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Klinik und Poliklinik für Epileptologie
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Über die Beeinflussung dynamischer epileptischer Hirnnetzwerke durch die transkutane aurikuläre Vagusnervstimulation

Habilitationsschrift
zur Erlangung der venia legendi
der Hohen Medizinischen Fakultät
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„Neurologie“

Vorgelegt von
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Für Philip, Gero und York.

Die folgenden aufgelisteten vier Originalarbeiten liegen der kumulativen Habilitationsschrift zu Grunde, welche die wesentlichen Ergebnisse der Publikationen zusammenfasst und diskutiert.

von Wrede R, Rings T, Schach S, Helmstaedter C, and Lehnertz K. (2021). Transcutaneous auricular vagus nerve stimulation induces large scale alterations in epileptic brain networks: towards understanding the effect of taVNS in subjects with epilepsy. *Sci Rep.* 2021 Apr 12;11(1):7906. doi: 10.1038/s41598-021-87032-1. PMID: 33846432; PMCID: PMC8042037.

Rings T*, **von Wrede R*,** Bröhl T*, Helmstaedter C, and Lehnertz K. (2021). Impact of transcutaneous auricular vagus nerve stimulation on large-scale functional brain networks: from local to global. *Front Physiol.* 2021 Aug 20;12:700261. doi: 10.3389/fphys.2021.700261. PMID: 34489724; PMCID: PMC8417898. *geteilte Erstautorenschaft

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von Wrede R, Rings T, Bröhl T, Pukropski J, Schach S, Helmstaedter C, and Lehnertz K. (2022). Transcutaneous auricular vagus nerve stimulation differently modifies functional brain networks of subjects with different epilepsy types. *Front Human Neurosc. 16, 867563.* DOI: 10.3389/fnhum.2022.867563

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1. Inhaltsverzeichnis

2. Einleitung

Epilepsien und ihre Behandlung

Mit einer Prävalenz von 0,5 bis 1 % sind Epilepsien eine der häufigsten neurologischen Erkrankungen mit über 50 Millionen Patient*innen weltweit (GBD 2016 Neurology Collaborators., 2019; World Health Organization, 2019). Nach dem jüngsten Vorschlag der Internationalen Liga gegen Epilepsie liegt eine Epilepsie vor, wenn mindestens zwei unprovozierte Anfälle oder ein unprovozierter Anfall und eine Wahrscheinlichkeit von mindestens 60 % für das Auftreten weiterer Anfälle innerhalb der nächsten 10 Jahre vorliegt oder die Diagnose eines Epilepsiesyndroms gestellt werden kann (Fisher et al., 2014). Ein epileptischer Anfall selbst ist definiert als ein vorübergehendes Auftreten von Symptomen, die auf eine abnorme übermäßige oder synchrone neuronale Aktivität im Gehirn zurückzuführen sind (Fisher et al., 2005).

Zur Behandlung erhalten Patient*innen mit Epilepsie in der Regel anfallssupprimierende Medikamente (antiseizure medication = ASM), die das Risiko weiterer Anfälle reduzieren. Während zwei Drittel der Betroffenen mit dem ersten oder zweiten ASM Anfallsfreiheit erreichen, benötigt das andere Drittel umfangreiche Therapieversuche, um Anfallsfreiheit oder zumindest eine akzeptable Anfallskontrolle zu erlangen (Kwan and Brodie, 2000). Gelingt es nicht, mit zwei verträglichen und angemessen ausgewählten ASM (ob als Monotherapie oder in Kombination) eine dauerhafte Anfallsfreiheit zu erreichen, so besteht nach der aktuellen Definition eine pharmakotherapieresistente Epilepsie (Kwan et al., 2010). Die Entwicklung neuer ASM hat nicht zu einer signifikanten Verbesserung der Anfallskontrolle bei Patient*innen mit Epilepsie geführt, wenngleich die Verträglichkeit und das Interaktionsprofil vorteilhafter geworden sind (Chen et al., 2018). Zusätzliche nicht-medikamentöse Therapieverfahren sind somit notwendig.

Für einige Patient*innen birgt die resektive Epilepsiechirurgie eine Hoffnung auf Anfallsfreiheit oder zumindest Anfallsreduktion (Baud et al., 2018), allerdings müssen Anästhesie- und Operationsrisiken sowie potentielle postoperative Defizite berücksichtigt werden. Für diejenigen, die für einen resektiven chirurgischen Eingriff nicht geeignet sind oder einen solchen nicht wünschen, oder Patient*innen, bei denen ein chirurgischer Eingriff

fehlgeschlagen ist, sind alternative Behandlungsmöglichkeiten, wie zum Beispiel neuromodulierende Verfahren, zu erwägen. Es stehen verschiedene Methoden der Neurostimulation zur Anfallskontrolle zur Verfügung. Invasive Methoden wie die tiefe Hirnstimulation des vorderen Thalamus (DBS) (Fisher et al., 2010), die responsive (closed loop) Neurostimulation (RNS) (Nair et al., 2020) und die invasive (klassische) Vagusnervstimulation (iVNS) wurden in mehreren Studien untersucht und hinsichtlich Nutzen und Risiken bewertet (Übersicht in (Boon et al., 2018)). Aufgrund ihres invasiven Charakters sind diese Behandlungsmöglichkeiten jedoch auch mit einem Anästhesie- und Operationsrisiko verbunden. Darüber hinaus ist bei einem Misserfolg eine Re-Operation, im Sinne einer Explantation des Gerätes, erforderlich, bei der jedoch z. T. Anteile der Implantate in situ verbleiben.

Vagusnervstimulation als Therapieoption

Die Vagusnervstimulation (VNS) ist eine etablierte Methode der Hirnstimulation bei verschiedenen Erkrankungen, darunter auch bei Epilepsie (Adair et al., 2020). Die invasive Vagusnervstimulation (iVNS) wurde bereits in den 1990er Jahren zugelassen und bis heute wurde bei mehr als 100000 Patient*innen mit einer Epilepsie ein Vagusnervstimulator implantiert (Fisher et al., 2020); die Sicherheit des iVNS wurde in über 20 Studien nachgewiesen, so dass eine umfangreiche experimentelle und klinische Erfahrung vorliegt. Die Wirksamkeit der invasiven Vagusnervstimulation, bestimmt durch die sogenannte Responder-Rate, also durch die Anzahl der Patient*innen, bei denen die Anfallshäufigkeit um mehr als 50 % reduziert werden konnte, beträgt bis zu 60 %. Anfallsfreiheit konnte bei bis zu 8 % der implantierten Patient*innen mit einer Epilepsie erreicht werden (Elliott et al., 2011; Morris et al., 2013; Englot et al., 2016). Die Gesamtkomplikationsrate liegt zwischen 2,5 und 12 %; diese kann technischer (wie Kabelbruch) oder chirurgischer Natur sein (z. B. Infektion, Hämatom, Stimmbandlähmung); die chirurgische Komplikationsrate beträgt bis zu 8,6 % (Révész et al., 2016), erfreulicherweise erholen sich die meisten Patient*innen von den Komplikationen gut. Neben dem Operationsrisiko und der Komplikationsrate bedingt die iVNS Einschränkungen bei der Magnetresonanztomografie (MRT)-Tauglichkeit. Zudem erfordert diese Behandlung regelmäßige Vor-Ort-Termine zur Therapie- und Gerätekontrolle. Angesichts dieser Einschränkungen und Risiken ist die transkutane Vagusnervstimulation (tVNS), die neuartige nicht-invasive externe Stimulationsmethode, eine interessante Alternative.

Transkutane Vagusnervstimulation

Für die externe Vagusnervstimulation gibt es verschiedene Methoden: die transkutane zervikale VNS (tcVNS), perkutane aurikuläre VNS (paVNS) und transkutane aurikuläre VNS (taVNS). Alle bislang bei Epilepsie durchgeführten Studien wurden mit einem taVNS durchgeführt. Die transkutane aurikuläre Vagusnervstimulation ist die nicht-invasive externe Stimulation des aurikulären Zweigs des Nervus vagus. In Deutschland sind bei Epilepsien aktuell folgende Geräte (siehe Abb. 1) zur taVNS zugelassen, international werden auch anderweitige taVNS-Geräte eingesetzt.

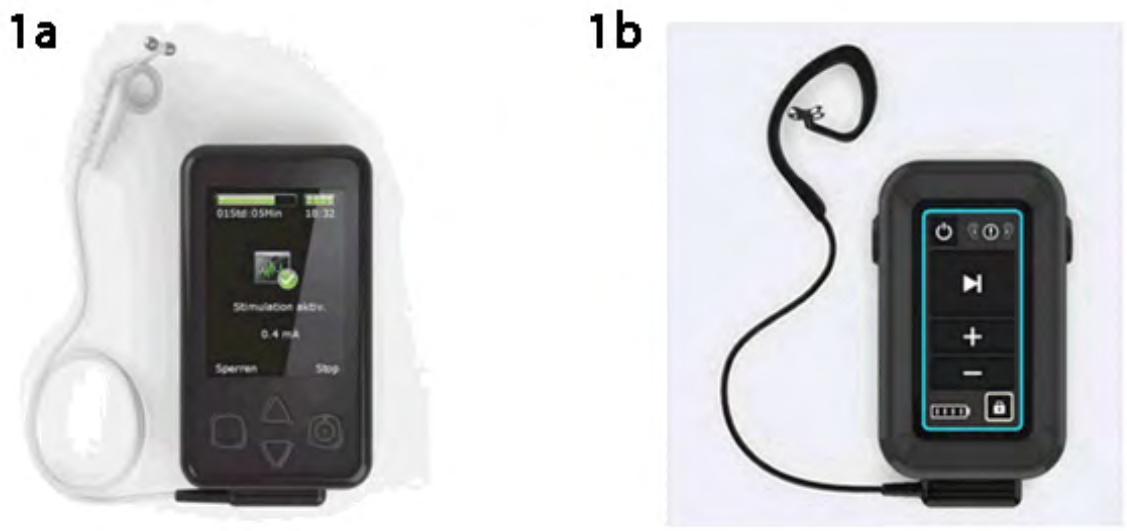

Abb. 1a: Transkutaner aurikulärer Vagusnervstimulator (tVNS-technology, 1. Generation Nemos®). Aktives Medizinprodukt der Klasse IIb, CE zertifiziert. **Abb. 1b:** Transkutaner aurikulärer Vagusnervstimulator (tVNS-technology, 2. Generation tVNS®L). Zulassung für **Epilepsie** und weitere Erkrankungen und Symptome (Migräne, M. Parkinson, Schlaganfall, Depression, Autismus, Angststörungen, kognitive Beeinträchtigung, Tinnitus, Prader-Willi-Syndrom, Schlafstörungen, Fibromyalgie, Asthma, Vorhofflimmern, Morbus Crohn, Entzündung). CE zertifiziert. Aktuell Kostenübernahme nur nach individuellem Einzelantrag. (Bilder mit freundlicher Überlassung von Prof. Dr. A. Bolz)

Die Stimulation erfolgt über zwei hemisphärische Titanelektroden, die in die linke Cymba conchae eingesetzt werden. Es werden folgende nicht verstellbare Stimulationsparameter verwendet: biphasische Signalform, Impulsdauer 20 Sekunden, Impulspause 30 Sekunden, Impulsfrequenz 25 Hz. Die Stärke der Stimulation wird individuell in mA eingestellt und

langsam erhöht, bis ein Kribbeln, jedoch kein Schmerz, im Bereich des Stimulationsortes wahrgenommen wird.

Für die Beurteilung der Wirksamkeit einer Behandlungsmethode muss berücksichtigt werden, dass die individuellen Anfallsfrequenzen meist unstet sind und großen Schwankungen unterliegen. Darüber hinaus sollte die Selbstdokumentation von Anfällen, welche die übliche Bestimmung der Anfallsfrequenz ist, hinsichtlich ihrer Aussagefähigkeit kritisch hinterfragt werden (Karoly et al., 2018). Anfälle können ggf. aus einer Erwartungshaltung heraus fehlerhaft dokumentiert werden bzw. in Abhängigkeit von der Art der Anfälle und dem Zeitpunkt des Auftretens in bis zu 86 % der Anfälle von den Patient*innen selbst nicht wahrgenommen oder erinnert werden (Hoppe et al., 2007). Weitere Untersuchungen belegen einen fehlenden Zusammenhang zwischen den selbstdokumentierten und den in der Elektroenzefalografie (EEG) aufgezeichneten Anfällen (Cook et al., 2013). Hieraus resultiert eine kritische Diskussion zu Anfallsfrequenzbewertung in Studien (Goldenholz et al., 2017; Karoly et al., 2019) sowie ein großer Bedarf an Methoden der Anfallsobjektivierung und -zählung für die individuelle Wirksamkeitsbeurteilung.

Für die Wirksamkeit der taVNS liegen sechs prospektive Studien an 155 Patient*innen und drei randomisiert-kontrollierte Studien an 280 Patient*innen mit pharmakotherapieresistenten Epilepsien vor. Die Heterogenität der Studienprotokolle hinsichtlich der Patient*innenmerkmale, der verwendeten Geräte, der Stimulationsparameter, der Studiendauer und der Beurteilungskriterien ist groß. Die Anfallsreduktion betrug bis zu 64 %, die Responderrate bis zu 65 %. Anfallsfreiheit wurde bei bis zu 31 % der Patient*innen erreicht. In vier Studien wurden Scores zur Anfallsschwere erhoben, wobei in zwei Studien eine signifikante Verbesserung der Anfallsschwere festgestellt wurde. Unerwünschte Nebenwirkungen waren vor allem Kopfschmerzen, Ohrenschmerzen und Hautveränderungen und wurden als leicht bis mittelschwer eingestuft. Ein Teil der Patient*innen berichtete über Müdigkeit, welche von der Stimulationsintensität abhängig zu sein schien ((Übersicht in (von Wrede and Surges, 2021); Sabers et al., 2021)).

