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BLUE ENRICHED LIGHT MODULATES PUPIL DILATION INDUCED BY TRANSCUTANEOUS VAGUS NERVE STIMULATION

Abbreviated title: Blue Light Modulates tVNS-Induced Pupil Dilation

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Abstract

Introduction

Transcutaneous vagus nerve stimulation (tVNS) targets the auricular branch of the vagus nerve and can modulate brainstem arousal systems, including the locus coeruleus–noradrenaline (LC-NE) pathway. Blue-enriched light also affects LC activity and can enhance alertness and cognition. However, no study has tested whether combining tVNS with blue light could further boost noradrenergic activity in humans. We therefore measured pupil responses, a well-established marker of LC-NE function, to assess how light and tVNS interact.

Methods

We conducted a randomized, within-subject block design with twenty-five healthy adults (13 men, 12 women, aged 18–34) who received short (3.4 s) bursts of tVNS or sham stimulation (cymba conchae vs earlobe). Pupil size was recorded under four light conditions: high intensity-blue, low intensity-blue, orange, and dim.

Results

TVNS increased pupil diameter relative to sham across all light conditions. Pupil dilation was largest and most sustained under low-blue light compared with dim or high-blue conditions. These results indicate that moderate tonic LC activation (e.g., low-blue light) enhances phasic responses to stimulation (e.g., tVNS), consistent with an inverted U-shaped relationship between tonic and phasic LC activity.

Conclusion

Overall, our findings provide causal evidence that light, particularly low intensity-blue, modulates the effects of tVNS in humans, and combining both increases noradrenergic activity, as highlighted by increased pupil dilation, suggesting a simple way to enhance vagal-based therapies.

Keywords: transcutaneous vagus nerve stimulation, blue light, pupillometry, locus coeruleus, noradrenalin

INTRODUCTION

Transcutaneous Vagus Nerve Stimulation (tVNS) is an innovative non-invasive technique targeting the vagus nerve. It is increasingly used in research on neurological and psychiatric disorders such as epilepsy and depression [1, 2]. The primary target of tVNS is the auricular branch of the vagus nerve (ABVN) which innervates parts of the external ear. The ABVN project to the Nucleus Tractus Solitarius (NTS) which has strong connections to the Locus Coeruleus (LC), the main source of noradrenaline (NE) in the brain [3]. Although the cymba conchae is considered the primary stimulation site, other regions such as the cavum conchae, internal acoustic meatus and tragus, have also been explored [1, 4].

Functional Magnetic Resonance Imaging (fMRI) studies showed that tVNS induces activation key brain regions, including the ipsilateral NTS, dorsal raphe nucleus (DRN), LC, amygdala, and nucleus accumbens, compared with sham stimulation applied to the earlobe [5]. These findings suggest that tVNS engages the LC and the noradrenergic system.

A growing body of evidence indicates that tVNS can modulate cognitive and affective processes, namely, learning[6], memory[7] and emotion processing[8], likely through LC-NE driven modulation. In addition, tVNS has been shown to improve attention[9, 10] and cognitive control[11], consistent with the role of the LC–NE system in optimizing neural gain and promoting efficient allocation of cognitive resources[12].

According to the adaptive-gain model of the LC–NE system (Supplementary Figure S1), phasic response follows an inverted-U relationship with tonic LC activity, being maximal at intermediate tonic levels and reduced at both extremes. This model provides a theoretical framework for understanding how variations in LC tone can shape arousal, attention, and cognitive performance.

While direct measurement of LC-NE activity in humans is currently unavailable, pupil size has emerged as an indirect measure of phasic LC activity in human and animal studies [9, 13]. Anatomically, pupil size is regulated by both the parasympathetic, driving the pupil constriction and the sympathetic system, involving the LC, resulting in pupil dilation. Experiments in monkeys confirmed that stimulating the LC induced a transient pupil dilation [14]. Recent studies have shown that tVNS induces significantly larger pupil dilation compared to sham stimulation applied to the earlobe [15-17].

