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ORIGINAL ARTICLE

Effect of transcutaneous vagus nerve stimulation on stress-reactive neuroendocrine measures in a sample of persons with temporal lobe epilepsy

Johanna M. Doerr[1] | **Martin Juenemann**[1] | **Lukas Hakel**[2] | **Laura Schmidt**[2] | **Katja Menzler**[2] | **Kristina Krause**[2] | **Louise Linka**[2] | **Nadine Skoluda**[3] | **Urs M. Nater**[3] | **Susanne Knake**[2,4]

[1]Department of Neurology, University Hospital Gießen and Marburg, Gießen, Germany

[2]Epilepsy Center Hessen, Department of Neurology, Philipps-University Marburg, Marburg, Germany

[3]Department of Clinical and Health Psychology, Faculty of Psychology, University of Vienna, Vienna, Austria

[4]Center for Mind, Brain and Behavior (CMBB), Philipps-University Marburg, Marburg, Germany

Correspondence
Susanne Knake, Philipps-University Marburg, Dept. of Neurology, Baldingerstr., 35043 Marburg, Germany.
Email: knake@med.uni-marburg.de

Abstract

Objective: Dysregulation of stress-reactive neuroendocrine measures, as well as subjective stress, have been found to worsen epilepsy. Transcutaneous vagus nerve stimulation (tVNS) is a relatively new treatment option for epilepsy. We were interested in its effect on the activity of the hypothalamic–pituitary–adrenal (HPA) axis and autonomic nervous system (ANS) as well as subjective stress and tiredness in patients with temporal lobe epilepsy (TLE).

Methods: Twenty patients (age 44 ± 11 years, 13 women) were enrolled in the study. They were free of seizures for more than 1 year. All took part in two sessions with 4 h of stimulation (tVNS vs. sham) in a randomized order. Saliva samples and subjective stress and tiredness levels were measured at five time points each session (before and after stimulation and three time points every hour in between). Data were analyzed using repeated measures analysis of variance as well as paired *t*-tests.

Results: There was a dampened salivary cortisol (sCort) decrease during tVNS (time × condition effect: $F_{[2.38,\ 38.15]} = 6.50$, $P = 0.002$, partial $\eta^2 = 0.29$). Furthermore, we detected a dampened increase in salivary flow rate during tVNS (time × condition effect: $F_{[3.28,\ 55.67]} = 2.82$, $P = 0.043$, partial $\eta^2 = 0.14$). There was neither a difference in overall sCort or salivary alpha-amylase (sAA) levels nor in subjective stress or tiredness levels between conditions. sAA levels at the last measurement point were slightly higher during tVNS ($t_{(19)} = 2.26$, $P = 0.035$, $d = 0.51$), but this effect failed to reach significance when controlled for multiple comparisons.

Significance: Our results partially support that tVNS influences the regulation of stress-reactive neuroendocrine systems (namely the HPA axis and ANS) in epilepsy. More research with larger samples is needed on the difference between short-term and repeated long-term stimulation.

KEYWORDS

autonomic nervous system, cortisol, epilepsy, HPA axis, stress, transcutaneous vagus nerve stimulation, VNS

1 | INTRODUCTION

Subjective stress has been reported to be the most prominent trigger of epileptic seizures reported by patients.[1–3] The influence of stress on negative health outcomes is hypothesized to be caused by dysregulation of the stress-reactive hypothalamic–pituitary–adrenal (HPA) axis or the autonomic nervous system (ANS).[4]

Patients with epilepsy display higher basal levels of serum and salivary cortisol (sCort), which is a measure of HPA axis activity.[5–7] Moreover, a flattened cortisol recovery after stress, which is also associated with seizure frequency,[5] has been found. It seems likely that the occurrence of seizures is at least partly mediated by HPA axis dysfunction (for an overview see Ref.[8]), which might be caused by structural changes in the temporal lobe (including the limbic system) and prefrontal structures. Difficulties in psychobiological stress regulation facilitate the occurrence of seizures, which in turn, cause recurring psychobiological stress reactions as part of a vicious cycle.[9]

Next to HPA axis dysregulation, ANS dysregulation has been found in patients with epilepsy.[10] Studies show a higher interictal sympathetic tone and lower parasympathetic tone in cardiovascular measures as well as impaired vagal cardiovascular control.[11,12] However, studies on endocrine markers of ANS activity are rare. One study found a stronger increase of salivary alpha-amylase (sAA, a surrogate marker of sympathetic ANS activity[13]) due to blood sampling (venipuncture) in children with epilepsy compared with healthy children.[14]