Patient*innen mit einer Epilepsie sind nicht nur durch die epileptischen Anfälle, sondern auch durch Nebenwirkungen der ASM, weitere Komorbiditäten und sozialmedizinische Folgen der Erkrankungen belastet. Daher spiegelt das bloße Zählen von Anfällen nicht den Nutzen oder

das Risiko einer Intervention wider. Als Maß für die Lebensqualität können evaluierte Fragebögen, wie der QOLIE-89 und seine Kurzform QOLIE-31, eingesetzt werden. Diese erfassen sowohl emotionale, psychologische als auch medizinische und soziale Auswirkungen der Erkrankung (Cramer et al., 1998; Devinsky et al., 1995). Drei Studien zeigten eine signifikante Verbesserung der Lebensqualität im Rahmen der taVNS (Rong et al., 2014b; 2014a; Aihua et al., 2014), in zwei weiteren Studien wurde ein Trend zu einer verbesserten Lebensqualität festgestellt (Bauer et al., 2016; Liu et al., 2018), während in zwei anderen Studien keine Veränderungen oder Trends in der Lebensqualität nach der taVNS-Behandlung im Vergleich zum Ausgangswert berichtet wurden (Barbella et al., 2018; Song et al., 2018). Zusammenfassend ist der Einfluss von taVNS auf die Lebensqualität bei Patient*innen mit einer Epilepsie noch nicht endgültig geklärt, vorläufige Ergebnisse deuten jedoch auf einen positiven Einfluss hin. Daher sollten in zukünftigen klinischen Studien zur Wirksamkeit und Verträglichkeit der taVNS bei Patient*innen mit einer Epilepsie auch die Beeinflussung der Lebensqualität miterfasst werden.

Die Adhärenz, d.h. das Ausmaß, in dem das Verhalten einer Person einer vereinbarten Empfehlung eines Gesundheitsdienstleisters entspricht (World Health Organizsation, 2003), ist unter anderen von der Praktikabilität der vereinbarten Gesundheitsmaßnahme abhängig. Dies gilt insbesondere für Maßnahmen, die eine aktive Mitarbeit erfordern. In einer prospektiven multizentrischen Beobachtungsstudie (Sabers et al., 2021), welche aufgrund von Rekrutierungsschwierigkeiten und hohen Abbruchraten vorzeitig beendet werden musste, wurden Handhabung und Tragekomfort des taVNS-Gerätes von der Mehrheit der Patient*innen als positiv eingeschätzt. Die Ohrelektroden wurden zum Teil kritisch bewertet und ein Großteil der Patient*innen schlug Verbesserungen für das Gerät vor (die Studie wurde mit Geräten der ersten Generation durchgeführt, Geräte der zweiten Generation zeigen insbesondere in Bezug auf die Elektrodenanlage deutliche Besserungen). Die Mehrheit der Patient*innen war zufrieden mit der täglichen Stimulationsdauer, wenngleich die Zufriedenheit über die Zeit abnahm. Die optimale Stimulationsdauer pro Einsatz und pro Tag sowie der optimale Tageszeitpunkt für die Stimulation sind bislang nur unzureichend untersucht. Die tageszeitliche Schwankung der vagalen Aktivität ist ein bekanntes Phänomen (Huikuri et al., 1990). Eine Bindung an bestimmte Tageszeiten wurde für Erkrankungen, die mit dem Vagustonus oder der vagalen Aktivität in Verbindung gebracht werden, umfangreich

gezeigt (Borniger et al., 2018; Chow et al., 2014; van Dijk et al., 2007; Huikuri et al., 1990; Morris et al., 2012). Ein Konzept des Wirkungsmechanismus der transkutanen Vagusnervstimulation ist, dass die taVNS die beeinträchtigte oder fehlende vagale sensorische Rückkopplung zum Gehirn nachahmt (Kaniusas et al., 2019). Folgt man diesem Konzept und berücksichtigt tageszeitliche Schwankungen der Vagusaktivität, ist ein tageszeitabhängiger Effekt der taVNS zu erwarten.

Unter einer Neuromodulation versteht man eine Veränderung der Nervenaktivität durch gezielte Abgabe eines Reizes, z. B. durch elektrische Stimulation oder chemische Wirkstoffe, (International Neuromodulation Society, 2022). Die durch eine Hirnstimulation bedingte Neuromodulation kann indirekt über therapeutische, häufig subjektiv geprägte Effekte gemessen werden, objektivierbare und reproduzierbare Methoden sind jedoch für die endgültige Bewertung des neuromodulierenden Verfahrens notwendig. Unmittelbare taVNS-induzierte Veränderungen auf die Hirnaktivität bei Gesunden und bei Patient*innen mit verschiedenen Krankheitsbildern wurden bereits mittels verschiedener Methoden wie EEG, Magnetenzefalografie (MEG) und funktionelle Magnetresonanztomografie (fMRT) berichtet (Mao et al., 2022; Schuerman et al., 2021; Zhang et al., 2021; Badran et al., 2018). Für anhaltende Effekte (Stunden, Wochen, Monate und Jahre) liegen zur Zeit nur spärliche Ergebnisse vor (Wu et al., 2021; Zhang et al., 2022; Rong et al., 2014; Barbella et al., 2018; Stefan et al., 2012; Liu et al., 2018); diese Studien sind jedoch durch eine wiederholte taVNS-Stimulation und mögliche andere Einflussfaktoren, z.B. pharmakologische Behandlung, gekennzeichnet. Das Wissen über unmittelbare, mittelfristige und länger anhaltende VNS-bedingte Veränderungen der Hirnaktivität steckt somit in den Kinderschuhen.

Komplexe Hirnnetzwerke und Netzwerk-Kenngrößen

Das menschliche Hirn ist ein hoch komplexes Netzwerk, das, bestehend aus lokalen, teils hochgradig vernetzten Netzwerken, zu größeren, teils überlappenden Netzwerken zusammengefügt ist. Es ist somit als ein Netzwerk von Netzwerken zu verstehen (Bröhl et al., 2020). Diese Netzwerkstrukturen sind über die räumlichen Verbindungen hinaus als funktionelle Netzwerke zu verstehen, für die insbesondere zeitliche Dynamiken und

Interaktionen berücksichtigt werden müssen. Die Methoden zum Erfassen von Hirnnetzwerken und ggf. ihrer Unterschiede bei verschiedenen Populationen bzw. ihre Änderungen unter bestimmten Bedingungen unterscheiden sich hinsichtlich ihrer räumlichen und zeitlichen Auflösungstiefe. Die fMRT hat eine hohe räumliche Auflösung bei schlechter zeitlicher Auflösung, während sich bei EEG-Methoden eine sehr gute zeitliche Auflösung bei jedoch durch die Elektrodenplazierung limitierter räumlicher Auflösung sowie reduzierter räumlicher Abdeckung ergibt (Bullmore and Sporns, 2009; Bröhl et al., 2020). Beide Techniken sind somit als ergänzend zu werten, wobei für die Messung von Veränderungen über längerfristige Zeiträume das EEG zum jetzigen Zeitpunkt die einzig sinnvoll einsetzbare Methode ist.

Grundsätzlich kann ein Netzwerk durch Knoten (nodes) und die diese verbindenden Kanten (edges) dargestellt werden (siehe Abbildung 2). Bei der Anwendung der Netzwerkkonzepte auf Untersuchungen zu Hirnfunktionen und -dynamiken, sowie in der vorliegenden Arbeit der Einfluss von Interventionen auf letzteres, sind funktionelle Netzwerke entscheidend. Hierbei werden Netzwerk-Knoten mit Hirnregionen, die durch die EEG-Elektrodenkontakte abgetastet werden, und Netzwerk-Kanten mit Interaktionen zwischen Paaren dieser Hirnregionen, unabhängig von ihrer anatomischen Konnektivität, assoziiert. Das Hirnnetzwerk kann also als ein vollständig verbundener und gewichteter, d.h. durch die Interaktionsstärke der Netzwerkbestandteile geprägter, Graph dargestellt werden.

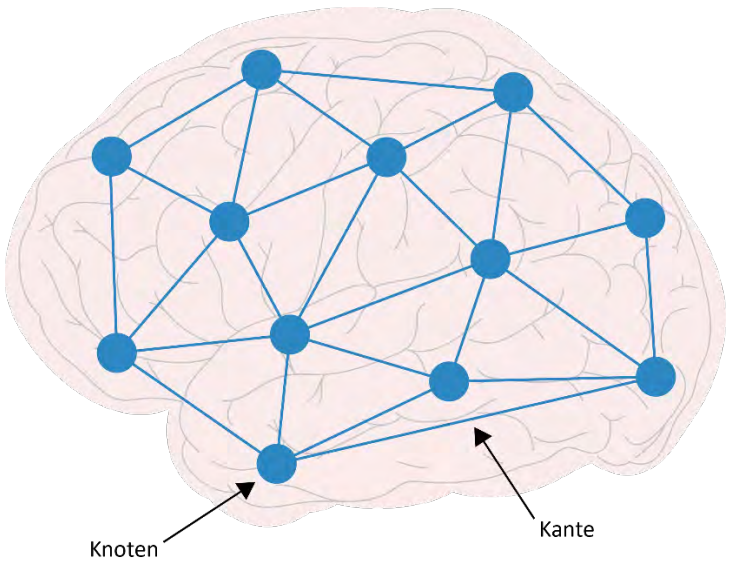

Abb. 2: schematische Darstellung von Hirnnetzwerken (Knoten und Kanten)

Mittels Zeitreihenanalysen können verschiedene Netzwerkkenngrößen und insbesondere ihre Veränderung über die Zeit ermittelt werden. Aufgrund der zu postulierenden Nichtstationarität von Hirnnetzwerken wird hier das Gleitfensterverfahren (Kuhnert et al., 2010; Dickten et al., 2016; Rings et al., 2019) verwendet, in dem für kurze nicht-überlappende Fenster eine approximative Stationarität des Systems Gehirn angenommen werden kann. Dies ermöglicht den Synchronisationsindex der mittleren Phasenkohärenz *R* (Mormann et al., 2000) zwischen Phasenzeitreihen von allen Paaren von Hirnregionen zu berechnen (für weitere Details, siehe (Rings et al., 2021)); der Synchronisationsindex bewegt sich im Intervall von 1 (vollständige Phasensynchronisation) und 0 (keine Phasensynchronisation) und wurde wiederholt als Indikator für die Stärke von Interaktionen in funktionellen Hirnnetzwerken beschrieben (Übersicht in (Dickten et al., 2016)). Durch Verknüpfung der Netzwerkknoten mit den abgetasteten Hirnregionen und der Netzwerkkanten mit den Synchronisationsindexwerten zwischen einem beliebigen Paar von Knoten können somit sich über die Zeit ändernde (evolving), vollständig verbundene und gewichtete Netzwerke abgeleitet und verschiedene lokale und globale Netzwerkkenngrößen bestimmt werden. Mit der mathematischen Graphentheorie lassen sich somit aus elektrophysiologischen Daten Hirnnetze sowohl auf lokaler als auch auf globaler Ebene charakterisieren.

Für die Charakterisierung eines Netzwerkes respektive dessen Änderung über die Zeit oder unter einer Intervention ist auf lokaler Netzwerkebene die Wichtigkeit von einzelnen Knoten von großer Relevanz. Auf dieser Ebene liegen verschieden Zentralitätskonzepte vor, durch die die Wichtigkeit (Zentralität) verschiedener Knoten und Kanten geschätzt werden kann. Ein relativ einfacher nachbarschaftsbasierter Zentralitätsindex ist der Grad (degree) eines Knotens, welcher in binären Netzen durch die Anzahl der anliegenden Kanten berechnet wird. Er ist ein Indikator für die Eingebundenheit des Knoten in das Netzwerk: je mehr Verbindungen ein Knoten hat, desto größer ist sein Einfluss auf das Netzwerk. Ein weiteres Maß für die Eingebundenheit eines Knotens in ein Netzwerk ist die Zentralität der Nähe (closeness centrality), bei der der „Abstand“ des Knotens zu allen anderen Knoten bestimmt wird. Es handelt sich somit um einen pfadlängenbasierten Zentralitätsindex. Eine hohe closeness centrality beschreibt somit einen Knoten, der „in der Mitte“ des Geschehens liegt (Überblick in (Lü et al., 2016)).

