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Stimulate to Remember? The Effects of Short Burst of Transcutaneous Vagus Nerve Stimulation (taVNS) on Memory Performance and Pupil Dilation

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ABSTRACT

The decline in noradrenergic (NE) locus coeruleus (LC) function in aging is thought to be implicated in episodic memory decline. Transcutaneous auricular vagus nerve stimulation (taVNS), which supports LC function, might serve to preserve or improve memory function in aging. However, taVNS effects are generally very heterogeneous, and it is currently unclear whether taVNS has an effect on memory. In this study, an emotional memory task with negative events involving the LC-NE system was combined with the short burst of event-related taVNS (3 s) in younger adults ($N = 24$). The aim was to investigate taVNS-induced changes in pupil dilation during encoding and possible taVNS-induced improvements in (emotional) memory performance for early and delayed (24 h) recognition. Negative events were associated with increased pupil dilation and better memory performance. Additionally, real as compared to sham or no stimulation selectively increased memory for negative events. Short bursts of stimulation, whether real or sham, led to an increase in pupil dilation and an improvement in memory performance over time, likely due to the attention-inducing sensory modulation of electrical stimulation.

1 | Introduction

Episodic memory is important for encoding and retrieval of events (Tulving 2002) with emotional events generally better remembered than neutral ones (Bradley et al. 1992; Cahill et al. 1995), showing better encoding (Erk et al. 2003) as well as better retrieval (Burke, Heuer, and Reisberg 1992; Dolan et al. 2000; Sterpenich et al. 2006). Human and animal studies have shown that the noradrenergic system of the locus coeruleus (LC-NE) in particular is involved in the processing and encoding of negative events. In humans, higher LC activation during the encoding of negative events (Sterpenich et al. 2006; Ludwig et al. 2024a) and higher LC integrity was related to better memory for negative events (Shibata et al. 2006; Clewett et al. 2016; Hämmerer et al. 2018). In animals, arousal-related NE release from the LC can support memory encoding via ß-adrenoceptors in the hippocampus and amygdala, which are important brain structures for the consolidation of emotional memories (Luo et al. 2015; Strange and Dolan 2004), while higher levels of NE release can be achieved by phasic stimulation of the LC (Florin-Lechner et al. 1996). Furthermore, pupil diameter measurements, though not exclusively tied to underlying LC activity, showed increased pupil dilation with emotionally negative events (Hämmerer et al. 2018, 2017) and can serve

as an indirect measurement for LC activation, as animal studies have shown that phasic LC stimulation causes an increase in pupil dilation, while research in humans has shown that pupil dilation is related to LC activity (Aston-Jones and Cohen 2005; Joshi et al. 2016; Reimer et al. 2016; Murphy et al. 2011, 2014).

During aging, episodic memory declines and it appears that the system of neuromodulatory nuclei, such as the LC, is involved in this decline (Engels-Domínguez et al. 2023; Ehrenberg et al. 2023), prompting research into the preservation or improvement of memory functions and cognition via targeting the LC. Indeed, research on invasive vagus nerve stimulation (iVNS) in rats revealed that iVNS may improve memory by activating vagal afferents (Clark et al. 1998) that project via the nucleus tractus solitarius (NTS), which is involved in memory modulation (Williams and McGaugh 1993; Roozendaal and McGaugh 2011) to the LC. Likewise, iVNS in rats increased NE discharge in a dose-dependent manner and resulted in an increased pupil dilation (Collins et al. 2021; Mridha et al. 2021; Hulsey et al. 2017; Roosevelt et al. 2006). IVNS as antiepileptic or antidepressant therapy in patients indicated an improvement in various areas of cognition such as attention, working memory, short-term memory, verbal memory recognition, memory consolidation induced by iVNS (Clark et al. 1998; Vonck et al. 2014; Sun et al. 2017; Broncel et al. 2020; Olsen et al. 2023). A non-invasive approach to modulate brainstem nuclei such as the NTS and the LC via the auricular branch and the other nerve fiber bundles of the vagus nerve is offered by transcutaneous auricular vagus nerve stimulation (taVNS) (Butt et al. 2019; Ruffoli et al. 2011). It has already been shown in humans, that short bursts of taVNS led to an increase in pupil dilation (Sharon, Fahoum, and Nir 2021; Lloyd and Wurm 2023; D'Agostini et al. 2023; Skora, Marzecová, and Jocham 2024) and that taVNS has the potential to improve the number of hits in a face-name association memory task in healthy older adults (Jacobs et al. 2015). Likewise, taVNS increased the recollection-based hit rates for emotional but not for neutral events compared to sham stimulation in healthy younger adults (Ventura-Bort et al. 2021). However, due to the high heterogeneity in taVNS study designs (Ludwig et al. 2021; Farmer et al. 2021), there is a lack of further taVNS studies investigating emotional memory performance including additional supportive indirect outcome measures such as pupillometry.

Therefore, the study focused on younger healthy adults ($N = 24$), as this allows for a more detailed investigation of the mechanisms of taVNS in a cognitively stable population, as older individuals may exhibit fluctuations in cognitive function. We combined an emotional memory task which is assumed to involve the LC-NE system (Ludwig et al. 2024a; Hämmerer et al. 2017, 2018) with pupillometry and event-related taVNS. In contrast to other taVNS studies, stimulation was not applied continuously, but based on short bursts of stimulation during the encoding phase, since phasic stimulation of the LC in animals was associated with improved cognitive functions such as improved encoding of salient stimuli and better memory performance (Aston-Jones and Cohen 2005; Wilmot et al. 2024; Vazey, Moorman, and Aston-Jones 2018; Hansen 2017). Moreover, our taVNS design allowed for an event-related decorrelation of stimulation on and off effects on a shorter time scale, providing an opportunity to assess how short bursts of stimulation (real vs. sham stimulation), compared to no stimulation, potentially contributes to improvements in emotional memory performance for early and delayed (24 h) recognition and increased pupil dilation during an encoding task.

2 | Methods

2.1 | Subjects

Twenty-four younger healthy subjects (12 females; 22.96 ± 2.24 years) were recruited through advertisements via university's mailing list as well as flyer distributions in Magdeburg. Subjects were included if they were between 20 and 30 years old, German speaking, had a BMI < 27, with low levels of alcohol and cigarette consumption. In addition, subjects were stratified into sporty (> 3 times a week sport in the last 4 weeks) versus non-sporty (< 2 times a week sport in the last 4 weeks) as the experiment also included the acquisition of heart-rate variability (HRV) which varies in athletes compared to non-athletes (Kiss et al. 2016). Exclusion criteria included cold symptoms, neurological (stroke, epilepsy, traumatic brain injury, syncope) as well as psychiatric (eating disorder, major depressive disorder, schizophrenia, bipolar disorder, any anxiety disorder, posttraumatic stress disorder) and other disorders (e.g., diabetes, alcohol dependence and/or drug use) as well as heart and eye diseases. Telephone screenings were conducted to verify the eligibility of those interested in the study. Subjects were asked to eat a light, healthy breakfast (no industrial sugar), not to drink caffeine and not to smoke on the day of the experiment, as well as not to drink alcohol on the day of the experiment and the day before. For determination of sample size see Data S1.

2.2 | Materials and Stimuli

The stimuli for the emotional memory task consisted of 288 indoor and outdoor images representing emotionally negative or neutral events taken from the International Affective Picture System Datenbank (IAPS (Lang 1995)) (272 images) and Geneva affective picture (GAPED (Dan-Glauser and Scherer 2011)) database (16 images) to allow for categorization of indoor and outdoor stimuli as a cover task while assessing effects of emotional stimulus materials (neutral indoor (72), neutral outdoor (72), emotional indoor (72), emotional outdoor (72)). Stimulus conditions were furthermore balanced with respect to stimulation conditions and early and delayed recognition. This means that the same proportion of the four stimulus categories was present for real stimulation, sham stimulation and off stimulation trials, as well as for early and delayed memory task (for more details see Table S1). This procedure not only ensured that the distribution of images for all conditions was randomized between subjects and also balanced within, but also allowed analyses to clearly separate memory and pupil effects related to stimulus types (emotional or neutral) and stimulation conditions (real, sham or no stimulation (off)).

2.2.1 | Visual Analog Scale (VAS)

After stimulation, subjects rated each stimulation session on a visual analog scale (VAS) (Yeung and Wong 2019) from (1) very

pleasant to (10) very unpleasant with respect to the sensations of the stimulation. Since it has been shown that the perception of sensations differs between real and sham stimulation, the VAS rating is often kept constant as a controlling factor in many studies and the individual intensity is allowed to vary for each subject based on for example, a "tingling" sensation below the pain threshold (Farmer et al. 2021; Ferstl et al. 2022; Müller et al. 2022). We could not keep VAS ratings constant because we systematically evaluated a predetermined range of intensities (see Ludwig et al. 2024b), but we documented how the different parameters affected perception of sensations (Supporting Information Section 1.3 and Figure S1). Specifically, subjective perception of sensations was higher for real stimulation (M ± SD: 5.78 ± 0.41) compared to sham stimulation (M ± SD: 4.86 ± 0.35), $F(1, 20) = 4.31$, $p = 0.05$.

