Brain Stimulation 17 (2024) 678–680

Contents lists available at ScienceDirect

Brain Stimulation

journal homepage: www.journals.elsevier.com/brain-stimulation

External stimulation of the trigeminal nerve causes pupil dilation in healthy volunteers, suggesting locus coeruleus modulation

ARTICLE INFO

Keywords
External trigeminal nerve stimulation
Pupillometry
Locus coeruleus
Norepinephrine

Dear Editor,

External trigeminal nerve stimulation (eTNS) is a non-invasive neuromodulation method that delivers electrical current via patch electrodes on the forehead, cheek or jaw to target a branch of the trigeminal nerve. eTNS has proven effective for treating migraines [1], trigeminal neuralgia [2] and ADHD in children [3]. In addition, eTNS is under investigation for the treatment of a wide range of diseases such as epilepsy and stress-related disorders [4]. Despite these therapeutic benefits, the working mechanism is poorly understood. The trigeminal nerve transmits pain and tactile information to the thalamus, while also extending projections to brainstem nuclei including the nucleus of the solitary tract and the locus coeruleus (LC) [5]. Importantly, the LC is the main nucleus of norepinephrine (NE) release in the brain [5]. NE is part of the sympathetic nervous system involved in processes such as arousal [6] and bodily response to threat [7]. These processes regulated by the LC-NE system can be characterized by pupil dilation. Pupil dilation is controlled by the iris dilator muscle which is regulated by the sympathetic nervous system and connects to a subcortical pathway that starts in the hypothalamus and the LC. Additionally, recent studies have shown that pupil diameter can serve as a measure of LC activity. In animals, direct evidence showed that LC activation closely matches changes in pupil size [8], while in humans, pupil diameter correlated positively with the BOLD activity in the LC [9].

Thus, eTNS may exert its therapeutic effect by activating the LC and regulating NE levels. Therefore, we investigated the effect of eTNS on pupil dilation, a marker for LC activity and arousal. We hypothesized that if eTNS would activate the LC-NE system, it should lead to pupil dilation due to the positive relation between LC activity and pupil diameter.

Twenty volunteers received stimulation while pupil diameter was recorded. For eTNS, electrodes were placed on the chin to stimulate the mandibular branch of the trigeminal nerve (Fig. 1a). Stimulation consisted of a symmetric biphasic pulse train (200μs per phase, interphase delay = 0) with a frequency of 1500Hz, 15 ms duration and were repeated every 4000 ms. Stimulation intensity was determined for each participant individually (see supplementary methods). In addition to sham/no stimulation, Median Nerve Stimulation (MNS) was administered as an extra control condition since it does not activate the LC-NE pathway, and to control for a startle response to stimulation. For MNS, electrodes were placed over the right forearm and stimulation intensity was also determined individually. All other stimulation parameters were identical to eTNS. The stimulation conditions (eTNS, SHAM and MNS) were applied alternatingly in a random order for 20 minutes.

The average pupil response of all subjects to the stimulation conditions was computed (Fig. 1b). As primary outcomes, the area under the curve (AuC), representing pupil response size, and the percentage of pupil dilations were calculated. The effect of the different stimulation conditions on both metrics was investigated using linear mixed effect models (see supplementary methods). Additionally, the relation between baseline pupil diameter and pupil response size was investigated using Pearson's correlation coefficients.

Stimulation intensities ranged from 2 to 10 mA across stimulation conditions (Fig. 1c), and were higher for eTNS compared to MNS. Statistical analyses showed no effect of stimulation intensity on AuC or percentage of pupil dilations ($p = 0.800$ and $p = 0.223$, respectively). In addition, the statistical model with stimulation intensity included did not add any predictive value to the model (Bayesian Information Criterion: AuC: −62.40 vs. −58.43; Percentage of pupil dilations: 98.79 vs. 118.1; without vs. with stimulation intensity). Both eTNS and MNS were well tolerated and caused minor to no side effects (see Table 1 in supplementary material). Fig. 1d shows that eTNS caused larger pupil responses compared to MNS and SHAM stimulation ($p < 0.001$ and $p < 0.001$, respectively). MNS also elicited pupil responses with a larger AuC than SHAM ($p < 0.001$). Fig. 1e depicts the percentage of pupil dilations in every stimulation condition. Statistical analysis revealed that the percentage of dilations was significantly higher in eTNS conditions compared to MNS and SHAM ($p = 0.011$ and $p < 0.001$, respectively). Importantly, the number of pupil dilations did not differ between MNS and SHAM ($p = 0.051$). In addition, the size of the pupil response was correlated with the baseline pupil diameter, showing smaller pupil responses when baseline pupil response was higher (see Results and Figs. S1 and S2 in supplementary material).