In gewichteten Netzwerken, also unter Berücksichtigung der Interaktionsstärke, ist die Stärke (strength centrality) eines Knotens, die durch die Gewichte der anliegenden Kanten berechnet werden kann, als Indikator für die Eingebundenheit eines Knoten in das Netzwerk und damit die Einflussnahme auf das Netzwerk zu werten. Auch die Zentralität der Nähe (closeness centrality, (C^C)) lässt sich auf gewichtete Netzwerke übertragen, wobei hier der „Abstand“ durch die Interaktionsstärke beeinflusst wird und die "Länge" eines Pfades zwischen einem Paar von Knoten als der Kehrwert des Gewichts der Kante, die die Knoten verbindet, definiert und berechnet werden kann (Dijkstra, 1959; Lehnertz et al., 2014). Diese Zentralitätskonzepte lassen sich auch auf Kanten ausweiten (Bröhl and Lehnertz, 2019). Weitere Zentralitätskonzepte, für die entsprechende Zentralitätsindizes sowohl für Knoten als auch für Kanten verfügbar sind (Bröhl and Lehnertz, 2019), ermöglichen eine weiterführende Charakterisierung funktioneller Hirnnetzwerke. Sowohl mit pfadbasierten Zentralitätsindizes als auch interaktionsstärkebasierten Zentralitätsindizes konnte in der Vergangenheit gezeigt werden, dass sie nicht-redundante Informationen über wichtige Bestandteile von epileptischen Hirnnetzwerken liefern (Kuhnert et al., 2012; Geier und Lehnertz, 2017; Bröhl und Lehnertz, 2019; Bröhl und Lehnertz, 2020; Fruengel et al., 2020). Die Betweenness-Zentralität (C^B) ist ein auf dem kürzesten Pfad zwischen Paaren von Knoten oder zwischen Paaren von Kanten basierender Zentralitätsindex. Die Betweenness-Zentralität misst, wie häufig ein bestimmter Knoten bzw. eine bestimmte Kante auf dem kürzesten Weg zwischen zwei anderen Knoten bzw. Kanten liegt. Ein Knoten bzw. eine Kante mit hoher Betweenness-Zentralität ist wichtig, da dieser bzw. diese verschiedenen Regionen des Netzwerks wie eine Brücke verbindet und damit den Informationsfluss beeinflusst. Die Eigenvektor-Zentralität (C^E) hingegen ist ein auf der Interaktionsstärke basierender Zentralitätsindex. Ein Knoten bzw. eine Kante ist wichtig, wenn die mit diesem verbundenen Knoten bzw. mit dieser verbundenen Kanten ebenfalls wichtig sind. Die Eigenvektor-Zentralität spiegelt somit den Einfluss des Knotens bzw. der Kante auf das Netzwerk als Ganzes wider. Bei der Stärkezentralität (C^S)ist ein Knoten umso zentraler (wichtiger), je größer die Summe der Gewichte der benachbarten Kanten ist (Barrat et al., 2004; Yook et al., 2001). Für Kanten liegt ein neuartiges Konzept der Nearest neighbor-Zentralität (C^N) vor. Ein hoher Nearest neighbor-Zentralitätsindex hebt eine Kante als umso zentraler hervor, je größer ihr Gewicht, je ähnlicher und je stärker die verbundenen Knoten sind (Bröhl and Lehnertz, 2022). Folglich ist die Nearest neighbor-Zentralität weitgehend unabhängig von der Netzwerktopologie, da sie ausschließlich auf

lokalen Knoten- und Kanteneigenschaften beruht. So spiegelt eine Kante mit einer hohen Nearest neighbor-Zentralität einen lokalen Engpass wider, der möglicherweise mit globalen Engpässen übereinstimmt.

Neben lokalen Netzwerkkenngrößen können funktionelle Netze durch globale Kenngrößen charakterisiert werden. Auf der globalen Netzwerkebene können (u.a.) topologische, Stabilitäts- und Robustheits-Eigenschaften des Netzwerkes berechnet werden. Die Topologie des Netzwerkes, also die Konfiguration bzw. Anordnung von Koten und Kanten, wird u.a. anderen durch den durchschnittlichen Clustering-Koeffizienten *C* und die durchschnittlich kürzeste Pfadlänge *L* beschrieben. Der durchschnittliche Clustering-Koeffizient *C* beschreibt das Ausmaß, in dem Knoten in einem Netz dazu neigen, sich zu gruppieren, sog. Cliquenbildung. *C* charakterisiert die funktionelle Segregation des Netzes: je niedriger *C*, desto segregierter ist das Netz. Die durchschnittliche kürzeste Pfadlänge *L* ist definiert als die durchschnittliche Anzahl von Schritten entlang der kürzesten Wege für alle möglichen Paare von Netzwerkknoten und spiegelt daher die funktionelle Integration des Netzwerks wider: je kleiner *L*, desto integrierter ist das Netz (Überblick in (Rings et al., 2021). In diesem Modell spiegelt die funktionelle Segregation (Integration) unabhängige (abhängige) Informationsprozesse zwischen Hirnregionen wider (Tononi et al., 1994).

Weitere wesentliche globale Kenngrößen sind die Assortativität (assortativity) *A* und die Synchronisierbarkeit (synchronisability) *S*. Die Assortativität bewertet die Tendenz von Kanten, Knoten mit ähnlichen oder gleichen Eigenschaften zu verbinden (Newman, 2003; Bialonski und Lehnertz, 2013). Wenn Kanten bevorzugt Knoten mit ähnlichen (unähnlichen) Eigenschaften verbinden, werden solche Netzwerke assortativ (disassortativ) genannt. *A* ist per Definition auf das Intervall [-1, 1] beschränkt, wobei positive (negative) Werte ein assortatives (disassortatives) Netzwerk beschreiben. Disassortative Netzwerke sind anfälliger für Störungen und scheinen leichter zu synchronisieren zu sein als assortative Netzwerke. Assortative Netzwerke sind somit widerstandsfähiger und robuster, tendieren dem hingegen stärker zur Desintegration in verschiedene Gruppen als disassortative Netzwerke. Die Synchronisierbarkeit *S* beschriebt die Stabilität eines synchronisierten Zustands: je höher *S*, desto leichter kann der synchronisierte Zustand gestört werden (Pecora and Carroll, 1998; Barahona and Pecora, 2002; Atay et al., 2006).

Änderungen der funktionellen Hirnnetzwerke durch die aurikuläre Vagusnervstimulation

Die mathematische Berechnungen von Netzwerkkenngrößen (für die Algorithmen siehe Zusammenfassung in (Rings et al., 2021)) im Rahmen der Netzwerktheorie bieten somit die Möglichkeit, wichtige Netzwerkmerkmale wie topologische, Stabilitäts- und Robustheits-Eigenschaften sowie Wichtigkeiten von lokalen Hirnregionen und den Interkationen zwischen diesen zu bestimmen. Die Untersuchung von Netzwerkcharakteristika im Zeitverlauf ermöglicht, stimulationsbedingte Veränderungen der Hirnnetzwerke zu identifizieren und zu beschreiben.

Im Gegensatz zu anderen, lokal spezifischeren Stimulationsmethoden wie der tiefen Hirnstimulation (DBS) (Boon et al., 2018) oder der responsiven Neurostimulation (RNS)(Jarosiewicz and Morrell, 2020) wird allgemein angenommen, dass die VNS zu einer eher unspezifischen globalen Aktivierung verschiedener Hirnstrukturen führt. Sowohl für die iVNS als auch für die taVNS konnten ähnliche Projektionen afferenter Vagusnervenfasern zum Nucleus des Tractus solitarius gezeigt werden und die durch iVNS und taVNS induzierten zerebralen Aktivierungsmuster ähneln einander. Die Ergebnisse der in der Literatur vorliegenden fMRT-Studien, welche die durch taVNS sekundär aktivierten Hirnregionen untersuchen, sind ausgesprochen heterogen. Dies ist zum Teil auf die Unterschiede in der Studienpopulation, den Stimulationsfrequenzen und den Auswerteprotokollen zurückzuführen. Nichtsdestotrotz verwiesen die Ergebnisse auf weitreichende Aktivierungen, u.a. des Nucleus caudatus, des linken präfrontalen Kortex, Thalamus, Cingulum und Kleinhirn (für einen Überblick siehe (Ellrich, 2019; Butt et al., 2020)). Diese lokale Unspezifität spiegelt sich auch in widersprüchlichen Befunden im EEG wider: Während einige Autoren von einer Veränderung der epileptiformen Aktivität berichten (Koo, 2001), weisen quantitative EEG-Studien auf gegensätzliche Phänomene (z.B. Synchronisation vs. Desynchronisation) (Rutecki, 1990) sowie auf uneindeutige Veränderungen in relevanten EEG-Frequenzbändern hin (Übersicht in (de Vos et al., 2011)).

Hypothesen und Fragestellungen der Habilitationsarbeiten

Aus den oben beschriebenen Arbeiten lässt sich die Hypothese formulieren, dass eine externe Stimulation des Nervus vagus zu einer Veränderung von funktionellen Hirnnetzwerken führen

könnte. Sollte ein solcher Effekt nachweisbar und quantifizierbar sein, könnten in einem späteren Schritt solche Veränderungen mit dem klinischen Ansprechen auf eine taVNS korreliert werden und ggf. als Prädiktor der Therapieantwort herangezogen werden. In einem ersten Schritt soll hier zunächst mit der Methodik der Netzwerkanalyse basierend auf dem Konzept des Gehirns als funktionelles komplexes Netzwerk untersucht werden, ob und wie eine kurzzeitige taVNS messbare unmittelbare und anhaltende Veränderungen der funktionellen Hirnnetzwerke bei Proband*innen mit und ohne Epilepsie hervorruft.

Diese Herangehensweise ermöglicht die Überprüfung der möglichen Veränderungen auf der lokalen sowie globalen, d.h. das gesamte Hirnnetzwerk betreffenden, Ebene und hebt sich damit sowohl von den anatomisch gebundenen als auch von den fokussierten Untersuchungsmethoden ab. Die auf der Graphentheorie basierende Untersuchungsmethode bildet somit alle Ebenen der Hirnnetzwerke ab und ist in der Lage mittels elektrophysiologischer Daten standardisierbare, reproduzierbare und objektivierbare Ergebnisse zur Neuromodulation mittels des transkutanem aurikulären Vagusnervstimulation zu erheben.

3. Ergebnisteil

von Wrede R, Rings T, Schach S, Helmstaedter C, Lehnertz K. (2021). Transcutaneous auricular vagus nerve stimulation induces large scale alterations in epileptic brain networks: towards understanding the effect of taVNS in subjects with epilepsy. *Sci Rep.* 2021 Apr 12;11(1):7906.

Zielsetzung der Arbeit: Die vorliegende Arbeit ist die Proof-of-Concept Studie zur Untersuchung der Beeinflussung von epileptischen Hirnnetzwerken durch die transkutane aurikuläre Vagusnervstimulation.

Methoden und Ergebnisse: 14 Proband*innen mit einer fokalen Epilepsie durchliefen das Studienprotokoll mit einer kontinuierlichen mindestens 3-stündigen EEG-Vielkanal-Aufzeichnung, welche von einer standardisierten neuropsychologischen Untersuchung begleitet wurde. Aus einer zeitaufgelösten Synchronisationsanalyse der EEG-Aufzeichnung wurden sich entwickelnde, vollständig verbundene und gewichtete Netzwerke abgeleitet und verschiedene globale Netzwerkkenngrößen und deren Veränderungen in Bezug auf die taVNS erhoben. Bei der Mehrheit der Proband*innen führte die taVNS zu unmittelbaren, stimulationsinduzierten Veränderungen in der Gesamtstärke der funktionellen Interaktionen (globaler Synchronisationsgrad R), der topologischen Eigenschaften (durchschnittliche kürzeste Pfadlänge *L* und durchschnittlicher Clustering-Koeffizient *C*) sowie der Stabilität (Snychronisierbarkeit *S*) und der Robustheit (Assortativität *A*) des Netzwerks; bei 30 - 50 % der Proband*innen zeigte die taVNS eine anhaltende Wirkung. Für *C* zeigte sich ein immediater Effekt einer Segregationszunahme und anhaltender Effekt einer Segregationsabnahme. Für *L* wurde ein ähnliches, wenn auch invertiertes Muster mit einem immediaten Effekt der Integrationsabnahme und anhaltenden Effekt der Integrationszunahme beobachtet. Für *S* zeigte sich eine geringe immediate stimulationsbedingte Erhöhung sowie ein anhaltender Effekt der Abnahme der Stabilität eines potenziell synchronisierten Zustands der Netzwerke (hier noch Nutzung der inverten Definition von *S*; Adaptation in Nachfolgeartikeln). Die deutlichsten Hinweise auf eine präventive Wirkung des taVNS zeigten sich bei den Veränderungen der Assortativität *A* der Netzwerke. Bereits vor der Stimulationsphase zeigten sich die Hirnnetzwerke assortativ (A = 0,37 ± 0,15), in der Studie konnte eine weitere Zunahme der Assortativität beobachtet werden, was auf eine sowohl immediate als auch anhaltende robustheitssteigernde Wirkung von taVNS hinweist.

Für zwei Proband*innen zeigten sich nach der Stimulation signifikante intraindividuelle Verbesserungen der exekutiven Funktionen. Alle anderen kognitionsbezogenen Variablen blieben bei diesen und den anderen Proband*innen unbeeinflusst, auch wurden keine signifikanten selbst wahrgenommenen Veränderungen in den Bereichen Kognition, Verhalten und physiologische ZNS-Symptome angegeben. Signifikanten Beziehungen zwischen den neuropsychologischen Variablen und den Merkmalen der epileptischen Hirnnetzwerke bestanden nicht.
Die Handhabung und der Tragekomfort des Geräts wurden als gut oder sehr gut bewertet. Die Mehrheit der Proband*innen fühlten sich in der Fortsetzung ihrer Aktivitäten durch die Stimulation nicht beeinträchtigt. Einige Proband*innen gaben jedoch an, dass das Gerät für eine langfristige Verwendung während des Tages oder eine wiederholte Verwendung innerhalb eines Tages eher schlecht geeignet sei. Es wurden weder Nebenwirkungen berichtet noch beobachtet.

Schlussfolgerungen: Eine kurzzeitige taVNS hat eine Topologie- und Robustheitsverändernde und Stabilitätssteigernde Wirkung auf epileptische Hirnnetzwerke ohne nachteilige Auswirkungen auf Kognition und Verhalten und wurde von den Proband*innen mit Epilepsie gut vertragen.