2.2.2 | State of Health

The state of health was queried for each subject after stimulation to control for potential side effects (Table S2). The following items were asked: (1) headache, (2) nausea, (3) tiredness, (4) dizziness, (5) tingling sensation at the previously stimulated area, (6) feeling of heat at the previously stimulated area, (7) reddening of the skin at the previously stimulated area, (8) skin irritation at the previously stimulated site, (9) impaired concentration, (10) itching at the previously stimulated area. Subjects indicated on a 4-point scale (0: not at all—3: strong) to what extent they perceived potential side effects. The reported sensations did not differ between real (M ± SD: 0.20 ± 0.04) and sham (M ± SD: 0.19 ± 0.04) stimulation ($F(1, 9) = 0.06$, $p = 0.81$) (Supporting Information Section 1.5 and Table S2). Overall, it can be concluded that the stimulation did not cause any side effects and can therefore be considered safe, which is in line with previous reports (Farmer et al. 2021).

2.3 | Procedure

The study was conducted as a sham-controlled, single-blind, within-subject, counterbalanced, randomized design using a one-day stimulation protocol. At the beginning of each session subjects underwent an HRV baseline measurement, which was repeated halfway through the whole and at the end of the experiment (Figure 1). Subsequently, the subjects were permitted to try out the taVNS to familiarize themselves with the device and to modify the highest stimulation intensity (see Section 2.4) with an additional subjective evaluation of the perception using a visual analog scale (VAS) (see Section 2.2.1). Regarding the stimulation, it was instructed at the beginning that the stimulation of the ear can be perceived as a harmless tingling sensation in various areas. In addition, the entire ear was cleaned and not just a specific stimulation area and the repositioning of the electrodes was covered up with the story that the cream dries on the electrode after a certain time. This procedure ensured that the test subjects did not question why the electrodes were reapplied during the real and sham stimulation. The study consisted of two parts: (1) emotional memory task and (2) resting state task (Ludwig et al. 2024b). During the performance of the emotional memory task, subjects received real and sham stimulation while changes in pupil dilation and HRV were recorded in parallel. Immediately after the encoding sessions of the emotional memory task, an early recognition task was performed on the same day, and a delayed recognition task was performed 24 h later, both without stimulation. Importantly, subjective perceptions of sensations (VAS rating) as well as query of the state of health (potential side effects) (Table S2) were assessed after each stimulation session. The present article focuses on the changes in pupil dilation and memory performance due to taVNS during the emotional memory task.

2.4 | Transcutaneous Auricular Vagus Nerve Stimulation

TaVNS was delivered using tVNS Technologies nextGen research device (tVNS R, tVNS Technologies GmbH). The electrodes were placed on the **left ear** (Figure 2): At the **cymba conchae** for **real stimulation**, which is assumed to be innervated exclusively by the auricular branch of the vagus nerve

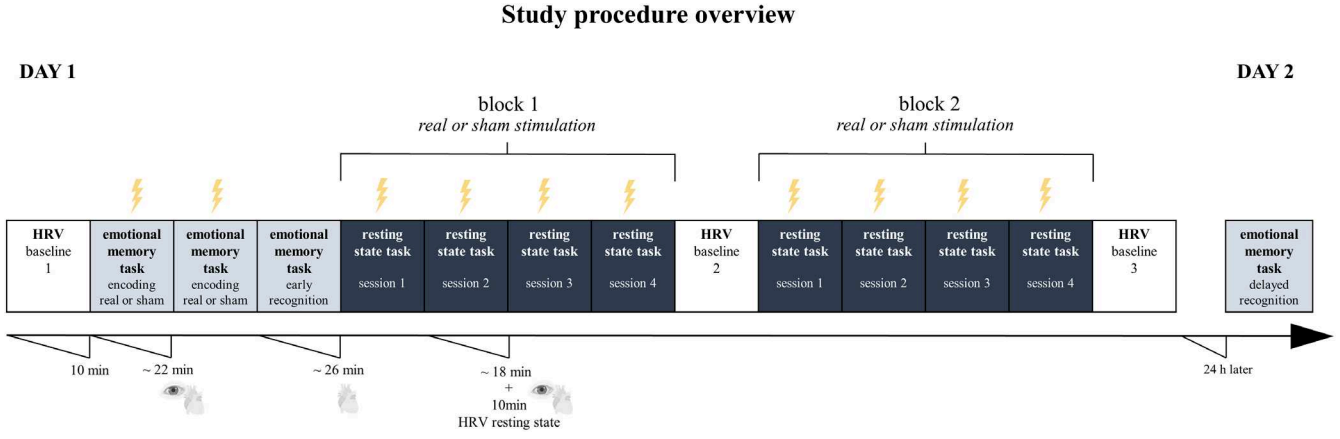

FIGURE 1 | The study was conducted using a sham-controlled, single-blind, within-subject, counterbalanced, randomized design featuring a one-day stimulation protocol. During the emotional memory task, real and sham stimulations were applied using the highest stimulation parameters (5 mA, 25 Hz). In the resting state task, four different parameter combinations were systematically tested (3 mA and 5 mA with 10 Hz and 25 Hz) across two blocks, comparing real and sham stimulation. Additionally, heart rate variability (HRV) and changes in pupil dilation during taVNS were recorded. This figure was reproduced with permission from Ludwig et al. (2024b).

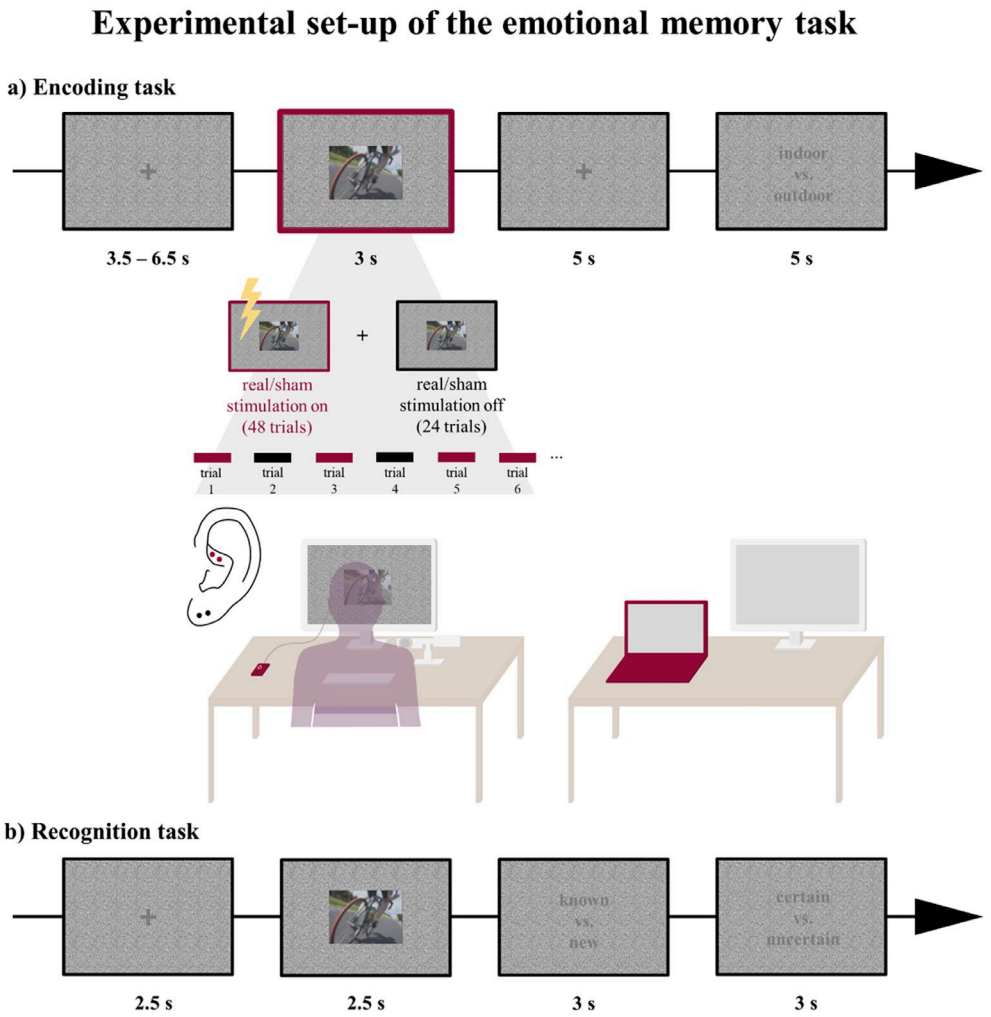

FIGURE 2 | The experimental set-up enabled a time-synchronous short bursts of stimulation (left ear: At the **cymba conchae** for **real taVNS** (red dots) and at the **earlobe** for **sham taVNS** (black dots)) during the (a) **encoding task** (72 trials) while changes pupil dilation (eyetracker camera, left) were recorded in parallel on one computer (red laptop, right). Each trial of the (a) **encoding task** began with a gray fixation cross (jitter between 3.5–6.5 s), followed by an image (3 s) showing either indoor or outdoor scene with a negative or neutral valence, while stimulation was applied for 2/3 of the images (see Section 2.5.1). Afterwards a gray fixation cross was presented (5 s) and subsequently subjects were asked to classify the image as outdoor (by pressing key A) or indoor (by pressing key L) as quickly but also as precisely as possible (time limitation of 5 s). During the (b) **recognition task** (144 trials) no stimulation was applied. Each trial began with a gray fixation cross (2.5 s) followed by an image presentation (2.5 s) with a subsequent question (time limit of 3 s) to classify the image as known (by pressing the A key) or new (by pressing the L key). This was followed by a question (time limit of 3 s) about how certain or uncertain the subjects were in their recognition. For all subjects, the same constant ambient light continued to be applied throughout the whole experiment and background's and images brightness variations were controlled with a grayish background image to prevent interference of luminance changes with pupillometric recordings.

