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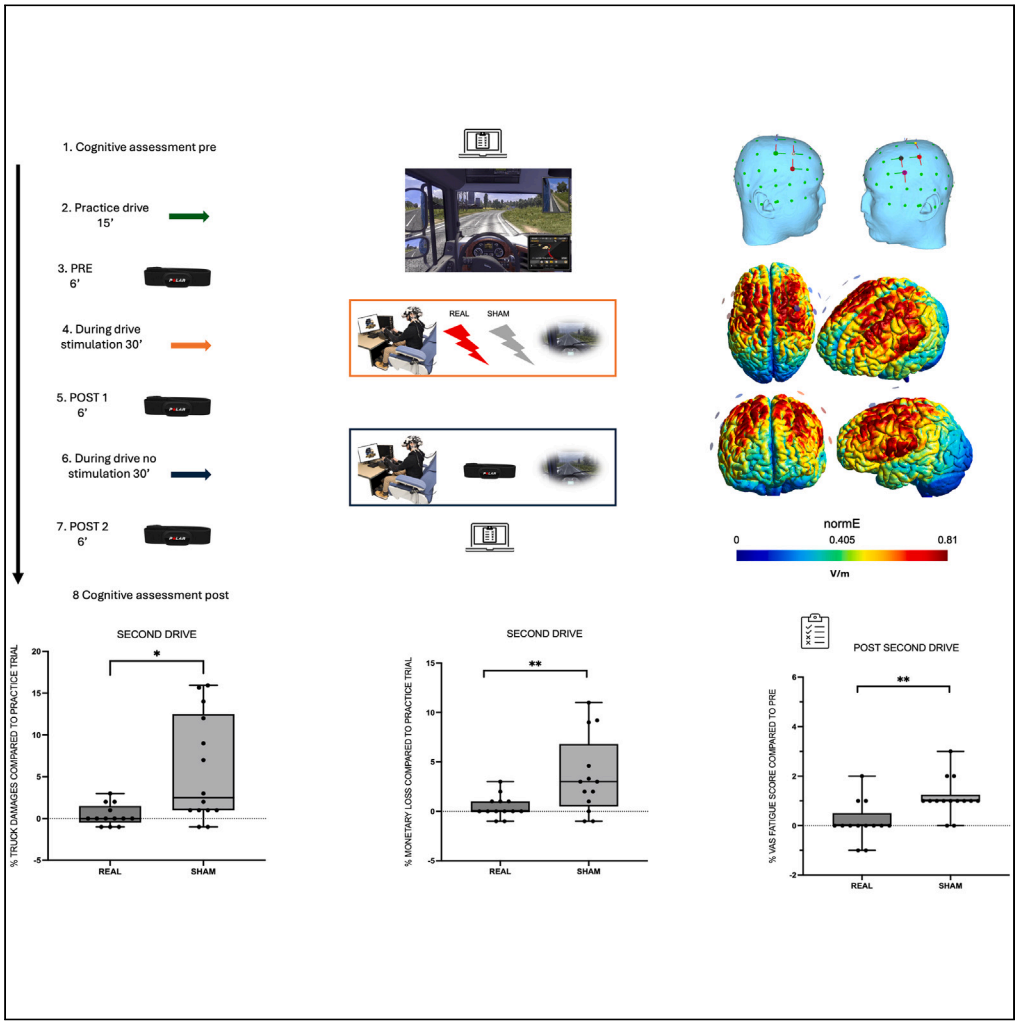

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Highlights

Cognitive fatigue (CF) lowers performance, affecting everyday activities

Transcranial random noise stimulation (tRNS) was applied on the "anti-fatigue network"

tRNS proved effective in reducing the CF perception and improving performance

Results can be used in daily life and neurological disorders characterized by fatigue

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Reduction of cognitive fatigue and improved performance at a VR-based driving simulator using tRNS

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SUMMARY

Cognitive fatigue (CF) increases accident risk reducing performance, especially during complex tasks such as driving. We evaluated whether transcranial random noise stimulation (tRNS) could mitigate CF and improve driving performance. In a double-blind study, thirty participants performed a virtual reality truck driving task during real (*n* = 15) or sham (*n* = 15) tRNS applied bilaterally on the "anti-fatigue network". They completed two 30-min driving sessions while their driving performances were constantly monitored; heart rate was also monitored to evaluate arousal (Root-Mean-Square of successive R-R difference). tRNS was applied only during the first driving session to evaluate both online and offline stimulation effects. The primary outcome was CF reduction and performance improvement in the second (non-stimulated) driving session. Real tRNS significantly improved driving performances in the second driving session and reduced perceived CF. These results might also lead to the use of tRNS in those neurological disorders characterized by fatigue.

INTRODUCTION

Cognitive fatigue (CF) is a condition characterized by progressive feelings of tiredness and disconnection from the task at hand, resulting in compromised cognitive and behavioral performance.[1] It poses a significant threat in modern society, contributing to accidents involving drivers and medical professionals.[2,3] Fatigue is a natural signal for the body to demand rest and regain homeostasis,[4] but its uncontrolled escalation is detrimental and risky. Consequently, there is a growing emphasis on comprehending the pathophysiological underpinnings of fatigue to counteract associated issues effectively. CF arises from mental workload, which is defined as the cognitive effort required to complete dynamic decision-making tasks.[5,6] It is closely tied to a prolonged high workload, which, in turn, depends on the task's duration and complexity.[5] Prolonged work contributes to increased mental fatigue, impacting worker efficiency. Accurate assessment of mental workload enhances employers' productivity and bolsters workplace safety by reducing accidents and boosting production.[7] While numerous studies delve into the neuroimaging aspects of the fatigue symptoms in neurological disorders such as multiple sclerosis, characterized by central fatigue,[8] limited research focuses on healthy subjects.

Recent functional neuroimaging studies in healthy subjects have identified specific brain regions, including the striatum, a nucleus of the basal ganglia (BG), dorsolateral prefrontal cortex (DLPFC), dorsal anterior cingulate cortex (dACC), and ventromedial prefrontal cortex (vmPFC), forming a sort of cortico-subcortical "fatigue network" associated with CF. For example, functional connectivity between these regions decreases as CF intensifies.[9] The DLPFC, insula, and vmPFC are key nodes within this fatigue network, with the anterior cingulate cortex (ACC) collaborating with the ventral striatum to mediate cost-benefit decisions and goal persistence.[10] Interestingly, the primary nodes of the fatigue network are also integral components of other networks critical for behavioral control, such as the salience network (SN), encompassing the anterior insula, the reward network (RN), comprising the striatum and vmPFC, and the cognitive control network (CCN), including the DLPFC and anterior insula.[9] An important contribution regarding the brain areas involved in maintaining good performance in response to workload-induced fatigue comes from what Ishii et al. (2014) called the "facilitation system." The authors observed that subjects' performance