While several studies support the use of pupil dilation, P3 amplitude, and salivary alpha-amylase (sAA) as reliable biomarkers of LC–NE activation, findings across studies have been somewhat inconsistent, likely reflecting differences in stimulation parameters, timing, or experimental design[18-22]. A recent meta-analysis has aimed to clarify these discrepancies (e.g., using sAA as an objective noradrenergic index)[21]. Continued efforts to better characterize these physiological indicators are essential for interpreting tVNS-related effects.

Light therapy, has been used in other clinical contexts such as mood disorders (e.g., seasonal affective disorder) and circadian rhythm regulation [23-25]. Besides its role in vision, light also exerts well-documented non-image-forming (NIF) biological functions. These include regulation of physiological and cognitive processes, such as melatonin production, alertness, mood regulation, body temperature, cortisol secretion, and pupil constriction [26-28]. These effects are mediated by intrinsically photosensitive retinal ganglion cells (ipRGCs), which express the melanopsin photopigment most sensitive to blue light, around 480 nm [29]. IpRGCS project to the suprachiasmatic nucleus (SCN), the

master of the circadian clock which can indirectly activate the LC. Neuroimaging studies suggest that light exposure modulates anterior hypothalamus activity near the SCN and may trigger LC activation [30, 31]. According to the adaptive-gain model, light exposure could shift tonic LC activity along an inverted-U function, with low luminance associated with reduced tonic activity and arousal, and high luminance with elevated tonic activity and heightened arousal (See Figure S1 – Supplementary Material). FMRI studies showed that blue light exposure enhances brain activity in areas involved in working memory, attention, and emotional processing, including the thalamus, hippocampus, amygdala, prefrontal cortex, parietal, temporal, and insular regions. Notably, these regions overlap with the anatomical targets of tVNS, including the LC [32, 33].

IpRGCs further project to the olivary pretectal nucleus contributing to pupil size regulation, to trigger the "pupil light reflex" [34], resulting in small pupil under high illuminance. A recent study demonstrated that exposure to blue-enriched white light produced a stronger task-related pupil dilation in response to auditory stimulation, despite smaller pupil size during bright blocks, likely via LC recruitment [31], as evidenced by pharmacological modulation of the Superior Cervical ganglion (SCG) α1 receptors and the Edinger Westphal (EWN) α2 receptors [35], resulting in larger pupil size under light exposure.

Although both tVNS and blue-enriched light are known to modulate the LC–NE system through distinct neural pathways, their potential interaction has not been directly investigated. Demonstrating that simultaneous activation of vagal and photic inputs can enhance LC-driven responses would provide novel insights into how neuromodulatory systems can be jointly leveraged to optimize noradrenergic tone. The present study addresses this gap by testing whether blue-enriched light amplifies the tVNS induced pupil dilation in healthy participants.

Considering these evidences, we posit that light administration may enhance tVNS effects on the LC and its impact on pupil size. Specifically, we hypothesized that, compared to a control light condition,

exposure to blue-enriched white light would amplify the adrenergic tone initially activated by tVNS, resulting in a larger pupil.

MATERIEL & METHODS

Participants

A total of 27 healthy participants (14 men, 13 women) aged between 18 and 34 years (mean age: 25 +/- 4.6 years) were recruited at the UCLouvain University (Alma campus), Brussels, Belgium, from July to October 2023. Based on previous studies showing robust pupil dilation after tVNS in 19–25 healthy participants [16, 36], a sample size of 25 participants was deemed sufficient to detect an effect on pupil size in our study. Two participants were excluded from the analyses as they were unable to complete the experiments (one participant due to a headache, while the other was overly sensitive to the high-intensity blue light) so that 25 individuals were included in the analyses (Table S1, Supplementary Material). All participants were free of any past or present neurological or psychiatric disorders, as well as substance abuse or any eye disease. On the day of the experiment, participants were instructed to refrain from smoking or consuming tea or coffee. Semi-structured interviews and questionnaires were administered to assess clinical levels of depression (Beck Depression Inventory)[37], anxiety (STAI-ETAT and STAI-TRAIT)[38] and sleep quality using the Leeds Sleep Evaluation Questionnaire. Exposure to 30 minutes of blue-enriched light is safe and does not affect sleep, as all experiments were conducted between 8:00 AM and 1:00 PM. The Karolinska Sleepiness Scale (KSS) was administered repeatedly throughout the experiment to monitor sleepiness during the experiments. The study was approved by the CUSL Ethical Committee (2023/06FEV/060) and was registered at ClinicalTrials.gov (N°NCT06304389), on 18/04/2023. All methods were carried out in accordance with relevant guidelines and regulations, and adhered to the