(ABVN) (Peuker and Filler 2002) and at the **earlobe** for **sham stimulation**, which is not innervated by the ABVN (Butt et al. 2019; Burger et al. 2020; Yakunina, Kim, and Nam 2017) and seems to not induce functional activation in the target brain areas, like LC and NTS (Yakunina, Kim, and Nam 2017). For both stimulation conditions, the anode was placed more rostrally. Prior to the electrode placement, the ear was cleaned with disinfectant alcohol and subsequently a small amount of EC2+, Grass electrode conductive cream (https://www.cnsac-medshop.com/de/ec2-elektrodenleitcreme/) on the electrodes was used to assure optimal conductance. Subjects were then able to test the taVNS themselves with a frequency of 25 Hz, a pulse width of 250 μs and a stimulation cycle of 5 s on vs. off stimulation. The intensity started at 1 mA and subjects were allowed to go as high as possible at a reasonable pace. At the highest level, subjects rated the subjective intensity on a VAS (see Section 2.2.1). Since during the (2) resting state task (see Ludwig et al. 2024b) low and high intensities (3 vs. 5 mA) as well as frequencies (10 vs. 25 Hz) were tested systematically within subjects, it was determined a priori that subjects who did not reach 5 mA as the highest intensity would receive 3 mA as highest **intensity** to map the linear trend of intensity gradations. In the end 3 mA as highest intensity was applied to 7 out of 24 subjects. A biphasic square-wave pulse with a stimulus phase pulse width of 250 μs and a recovery phase at half the amplitude and double the duration, delivered at a frequency of 25 Hz, was applied during short bursts of stimulation of 3 s on and 15 s off stimulation.

2.5 | Emotional Memory Task

The emotional memory task consisted of an encoding task during which real and sham stimulation was applied and an early and delayed recognition task. The experiment was controlled by custom MATLAB code (Math Works, www.mathworks.com) using Psychtoolbox 3 (www.psychtoolbox.org), while during the encoding task stimulation could be controlled in a time-synchronized manner with the experiment via Bluetooth Low Energy (BLE) connection, while an HTTPS POST request was used to control the 'tVNS manager'. Thus, messages were forwarded via the 'tVNS Manager', which were integrated within the MATLAB code, so that the stimulation (without ramp-up) was either switched on or off per trial event within a loop.

2.5.1 | Encoding Task

The emotional memory task (see Section 2.2) was based on the task published by Hämmerer et al. (2017), but differed in the following aspects: The encoding task was divided into two sessions for real and sham stimulation, each with 72 trials, and electrodes were repositioned between sessions. As a cover task to ensure attentive processing of stimuli, subjects had to indicate whether the stimuli depicted indoor or outdoor scenes. Each trial began with a gray fixation cross (jitter between 3.5 and 6.5 s), followed by an image (3 s) showing either indoor or outdoor scene with a negative or neutral valence. During the image presentation the stimulation was on for 48 trials and off for 24 trials (Figure 2). The number of images for *outdoor* versus *indoor* and *negative* versus *neutral* as well as *real stimulation* and *sham stimulation* condition was balanced for on and off stimulation and resulted in 12 stimulation on trials and 6 stimulation off trials across the four conditions (i.e., outdoor neutral, outdoor negative, indoor neutral and indoor negative). After the image a gray fixation cross was presented again (5 s) and subsequently subjects were asked to classify the image as outdoor (by pressing key A) or indoor (by pressing key L) as quickly but also as precise as possible (time limitation of 5 s) as a cover task to ensure attentional processing of the stimuli. During the encoding task changes in pupil dilation were measured in parallel. In total, subjects performed 72 trials of 18 s duration, resulting in a total task duration of ~22 min per session, with the stimulation lasting a total of 2.4 min (3 s × 48 trials) during each session.

2.5.2 | Early and Delayed Recognition Task

The recognition tasks consisted of 144 trials each. Each task consisted of 72 old images (from the encoding task) and 72 completely new images balanced for *outdoor*, *indoor*, *negative*, *neutral scenes* and *real stimulation*, *sham stimulation* as well as *on* and *off stimulation*. The presentation of a gray fixation cross (2.5 s) was followed by an image presentation (2.5 s) with a subsequent question (time limit of 3 s) to classify the image as known (by pressing the A key) or new (by pressing the L key). This was followed by a question (time limit of 3 s) about how certain (1) or uncertain (0) the subjects were in their recognition (Figure 2). Thus, subjects saw images that were either associated with stimulation during encoding or completely new images. Both the question and the certainty had to be completed as quickly but also as precisely as possible. The total task duration per recognition task was 26.40 min (11 s × 144 trials).

2.6 | Pupil Data Acquisition

A desk-mounted infrared EyeLink 1000 eyetracker (SR Research, www.sr-research.com) with a chin rest was used to continuously record changes in pupil diameter monocularly from the left eye at a sampling rate of 1000 Hz. To provide more precise estimates of changes in pupil dilation over time, the centroid measure of pupil change was selected. Custom scripts in MATLAB 2020b (Math Works, www.mathworks.com) using Psychtoolbox 3 (www.psychtoolbox.org) and the Eyelink add-in toolbox for eyetracker control were used to control the pupillometry recording. Throughout the whole experiment, the same constant ambient light was applied to each subject. Five-point calibration was used to calibrate the camera at the beginning of the experiment.

2.7 | Pupil Data Analysis

Pupil data were pre-processed and analyzed using custom-made scripts in MATLAB 2020b (Math Works, www.mathworks.com). For pre-processing, pupil data were segmented 200 ms (Hämmerer et al. 2017; Mathôt et al. 2018; Mathôt and Vilotijević 2022) before and 9 s after stimulus onset. To clean pupil data from artifacts and blinks, the data was further processed following recommendations in Mathot (Mathôt 2013). First, the signal was smoothed using a moving Hanning window (15 ms) average. A velocity profile was then created based on the smoothed signal to detect, using a threshold of mean-standard deviation, to identify the beginning (velocity is below a threshold) and the end of a blink (velocity is above a threshold) as well as closed eyes (velocity is zero). Since the blink period can be underestimated (Mathôt 2013) 40 ms were additionally subtracted from the beginning time and added to the end time. All defined artifacts and blinks were set to NaN, summarized and then linearly interpolated. For the analyses, only trials whose raw signal was 70% free of blinks and artifacts, allowing 30% for interpolated data were included. Variations in trial numbers per condition were observed following artifact correction. Finally, all trials were also quality controlled by visual inspection. More trials survived artifact correction in sham stimulation (M ± SD: 70.92 ± 2.43) as compared to real stimulation (M ± SD: 66.54 ± 7.52), F (1, 23) = 14.48, $p < 0.001$. Pupil data were baseline-corrected (200 ms before stimulation onset) as well as individually z-scored to allow comparison of task conditions independent of individual differences in pupil dilation size (Hämmerer et al. 2017, 2018). The z standardized and baseline corrected data were analyzed in a time window between 0.8–3.8 s (see results Figure 5).

2.8 | Statistical Analysis

Statistical analyses were conducted in R version 4.2.2 (R Core Team, 2022) using RStudio version (RStudio Team, 2022) and graphs were created using the package ggplot2 (Wickham et al. 2023). For the behavioral analysis, RTs, hit rate (old images) (hits/(hits + misses)), false alarm (FA) rate (new images)

(FA/(FA + correct rejections)), hit-FA rate were calculated for early and delayed recognition task, while RTs were also assessed for the encoding task, by using aov_ez() function for repeated-measures ANOVA ({afex} package (Singmann et al. 2023) and emmeans() function ({emmeans} package (Lenth 2023)). RTs ± 2 standard deviations from the mean were excluded from RT analyses. RT analyses during the encoding task were based on 4 experimental levels of stimulation ((*real on stimulation* (1) vs. *real off stimulation* (2) vs. *sham on stimulation* (3) vs. *sham off stimulation* (4)). Detailed behavioral and RT analyses for the recognition task were based on 3 levels of stimulation (real (1) vs. sham (2) vs. off (0) stimulation), since there was no significant difference between real off stimulation and sham off stimulation (Supporting Information Section 2.1). Specifically, the analyses included two (encoding task) or three (recognition task) within-subject factors: stimulation (with levels indicating real vs. sham vs. off conditions), valence (representing negative and neutral valence), and timepoint (indicating early vs. delayed recognition). Furthermore, the mean value of the respective items for potential side effects (state of health) as well as the perception of sensations (VAS rating) were analyzed across all subjects by using aov_ez() function for repeated-measures ANOVA ({afex} package (Singmann et al. 2023) and lsmeans() function ({emmeans} package (Lenth 2023)). Additionally, Pearson correlation coefficients between VAS/memory performance (hit-FA)/reaction times (RTs) and pupil dilation (averaged per subject across trials) were calculated by using rcorr() function ({Hmisc} package (Harrell Jr and Dupont 2024)) and corrected for outliers based on interquartile range (1.5*IQR).

Changes in pupil dilation were analyzed using a linear mixed-effects (LMM) model, implemented with the {lme4} package (Bates et al. 2015), to account for repeated measurements and individual-level variability, including 'trials' as a fixed effect to model the overall time-on-task effect across all subjects, following a forward model selection approach. Model comparisons were conducted using the anova() function ({lme4} package (Bates et al. 2015) with likelihood-ratio chi-squared tests and models were fit using maximum likelihood (ML) estimation to ensure valid comparisons between models with different fixed effects. AIC (Akaike Information Criterion) values of the best model for statistical modeling and model selection were reported. In general, models with lower AIC values are indicative of a superior trade-off between data explanation and prevention of overfitting, in comparison to alternative assessed models (Vrieze 2012). To assess the relevant assumptions of LMM, check_model() function ({performance} package (Lüdecke et al. 2021)) was used to investigate linearity, homogeneity of variance, influential observations, collinearity, normality of residuals and of random effects (https://osf.io/xuwsm/).