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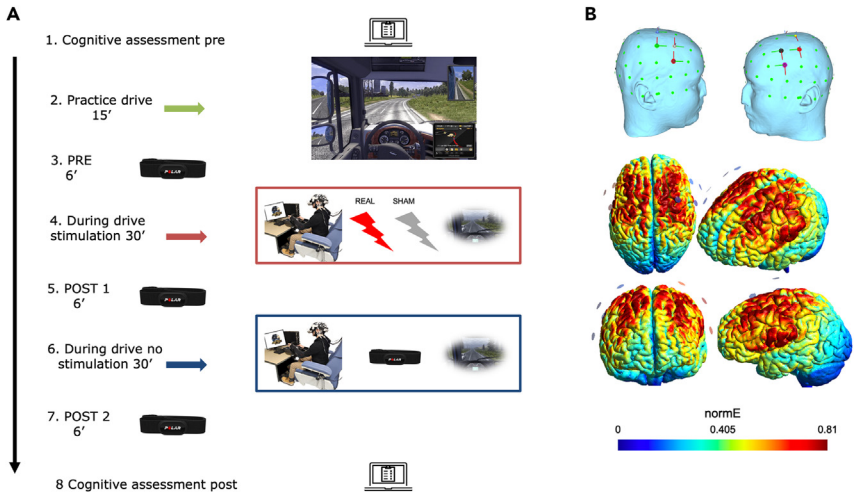

Figure 1. Study design

(A) Timeline of the study. Initially, tests used for cognitive assessment were administered (applied before and after driving tests); subjects were driving while wearing a VR headset (either during the first drive with real or placebo stimulation or during the second drive).

(B) tRNS montage with 8 electrodes chosen to stimulate bilaterally supplementary motor area (SMA) (MNI coordinates left: −28; −2; 52 right: 28; 1; 52), bilaterally middle frontal gyrus (MFG) (MNI coordinates left: 45; 53; −7 right: 44; 34; 20), and primary motor cortex (M1) (MNI coordinates left: −36; −19; 48; right = 38; 18; 45): C1 (0.85mA), C2 (−0.85mA), C3 (1.3mA), C4 (−1.3mA), FC3 (0.75mA), FC4 (−0.75mA), FC5 (1.1mA), and FC6 (−1.1mA). The figure graphically shows the arising E-field (represented in NormE) resulting from the montage in V/m on a healthy example subject.

levels in stimulating and demanding tasks were elevated when there was greater activation of the limbic system, BG, thalamus (TH), orbitofrontal cortex (OFC), DLPFC, ACC, premotor area (PM), supplementary motor area (SMA) and primary motor cortex (M1), and subjects were adequately motivated.[11] In addition, the impact of the reward system and motivational drive in reducing the perception of fatigue is also confirmed by other neuroimaging studies showing the involvement of the middle frontal gyrus (MFG), insula, and ACC.[12,13]

CF is also associated with an increased parasympathetic nervous system (PSNS) activation, as indexed by an elevation in the Root-Mean-Square of successive RR interval differences (RMSSD),[14] a time-domain parameter of heart rate variability (HRV),[15] following cognitive task disengagement. RMSSD reflects the vagal control of heart rate[16] and is poorly influenced by respiratory factors.[17,18]

We aimed to investigate the efficacy of a transcranial random noise electrical stimulation (tRNS)—a non-invasive brain stimulation (NiBS) technique able to increase cortical excitability of the stimulated area/network[19] - in reducing CF[20] when targeted on central nodes of the "anti-fatigue network", composed bilaterally by supplementary motor area, middle frontal gyrus, and primary motor cortex, while maintaining adequate performance levels at a virtual reality (VR) truck driving task. VR creates high levels of immersiveness, affecting the CF system. Additionally, we utilized a peripheral index of neurovegetative activity, HRV, to further elucidate tRNS's impact on CF. Details of the study design are available in Figure 1 and the STAR methods section of the manuscript.

RESULTS

Normal distribution was violated for all behavioral and physiological variables. Scores above or below two standard deviations from the mean were identified and eliminated using JAMOVI v.2.4 software. Comparing both behavioral results and physiological values, tRNS did not affect both truck damages and monetary loss for violations of the driving code (i.e., the two main outcome measures addressing performance) between the two subjects' groups during the practical trial, pointing out an equal baseline level among them (full details reported in Table 1); no tRNS effects on each single cognitive test (full details reported in Table 2). Instead, significant tRNS improvements among participants who

Table 1. Damages and monetary loss (US dollars)

	Sham	Real	p	ε^2
practice drive damages	2%	6%	0.236	0.005
pratice drive loss	$806	$1227.5	0.548	0.013
first drive damages	4.6%	1.8%	0.765	0.004
first drive loss	6.9%	−1.5%	0.137	0.088
second drive damages	5.75%	0.38%	0.011	0.246
second drive loss	3.54%	0.26%	0.009	0.272

Table 2. Cognitive test

	Sham	Real	p	ε^2
SRT reaction time	6.20 ms	−0.02 ms	0.322	0.036
GNG accuracy	0%	0%	0.678	0.006
GNG reaction time	−8.5 ms	−16.4 ms	0.141	0.080
UFOV reaction time	−244.5 ms	−304.3 ms	0.604	0.000
UFOV accuracy	0%	0%	0.604	0.009
Visual search reaction time	−183.4 ms	−191.6 ms	0.144	0.082
Visual search accuracy	0%	0%	0.438	0.023

Note: SRT= Simple Reaction Time; GNG= Go No Go; UFOV= Useful Field of View.

received real stimulation compared with the group who received sham stimulation (during the first drive) were revealed only during the second drive trial (without stimulation) and included both truck damage and monetary loss (full details reported in Table 1) and visual analog scale of fatigue (VAS-F) (full details reported in Table 3); all significant differences are shown in Figure 2 tRNS effects were not found either about RMSSD (full details reported in Table 4) or to SSQ scores (full details reported in Table 5).

Supplemental information include graphs (Figure S1) with the distributions of the nonsignificant comparisons of RMSSD, VAS-F after the first drive, and performance relative to first drive, respectively; additionally, graphs with plots with comparisons regarding the driving performance differences between the first and second drive for both conditions are shown in Figure S2 (outcome values are in Table S1), whereas graphs with comparisons against both RMSSD and VAS-F differences between the first and second guides for both conditions are shown in Figure S3 (outcome values are in Table S2).

DISCUSSION

Despite being a growing issue in the demanding modern society, CF is still understudied and there is a need for better characterization of the problem to develop possible countermeasures. There are no drugs to counteract CF in healthy individuals other than natural remedies with mixed effects.[21] Drugs counteracting fatigue are those used to reduce fatigue during diseases or therapies such as cancer therapy; they might have toxic effects[22] and hence their use is not justified in healthy people.

The primary aim of the current study was to assess the feasibility and effectiveness of tRNS in mitigating CF and enhancing performance in healthy individuals within a real-world ecological context, specifically during a VR truck driving experience. The choice of tRNS over other NiBS techniques was mainly dictated by two reasons: tRNS' effects on cortical excitability have been shown to be stronger than those of transcranial direct current stimulation (tDCS),[23] and longer-lasting after effects than tDCS have been shown as well.[19,24,25] Last, the use of tRNS has been associated with improvements in performance in complex cognitive tasks relevant to the task used in the present study.[26] Repetitive transcranial magnetic stimulation was not considered given the limitations of using such a solution outside the laboratory settings.

