta oscillatory activity in key nodes of the dorsal attention network, as well as upregulated noradrenaline activity and downregulated cytokine activity.

Specifically, in Experiment 1, participants were randomly assigned to receive 40 Hz NITESGON (active condition) or 1 Hz NITESGON (active-control) during a word-association task, while theta activity in key nodes of the dorsal attention network (i.e. bilateral frontal eye fields and intraparietal sulcus) and changes in IL-6 cytokine were measured. Experiment 2 built on these findings, with participants randomly assigned to one of three groups (i.e. 40 Hz NITESGON, 1 Hz NITESGON and sham NITESGON). In addition to theta activity in the dorsal attention network and levels of IL-6, in Experiment 2 changes in IL-1 β and TNF- α were also measured, as were changes in salivary alpha-amylase (as a proxy for noradrenaline activity).

2. Experiment 1

2.1. Methods

This experiment was designed as a double-blinded, placebo-controlled, randomized, parallel group study, in accordance with the ethical standards of the Helsinki declaration (1964) and approved by the Institutional Review Board of the University of Texas at Dallas (#18–42). Participants were screened to ensure no history of medical, neurological, psychiatric disorders or any tACS contraindications,

including previous history of epileptic insult, head injury, diagnosis of neuropsychiatric disorders, taking neuropsychiatric medications or prescription stimulants, or chronic use of illicit drugs. All participants had similar educational background (i.e. students enrolled in UT Dallas), and had normal or corrected-to-normal vision, and no participants were taking medication or other drugs. Participants were native-English speakers, with no prior familiarity of or exposure to Arabic language or Swahili (due to the task stimuli).

The sample size in the present study was estimated based on our previous research using the word association task [47]. We calculated the sample size by assuming an α level of .05 (one-sided), power of 80 %, and a strong effect size for the word association test during Visit 2. This resulted in a sample size of 30.

Thirty healthy, right-handed participants completed this experiment (15 males and 15 females; mean age 23 years, SD = 4.64 years). Prior to the first session, participants were instructed to abstain from: dental work for 48hrs, alcohol for 24hrs, caffeine and nicotine for 16hrs, and hair styling products the day of. On the day of the study, participants provided written, informed consent.

2.1.1. Procedure

Participation involved two visits to the lab in UT Dallas. On Visit 1: control assessments incl. demographic items, the Mini Mental State Exam (MMSE), Beck Anxiety Inventory (BAI), and Beck Depression Inventory (BDI), Visual Analog Scale (VAS) and Profile of Mood States (POMS) were completed; stimulation and a computer-based word-association task were administered; changes in rs-EEG and salivary IL-6 cytokine were measured pre-and-post stimulation, and a stimulation exit questionnaire was completed. 30 participants were randomly

assigned to receive either 40 Hz NITESGON (active condition) or 1 Hz NITESGON (active-control) during the word-association task. A second researcher administered the stimulation, ensuring that the researcher who instructed the participant and conducted the analysis was blind to the stimulation protocol. Participants returned exactly 1 week later for visit 2 to test memory recall performance.

2.1.2. NITESGON

Alternating current (AC) was transmitted via a pair of saline-soaked (1.3 % saline) sponges (5 cm × 7 cm) and was delivered by a specially-developed, battery-driven, alternating current stimulator with a maximum output of 10 mA (device name ‘DC Stimulator Plus’ <http://www.neuroconn.de>). Electrodes were placed over the left and right C2 nerve dermatome, and impedance under each electrode was maintained under 10 k Ω . NITESGON was applied using a current of ± 1.5 mA peak intensity (3 mA peak to peak; current density 21.43 μ A/cm²) for each of the 3 study phases (3 × 375s) only, for a total of 18.75 min. No stimulation was applied during rest or testing phases (see Fig. 1). The stimulation was phasic with a sinusoidal waveform. Participants were randomly-assigned to one of two groups: NITESGON delivered stimulation at a frequency of 40 Hz with 0° phase difference (active), or at a frequency of 1 Hz with 0° phase difference (active-control). In both conditions, the stimulation ramped up and down for 30s each.

2.1.3. Word-association task

Participants completed a computerized 75-pair Swahili-English word association task, adapted from a well-established paradigm by Karpicke & Roediger [48], and following a similar format to previous studies conducted by our lab [34,35,38]. The task was presented on a computer

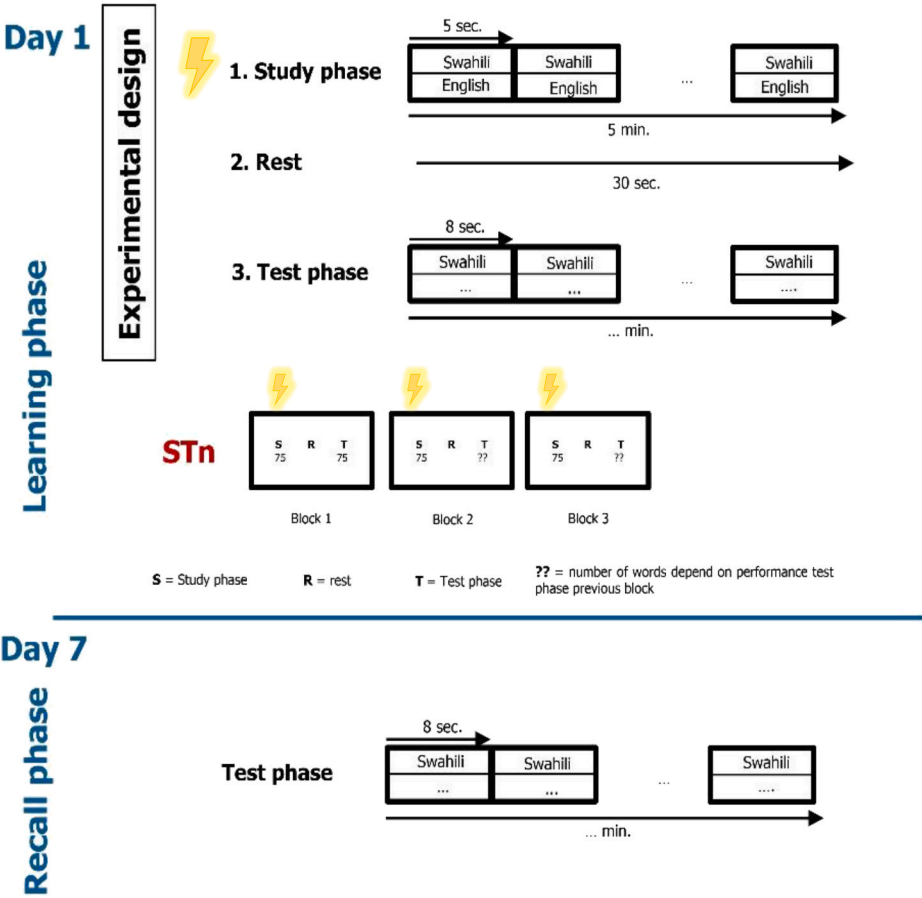


Fig. 1. | Experiment 1 and 2 - Swahili-English word-association paradigm design. The learning phase on Day 1 consisted of 3 blocks (with a study phase, rest, and test phase) of a Swahili-English word association task. Memory was tested when participants returned 1 week later for 1 block of a cued recall phase of this task.

with a 27-inch screen positioned at eye level, with a refresh rate of 60 Hz. The task was generated using Visual Studio software using C#. It involved 3 blocks, each with a study and test phase. In the study phase, each Swahili-English word pair (e.g. Bustani – Garden) were shown on screen for 5 s at a time, with the Swahili word on top, and English translation underneath (5s x 75 pairs = 375s). Words were presented in black, on a white background. A 30 s rest separated the study phase from the test phase. In the test phase, each Swahili cue word was presented on screen for 8 s, and the participant was tasked with typing the English translation. If no response was given, the computer program automatically moved onto the next Swahili cue. This cycle was repeated twice more. Any correctly recalled pairs from the 1st and 2nd testing phases were dropped from being tested in subsequent test phases (but were still presented in all 3 study phases). This meant that the study phase was always 375s per block (i.e. 5s x 75 pairs), with 3 blocks in total. However, the length of the test phase did vary for each participant depending on how many words they had correctly learned in the previous block. Word order was randomized in each phase and block. Cumulative rates of learning were calculated across the 3 test phases.

When participants returned for the 2nd visit a week later, they completed only 1 testing phase where each of the 75 Swahili word cues were shown on screen one at a time and their task was to type the correct English translation. There was no review of word-pairs and no stimulation on this visit. The structure of this word-association paradigm is presented in Fig. 1.

2.1.4. Rs-EEG

Resting-state EEG data were collected from each participant pre-and-post stimulation/task. The data were collected using a 64-channel Neuroscan SynAmps 2 Quick Cap (configured using the International 10–20 placement system with the midline reference located at the vertex and the ground electrode located at AFz using the Neuroscan Scan 4.5 software). Impedance on each electrode was maintained at < 5 k Ω . The data were sampled using the Neuroscan Synamps² amplifier at 500Hz with online band-pass filtering at 0.1–100 Hz.

Eyes-closed recordings (sampling rate = 1 kHz, band passed DC–200Hz) for approximately 5 min were obtained while participants sat upright in a comfortable chair in a dark/dimly lit room. Participants were instructed not to drink alcohol (24hrs prior) or caffeinated beverages (1hr prior) to EEG recording. To prevent drowsiness-related enhanced theta power during EEG recording, the alertness of participants was checked by monitoring both slowing of the alpha rhythm and appearance of spindles in the EEG stream [49].

For EEG preprocessing, the data was resampled to 128 Hz, band-pass filtered (Finite Impulse Response filter) to 2–44 Hz, and re-referenced to the average reference using EEGLAB 14_1_1b [50]. The EEG data was then plotted for a careful inspection for artifacts. All episodic artifacts suggestive of eye blinks, eye movements, jaw tension, teeth clenching, or body movements were manually removed from the EEG stream. In addition, an independent component analysis (ICA) was conducted to further verify whether all artifacts were excluded. Standardized low-resolution brain electromagnetic tomography (sLORETA) was used to estimate the intracerebral electrical sources that generated the scalp-recorded activity in each of the theta frequency bands (4–7.5 Hz) [51]. sLORETA computes neuronal activity as current density (A/m²) without assuming a predefined number of active sources. The sLORETA solution space consists of 6,239 voxels (voxel size: 5 × 5 × 5 mm) and is restricted to cortical gray matter and hippocampi, as defined by the digitized Montreal Neurological Institute (MNI) 152 template [52]. Scalp electrode coordinates on the MNI brain are derived from the international 10–20 system [53]. We used a standard head model, but even without utilizing an individual patient's MRI anatomical scans, research has demonstrated that with as many as 19-electrodes, and using the approximate three-shell head model registered to the Talairach human brain, localization accuracy of EEG is 10 mm, for worst cases [54]. By adding the expected localization error, as a result of the head

model, the average error is not expected to exceed 2 cm in the final LORETA source localization results. The tomography of sLORETA has received considerable validation from studies combining sLORETA with other more established spatial localization methods such as fMRI [55, 56], structural MRI [57], and PET [58–60]. Further sLORETA validation is based on accepting as ground truth that the localization findings obtained from invasive, implanted depth electrodes, of which there are several studies in epilepsy [61,62] and cognitive ERPs [63]. A region of interest analysis was also conducted. The log-transformed electric current densities were calculated for the regions of interest (ROIs) for the theta (4–7.5 Hz) frequency band. The ROIs in the present study are the right and left frontal eye fields and intraparietal sulcus (key nodes of the dorsal attention network [5,7,64]).