The significance of predictors on the goodness of fit of the model was assessed using Anova() function ({car} package (Fox et al. 2023)), which computes type-II analysis-of-variance tables for mixed-effects models and provides likelihood-ratio Chi-Square statistics. The significance of the deviance of individual groups from the intercept was assessed using summary() function ({lmerTest} package (Kuznetsova, Brockhoff, and Christensen 2017)), which calculates model's coefficients, standard errors, *t*-values, and *p*-values associated with each coefficient.

The forward model selection approach included dummy coded variables identifying real and sham '**stimulation**' *[real* (1) vs. *sham* (2) vs. *off stimulation* (0)] and the differences in '**valence**' [*negative* (1) vs. *neutral* (0)] (Table S3), after ruling out any significant differences in the off stimulation condition in an initial analysis across real and sham stimulation [*real on stimulation* (1) vs. *real off stimulation* (2) vs. *sham on stimulation* (3) vs. *sham off stimulation* (4)] (Supporting Information Section 1.6). Furthermore, a random intercept 'ID' was included to account for inter-individual variations in the mean pupil change, and the variable "trials" was included to capture the impact of "time-on-task" on pupil dilations. Model comparisons were conducted (*anova(m0, m1, m2, m3, m4)*) and revealed that the best model was **model m_3** (AIC = 10,847 ($\chi^2 = 5.71$, $p = 0.02$), see Supporting Information Section 1.7 and Table S3):

- StimValence-LMM

$$pupil\ dilation \sim trials + stimulation + valence + (1|\,ID)$$

Second, based on **model m_3** the following factors were added stepwise: '***VAS***' ratings as a measure of subjective perception of sensations due to stimulation, '***sensitivity***' [*sensitive* (1) vs. *not sensitive* (0)] differentiating whether subjects received 3 and 5 mA, whether subjects received real stimulation first '***real_first***' [*counterbalanced*: *real* (1) *before sham* (0) *stimulation*], ***gender*** [*female* (1) vs. *male* (0)] and ***sporty*** [*sporty* (1) vs. *non-sport* (0)]. Subsequently model comparisons were conducted again based on all models (*anova(m0, m1, m2, m3, m3_1, m3_2, m3_3, m3_4, m3_5, m4_6)*) and the best fitting model from the second step for was **model m3_3** (AIC = 10,839 ($\chi^2 = 8.01$, $p = 0.005$), see Tables S3 and S4):

- StimValence-VAS-LMM

$$pupil\ dilation \sim trials + stimulation + valence + VAS + sensitivity + real\_first + (1|\,ID)$$

Consequently, statistical analyses regarding changes in pupil dilation are based on the '**StimValence-VAS-LMM**' model.

Additionally, two theory-driven exploratory analyses were conducted to investigate to what extent (a) stimulation had an additional benefit to the effect of stimulus valence on pupil dilation and (b) how sensitivity affects the subjective perception of stimulation on pupil dilation. Based on the best model for the pupil analysis, interactions between (a) stimulation and valence, and (b) valence and sensitivity were incorporated:

- Stim*Valence-VAS-LMM

$$pupil\ dilation \sim trials + stimulation{*}valence + VAS + sensitivity + real\_first + (1|ID)$$

- Sensitivity*VAS-LMM

$$pupil\ dilation \sim trials + stimulation + valence + VAS{*}sensitivity + real\_first + (1|ID).$$

Finally, to explore potential effects of subjective sensory perception caused by stimulation on memory performance a comparable model for the behavioral data based on the average memory

performance (averaged per subject across trials) was performed (cf. Table S5; Supporting Information Section 2.7).

Emmip() and emtrends() functions ({emmeans} package (Lenth 2023)) were utilized to analyze interaction effects, such as interactions of VAS and stimulation conditions. The function emmip() generates an interaction plot to see how the categorical variable affects the variable over its entire range. The function emtrends() calculates estimated marginal means for different levels of the categorical variable.

Ethics approval, written informed consent, and compensation. The study was approved by the Ethics Committee of medical faculty at the Otto von Guericke University of Magdeburg (reference no. 107/20) and was carried out in accordance with the ethical standards of Helsinki. A written informed consent was obtained from each subject before participation, and subjects received 90 Euro reimbursement.

3 | Results

3.1 | Behavioral Results—taVNS During Encoding Task

During the cover task (indoor-outdoor classification), subjects showed high accuracy ratings on indoor (M ± SD: 0.96 ± 0.18) and outdoor (M ± SD: 0.98 ± 0.14) scenes, indicating that all subjects followed the instructions of the cover task relevant for incidental encoding. With regard to main effects of response times during the **encoding task**, subjects did not show faster reactions times (RTs) when classifying whether an image was "inside or outside" for negative (M ± SD: 0.79 ± 0.47) as compared to neutral (M ± SD: 0.79 ± 0.50) events, F (1, 23) = 0.12, p = 0.73. RTs also did not differ between real (M ± SD: 0.76 ± 0.47) as compared to sham (M ± SD: 0.82 ± 0.49) stimulation sessions (collapsed across on or off stimulation trials within session), F (1, 23) = 2.29, p = 0.14 or during on (M ± SD: 0.81 ± 0.48) as compared to off (M ± SD: 0.77 ± 0.49) stimulation, F (1, 23) = 3.02, p = 0.1. There was no interaction between 'real vs sham' stimulation and valence (F (1, 23) = 2.05, p = 0.17), 'real vs. sham' stimulation and 'on vs off' stimulation (F (1, 23) = 0.0003, p = 0.1), valence and 'on vs. off' stimulation (F (1, 23) = 0.21, p = 0.65) for RTs during encoding task.

3.2 | Behavioral Results—Early and Delayed Recognition Task

3.2.1 | Better Memory Performance (hit-FA) for Negative Events and due to Stimulation

Consistent with studies showing that **negative events** improve memory performance (Hämmerer et al. 2017, 2018) subjects showed a higher recognition accuracy (hit-FA rate) for negative events (M ± SD: 0.63 ± 0.26) compared to neutral events (M ± SD: 0.55 ± 0.25) (Figure 3c), which underpins the impact of negative emotionality on memory performance, F (1, 23) = 18.68, p < 0.001. Likewise, as expected, memory performance was decreased on the delayed (M ± SD: 0.53 ± 0.23) as compared to the early (M ± SD: 0.65 ± 0.26) recognition task, F (1, 23) = 54.79, p < 0.001 (Figure 3e). There was no interaction between timepoint and valence, F (1, 23) = 0.27, p = 0.61.

We also observed that the **stimulation** per se, but not the stimulation condition (real vs. sham stimulation), had an influence on memory performance as indicated by hit-FA, F (2, 46) = 509, p < 0.001 (Figure 3d). Subjects, in particular, showed better memory performance during real (M ± SD: 0.73 ± 0.15) as compared to off (M ± SD: 0.31 ± 0.10) stimulation (off-real: β = −0.42 (SE = 0.01; t = −38.37, p < 0.001)) and during sham (M ± SD: 0.74 ± 0.13) as compared to off (M ± SD: 0.31 ± 0.10) stimulation (off-sham: β = −0.42 (SE = 0.02; t = −26.30, p < 0.001)); however memory performance was not better during real as compared to sham stimulation (real-sham: β = −0.01 (SE = 0.02; t = −0.54, p = 1)).

Interestingly, there was a significant ordinal interaction between stimulation and valence, F (2, 46) = 6.46, p = 0.003 (Figure 3a). Specifically, the effect that negative events were better remembered than neutral events was more pronounced during real stimulation than during sham stimulation (emo-neu real-sham: β = 0.07 (SE = 0.03; t = 2.64, p = 0.01)) and during off stimulation (emo-neu off-real: β = −0.08 (SE = 0.02; t = −3.89, p = 0.007)). There was no interaction between valence and 'off vs. sham' stimulation (emo-neu off-sham: β = −0.02 (SE = 0.03; t = −0.59, p = 0.56)). Additionally, there was a significant ordinal interaction between stimulation and timepoint, F (2, 46) = 5.54, p = 0.007 (Figure 3b), indicating that during off stimulation memory performance during early and delayed recognition was worse than during real stimulation (delay-early off-real: β = 0.1 (SE = 0.03; t = 3.77, p = 0.001)) and sham stimulation (delay-early off-sham: β = 0.07 (SE = 0.03; t = 2.25, p = 0.03)). However, there was no significant interaction between timepoint and real compared to sham stimulation (delay-early real-sham: β = −0.02 (SE = 0.03; t = −0.70, p = 0.49)). This suggests that stimulation per se had stronger effects on memory performance than off stimulation. An additional exploratory analysis of memory performance was conducted to investigate individual factors associated with taVNS, such as subjects' sensitivity and gender (Supporting Information Section 2.8), which revealed no significant effects. Additionally, there were no correlations between subjective perception of sensation due to stimulation and memory performance (Supporting Information Section 2.9).

3.2.2 | Better Correct Recognition (hit) for Negative Events and due to Stimulation

Subjects showed higher number of hits for **negative events** (M ± SD: 0.73 ± 0.25) compared to neutral events (M ± SD: 0.66 ± 0.24), F (1, 23) = 25.67, p < 0.001. Likewise, hits were fewer after delayed (M ± SD: 0.65 ± 0.24) as compared to early (M ± SD: 0.73 ± 0.25) recognition task, F (1, 23) = 39.12, p < 0.001. There was a tendency for an interaction between timepoint and valence, F (2, 46) = 4.0, p = 0.06 (Figure S3).