To achieve our goal, the application of tRNS was biophysically modeled to target what is referred to as the cortico-subcortical "anti-fatigue network," which includes regions such as the bilateral SMA, the MFG, and the M1.[9] Previous research in the field of neuroenhancement highlighted a significant advantage of NiBS: its functional effects persist for up to 60 min after stimulation.[27] The experimental design was thought to allow better evaluation of subjects both during and after stimulation.

Results confirm that the neurophysiological effects described for tRNS on cortical excitability can be translated at a behavioral level. Indeed, during the initial VR drive, no significant differences were observed between the sham group and those who received real tRNS. More importantly, significant performance improvements, in terms of reduced truck damages and less monetary expenses for violations of the driving code, emerged during the second drive, only in individuals who had previously undergone the driving trial during real tRNS. This suggests no immediate effects of tRNS (i.e., during the first drive), but rather a prolonged neuromodulatory after-effect, that is particularly relevant from a translational point of view.

Such lasting behavioral benefits, that overall reflect improved allocation of spatiotemporal and attentional resources and more efficient reactivity, are not associated with a significant reduction of the HRV-RMSSD parameter. However, we observed a gradual reduction from the first to the second drive that might reach statistical significance by expanding the sample size, given that there are distinct

Table 3. Perceived fatigue

	Sham	Real	p	ε^2
VAS-F pre	3.2	4	0.074	0.110
VAS-F post first drive	0.9%	0.6%	0.127	0.090
VAS-F post second drive	*1.14%*	*0.15%*	*0.003*	*0.346*

Note: VAS-F= Visual Analog Scale Fatigue.

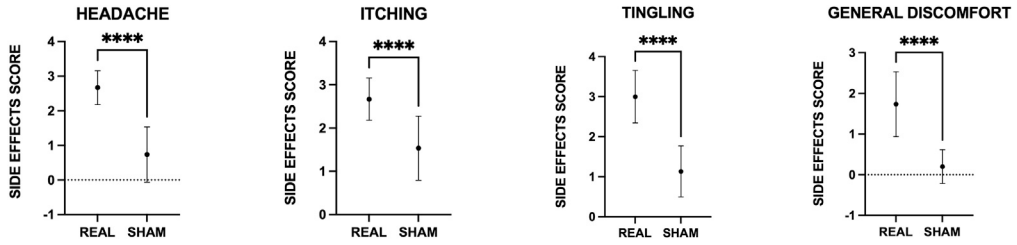

Figure 2. tRNS side effects

This figure presents the disparities in side effects between the two conditions, revealing elevated scores for all items in the real condition. Boxplots represent the confidence (upper and lower bounds), and participants (black dots), and the black line inside the graphs represents the median. ****$p < 0.0001$.

patterns in the parasympathetic system activity following real and sham stimulation. However, physiological relationships between CF and neurovegetative activity[17] are complex and often discordant; when measured by RMSSD, some argue that a decrease in parasympathetic tone is associated with high CF,[28,29] while others conclude that a decrease in parasympathetic tone corresponds to an increased state of CF.[30,31]

Our result, however, would indicate that after real stimulation, subjects report a decrease in parasympathetic tone indicative of an increase in alertness[32] useful in real-life situations without experiencing fatigue, as demonstrated by scores on the VAS-F scale. This reduction could also be due to the nature of the task itself, as it is very stimulating, which (as explained in the introduction) could lead to a decrease in RMSSD. Although the subjects did not report negative experiences related to the stimulation after the first drive, significant differences emerged from the side effects questionnaire between the sham and real stimulation, and this latter result could explain the consequent decrease in parasympathetic tone[33] during the second drive. The fact that side effects were greater (albeit still small) during real stimulation than during sham (Figure 3) reinforces the importance of the beneficial effects of tRNS during drive performances.

Even though the results indicate differences between the two groups, these did not show significant changes in the level of general cognitive performance at the final assessments. This suggests that the effects of tRNS stimulation while driving are independent of changes related to the overall subjects' cognitive abilities, at least those investigated by the employed tests. This could potentially lead to the standardization of tRNS administrations based solely on the required duration of the effect, irrespective of the individual cognitive ability level, thus opening the door to large-scale practical applications in various settings with imbalances in mental workload.

CF is not merely a disruption in cognitive functioning, but rather a multifaceted symptom arising from the concurrent activation and deactivation of overlapping neural networks.[9] It is noteworthy that the regions identified as part of the cortico-subcortical anti-fatigue network also play a role in the reward network, reinforcing the notion that CF is strongly linked to an imbalance between effort and reward.[34] Furthermore, the results underscore the pivotal role of motivation and reward in balancing the mental facilitation and inhibition systems. Motivational input, primarily through enhanced dopaminergic and norepinephrine tone,[35] activates the facilitation system, effectively compensating for the effects of CF.[11] A last point to consider is the cybersickness symptom, one of the major VR side effects. The use of NiBS techniques has already yielded positive results in reducing this symptom as shown by the study[36] where the transcranial alternating current stimulation at 10 Hz applied on the vestibular cortex significantly reduced cybersickness in healthy subjects undergoing a VR-based rollercoaster ride. In the current study, however, considering that both the type of stimulation and target areas were different, participants' cybersickness remained unchanged throughout driving trials (Figure 2). The trend of slightly increased cybersickness in the real group compared with the sham group did not affect driving performance.

In conclusion, this study presents a novel approach involving non-invasive brain stimulation to sustain high levels of focus, particularly in occupations characterized by substantial workloads, such as professional drivers. The implications of these findings extend beyond this specific context and hold significant potential for enhancing performance and safety in other demanding professions. Considering also the strong link between CF and physical fatigue,[37,38] and the existing utilization of noninvasive brain stimulation techniques like tDCS in the context of physical fatigue,[39–41] the findings of this study open the door to the prospect of introducing a novel treatment approach and launching further research endeavors aimed at improving athletic performance in endurance activities like marathons or triathlons. The proposed approach is also worth testing in neurological disorders where fatigue is a central symptom, such as multiple sclerosis or Parkinson's disease, to accelerate their rehabilitation program.

Table 4. Cardiac parasympathetic activity

	Sham	Real	p	ε^2
RMSSD pre	37.2	47.6	0.430	0.022
RMSSD during first drive	−6.4%	−19.3%	0.068	0.145
RMSSD during second drive	*−0.9%*	*−20.6%*	*0.053*	*0.139*

Note: RMMSD= Root-Mean-Square of Successive RR interval differences.

Table 5. Perceived cyber sickness

	Sham	Real	p	ε^2
SSQ post first drive	5.6	9.5	0.341	0.457
SSQ post second drive	6.6	9.8	0.440	0.155
SSQ total	6.3	9.6	0.420	0.247

Note: SSQ = Simulator Sickness Questionnaire.

The latest field of potential translational application may be the gaming and e-sport world; demanding games such as first-person shooter, racing, and real-time strategy games. The last area of potential translational application may be the world of gaming and e-sports; challenging games, such as first-person shooters, racing, and real-time strategy games. By decreasing fatigue perception, this stimulation technique would maintain both a high focus on the right target as well as good visuomotor coordination.