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Transcutaneous auricular vagus nerve stimulation induces stabilizing modifications in large-scale functional brain networks: towards understanding the effects of taVNS in subjects with epilepsy

Randi von Wrede[1]✉, Thorsten Rings[1,2], Sophia Schach[1], Christoph Helmstaedter[1] & Klaus Lehnertz[1,2,3]

Transcutaneous auricular vagus nerve stimulation (taVNS) is a novel non-invasive brain stimulation technique considered as a potential supplementary treatment option for subjects with refractory epilepsy. Its exact mechanism of action is not yet fully understood. We developed an examination schedule to probe for immediate taVNS-induced modifications of large-scale epileptic brain networks and accompanying changes of cognition and behaviour. In this prospective trial, we applied short-term (1 h) taVNS to 14 subjects with epilepsy during a continuous 3-h EEG recording which was embedded in two standardized neuropsychological assessments. From these EEG, we derived evolving epileptic brain networks and tracked important topological, robustness, and stability properties of networks over time. In the majority of investigated subjects, taVNS induced measurable and persisting modifications in network properties that point to a more resilient epileptic brain network without negatively impacting cognition, behaviour, or mood. The stimulation was well tolerated and the usability of the device was rated good. Short-term taVNS has a topology-modifying, robustness- and stability-enhancing immediate effect on large-scale epileptic brain networks. It has no detrimental effects on cognition and behaviour. Translation into clinical practice requires further studies to detail knowledge about the exact mechanisms by which taVNS prevents or inhibits seizures.

Epilepsy is one of the most common neurological disorders and is defined by recurrent epileptic seizures. Although two thirds of affected subjects achieve seizure-freedom with the first two appropriately chosen antiseizure medications (ASM)[1], the other third requires extensive therapy attempts in order to achieve seizure-freedom or at least an acceptable seizure situation. Even the development of new ASM has not led to a significant improvement of seizure outcome, though tolerability and interaction profile have become more advantageous[2]. Thus, there is a strong need for alternative or complementary treatment options. Vagus nerve stimulation (VNS) is an established method of brain stimulation in several diseases, including epilepsy[3]. Invasive vagus nerve stimulation (iVNS) was first approved as early as in the 1990s. It has been extensively studied and its safety has been demonstrated in more than 20 studies. Its effectiveness is assumed with a responder rate (subjects in whom seizure frequency is reduced by more than 50%) of approximately 50%[4,5]. However, it is an invasive method with need of anaesthesia and surgical risk. Transcutaneous auricular vagus nerve stimulation (taVNS) is a non-invasive external stimulation (of the auricular branch of the vagus nerve) and seems to be an interesting alternative. Good

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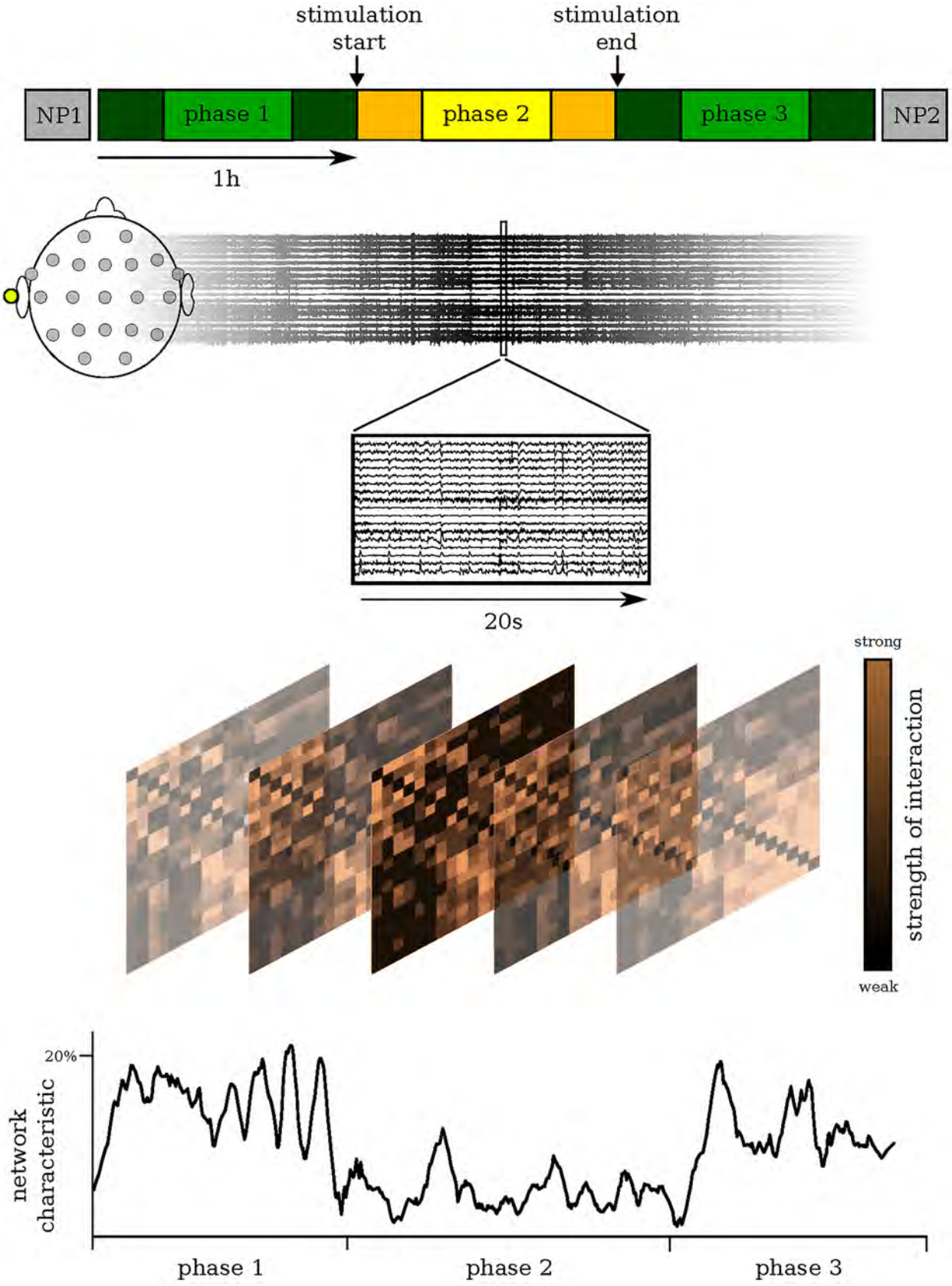
stimulation start
stimulation end
NP1
phase 1
phase 2
phase 3
NP2
1h
20s
strong
strength of interaction
weak
20%
network characteristic
phase 1
phase 2
phase 3

◂**Figure 1.** Examination schedule: Probing for taVNS-induced changes in epileptic brain networks. Our examination schedule consisted of a 3-h EEG recording (Methods) that covered a stimulation phase (phase 2; continuous stimulation of the left cymba conchae) and a pre- and post-stimulation phase (phase 1 and phase 3, resp.). In our analyses, we neglected data from the first and last 15 min of each phase (darker colours) in order to remove possible transient effects. The EEG recording was preceded and followed by a standardized neuropsychological assessment (NP1 and NP2, resp. 30 min; Methods). We derived evolving epileptic brain networks from the EEG recording using a sliding-window approach (Methods), assessed important global characteristics of each network (Methods), and tracked their changes over time.

tolerability and effectiveness have been demonstrated for taVNS[6–10]. For both iVNS and taVNS, similar projections of afferent vagus nerve fibres to the nucleus of the solitary tract could be shown[11] and cerebral activation patterns induced by iVNS and taVNS resemble each other (for overview see[12]).

Knowledge about immediate and longer-lasting VNS-related changes of brain activity is sparse. In contrast to other, locally specific stimulation methods such as deep brain stimulation (DBS)[13] or responsive neurostimulation (RNS)[14], it is generally assumed that VNS leads to a rather unspecific, global activation of various brain structures (including thalamus, limbic system, insular cortex)[15,16]. This local unspecificity is also reflected in contradicting findings on the EEG: while some authors report a modification of epileptiform activity[17], quantitative EEG studies point to opposing phenomena (e.g., synchronisation vs. desynchronisation[16]) as well as to ambiguous changes in relevant EEG frequency bands[18].

We hypothesized that the impact of the global, apparently unspecific activation can be suitably assessed with a global analysis approach which makes use of the EEG derived so-called evolving functional brain networks[19,20]. The powerful mathematical framework of network theory provides means to determine important network characteristics such as their topological, stability, and robustness properties. Tracking network characteristics over time would allow one to identify and delineate stimulation-related changes of EEG activity. Accompanying such an investigation with an examination of cognitive functions may provide important insights into their possible relationships with the aforementioned network characteristics[21] and could help to improve understanding of whether and how VNS may impact cognition[22,23]. We tested this hypothesis by investigating whether short-term taVNS induces measurable immediate modifications of functional brain network in subjects with epilepsy and whether modifications are accompanied by changes of cognition and behaviour (see Fig. 1).

Results

Stimulation-related modifications of evolving epileptic brain networks. Evolving epileptic brain networks are functional networks[19] that can be derived from EEG recordings by associating network vertices with brain regions sampled by electrode contacts and network edges with the time-varying estimates of the strength of interactions between pairs of brain regions[20] (Methods). We derived such evolving, fully connected and weighted networks from a time-resolved synchronisation analysis of the 3-h EEG recording, used various measures (Methods) to assess important characteristics of each network and tracked their changes over time. In order to characterise the network's global topological properties, we estimated its average shortest path length L and its average clustering coefficient C. In addition, we assessed the network's stability and robustness properties by estimating its synchronisability S and its assortativity A. The average shortest path length characterises the network's functional integration; the lower L, the more integrated is the network. The average clustering coefficient characterises the network's functional segregation; the lower C, the more segregated is the network. Synchronisability assesses the network's propensity (or vulnerability) to get synchronised by an admissible input activation: the lower S, the more easily can the synchronised state be perturbed. Assortativity assesses the tendency of edges to connect vertices with similar or equal properties. If edges preferentially connect vertices of similar (dissimilar) property, such networks are called assortative (disassortative). Disassortative networks are more vulnerable to perturbations and appear to be easier to synchronise than assortative networks. The latter show a stronger tendency to disintegrate into different groups than disassortative networks.

In the majority of subjects (Fig. 2), taVNS led to immediate, stimulation-related alterations in the overall strength of functional interactions (global synchronisation level R) in epileptic brain networks. Their average shortest path length L, average clustering coefficient C, synchronisability S, and assortativity A were seen to be modified in a similar number of subjects. Interestingly, taVNS appeared to have a persistent effect in about 30–50% of subjects, as seen with most network characteristics (for those subjects, for which we achieved significant differences between phases ($p < 0.05$ after Bonferroni correction, Mann–Whitney U values ranged between 173 and 3187 (phase 1 $\rightarrow$ 2), between 23 and 3231 (phase 2 $\rightarrow$ 3) and between 585 and 3301 (phase 1 $\rightarrow$ 3); ranges are reported for all network characteristics; the number of degrees amounted to 90 for each phase).

We provide a more detailed picture of stimulation-related alterations of network characteristics in Fig. 3, where we plot the distributions of their relative changes for networks transiting between the different phases. The global synchronisation level R slightly decreased from the pre-stimulation to the stimulation phase (desynchronisation; phase 1 $\rightarrow$ 2: −5%; we report the median values in the following) but it increased when networks transit from the stimulation to the post-stimulation phase (re-synchronisation; phase 2 $\rightarrow$ 3: 10%). We observed only slight differences between the pre- and post-stimulation phase (phase 1 $\rightarrow$ 3: 4%). Together with the high interindividual variability, these findings partly confirm previous observations with long-term iVNS[24,25] or immediate iVNS[26].

For the average shortest path length L, we attained a similar though inverted patterning (which is to be expected given the definition of a path length in a weighted network): a slight increase of L from the pre-stimulation to the stimulation phase (phase 1 $\rightarrow$ 2: 6%), a slight decrease from the stimulation to the post-stimulation

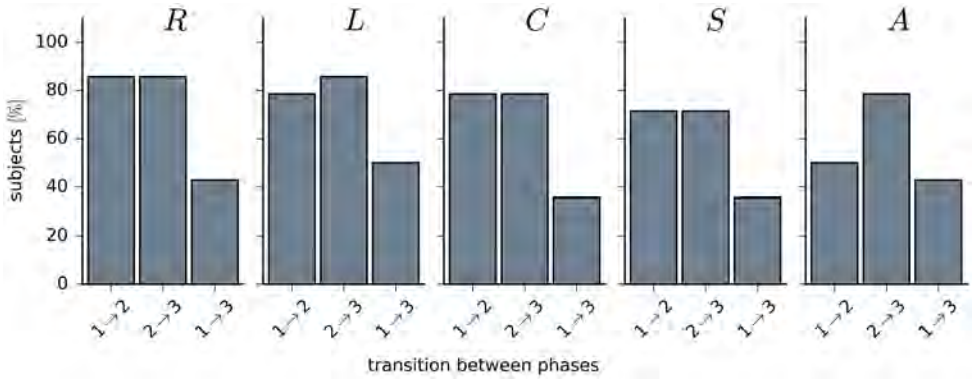

Figure 2. Percentage of subjects for which taVNS led to significant differences (Methods) between networks characteristics from phases 1, 2, and 3; global synchronization level R, average shortest path length L, average clustering coefficient C, synchronisability S, and assortativity A.