Thus, there is a need for treatment options that influence subjective stress as well as HPA axis and ANS activity in patients with epilepsy. There is some research showing an influence of vagus nerve stimulation (VNS[15]) on measures in both the HPA axis and ANS. The influence of VNS on HPA axis functioning likely originates in afferent fibers that transfer signals to the brainstem. In the nucleus coeruleus of the brain stem, central norepinephrine is released, which, in turn, binds to α_2 receptors in the hypothalamus. Through these connections, VNS can activate a hypothalamic–pituitary–adrenal (HPA) axis as well as an autonomic nervous system (ANS) response via the hypothalamus.[16,17] O'Keane et al,[18] for instance, found normalization in the form of reductions in both adrenocorticotropic hormone (ACTH) and serum cortisol levels in response to corticotropin-releasing hormone (CRH) challenge after 3 months of VNS in chronic depression. Similarly, Majoie et al[7] found a normalization of formerly heightened cortisol levels in the peripheral blood of persons with epilepsy compared with a healthy control group after 27 weeks of VNS. We are aware of only one study that investigated the acute effect of VNS on sCort in a within-subject controlled design. This study found an attenuated sCort decline with tVNS (transcutaneous VNS, which activates afferent fibers from the ear to the brain stem[19]) compared with sham stimulation in a healthy sample.[20] tVNS might be preferable to VNS as a treatment because of its less invasive nature.

Concerning ANS measures, research suggests a decrease in sympathetic control and an increase in parasympathetic control, that is, an overall reduced activity, during tVNS, using cardiovascular measures.[21] There are, however, also two studies that found increased sAA levels due to tVNS stimulation using laboratory designs.[20,22]

Taken together, tVNS might be able to influence the HPA axis as well as ANS functioning in patients with epilepsy, which should reflect in changes in both salivary and subjective stress measures. However, this has not been shown in a randomized controlled design under laboratory conditions. We hypothesized that tVNS will regulate (i.e., decrease) subjective stress measures and the activities of both the HPA axis and ANS (reflected in reduced sCort and sAA levels) in patients with temporal lobe epilepsy (TLE).

Key Points

- Transcutaneous vagus nerve stimulation (tVNS) influences endocrine stress measures in persons with epilepsy in a design with several hours of intermitted tVNS.
- tVNS flattens the decrease in hypothalamic–pituitary–adrenal axis activity.
- tVNS augments autonomous activity (salivary flow rate and alpha-amylase activity).
- tVNS does not influence subjective stress measures in this design.

2 | METHODS

Participants were recruited via the Epilepsy Center Hessen, University Hospital Marburg, Germany. Inclusion criteria comprised a neurologist-confirmed diagnosis of TLE, free of seizures for at least 1 year, age ≥18 years, being cognitively and physically able to give written informed consent, being willing and physically able to receive tVNS. Exclusion criteria comprised neurological diseases other than epilepsy, any current psychiatric disorder, taking medication (other than antiseizure drugs) known to affect neuroendocrine measures, severe endocrinological diseases, cardiovascular diseases or abnormal ECG, pregnancy or breast-feeding, chronic alcohol or drug misuse, and enrollment in other studies. Criteria were assessed via interview and medical history data as well as an ECG recording. The study was approved by the local IRB (Medical Department of the University of Marburg). All participants gave written informed consent. The STROBE guidelines were followed to minimize methodical bias.

2.1 | Experimental design

Participants completed two (tVNS vs. sham stimulation) 4-h stimulation sessions (5-h laboratory visits) in a randomized condition order with at least 2 weeks (range 14-56 days, mean ± SD: 27 ± 15 days) between sessions. They did not eat or drink anything but water during the study visits. Several measures and paradigms were completed during the sessions, which are reported elsewhere.[23] For the current analyses, we report data on salivary measures and subjective stress and tiredness ratings taken at five time points each session (before and after stimulation and on three time points every hour in between). Participants were naïve to VNS. They were told that two different stimulation strategies of VNS were compared, and thus unaware of the study condition. Furthermore, an independent clinician attached the stimulation device and concealed it with a headband covering the entire ear, resulting in a double-blind design.

2.2 | tVNS

Patients were stimulated in intervals of 30 seconds (30 seconds off and 30 seconds on) via auricular tVNS (Cerbomed, Nemos, Cerbomed GmbH) for 4 hours (Figure 1). Stimulation amplitude was set in between the individual sensory and the individual pain threshold of each patient (mean ± SD stimulation amplitude: tVNS 1.20 ± 0.52 mV; sham: 1.09 ± 0.41 mV).[24] In the tVNS condition, the probe was applied to the left cymba conchae according to the guidelines of the manufacturer in order to stimulate the auricular branch of the vagus nerve.[25] In the sham condition, the probe was applied to the center of the left earlobe, an area known not to evoke potentials in the auricular branch of the vagus nerve[26] and not to elicit vagus sensory evoked potentials in the brain stem.[27]