Declaration of Helsinki. All participants provided written informed consent before the experiments and were financially compensated for their participation.

tVNS administration and experimental design

tVNS was applied to the left ear, targeting the cymba conchae (anode) and tragus (cathode), as illustrated in Figure 1. This electrode configuration was selected to optimize activation of the auricular branch of the vagus nerve, based on recent studies[39, 40]. Custom electrodes were crafted by trimming standard electrodes (AXION, Germany) to fit the ear anatomy of each participant. Ear paste was used to ensure electrodes remained in place throughout the experiment. A Master 8 device and two external stimulators were used to deliver both tVNS and sham stimulations. Sham stimulation was delivered to the earlobe, where the electrode was attached using an ear clip. Stimulation intensity was individually calibrated for each participant using a two-direction (ascending and descending) staircase procedure, performed twice to ensure perceptual stability. Starting from 0.1 mA, each stimulation intensity was delivered for 5 seconds, after which participants rated the perceived sensation on a 0–9 scale (0 = “I don’t feel anything”; 9 = “painful”). The current was then increased in 0.2-mA steps until participants rated the sensation as 9 (“painful”). The intensity was then decreased until a rating of 6 was reached, yielding two intensity points rated as 8 (“just below painful”). The entire ascending–descending sequence was repeated once more, resulting in four intensities rated as 8 in total. The mean of these four values was used as the individual tVNS intensity for the experiment. For the sham condition, no staircase was applied; instead, current amplitude was manually adjusted so that participants explicitly matched the perceived sensation to that of the finalized tVNS intensity, ensuring comparable subjective perception. This procedure ensured blinding of participants to the stimulation condition. TVNS is non-invasive and well-tolerated; participants felt mild tingling at the stimulation site. Although skin irritation from electrode placement was possible,

none was observed. For each light condition, participants received three blocks of tVNS and three blocks of sham stimulation, resulting in a total of 24 blocks per participant. Each block, whether tVNS or sham, consisted of 11 trials lasting 3.4 seconds each (pulse width: 200 µs, frequency: 25 Hz), separated by 26.6-second OFF intervals. Two minutes pause were done after each stimulation blocks. The allocation of stimulation block types was randomized by the experimenter. They were administered under four different lighting conditions: high and low-intensity blue-enriched white light, control orange light, and dim light (Figure 2). The order of light exposure was counterbalanced across participants.

Pupil size measure

The pupil diameter response (PDR) of the left eye was continuously recorded using an infrared eye tracker (EyeLink 1000; SR Research Ltd, Kanata, ON, Canada) at a sampling rate of 1000 Hz. Participants were instructed to fixate on a small white cross displayed on a monitor positioned at 70 cm. To ensure stability and minimize movement artifacts, participants rested their chin on a chinrest, maintaining a consistent distance of 40 cm from the EyeLink infrared camera for optimal tracking accuracy.

The recorded data were exported to MATLAB 2022a (The MathWorks Inc., MA, USA) for processing. Blink artifacts were detected and removed using linear interpolation, with a window of 150 ms before and after each blink occurrence. The data were low-pass filtered (10 Hz) and segmented from 1 second before to 30 seconds after the onset of each stimulation. Baseline pupil diameter was calculated as the mean pupil diameter during the 1-second period preceding stimulation onset. Pupil size data were normalized by subtracting, for each participant and condition, the mean baseline pupil diameter from all subsequent samples. The transient pupil response was then expressed as the percentage change in pupil diameter from baseline. Blinks with eye closure exceeding 120 ms were considered non-physiological (prolonged eye closures). Given the ±150 ms interpolation window, such events would have resulted in approximately 420 ms of interpolated data within the 1-second baseline period and were therefore

excluded. Trials with missing data periods (e.g. signal loss) exceeding the stimulation window were excluded. Moreover, if more than 1 second within the 3.4 second stimulation period required interpolation, the trial was discarded. Additionally, any block in which more than 50% of trials were discarded based on these criteria was excluded from further analysis. In total, 662 out of the 6435 trials were removed, evenly distributed across light and stimulation conditions (dim light = 177, high blue = 145, orange = 183, low blue = 157 trials).