2.1.5. Saliva collection

Saliva was collected immediately before and after stimulation using the passive drool technique, and *Salimetrics*' collection aid mouthpiece. See Supplementary Methods for details on instructions given to participants ahead of saliva collection, as well as guidelines given to participants on the passive drool technique. Saliva was collected to measure changes in levels of IL-6 pre-and-post stimulation. Prior studies have shown the utility of inflammatory markers (i.e. cytokines) in saliva at detecting changes from baseline in response to acute stress [65–67], see Supplementary Methods for further details. To ensure the maximum reliability of saliva samples collected, “gold standard” collection practices were followed [68]. These included collecting resting baseline measures of saliva, using the passive-drool collection method, and samples being stored in a –80 °C laboratory freezer.

2.1.6. Control measures

Demographic items (age, sleep and memory expectations) and neuropsychological questionnaires were collected for each participant. Validated measures including the MMSE, BAI, and BDI were administered to each participant at the start of visit one to confirm eligibility and to measure cognition, anxiety or depression levels. Pre-and-post stimulation, participants also completed the VAS and POMS to measure levels of stress, alertness, tension, depression, anger, fatigue, confusion, or vigour. Given that these factors can impact learning and memory outcomes, these control measures were recorded to exclude the possibility that any behavioural differences between the groups were due to underlying differences anxiety, depression, cognition or mood levels etc., and to ensure that any between-group behavioural differences were specifically due to the effects of the stimulation.

2.1.7. tES exit questionnaire

To assess possible side effects of tACS, post-stimulation participants completed the tES Exit Questionnaire [69]. It asked participants to rate (using a 4-point scale from 1 = absent to 4 = severe) the extent to which they might have felt symptoms (incl. headache, neck pain, scalp pain, tingling, itching, burning sensation, skin redness, sleepiness, trouble concentrating, acute mood changes, or others). If any were experienced, participants were asked to indicate (yes/no) whether they believed each symptom was related to the application of tACS. To determine if the stimulation was well-blinded, an item asked participants to guess, based on their experience, which stimulation they believed they had received (active or non-active).

2.1.8. Statistical analysis

1. Word-Association Task

Cumulative learning rate (% of correctly recalled words on Day 1 from blocks one to three) was analysed using a one-way analysis of variance (ANOVA) with stimulation group (active or active-control) as the independent variable and % of correctly recalled words as the dependent variable. To analyse memory recall 7 Days later, a one-way

ANOVA was conducted with stimulation groups as the between-subject variable and correctly recalled words on Day 2 as the dependent variable. Pearson correlations were obtained between % correctly learned on Day 1 and % of correctly recalled words on Day 7. A one-way ANOVA was used to compare the rate of forgetting between groups, from Day 1 to Day 7.

2. EEG

The methodology used a non-parametric permutation test based on estimating, via randomization, the empirical probability distribution for the max-statistic under the null hypothesis comparisons [70]. This methodology corrects for multiple testing (i.e., for the collection of tests performed for all voxels, and for all frequency bands). Due to the non-parametric nature of this method, its validity does not rely on any assumption of Gaussianity [70]. These whole brain comparisons were performed by sLORETA through multiple voxel-by-voxel comparisons using a logarithm of F -ratio. The significance threshold for all tests was based on a permutation test with 5000 permutations. Comparisons were made between the active and active-control subject groups, and between before and after stimulation on Day 1.

A repeated measure ANOVA was conducted with before and after stimulation as within-subjects variable, and group (1 vs 40 Hz) as between-subjects variable for the log-transformed current density for the right frontal eye fields and the right intraparietal sulcus. These activity changes (post-pre stimulation) were then correlated with the % of words learned on Day 1 and those correctly recalled on Day 7, using a Pearson correlation for right frontal eye fields and the right intraparietal sulcus. The same statistical analyses were also conducted for the left frontal eye fields and intraparietal sulcus.

3. Salivary IL-6

Utilizing the passive drool method and following *Salimetrics'* protocols and determination techniques, the flow rate was calculated using *Salimetrics'* formula (amount of saliva (ml)/time to pool (min)). The tubes were weighed; with the weight of the saliva being the difference between the weights of the full tube and the empty tube.

To test any difference in saliva collection procedures, a repeated measures ANOVA was run with stimulation groups (active, active-control) as the between-subject variable, and volume (ml) of saliva collected (pre-and-post stimulation) as the within-subjects variable. Another repeated measures ANOVA was run with the same between-subject variable, but with time (mins) to collect saliva (pre-and-post stimulation) as the within-subject variable.

A Wald Chi-Squared test was conducted to compare changes in IL-6 between the stimulation groups, using a post-stimulation and baseline-corrected measure of IL-6 as the dependent variable, and stimulation groups (active and active-control) as the independent variable. Pearson correlations were calculated to test whether there was an association between changes in IL-6 levels (pre and post stimulation) with (a) % correctly learned words on Day 1, and (b) % correctly recalled words 7 days after stimulation. Pearson correlations were also run to test whether there was an association between changes in IL-6 levels (pre and post stimulation) and activation of the left or right intraparietal sulci and frontal eye fields.

4. Control Measures

ANOVAs were conducted to examine whether there was a difference between the stimulation groups (active vs active-control) for age (years), average sleep (hours), expected memory performance, and MMSE, BDI, and BAI scores. Chi-square (χ^2) tests were calculated to compare if there was a difference between the active and active-control group for gender.

In addition, multivariate analyses of variance (MANOVAs) were computed to analyse whether VAS Stress, VAS Alertness, and POMS

subscales (Tension, Depression, Anger, Fatigue, Confusion, Vigour) differed between stimulation groups (active vs active-control) and pre-and-post-stimulation.

5. tES Exit Questionnaire

To analyse whether participants were blinded to their stimulation group, Chi-square (χ^2) tests compared the stimulation participants received (active vs active-control) to what they thought they received. A MANOVA was conducted to compare whether there was any difference between groups for experiencing common stimulation side-effects.

2.2. Results

2.2.1. Learning and memory performance

As expected, on Day 1 a significant difference in the % of words correctly learned between the two stimulation groups was found ($F = 6.20, p = .019, \eta^2 = .18$), with the 40 Hz NITESGON group ($M = 65.99, Sd = 18.28$) learning significantly more words on Day 1 in comparison to the 1 Hz group ($M = 44.83, Sd = 27.39$) (see Fig. 2a). Similarly, a significant effect for memory recall was obtained between the groups on Day 7 ($F = 4.67, p = .039, \eta^2 = .14$), indicating that the 40 Hz group ($M = 38.67, Sd = 11.60$) recalled more words in comparison to the 1 Hz group ($M = 27.37, Sd = 16.38$) (see Fig. 2b). A Pearson correlation demonstrated a significant positive association ($r = .48, p = .008$) between the percentage of correctly learned words on Day 1 and recalled words on Day 7 (see Fig. 2c). When comparing how many words participants forgot between Day 1 and Day 7, no significant difference was obtained between the two groups (1 versus 40 Hz stimulation) ($F = .85, p = .36, \eta^2 = .02$), suggesting no difference in memory consolidation processes between the groups. These findings support that 40Hz NITESGON's sustained memory performance on Day 7 stemmed from greater learning on Day 1, with the more words learned during Day 1, associated with correctly remembering more on Day 7.

2.2.2. Theta activity in dorsal attention regions

As expected, for the 40 Hz NITESGON group, there was a significant increase in theta frequency activity post-stimulation in comparison to pre-stimulation, in the right intraparietal sulcus and the dorsal anterior cingulate cortex ($F = 4.56, p < .05, \eta^2 = .14$) (see Fig. 2d). No effect was obtained for the delta, alpha, beta or gamma frequency band. Furthermore, no effect was obtained when comparing pre- and post-stimulation for the 1 Hz group for the delta, theta, alpha, beta or gamma frequency bands.

A region of interest (ROI) analysis using log-transformed current density for the right frontal eye field at the theta frequency band with EEG (before and after stimulation) as the within-subjects variable and group (1 versus 40 Hz) as the between-subjects variable revealed a significant interaction effect ($F = 5.21, p = .033, \eta^2 = .15$). A simple contrast analysis revealed that before stimulation no significant difference ($F = .41, p = .52, \eta^2 = .04$) was obtained between the 1 Hz ($M = .16, Sd = .07$) and 40 Hz group ($M = .14, Sd = .11$). After stimulation a significant difference ($F = 4.48, p = .043, \eta^2 = .13$) was obtained demonstrating increased activity in the right frontal eye field at the theta frequency band for the 40 Hz group ($M = .29, Sd = .18$) in comparison to the 1 Hz group ($M = .18, Sd = .11$). Furthermore, a comparison between before and after stimulation for the 1 Hz group revealed no significant effect ($F = .12, p = .74, \eta^2 = .03$). However, a comparison of before and after stimulation for the 40 Hz stimulation group revealed a significant effect ($F = 12.75, p < .001, \eta^2 = .18$), indicating that after stimulation, increased activity was obtained in the right frontal eye field for the theta frequency band (see Fig. 2e).

For the right intraparietal sulcus, a ROI analysis for the theta frequency band with EEG (before and after stimulation) as the within-subjects variable and group (1 versus 40 Hz) as the between-subjects variable revealed a significant interaction effect ($F = 4.61, p = .041$,