2.3 | Salivary measures

Saliva samples were collected using the SaliCap® system (IBL-Tecan). Participants accumulated saliva in their mouths for 2 minutes and subsequently salivated into a prelabeled polypropylene tube via a straw. Samples were kept frozen at −30°C, and with an uninterrupted cooling chain, shipped frozen (dry ice) to the Biochemical Laboratory of the Department of Clinical and Health Psychology at the University of Vienna, Austria, for biochemical analysis. sCort levels were measured using a commercially available enzyme-linked immunoassay (IBL-Tecan). Cortisol is seen as the gold standard marker of HPA axis activity, with salivary cortisol being of special relevance in psychoneuroendocrine research due to its ability to capture shorter-term dynamics of HPA axis activity.[28] As a measure of ANS activity, sAA

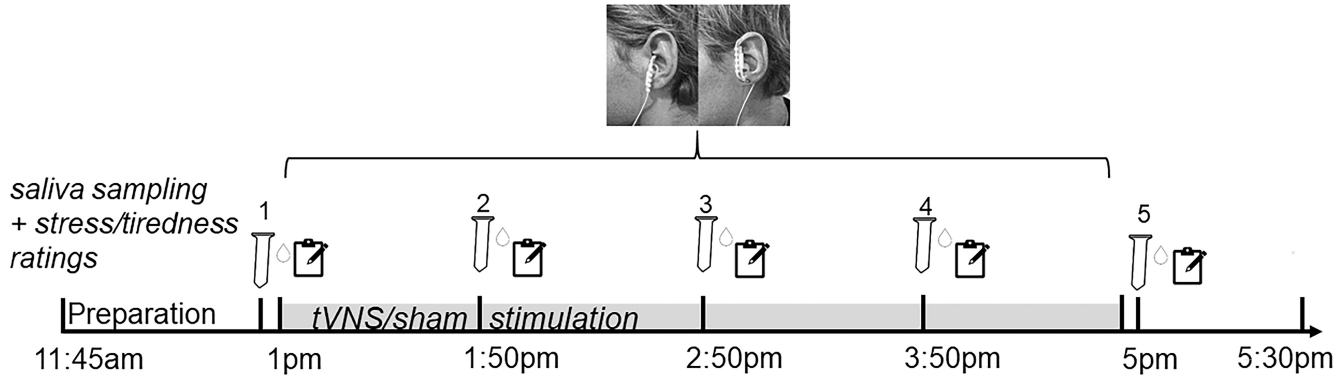

FIGURE 1 Laboratory protocol of relevant measurements.

was extracted from saliva samples using a kinetic colorimetric test and reagents from DiaSys (DiaSys Diagnostic Systems). SAA reflects ANS activity because of noradrenergic binding to the adrenergic receptors on the acinar cells of the salivary glands, which stimulates the production and release of sAA into the oral cavity. It has become a well-established marker used in behavioral medical research during the past two decades.[29] The inter- and intra-assay variance for both assays was below 10%. Additionally, salivary flow rate (difference of pre- to post-weight of saliva samples in mg/2 minutes of saliva accumulation) was assessed as a measure of parasympathetic ANS activity.[30]

2.4 | Subjective stress measures

Momentary stress levels were measured using the item "At the moment, I feel stressed out" from 0 (not at all) to 100 (very) on a visual analog scale, an item that has successfully been used in previous studies.[31] There is also evidence that VNS improves daytime sleepiness and arousal in persons with epilepsy after several weeks or months of stimulation.[32,33] As feelings of tiredness are closely related to feelings of stress,[34] this might be another important subjective stress outcome in the tVNS research. To assess changes in feelings of tiredness, participants therefore equivalently rated their tiredness on a scale from 0 (not at all) to 100 (very much) on a visual analog scale.[35]

2.5 | Statistical analysis

Statistical analyses were performed using SPSS version 22.0 (IBM). We used repeated measures analyses of variances (ANOVAs) for the assessment of differences in the levels of sCort and sAA, salivary flow rate, and subjective stress and tiredness ratings between conditions (tVNS vs. sham). For the analyses of differences in the slopes of sCort and sAA activities and between single time points, paired *t*-tests and univariate ANOVAs were calculated. Analyses were controlled for age (in years) and gender (1 = men, 2 = women). We report the results after Greenhouse-Geiser correction and used partial eta square as a measure of effect size in statistically significant results. For *t*-tests, we report Cohen's *d* as an effect size measure.

3 | RESULTS

Twenty persons with TLE (mean ± SD age: 44 ± 11 years, 13 women, and 12 with left TLE) were enrolled in the study. Five measurement time points in two conditions in 20 persons led to a possible maximum of 200 measurements. There were no missing values with regard to both sCort and sAA levels. One cortisol value in one person was, however, deleted post hoc because of an intra-individually implausibly high value (28.5 nmol/L compared with the mean of 1.71 nmol/L for the remaining time points). Furthermore, one subjective stress and tiredness rating was missing. This led to 0.05% missing values in the sCort and subjective ratings. For descriptive values of the outcome variables, see Table 1.