Limitations of the study

The findings suggest that tRNS when precisely targeted to the cortical areas of the brain "anti-fatigue" network, could represent a novel strategy for alleviating CF and enhancing performance compared to placebo stimulation. This study, however, has several limitations, primarily related to the relatively limited sample size, which could be expanded in future research endeavors. Due to experimental time constraints, EEG analysis could not be conducted, but assessing EEG activity before, during, and after stimulation could offer further validation of the positive effects of tRNS on CF by visualizing cortical activity dynamics. The stimulation method may have an impact on changing cardiac activity,[42] so in a future phase of the study, it will be appropriate to make a recording of the RMSSD only during stimulation to obtain an individual baseline during stimulation to adjust the parameter obtained during the task with its stimulation-related baseline. Additionally, future research could benefit from longer-term follow-ups to investigate the eventual sustained effects of tRNS. Since these findings emanated from a single-day experiment and a single session of stimulation, future studies should investigate whether subjects undergoing sequential

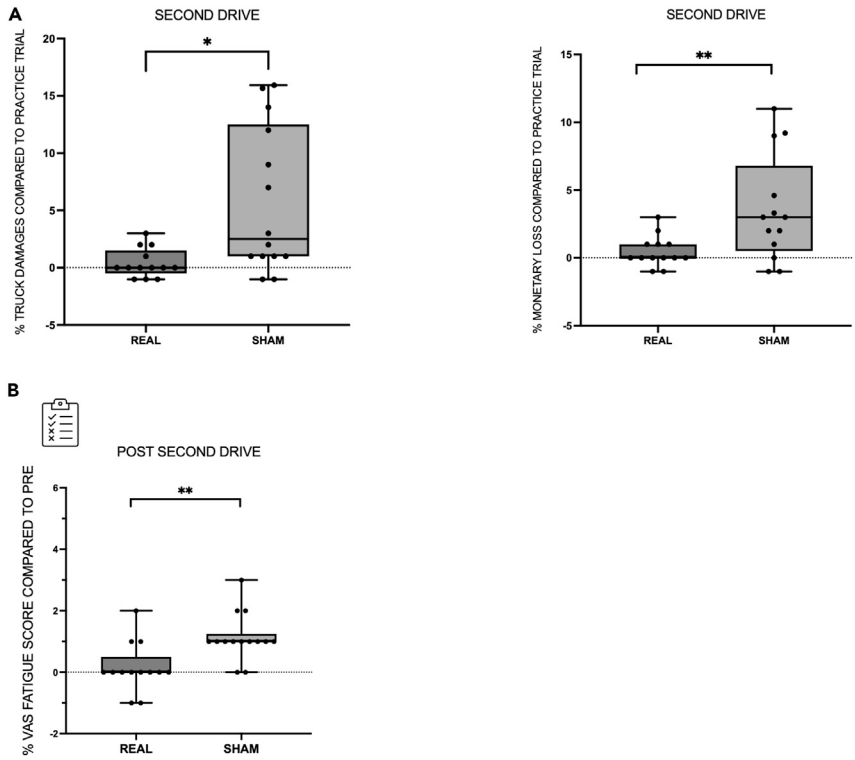

Figure 3. tRNS effects on driving performance and fatigue perception related to second driving session

(A): the graph shows the significant score differences between the sham and real condition regarding both damages (left) and monetary loss (right).

(B): shows significant differences between fatigue perception comparing sham and real condition. Boxplots represent the confidence (upper and lower bounds), and participants (black dots), and the black line inside the graphs represents the median. *$p < 0.05$; **$p < 0.01$. Note: the plot whiskers represent min to max values.

stimulations over an extended period could yield even more pronounced performance improvements. It is also important to acknowledge the subjective nature of reporting CF as a limitation, which is inherent in CF evaluations; however, the observed changes in parasympathetic activity mitigate the intrinsic weakness of the subjective report. Subsequent investigations could explore the effects of tRNS without practical driving tests during stimulation to determine if the behavioral and physiological responses observed in this study remain consistent or undergo changes.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2024.110536.

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AUTHOR CONTRIBUTIONS

E.S. and S.R. conceived the project; E.S. and A.B. designed the experiment; E.S., A.B., C.M., and F.N. implemented the experiment; A.B., C.M., F.N., A.C., A.G., F.L., and A.S., conducted the experiment; E.S., S.M.R., and A.B. designed the biophysical modeling; A.B. and S.P. analyzed data; A.B and C.M. wrote the first draft of the paper; A.B., C.M., E.S., S.R., F.N., A.G., F.L., A.S., S.M.R., and R.M.Z. contributed to the final draft of the paper.

DECLARATION OF INTERESTS

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REFERENCES

1. Qi, P., Ru, H., Gao, L., Zhang, X., Zhou, T., Tian, Y., Thakor, N., Bezerianos, A., Li, J., and Sun, Y. (2019). Neural Mechanisms of Mental Fatigue Revisited: New Insights from the Brain Connectome. Engineering *5*, 276–286. https://doi.org/10.1016/j.eng.2018.11.025.
2. Lim, J., and Dinges, D.F. (2010). A meta-analysis of the impact of short-term sleep deprivation on cognitive variables. Psychol. Bull. *136*, 375–389. https://doi.org/10.1037/a0018883.
3. Ma, Y., Chen, B., Li, R., Wang, C., Wang, J., She, Q., Luo, Z., and Zhang, Y. (2019). Driving Fatigue Detection from EEG Using a Modified PCANet Method. Comput. Intell. Neurosci. *2019*, 4721863. https://doi.org/10.1155/2019/4721863.
4. Ishii, A., Tanaka, M., Yoshikawa, T., and Watanabe, Y. (2017). Evidence for unconscious regulation of performance in fatigue. Sci. Rep. *7*, 16103. https://doi.org/10.1038/s41598-017-16439-6.
5. Gupta, A., Siddhad, G., Pandey, V., Roy, P.P., and Kim, B.-G. (2021). Subject-Specific Cognitive Workload Classification Using EEG-Based Functional Connectivity and