Light application

Participants were exposed to four lighting conditions: dim light (<10 lux), high-intensity blue light (500 lux), low-intensity blue light (80 lux), and orange light (76 lux) (see S2 and S3: supplementary material for more details about lights specifications). Each light was administered during 30 minutes in total (15 min x 2). Light was emitted by LEDs embedded in a head-mounted light device (Luminette, Lucimed, Belgium), directing light toward the participants' eye. The intensity and spectral properties of the light were confirmed using an illuminance spectrophotometer. The peak wavelength was 456 for low blue and 471 nm for high blue light and the photon flux was between 6×10^{13} and 7×10^{13} photons/s/cm^2 at the retina. Additionally, participants were exposed to a monochromatic orange light. The peak wavelength was 629 nm and the photon flux were identical to blue lights. Orange light minimally activates ipRGCs, and serves as a control condition. Periods of 10 minutes of dim light were presented before each lighting condition.

Data analysis- Statistics

1- Demographic data, self-reported measures

A paired t-test was performed to compare the mean stimulation intensities of tVNS and sham conditions. The Karolinska Sleepiness Scale (KSS) was administered 13 times before and after each light condition to evaluate subjective sleepiness.

2- Pupil Dilation Responses: tVNS vs. Sham

We computed the mean pupil response during the stimulation period (from 1 second after the onset of stimulation to the end of the 3.4-second stimulation). To address our hypothesis regarding the differential effects of tVNS across lighting conditions compared to sham, we performed separate paired t-tests per light condition. The Benjamini-Hochberg (BH) correction was applied within each analysis across the four light condition to control for multiple comparisons. Although a combined model was considered, this targeted approach offered clearer interpretation.

3- Pupil Dilation Responses: Effect of light on tVNS

To investigate which lighting condition elicited the largest pupil dilation during tVNS, we used a linear mixed model (LMM) fit by restricted maximum likelihood (REML), which is suited for repeated measures data as it accounts for both fixed and random sources of variation. The model included light type (high-intensity blue, low-intensity blue, orange, and dim light) as fixed effects, with participants modeled as a random effect to account for inter-individual variability. Post hoc pairwise comparisons were performed to identify significant differences between conditions, with Tukey's correction applied to account for multiple testing. To explore the influence of each covariate such as age, sex, anxiety (STAI-ETAT, STAI-

TRAIT), depression (BDI), and sleep quality (Leeds) on pupil dilation, we first fitted separate models including each covariate individually. A final model was fitted, including all covariates simultaneously, in order to estimate adjusted effects. Generalized Variance inflation factors (VIFs) were used to assess multicollinearity (Table 1).

4- Pupil Dilation persistent effect

During our analysis, we incidentally observed that the pupil dilation response induced by tVNS persisted beyond the stimulation period in certain lighting conditions compared to sham. To investigate this phenomenon, we analyzed pupil dilation across the 5 to 6 seconds post-stimulation for each light condition. Statistical analyses were conducted using paired t-tests for each light condition separately. BH correction was applied to control for multiple comparisons.

RESULTS

1) Demographic data, self-reported measures & stimulation intensities

We calculated the scores from each questionnaire (BDI, STAI, Leeds) to assess individual differences in psychopathological measures, as detailed in Table S1 (See Supplementary Material). The BDI scores among participants ranged from 0 to 10, with a mean of 3.32 ± 2.84. Overall, participants showed low levels of depressive symptoms, although 2 participants scored slightly higher (10 out of a total of 31), reflecting some interindividual variability. The STAI-ETAT scores ranged from 20 to 49, with a mean of 29.84 ± 7.72, indicating that most participants exhibited low to moderate levels of state anxiety, though 2 participants scored higher (42 and 49 out of a total of 80), reflecting elevated anxiety during the

experiment. The STAI-TRAIT scores ranged from 25 to 57, with a mean of 38.95 ± 10.14, suggesting moderate baseline levels of trait anxiety on average, with 8 participants showing higher levels. The Leeds Sleep Evaluation scores ranged from 37 to 88, with a mean of 54.53 ± 13.20, reflecting moderate perceived sleep quality overall. The mean stimulation intensities for tVNS and sham conditions were compared and showed a significant difference (tVNS: 2.29 ± 1.13 mA; sham: 4.60 ± 1.80 mA; $p < 0.0001$).