Individual stimulation intensities for eTNS and MNS were used,

https://doi.org/10.1016/j.brs.2024.05.008
Received 21 February 2024; Received in revised form 2 April 2024; Accepted 14 May 2024
Available online 17 May 2024

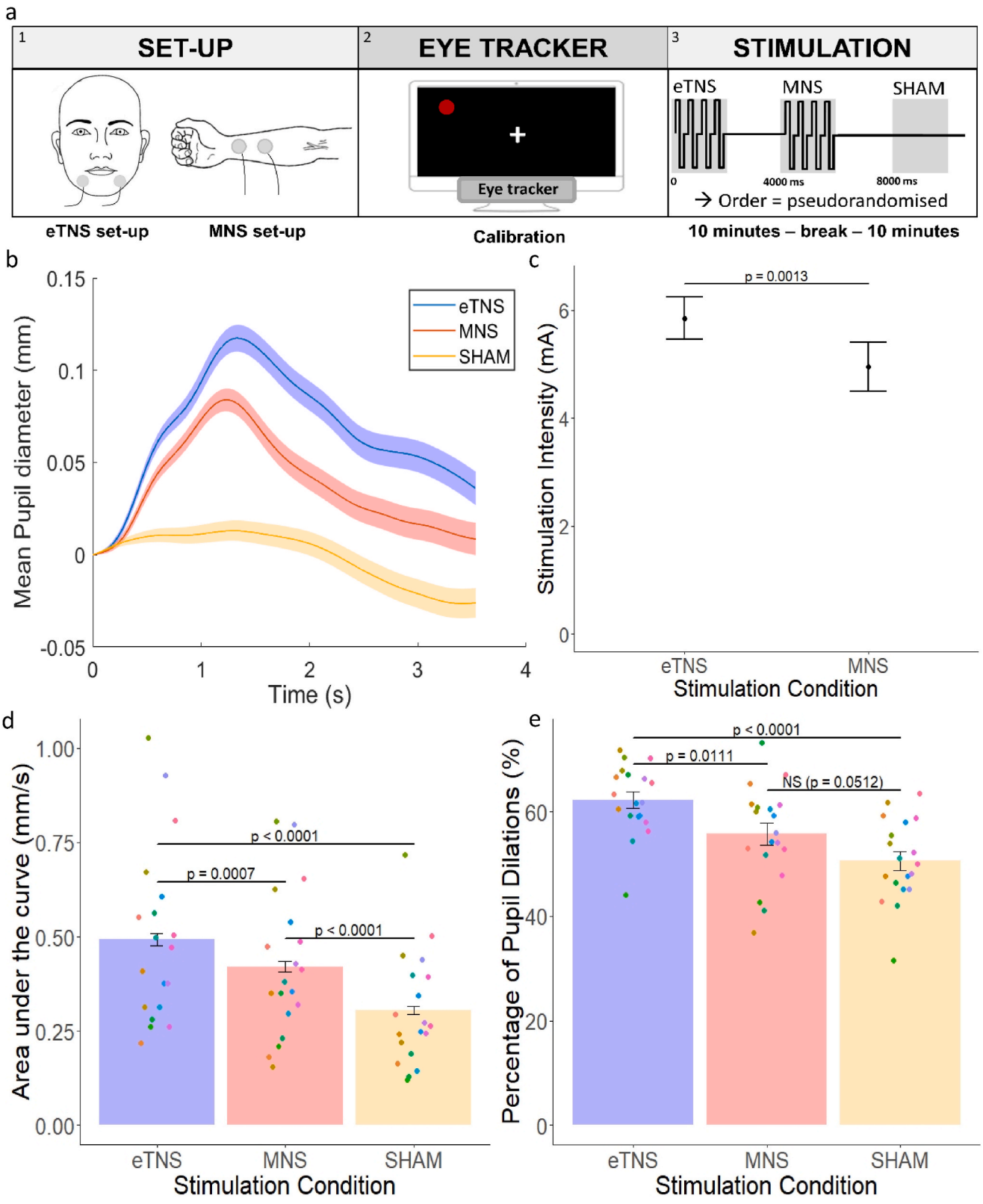

Fig. 1. a) Experimental procedure. 1. Stimulation electrodes were attached, and stimulation intensity was determined on the sensory threshold and kept under the pain threshold. 2. A 9-point calibration was conducted, and subjects were instructed to focus on the cross on the screen. 3. Pupil diameter was recorded while stimulation was applied alternatingly. The experiment consisted of two blocks of 10 min each, with a short break between the blocks. b) Average pupil response of all participants for every stimulation condition. The shaded area is the 95 % confidence interval. Every stimulation condition consisted of 100 repetitions per subject. c) The mean stimulation intensity for eTNS and MNS (5.85 and 4.95 mA, respectively). d) The average area under the curve for every stimulation condition. e) The percentage of pupil dilations in every stimulation condition. eTNS = external trigeminal nerve stimulation; MNS = median nerve stimulation. Error bars represent standard errors and colored dots represent individual subjects.

showing higher currents were necessary for eTNS to achieve a similar sensation with MNS. However, the analysis showed no effect of stimulation intensity on any of the pupil metrics, and the statistical models without intensity had a higher predictive value. A possible explanation to why eTNS and MNS elicited larger pupil responses compared to SHAM, is that both might activate similar sensory pathways since participants feel the stimulation. Moreover, we introduced MNS to control for the startle response, an automatic and mostly unconscious reaction to abrupt or unfamiliar stimuli, which can also result in pupil dilation. Additionally, we observed 4s was not always sufficient for the pupil diameter to return to baseline, potentially causing a carry-over effect from the previous condition. However, this is minimized by pseudo-randomizing conditions.