3.1 | Differences between tVNS and sham stimulation

Cortisol levels did not differ between conditions ($F_{[1.00, 16.00]} = 1.79$, $P = 0.46$). There was, however, a significant time × condition effect on cortisol ($F_{[2.38, 38.15]} = 6.50$, $P = 0.002$, partial $\eta^2 = 0.29$ [large effect]), suggesting a difference in the trajectory of cortisol output throughout the two sessions. This effect was based on a dampening of the decrease from time point 3 to 5 in the tVNS condition, whereas sCort steadily decreased during sham stimulation (Figure 2). However, the overall decline (i.e., slope) in sCort throughout the sessions did not differ between conditions ($F_{(1, 36)} = 0.07$, $P = 0.79$).

There was no difference in overall sAA activity between tVNS and the sham condition ($F_{[1.00, 17.00]} = 0.04$, $P = 0.84$) and no time × condition interaction ($F_{[1.00, 47.73]} = 0.09$, $P = 0.96$). When each measurement point was compared directly, values at the last measurement point (after stimulation) differed between conditions with lower values in the tVNS condition, with mean (SD) sAA activity

TABLE 1 Descriptive values of salivary and subjective measures.

	tVNS	Sham
Condition	**M (SD)**	**M (SD)**
sCort in nmol/L	2.48 (1.03)	2.32 (1.09)
sCort slope	−0.005 (0.01)	−0.004 (0.01)
sAA activity in U/mL	129.91 (120.80)	145.58 (129.37)
sAA activity slope	0.05 (0.25)	0.15 (0.36)
Salivary flow rate in mg/min	0.88 (0.43)	0.91 (0.42)
Salivary flow rate slope	−0.0004 (0.001)	0.0004 (0.002)
Subjective stress	21.49 (20.26)	22.15 (21.56)
Subjective tiredness	49.85 (25.14)	50.34 (25.43)

Note: Stress and tiredness were measured on a scale from 0 to 100; M (SD) = mean and standard deviation across all time points.

Abbreviations: sAA, salivary alpha-amylase; sCort, salivary cortisol; tVNS, transcutaneous vagus nerve stimulation.

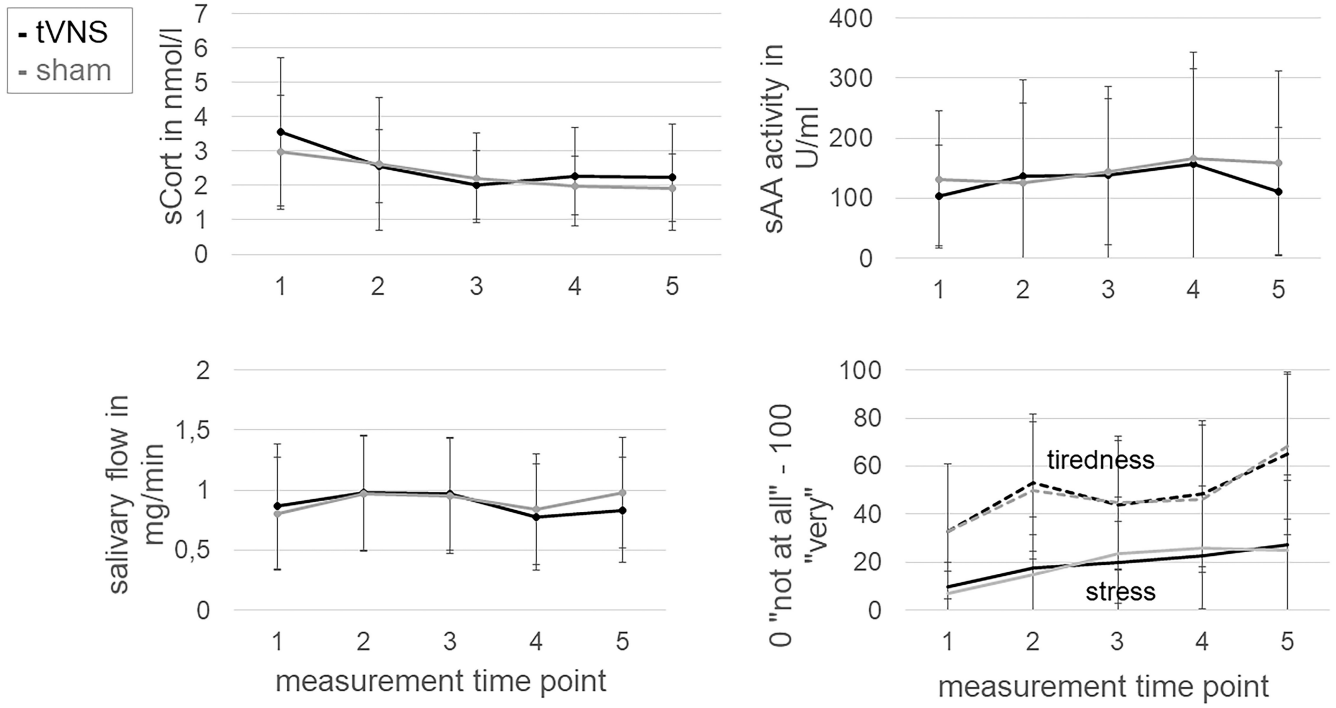

FIGURE 2 Values (M ± SD) of the outcomes by time point during transcutaneous vagus nerve stimulation (black) and sham (gray) conditions.