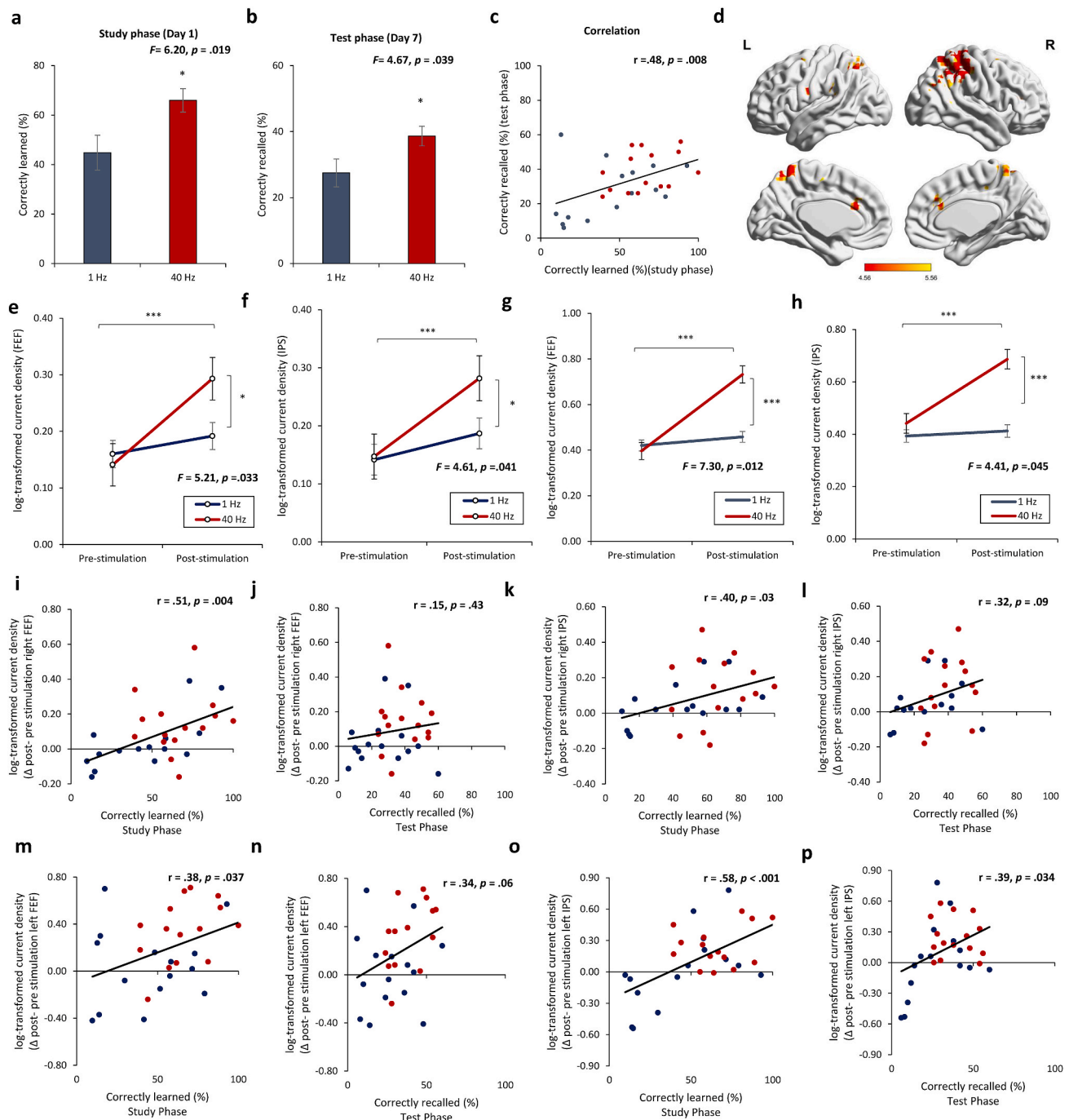


Fig. 2. | Experiment 1 – NITESGON (40 Hz or 1 Hz), learning, memory, and theta-frequency dorsal attention network activity.

a Participants receiving 40 Hz NITESGON learned significantly more words on Day 1 than those receiving 1 Hz **b** Similarly, the 40 Hz group recalled significantly more words on Day 7 than the 1 Hz group. **c** The percentage of words correctly learned on Day 1 positively correlated with the percentage of words correctly recalled on Day 7. **d** Source-reconstructed resting-state EEG analysis shows increased synchronization for the theta frequency band in key dorsal attention regions (FEE and IPS) for the 40 Hz group relative to the 1 Hz group (after stimulation, corrected for baseline activity). L; left hemisphere, R; right hemisphere. **e** A region of interest (ROI) analysis using log-transformed current density showed a significant interaction effect in the theta frequency band at the right FEE between the two stimulation groups after stimulation, and for the 40 Hz group when comparing pre-to post-stimulation. **f** A ROI analysis using log-transformed current density showed a significant interaction effect in the theta frequency band at the right IPS between the two stimulation groups after stimulation, and for the 40 Hz group when comparing pre-to post-stimulation. **g** A ROI analysis using log-transformed current density showed a significant interaction effect in the theta frequency band at the left FEE between the two stimulation groups after stimulation, and for the 40 Hz group when comparing pre-to post-stimulation. **h** A ROI analysis using log-transformed current density showed a significant interaction effect in the theta frequency band at the left IPS between the two stimulation groups after stimulation, and for the 40 Hz group when comparing pre-to post-stimulation. **i – p** When assessing the difference (post-pre stimulation) in log-transformed current density in dorsal attention regions (FEE and IPS) and how these correlated with learning and memory outcomes: significant correlations were found between learning on Day 1 and **i** the right FEE, **m** the left FEE, **k** the right IPS, and **o** the left IPS. Similarly, significant correlations were found between memory recall on Day 7 and **n** the left FEE, **l** the right IPS, and **p** the left IPS, but not **j** the right FEE. Error bars represent standard error mean. Asterisks represent significant differences ($*p < .05$, $***p < .001$). Abbreviations include Frontal Eye Field (FEE) and Intraparietal Sulcus (IPS).

$\eta^2 = .14$) (see Fig. 2f). A simple contrast analysis yielded no significant difference ($F = .11, p = .75, \eta^2 = .03$) between the 1 Hz ($M = .14, Sd = .08$) and 40 Hz group ($M = .15, Sd = .12$). After stimulation a significant difference ($F = 6.58, p = .016, \eta^2 = .14$) was obtained, demonstrating increased theta frequency activity in the right intraparietal sulcus for the 40 Hz group ($M = .30, Sd = .15$) in comparison to the 1 Hz group ($M = .17, Sd = .13$). Furthermore, a comparison between before and after stimulation for the 1 Hz stimulation group revealed no significant effect ($F = .55, p = .46$). However, a comparison between before and after stimulation for the 40 Hz group revealed a significant effect ($F = 14.29, p < .001, \eta^2 = .19$), indicating that after stimulation increased theta frequency activity was obtained in the right intraparietal sulcus.

For the left frontal eye fields, a ROI analysis for the theta frequency band with EEG (before and after stimulation) as the within-subjects variable and group (1 versus 40 Hz) as the between-subjects variable revealed a significant interaction effect ($F = 7.30, p = .012, \eta^2 = .21$) (see Fig. 2g). A simple contrast analysis showed no significant difference ($F = .06, p = .80, \eta^2 = .002$) between the 1 Hz ($M = .42, Sd = .33$) and 40 Hz group ($M = .40, Sd = .16$) before stimulation. After stimulation a significant difference ($F = 14.64, p < .001, \eta^2 = .34$) was obtained, demonstrating increased theta frequency activity in the left frontal eye fields for the 40 Hz group ($M = .73, Sd = .18$) in comparison to the 1 Hz group ($M = .45, Sd = .21$). Furthermore, a comparison between before and after stimulation for the 1 Hz stimulation group revealed no significant effect ($F = .23, p = .64, \eta^2 = .008$). However, a comparison between before and after stimulation for the 40 Hz group revealed a significant effect ($F = 18.50, p < .001, \eta^2 = .40$), indicating that after stimulation increased theta frequency activity was obtained in the left frontal eye fields.

A region of interest (ROI) analysis using log-transformed current density for the left intraparietal sulcus at the theta frequency band with EEG (before and after stimulation) as the within-subjects variable and group (1 versus 40 Hz) as the between-subjects variable revealed a significant interaction effect ($F = 4.41, p = .045, \eta^2 = .14$) (see Fig. 2h). A simple contrast analysis revealed that before stimulation no significant difference ($F = .32, p = .57, \eta^2 = .011$) was obtained between the 1 Hz ($M = .39, Sd = .27$) and 40 Hz group ($M = .44, Sd = .18$). After stimulation a significant difference ($F = 12.72, p = .001, \eta^2 = .31$) was obtained demonstrating increased activity in the left intraparietal sulcus at the theta frequency band for the 40 Hz group ($M = .69, Sd = .18$) in comparison to the 1 Hz group ($M = .41, Sd = .23$). Furthermore, a comparison between before and after stimulation for the 1 Hz group revealed no significant effect ($F = .07, p = .79, \eta^2 = .002$). However, a comparison of before and after stimulation for the 40 Hz stimulation group revealed a significant effect ($F = 10.44, p = .003, \eta^2 = .27$), indicating that after stimulation, increased activity was obtained in the left intraparietal sulcus for the theta frequency band (see Fig. 2e).

A correlation was obtained between the difference in log-transformed current density in the frontal eye fields after stimulation compared to before stimulation and the percentage of correctly learned words during Day 1 (right: $r = .51, p = .004$ | left: $r = .38, p = .037$), but not for memory recall at Day 7 (right: $r = .15, p = .4$ | left: $r = .34, p = .059$) (see Fig. 2i, j, 2m and 2n). Similarly, a significant correlation was obtained between the difference in log-transformed current density for the intraparietal sulcus after stimulation in comparison to before stimulation and the percentage of correctly recalled words for Day 1 (right: $r = .40, p = .03$ | left: $r = .58, p < .001$) (see Fig. 2k and o). However, for Day 7, a positive correlation was found between memory recall and log-transformed current density in the left intraparietal sulcus after stimulation in comparison to before ($r = .39, p = .034$), but not the right intraparietal sulcus ($r = .32, p = .09$) (see Fig. 2l and p). These results highlight how enhanced encoding and learning on Day 1, is specifically associated with upregulated theta activity in bilateral dorsal attention regions (frontal eye field and intraparietal sulcus). However, memory recall on Day 7 was only significantly associated with upregulated theta activity in the left intraparietal sulcus.

2.2.3. Cytokine IL-6 modulation

No significant differences were found for saliva collection procedures (see Fig. 3a, and Supplementary Results for details). Average pre-stimulation baseline IL-6 levels are reported in Supplementary Table 2. After controlling for baseline levels, a significant difference ($\chi^2 = 6.33, p = .012$) in IL-6 levels post-stimulation was found for the 40 Hz NITESGON group ($M = 4.11, Sd = 3.56$) in comparison to the 1 Hz group ($M = 9.73, Sd = 15.23$) (see Fig. 3b). For Day 1, there were no correlations between the difference in IL-6 after stimulation in comparison to before stimulation and the number of words the participant learned ($r = -.18, p = .34$) (Fig. 3c). However, seven days after stimulation, there was a positive correlation between the difference in IL-6 after stimulation in comparison to before stimulation and the number of words that participant recalled ($r = .44, p = .015$) (Fig. 3d).

An additional analysis correlating differences in IL-6 levels with the left and right frontal eye field as well as the intraparietal sulcus did not show any significant correlations (range $r = .13 - .21$, range $p = .50$ to $.22$).

2.2.4. Control measures

When comparing mood changes (before and after stimulation) between the 1 and 40 Hz stimulation groups, no significant effect was found for participants' reported levels of VAS stress ($F = .94, p = .34, \eta^2 = .032$), VAS Alertness ($F = 3.09, p = .09, \eta^2 = .099$), or on the POMS subscales including Tension ($F = .17, p = .70, \eta^2 = .006$), Depression ($F = .01, p = .99, \eta^2 = .001$), Anger ($F = .20, p = .66, \eta^2 = .007$), Fatigue ($F = 1.37, p = .25, \eta^2 = .047$) or Vigour ($F = 2.57, p = .12, \eta^2 = .084$). However, for the subscale Confusion ($F = 6.55, p = .016, \eta^2 = .19$) we found a significant interaction effect, indicating that post stimulation ($M = 1.93, Sd = 1.68$), participants assigned to the 40 Hz group had a significant reduction in comparison to before stimulation ($M = 4.07, Sd = 2.89; F = 9.79, p = .004, \eta^2 = .15$). For the 1 Hz group, no difference was obtained when comparing pre versus post stimulation ($F = .24, p = .63, \eta^2 = .012$). However, after correction for multiple comparison the effect did not survive. See Supplemental Figs. 1a–1h.

No significant effect was shown for how many words participants expect to recall on day 7 ($F = .08, p = .78, \eta^2 = .006$), or the average of hours of sleep ($F = 1.04, p = .32, \eta^2 = .011$) between the 1 and 40 Hz stimulation group (see Supplemental Figs. 1k and 1l). Between the 1 and 40 Hz NITESGON groups, there was no significant difference in the numbers of males and females ($\chi^2 = .13, p = .72$) (Supplemental Fig. 1i), age ($F = .49, p = .49, \eta^2 = .009$), or scores on the MMSE ($F = 2.15, p = .15, \eta^2 = .019$), BDI ($F = .07, p = .79, \eta^2 = .008$) or BAI ($F = 2.57, p = .12, \eta^2 = .087$) measures. See Table 1 for details on average age, MMSE, BDI and BAI scores for each group.