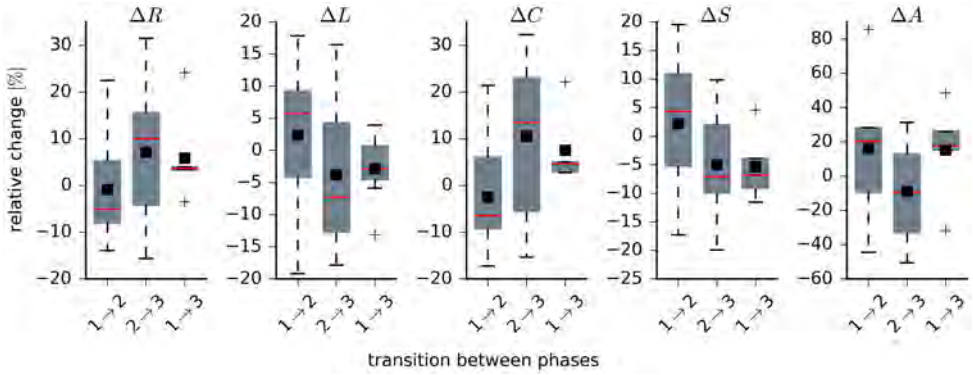

Figure 3. Distributions of taVNS-related alterations in network characteristics. Boxplots of relative changes Δ in network characteristics. Relative changes calculated as $\Delta = (M_l - M_k)/M_k$, where M_k and M_l denote placeholders for the temporal means of the respective characteristics from phase k and phase l (global synchronization level R, average shortest path length L, average clustering coefficient C, synchronisability S, and assortativity A). During phase 1, network characteristics attained the following values: $R = 0.31 \pm 0.02$, $L = 3.43 \pm 0.29, C = 3.33 \pm 0.02, S = 3.15 \pm 0.49$, and $A = 0.37 \pm 0.15$. Bottom and top of a box are the first and third quartiles, and the red band and the black square are the median and the mean of the distribution. The ends of the whiskers represent the interquartile range of the data. Outliers are marked by a + sign.

phase (phase 2 → 3: −7%), and slight undershoot effect when comparing the pre- and post-stimulation phase (phase 1 → 3: −3%).

We can derive similar indications with changes of the average clustering coefficient C, for which we observed a patterning that compares to the one seen for the global synchronisation level, although changes were slightly more pronounced (phase 1 → 2: −6%; phase 2 → 3: 14%; phase 1 → 3: 5%).

Synchronisability S slightly increased from the pre-stimulation to the stimulation phase (phase 1 → 2: 4%) and it decreased when networks transit from the stimulation back to the post-stimulation phase (phase 2 → 3: −7%). A similar decrease was observed when comparing the pre- and post-stimulation phase (phase 1 → 3: −7%).

Interestingly, we obtained strongest indications for a preventive effect of taVNS with changes in the networks' assortativity A. Already during the pre-stimulation phase, epileptic brain networks were seen to be assortative ($A = 0.37 \pm 0.15$). taVNS even increased their assortativity (phase 1 → 2: 20%). Although networks experienced a slight decrease of their robustness when transiting from the stimulation back to the post-stimulation phase (phase 2 → 3: −7%), the comparably strong increase seen between the pre- and post-stimulation phase (phase 1 → 3: 18%) would point to an enduring robustness-enhancing effect of taVNS.

Stimulation-related modifications of cognition and behaviour. Prior to stimulation, 35% of subjects presented with impaired executive functions. Mild or severe impairment in verbal memory was seen in 82%, and a relevant depressive symptomatology in 43% of subjects. After stimulation, we observed in two subjects a significant intraindividual improvement of executive functions. All other cognition-related variables remained unaffected in these and the other subjects (Mann Whitney U values ranged between 64 and 98 for the different domains; the number of degrees of freedom amounted to 14; n.s.). No significant self-perceived changes in the

evaluated domains cognition, behaviour and physiological symptoms were observed; one subject reported an improvement in anxiety after taVNS. There were no significant relationships between neuropsychological variables and characteristics of epileptic brain networks.

Evaluation of the device: side effects and usability. No local side effects were complained or detected by clinical check-up. All subjects rated the handling of the device as good or very good. 86% felt that the continuation of their activities was not affected by the stimulation. The majority rated the wearing comfort as good or very good (79%). However, some subjects stated that the device is rather poorly suited for long-term use during the day (43%) or repeated use within one day (29%).

Discussion

With our prospective trial, we investigated whether short-term transcutaneous auricular vagus nerve stimulation (taVNS) induces measurable immediate modifications of functional brain networks in fourteen subjects with epilepsy and whether modifications are accompanied by changes of cognition and behaviour. Our findings reveal that taVNS has stabilising effects on networks in the majority investigated subjects and these effects persist in up to 50% of subjects. In contrast, cognition and behaviour are not affected by the stimulation.

The stimulation-related alterations seen for network characteristics average shortest path length and clustering coefficient indicate that taVNS modifies the network's topological organisation, which is reflected in a more integrated and less segregated network. Similar findings could be achieved only recently also with long-term invasive vagus nerve stimulation[27]. In addition to modifications of network topology, short-term taVNS can enhance stability and robustness of epileptic brain networks. The alterations seen for synchronisability indicate an increase of the network's stability against perturbations, i.e., a more resilient brain network. Moreover, the observed similar decrease in synchronisability when comparing the pre- and post-stimulation phases would point to an enduring stabilising effect of taVNS. Interestingly, we obtained strongest indications for a preventive effect of taVNS with changes in the networks' assortativity. Already prior to stimulation, epileptic brain networks were seen to be assortative, which confirms previous observations[28,29], and taVNS even increased their assortativity. Although networks experienced a slight decrease of their robustness when transiting from the stimulation back to the post-stimulation phase, the comparably strong increase seen between the pre- and post-stimulation phase would point to an enduring robustness-enhancing effect of taVNS.

There were no detrimental effects of taVNS on cognition and behaviour in our subjects with epilepsy. Similar observations were made recently in healthy subjects[30], even when stimulating the brain during the memory consolidation phase. However, findings need to be taken with care, given that research into the impact of taVNS on cognition is still in its infancy[31,32]. Previous studies revealed that long-term iVNS can enhance recognition memory in subjects with epilepsy in comparison to sham stimulation and depending on stimulation intensity[33]. Detrimental effects, however, were reported for acute high-intensity iVNS on figural memory but not on verbal memory in subjects with epilepsy[22]. Future studies would need to further elucidate the influence of taVNS on cognition and behaviour.

Short-term taVNS was well tolerated by our subjects with epilepsy, and no local side effects occurred. These results are in par with expected results from long-term studies[34]. The usability of the device was rated good and very good in terms of handling, management, comfort, and possibility of continuation of one's activities. However, rating for suitability for long-term or repeated use was viewed critically by some subjects. Complaints about the duration of a daily stimulation of 4 h were given by subjects with epilepsy in treatment settings before and recently led to an evaluation of the effects of reduced stimulation times[9].

Brain stimulation is a rapidly evolving field and is considered as a supplementary treatment option for subjects with refractory epilepsy. Invasive VNS is accompanied with perioperative risks involved with device implantation and is thus limited to the treatment of more severe, drug-resistant cases. taVNS is a non-invasive brain stimulation technique and clinical data about efficacy and tolerability indicate this approach to be an interesting alternative. Nevertheless, we still lack detailed knowledge about the exact mechanisms—from the molecular[35] to the brain level and to other organs (e.g. heart[36])—by which taVNS prevents or inhibits seizures which currently hinders the translation into clinical practice[37]. Our findings point to a topology-modifying, robustness- and stability-enhancing immediate effect of short-term taVNS on large-scale epileptic brain networks. At least on the time scale considered in our study (few hours), these network modifications did not impact on the investigated variables of cognition and behaviour. Our approach thus opens new perspectives towards improving our understanding of the dynamics of large-scale epileptic brain networks as well as towards deciphering the mechanism of action of taVNS.

Future studies should investigate the impact of long-term transcutaneous auricular vagus nerve stimulation on brain networks as well as long-term effects of the stimulation to deepen understanding of the mechanism of action and the potential efficacy of taVNS. By the same token, future studies should also investigate the impact of stimulation on local and/or medium-scale properties of epileptic brain networks (such as centralities of vertices and edges, cores, motifs, or community structures) as this could help in optimizing stimulation parameters which are currently selected rather heuristically. Comparing the effects of iVNS and taVNS using the same study design could reveal similarities and differences of these stimulation approaches with regard to large-scale epileptic networks. And finally, evaluation of subjects with different epilepsy syndromes and different severities could help translating this brain stimulation approach into clinical use.

There are some limitations of our prospective investigations. Since we avoided as much confounders on the EEG-evaluation as possible (e.g. by activation methods, change of ASM, seizures before study), we generated a high exclusion rate that led to a small number and higher heterogenicity of investigated subjects with epilepsy. A larger group size as well as more homogeneous groups could be interesting. The device we used for taVNS

	Sex	Age	Dur.	Lat.	Loc.	Hand.	MRI lesion	Drug res.	ASM	Stim.
1	f	50	1	Right	Insula	Right	Yes	No	LEV	3.0
4	f	19	0	Left	Frontal	Right	No	No		0.9
5	m	18	0	Right	Temporal	Ambidexter	No	No	LEV	0.9
6	m	25	1	Unknown	Unknown	Right	No	No		3.5
7	f	22	7	Right	Frontal	Right	No	Yes	LCM	0.6
9	f	55	4	Right	Temporal	Right	No	Yes	LEV, TPM	3.0
11	f	24	12	Bilateral	Temporal	Right	No	Yes	BRV, LTG, LCM	3.0
12	f	70	60	Right	Temporal	Right	Yes	Yes	LTG, VPA, PB	0.9
14	m	71	1	Left	Temporal	Right	No	No	LEV	1.4
15	m	26	19	Left	Frontal	Right	Yes	Yes	LEV, LTG, VPA, OXC	1.9
17	f	25	5	Right	Frontal	Right	No	Yes	CBZ	2.9
18	m	77	2	Left	Temporal	Right	No	No	LEV	1.6
19	f	53	34	Left	Temporal	Right	No	Yes	LTG, ZON	1.9
20	m	40	17	Right	Temporal	Right	No	Yes	LEV, OXC	2.7

Table 1. Patient demographics. Dur.: duration of disease in years; lat. = lateralization; loc. = localisation; hand. = handedness; drug res. = drug resistance according to ILAE[40]; ASM = antiseizure medication; LEV = levetiracetam, LTG = lamotrigine; LCM = lacosamide; TPM = topiramate; BRV = brivaracetam; VPA =valproate; PB = phenobarbital, OXC = oxcarbazepine, CBZ = carbamazepine; ZON = zonisamide; stim. = stimulus intensity in mA.

has non-adjustable stimulation parameters. However, in iVNS, adjusting parameters individually is not only crucial for an effective treatment of epilepsy[38,39], but might also impact on topological and robustness properties of epileptic brain networks. Evaluating the impact of varying stimulation parameters could contribute to the understanding of the mechanism of actions of taVNS and, in the long run, help to optimize its clinical use.

To conclude, short-term taVNS has a topology-modifying, robustness- and stability-enhancing immediate effect on large-scale epileptic brain networks. It has no detrimental effects on cognition and behaviour and was well tolerated by our subjects with epilepsy. There are similarities between taVNS and iVNS that emphasise the necessity of further research on taVNS as the less complicated way of brain stimulation via the vagus nerve.

Methods

Subjects. Between March 25 and September 19 of 2020, 472 subjects were admitted to our ward and were screened for suitability for our study. Exclusion criteria were unclear diagnosis, progressive disease, previous resective brain surgery, actual or previous vagus nerve stimulation or deep brain stimulation, insufficient German language capability, mental disability and incompetence to follow instructions. Inclusion criteria were clinical necessity for long-term video EEG-recording and proven diagnosis of epilepsy. Of the 36 eligible subjects, 22 declined participation. Fourteen subjects signed informed consent after being provided with written information and being given the opportunity to ask further questions; these subjects were included in the study. The study protocol had been approved by the ethics committee of the University of Bonn before the study has started. All experiments were performed in accordance with relevant guidelines and regulations.

Fourteen subjects with epilepsy (8 females; age 18–77 years, median 41 years; Table 1) were included in the study. Eight subjects had a drug-resistant epilepsy according to the definition of the International League against Epilepsy[40]. We applied taVNS with individualized stimulation intensities (range: 0.6–3.5 mA. mean 2.0, SD ±1.0, Methods) for 1 h in the early afternoon while subjects underwent a continuous 3-h EEG recording (see Fig. 1). No activation methods (such as change in ASM, hyperventilation or sleep deprivation) were applied at least 24 h before stimulation. The EEG recording was preceded and followed by a standardized neuropsychological assessment which involved measures of executive functions, verbal memory, mood, and the rating of subjective changes of the subjects' cognitive, psychiatric and somatic condition. To reduce potential practice effects, parallel test versions were applied for examining executive functions and verbal memory. No side effects were reported or observed.

Transcutaneous auricular vagus nerve stimulation. Stimulation was carried out with two hemispheric titanium electrodes of a NEMOS device (tVNS Technologies GmbH, Erlangen, Germany) fitted in the left cymba conchae and using a common set of non-adjustable parameters (biphasic signal form, impulse duration 20 s, impulse pause 30 s, impulse frequency 25 Hz). Intensity of stimulation was adjusted individually and was raised slowly until the subject noticed a "tingling", but no pain.

Details of neuropsychological assessment. *Attention and executive functions*. The EpiTrack 3rd edition[41] is a screening tool consisting of six subtests assessing response inhibition, visuo-motor speed, mental flexibility, visuo-motor planning, verbal fluency, and verbal working memory. It can be completed in 15 min. The performance in each subtest results in an age-corrected total score with a maximum score of 49 points (after

age-correction). Mild impairment is reflected by a total score in the range of 29 to 31, the cut-off score for severe impairment is $\leq$ 28 points ($>$ 2 SD below the normative sample). A significant intraindividual change in the total scores between two assessments is indicated by a gain of $\geq$ 4 points or the loss of $\geq$ 3 points.