at the last measurement point: tVNS 110.84 (106.72) U/mL, sham 158.80 (152.52) U/mL and $t_{(19)} = 2.26$, $P = 0.035$, (95% CI [3.62; 92.29]), $d = 0.51$ (medium effect), and paired *t*-test (Figure 2). This difference, however, failed to reach statistical significance when controlled for age and gender ($F_{[1.00,\ 36.00]} = 1.46$, $P = 0.24$), as well as when corrected for multiple comparisons (then $P = 0.175$). Again, the sAA activity slope did not differ between conditions ($F_{[1.00,\ 36.00]} = 1.21$, $P = 0.28$).

There was also no difference in the overall salivary flow rate ($F_{[1.00,\ 17.00]} = 0.06$, $P = 0.81$). Results did, however, show a significant time × condition (tVNS vs. sham) effect ($F_{[3.28,\ 55.67]} = 2.82$, $P = 0.04$, partial $\eta^2 = 0.14$ [large effect]) on the salivary flow rate. This effect is based on a steeper increase in salivary flow rate from time point 4 to time point 5 during the sham condition compared with that during the tVNS condition (Figure 2). The slope of the salivary flow rate did not differ significantly between conditions, but analyses showed a trend toward significance ($F_{[1.00,\ 36.00]} = 3.39$, $P = 0.07$).

Controlling sAA activity for the salivary flow rate, as has been suggested in previous research,[36,37] did not change the results (i.e., there was no main effect of condition, but there was a significant difference in sAA [U/min] between conditions at the last measurement time point, which failed to reach statistical significance when controlled for age and gender as well as for multiple comparisons [data not shown]).

There was no difference in subjective stress ratings between tVNS and the sham condition ($F_{[1.00,\ 16.00]} = 0.20$, $P = 0.66$) and no time × condition interaction ($F_{[1.00,\ 41.63]} = 0.20$, $P = 0.85$) on subjective stress ratings (Figure 2). Furthermore, there was no difference in subjective tiredness ratings between tVNS and the sham condition ($F_{[1.00,\ 16.00]} = 0.47$, $P = 0.50$) and no time × condition interaction ($F_{[2.70,\ 43.13]} = 1.44$, $P = 0.23$).

4 | DISCUSSION

As data acquisition took place in the afternoon, a decrease in sCort (as seen during the sham condition) reflects the regular diurnal rhythm of HPA axis activity.[38,39] The dampening of the sCort decrease during the tVNS condition is in line with results reported by Warren et al,[20] using a similar design in healthy participants. Therefore, this effect does not seem to differ between patients with TLE and healthy participants. On the other hand, studies using a design of long-term (several months) VNS report a reduction, not an increase, in cortisol levels in epilepsy and chronic depression.[7,18] This possibly reflects a difference between the acute effects and long-term effects of VNS and as such an adaptive response of the HPA axis to VNS. However, differences might also be due to different study designs and measurement methods (repeated saliva sampling vs. pre−/postblood sampling, laboratory

vs. ambulatory, and tVNS vs. VNS). As studies generally suggest that the effect of VNS treatment becomes optimal after repeated stimulation for about 6 months (for review see Ref.[40]), studies are now needed that investigate acute and long-term changes in HPA axis activity due to tVNS. These studies should use a long-term design and ideally multiple time points per day with several measurement periods spread across several months.

Similar to sCort, the increases in sAA activity and in salivary flow rate during the sham condition reflect the regular diurnal rhythm of ANS activity.[41,42] The finding that tVNS did not influence sAA activity (and, by trend, reduced sAA activity at the last measurement time point) contradicts studies that showed an increase in sAA activity during tVNS in similar designs.[20,22] As these studies included healthy participants, the different results might be due to the fact that sympathetic control is reduced in persons suffering from epilepsy. This has been found concerning cardiovascular control,[43] but it might also hold true for sympathetic control of the salivary glands. On the other hand, a dampened increase in the salivary flow rate, which suggests dampened parasympathetic activity, can be seen in our results. This is in contradiction to studies showing higher parasympathetic activity due to VNS, which is mainly based on research using heart rate variability (HRV) measures.[44] Results on this matter are, however, mixed.[17] We are not aware of any study investigating the influence of tVNS on the salivary flow rate. Clearly, more research is needed that investigates if sAA activity and salivary flow rate parallel changes in cardiovascular ANS measures during tVNS.