2.2.5. tES exit questionnaire

A MANOVA revealed a non-significant main effect of stimulation group (1 versus 40 Hz) for the various tES side effects ($F = .42, p = .91, \eta^2 = .003$). All stimulation sessions were well tolerated, and no major stimulation related complications were noted. Participants were not able to guess what group they were assigned to, as no significant difference was obtained in the number of participants indicating what thought they received (1 or 40 Hz) in comparison what they did receive ($\chi^2 = 2.40, p = .12$) (Supplemental Fig. 1j).

2.2.6. Experiment 1 summary

Experiment 1 found that 40Hz NITESGON's sustained memory performance on Day 7 stemmed from greater learning on Day 1, with the more words learned during Day 1, associated with correctly remembering more on Day 7. Furthermore, as expected, for the 40 Hz group (but not 1 Hz NITESGON), upregulated theta activity was found in bilateral frontal eye fields and intraparietal sulcus regions. Interestingly, while this activation was positively correlated with learning on Day 1, only theta activity in the left intraparietal sulcus was positively correlated with memory recall on Day 7. In terms of cytokine modulation, as

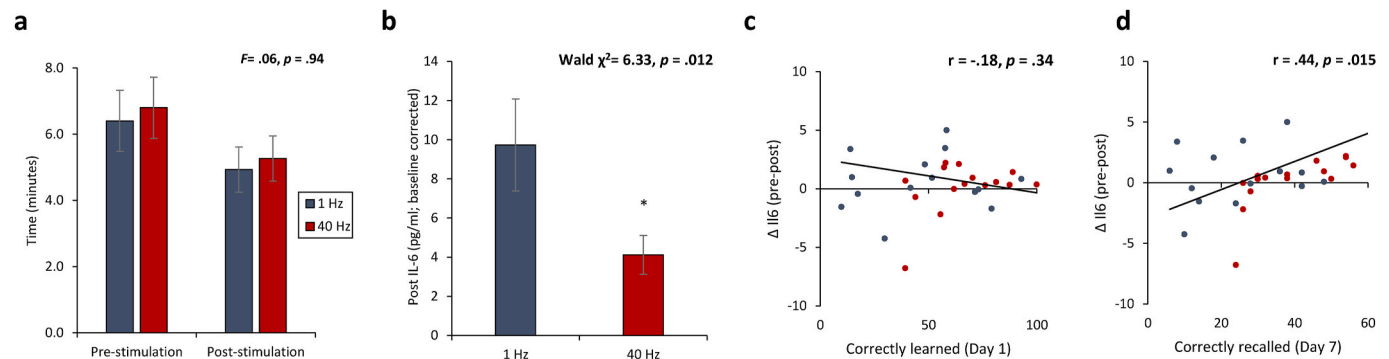


Fig. 3. | Experiment 1 – Salivary IL-6 and its correlation with memory recall, but not learning.
a There were no significant differences in the time taken to collect the salivary measures between the stimulation groups. **b** The 40 Hz group had significantly lower IL-6 levels post-stimulation (corrected for baseline) compared to the 1Hz group. **c & d** On Day 1, there were no significant correlations between learning and changes (post-stimulation compared to baseline) in the IL-6 levels. However, on Day 7, the difference in IL-6 levels was positively correlated with memory recall. Error bars represent standard error mean. Asterisks represent significant differences ($*p < .05$).

Table 1					
Average age, MMSE, BDI and BAI scores of the stimulation groups.					
	sham	1 Hz	40 Hz	F	p
Experiment 1					
Age		23.6 (4.53)	22.4 (4.82)	0.49	0.49
MSSE		27.5 (2.23)	28.5 (1.41)	2.15	0.15
BDI		2.3 (2.28)	2.60 (3.24)	0.07	0.79
BAI		1.3 (1.22)	2.7 (3.33)	2.57	0.12
Experiment 2					
Age	19.7 (2.29)	19.9 (3.02)	19.3 (1.06)	0.22	0.81
MSSE	29.2 (0.80)	29.3 (0.90)	29.6 (0.51)	0.62	0.54
BDI	4.0 (2.95)	3.00 (2.52)	2.7 (2.91)	0.50	0.61
BAI	2.6 (2.79)	2.4 (1.85)	2.1 (2.18)	0.09	0.91

Abbreviations include: Mini Mental State Exam (MMSE), Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI).

expected, the 40 Hz group had significantly reduced levels of IL-6 post-stimulation, and notably these changes correlated with memory recall on Day 7.

Given IL-6's known impact on synaptic plasticity processes, it is possible that if applying stimulation during learning is associated with reduced IL-6 post-learning, that the benefits of this reduction in pro-inflammatory IL-6 cytokine subsequently supports consolidation stages. In fact, this is in line with earlier research which highlighted how cytokines can act as humoral factors in regulating memory consolidation [22,24]. This finding and the literature informed the decision to include additional cytokine measures in Experiment 2.

3. Experiment 2

Given the significant findings in Experiment 1, and given prior research by our lab and others [32,34,35,37] which demonstrate how peripheral nerve stimulation can exert behavioural effects through locus coeruleus – noradrenaline activity, the second study aimed to expand on our first experiment's findings by measuring additional cytokines (IL-1 β and TNF- α , alongside IL-6) as well as salivary alpha-amylase (as a proxy for noradrenaline) and theta activity through rs-EEG. The aim of this second study was to add further insight into the mechanisms of the original study, as we hypothesised that noradrenaline activity would be modulated alongside the cytokines and would likewise impact the behavioural outcomes. In addition, when analysing the results of the first experiment, because our sham group could be considered an active-control group, the possibility (albeit unlikely) that 1 Hz (active-control) stimulation negatively impacted performance, rather than 40 Hz stimulation (active group) enhancing behavioural

performance, could not be excluded. As such, Experiment 2 also sought to investigate this, by randomizing participants to one of three groups, where an additional arm acted as a true sham condition alongside the active and active-control groups, while maintaining the double-blind conditions.

3.1. Methods

3.1.1. Participants

Similar to Experiment 1, the sample was estimated based on our previous research using the word association task [47]. We calculated our sample size based on the idea that we have two control groups (sham and 1 Hz) and one active group (40 Hz) by assuming an α level of .05 (one-sided), power of 80 %, and a strong effect size on the word association test during Visit 2. This resulted in a sample size of 30.

Participants were 30 healthy, right-handed adults (12 males and 19 females; mean age 19.74 years, SD = 2.26 years) with a similar education background (i.e., undergraduate students at UT Dallas). Inclusion criteria followed Experiment 1. This double-blinded, randomized, parallel group study, was conducted in accordance with the relevant guidelines and the Declaration of Helsinki. Each participant provided written informed consent.

3.1.2. Procedure

Experiment 2 followed the same procedure and methods as Experiment 1, with some slight variations. The only elements that differed were the stimulation groups, and additional salivary measures recorded. The details of these are outlined below.

3.1.3. NITESGON

30 participants were randomly-assigned to one of these three stimulation groups. The NITESGON details for groups 1 and 2 in this Experiment directly replicated the conditions of Experiment 1. A third NITESGON condition was added to serve as a sham/baseline comparison. For this sham stimulation group, the NITESGON electrode montage and equipment were the same as groups 1 and 2. However, sham stimulation differed in that it was delivered at a frequency of 1 Hz with 0° phase difference, for 70 s only. This consisted of a 30s ramp-up, 10s duration, and 30s ramp down time immediately prior to the start of each study phase. The sham stimulation aimed to mimic the sensation of stimulation, without exerting the same physiological or behavioural effects.

3.1.4. Word-Association Task

Same as Experiment 1.

3.1.5. Rs-EEG

Same as Experiment 1.

3.1.6. Saliva collection

Saliva collection methods followed the same process as Experiment 1. However, in addition to IL-6, pre-and-post stimulation levels in IL-1 β , TNF- α , and salivary α -amylase (sAA) were measured. The validity of salivary measures of IL-1 β and TNF- α for acute inflammation has been confirmed alongside IL-6 [65–67]. sAA, a marker of endogenous NA activity [71,72], was used to test the effect of NITESGON on the LC-NA pathway. Prior research validates sAA as an inexpensive and non-invasive proxy for LC-NA activity [73], see Supplementary Methods for details.

3.1.7. Control measures and tES exit questionnaire

Same as Experiment 1.

3.1.8. Statistical analysis

1. Word Association Task, EEG, Control Measures and tES Exit Questionnaire

Statistical analyses of these measures were the same as in Experiment 1, except they also included the additional sham group (alongside the 1 and 40 Hz groups) for comparison.

2. Salivary IL-6, IL-1 β and TNF- α

Measurements, calculation procedures and statistical analyses of IL-6, IL-1 β and TNF- α followed the same steps as outlined for IL-6 in Experiment 1, but included the additional sham group for comparison. Additionally, Pearson correlations were calculated to test the association between changes in IL-6 and IL-1 β , in IL-6 and TNF- α , and IL-1 β and TNF- α , and between changes in each of the cytokines and theta activity in the left and right intraparietal sulci and frontal eye fields.

3. Salivary α -Amylase (sAA)

sAA levels were measured using the passive drool method and analysed following *Salimetrics* protocols and determination techniques. See Supplementary Methods for details on how collection procedures (saliva time and volume) were measured and calculated.

To test any difference in saliva collection procedures between stimulation groups, repeated measures ANOVAs were performed comparing the flow rate, and time to collect saliva.

A repeated measures ANOVA with sAA (before and after stimulation) as the within-subjects variable and group (sham, 1 or 40 Hz) as the between-subjects variable was conducted to test for any interaction in pre- and post-stimulation sAA levels and group. A simple contrast analysis was applied to compare the different conditions using a Bonferroni correction. To test the differences of the change in sAA levels after stimulation, compared to before, between the three groups, a repeated measures ANOVA was conducted with groups as the between-subjects variable and change in sAA as within-subjects variable. A simple contrast analysis was applied to compare the different conditions using a Bonferroni correction. Given the inter-individual variability that typically occurs with sAA data, the above analyses were also conducted using the log transformed sAA data. A Pearson correlation was computed to investigate the association between changes in sAA levels and learning on Day 1, and memory recall on Day 7. Correlations were also conducted to test the association between sAA changes and theta activity in the left and right intraparietal sulci and frontal eye fields, as well as with changes in cytokine levels.

3.2. Results

3.2.1. Learning and memory performance

Replicating Experiment 1's findings, on Day 1 a significant difference in the percentage of words correctly learned between the two stimulation groups was found ($F = 5.56, p = .009, \eta^2 = .292$) (Fig. 4a), with the 40 Hz NITESGON group ($M = 87.80, Sd = 14.68$) learning significantly more words on Day 1 in comparison to the 1 Hz ($M = 59.91, Sd = 19.89; p < .008$) and sham group ($M = 59.81, Sd = 22.71; p < .035$). There was no significant difference between the 1 Hz and sham group ($p = .97$).

Similarly, a significant effect for memory recall was obtained between the groups on Day 7 ($F = 4.16, p = .027, \eta^2 = .236$) (Fig. 4b), with the 40 Hz group ($M = 46.00, Sd = 14.67$) remembering significantly more words in comparison to the 1 Hz ($M = 30.91, Sd = 16.71; p < .033$) and sham group ($M = 26.00, Sd = 16.31; p < .015$). No significant effect was obtained between the 1 Hz and sham group ($p = .95$). A Pearson correlation found a significant positive association ($r = .72, p < .001$) between the percentage of correctly learned words on Day 1 and recalled words on Day 7 (Fig. 4c). When comparing how many words participants forgot between Day 1 and Day 7, no significant differences were obtained between the three groups (sham, 1 or 40 Hz stimulation) ($F = 2.31, p = .12$), suggesting no difference in memory consolidation processes between the groups. These findings validate Experiment 1's results and again highlight how frequency-specific NITESGON induces encoding-stage benefits that lead to enhanced long-term memory performance.