Verbal memory. Verbal memory was assessed using a short version of the Verbal Learning and Memory Test (VLMT[42]) which is the German adaptation of the Rey Auditory Verbal Learning Test (RAVLT). The shortened VLMT version includes two consecutive trials of word list learning (15 words) with immediate free recall. After the two learning trials, the EpiTrack was performed, followed by the delayed free recall of the word list. Thus, the EpiTrack provided a distraction for memory testing. Age-correction was based on normative data of 383 healthy subjects. Scores for learning, memory and loss over time were transformed into a scale ranging from 1 to 7 according to the normative sample and converted into a total memory score ranging from 3 to 21. After age correction, total memory scores from 14 to 18 are rated as normal, scores $>$ 18 as above average, scores from 11 to 13 as mild impairment, and scores of $\leq$ 10 are considered a significant impairment. A significant change is indicated by a gain of $>$ 3 points or a loss of $>$ 5 points.

Mood/Depression. The Neurological Disorders Depression Inventory for Epilepsy (NDDI-E[43]) is a brief self-report questionnaire used as a screening tool for detecting depression in people with epilepsy. This 6-item screening instrument specifically focuses on symptoms of depression that cannot be explained by adverse effects of antiseizure medication. All items are rated on a four-tiered scale (1—never, 2—rarely, 3—sometimes, 4—always or often). A total score above 15 indicates a relevant depressive symptomatology.

Subjective measures. A modified version of the Adverse Events Profile was used before and after stimulation to assess self-perceived changes in three domains: (1) cognition (vigilance, energy, psychomotor speed, attention/ability to concentrate, fluent speech, verbal comprehension, word finding, remote memory), (2) behaviour (depression, anxiety, aggression, restlessness), (3) physiological symptoms (dizziness, drowsiness, nervousness, tremor, headache, nausea, dermatological symptoms, vision problems/double vision). Subjects were asked to rate the presence and severity of impairments on a four-tiered scale ranging from very good (0) to very bad (3). Total scores for each domain were calculated.

Questionnaire on the evaluation of the device. Seven ordinal questions were asked concerning handling, possibility to continue activities while using the device, feeling while using the device, comfort, suitability for long-term and repeated use.

EEG recordings and data pre-processing. We recorded electroencephalograms (EEG) from 19 electrode sites according to the 10–20 system and Cz served as physical reference. EEG data were sampled at 256 Hz using a 16 bit analogue-to-digital converter and were band-pass filtered offline between 1–45 Hz (4th order Butterworth characteristic). Additionally, a notch filter (3rd order) was used to suppress contributions at the line frequency (50 Hz). We visually inspected all recordings for strong artefacts such as subject movements, amplifier saturation, or stimulation artefacts. Such data were excluded from further analyses.

We used a sliding-window approach[44–46] to calculate a synchronisation index r_{ij} (mean phase coherence[47]) between phase time series (derived adaptively with Hilbert transform[48]) from all pairs of brain regions (i, j) sampled by the EEG electrodes. Non-overlapping windows had duration of 20 s (5120 data points), which represents a compromise between the required statistical accuracy for the calculation of r_{ij} and approximate stationarity within a window length.

The synchronisation index serves as an indicator for the strength of functional interactions in the epileptic brain network[45] and is confined to the unit interval: $r_{ij} = 1$ indicates fully phase-synchronised brain regions and $r_{ij} = 0$ indexes no phase synchronisation. For subsequent analyses, we associated the sampled brain regions with network vertices and the calculated phase synchronisation indices between any pair of vertices with network edges. This resulted in a time-dependent sequence of weighted and fully connected brain networks.

Network characteristics. In addition to global synchronisation level R (mean over all non-redundant pairwise synchronisation indices), we assessed four relevant global characteristics for each network that we derived from the time-resolved synchronisation analysis of the 3-h EEG recording prior to (phase 1), during (phase 2), and after taVNS (phase 3): average shortest path length L, average clustering coefficient C, synchronisability S, and assortativity A. In order to remove possible transient effects, we neglected data from the first and last 15 min of each phase.

The average shortest path length L is defined as the average number of steps along the shortest paths for all possible pairs of network vertices. For our weighted networks, we defined the 'length' of a path between a pair of vertices as the inverse of the weight of the edge that connects the vertices[20] and used an algorithm proposed by Dijkstra[49] to compute L. The clustering coefficient is a measure of the degree to which vertices in a network tend to cluster together. We made use of a definition of the clustering coefficient in a weighted network[50] and calculated the average clustering coefficient C as the mean of clustering coefficients computed for all vertices. Synchronisability S is a measure of the stability of the network's synchronised state[51,52]. We computed S from the ratio of the largest and smallest non-vanishing eigenvalue that we calculated for the network's Laplacian[53]. To assess assortativity A of the networks[54], we estimated the Pearson correlation coefficient between the degrees of vertices at both ends of an edge[55]. To this end, we derived a connected binary network from the weighted network by thresholding thereby requiring a constant edge density. A is confined to the interval $[-1, 1]$ by definition. Positive (negative) values of A indicate an assortative (disassortative) network.

Statistical analyses. Differences between network characteristics from the three phases (phase 1: pre-stimulation; phase 2: during stimulation; phase 3: post-stimulation; see Fig. 1) were investigated on a per-subject basis using the Mann–Whitney U-test (phase 1 vs. phase 2, phase 1 vs. phase 3, and phase 2 vs. phase 3). For

downstream network analyses, we only considered data from subjects for whom we attained significant differences after Bonferroni correction ($p < 0.05$). Group level (all subjects) differences between neuropsychological variables from the phases prior to and after the EEG recording (NP1 vs. NP2; see Fig. 1) were investigated using the Mann–Whitney U-test ($p < 0.05$). Finally, we probed for possible relationships between the aforementioned changes in neuropsychological variables and (a) network characteristics (temporal means) from the three phases and (b) relative changes of network characteristics between the three phases (relative changes calculated as $\Delta = (M_l - M_k)/M_k$, where M_k and M_l denote placeholders for the temporal means of the respective characteristics from phase k and phase l). Relationships were deemed significant after Bonferroni correction (Pearson correlation coefficient; $p < 0.05$).

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request. The data are not publicly available as they contain information that could compromise the privacy of research participants.

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References

1. Kwan, P. & Brodie, M. J. Early Identification of Refractory Epilepsy. *N. Engl. J. Med.* **342**, 314–319 (2000).
2. Chen, Z., Brodie, M. J., Liew, D. & Kwan, P. Treatment outcomes in patients with newly diagnosed epilepsy treated with established and new antiepileptic drugs a 30-year longitudinal cohort study. *JAMA Neurol.* **75**, 279–286 (2018).
3. Adair, D. *et al.* Electrical stimulation of cranial nerves in cognition and disease. *Brain Stimul.* **13**, 717–750 (2020).
4. Elliott, R. E. *et al.* Vagus nerve stimulation in 436 consecutive patients with treatment-resistant epilepsy: Long-term outcomes and predictors of response. *Epilepsy Behav.* **20**, 57–63 (2011).
5. Morris, G. L. *et al.* Evidence-based guideline update: vagus nerve stimulation for the treatment of epilepsy: Report of the Guideline Development Subcommittee of the American Academy of Neurology. *Neurology* **81**, 1453–1459 (2013).
6. Stefan, H. *et al.* Transcutaneous vagus nerve stimulation (t-VNS) in pharmacoresistant epilepsies: A proof of concept trial. *Epilepsia* **53**, e115–e118 (2012).
7. Bauer, S. *et al.* Transcutaneous vagus nerve stimulation (tVNS) for treatment of drug-resistant epilepsy: a randomized, double-blind clinical trial (cMPsE02). *Brain Stimul.* **9**, 356–363 (2016).
8. Liu, A. *et al.* Efficacy and safety of treatment with transcutaneous vagus nerve stimulation in 17 patients with refractory epilepsy evaluated by electroencephalogram, seizure frequency, and quality of life. *Med. Sci. Monit.* **24**, 8439–8448 (2018).
9. Barbella, G. *et al.* Transcutaneous vagal nerve stimulatio (t-VNS): An adjunctive treatment option for refractory epilepsy. *Seizure* **60**, 115–119 (2018).
10. von Wrede, R., Moskau-Hartmann, S., Rüber, T., Helmstaedter, C. & Surges, R. Sustained seizure freedom with transcutaneous vagal nerve stimulation in drug-resistant epilepsy caused by subcortical band heterotopias. *Seizure* **70**, 25–26 (2019).
11. Butt, M. F., Albusoda, A., Farmer, A. D. & Aziz, Q. The anatomical basis for transcutaneous auricular vagus nerve stimulation. *J. Anat.* **236**, 588–611 (2020).
12. Ellrich, J. Transcutaneous auricular vagus nerve stimulation. *J. Clin. Neurophysiol.* **36**, 437–442 (2019).
13. Boon, P., De Cock, E., Mertens, A. & Trinka, E. Neurostimulation for drug-resistant epilepsy: a systematic review of clinical evidence for efficacy, safety, contraindications and predictors for response. *Curr. Opin. Neurol.* **31**, 198–210 (2018).
14. Jarosiewicz, B. & Morrell, M. The RNS system: Brain-responsive neurostimulation for the treatment of epilepsy. *Expert Rev. Med. Devices* **2020**, 1–10 (2020).
15. Ben-Menachem, E. Vagus-nerve stimulation for the treatment of epilepsy. *Lancet Neurol.* **1**, 477–482 (2002).
16. Rutecki, P. Anatomical, physiological, and theoretical basis for the antiepileptic effect of vagus nerve stimulation. *Epilepsia* **31**, S1–S6 (1990).
17. Koo, B. EEG changes with vagus nerve stimulation. *J. Clin. Neurophysiol.* **18**, 434–441 (2001).
18. de Vos, C. C. *et al.* Predicting success of vagus nerve stimulation (VNS) from interictal EEG. *Seizure* **20**, 541–545 (2011).
19. Bullmore, E. & Sporns, O. Complex brain networks: Graph theoretical analysis of structural and functional systems. *Nat. Rev. Neurosci.* **10**, 186–198 (2009).
20. Lehnertz, K. *et al.* Evolving networks in the human epileptic brain. *Physica D* **267**, 7–15 (2014).
21. Kuhnert, M.-T. *et al.* Incidental and intentional learning of verbal episodic material differentially modifies functional brain networks. *PLoS ONE* **8**, e80273 (2013).
22. Helmstaedter, C., Hoppe, C. & Elger, C. E. Memory alterations during acute high-intensity vagus nerve stimulation. *Epilepsy Res.* **47**, 37–42 (2001).
23. Chan, A. Y., Rolston, J. D., Rao, V. R. & Chang, E. F. Effect of neurostimulation on cognition and mood in refractory epilepsy. *Epilepsia Open* **3**, 18–29 (2018).
24. Fraschini, M. *et al.* VNS induced desynchronization in gamma bands correlates with positive clinical outcome in temporal lobe pharmacoresistant epilepsy. *Neurosci. Lett.* **536**, 14–18 (2013).
25. Bodin, C. *et al.* Responders to vagus nerve stimulation (VNS) in refractory epilepsy have reduced interictal cortical synchronicity on scalp EEG. *Epilepsy Res.* **113**, 98–103 (2015).
26. Bartolomei, F. *et al.* How does vagal nerve stimulation (VNS) change EEG brain functional connectivity?. *Epilepsy Res.* **126**, 141–146 (2016).
27. Wang, Z.-J. *et al.* Alteration in brain connectivity in patients with Dravet syndrome after vagus nerve stimulation (VNS): exploration of its effectiveness using graph theory analysis with electroencephalography. *J. Neural Eng.* **17**, 36014 (2020).
28. Geier, C., Lehnertz, K. & Bialonski, S. Time-dependent degree-degree correlations in epileptic brain networks: from assortative to dissortative mixing. *Front. Hum. Neurosci.* **9**, 462 (2015).
29. Chiosa, V. *et al.* Large-scale network architecture and associated structural cortico-subcortical abnormalities in patients with sleep/awake-related seizures. *Sleep* **42**, zsz006 (2019).
30. Mertens, A. *et al.* Transcutaneous vagus nerve stimulation does not affect verbal memory performance in healthy volunteers. *Front. Psychol.* **11**, 551 (2020).
31. Vonck, K. *et al.* Vagus nerve stimulation... 25 years later! What do we know about the effects on cognition?. *Neurosci. Biobehav. Rev.* **45**, 63–71 (2014).
32. Colzato, L. & Beste, C. A literature review on the neurophysiological underpinnings and cognitive effects of transcutaneous vagus nerve stimulation: challenges and future directions. *J. Neurophysiol.* **123**, 1739–1755 (2020).