Deep Learning. Sensors *21*, 6710. https://doi.org/10.3390/s21206710.
6. Paas, F., Renkl, A., and Sweller, J. (2003). Cognitive Load Theory and Instructional Design: Recent Developments. Educ. Psychol. *38*, 1–4. https://doi.org/10.1207/S15326985EP3801_1.
7. Li, G., Huang, S., Xu, W., Jiao, W., Jiang, Y., Gao, Z., and Zhang, J. (2020). The impact of mental fatigue on brain activity: a comparative study both in resting state and task state using EEG. BMC Neurosci. *21*, 20. https://doi.org/10.1186/s12868-020-00569-1.
8. Nakagawa, S., Takeuchi, H., Taki, Y., Nouchi, R., Kotozaki, Y., Shinada, T., Maruyama, T., Sekiguchi, A., Iizuka, K., Yokoyama, R., et al. (2016). Basal ganglia correlates of fatigue in young adults. Sci. Rep. *6*, 21386. https://doi.org/10.1038/srep21386.
9. Wylie, G.R., Yao, B., Genova, H.M., Chen, M.H., and DeLuca, J. (2020). Using functional connectivity changes associated with cognitive fatigue to delineate a fatigue network. Sci. Rep. *10*, 21927. https://doi.org/10.1038/s41598-020-78768-3.
10. Rushworth, M.F.S., Walton, M.E., Kennerley, S.W., and Bannerman, D.M. (2004). Action sets and decisions in the medial frontal cortex. Trends Cognit. Sci. *8*, 410–417. https://doi.org/10.1016/j.tics.2004.07.009.
11. Ishii, A., Tanaka, M., and Watanabe, Y. (2014). Neural mechanisms of mental fatigue. Rev. Neurosci *25*, 469–479. https://doi.org/10.1515/revneuro-2014-0028.
12. Darnai, G., Matuz, A., Alhour, H.A., Perlaki, G., Orsi, G., Arató, Á., Szente, A., Áfra, E., Nagy, S.A., Janszky, J., and Csathó, Á. (2023). The neural correlates of mental fatigue and reward processing: A task-based fMRI study. Neuroimage *265*, 119812. https://doi.org/10.1016/j.neuroimage.2022.119812.
13. Müller, T., and Apps, M.A.J. (2019). Motivational fatigue: A neurocognitive framework for the impact of effortful exertion on subsequent motivation. Neuropsychologia *123*, 141–151. https://doi.org/10.1016/j.neuropsychologia.2018.04.030.
14. Csathó, Á., Van Der Linden, D., and Matuz, A. (2024). Change in heart rate variability with increasing time-on-task as a marker for mental fatigue: A systematic review. Biol. Psychol. *185*, 108727. https://doi.org/10.1016/j.biopsycho.2023.108727.
15. Shaffer, F., and Ginsberg, J.P. (2017). An Overview of Heart Rate Variability Metrics and Norms. Front. Public Health *5*, 258. https://doi.org/10.3389/fpubh.2017.00258.
16. (1996). Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Circulation *93*, 1043–1065.
17. Laborde, S., Mosley, E., and Thayer, J.F. (2017). Heart Rate Variability and Cardiac Vagal Tone in Psychophysiological Research – Recommendations for Experiment Planning, Data Analysis, and Data Reporting. Front. Psychol *8*, 213. https://doi.org/10.3389/fpsyg.2017.00213.
18. Penttilä, J., Helminen, A., Jartti, T., Kuusela, T., Huikuri, H.V., Tulppo, M.P., Coffeng, R., and Scheinin, H. (2001). Time domain, geometrical and frequency domain analysis of cardiac vagal outflow: effects of various respiratory patterns: Assessment of cardiac vagal outflow. Clin. Physiol. *21*, 365–376. https://doi.org/10.1046/j.1365-2281.2001.00337.x.
19. Terney, D., Chaieb, L., Moliadze, V., Antal, A., and Paulus, W. (2008). Increasing Human Brain Excitability by Transcranial High-Frequency Random Noise Stimulation. J. Neurosci. *28*, 14147–14155. https://doi.org/10.1523/JNEUROSCI.4248-08.2008.
20. Moret, B., Donato, R., Nucci, M., Cona, G., and Campana, G. (2019). Transcranial random noise stimulation (tRNS): a wide range of frequencies is needed for increasing cortical excitability. Sci. Rep. *9*, 15150. https://doi.org/10.1038/s41598-019-51553-7.
21. Son, M.J., Im, H.-J., Kim, Y.-E., Ku, B., Lee, J.-H., and Son, C.-G. (2016). Evaluation of the anti-fatigue effects of a traditional herbal drug, Gongjin-dan, under insufficient sleep conditions: study protocol for a randomised controlled trial. Trials *17*, 418. https://doi.org/10.1186/s13063-016-1542-7.
22. Luo, C., Xu, X., Wei, X., Feng, W., Huang, H., Liu, H., Xu, R., Lin, J., Han, L., and Zhang, D. (2019). Natural medicines for the treatment of fatigue: Bioactive components, pharmacology, and mechanisms. Pharmacol. Res. *148*, 104409. https://doi.org/10.1016/j.phrs.2019.104409.
23. Inukai, Y., Saito, K., Sasaki, R., Tsuiki, S., Miyaguchi, S., Kojima, S., Masaki, M., Otsuru, N., and Onishi, H. (2016). Comparison of Three Non-Invasive Transcranial Electrical Stimulation Methods for Increasing Cortical Excitability. Front. Hum. Neurosci. *10*, 668. https://doi.org/10.3389/fnhum.2016.00668.
24. Potok, W., Bächinger, M., Van Der Groen, O., Cretu, A.L., and Wenderoth, N. (2021). Transcranial Random Noise Stimulation Acutely Lowers the Response Threshold of Human Motor Circuits. J. Neurosci. *41*, 3842–3853. https://doi.org/10.1523/JNEUROSCI.2961-20.2021.
25. Sánchez-León, C.A., Cordones, I., Ammann, C., Ausín, J.M., Gómez-Climent, M.A., Carretero-Guillén, A., Sánchez-Garrido Campos, G., Gruart, A., Delgado-García, J.M., Cheron, G., et al. (2021). Immediate and after effects of transcranial direct-current stimulation in the mouse primary somatosensory cortex. Sci. Rep. *11*, 3123. https://doi.org/10.1038/s41598-021-82364-4.
26. Chenot, Q., Hamery, C., Lepron, E., Besson, P., De Boissezon, X., Perrey, S., and Scannella, S. (2022). Performance after training in a complex cognitive task is enhanced by high-definition transcranial random noise stimulation. Sci. Rep. *12*, 4618. https://doi.org/10.1038/s41598-022-08545-x.
27. Antal, A., Luber, B., Brem, A.-K., Bikson, M., Brunoni, A.R., Cohen Kadosh, R., Dubljević, V., Fecteau, S., Ferreri, F., Flöel, A., et al. (2022). Non-invasive brain stimulation and neuroenhancement. Clin. Neurophysiol. Pract. *7*, 146–165. https://doi.org/10.1016/j.cnp.2022.05.002.
28. Melo, H.M., Nascimento, L.M., and Takase, E. (2017). Mental fatigue and heart rate variability (HRV): The time-on-task effect. Psychol. Neurosci. *10*, 428–436. https://doi.org/10.1037/pne0000110.
29. Escorihuela, R.M., Capdevila, L., Castro, J.R., Zaragozà, M.C., Maurel, S., Alegre, J., and Castro-Marrero, J. (2020). Reduced heart rate variability predicts fatigue severity in individuals with chronic fatigue syndrome/myalgic encephalomyelitis. J. Transl. Med. *18*, 4. https://doi.org/10.1186/s12967-019-02184-z.
30. Matuz, A., van der Linden, D., Kisander, Z., Hernádi, I., Kázmér, K., and Csathó, Á. (2021). Enhanced cardiac vagal tone in mental fatigue: Analysis of heart rate variability in Time-on-Task, recovery, and reactivity. PLoS One *16*, e0238670. https://doi.org/10.1371/journal.pone.0238670.
31. Rosa, E., Lyskov, E., Grönkvist, M., Kölegård, R., Dahlström, N., Knez, I., Ljung, R., and Willander, J. (2022). Cognitive performance, fatigue, emotional, and physiological strains in simulated long-duration flight missions. Mil. Psychol. *34*, 224–236. https://doi.org/10.1080/08995605.2021.1989236.
32. Lok, R., Smolders, K.C.H.J., Beersma, D.G.M., and De Kort, Y.A.W. (2018). Light, Alertness, and Alerting Effects of White Light: A Literature Overview. J. Biol. Rhythm. *33*, 589–601. https://doi.org/10.1177/0748730418796443.
33. Schmaußer, M., Hoffmann, S., Raab, M., and Laborde, S. (2022). The effects of noninvasive brain stimulation on heart rate and heart rate variability: A systematic review and meta-analysis. J. Neurosci. Res. *100*, 1664–1694. https://doi.org/10.1002/jnr.25062.
34. Dobryakova, E., and Tricomi, E. (2013). Basal ganglia engagement during feedback processing after a substantial delay. Cognit. Affect Behav. Neurosci. *13*, 725–736. https://doi.org/10.3758/s13415-013-0182-6.
35. Hopstaken, J.F., Van Der Linden, D., Bakker, A.B., and Kompier, M.A.J. (2015). The window of my eyes: Task disengagement and mental fatigue covary with pupil dynamics. Biol. Psychol. *110*, 100–106. https://doi.org/10.1016/j.biopsycho.2015.06.013.
36. Benelli, A., Neri, F., Cinti, A., Pasqualetti, P., Romanella, S.M., Giannotta, A., De Monte, D., Mandalà, M., Smeralda, C., Prattichizzo, D., et al. (2023). Frequency-Dependent Reduction of Cybersickness in Virtual Reality by Transcranial Oscillatory Stimulation of the Vestibular Cortex. Neurotherapeutics *20*, 1796–1807. https://doi.org/10.1007/s13311-023-01437-6.
37. Van Cutsem, J., Marcora, S., De Pauw, K., Bailey, S., Meeusen, R., and Roelands, B. (2017). The Effects of Mental Fatigue on Physical Performance: A Systematic Review. Sports Med. *47*, 1569–1588. https://doi.org/10.1007/s40279-016-0672-0.
38. Alix-Fages, C., Jiménez-Martínez, P., De Oliveira, D.S., Möck, S., Balsalobre-Fernández, C., and Del Vecchio, A. (2023). Mental fatigue impairs physical performance but not the neural drive to the muscle: a preliminary analysis. Eur. J. Appl. Physiol. *123*, 1671–1684. https://doi.org/10.1007/s00421-023-05189-1.
39. Angius, L., Pascual-Leone, A., and Santarnecchi, E. (2018). Brain stimulation and physical performance. Prog. Brain Res. *240*, 317–339. https://doi.org/10.1016/bs.pbr.2018.07.010.
40. Angius, L., Santarnecchi, E., Pascual-Leone, A., and Marcora, S.M. (2019). Transcranial Direct Current Stimulation over the Left Dorsolateral Prefrontal Cortex Improves Inhibitory Control and Endurance Performance in Healthy Individuals. Neuroscience *419*, 34–45. https://doi.org/10.1016/j.neuroscience.2019.08.052.
41. Vitor-Costa, M., Okuno, N.M., Bortolotti, H., Bertollo, M., Boggio, P.S., Fregni, F., and Altimari, L.R. (2015). Improving Cycling Performance: Transcranial Direct Current Stimulation Increases Time to Exhaustion in