3.2.2. Theta activity in dorsal attention regions

Supporting Experiment 1's findings, for the 40 Hz NITESGON group, there was a significant increase in theta frequency activity post-stimulation in comparison to pre-stimulation, in the right intraparietal sulcus ($F = 3.96, p < .05, \eta^2 = .18$) (Fig. 4d). No effect was obtained for the delta, alpha, beta or gamma frequency band. Furthermore, no effect was obtained when comparing pre versus post stimulation for the sham or 1 Hz groups for the delta, theta, alpha, beta or gamma frequency bands.

A ROI analysis using log-transformed current density for the right frontal eye field at the theta frequency band with EEG (before and after stimulation) as within-subjects variable and group (sham, 1 or 40 Hz) as between-subjects variable revealed a significant interaction effect ($F = 3.68, p = .039, \eta^2 = .214$; see Table 2 and Fig. 4e). A simple contrast analysis revealed that before stimulation no significant difference was obtained between the 40 Hz group, in comparison to the sham and 1 Hz group. After stimulation a significant difference was obtained, demonstrating increased theta frequency activity in the right frontal eye field for the 40 Hz group in comparison to the sham and 1 Hz groups. No significant difference was found between the sham and 1 Hz group. Furthermore, for the 40 Hz group, a significant effect was found indicating increased theta frequency activity after stimulation (in comparison to before) in the right frontal eye field.

For the right intraparietal sulcus, a ROI analysis for the theta frequency band with EEG (before and after stimulation) as within-subjects variable and group (sham, 1 and 40 Hz) as between-subjects variable revealed a significant interaction effect ($F = 4.02, p = .030, \eta^2 = .230$; see Table 2 and Fig. 4f). A simple contrast analysis yielded no significant difference between the 40 Hz and sham group, and the 1 Hz group before stimulation. After stimulation, there was a significant difference with increased theta frequency activity in the right intraparietal sulcus for the 40 Hz group, in comparison to the sham and 1 Hz group. No significant difference was found between the sham and 1 Hz group. Furthermore, a comparison between before and after stimulation for the sham and 1 Hz stimulation group revealed no significant effect in the right intraparietal sulcus at the theta frequency band.

A ROI analysis using log-transformed current density for the left frontal eye field at the theta frequency band with EEG (before and after stimulation) as within-subjects variable and group (sham, 1 or 40 Hz) as

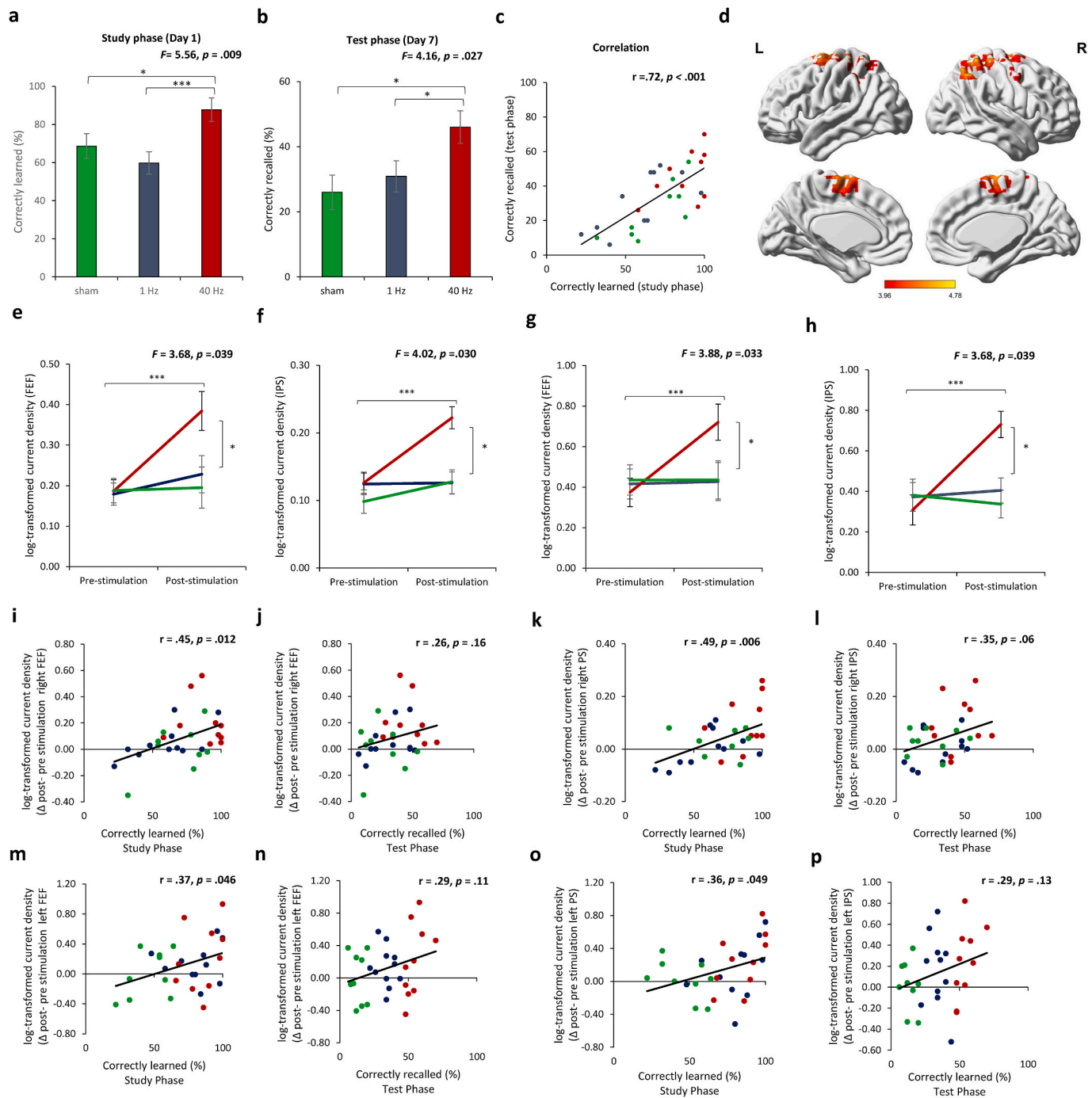


Fig. 4. | Experiment 2 – NITESGON (40 Hz or 1 Hz or sham), learning, memory, and theta-frequency dorsal attention network activity

a Participants receiving 40 Hz NITESGON learned significantly more words on Day 1 than those receiving 1 Hz or sham. **b** Similarly, the 40 Hz group recalled significantly more words on Day 7 than the 1 Hz or sham groups. **c** The percentage of words correctly learned on Day 1 positively correlated with the percentage of words correctly recalled on Day 7. **d** Source-reconstructed resting-state EEG analysis shows increased synchronization for the theta frequency band in key dorsal attention regions (FEF and IPS) for the 40 Hz group relative to the 1 Hz group (after stimulation, corrected for baseline activity), L; left hemisphere, R; right hemisphere. **e** A region of interest (ROI) analysis using log-transformed current density showed a significant interaction effect in the theta frequency band at the right FEF between the three stimulation groups after stimulation, with increased activity in the 40 Hz group compared to the 1 Hz and sham groups, and increased activity for the 40 Hz group when comparing pre-to post-stimulation. **f** A ROI analysis using log-transformed current density showed a significant interaction effect in the theta frequency band at the right IPS between the three stimulation groups after stimulation, with increased activity in the 40 Hz group compared to the 1 Hz and sham groups, and increased activity for the 40 Hz group when comparing pre-to post-stimulation. **g** A ROI analysis using log-transformed current density showed a significant interaction effect in the theta frequency band at the left FEF between the three stimulation groups after stimulation, and for the 40 Hz group when comparing pre-to post-stimulation. **h** A ROI analysis using log-transformed current density showed a significant interaction effect in the theta frequency band at the left IPS between the three stimulation groups after stimulation, and for the 40 Hz group when comparing pre-to post-stimulation. **i** – **p** When assessing the difference (post-pre stimulation) in log-transformed current density in dorsal attention regions (FEF and IPS) and how these correlated with learning and memory outcomes: significant correlations were found between learning on Day 1 and **j** the right FEF, **m** the left FEF, **k** the right IPS and **o** the left IPS. Additionally, a significant correlation was found between memory recall on Day 7, and **l** the right IPS, but not with **j** the right FEF, **n** the left FEF, or **p** the left IPS. Error bars represent standard error mean. Asterisks represent significant differences (* $p < .05$, ** $p < .01$, *** $p < .001$). Abbreviations include Frontal Eye Field (FEF) and Intraparietal Sulcus (IPS).

Table 2
Region of interest analysis results for experiment 2.

	Sham		1 Hz		40 Hz	
	<i>M</i>	<i>Sd</i>	<i>M</i>	<i>Sd</i>	<i>M</i>	<i>Sd</i>
<i>FEF</i>						
<i>right</i>						
Before stimulation	.184 ^a	.147	.188 ^a	.062	.186 ^a	.037
After stimulation	.195 ^a	.118	.228 ^a	.130	.384 ^b	.195
<i>left</i>						
Before stimulation	.435 ^a	.288	.416 ^a	.160	.374 ^a	.215
After stimulation	.436 ^a	.334	.427 ^a	.288	.720 ^b	.218
<i>IPS</i>						
<i>right</i>						
Before stimulation	.093 ^a	.047	.124 ^a	.047	.126 ^a	.062
After stimulation	.127 ^a	.036	.126 ^a	.051	.222 ^b	.067
<i>left</i>						
Before stimulation	.353 ^a	.230	.373 ^a	.219	.308 ^a	.164
After stimulation	.337 ^a	.240	.407 ^a	.225	.730 ^b	.138

a vs b indicates a significant difference. Abbreviations include: Frontal Eye Field (FEF) and Intraparietal Sulcus (IPS).

between-subjects variable revealed a significant interaction effect ($F = 3.88, p = .033, \eta^2 = .22$; see Table 2 and Fig. 4g). A simple contrast analysis revealed that before stimulation no significant difference was obtained between the 40 Hz group, in comparison to the sham and 1 Hz group. After stimulation a significant difference was obtained, demonstrating increased theta frequency activity in the left frontal eye field for the 40 Hz group in comparison to the sham and 1 Hz groups. No significant difference was found between the sham and 1 Hz group. Furthermore, for the 40 Hz group, a significant effect was found indicating increased theta frequency activity after stimulation (in

comparison to before) in the left frontal eye field.
For the left intraparietal sulcus, a ROI analysis for the theta frequency band with EEG (before and after stimulation) as within-subjects variable and group (sham, 1 and 40 Hz) as between-subjects variable revealed a significant interaction effect ($F = 9.41, p < .001 \eta^2 = .41$; see Table 2 and Fig. 4h). A simple contrast analysis yielded no significant difference between the 40 Hz and sham group, and the 1 Hz group before stimulation. After stimulation, there was a significant difference with increased theta frequency activity in the left intraparietal sulcus for the 40 Hz group, in comparison to the sham and 1 Hz group. No significant difference was found between the sham and 1 Hz group. Furthermore, a comparison between before and after stimulation for the sham and 1 Hz stimulation group revealed no significant effect in the left intraparietal sulcus at the theta frequency band.
A correlation was obtained between the difference in log-transformed current density in the frontal eye fields after stimulation compared to before stimulation and the percentage of correctly learned words during Day 1 (right: $r = .45, p = .012$ | left: $r = .37, p = .046$), but not for memory recall at Day 7 (right: $r = .26, p = .16$ | left: $r = .29, p = .11$)(Fig. 4i, 4m, 4j and 4n). A significant correlation was revealed between the difference in log-transformed current density for the intraparietal sulcus after stimulation in comparison to before stimulation and the percentage of correctly learned words during Day 1 (right: $r = .49, p = .00$ | left: $r = .36, p = .049$), but not for memory recall on Day 7 (right: $r = .35, p = .06$ | left: $r = .28, p = .13$)(Fig. 4k, 4o, 4l and 4p). Building on Experiment 1, these findings replicate how enhanced learning on Day 1 is associated with upregulated theta activity in key dorsal attention regions (bilateral frontal eye field and intraparietal sulcus). Interestingly, however theta activity in neither regions was associated with memory recall on Day 7.