Our finding that tVNS did not influence stress and tiredness is in line with the results reported by Tona et al.[45] It is, however, contradictory to other studies showing positive effects on mood (including arousal levels) in short-term[46] and longer-term[33,47] designs. Again, the difference between our results and those of the longer-term studies might stem from differences in the duration and setting. As mentioned before, tVNS likely exerts its influence on the HPA axis as well as autonomous functioning via the brain stem (more specifically: via the release of central norepinephrine from the locus coeruleus and noradrenergic influence on the HPA axis and ANS).[16,17] Therefore, it is perceivable that biological alterations can be seen before they influence mood. Additionally, in comparison to Kraus et al,[46] where participants lay still during stimulation, they participated in several psychophysiological tasks in our study and in the study by Tona et al.[45] Therefore, we might suspect that the ability of tVNS to improve mood is not very strong while performing distracting tasks, and increases with days of stimulation.

This study is the first to investigate the short-term influence of tVNS on psychoneuroendocrinological stress measures in patients with epilepsy using a randomized within-subject study design. Further strengths are the use of sham stimulation (instead of no stimulation) and having the participant and the examiner blind to the condition (double-blind design). Therefore, we are confident that differences between conditions are attributable to tVNS.

The first limitation of our study is the small sample size of 20 participants. We did, however, find large effect sizes in our repeated measures analyses and, therefore, achieved statistical power between 70% and 99%,[48] which is satisfactory. Still, smaller effects might have stayed undetected, which warrants a repetition of the study using a larger sample size. Furthermore, we exclusively included persons with TLE that were free of seizures for at least 1 year to minimize the effect of seizures. Therefore, results are not necessarily applicable to patients with other forms of epilepsy and those with higher seizure frequency. Furthermore, the missing healthy control group does impede direct conclusions on differences between persons with epilepsy and those without. We tried to minimize the influence of the different tasks performed by using the same tasks in both conditions. Furthermore, each parameter (including stress[34] as well as tiredness[49,50]) showed a regular diurnal pattern during the sham condition. Therefore, we assumed that the tasks did not influence our results. It would, however, be of great interest to investigate how the measures used in the present study are influenced by a laboratory stress task while tVNS (not only to investigate the activities of stress systems but also to investigate their reactivities). This kind of design should be considered in future research.

In summary, we found some indication that a 4-h-long tVNS dampens the decrease in HPA axis activity and reduces ANS activity in patients with TLE. As changes in these parameters were not in the expected direction, there is now need for microlongitudinal studies to further investigate adaptive processes to prolonged tVNS and, as such, its potential to reverse adverse effects of the dysregulation of these systems. However, tVNS did not influence subjective stress or tiredness levels. Results differ in their direction from those of studies using repeated longer-term (daily stimulation for weeks to months) tVNS as discussed above. Therefore, the influence of tVNS on the activities of both the HPA axis and ANS might change over time, with beneficial health effects (including influences on subjective stress and tiredness) being achieved across longer periods of stimulation. To further investigate the mechanisms behind the positive effect of tVNS in epilepsy patients, studies are now needed to track the dynamic of these associations across time. Furthermore, the influence of tVNS on the stress-reactive capacity of both the HPA axis and ANS in persons with epilepsy should be

investigated using standardized stress-inducing laboratory protocols.

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CONFLICT OF INTEREST STATEMENT

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

AUTHOR CONTRIBUTORS

SK and KK conceptualized the study design. JMD analyzed and interpreted the data and drafted the manuscript. MJ made substantial contributions to data analysis and interpretation. LH and LS led the data acquisition. LB and KM made substantial contributions to data acquisition. NS and UMN made substantial contributions to study design and supervised biochemical analyses. All authors provided critical feedback on the manuscript and approved the final version of the manuscript.

ORCID

Johanna M. Doerr https://orcid.org/0000-0002-8170-1655
Louise Linka https://orcid.org/0000-0003-1711-7072
Susanne Knake https://orcid.org/0000-0002-6298-0513