33. Clark, K. B., Naritoku, D. K., Smith, D. C., Browning, R. A. & Jensen, R. A. Enhanced recognition memory following vagus nerve stimulation in human subjects. *Nat. Neurosci.* **2**, 94–98 (1999).
34. Redgrave, J. *et al.* Safety and tolerability of Transcutaneous Vagus Nerve stimulation in humans; a systematic review. *Brain Stimul.* **11**, 1225–1238 (2018).
35. Follesa, P. *et al.* Vagus nerve stimulation increases norepinephrine concentration and the gene expression of BDNF and bFGF in the rat brain. *Brain Res.* **1179**, 28–34 (2007).
36. Cadeddu, C. *et al.* Cardiovascular modulation during vagus nerve stimulation therapy in patients with refractory epilepsy. *Epilepsy Res.* **92**, 145–152 (2010).
37. Yap, J. Y. Y. *et al.* Critical review of transcutaneous vagus nerve stimulation: challenges for translation to clinical practice. *Front. Neurosci.* **14**, 284 (2020).
38. Naritoku, D. K., Terry, W. J. & Helfert, R. H. Regional induction of fos immunoreactivity in the brain by anticonvulsant stimulation of the vagus nerve. *Epilepsy Res.* **22**, 53–62 (1995).
39. Musselman, E. D., Pelot, N. A. & Grill, W. M. Empirically based guidelines for selecting vagus nerve stimulation parameters in epilepsy and heart failure. *Cold Spring Harb. Perspect. Med.* **9**, 2019 (2019).
40. Kwan, P. *et al.* Definition of drug resistant epilepsy: consensus proposal by the ad hoc Task Force of the ILAE Commission on Therapeutic Strategies. *Epilepsia* **51**, 1069–1077 (2010).
41. Helmstaedter, C. *EpiTrack® - Veränderungssensitives kognitives Screening zur Beurteilung der Aufmerksamkeit und der Exekutivfunktionen für die Qualitäts- und Ergebniskontrolle der Behandlung von Patienten mit Epilepsie* (Eisai GmbH, Frankfurt a. M., 2019).
42. Helmstaedter, C. & Lendt, M., & Lux, S. VLMT. Verbaler Lern-und Merkfähigkeitstest (Beltz Test GmbH, Göttingen, 2001).
43. Gilliam, F. G. *et al.* Rapid detection of major depression in epilepsy: A multicentre study. *Lancet Neurol.* **5**, 399–405 (2006).
44. Kuhnert, M.-T., Elger, C. E. & Lehnertz, K. Long-term variability of global statistical properties of epileptic brain networks. *Chaos* **20**, 043126 (2010).
45. Dickten, H., Porz, S., Elger, C. E. & Lehnertz, K. Weighted and directed interactions in evolving large-scale epileptic brain networks. *Sci. Rep.* **6**, 34824 (2016).
46. Rings, T., von Wrede, R. & Lehnertz, K. Precursors of seizures due to specific spatial-temporal modifications of evolving large-scale epileptic brain networks. *Sci. Rep.* **9**, 10623 (2019).
47. Mormann, F., Lehnertz, K., David, P. & Elger, C. E. Mean phase coherence as a measure for phase synchronization and its application to the EEG of epilepsy patients. *Physica D* **144**, 358–369 (2000).
48. Osterhage, H., Mormann, F., Staniek, M. & Lehnertz, K. Measuring synchronization in the epileptic brain: A comparison of different approaches. *Int. J. Bifurc. Chaos Appl. Sci. Eng* **17**, 3539–3544 (2007).
49. Dijkstra, E. W. A note on two problems in connexion with graphs. *Numer. Mat.* **1**, 269–271 (1959).
50. Onnela, J. P., Saramäki, J., Kertész, J. & Kaski, K. Intensity and coherence of motifs in weighted complex networks. *Phys. Rev. E* **71**, 065103 (2005).
51. Pecora, L. M. & Carroll, T. L. Master stability functions for synchronized coupled systems. *Phys. Rev. Lett.* **80**, 2109–2112 (1998).
52. Barahona, M. & Pecora, L. M. Synchronization in small-world systems. *Phys. Rev. Lett.* **89**, 54101 (2002).
53. Atay, F. M., Bıyıkoğlu, T. & Jost, J. Network synchronization: Spectral versus statistical properties. *Physica D* **224**, 35–41 (2006).
54. Newman, M. E. J. Mixing patterns in networks. *Phys. Rev. E* **67**, 026126 (2003).
55. Bialonski, S. & Lehnertz, K. Assortative mixing in functional brain networks during epileptic seizures. *Chaos* **23**, 033139 (2013).

Author contributions

All authors designed the study, analysed the data and wrote the manuscript. All authors reviewed the manuscript.

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Competing interests

RvW received once a fee for lecture from Cerbomed in 2016. The other authors declare that they have no competing interests.

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Rings T*, **von Wrede R*;** Bröhl T*, Helmstaedter C, Lehnertz K. (2021). Impact of transcutaneous auricular vagus nerve stimulation on large-scale functional brain networks: from local to global. *Front Physiol.* 2021 Aug 20;12:700261.

Zielsetzung der Arbeit: In dieser Arbeit wurde der Einfluss der taVNS auf sich entwickelnde funktionelle Hirnnetzwerke auf lokaler und globaler Netzwerkebene untersucht.

Methoden und Ergebnisse: 30 Proband*innen mit und ohne Erkrankungen des zentralen Nervensystems (ZNS) durchliefen ein Studienprotokoll mit einer kontinuierlichen mindestens 3-stündigen EEG-Vielkanal-Aufzeichnung. Aus der zeitaufgelösten Synchronisationsanalyse dieser EEG-Vielkanal-Aufzeichnung wurden sich entwickelnde, vollständig verbundene und gewichtete Netzwerke abgeleitet und verschiedene lokale (Betweenness-Zentralität C^B und Eigenvektor-Zentralität C^E) und globale (C, L, A, S) Kenngrößen erhoben. Im Allgemeinen bewirkte die taVNS bei fast allen Knoten und Kanten Veränderungen der Zentralitätswerte, allerdings ohne ein klares räumliches Muster, was auf taVNS-induzierte Umstrukturierungen des globalen funktionellen Netzwerks verweist. Die höhere Anzahl signifikanter taVNS-induzierter Veränderungen von Knoten und Kanten bei der Eigenvektor-Zentralität legt nahe, dass die Umstrukturierungen innerhalb des globalen funktionellen Netzwerks höchstwahrscheinlich eher mit Veränderungen der Interaktionsstärke von Netzwerkbestandteilen als mit einer Modifikation der Pfadstruktur des Netzwerks zu erklären ist. TaVNS-induzierte Veränderungen der wichtigsten Knoten und Kanten konnten nicht beobachtet werden, so dass die oben benannten Veränderungen somit nicht die Wichtigkeitshierarchie von Knoten und Kanten zu beeinflussen scheinen.

Die taVNS-induzierten Veränderungen der Kenngrößen globaler Netzwerkeigenschaften zwischen den einzelnen Phasen zeigten für die topologische Kenngröße der Netzwerksegregation C sowohl einen immediaten als auch einen anhaltenden Effekt der Abnahme der Segregation; für die topologische Kenngröße der Netzwerkintegration L ein ähnliches, wenn auch invertiertes Muster wie bei C (sowohl immediater als auch anhaltender Effekt mit Zunahme der Integration). Für die Assortativität A ließ sich eine geringe immediate stimulationsbedingte Abnahme sowie eine anhaltende Robustheitsverbesserung bei jedoch nur einer geringen Proband*innenzahl beobachten. Für die Synchronisierbarkeit S zeigte sich eine geringe immediate stimulationsbedingte Erhöhung der Stabilität sowie eine anhaltende Abnahme der Stabilität eines potenziell synchronisierten Zustands der Netzwerke.

Schlussfolgerungen: Die Ergebnisse deuten auf unterschiedliche, auf den ersten Blick kontraintuitive, taVNS-vermittelte Veränderungen lokaler und globaler topologischer Netzwerkcharakteristika hin, die zu einer Rekonfiguration von Hirnnetzwerken und einer Veränderung ihrer Stabilitäts- und Robustheitseigenschaften führen. Es wird ein Modell der stimulationsbedingten „Dehnung“ und „Komprimierung“ von sich entwickelnden funktionellen Netzwerken vorgeschlagen, um die Ergebnisse zu lokalen und globalen Veränderungen erklären zu können.

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Impact of Transcutaneous Auricular Vagus Nerve Stimulation on Large-Scale Functional Brain Networks: From Local to Global

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Transcutaneous auricular vagus nerve stimulation (taVNS) is a novel non-invasive brain stimulation technique considered as a potential supplementary treatment option for a wide range of diseases. Although first promising findings were obtained so far, the exact mode of action of taVNS is not fully understood yet. We recently developed an examination schedule to probe for immediate taVNS-induced modifications of large-scale epileptic brain networks. With this schedule, we observed short-term taVNS to have a topology-modifying, robustness- and stability-enhancing immediate effect on large-scale functional brain networks from subjects with focal epilepsies. We here expand on this study and investigate the impact of short-term taVNS on various local and global characteristics of large-scale evolving functional brain networks from a group of 30 subjects with and without central nervous system diseases. Our findings point to differential, at first glance counterintuitive, taVNS-mediated alterations of local and global topological network characteristics that result in a reconfiguration of networks and a modification of their stability and robustness properties. We propose a model of a stimulation-related stretching and compression of evolving functional brain networks that may help to better understand the mode of action of taVNS.

Keywords: evolving functional brain network, brain stimulation, network reconfiguration, network characteristics, centrality, EEG

1. INTRODUCTION

Vagus nerve stimulation (VNS) is an established method of brain stimulation in several diseases, including depression and epilepsy. Studies on invasive vagus nerve stimulation (iVNS) have demonstrated its effectiveness in these diseases (Elliott et al., 2011; Bottomley et al., 2020). However, being an invasive method it needs anesthesia and comprises surgical risks. Therefore, transcutaneous auricular vagus nerve stimulation (taVNS), a non-invasive external stimulation of the auricular branch of the vagus nerve, seems to be an interesting alternative. Efficacy of taVNS could be demonstrated for refractory epilepsy (Bauer et al., 2016; Liu et al., 2018; von Wrede et al., 2019) and depression (Hein et al., 2013; Fang et al., 2016; Kong et al., 2018; Tu et al., 2018). Good tolerability and usability have been demonstrated for taVNS in different health

conditions (Redgrave et al., 2018). Non-invasiveness, reversibility and the possibility of a rapid start of therapy broaden the spectrum of symptoms and diseases that can be treated. Clinical applications and investigations span from cardiovascular and digestive system diseases (Kaniusas et al., 2019; Wang et al., 2020), insomnia (Wu et al., 2021), tinnitus (Stegeman et al., 2021), pain (Kaniusas et al., 2019), migraine (Straube et al., 2015) as well as to disorders of consciousness (Briand et al., 2020), aging (Bretherton et al., 2019), and cognitive impairment (Colzato and Beste, 2020).

Apart from more clinically-oriented questions on efficacy and safety there is a growing body on basic research that investigates the mode of action of taVNS in healthy subjects as well as in subjects with different diseases. For taVNS and iVNS similar projections to the nucleus of the solitary tract (NTS) and resembling pattern of brain activation could be shown (Ellrich, 2019). Studies on taVNS show widespread activity in expected vagal projections areas, including NTS, locus coeruleus, hypothalamus, thalamus, amygdala, hippocampus, as well as the prefrontal cortex and other widespread areas (Yap et al., 2020), though interpretation is difficult due to different study protocols and investigated subjects. Given these observations, we hypothesized that the impact of the global, apparently unspecific VNS-mediated activation of the brain can be suitably assessed with an analysis approach which makes use of EEG-derived, evolving functional brain networks (Bullmore and Sporns, 2009; Lehnertz et al., 2014). In a previous study (von Wrede et al., 2021), we could demonstrate that short-term taVNS has a topology-modifying, robustness- and stability-enhancing immediate effect on such brain networks derived from subjects with focal epilepsy. We here extend our investigations on short-term, taVNS-mediated modifications of global network characteristics beyond focal epilepsies and by considering local aspects related to possible modifications of individual network constituents.

2. MATERIALS AND METHODS

2.1. Subjects

We investigated evolving functional brain networks from 30 subjects (20 females; age 18–55 years; median 31 years) with and without central nervous system (CNS) diseases. All subjects volunteered to participate and signed informed consent after being provided with written information and being given the opportunity to ask further questions. The study protocol had been approved by the ethics committee of the University of Bonn before the study has started. All experiments were performed in accordance with relevant guidelines and regulations. For subjects that received CNS medication, this was kept stable, and no activation methods (such as change in medication, hyperventilation, or sleep deprivation) were applied at least 24 h before stimulation.

2.2. Transcutaneous Auricular Vagus Nerve Stimulation and EEG Recording

Following von Wrede et al. (2021), we applied taVNS with individualized stimulation intensities (range: 0.5–5.0 mA, mean 2.2, SD ±1.1) for 1 h in the early afternoon while subjects underwent a continuous 3 h EEG recording. The stimulation phase ("S"; continuous stimulation of the left cymba conchae) was preceded and followed by 1-h pre- and post-stimulation phase (baseline phases "B1" and "B2"). Stimulation was carried out with two hemispheric titanium electrodes of a NEMOS device (tVNS Technologies GmbH, Erlangen, Germany) fitted in the left cymba conchae and using a common set of non-adjustable parameters (biphasic signal form, impulse duration 20 s, impulse pause 30 s, impulse frequency 25 Hz). Intensity of stimulation was adjusted individually and was raised slowly until the subject noticed a "tingling," but no pain.

We recorded electroencephalograms (EEG) from electrode sites according to the 10-20 system and Cz served as physical reference. EEG data were sampled at 256 Hz using a 16 bit analog-to-digital converter and were band-pass filtered offline between 1 and 45 Hz (4th order Butterworth characteristic). Additionally, a notch filter (3rd order) was used to suppress contributions at the line frequency (50 Hz). We visually inspected all recordings for strong artifacts such as subject movements, amplifier saturation, or stimulation artifacts. Such data were labeled for further analyses.