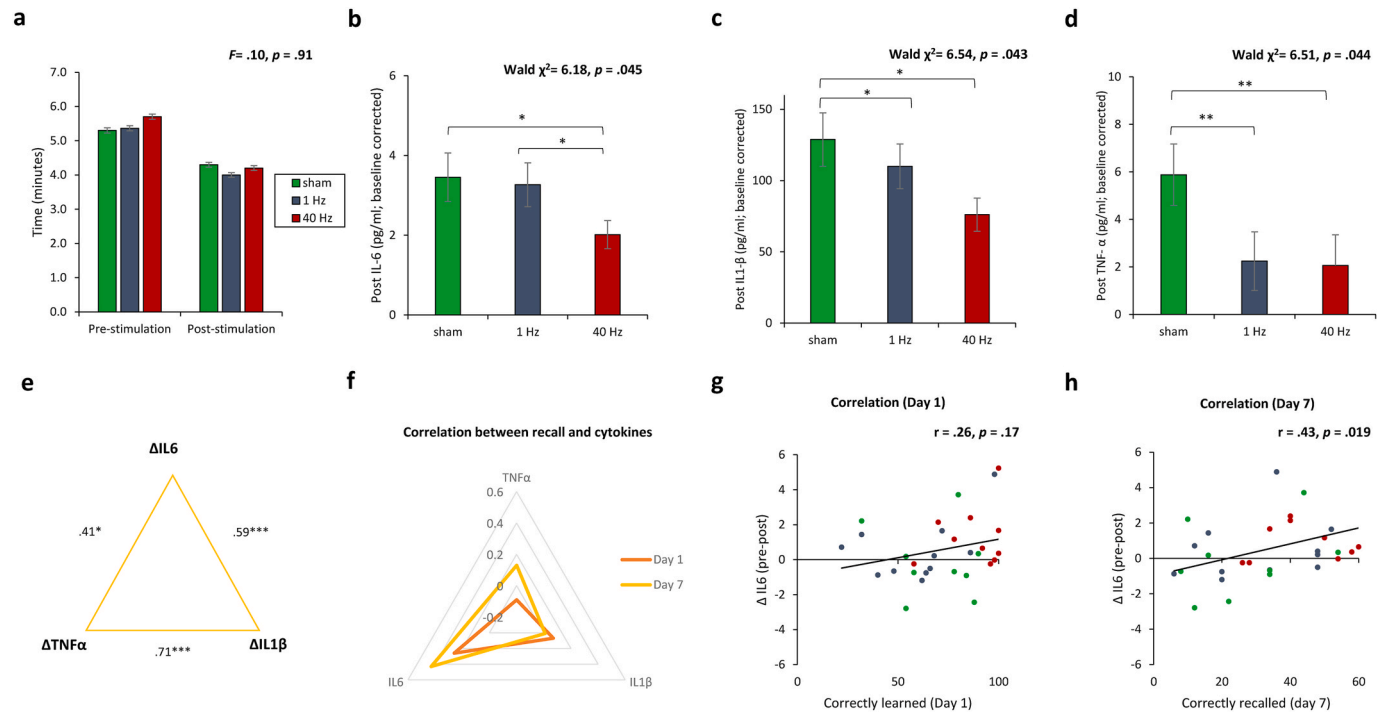


Fig. 5. | Experiment 2 – Salivary cytokine measures and correlation with memory recall.
a There were no significant differences in the time taken to collect the salivary measures between the stimulation groups. **b** The 40 Hz group had significantly lower IL-6 levels post-stimulation (corrected for baseline) compared to the 1 Hz and sham group. **c** The sham group had significantly higher IL-1β levels post-stimulation (corrected for baseline) compared to the 40 Hz and the 1 Hz groups. **d** The sham group had significantly higher TNF-α levels post-stimulation (corrected for baseline) compared to the 1 Hz and 40 Hz groups. **e** There were significant correlations between the changes in IL-6, IL-1β and TNF-α. **f** to **h** On Day 1, there were no significant correlations between learning and changes (post-stimulation compared to baseline) in the IL-6, IL-1β or TNF-α levels. However, on Day 7, only the difference in IL-6 levels was positively correlated with memory recall. Error bars represent standard error mean. Asterisks represent significant differences (* $p < .05$, ** $p < .01$, *** $p < .001$).

3.2.3. Cytokine modulation (IL-6, IL-1 β and TNF- α)

No significant differences were found for saliva collection procedures (see Fig. 5a, and Supplementary Results for details). Average baseline cytokine levels are reported in Supplementary Tables 2 and 3. When comparing cytokine levels (post stimulation corrected for baseline) for the sham, 1 and 40 Hz stimulation groups, a significant effect was obtained for IL-6 ($\chi^2 = 6.18, p = .045$), IL-1 β ($\chi^2 = 6.54, p = .043$) and TNF- α ($\chi^2 = 6.51, p = .044$) (see Fig. 5b–d). For IL-6, a simple contrast analysis revealed that the 40 Hz group ($M = 2.01, Sd = 1.17$) had a significantly lower score in comparison to the sham ($M = 3.45, Sd = 2.77; p = .041$) and 1 Hz group ($M = 3.27, Sd = 2.26; p = .045$). There was no significant difference between the sham and 1 Hz group ($p = .82$). The results for IL-1 β and TNF- α followed a different pattern. For IL-1 β , a simple contrast analysis found the 40 Hz group ($M = 76.05, Sd = 52.21$) had a significantly lower score compared to the sham ($M = 128.78, Sd = 109.14; p = .045$), but not the 1 Hz group ($M = 60.97, Sd = 37.63; p = .47$). A significant effect was obtained between the sham and the 1 Hz group ($p = .026$). For TNF- α , a simple contrast analysis showed that the sham group ($M = 5.88, Sd = 7.38$) had a significantly higher score compared to the 1 Hz ($M = 2.24, Sd = .1.19; p = .008$) and 40 Hz groups ($M = 2.067, Sd = 1.18; p = .005$). No significant effect was obtained between the 1 and 40 Hz group ($p = .77$). These results suggest no differences between the active and active control conditions for IL-1 β and TNF- α levels post-stimulation, with both groups reporting significantly lower post-stimulation levels than the sham group.

A significant correlation was obtained between the difference in IL-6, and IL-1 β ($r = .59, p < .001$) and TNF- α ($r = .41, p < .016$), respectively. Also, a significant correlation was obtained between IL-1 β and TNF- α ($r = .71, p < .001$), suggesting synergistic modulation of these three cytokines (see Fig. 5e). On Day 1, there were no correlations between the changes (post-stimulation compared to pre) in IL-6 ($r = .26, p = .17$), IL-1 β ($r = .007, p = .97$), or TNF- α ($r = -.09, p = .64$) levels and the number of words correctly learnt (Fig. 5f and g). However, seven days after stimulation, there was a significant correlation between changes in IL-6 (post-stimulation compared to pre) and the number of words correctly remembered ($r = .43, p = .019$) (see Fig. 5f and h). However, no significant effect was obtained for IL-1 β ($r = .10, p = .61$) or TNF- α ($r = .13, p = .51$) (Fig. 5f).

An additional analysis correlating differences in IL-6 levels with the left and right frontal eye field as well as the interparietal sulcus did not show any significant correlations (range $r = .002 - .32$, range $p = .99$ to $.08$). A similar analysis for both for TNF- α (range $r = .009 - .22$, range $p = .96$ to $.24$) and IL-1 β (range $r = .15 - .20$, range $p = .44$ to $.29$) also showed no significant correlations.

These results reiterate Experiment 1's findings, and highlight how 40 Hz NITESGON is specifically associated with reduced IL-6 levels, and that these reduced levels likely play a role in and facilitate subsequent consolidation stages of memory, rather than encoding stages. However, for TNF- α and IL-1 β , the changes in cytokine levels were observed in both the 1 Hz and 40 Hz NITESGON groups, meaning these changes were not frequency specific. Stimulation had a synergistic effect on these inflammatory cytokines, with moderate-to-strong correlations found between changes in salivary IL-6, IL-1 β and TNF- α cytokine levels. These findings highlight that peripheral nerve stimulation's effect is accompanied by interrelated changes in several immune system chemical messengers. Additionally, the results support the known "cross-inducibility" of cytokines [74] and their network interactions [23,75].

3.2.4. LC-NA upregulation (sAA)

No significant differences were found for saliva collection procedures (see Supplementary Results for details). A repeated measures ANOVA with sAA (before and after stimulation) as within-subjects variable and group (sham, 1 or 40 Hz) as between-subjects variable revealed a significant interaction effect ($F = 3.77, p = .035, \eta^2 = .21$). A simple contrast analysis revealed a significant mean increase from 59.58 ($Sd = 32.93$) to 77.95 ($Sd = 47.20$) ($F = 3.41, p = .045, \eta^2 = .17$) for 40 Hz

stimulation. No significant difference was obtained for the 1 Hz ($F = .16, p = .69, \eta^2 = .006$) or the sham group ($F = 3.06, p = .085, \eta^2 = .09$) when comparing before (1 Hz $M = 59.76, Sd = 66.37$; Sham $M = 119.13, Sd = 123.96$) versus after stimulation (1 Hz $M = 63.57, Sd = 48.85$; Sham $M = 99.21, Sd = 112.52$).

Additionally, when comparing the differences in sAA levels (post-stimulation minus pre between the groups, there was a significant difference in salivary α -amylase (sAA) levels for the three groups (sham, 1 Hz and 40 Hz) ($F = 3.69, p = .038, \eta^2 = .189$) (see Fig. 6a). A simple contrast analysis revealed the 40 Hz group ($M = 18.37, Sd = 20.69$) had significantly increased levels compared to the sham group ($M = -19.92, Sd = 41.83; p = .034$). No significant effect was obtained between 1 Hz ($M = -1.67, Sd = 41.83; p = .59$) and sham, or 40 Hz ($p = .47$) respectively. See the Discussion section for a hypothesis about this finding.

There was a positive correlation between changes in sAA (post-stimulation compared to pre) and both learning during Day 1 ($r = .38, p = .036$) as well as recall during Day 7 ($r = .47, p = .010$) (see Fig. 6b and c). These results were in line with our expectations and prior literature [38] that 40 Hz stimulation would be associated with increased circulating noradrenaline (as a proxy for LC-NA upregulation), with LC-NA activity being associated with both learning and memory.