REFERENCES

1. Nakken KO, Solaas MH, Kjeldsen MJ, Friis ML, Pellock JM, Corey LA. Which seizure-precipitating factors do patients with epilepsy most frequently report? Epilepsy Behav. 2005;6(1):85–9.
2. Haut SR, Hall CB, Masur J, Lipton RB. Seizure occurrence: precipitants and prediction. Neurology. 2007;69(20):1905–10.
3. Thapar A, Kerr M, Harold G. Stress, anxiety, depression, and epilepsy: investigating the relationship between psychological factors and seizures. Epilepsy Behav. 2009;14(1):134–40.
4. McEwen BS. Stress, adaptation, and disease: allostasis and allostatic load. Ann NY Acad Sci. 1998;840:33–44.
5. Allendorfer JB, Heyse H, Mendoza L, Nelson EB, Eliassen JC, Storrs JM, et al. Physiologic and cortical response to acute psychosocial stress in left temporal lobe epilepsy—a pilot cross-sectional fMRI study. Epilepsy Behav. 2014;36:115–23.
6. Afifi S, Fadel W, Morad H, Eldod A, Gad E, Arfken CL, et al. Neuroendocrinal study of depression in male epileptic patients. J Neuropsychiatry Clin Neurosci. 2011;23(2):163–7.
7. Majoie HJ, Rijkers K, Berfelo MW, Hulsman JA, Myint A, Schwarz M, et al. Vagus nerve stimulation in refractory epilepsy: effects on pro- and anti-inflammatory cytokines in peripheral blood. Neuroimmunomodulation. 2011;18(1):52–6.
8. Cano-López I, González-Bono E. Cortisol levels and seizures in adults with epilepsy: a systematic review. Neurosci Biobehav Rev. 2019;103:216–29.
9. Maguire J, Salpekar JA. Stress, seizures, and hypothalamic-pituitary-adrenal axis targets for the treatment of epilepsy. Epilepsy Behav. 2013;26(3):352–62.
10. Surges R, Sander JW. Sudden unexpected death in epilepsy: mechanisms, prevalence, and prevention. Curr Opin Neurol. 2012;25(2):201–7.
11. Mukherjee S, Tripathi M, Chandra PS, Yadav R, Choudhary N, Sagar R, et al. Cardiovascular autonomic functions in well-controlled and intractable partial epilepsies. Epilepsy Res. 2009;85(2–3):261–9.
12. Ansakorpi H, Korpelainen JT, Huikuri HV, Tolonen U, Myllylä VV, Isojärvi JI. Heart rate dynamics in refractory and well controlled temporal lobe epilepsy. J Neurol Neurosurg Psychiatry. 2002;72(1):26–30.
13. Ali N, Nater UM. Salivary alpha-amylase as a biomarker of stress in behavioral medicine. Int J Behav Med. 2020;27(3):337–42.
14. Ferrara P, Bottaro G, Angeletti S, Gatto A, Vitelli O, Battaglia D, et al. Salivary alpha-amylase: a new non-invasive biomarker for assessment of pain perception in epileptic children. Acta Neurol Belg. 2013;113(3):279–83.
15. Beekwilder JP, Beems T. Overview of the clinical applications of vagus nerve stimulation. J Clin Neurophysiol. 2010;27(2):130–8.
16. Bonaz B, Sinniger V, Pellissier S. Anti-inflammatory properties of the vagus nerve: potential therapeutic implications of vagus nerve stimulation. J Physiol. 2016;594(20):5781–90.
17. Burger AM, D'Agostini M, Verkuil B, Van Diest I. Moving beyond belief: a narrative review of potential biomarkers for transcutaneous vagus nerve stimulation. Psychophysiology. 2020;57(6):e13571.
18. O'Keane V, Dinan TG, Scott L, Corcoran C. Changes in hypothalamic-pituitary-adrenal axis measures after vagus nerve stimulation therapy in chronic depression. Biol Psychiatry. 2005;58(12):963–8.
19. Ellrich J. Transcutaneous vagus nerve stimulation. Eur Neurol Rev. 2011;6(4):254–6.
20. Warren CM, Tona KD, Ouwerkerk L, van Paridon J, Poletiek F, van Steenbergen H, et al. The neuromodulatory and hormonal effects of transcutaneous vagus nerve stimulation as evidenced by salivary alpha amylase, salivary cortisol, pupil diameter, and the P3 event-related potential. Brain Stimul. 2019;12(3):635–42.
21. Zamotrinsky AV, Kondratiev B, de Jong JW. Vagal neurostimulation in patients with coronary artery disease. Auton Neurosci. 2001;88(1–2):109–16.
22. Ventura-Bort C, Wirkner J, Genheimer H, Wendt J, Hamm AO, Weymar M. Effects of transcutaneous vagus nerve stimulation (tVNS) on the P300 and alpha-amylase level: a pilot study. Front Hum Neurosci. 2018;12:202.
23. Weber I, Niehaus H, Krause K, Molitor L, Peper M, Schmidt L, et al. Trust your gut: vagal nerve stimulation in humans improves reinforcement learning. Brain Commun. 2021;3(2):fcab039.
24. Treutwein B. Adaptive psychophysical procedures. Vision Res. 1995;35(17):2503–22.
25. Jongkees BJ, Immink MA, Finisguerra A, Colzato LS. Transcutaneous vagus nerve stimulation (tVNS) enhances response selection during sequential action. Front Psychol. 2018;9:1159.