Additionally, subjects showed higher number of hits during real (M ± SD: 0.83 ± 0.14) as compared to off (M ± SD: 0.41 ± 0.08) stimulation (off-real: β = −0.42 (SE = 0.01; t = −38.37, p < 0.001)) and during sham (M ± SD: 0.84 ± 0.11) as compared to off (M ± SD: 0.41 ± 0.08) stimulation (off-sham: β = −0.43 (SE = 0.02; t = −26.30, p < 0.001)); however number of hits were not higher during real

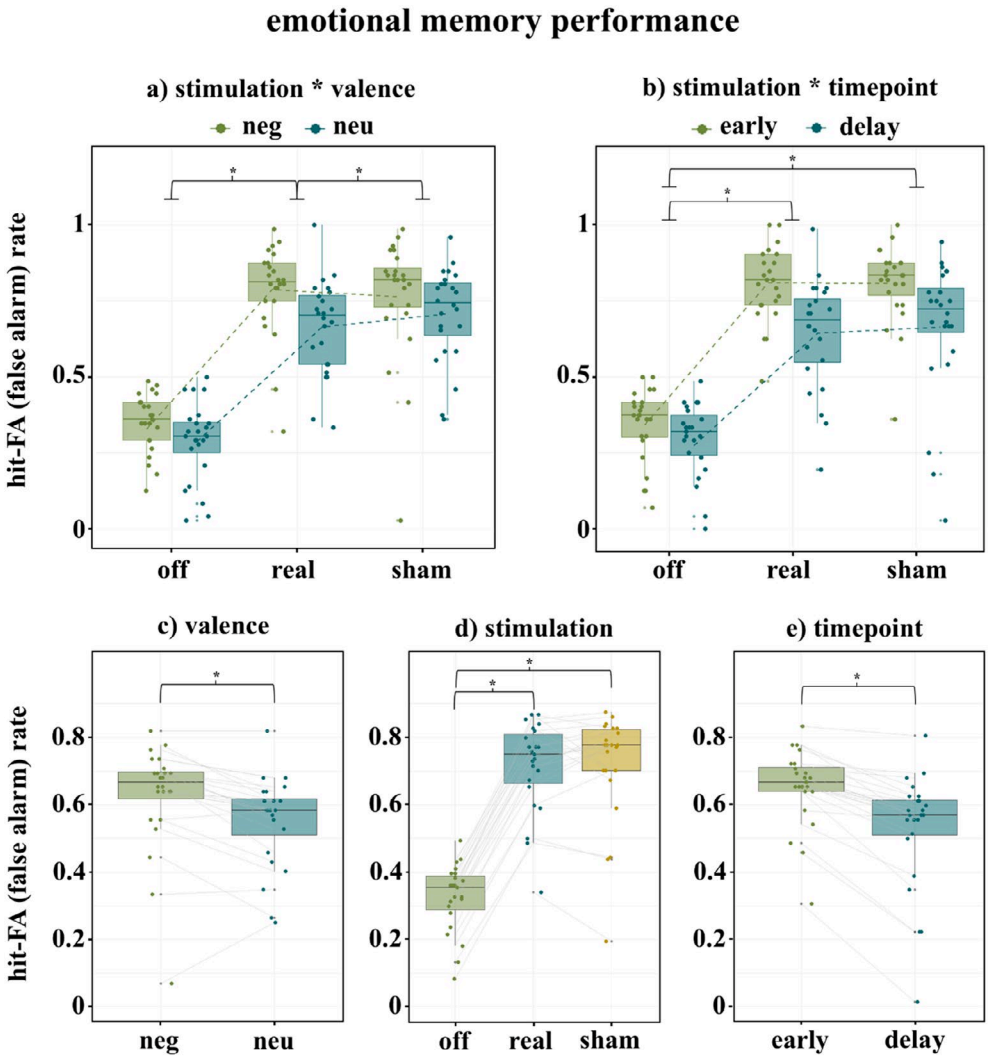

FIGURE 3 | Memory performance (hit-FA (false alarms)) is shown here aggregated at the subject level ($N = 24$) using boxplots. Panel (a) shows a significant interaction between stimulation (off, real, and sham) and valence (negative (green) and neutral (turquoise)), $F(2, 46) = 6.46$, $p = 0.003$, indicating that negative events specifically benefitted from real stimulation. Panel (b) shows a significant interaction between stimulation (off, real, and sham) and timepoint (early (green) and delayed (turquoise)), $F(2, 46) = 5.54$, $p = 0.007$. Panel (c) depicts valence effects, showing better memory performance for negative (green) compared to neutral (turquoise) events, $F(1, 23) = 18.68$, $p < 0.001$. Panel (d) presents the effects of stimulation, indicating significantly improved memory performance for real stimulation compared to off stimulation, and sham stimulation compared to off stimulation, $F(2, 46) = 509$, $p < 0.001$. Panel (e) demonstrates better memory performance during early (green) compared to delayed (turquoise) recognition tasks, $F(1, 23) = 54.79$, $p < 0.001$. Each point represents an individual subject, and light gray lines connect the conditions for each subject. Significant differences or interaction effects are indicated by asterisks.

as compared to sham stimulation (real-sham: $\beta = -0.01$ (SE = 0.02; $t = -0.54$, $p = 1$)), $F(2, 46) = 509$, $p < 0.001$. There was a significant ordinal interaction between valence and stimulation, $F(2, 46) = 6.46$, $p = 0.003$ (Figure S3d). Specifically, during real stimulation the difference between negative and neutral events was more pronounced than during sham stimulation (emo-neu real-sham: $\beta = 0.07$ (SE = 0.03; $t = 2.64$, $p = 0.01$)) and during off stimulation (emo-neu off-real: $\beta = -0.08$ (SE = 0.02; $t = -3.89$, $p = 0.007$)). There was no interaction between valence and 'off vs. sham stimulation (emo-neu off-sham: $\beta = -0.02$ (SE = 0.03; $t = -0.59$, $p = 0.56$)). Additionally, there was a significant ordinal interaction between timepoint and stimulation, $F(2, 46) = 5.54$, $p = 0.007$ (Figure S3e), indicating that during off stimulation the difference in number of hits between early and delayed recognition was higher than during real stimulation (delay-early off-real: $\beta = 0.1$ (SE = 0.03; $t = 3.77$, $p = 0.001$)) and sham stimulation (delay-early off-sham: $\beta = 0.07$ (SE = 0.03; $t = 2.25$, $p = 0.03$)). However, there was no significant interaction between timepoint and 'real vs. sham' stimulation (delay-early real-sham: $\beta = -0.02$ (SE = 0.03; $t = -0.70$, $p = 0.49$)). For results on certainty ratings for correctly identified images see Supporting Information Section 2.5. The effects for hits were generally consistent with the effects for hit-FAs, suggesting that the memory effects were primarily due to encoding rather than response biases.

3.2.3 | Less False Alarms (FA) During Early Recognition Task

Subjects showed fewer false alarms (FA) during early (M ± SD: 0.08 ± 0.02) as compared to delayed (M ± SD: 0.13 ± 0.04) recognition task, $F(1, 23) = 10.44$, $p = 0.004$. There was no

significant difference between negative (M ± SD: 0.11 ± 0.03) and neutral (M ± SD: 0.10 ± 0.03) events for FA, F (1, 23) = 0.07, p = 0.79. However, there was a significant ordinal interaction between timepoint and valence, F(1, 23) = 4.18, p = 0.05 (Figure S4), indicating in FA-rate that the negative events may be more strongly influenced by timing (delayed vs. early) compared to the neutral events (delay-early emo-neu: β = 0.03 (SE = 0.02; t = 2.04, p = 0.05)). For results on certainty ratings for incorrect identified images (FA) Supporting Information Section 2.5 and for certainty ratings for FA RTs see Supporting Information Section 2.6.

3.3 | Behavioral Results—RTs During Early and Delayed Recognition Task

In general, subjects showed longer RTs for new (M ± SD: 1.03 ± 0.34) compared to old (M ± SD: 0.96 ± 0.31) images (averaged across correct and incorrect responses), F (1, 23) = 27.85, p < 0.001, which is in line with previous RTs during emotional memory task (see Hämmerer et al. (2017). Additionally, subjects showed no difference in RTs between negative (M ± SD: 0.99 ± 0.33) and neutral (M ± SD: 0.99 ± 0.32) events, F (1, 23) = 0.20, p = 0.65, and no RTs differences between early (M ± SD: 1.02 ± 0.34) and delayed (M ± SD: 0.96 ± 0.31) recognition, F(1, 23) = 3.14, p = 0.09. However, there was a significant interaction between "old vs. new" images and valence, F (1, 23) = 6.40, p = 0.02, indicating that RTs for negative events were generally longer for new images compared to old images (Figure S5). There were no significant interactions between 'old vs. new' images and timepoint, F (1, 23) = 0.65, p = 0.43 and between valence and timepoint, F (1, 23) = 2.21, p = 0.15.