Cycling. PLoS One *10*, e0144916. https://doi.org/10.1371/journal.pone.0144916.

42. Matsumoto, H., and Ugawa, Y. (2017). Adverse events of tDCS and tACS: A review. Clin. Neurophysiol. Pract. *2*, 19–25. https://doi.org/10.1016/j.cnp.2016.12.003.
43. Faul, F., Erdfelder, E., Buchner, A., and Lang, A.-G. (2009). Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. Behav. Res. Methods *41*, 1149–1160. https://doi.org/10.3758/BRM.41.4.1149.
44. Morizio, C., Compagnat, M., Boujut, A., Labbani-Igbida, O., Billot, M., and Perrochon, A. (2022). Immersive Virtual Reality during Robot-Assisted Gait Training: Validation of a New Device in Stroke Rehabilitation. Medicina *58*, 1805. https://doi.org/10.3390/medicina58121805.
45. Thielscher, A., Antunes, A., and Saturnino, G.B. (2015). Field modeling for transcranial magnetic stimulation: A useful tool to understand the physiological effects of TMS? In 2015 37th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC) (IEEE), pp. 222–225. https://doi.org/10.1109/EMBC.2015.7318340.
46. Saturnino, G.B., Siebner, H.R., Thielscher, A., and Madsen, K.H. (2019). Accessibility of cortical regions to focal TES: Dependence on spatial position, safety, and practical constraints. Neuroimage *203*, 116183. https://doi.org/10.1016/j.neuroimage.2019.116183.
47. Windhoff, M., Opitz, A., and Thielscher, A. (2013). Electric field calculations in brain stimulation based on finite elements: An optimized processing pipeline for the generation and usage of accurate individual head models. Hum. Brain Mapp. *34*, 923–935. https://doi.org/10.1002/hbm.21479.
48. Linnhoff, S., Wolter-Weging, J., and Zaehle, T. (2021). Objective electrophysiological fatigability markers and their modulation through tDCS. Clin. Neurophysiol. *132*, 1721–1732. https://doi.org/10.1016/j.clinph.2021.02.391.
49. Lattari, E., Rosa Filho, B.J., Fonseca Junior, S.J., Murillo-Rodriguez, E., Rocha, N., Machado, S., and Maranhão Neto, G.A. (2020). Effects on Volume Load and Ratings of Perceived Exertion in Individuals' Advanced Weight Training After Transcranial Direct Current Stimulation. J. Strength Condit Res. *34*, 89–96. https://doi.org/10.1519/JSC.0000000000002434.
50. Pope, P.A., Brenton, J.W., and Miall, R.C. (2015). Task-Specific Facilitation of Cognition by Anodal Transcranial Direct Current Stimulation of the Prefrontal Cortex. Cerebr. Cortex *25*, 4551–4558. https://doi.org/10.1093/cercor/bhv094.
51. Tarvainen, M.P., Niskanen, J.-P., Lipponen, J.A., Ranta-aho, P.O., and Karjalainen, P.A. (2014). Kubios HRV – Heart rate variability analysis software. Comput. Methods Progr. Biomed. *113*, 210–220. https://doi.org/10.1016/j.cmpb.2013.07.024.
52. Lipponen, J.A., and Tarvainen, M.P. (2019). A robust algorithm for heart rate variability time series artefact correction using novel beat classification. J. Med. Eng. Technol. *43*, 173–181. https://doi.org/10.1080/03091902.2019.1640306.
53. Shahid, A., Wilkinson, K., Marcu, S., and Shapiro, C.M. (2011). Visual Analogue Scale to Evaluate Fatigue Severity (VAS-F). In STOP, THAT and One Hundred Other Sleep Scales, A. Shahid, K. Wilkinson, S. Marcu, and C.M. Shapiro, eds. (Springer New York), pp. 399–402. https://doi.org/10.1007/978-1-4419-9893-4_100.
54. Brunoni, A.R., Amadera, J., Berbel, B., Volz, M.S., Rizzerio, B.G., and Fregni, F. (2011). A systematic review on reporting and assessment of adverse effects associated with transcranial direct current stimulation. Int. J. Neuropsychopharmacol. *14*, 1133–1145. https://doi.org/10.1017/S1461145710001690.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
G*Power 3.1	Universität Düsseldorf: Psychologie - HHU	https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower
SimNIBS v3.2	Danish Research Center for Magnetic Resonance (Copenhagen, Denmark), Technical University of Denmark (Kgs Lyngby, Denmark)	https://simnibs.github.io/simnibs/build/html/index.html
DSI Studio	Frank Yeh, US	https://dsi-studio.labsolver.org
Kubios HRV, Standard Version	Kubios Oy, Finland	https://www.kubios.com
JAMOVI® v.2.4 software	Jamovi project, 2023	https://www.jamovi.org
Other		
Starstim 32	Starstim; Neuroelectrics, Barcelona, Spain	https://www.neuroelectrics.com/solutions/starstim/32
Polar H10	Polar, Finland	https://www.polar.com/us-en/sensors/h10-heart-rate-sensor
Euro Truck Simulator 2	SCS Software, Cech Republic	https://eurotrucksimulator2.com
Oculus Quest 2	Meta Platforms Technologies, US	https://www.meta.com/quest/products/quest-2/
Participants demographic details	Table S3.	