In conclusion, we investigated the effects of eTNS on pupil dilation metrics in healthy volunteers. We found that eTNS induced larger pupil dilations than MNS and SHAM stimulation. In addition, the number of pupil dilations was higher after eTNS than after the MNS-control conditions. These results support the hypothesis that eTNS may activate noradrenergic pathways such as the LC-NE system. This knowledge on the effect of eTNS and its underlying working mechanism is crucial for optimizing eTNS protocols, refining treatment approaches, and expanding the potential clinical applications of this promising neuromodulatory technique. Moreover, the use of non-invasive neuromodulation methods has grown over the past decade. In the clinic, a small number of eTNS therapies have received approval [3], while some are in the clinical trial stage [4]. Additionally, there is a growing interest in investigating the involvement of the LC-NE system in several aspects of human cognition, including learning and memory processes, decision making, as well as aging and neurodegeneration. In this work, we looked at pupil dilation, an indirect measure of LC activity. Although pupil dilation has been widely used as an indicator of LC activity [8,9], future work could explore additional metrics, such as EEG, fMRI, and salivary α-amylase which could provide further insights into the physiological and neurobiological processes associated with eTNS.

Funding sources for study

This work was supported by National Institutes of Health [grant number 1R01MH123508-01] and Fonds Wetenschappelijk Onderzoek [grant number G0B4520 N].

CRediT authorship contribution statement

Nina Seminck: Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Ahmad Khatoun:** Visualization, Methodology, Formal analysis, Data curation. **Silke Kerstens:** Writing – review & editing, Methodology, Conceptualization. **Bart Nuttin:** Writing – review & editing, Supervision, Methodology. **Myles Mc Laughlin:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT in order to improve language and readability. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We further confirm that any aspect of the work covered in this manuscript that has involved human participants has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). He/she is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author and which has been configured to accept email from (nina.seminck@kuleuven.be).

Declaration of competing interest

No disclosures relevant to the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2024.05.008.

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