As there are enormous inter-individual variability typically in sAA data, as well in our own data we log-transformed our sAA data. A repeated measures ANOVA with the log-transformed sAA data (before and after stimulation) as the within-subjects variable and group (sham, 1 or 40 Hz) as the between-subjects variable revealed a significant interaction effect ($F = 3.09, p = .045, \eta^2 = .20$). A simple contrast analysis revealed a significant mean increase from 1.56 ($Sd = .36$) to 1.81 ($Sd = .34$) ($F = 3.41, p = .045, \eta^2 = .17$) for 40 Hz stimulation. No significant difference was obtained for the 1 Hz ($F = 3.45, p = .07, \eta^2 = .110$) or the sham group ($F = 1.25, p = .27, \eta^2 = .04$) when comparing before (1 Hz $M = 1.67, Sd = .37$; Sham $M = 1.92, Sd = .43$) versus after stimulation (1 Hz $M = 1.73, Sd = .26$; Sham $M = 1.81, Sd = .38$). When comparing the difference in log-transformed sAA levels between the sham, 1 and 40 Hz group, this was also significant ($F = 3.07, p = .046, \eta^2 = .19$) confirming the results of the raw data.

An additional analysis correlating the difference in sAA levels with the left and right frontal eye field as well as the interparietal sulcus did not show any significant correlations (range $r = .035 - .30$, range $p = .85$ to $.11$). However, a significant correlation was found between changes in sAA and IL-6 ($r = .40, p < .05$) (but not between sAA and IL-1 β or TNF- α). These results are included in Supplementary Table 4.

3.2.5. Control measures

When comparing mood changes (before and after stimulation) between the sham, 1 Hz and 40 Hz stimulation groups, no significant effect was found for participants' reported levels of VAS stress ($F = .05, p = .95, \eta^2 = .002$), VAS Alertness ($F = 1.53, p = .23, \eta^2 = .012$), or on the POMS subscales including Tension ($F = .03, p = .97, \eta^2 = .001$), Depression ($F = .22, p = .81, \eta^2 = .011$), Anger ($F = .16, p = .86, \eta^2 = .010$), Fatigue ($F = 1.05, p = .37, \eta^2 = .015$), Confusion ($F = .25, p = .78, \eta^2 = .006$) or Vigor ($F = 2.15, p = .14, \eta^2 = .066$) (see Supplemental Figs. 2a–2h).

No difference was revealed between the numbers of males of females assigned to the sham, 1 or 40 Hz stimulation group ($\chi^2 = .383, p = .15$) (Fig. 2i). When comparing the three stimulation groups (sham, 1 Hz or 40 Hz), no significant effect was yielded for how many words participants expect to recall on day 7 ($F = .80, p = .46, \eta^2 = .017$) or average of hours of sleep ($F = .40, p = .67, \eta^2 = .023$). Participants assigned to the sham, 1 or 40 Hz stimulation group did not significantly differently on age ($F = .22, p = .81$), the MMSE ($F = .62, p = .54, \eta^2 = .032$), BDI ($F = .50, p = .61, \eta^2 = .034$) or BAI measures ($F = .09, p = .91, \eta^2 = .003$) (see Table 1).

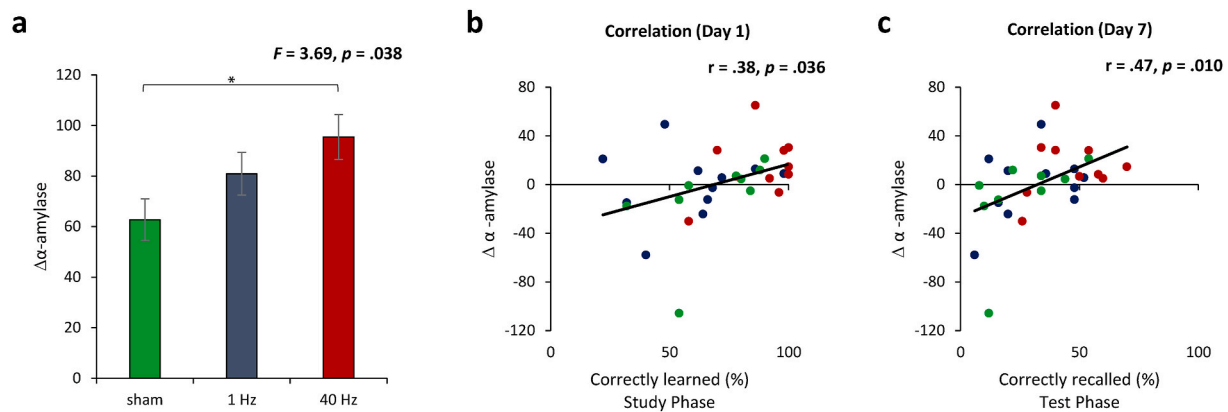


Fig. 6. | Experiment 2 – Salivary α-amylase (sAA) and correlation with learning memory recall.

a The 40 Hz group had a significant difference in salivary α-amylase (sAA) levels (post-stimulation compared to pre) compared to the sham group, but not the 1 Hz group. **b & c** Changes in sAA levels significantly correlated with both learning on Day 1 and memory recall on Day 7. Error bars represent standard error mean. Asterisks represent significant differences ($*p < .05$).

3.2.6. tES exit questionnaire

A MANOVA revealed a non-significant main effect on stimulation group (sham, 1 or 40 Hz) for the various tES side effects ($F = .79, p = .71, \eta^2 = .014$). All stimulation sessions were well tolerated, and no major stimulation related complications were noted. No significant difference was obtained in the number of participants indicating they think they received sham, 1 or 40 Hz in comparison to what they actually received ($\chi^2 = .81, p = .65$) (see Fig. 2j).

4. Experiment 1 and 2 post-hoc analyses

Given that some of the correlations were significant at trend level, we pooled the data for the behavioural, EEG and IL-6 cytokine results for the 2 studies together and conducted Pearson correlations on the combined data. The majority of these results were in line with the results of the individual experiments. However, interestingly, there were novel significant findings when the association between activity changes in these dorsal attention regions and memory recall on Day 7 were tested. The results of these post-hoc statistical analyses are outlined in more detail in the supplemental results and [Supplementary Table 5](#).

5. Experiment 2 summary

Experiment 2 aimed to replicate Experiment 1, with the inclusion of a true sham condition, and to further investigate the potential mechanisms involved with peripheral nerve stimulation. As expected, the 40 Hz NITESGON group learned more words on Day 1 and recalled more on Day 7, with a positive correlation found between learning and memory outcomes. Additionally, as in Experiment 1, there were no differences in rates of forgetting between the groups, suggesting the memory benefit for the 40 Hz group stemmed from upregulated encoding processes.

Replicating Experiment 1, the 40 Hz group had increased theta activity in bilateral frontal eye fields and intraparietal sulcus regions. Additionally, learning on Day 1 was positively correlated with theta activity in these regions. Notably however, while theta activity in the left intraparietal sulcus was correlated with memory recall on Day 7 in Experiment 1, this was not the case in Experiment 2. In Experiment 2, neither right nor left dorsal attention regions were correlated with memory recall.

Building on Experiment 1, the 40 Hz stimulation group had reduced IL-6 post-stimulation compared to the sham and 1 Hz groups, which correlated with memory recall on Day 7. However interestingly, while both 1 Hz and 40 Hz were associated with reduced IL-1β and TNF-α levels (suggesting modulation of these cytokines might not be frequency specific), neither of these reductions were associated with learning or

memory outcomes. Additionally, moderate to strong correlations were found between changes in IL-6, IL-1β and TNF-α, suggesting synergistic modulation of these cytokines in response to peripheral nerve stimulation.

Combined results from the two experiments highlight 40 Hz NITESGON's demonstrable effects on the encoding stage of memory formation. Interestingly, while this learning effect was found to be in association with changes to theta oscillatory activity in key dorsal attention regions in the brain as expected, the patterns of modulation of both noradrenaline and immune system activity were associated with learning and memory to different extents.

6. Discussion

Episodic memory relates to our ability to refer to prior events in our life. It defines our sense of self and supports our decision-making by enabling us to draw on our past experiences. Attention plays a central role in our ability to learn and retain information over time, however an important question remains as to whether this avenue can be targeted via peripheral nerve stimulation as a means of bolstering long-term memory performance. These two double-blinded experiments firstly highlight how encoding-stage processes can be modulated by frequency-specific peripheral nerve stimulation, that activates the dorsal attention network. Additionally, they further elucidate how in response to this stimulation, changes in circulating chemical messengers of the CNS and immune systems are differentially associated with learning and memory outcomes. Understanding how attention plays a central role in learning and memory processes is relevant for our mechanistic knowledge of these processes, but also for potential treatment avenues for a variety of pathological conditions such as ADHD, Alzheimer's or PTSD.

Both experiments found that the 40 Hz NITESGON group learned significantly more words on Day 1, with learning outcomes positively correlating with memory recall a week later. Additionally, they demonstrate that 40 Hz NITESGON (but not 1 Hz or sham), enhances the encoding stage of memory, with sustained long-term memory performance stemming from enhanced initial learning. These frequency-specific effects evidently targeted encoding stages, as there were no differences in memory consolidation between the groups over the course of the week. Memory consolidation is the stage at which information is transferred from short-term to long-term storage [1–3]. We were able to show that 40 Hz NITESGON's memory effect was *not* consolidation-driven, as there was no significant difference in the rate of forgetting from Day 1 to Day 7, between the two groups. In other words, there was no difference in the percentage of words that were successfully transferred to long-term memory storage between the groups. Given that

the 40 Hz NITESGON group exhibited greater learning, with sustained memory benefits stemming from this, and given this enhanced memory performance was not driven by boosting consolidation, these results add further evidence to earlier research which suggested that this stimulation protocol enhances episodic memory processes by specifically supporting the encoding stages [38].

The point at which information is encoded or learnt, is critically impacted by attention processes [4]. According to a well-researched neurobiological model (the Dual-Attention Theory), a ventral-PPC pathway underlies bottom-up, or externally-driven attention processes, while a dorsal-PPC network facilitates top-down control, in which orientation by internal goals enables successful task execution [4]. A recent study has shown how the dorsal attention network influences encoding in a working memory task [14], whilst two separate studies in healthy [9] and clinical [7] samples demonstrate that theta-frequency activity in the dorsal attention network is functionally-related to goal-directed attention performance. Both of our experiments show for the first time, that 40 Hz stimulation targeting the greater occipital nerve can selectively upregulate theta activity in central regions in the dorsal attention network (the left and right intraparietal sulcus and frontal eye fields). Moreover, the two experiments provide direct evidence that activation in these regions are positively correlated with the encoding stages of memory, supporting learning of information on Day 1, which leads to sustained memory benefits. Thus, our findings connect these parallel streams of research by reporting that 40 Hz NITESGON likely enhances episodic memory performance by supporting goal-directed attention during memory-encoding, as this stimulation-induced enhanced encoding of information was associated with upregulated resting-state theta activity in dorsal attention regions.