3.3.1 | RTs For Correct Responses During Stimulation

hit RTs (averaged speed of correct responses to target image) revealed faster RTs during real (M ± SD: 0.87 ± 0.12) as compared to off (M ± SD: 0.93 ± 0.16) stimulation (off-real: β = 0.05 (SE = 0.01; t = 3.80, p = 0.002)) and during sham (M ± SD: 0.87 ± 0.11) as compared to off (M ± SD: 0.92 ± 0.16) stimulation (off-sham: β = 0.05 (SE = 0.01; t = 3.81, p = 0.002)); but RTs were not faster during real as compared to sham stimulation (real-sham: β = −0.003 (SE = 0.02; t = −0.15, p = 1)), F (2, 46) = 6.95, p = 0.002 (Figure 4b). hit RTs were not different between negative (M ± SD: 0.89 ± 0.13) and neutral (M ± SD: 0.89 ± 0.13) events, F (1, 23) = 0.01, p = 0.92 (Figure 4a). Furthermore, there was a tendency for hit RTs during early recognition (M ± SD: 0.92 ± 0.1) to be slower compared to delayed recognition (M ± SD: 0.86 ± 0.12), F(1, 23) = 3.54, p = 0.07 (Figure 4c). There were no significant hit RTs difference neither between stimulation and valence, F (2, 46) = 0.008, p = 0.99, stimulation and timepoint (F (2, 46) = 0.49, p = 0.61), nor valence and timepoint, F(1, 23) = 0.05, p = 0.82 (Figure S6). For results on certainty ratings for hit RTs see Supporting Information Section 2.6.

3.4 | Pupillometry Results

In accordance with increased pupil dilations observed during **emotionally salient events** (Joshi et al. 2016; Hämmerer et al. 2018), pupil dilation was increased during the presentation of negative (M ± SE: 0.44 ± 0.11) as compared to neutral (M ± SE: 0.34 ± 0.11) events (χ^2 = 5.65, p = 0.02) (Figure 5).

Regarding the influence of the **stimulation conditions** (χ^2 = 44.47, p < 0.001), pupil dilation was increased during real (M ± SE: 0.49 ± 0.11) as compared to off (M ± SE: 0.19 ± 0.11) stimulation (off-real: β = −0.31 (SE = 0.05; t = −5.68, p < 0.001)) and during sham (M ± SD: 0.49 ± 0.11) as compared to off (M ± SE: 0.19 ± 0.11) stimulation (off-sham: β = −0.31 (SE = 0.05; t = −5.65, p < 0.001)); however pupil dilation was not increased during real as compared to sham stimulation

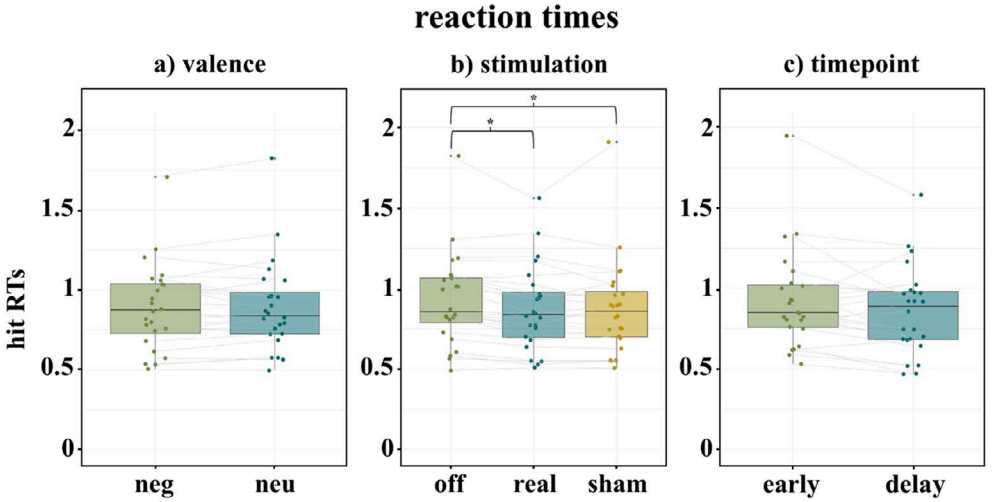

FIGURE 4 | Averaged speed of correct responses to target image (hit RTs) is shown here aggregated at the subject level (N = 24) using boxplots. Panel (a) depicts valence effects (negative (green) > neutral (turquoise)), F (1, 23) = 0.01, p = 0.92, indicating no significant effects on RTs. Panel (b) presents the effects of stimulation, indicating significant faster RTs for real stimulation compared to off stimulation and sham stimulation compared to off stimulation, F (2, 46) = 6.95, p = 0.002. Panel (c) shows timepoint effects (early (green) > delay (turquoise)), F (1, 23) = 3.54, p = 0.07 indicating no significant effects on RTs. Each point represents an individual subject, and light gray lines connect the conditions for each subject. Significant differences are indicated by asterisks.

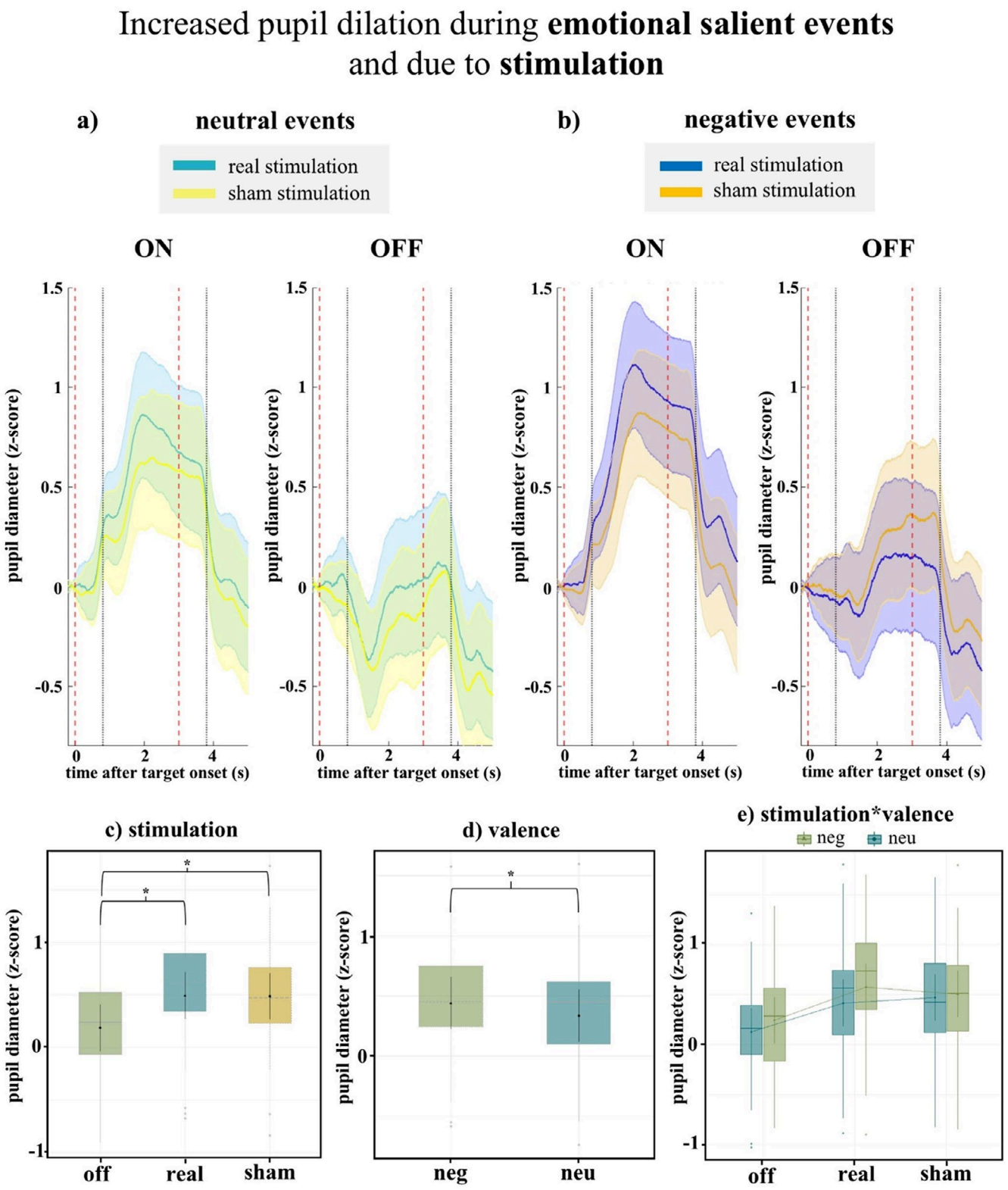

FIGURE 5 | Pupil diameters (z-scores) are shown for (a) neutral events (left) under real stimulation (light blue) and sham stimulation (yellow), and (b) negative events (right) under real stimulation (dark blue) and sham stimulation (orange), during both on (3 s) and off (15 s) periods of stimulation. The shaded areas represent the standard error across subjects ($N = 24$). Dashed red vertical lines indicate the time window when stimulation was applied, while dashed black vertical lines mark the analysis window between 0.8 and 3.8 s. In (c) stimulation, boxplots show significantly increased pupil dilation for both real stimulation and sham compared to off stimulation ($\chi^2 = 44.47$, $p < 0.001$). In (d) valence, boxplots significantly increased pupil dilation for negative compared to neutral events ($\chi^2 = 5.65$, $p = 0.02$). (e) Shows the boxplot for the interaction between stimulation and valence, which was not significant ($\chi^2 = 1.44$, $p = 0.49$; model: *pupil dilation ~ trials + stimulation*valence + VAS + sensitivity + real_first* + (1|*ID*)). Significant differences or interaction effects are indicated by asterisks.

(real-sham: $\beta = 0.006$ (SE = 0.06; $t = 0.12$, $p = 1$)) (Figure 5; Table S5).