The results of the 13 KSS (Figure 3) were averaged throughout the experiment and indicate a mean score between 4 (rather awake) and 6 (some signs of drowsiness), on a scale ranging from 1 (extremely alert) to 9 (extremely sleepy). This reflects a moderate to slightly low level of subjective sleepiness experienced by participants across all light conditions.

2) Pupil Dilation Responses: tVNS vs. Sham

We first aimed to reproduce tVNS-induced pupil dilation, as described previously in literature[15-17]. Figure 4 (left panel) illustrates the average PDR induced by tVNS and sham stimulation across our 25 participants in the dim light condition. The shaded grey area indicates the stimulation period (3.4 seconds), and the green arrow marks the mean value used for comparing conditions. tVNS elicited significantly larger pupil dilations compared to sham in all light conditions (Figure 4- Right panel).

3) Pupil Dilation Responses: Effect of light on tVNS

Figure 5 shows the average tVNS-induced PDR across all participants. The linear mixed model's random intercept variance for participants was 9.14 (SD = 3.02), reflecting substantial inter-individual variability. A total of 595 observations from 25 participants were analyzed. Consistent with our hypothesis, tVNS elicited the largest pupil dilation under the low-intensity blue light condition compared to the high-intensity blue (Cohen's d: 0.53, 95% CI [0.21, 0.87]) and dim light conditions (Cohen's d: 0.53, 95% CI [0.20, 0.86]) ($p < 0.001$, Figure 5). However, the comparison between low-intensity blue light and orange light conditions reached statistical trend level but did not reach significance (Cohen's d: 0.28, 95% CI [-0.05, 0.61]) ($p = 0.1$, Figure 5). Interestingly, the PDR induced by tVNS under high-intensity blue light was comparable to that observed under dim light, despite notable differences in baseline pupil size between these two conditions, as high intensity blue light induces a higher pupil constriction.

A power analysis using Monte Carlo simulations (1,000 iterations; *simr* package, R 4.4) indicated that our sample size (N = 25) provided 82% power (95% CI = 79.9–84.7%) to detect a medium main effect of light type (Cohen's $f^2 = 0.17$, $\alpha = 0.05$). These analyses suggest that the study had adequate sensitivity to detect medium-to-large effects of tVNS and lighting conditions on pupil dilation.

Further analysis incorporating covariates such as age, sex, anxiety, depression, and sleep quality confirmed that these factors did not influence the observed results, whether added individually in the model or simultaneously (Table 1). No covariate exceeded conventional thresholds (all adjusted GVIFs < 2), indicating no multicollinearity of covariates.

A global LMM including Stimulation Type, Light Condition, and their interaction was initially tested but did not reveal a significant interaction (all $p > 0.17$). Therefore, to focus specifically on the effect of light during tVNS, a separate LMM restricted to the tVNS condition was performed.

4) Pupil Dilation persistent effect

We incidentally noticed that, in the low blue light and dim light conditions, tVNS-induced pupil dilation remained elevated for up to ~6 seconds post-stimulation compared to sham, indicating a prolonged effect (highlighted by the red arrow, Figure 6, $p<0.01$). No sustained post stimulation pupil dilation between sham and tVNS was observed in orange nor high blue light condition.

DISCUSSION

In this study, we evaluated the effect of tVNS on pupil size in 25 healthy subjects exposed to four different lights conditions. We hypothesized that, compared to a control light, blue light exposure would modulate adrenergic response induced by tVNS, resulting in greater pupil dilation. Consistent with prior literature, tVNS induced significantly greater pupil dilation compared to sham stimulation, supporting the involvement of a phasic activation of the LC-NE system [4, 15-17]. In addition, as in previous studies, sham stimulation also induced a relatively weak pupil dilation. While this site is not thought to activate vagal afferents directly, the response likely reflects general arousal triggered by tactile sensation itself, possibly via sympathetic pathways [41, 42].