26. Burger AM, Van der Does W, Brosschot JF, Verkuil B. From ear to eye? No effect of transcutaneous vagus nerve stimulation on human pupil dilation: a report of three studies. Biol Psychol. 2020;152:107863.
27. Polak T, Markulin F, Ehlis AC, Langer JB, Ringel TM, Fallgatter AJ. Far field potentials from brain stem after transcutaneous vagus nerve stimulation: optimization of stimulation and recording parameters. J Neural Transm (Vienna). 2009;116(10):1237–42.
28. Kirschbaum C, Hellhammer DH. Salivary cortisol in psychoneuroendocrine research: recent developments and applications. Psychoneuroendocrinology. 1994;19(4):313–33.
29. Nater UM, Rohleder N. Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: current state of research. Psychoneuroendocrinology. 2009;34(4):486–96.
30. Proctor GB, Carpenter GH. Regulation of salivary gland function by autonomic nerves. Auton Neurosci. 2007;133(1):3–18.
31. Doerr JM, Nater UM, Feneberg AC, Mewes R. Differential associations between fatigue and psychobiological stress measures in women with depression and women with somatic symptom disorder. Psychoneuroendocrinology. 2021;132:105343.
32. Malow BA, Edwards J, Marzec M, Sagher O, Ross D, Fromes G. Vagus nerve stimulation reduces daytime sleepiness in epilepsy patients. Neurology. 2001;57(5):879–84.
33. Klinkenberg S, Majoie HJ, van der Heijden MM, Rijkers K, Leenen L, Aldenkamp AP. Vagus nerve stimulation has a positive effect on mood in patients with refractory epilepsy. Clin Neurol Neurosurg. 2012;114(4):336–40.
34. Doerr JM, Ditzen B, Strahler J, Linnemann A, Ziemek J, Skoluda N, et al. Reciprocal relationship between acute stress and acute fatigue in everyday life in a sample of university students. Biol Psychol. 2015;110:42–9.
35. Stone AA, Broderick JE, Porter LS, Kaell AT. The experience of rheumatoid arthritis pain and fatigue: examining momentary reports and correlates over one week. Arthritis Care Res. 1997;10(3):185–93.
36. Bosch JA, Veerman EC, de Geus EJ, Proctor GB. α-Amylase as a reliable and convenient measure of sympathetic activity: don't start salivating just yet! Psychoneuroendocrinology. 2011;36(4):449–53.
37. Beltzer EK, Fortunato CK, Guaderrama MM, Peckins MK, Garramone BM, Granger DA. Salivary flow and alpha-amylase: collection technique, duration, and oral fluid type. Physiol Behav. 2010;101(2):289–96.
38. Adam EK, Quinn ME, Tavernier R, McQuillan MT, Dahlke KA, Gilbert KE. Diurnal cortisol slopes and mental and physical health outcomes: a systematic review and meta-analysis. Psychoneuroendocrinology. 2017;83:25–41.
39. Pruessner JC, Wolf OT, Hellhammer DH, Buske-Kirschbaum A, von Auer K, Jobst S, et al. Free cortisol levels after awakening: a reliable biological marker for the assessment of adrenocortical activity. Life Sci. 1997;61(26):2539–49.
40. Toffa DH, Touma L, El Meskine T, Bouthillier A, Nguyen DK. Learnings from 30 years of reported efficacy and safety of vagus nerve stimulation (VNS) for epilepsy treatment: a critical review. Seizure. 2020;83:104–23.
41. Nater UM, Rohleder N, Schlotz W, Ehlert U, Kirschbaum C. Determinants of the diurnal course of salivary alpha-amylase. Psychoneuroendocrinology. 2007;32(4):392–401.
42. Dawes C. Rhythms in salivary flow rate and composition. Int J Chronobiol. 1974;2(3):253–79.
43. Hilz MJ, Devinsky O, Doyle W, Mauerer A, Dütsch M. Decrease of sympathetic cardiovascular modulation after temporal lobe epilepsy surgery. Brain. 2002;125(Pt 5):985–95.
44. De Couck M, Cserjesi R, Caers R, Zijlstra WP, Widjaja D, Wolf N, et al. Effects of short and prolonged transcutaneous vagus nerve stimulation on heart rate variability in healthy subjects. Auton Neurosci. 2017;203:88–96.
45. Tona KD, Revers H, Verkuil B, Nieuwenhuis S. Noradrenergic regulation of cognitive flexibility: no effects of stress, transcutaneous vagus nerve stimulation, and atomoxetine on task-switching in humans. J Cogn Neurosci. 2020;32(10):1881–95.
46. Kraus T, Hösl K, Kiess O, Schanze A, Kornhuber J, Forster C. BOLD fMRI deactivation of limbic and temporal brain structures and mood enhancing effect by transcutaneous vagus nerve stimulation. J Neural Transm (Vienna). 2007;114(11):1485–93.
47. Bretherton B, Atkinson L, Murray A, Clancy J, Deuchars S, Deuchars J. Effects of transcutaneous vagus nerve stimulation in individuals aged 55 years or above: potential benefits of daily stimulation. Aging (Albany NY). 2019;11(14):4836–57.
48. Faul F, Erdfelder E, Lang A-G, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods. 2007;39(2):175–91.
49. Dahlgren A, Kecklund G, Akerstedt T. Different levels of work-related stress and the effects on sleep, fatigue and cortisol. Scand J Work Environ Health. 2005;31(4):277–85.
50. Buysse DJ, Thompson W, Scott J, Franzen PL, Germain A, Hall M, et al. Daytime symptoms in primary insomnia: a prospective analysis using ecological momentary assessment. Sleep Med. 2007;8(3):198–208.

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