Furthermore, some of the variance in pupil dilation was significantly explained by perception of stimulation (**VAS**) ($\chi^2 = 4.45$, $p = 0.03$), although this did not imply that stimulation effects could no longer be explained in the emotional memory task, as was the case in a further recording in this study not including an emotional memory task (compare results Ludwig et al. (2024b); see Table S3 model m_3 and m3_1). There was no significant sensitivity difference in pupil dilation between subjects receiving 3 mA (M ± SE: 0.37 ± 0.18) compared to subjects receiving 5 mA (M ± SE: 0.41 ± 0.12), $\chi^2 = 0.03$, $p = 0.87$. Additionally, although the **order of stimulation** was counterbalanced in the experiment, subjects who received real (M ± SE: 0.68 ± 0.15) stimulation before sham (M ± SE: 0.10 ± 0.14) stimulation showed generally larger pupil dilations ($\chi^2 = 8.33$, $p = 0.004$).

The theory-driven analysis to (a) assess the extent to which stimulation enhances the effect of stimulus valence on pupil dilation (model *pupil dilation ~ trials + stimulation*valence + VAS + sensitivity + real_first + (1|ID)*), revealed no significant interaction between stimulation and valence ($\chi^2 = 1.44$, $p = 0.49$) (Figure 5e), while stimulation ($\chi^2 = 44.46$, $p < 0.01$), valence ($\chi^2 = 5.65$, $p = 0.02$), VAS ($\chi^2 = 4.42$, $p = 0.04$) and the order of stimulation ($\chi^2 = 8.33$, $p = 0.004$) were significant and sensitivity was not significant ($\chi^2 = 0.03$, $p = 0.87$).

The theory-driven analysis to (b) assess how sensitivity influences the subjective perception of stimulation on pupil dilation (model *pupil dilation ~ trials + stimulation + valence + VAS*sensitivity + real_first + (1|ID)*), revealed a significant interaction between VAS rating and sensitivity ($\chi^2 = 7.13$, $p = 0.01$) (Figure 6), indicating that higher sensitivity to stimulation (3 mA) led to more pronounced pupil dilation as the perceived intensity of the stimulation increased (higher VAS ratings), while subjects with lower sensitivity (5 mA) showed a more stable and moderate pupil dilation response across VAS ratings. Stimulation ($\chi^2 = 44.46$, $p < 0.01$), valence ($\chi^2 = 5.65$, $p = 0.02$), VAS ($\chi^2 = 4.67$, $p = 0.03$) and the order of stimulation ($\chi^2 = 5.75$, $p = 0.02$) were significant and while sensitivity was not significant ($\chi^2 = 0.03$, $p = 0.88$). Additionally, there were no correlation between pupil dilation and memory performance (Supporting Information Section 2.9).

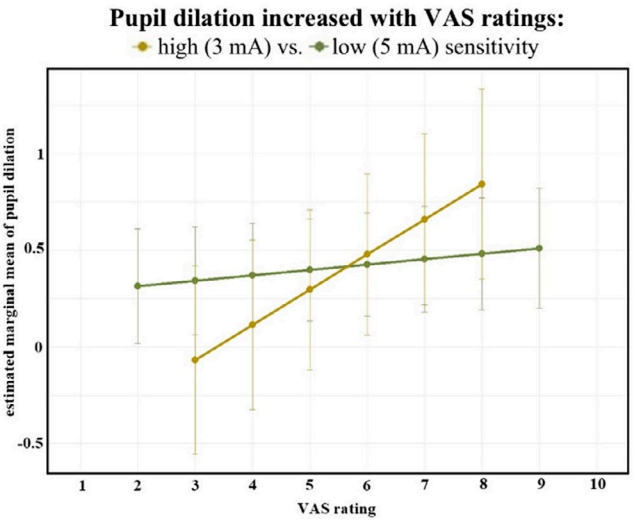

FIGURE 6 | Interaction between VAS rating and sensitivity on pupil dilation. The model *pupil dilation ~ trials + stimulation + valence + VAS*sensitivity + real_first + (1|ID)* revealed a significant interaction between VAS rating and sensitivity ($\chi^2 = 7.13$, $p = 0.01$). The estimated marginal means (emmeans) of pupil dilation are presented across VAS ratings for both the low sensitivity group (5 mA, green ($N = 17$)) and the high sensitivity group (3 mA, ochre ($N = 7$)). **VAS 2**: In the low sensitivity group, the emmean is 0.314 (SE = 0.147, 95% CI [0.017, 0.611]). **VAS 3**: The emmean in the low sensitivity group is 0.342 (SE = 0.137, 95% CI [0.062, 0.622]), while in the high sensitivity group it is −0.069 (SE = 0.241, 95% CI [−0.555, 0.418]). **VAS 4**: The emmean is 0.370 (SE = 0.130, 95% CI [0.101, 0.639]) in the low sensitivity group and 0.114 (SE = 0.214, 95% CI [−0.326, 0.553]) in the high sensitivity group. **VAS 5**: The low sensitivity group has an emmean of 0.398 (SE = 0.127, 95% CI [0.134, 0.662]), while the high sensitivity group shows 0.296 (SE = 0.200, 95% CI [−0.119, 0.711]). **VAS 6**: The low sensitivity group emmean is 0.426 (SE = 0.128, 95% CI [0.160, 0.692]), and the high sensitivity group is 0.478 (SE = 0.200, 95% CI [0.062, 0.894]). **VAS 7**: The emmean for the low sensitivity group is 0.454 (SE = 0.133, 95% CI [0.179, 0.729]), while the high sensitivity group shows 0.660 (SE = 0.216, 95% CI [0.218, 1.103]). **VAS 8**: In the low sensitivity group, the emmean is 0.482 (SE = 0.142, 95% CI [0.192, 0.772]), while the high sensitivity group emmean is 0.842 (SE = 0.243, 95% CI [0.351, 1.334]). **VAS 9**: The low sensitivity group emmean is 0.510 (SE = 0.154, 95% CI [0.200, 0.820]). This illustrates that as VAS ratings increased, pupil dilation generally increased more in the high sensitivity group than in the low sensitivity group.

4 | Discussion

In this study, an emotional memory task with negative events involving the LC-NE system (Hämmerer et al. 2017, 2018) was combined with short bursts of event-related taVNS in younger adults. While we refer to phasic stimulation in our study as short bursts of externally applied stimuli, it is important to distinguish this from the natural phasic activity of the LC, which involves much shorter, rapid bursts of NE release, typically lasting tens of milliseconds (Aston-Jones and Cohen 2005). Phasic taVNS was applied because previous phasic i/taVNS has been shown to modulate LC-NE activity in animal and human studies (Collins et al. 2021; Mridha et al. 2021; Hulsey et al. 2017, 2019; Sharon, Fahoum, and Nir 2021; Sclocco et al. 2019, 2020), while tonic stimulation has not always shown reliable effects on the LC-NE system (Ludwig et al. 2021; Keute et al. 2019). The purpose was to investigate to what extent real taVNS can enhance memory performance (immediate and delayed recognition task) and increase pupil dilation (encoding task), while varying the emotional content of the images.

The study confirmed that emotionally negative events were better remembered than neutral events, which is in line with previous studies on emotional memory performance (Hämmerer et al. 2017, 2018). Animal and human research suggest that the LC-NE system is a crucial part in the processing and encoding of emotionally negative events by releasing NE in LC target areas including the amygdala and hippocampus (Sterpenich

et al. 2006; Ludwig et al. 2024a; Luo et al. 2015; Hämmerer et al. 2018; Chen and Sara 2007; Tully and Bolshakov 2010). Likewise, as expected, the overall memory performance was better during the immediate recognition task compared to delayed recognition task (24 h) driven by both more frequent false alarms (FAs) and fewer correct recognitions (hits) in the delayed recognition. While the hit rate tended to decrease less over time for emotional than neutral events, FAs were more frequent for emotional than neutral events during the delayed recognition task. This may be consistent with results showing that arousal makes it more difficult to retain multiple representations of the same level in working memory (Mather and Sutherland 2011; Mather et al. 2016a).

In contrast to Hämmerer et al. (2017), we did not observe longer RTs for negative events during the encoding tasks or the recognition task. However, we replicated the effect of longer RTs for new compared to old images during the recognition task (cf. Hämmerer et al. 2017), which might be related to subjects focusing particularly on old images when they were given the task of classifying stimuli as old or new (Hämmerer et al. 2017; Kafkas and Montaldi 2018). Given our stimulation design, we were able to compare effects of real or sham and no stimulation on behavioral performance in an event-related manner, that is, examine 'stimulation on' versus 'stimulation off' effects distributed across events during the encoding task. In general, memory performance as assessed in hit-FA and correct recognition (hit) did not differ for real and sham stimulation but was improved during any type of stimulation (real or sham) as compared to no stimulation trials. Additional analyses also showed that this effect could not be explained by the subjective sensory perception of the stimulation, which was higher for real stimulation than compared to sham stimulation.