We further found that light modulated tVNS induced pupil dilation: exposure to low intensity blue light significantly increased pupil dilation compared to the high blue and dim light. However, the difference did not reach significance with orange control light. Surprisingly, the high illuminance blue-enriched white light did not differ from dim or orange light condition. In addition, we observed a sustained post stimulation pupil dilation difference between tVNS and sham stimulations in dim and low intensity blue light, which lasted up to 6 seconds after the stimulation.

TVNS has emerged as an interesting non-invasive tool to investigate vagus nerve stimulation. Recent investigations have demonstrated a phasic pupil dilation in healthy subjects using similar protocols [4, 15, 16]. Skora et al. extended the duration of tVNS stimulation to assess whether pupil dilation would be more tonic (sustained) but found instead a phasic dilation pattern [17]. These pupil dilations are thought to be mediated by LC activation, as intra-neuronal firing of LC neurons correlates with pupil dilation [14, 43]. Specifically, LC activation modulates the iris dilator muscle by activating the SCG and also inhibiting the EWN, a key component of the parasympathetic pathway, thereby promoting pupil dilation.

Effect of blue light on tVNS-induced pupil dilation

The LC exhibits two firing modes: phasic, associated with a strong burst of activity in response to salient stimulus [12], and tonic. Phasic LC responses enhance the encoding of salient information and support optimal performance on attention-demanding tasks[12]. In contrast, tonic activity is characterized by sustained levels of LC activation [44]. High tonic activity can reflect acute stress or high alertness, while low activity may correspond to drowsiness, sleep or a disorder of consciousness [44]. In our protocol, 3.4-second-long tVNS induced a phasic dilation, reflecting transient LC activation.

Simultaneously, continuous application of different light conditions likely engaged the tonic mode of activity of the LC, as blue-enriched white light exposure is known to increase alertness and modulate arousal. High tonic LC activity correlates with a larger resting pupil size [12, 14]. However, under constant light, this resting pupil size is masked by pupil constriction induced by the parasympathetic light reflex [45]. With tVNS, we affected this constriction and posit that we induced a phasic LC response, observed as a transient pupil dilation, followed by a return to baseline size. Although high tonic LC activation such as under 500 lux blue light resulted in greater pupil constriction, the tVNS-induced dilation was smaller under this condition, comparable to the pupil dilation induced in dim light. In dim light, the lower parasympathetic influence leads to larger baseline pupil size[34], potentially leaving less room for phasic LC activation to induce dilation. This was reflected in our results, where dim light showed the smallest transient dilation compared to low blue light condition. The lower dilation under high blue light and dim light could be therefore explained by an inverted-U relationship between LC tonic and phasic activity (Figure 7). In conditions of low arousal and low tonic activity, performance is generally weak, reflected by diminished phasic task-related responses in experimental contexts[46]. Conversely, studies have shown that an intermediate arousal state with moderate tonic LC activity provides optimal conditions for target detection by enabling a phasic increase in neural gain in response to stimuli [41, 43]. While these findings are grounded in task-related paradigms, we hypothesize that the inverted-U model can also

apply to resting-state conditions, which could, in our study, explain the interaction between blue light and tVNS. We speculate that dim light corresponds to low tonic activity and blue light is associated with higher tonic activity. Under low tonic LC activity (dim light), the phasic response induced by tVNS is weak, as the LC lacks sufficient tonic activation to support a strong phasic response. At intermediate tonic LC activation (low blue light), the phasic response is enhanced, enabling optimal pupil dilation. However, under high tonic LC activation, phasic responses diminish due to overactivation, consistent with the right side of the inverted-U curve. This hypothesis aligns with the recruitment of alpha-2 auto receptors present in the LC, during excessive LC activation, which diminishes noradrenaline release [47]. This could explain why pupil dilation is reduced under high blue light.