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Emiliano Santarnecchi (esantarnecchi@mgh.harvard.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

This study did not generate new datasets.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Experimental design, protocol, and participant data are given below.

METHOD DETAILS

Participants

In this exploratory, double-blind, parallel arm study, 32 healthy young adults (all right-handed; 19 males and 13 females; mean age: 25.3 ± 3.7 years; education: 14.6 ± 2.4 years) were recruited from students at the University Hospital of Siena, Italy. Two of them withdrew for personal reasons; therefore, analyses were carried out on 30 subjects (17 males and 13 females; mean age: 24.9 ± 3.5 years; education: 14.5 ± 2.3 years). Demographic details for all participants are provided in the supplemental information in Table S3. All subjects were licensed to drive and were inexperienced in operating a VR driving simulator. Each subject declared their willingness to participate in the study and signed a written informed consent; the Local Ethics Committee approved the research (Code: Brainsight 21-24).

Participants were assigned to the active group (9 males and 6 females; mean age: 24.5 ± 10.7) or sham group (8 males and 7 females; mean age: 25.2 ± 11.3) in a blind, and randomized manner. Taking into account the exploratory nature of the study, the sample size was defined using G*Power 3.1[43] on the following basis: since we were interested in one specific contrast (tRNS vs. Sham) alpha was set at 0.05/2=0.025. Power was set at 0.60, indicating that 30 subjects are essential to have a 60% probability of recognizing a difference between two conditions as statistically significant (at two-sided alpha 0.025).

Subjects with a history of epilepsy, sleep disturbances, migraine, psychiatric drug therapy, and history of other neurological or psychiatric disorders were excluded. Simulator Sickness Questionnaire (SSQ) was used to assess the degree of cybersickness resulting from the use of Virtual Reality.[44]

Experimental design

Participants engaged in the VR sessions were sitting on a chair, blind to the type of stimulation applied. Each participant also underwent two cognitive assessments (before the practice trial and after the second drive) by performing several tasks in a fixed order. Subjects were tested under the same experimental protocol within a randomized double-blinded study design. Blindness was deployed using the Matlab programming language, which randomly assigned a number for each condition with no knowledge of the experimenter. Using the same script again, the conditions were randomized equally among participants. The experiment was conducted in a quiet environment to minimize the influence of external stimulations. The protocol included 3 sequential drive trials (practice trial, first and second drive) with a truck driving experience (Euro Truck Simulator 2) by connecting the Steam gaming platform to head-mounted Oculus Quest 2. Driving routes were the same for all participants. The practice trial consisted of a 15-minute training session without any brain stimulation to practice driving in VR. The first 30-minute drive involved the administration of stimulation either tRNS (0Hz-500Hz) or Sham, applied in a double-blind and randomized controlled manner among participants; tRNS Stimulation represented the experimental condition and the Sham stimulation represented the control condition. The following 30-minute drive was performed without any stimulation, to verify the possible stimulation after effect on the driving performance, the main goal of the study. HRV recordings were made during the first and second drives, as well as between the practice trial and the first drive, and between the first and second drives. A third HRV recording was taken at the end of the second drive. We decided to perform all 3 trials on the same day to induce the maximal cognitive workload inducing more CF.

To evaluate cognitive abilities changes before and after the two drives we used different cognitive tasks: visual search task (in this task multiple objects are presented and a target is identified among them; subjects were asked to identify the target stimulus as fast as possible. Not only the number of correct responses is taken into account but also the speed of stimulus processing), and useful visual field task (in this task a stimulus was shown briefly and then made to disappear by asking the subject to return as quickly as possible to where she/he saw it; not only is the number of correct responses taken into account but also the speed of execution), Go-NoGo task (with this test, the ability to inhibit automatic responses is assessed as target stimuli are presented interspersed with confounding stimuli rapidly; the subject is asked to press as fast as possible when the target stimulus appears and not press when the confounding stimulus appears. Not only is the number of correct responses taken into account but also the speed of execution) and simple reaction time task (with this test, the speed processing is assessed; the subject is asked to press as fast as possible when the target stimulus appears. Only the execution speed is taken into account). Each task required approximately 3–5 min; they were executed using a desktop PC controlled by Presentation software (Versions 13 and 14, NeuroBehavioral Systems, Albany, CA, USA). Figure 3A shows the experimental setting and design.

Euro Truck Simulator 2

We chose Euro Truck Simulator 2 because it offers the opportunity to use real maps while driving a truck. The game has been customized to increase the smoothness of the steering wheel, and pedal pressure to recreate a real driving as much as possible. During the simulation, all participants ran 3 routes of different durations (as shown above). In the practical test section, of mild difficulty, the course was set with a reduced percentage of traffic, a sunny sky, and no abrupt behavior from other drivers. The second and third guides had a higher difficulty degree: rainy sky, high traffic, abrupt behavior by other drivers, and increased police presence to carry out checks. This distinctive setting was aimed at recreating a highly challenging environment. Participants were required to maintain a high level of attention while performing multiple tasks simultaneously: watch the road, maintain a safe speed within the limits, and pay attention to other drivers while following the route correctly as a navigator. Variables of interest extracted from the simulator were the driving performances obtained based on the damages caused to the truck and the cumulative monetary losses.

Transcranial random noise stimulation (tRNS)

tRNS was administered through a 32-channel hybrid EEG/tCS neurostimulation system (Starstim; Neuroelectrics, Barcelona, Spain). The device was connected by cable to the computer. Hybrid electrodes (NG Pistim) were used, consisting of an upper part containing the sintered Ag/AgCl core with a diameter of 12 mm, screwed onto a lower base such that a circular area of about 3.14 cm^2 was covered. The electrodes were placed on a 32-channel neoprene EEG headset with holes corresponding to the positions of the International 10-20 EEG System. The scalp portion below the electrode was prepared by inserting 15 ml of sterile sodium chloride solution (0.9%) to avoid uncomfortable sensations on the skin and reduce impedances, which were always kept below 20 kΩ. Gel (Signa, Parker Laboratories, Inc.) was applied to optimize signal conductivity and lower impedance. Electrode impedance was checked before starting each tRNS session to ensure safety and maximal efficacy of stimulation, as well as to ensure familiarization of participants with the tRNS-induced scalp sensations (e.g., slight tingling). tRNS was applied for 30 minutes with a maximum intensity of 2 mA on each electrode and of 4 mA total across all electrodes, preceded by a 30-s ramp-up period and followed by a 30-s ramp-down period, while research and clinical personnel carefully monitored for eventual side effects for the entire duration of each session. For Sham stimulation, only a ramp up and ramp down of 30-s were set, without any stimulation in between.