The presence of real or sham stimulation could therefore facilitate the initial processing and storage of the images in memory, making them easier to recall in the recognition task, which was also reflected in RTs, where the stimulation per se but not the stimulation condition led to a faster RT during the recognition task. Interestingly, while negative events were generally better remembered than neutral events, this effect was more pronounced for events encoded during real stimulation as compared to sham or no stimulation. This suggests that in addition to a general encoding enhancing effect of real or sham stimulation, real stimulation was able to further improve encoding in particular of negative events, which assumably rely more on the noradrenergic LC (Ludwig et al. 2024a; Hämmerer et al. 2018; Chen and Sara 2007; Manaye et al. 1995). These results are in line with increased recollection-based memory performance for emotional, but not neutral, images in a tonic tAVNS paradigm (Ventura-Bort et al. 2021). As neutral and negative events were mixed within real stimulation sessions, and sensory perception was independent of valence rendering, this effect is unlikely to be driven by differences in subjective sensory perception related to the stimulation. Instead, the encoding of emotionally negative events known to engage the noradrenergic system might have been further supported by a stronger engagement of the LC during real stimulation or by a stronger overall arousal in the real stimulation condition given higher ratings of subjective perception of sensations due to stimulation. Both notions would be in line with prior evidence suggesting that noradrenergic and glutamatergic processes interact in affecting cognition through a combination of attentional focus and arousal, (GANE) model (Mather et al. 2016b).

While an involvement of the vagus nerv (VN) and the LC appear to be involved in the processing of emotional memories (Ludwig et al. 2024a; Hämmerer et al. 2017, 2018; Mather et al. 2016a; McIntyre, McGaugh, and Williams 2012) the underlying mechanism involved in the possible improvement of emotional memory by taVNS appears to be complex. Since the VN endings are mainly connected to the NTS in the brainstem, which then engage the LC and via the LC further structures of the CNS, i/taVNS has the potential to modulate NE-levels and therefore promote long-term potentiation in the hippocampus and neuronal plasticity, which is important for memory formation (Williams and McGaugh 1993; Roozendaal and McGaugh 2011; McIntyre, McGaugh, and Williams 2012; George et al. 2000; Sara 2009). According to Vonck et al. (2014), VNS may increase hippocampal synaptic plasticity by influencing the trisynaptic circuit through adrenergic signaling mediated by the LC. Indeed, electrophysiological studies in rodents showed, that phasic stimulation increased LC-released NE, which could support memory encoding via ß-adrenoceptors in the hippocampus (Luo et al. 2015; Florin-Lechner et al. 1996; Roosevelt et al. 2006; Dorr and Debonnel 2006; Raedt et al. 2011). Additionally, iVNS studies in animals have shown that iVNS led to burst firing in the neurons of the LC (Dorr and Debonnel 2006), thus improving memory storage after avoidance learning (Clark et al. 1998) and increased NE-levels in the amygdala after stimulation (Hassert, Miyashita, and Williams 2004).

In this context, it is surprising to observe that short bursts of stimulation per se, also after controlling for condition differences in sensory perception effects, resulted in an increased pupil dilation as well as better memory performance compared to no stimulation. This is in contrast to previous short burst of taVNS studies which demonstrated increased pupil dilation during real compared to sham stimulation during resting-state task (Sharon, Fahoum, and Nir 2021; Lloyd and Wurm 2023; D'Agostini et al. 2023; Skora, Marzecova, and Jocham 2024). Additionally, our theory-driven pupillometry analysis showed no interaction between stimulation and valence, suggesting that pupil dilation was not differentially modulated by stimulus valence during real or sham stimulation, and that short bursts of stimulation per se led to increased pupil dilation. The lack of an effect between real and sham stimulation in our study could be partly explained by attentional factors. In the presence of subtle sensory differences between the stimulation conditions, attentional resources might be directed to the evaluation of these differences, independent of the subjective perception of sensation due to stimulation. Our concurrent task may have engaged these attentional resources, preventing differentiation between the perceptual aspects of real and sham stimulation that might otherwise be reflected in pupil dilation. Currently, these considerations remain speculative and require assessment in future studies that either eliminate or rigorously control for sensory differences between real and sham stimulation conditions.

Since sham stimulation applied to the earlobe should not directly affect the VN (Peuker and Filler 2002), an effect of

sham stimulation on the LC via the VN should be excluded. It is thus to be assumed that increased pupil dilation and memory encoding related to real or sham stimulation (as compared to no stimulation) might relate to attentional and/or arousal processes related to the sensory effects of stimulations. This would be in line with prior studies showing that pupil size and encoding performance can be modulated by attention or arousal (Kahneman 1973; Miller, Gross, and Unsworth 2019; Sara and Bouret 2012; Lee et al. 2018). Indeed, real versus sham differences in stimulation sensations did explain a significant portion of the explained variance in pupil dilation. In addition, subjects with 3 mA ($N = 7$) showed a more pronounced pupil dilation when the perceived intensity of stimulation increased, while subjects with 5 mA ($N = 17$) showed a more stable and moderate response to pupil dilation across VAS ratings. However, given the contrast between any type of sensory stimulations versus no stimulation and the fact that sensations of stimulation were only acquired after each stimulation session, a remaining general effect in arousal or attention related to stimulation should be assumed. Unlike prior studies, our event related design was able to investigate these cognitive and physiological effects related to stimulation sensations on a trial-by-trial basis, suggesting assumable attentional or arousal-related changes on a timescale of seconds related to sensory effects of stimulation. Interestingly, these sensory stimulation effects proved generally conducive rather than distractive for memory encoding (Pleger and Villringer 2013; Kong et al. 2005). Nonetheless, this suggests, that future taVNS stimulation studies should carefully control and assess not only sensory effects of different stimulation conditions but also potentially cognitively enhancing effects related to the sensation of stimulations per se. Furthermore, it reinforces the importance of keeping sensory perceptions related to real and sham stimulation as constant as possible (Ludwig et al. 2024b).

Interestingly, while controlling for subjective perception of sensations in the resting-state task without cognitive component in the same subjects (cf. Ludwig et al. (2024b), which explained a significant proportion of the stimulation effects on pupil dilation, in the present task, both behavioral and pupil effects of real versus sham stimulation could not be fully explained by stimulation condition differences in subjective perception of sensations (cf. Ludwig et al. (2024b); see Table S3 model m_3 and m3_1). This suggests that sensations of perception generally do not dominate the potentially arousal-related physiological and cognitive effects. For instance, attentional resources during an emotional memory as compared to a resting state task, might be additionally biased towards processing and encoding emotional information, while the negative events itself caused also significant pupil dilations independent of stimulation sensations. This may have contributed to the fact that the variance of pupil dilation in the emotional memory task compared to the resting state task was less explained by subjective sensory perceptions (cf. Ludwig et al. (2024b)).

Finally, independent of stimulation conditions, emotionally negative events resulted in larger pupil dilations than neutral events, confirming existing animal and human studies on pupil dilation during emotionally negative events (Hämmerer et al. 2017, 2018; Joshi et al. 2016). As changes in pupil dilation can serve as an indirect indicator of LC-NE activity (Joshi et al. 2016) increased pupil dilation due to emotionally negative events may reflect stimulus driven influences on the LC-NE system (Sara and Bouret 2012).

As a limitation, it should be mentioned that further research is needed to systematically investigate possible carry-over effects of individual taVNS sessions of varying duration on pupil dilation and emotional memory. Another possibility as to why we found no differences between real and sham stimulation on a physiological and behavioral level could also be that effects of real stimulation might have carried over into the following sham stimulation period. Although real and sham stimulation were balanced in our design across subjects, both sessions took place directly after each other (wash-out approx. 5–10 min) and might have thus resulted in a partial contamination through preceding stimulation effects. As there is currently no consensus regarding presence and temporal extent of aftereffects of short bursts of event-related stimulation designs, these suggestions should be verified in future studies investigating parallel interventions close in time as well as separated by a few days. Furthermore, to achieve more reliable model estimates and enhance generalizability, an increased sample size might be advantageous for future research.

Taken together, our study shows that real or sham taVNS stimulation could enhance encoding of events, while real stimulation in particular enhanced encoding of negative as compared to neutral events. This finding is in line with animal research showing an involvement of ascending vagal fibers in emotional memory (Clark et al. 1998; Williams and McGaugh 1993; Hulsey et al. 2017; McIntyre, McGaugh, and Williams 2012) and suggests that taVNS represents a promising method to influence emotional memory processes. Further research into its effects on certain types of memories—especially those that are also associated with positive emotions—would be valuable for future therapeutic applications. Furthermore, our results showed that emotional valence as well as stimulation per se increased pupil dilation. Therefore, our taVNS design, which facilitated event-related decorrelation of stimulation (real vs. sham) in comparison to a no-stimulation condition, may encourage other researchers to incorporate trials without stimulation to more effectively differentiate the effects of stimulation. While the role of the LC in the regulation of arousal and attention, also in the context of emotional events, is well known (Clewett et al. 2016; Aston-Jones and Cohen 2005; Hämmerer et al. 2018; Mather et al. 2016a; Sara and Bouret 2012; Lee et al. 2018; Samuels and Szabadi 2008; Jacobs et al. 2020; Bari et al. 2020; Berridge and Waterhouse 2003), the broader network of cognitive and sensory processes leading to pupil dilation under these conditions is complex and not fully understood. Factors such as VN activity, attentional capture and sensory perception may indirectly influence LC, but they might also affect pupil size and cognitive processes through additional pathways. Further research including functional magnetic resonance imaging (fMRI) during stimulations taVNS (see Ludwig et al. 2021 for review) is therefore needed to decipher these taVNS mechanisms and to clarify the specific contributions of the LC and other neuronal structures to pupil dilation (Reimer et al. 2016; Collins et al. 2021; Mridha et al. 2021; Hulsey et al. 2017).

Author Contributions

Mareike Ludwig: conceptualization, data curation, formal analysis, investigation, methodology, project administration, validation, visualization, writing – original draft, writing – review and editing. **Matthew J. Betts:** conceptualization, funding acquisition. **Dorothea Hämmerer:** conceptualization, funding acquisition, methodology, supervision, validation, writing – review and editing.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in OSF: https://osf.io/xuwsm/.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.