Based on prior research implicating the LC-NA pathway in the mechanism of peripheral nerve stimulation [33–38], Experiment 2 sought to further investigate whether 40 Hz NITESGON's impact learning and memory processes was indeed associated with upregulated LC-NA activity (measured via sAA). Notably, sAA levels were significantly elevated post-stimulation (in comparison to pre) for the 40 Hz NITESGON group compared to the sham group, whilst there was no significant difference between 1 Hz and sham, or 1 Hz and 40 Hz. Furthermore, the increase in sAA levels was positively associated with both learning on Day 1, and memory recall on Day 7. The fact that the 40 Hz NITESGON group had significantly higher sAA levels post-stimulation compared to sham is in line with prior research [38]. On the other hand, the finding that sAA levels were positively associated with both learning on Day 1 and memory recall on Day 7 is a novel finding, but one that gives further insight into previous research by our lab. For example, in a previous study where participants completed a similar Swahili-English word association task, our lab found that NITESGON using direct current resulted in increased sAA levels post-stimulation in comparison to pre [34]. This change in sAA levels was correlated with other markers of LC-NA activity (namely pupil diameter and the P3b ERP). However interestingly, this sAA change was only correlated with memory outcomes on day 7, and not initial learning [34].

Although this differs to the findings of the present study, it may come down to how NITESGON using direct or alternating current at 40 Hz could have different mechanistic impact on learning and memory outcomes. Specifically, a more recent study by our lab reported how using NITESGON to apply either 40 Hz alternating current or direct current, impacted long term memory outcomes differentially [38]. In that study both direct current and 40 Hz NITESGON led to enhanced memory recall 7 days later. The 40 Hz group's enhanced memory recall stemmed from greater learning on the first day (in line with the findings of the present study). However, while the direct current group also demonstrated significantly enhanced memory recall, this stemmed from offline consolidation benefits, and not enhanced initial learning. Moreover, only the direct current NITESGON group demonstrated significantly reduced forgetting over the 7 days [38]. Thus taken together,

NITESGON using direct current can be seen to enhance memory via consolidation [38], with long-term memory performance associated with increased sAA [34]. Whereas NITESGON using 40 Hz alternating current can enhance memory via upregulated encoding [38], with the present study highlighting how both learning and long-term memory performance are associated with increased sAA levels. However, in the present study it is interesting to note that there were no significant differences between sAA levels post-stimulation in the 40 Hz NITESGON and 1 Hz NITESGON group. Especially given that this effect was not observed in previous work by our lab [38], and that 1 Hz NITESGON was not associated with enhanced learning or memory, or activation of theta activity in the dorsal attention network in this current study.

It is known that LC neurons fire in two patterns - either tonically or phasically. Under tonic firing, LC neurons spike in irregular but continuous patterns that form its baseline activity [76]. Whereas under phasic firing, LC neurons exhibit short bursts of higher frequency spikes [76]. LC neural activity alternates between these two modes, with each mode associated with different attentional states. Phasic LC activity has been shown to be associated with focused and goal-directed behaviours, whilst tonic LC activity has been associated with greater distractibility, and poorer performance on selective attention tasks [19]. Given the role of phasic LC-NA activity in modulating goal-directed attentional processes [77], our data suggest that 40 Hz NITESGON could modulate LC-NA activity into a phasic firing state, to support goal-directed attention and facilitate encoding processes, leading to sustained memory performance. Conversely, it is possible that 1 Hz NITESGON, despite being a low frequency, was *sufficient* to stimulate the peripheral nerve mechanism and lead to a slight increase in NA release, whilst also being *insufficient* to shift LC firing from a tonic to phasic state. Although it remains to be specifically tested, this theory could explain why 1 Hz NITESGON was not associated with learning or memory outcomes here, as according to the Dual Attention Theory and LC-NA research, this would require phasic LC-NA activity.

Outside of this, one of the mechanisms through which LC-NA activity can influence learning and memory outcomes is through its input into synaptic plasticity processes, including mediating communication between the nervous and immune systems [20] and modulating various cytokines [29,75]. Preliminary human and rodent research demonstrates how peripheral nerve stimulation can either downregulate cytokine levels and improve cognitive responses [39,41], or improve learning, motor and memory performance by modulating LC-NA activity [32,34,36,37]. However, no research to date (to the best of our knowledge) has confirmed that stimulation of the greater occipital nerve can enhance learning by modulating both LC-NA activity and cytokine levels, despite the cross-talk that exists between the immune and central nervous systems.

In response to this, our findings highlight that peripheral nerve stimulation's effect is accompanied by interrelated changes in central nervous and immune system chemical messengers. From both experiments, we can specifically observe that 1 Hz or 40 Hz NITESGON has diverging effects on pro-inflammatory cytokines, with only the modulation of IL-6 associated with memory outcomes, and only in response to 40 Hz. Although this finding was not fully in line with our expectations, there are several potential explanations for these results. For example, although both 1 Hz and 40 Hz stimulation was associated with reduced IL-1 β and TNF- α but not learning or memory outcomes, it is conceivable that they were not reduced significantly enough to be associated with enhanced learning or memory. Put another way, it is plausible that these pro-inflammatory cytokine levels, although reduced, still remained high, so as to impair learning or memory. This dose-dependent effect of IL-1 β and TNF- α has been shown in empirical research in mice [78] and discussed in prior reviews [23,75]. For an additional hypothesis about what may underlie these cytokine results, see the Supplementary Discussion section.

Notably, while the 40 Hz NITESGON group post-stimulation had upregulated theta activity in dorsal attention regions, upregulated sAA

levels and downregulated IL-6, IL-1 β and TNF- α levels, with these changes associated with either enhanced learning and/or memory recall (as discussed above), there were no correlations found between noradrenaline levels and dorsal attention activation. In other words, changes in dorsal attention network activity were not associated with changes in sAA or cytokine levels. This is an interesting finding, and suggests perhaps that peripheral nerve stimulation's effect on learning and memory outcomes are associated with somewhat independent attention, and CNS system mechanisms. Although future research is needed to specifically test this interpretation. On the other hand, changes in sAA levels were correlated with changes in IL-6, which supports this idea of cross-talk that exists between the CNS and immune system. Moreover, it adds to our understanding of this peripheral nerve stimulation technique and suggests a shared potential mechanistic pathway behind its effect.

Incorporating the results of these two experiments together, applying 40 Hz NITESGON (but not 1 Hz or sham) during learning is associated with enhanced theta activity in the dorsal attention network, which is associated with augmented learning on Day 1. Learning on Day 1 was positively correlated with memory recall 1 week later, and there was no difference between the rates of forgetting between the active, active-control or sham groups (highlighting how the long-term memory benefits from 40 Hz NITESGON stemmed from enhanced encoding and not consolidation). sAA (a proxy for LC-NA activity) was elevated post-stimulation for both NITESGON groups but not the sham group, however it only positively correlated with learning and memory outcomes for the 40 Hz NITESGON group (and not the 1 Hz group). Finally, reduced IL-6 was observed in the 40 Hz group, and reduced IL-1 β and TNF- α were observed post-stimulation for both NITESGON groups but not sham. However, only changes in IL-6 were associated with memory outcomes for the 40 Hz NITESGON group. Taking these findings together, these experiments support the application of 40 Hz NITESGON for activating a noradrenaline – dorsal attention network, to facilitate goal-directed attention during encoding stages of a long-term memory task in association with neuroinflammatory changes. However, one limitation of this study is that participants were all young healthy individuals, as such the generalisability of the findings need to be confirmed with future research. This is especially the case in order to investigate the potential translational applicability of these results. For example, in one study of patients with traumatic brain injury and healthy controls, successful episodic memory encoding relied in part upon activation of central nodes of the dorsal attention network [79]. The authors further reported how episodic memory impairments in the patients stemmed from encoding failures due to abnormal dorsal attention network activation and default mode network activation [79]. Thus, future research could investigate if applying the peripheral nerve stimulation technique of the present study (40 Hz NITESGON) has clinically meaningful impact for patient groups such as those with traumatic brain injury.

The same is true for other clinical conditions such as Alzheimer's disease or ADHD. A systematic review and meta-analysis of 38 studies found abnormal functional alterations in dorsal attention network regions in those with mild cognitive impairment (a prodromal stage of Alzheimer's disease) [80]. Alzheimer's disease is characterised by deficits in memory function, yet to date, to the best of our knowledge, no study has attempted to investigate if neurostimulation can modulate dorsal attention nodes to positively impact learning and memory processes. Thus, future research is warranted to explore whether peripheral nerve stimulation specifically has clinical applicability here. This is an especially interesting area given how 40 Hz NITESGON not only influenced noradrenaline and learning and memory processes, but also modulated cytokine levels.

Notably, a meta-analysis of 40 studies reported elevated levels of peripheral cytokines incl. IL-6, IL-1 β and TNF- α in Alzheimer's disease patients in comparison to healthy controls [81]. These findings were further supported by a more recent systematic review and meta-analysis

of 175 studies [82]. Moreover, in that review, IL-6 levels in particular, were negatively correlated with cognitive function (as measured by the Mini-Mental State Examination) [82]. In light of the results of the present study, where 40 Hz NITESGON modulated levels of IL-6, IL-1 β and TNF- α , but only changes in IL-6 were correlated with memory outcomes, this suggests the potential for IL-6 to be a specific therapeutic target for both neuroinflammation and cognitive function. Although given how the meta-analyses found elevated levels of additional cytokines alongside the 3 investigated in the present study, there is also reason to investigate the potential impact of peripheral nerve stimulation on other cytokines.

Future research could likewise investigate the potential benefit of peripheral nerve stimulation at modulating attention for those with Attention Deficit Hyperactivity Disorder (ADHD). Altered dorsal attention network activation has been reported in those with ADHD. For example, one meta-analysis of 13 studies (2 of which were adult samples, and the remaining paediatric) which comprised 171 patients and 178 controls, analysed activation of participants' dorsal attention networks while they completed attention tasks [83]. Notably, the meta-analysis demonstrated reduced activity in the right hemisphere of dorsal attention regions in ADHD patients in comparison to controls [83]. Thus, it would be interesting to investigate whether 40 Hz NITESGON can increase activation in dorsal attention network regions in those with ADHD, and whether this has any potential cognitive benefit.

This raises another limitation of the present study. Here, 40 Hz NITESGON was shown to modulate encoding via upregulated attention (which was measured through changes in dorsal attention regions using rs-EEG). However, given the main aim of the present paper was to understand how attention-related brain activity impacted learning and memory outcomes, no specific attention-related task was employed to directly assess 40 Hz NITESGON's on attention performance. Given the significant findings here, a future point worth investigating is whether this peripheral nerve stimulation technique can modulate performance on goal-directed attention tasks such as Stop-Signal task, or Visual Search task, among others [84,85]. This would add to the findings of the present study and further strengthen the proposal that 40 Hz NITESGON can enhance performance in attention domains specifically, in association with increased activation of dorsal attention network regions.

In conclusion, applying 40 Hz NITESGON during learning in two double-blinded experiments, is associated with enhanced theta activity in the dorsal attention network, which is associated with enhanced memory outcomes that stem from an encoding, and not consolidation benefit. For those receiving 40 Hz NITESGON, sAA (a proxy for LC-NA activity) was elevated post-stimulation and was positively correlated with changes in IL-6, and learning and memory outcomes. Finally, for the 40 Hz group specifically, reduced IL-6 was observed post-stimulation and this was associated with memory outcomes. As such, we propose 40 Hz NITESGON as a viable, cost-effective and non-invasive means of modulating a noradrenaline dorsal attention network, to facilitate goal-directed attention during encoding stages of a long-term memory task in association with neuroinflammatory changes. These findings support the aims of future research investigating this technique for its potential therapeutic benefits in cases of, for example, traumatic brain injury, Alzheimer's disease or ADHD.

CRedit authorship contribution statement

Elva Arulchelvan: Writing – original draft, Methodology, Investigation. **Sven Vanneste:** Writing – original draft, Visualization, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare no competing interests.
No conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.brs.2025.01.008>.

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