Biophysical modeling

To determine the appropriate electrode montage for our target, an open-source simulation software (SimNIBS v3.2) was employed. Utilizing computational modeling through the Finite Element Method (FEM), SimNIBS incorporates magnetic resonance imaging (MRI) scan segmentation, mesh generation, and E-field computations. This allows for the projection of current distribution and accurate calculation of the electric field generated by various NIBS techniques.[45] The software presents a realistic volume conductor head model, defaulting to the FEM model generated from T1 - and T2 - weighted images and segmentation from the SimNIBS example dataset.[46] The data sample, acquired ethically from a healthy subject under the approval of the Ethics Committee of the Medical Faculty of the University of Tübingen,[47] comprises white matter, gray matter, cerebrospinal fluid, bone, and scalp tissue volumes for a comprehensive simulation.

Simulations were based on isotropic conductivities reported in Thielscher, Antunes, and Saturnino (2015), with values corresponding to gray matter (0.276 S/m), cerebrospinal fluid (1.790 S/m), bone (0.010 S/m), and scalp (0.250 S/m). The final mesh, encompassing gray and white matter, scalp, bone, and cerebrospinal fluid, consisted of approximately 200,000 nodes and 3.6 million tetrahedral elements (see Windhoff, Opitz, and Thielscher 2013) for further modeling details).

Drawing on literature data, we proposed an innovative stimulation montage targeting both cortical and subcortical areas directly and indirectly, respectively. Taking a cue from the study regarding the facilitation system by Ishii et al. (2014) and other studies that only considered DLPFC[48–50] as the target stimulation area, we decided to focus on the SMA (MNI coordinates left: -28; -2; 52 right: 28; 1; 52), bilaterally, MFG (MNI coordinates left: 45; 53; -7 right: 44; 34; 20) and M1 (MNI coordinates left: -36; -19; 48; right= 38; 18; 45).

Using the DSI Studio tractography software tool, subcortical projections of the cortical target region revealed fiber connections to subcortical structures such as the striatum, TH, and the caudate nucleus. Importing subcortical nuclei as ROIs from the ATAG_basal_ganglia Atlas in DSI Studio, we observed fibers passing through these ROIs using automatic fiber tracking. A connectivity matrix was then created by loading Brodmann's anatomical atlas to observe cortical areas reached by the fibers, including DLPFC, SMA, and M1.

Based on simulation results and the model, we identified a montage capable of targeting both regions. Specifically, we placed eight electrodes over C1, C2, C3, C4, FC3, FC4, FC5, and FC6. On C1 level, an intensity of 0.85 mA with a phase angle of 0° was used; on C2 level, an intensity of 0.85 mA with a phase angle of 180° was used; on C3 level, an intensity of 1.3 mA with a phase angle of 0° was used; on C4 level, an intensity of 1.3 mA with a phase angle of 180° was used; on FC3 level, an intensity of 0.75 mA with a phase angle of 0° was used; for a total of 2.5 mA; on FC4 level, an intensity of 0.75 mA with a phase angle of 180° was used; on FC5 level, an intensity of 1.1 mA with a phase angle of 0° was used; and on FC6 level, an intensity of 1.1 mA with a phase angle of 180° was used; for a total of 4 mA (Figure 3B).

HRV recording

In all subjects, Heart Rate Variability was measured by a Polar H10 heart rate monitor device placed on the chest. Specifically, we were interested in the detection of Root Mean Square of Successive RR interval differences (RMSSD) parameter, related to the activity of the vagal parasympathetic system. HRV was recorded before (6 minutes), during the first VR drive experience (30 minutes), after the first drive trial (6 minutes), during the second VR drive experience (30 minutes), and after the second drive trial (6 minutes). Measures of RMSSD variations were expressed in ms percentage. For the resting recording, subjects were asked to rest with their eyes open for the first 3 minutes and then to close their eyes for the following 3 minutes. They were asked not to think about anything in particular and to remain as relaxed as possible.

The Kubios HRV software, Standard Version,[51] was employed to conduct HRV analysis on individual cardiac traces and to identify outliers and artifacts (utilizing the automatic correction feature). An automatic beat correction algorithm was utilized to rectify erroneous Inter-Beat Intervals (IBIs).[52] This algorithm accurately identifies missed and extra beat detections with a 100% accuracy rate and ectopic beats with a 97% accuracy rate. The detected artifacts are rectified using cubic spline interpolation.

Outcome measures and data analysis

Drive Performance, RMSSD, VAS-F, Cyber Sickness and Side Effects. The primary aim was to verify fatigue scores after each trial in the different stimulation conditions. Through the[53] VAS-F, participants were asked to report their fatigue status by choosing a score from 0-10 (0=absence of fatigue; 10=worst possible fatigue). In addition to the level of fatigue, driving performance (damages to the truck and monetary losses incurred because of traffic violations) was compared.

Damage refers to the mechanical damage to the vehicle on 5 key components of the player's truck: engine, transmission, chassis, cab, and wheels. This parameter is quantified as the average percentage value of damage among these vehicle elements. Loss refers to monetary loss expressed in dollars and simulates both the penalties that the driver incurs due to traffic violations while driving and the money that is deducted to pay for repairs to the vehicle in the event of mechanical damage. RMSSD data refer to the variation between RMSSD recorded before, during, and after the VR drive for each condition. A standardized side-effects questionnaire addressing general discomfort, headache, itching, and tingling during tRNS[54] was also administered for each experimental condition. The practical trial values obtained and corrected all the results shown from the first and second drives, both behavioral and physiological. Cognitive test scores were made considering the differences between the first and second administrations.

Statistical analysis

JAMOVI® v.2.4 software (Jamovi project, 2023) was employed to analyze both behavioral differences in Drive Performance and VAS-F scores, the RMSSD cardiac parameter, SSQ (Simulator Sickness Questionnaire), and stimulation side effects questionnaire. Within the scope of

behavioral data analysis, the dependent variables consisted of self-reported Drive Performances (3 levels: practice trial, first drive performance, and second drive performance) and VAS-F (3 levels: VAS-F PRE, VAS-F POST 1 and VAS-F POST 2), while the selected independent variable was the stimulation condition (2 levels: tRNS and Sham). In analyzing physiological data, RMSSD (RMSSD PRE, RMSSD during the first drive, and RMSSD during the second drive) represented the dependent variables, and stimulation condition was the independent variable. Through the same procedure, we tested the levels of Cyber Sickness induced by VR and the stimulation Side effects. The Shapiro-Wilk test was used to check the normality of data distribution.

The study employed the nonparametric Kruskal-Wallis Test to examine differences between groups. The grouping variable was the condition (real or sham), and the dependent variables were RMSSD, VAS-F, damages, and loss. The effect size (ε^2) is reported. Two separate analyses were conducted, each considering the four variables of the first and second drives. The Bonferroni correction method was applied to each drive analysis, correcting α to 0.05/4 = 0.0125.