Additionally, an alternative explanation for the reduced pupil dilation under high blue light intensity could be that strong pupil constriction limits or masks transient dilation responses, making them difficult to detect. Hence, low intensity blue light, at moderate intensity, may represent an optimal level of tonic LC activation, allowing sufficient flexibility for phasic activation by tVNS. Since we measured pupil size rather than directly assessing LC activity, it is possible that afferent regions to the LC – such as the thalamus[48], or other subcortical structures[49] involved in arousal, like the frontal cortex[50] may modulate the phasic activity in response to tVNS.

Beyond these hypotheses, it is possible that the effects observed in this study were solely light intensity-dependent. Pan et al. reported similar findings to ours [51]. They examined the pupil size of 24 healthy subjects exposed to varying light illuminance levels ranging from 0.92 to 233.37 cd/m², while undergoing emotional tasks. Greatest modulation of pupil size occurred at low to mid-luminance levels, whereas minimal modulation was observed at high luminance, which aligns with our findings. The authors suggested that mid-luminance levels, ranging from 5 to 37 cd/m², may represent an optimal range for assessing the influence of cognitive arousal on pupil size. However, exact spectral composition and

wavelength characteristics of the light were not provided. In our study, light intensity (in lux) was matched between low-blue and orange light conditions. Although mean pupil dilation did not differ significantly between the two conditions, a trend emerged, warranting further investigation. A potential approach to address this question would be to introduce a more intense monochromatic orange light and evaluate the pupil response. If a significant effect is observed, it would suggest that tVNS-induced pupil dilation is primarily intensity-dependent rather than wavelength-dependent. In addition, a recent study reported that higher levels of blue-enriched white light elicited a stronger task-evoked pupil response to auditory stimulation. In this study, the authors quantified Melanopic Equivalent Daylight Illuminance (Mel EDI) lux, a measure of melanopic system activation. They found that brighter light conditions (37, 92, and 190 Mel EDI lux) induced greater pupil dilation during auditory tasks compared to monochromatic orange light (0.16 Mel EDI lux). Notably, no significant differences were observed between the brighter light conditions (37, 92 and 190), indicating a ceiling effect occurring immediately after low intensity (37 Mel EDI lux). This seems to indicate that higher activation of the melanopic system might not be proportionally linked to higher task evoked pupil dilation.

Pupil dilation persistent after effect

We exploratorily observed a persistent tVNS induced pupil dilation in the low blue and dim light conditions. Interestingly, this persistent effect was not observed under orange or high-intensity blue light. In contrast, we observed a prolonged effect under dim light, although shorter than under low blue light. While we cannot determine specific contribution of different photoreceptors, these exploratory differences in post-stimulation pupil dynamics may reflect varying levels of photoreceptor recruitment across light conditions. Most importantly, these results suggest that low-intensity blue light exerts a stronger modulatory effect on tVNS-induced responses.

Limitations

This study has several limitations. A larger sample size would clarify trends and reduce variability in the results. We also observed variability in the pupillary response among healthy participants, which may reflect individual differences in autonomic LC-NE system function or sensitivity to light. Moreover, the strong pupil constriction observed under high-intensity blue light could have partially masked transient phasic dilations, as discussed above. This potential physiological masking effect may represent a confounding factor that should be considered when interpreting the results. In addition, although stimulation intensities differed between tVNS and sham conditions, this difference reflects the distinct sensory characteristics of the stimulation sites. Because perceptual matching was applied to achieve comparable sensations, participants were effectively blinded to the stimulation condition, although explicit condition-guess data were not collected. Another limitation of the present study is that pupil dilation, while widely accepted as a reliable correlate of LC-NE activity, remains an indirect physiological marker. Direct evidence of LC engagement would require complementary neuroimaging or behavioral measures, such as fMRI or cognitive assessments, although LC-NE activation has been previously tightly linked to effects such as enhanced attention and memory[6, 7, 10]. Lastly, the experimental protocol lasted approximately five hours which may have contributed to variability [52].

Conclusion

This study provides insights into the interaction between blue light exposure and tVNS in modulating LC activity. Low-intensity blue light modulates tVNS-induced pupil dilation, likely by optimizing LC tonic activation, and offers potential for optimizing vagal stimulation therapies.

Due to privacy restrictions, the data are not publicly available but can be requested under specific conditions from the corresponding author.

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Declaration of Interest

The authors declare no competing interests.

Authors contribution

Inci Cakiroglu: Designed research, Methodology, performed research, Analyzed data, Visualization, Writing – original draft, Writing – review and editing. Enrique Germany Morisson: Software, Visualization, Writing – review and editing. Sarah Brisbois: performed research, Methodology. Venethia Danthine: Methodology, Visualization, Writing – review and editing. Ana Marta Dias: Writing – review and editing. Antoine Nonclercq: Visualization, Validation, Methodology, Writing – review and editing. Gilles Vandewalle: Validation, Methodology, Visualization, Writing – review and editing. Riëm El Tahry: Designed research, Methodology, Validation, Visualization, Writing – review and editing, Supervision, Funding acquisition, Resources.

During the preparation of this work, the authors used Chat GPT in order to improve the readability and language of the manuscript.

LEGENDS

Figure 1. tVNS and sham stimulations montages*:* **Left picture**- Master 8 and Digitimers. A Master 8 was used to guide the two external stimulators used to induce the stimulations. **Right picture**: tVNS was administered to the cymba conchae and to tragus zone. Sham was applied on the earlobe. Pupil size was recorded using an eye tracker (Eyelink 1000 Plus device).

Figure 2. Experimental design *:* Subjects were exposed to dim, high blue, orange and low intensity blue light, using Luminette devices. Blocks of 5 minutes of tVNS or Sham were composed of 11 trials of 3.4 seconds each, with 2 minutes pauses between blocks. The order of the blocks as well as lights were counterbalanced between participants. The experimental design was repeated one time, following a longer break, to collect 3 blocks of each stimulation in blue, orange and low blue light condition. In the second session, participants remained seated in dim light without receiving any additional tVNS or sham stimulation, as all six blocks had already been collected during the first session.

Figure 3 *: The Karolinska Sleepiness Scale (KSS) was administered 13 times throughout the experiment, specifically before and after each lighting condition, to assess subjective sleepiness. Mean KSS scores for each participant are presented along with their standard deviations (SD).*

Figure 4. Left - Average tVNS and sham-induced pupil dilation change (%) of 25 healthy subjects in the dim light condition: *The gray shaded area represents the stimulation period (3.4 seconds). The green arrow indicates the calculated mean used for comparison between conditions. Black and gray curves represent tVNS and sham-induced pupil dilations, respectively. tVNS induced higher pupil dilation (5.94 ± 5.16%) compared to sham (2.67 ± 3.41%). Only the dim light condition is represented in full.* ***DL-T****: Dim light tVNS;* ***DL-S****: Dim light sham.* ***Right - tVNS and sham induced pupil dilation across all conditions:*** *Boxplots show that tVNS induced higher pupil dilation compared to sham in blue (p<0.05), low blue (p<0.001), orange (p<0.05) & dim light (p<0.001).*

Figure 5 – Average tVNS induced pupil dilation change (%) of all participants in all light conditions *: tVNS induced the highest pupil dilation in low blue light compared to dim (p<0.001), and high intensity blue light (p<0.001) and showed a trend toward significance compared to orange light (p=0.1).*

Figure 6. Average tVNS and sham-induced pupil dilation change (%) of 25 healthy subjects in the low intensity blue light condition: *tVNS showed sustained pupil dilation compared to sham up to 6 seconds post-stimulation (represented by the red arrow; p<0.01, only in low blue and dim light condition).*

Figure 7. The inverted-U curve illustrates the relationship between tonic LC activation (x-axis, e.g., light condition) and phasic responses (y-axis, e.g., pupil dilation induced by tVNS). *The blue zone represents hypoactivation (e.g., DL-dim light), where phasic responses are weak due to low LC activity. The green zone represents optimal activation (e.g., LBL-low blue light), where moderate tonic activation enhances phasic responses. The red zone reflects hyperactivation (e.g., BL-blue light), where excessive tonic activity diminishes phasic responses. An optimal level of tonic activation seems crucial to maximize phasic responses.*

Table 1 Effects of individual covariates on tVNS-induced pupil dilation, as estimated from the LMM: Fixed effects include age, sex, anxiety (STAI-Etat and STAI-Trait), depression (BDI), and sleep quality (Leeds). No covariate showed a statistically significant association with pupil dilation.