2.3. Constructing Functional Brain Networks

We followed previous studies (Kuhnert et al., 2010; Dickten et al., 2016; Rings et al., 2019; Fruengel et al., 2020; von Wrede et al., 2021) and used a sliding-window approach to calculate a synchronization index r_{nm} [mean phase coherence (Mormann et al., 2000); see **Appendix A1** for details] between phase time series from all pairs of brain regions (n, m) sampled by the $N = 18$ EEG electrodes. We derived these phase time series adaptively with the Hilbert transform from the respective EEG time series (Osterhage et al., 2007). Non-overlapping windows (with index w) had a duration of 20 s (5,120 data points), which represents a compromise between the required statistical accuracy for the calculation of r_{nm} and approximate stationarity within a window length (Osterhage et al., 2007; Kuhnert et al., 2013; Fruengel et al., 2020). The synchronization index was repeatedly shown to serve as an indicator for the strength of interactions in functional brain networks and is confined to the unit interval: $r_{nm} = 1$ indicates fully phase-synchronized brain regions and $r_{nm} = 0$ indexes no phase synchronization. For subsequent analyses, we excluded windows containing artifacts (on average 22% of windows from B1, 12% from S, and 20% from B2) and eventually associated network vertices with the sampled brain regions and network edges with the synchronization index values between any pair of vertices. This resulted in a time-dependent sequence of weighted and fully connected brain networks.

2.4. Scale-Dependent Characterization of Functional Brain Networks

We here utilized various graph-theoretical concepts to characterize functional brain networks on the level of single vertices and edges to the level of the whole network. Tracking

the temporal evolution of these network characteristics allowed us to investigate possible taVNS-induced alterations at the local to the global network scale.

On the level of single vertices and edges, we utilized two opposing centrality concepts, for which corresponding centrality indices are available for both vertices and edges (Bröhl and Lehnertz, 2019) (see **Appendix A2** for details). Path-based centrality indices and interaction-strength-based centrality indices were both shown previously to provide non-redundant information about important network constituents (Kuhnert et al., 2012; Geier and Lehnertz, 2017; Bröhl and Lehnertz, 2019; Bröhl and Lehnertz, 2020; Fruengel et al., 2020). Betweenness centrality $\mathcal{C}^{\mathrm{B}}$ is a shortest-path-based centrality index, which requires the definition of "length" of a path between pairs of vertices or between pairs of edges. Following Lehnertz et al. (2014) (and references therein), we related the length of the shortest path between pairs of vertices/edges to the sum of the inverse weights of edges along this path. For a pair being connected to a same vertex/edge, we set the length to zero. In case of adjacent edges, i.e., edges connected by a single vertex, we also set the length to zero. Betweenness centrality $\mathcal{C}^{\mathrm{B}}$ measures how frequently a given vertex/edge falls on the shortest path between two other vertices/edges. A vertex/edge with a high betweenness centrality acts as a bridge between other parts of the network. Eigenvector centrality $\mathcal{C}^{\mathrm{E}}$ is an interaction-strength-based centrality index and considers the influence of a vertex/edge on the network as a whole. A vertex/edge is central if the vertices/edges connected to it are also central. In the following, we refer with $\mathcal{C}_{\mathrm{v}}^{\mathrm{B}}$ and $\mathcal{C}_{\mathrm{e}}^{\mathrm{B}}$ to the vertex and edge betweenness centrality, and with $\mathcal{C}_{\mathrm{v}}^{\mathrm{E}}$ and $\mathcal{C}_{\mathrm{e}}^{\mathrm{E}}$ to the vertex and edge eigenvector centrality.

On the global network scale, we utilized the global clustering coefficient C for weighted networks (Onnela et al., 2005) and the average shortest path length L to characterize a network's global topological properties. Moreover, we utilized synchronizability S and assortativity A to assess the network's stability and robustness properties (Arenas et al., 2008; Newman, 2018) (see **Appendix A2** for details). The global clustering coefficient is a measure of the degree to which vertices in a network tend to cluster together and characterizes the network's functional segregation; the lower C, the more segregated is the network. The average shortest path length is defined as the average number of steps along the shortest paths for all possible pairs of network vertices and characterizes the network's functional integration; the lower L, the more integrated is the network. Assortativity assesses the tendency of edges to connect vertices with similar or equal properties (Newman, 2003; Bialonski and Lehnertz, 2013). If edges preferentially connect vertices of similar (dissimilar) property, such networks are called assortative (disassortative). A is confined to the interval $[-1, 1]$ by definition, where positive (negative) values indicate an assortative (disassortative) network. Disassortative networks are more vulnerable to perturbations and appear to be easier to synchronize than assortative networks. The latter show a stronger tendency to disintegrate into different groups than disassortative networks. Synchronizability S assesses the network's propensity (or vulnerability) to get synchronized by an admissible input activation: the higher S, the more easily can the synchronized state be perturbed (Pecora and Carroll, 1998; Barahona and Pecora, 2002; Atay et al., 2006).

In our downstream analyses, we neglected data from the first and last 15 min of each phase in order to remove possible transient effects. Together with the artifact removal, this resulted in data from $N_w = 70$ windows from B1, $N_w = 79$ windows from S, and $N_w = 72$ windows from B2, on average.

2.5. Statistics

We investigated differences between network characteristics from the three phases (B1, S, B2) on a per-subject basis using the Mann-Whitney U-test (B1 vs. S, B1 vs. B2, and S vs. B2; $p < 0.05$; Bonferroni correction).

3. RESULTS

In order to facilitate the analysis of possible taVNS-induced network modifications from the local to the global scale, we first investigated whether alterations can be observed for the synchronization index, which we used to define edges of our evolving functional brain networks. Given that taVNS modulates disease-related symptoms in about 30–50% of cases (Hein et al., 2013; Bauer et al., 2016) and taking into account the inhomogeneity of subjects investigated here, we did not expect to identify significant alterations on a sample level. We therefore inspected, on a single-subject level and utilizing the average $\bar{R}(w) = \frac{1}{\nu}\sum_{n\neq m} r_{nm}(w)$ over all non-redundant ($\nu = N(N-1)/2$) pairwise synchronization indices for each phase ($w \in \{\mathrm{B1}, \mathrm{S}, \mathrm{B2}\}$ denotes the window number), the following scenarios:

- **a** *taVNS has no effect*; in this case, there should be no significant differences between values of $\bar{R}$ from phases B1 and S (immediate effect) as well as between values of $\bar{R}$ from phases B1 and B2 (enduring effect);
- **b** *taVNS has an immediate effect and a short-lasting enduring effect (fast relaxation)*; in this case we expect significant difference for values of $\bar{R}$ from phases B1 and S but no significant difference for values of $\bar{R}$ from phases B1 and B2;
- **c** *taVNS has an immediate effect and a long-lasting enduring effect (slow relaxation)*; in this case we expect significant difference for values of $\bar{R}$ from phases B1 and S and for values of $\bar{R}$ from phases B1 and B2.

We observed scenario **a** in seven subjects, scenario **b** in seven subjects, and scenario **c** in 16 subjects. Stimulation parameters did not differ between these groups of subjects. For all downstream analyses, we pooled the data from subjects identified in scenarios **b** and **c**. Exemplary time courses of local and global network characteristics are presented in **Figure 1**, and in **Table 1**, we report statistical moments of local and global network characteristics from phase B1 from the sample.

3.1. Impact on Local Network Characteristics

Having identified a group of subjects with taVNS-induced immediate and short-/long-lasting enduring alterations of edge

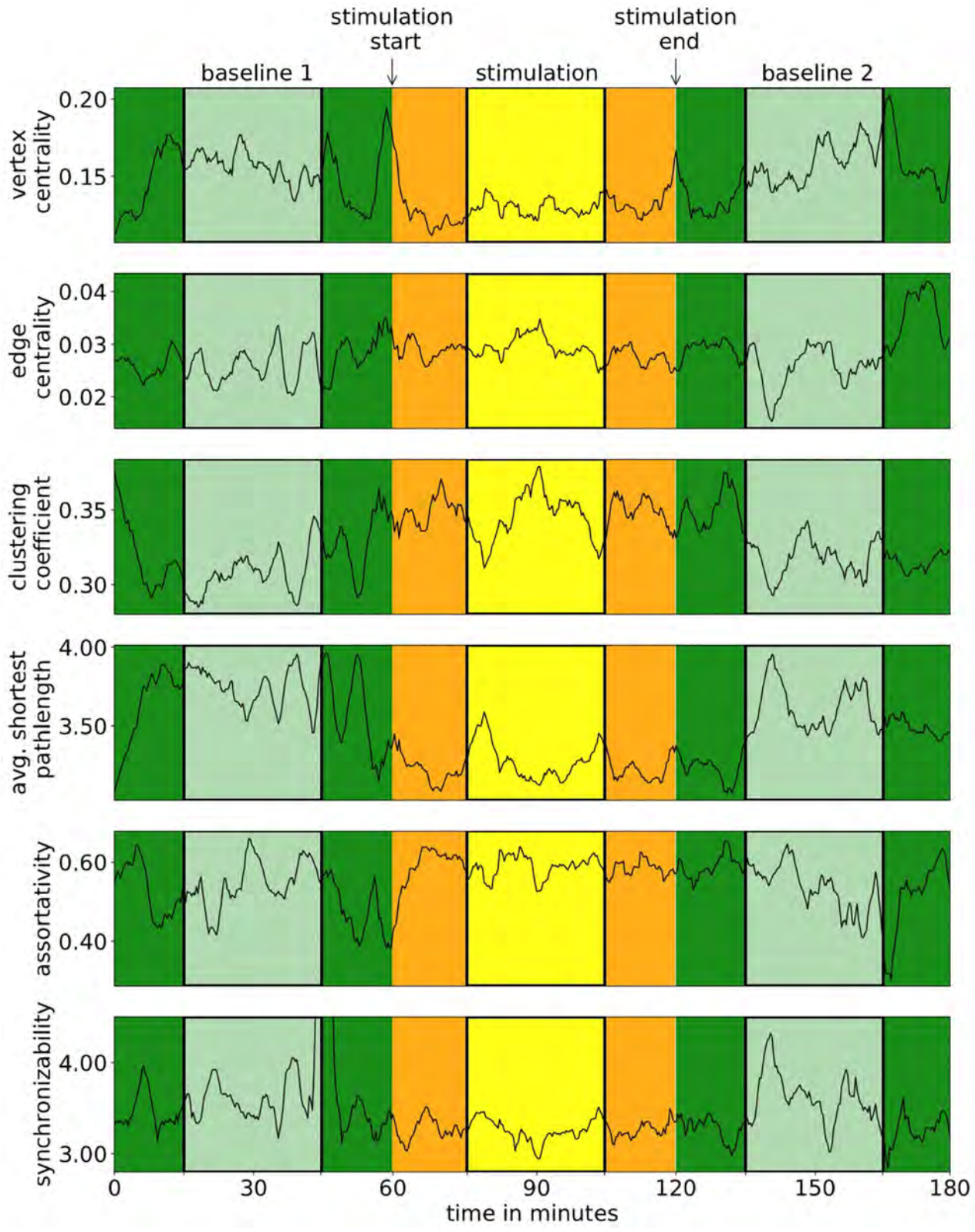

FIGURE 1 | Exemplary time courses of local and global network characteristics of one subject. In our analyses, we neglected data from the first and last 15 min of each phase (darker colors) in order to remove possible transient effects.

TABLE 1 | Descriptive statistic of local (spatially averaged betweenness centralities ($\overline{\mathcal{C}_v^B}$ and $\overline{\mathcal{C}_e^B}$) and eigenvector centralities ($\overline{\mathcal{C}_v^E}$ and $\overline{\mathcal{C}_e^E}$) and global network characteristics (global clustering coefficient C, average shortest path length L, assortativity A, and synchronizability S), from phase B1 for the $N_s = 23$ subjects with a taVNS-induced immediate effect and a short-/long-lasting enduring effect. For Gaussian distributed data, skewness and (excess) kurtosis would be zero with standard deviation $\sqrt{6/N_s} \approx 0.51$, resp. $\sqrt{24/N_s} \approx 1.02$.

	$\overline{\mathcal{C}_v^B}$	$\overline{\mathcal{C}_v^E}$	$\overline{\mathcal{C}_e^B}$	$\overline{\mathcal{C}_e^E}$	C	L	A	S
Mean	0.03	0.22	0.01	0.07	0.34	3.30	0.41	3.05
Median	0.03	0.22	0.01	0.07	0.34	3.28	0.46	3.01
Std. dev.	0.00	0.00	0.00	0.00	0.04	0.38	0.17	0.38
Skewness	−0.47	−0.09	−0.47	0.02	0.78	−0.16	−0.51	0.60
Kurtosis	0.23	−0.94	0.23	−0.98	0.53	−0.80	−0.43	−0.15

weights of their evolving functional brain networks, we next investigated the impact of taVNS on other local network characteristics, namely centralities of vertices and edges. In the upper part of **Figure 2**, we provide a detailed picture of stimulation-related alterations of centralities of single vertices and edges. We highlight those network constituents for which we obtained significant changes in their centralities on a per-subject base. Depending on the centrality concept used, we observed some vertices and edges to exhibit taVNS-induced alterations in a higher percentage of subjects. In general, however, taVNS apparently induced alterations in centrality values of almost all vertices and edges, and no clear-cut substructures could be observed. These findings point to taVNS-induced rearrangements of the larger functional network. Since we observed, in general, a higher number of significant taVNS-induced changes with eigenvector centrality, the rearrangements within the larger functional network most likely are associated with changes in strongly connected network constituents rather than with modifications of the network's path-structure.

To examine whether taVNS impacts on the importance of individual vertices and edges, we next regarded a network constituent with the highest centrality value as most important (Lü et al., 2016) and the one with the lowest centrality value as least important (in the case of equal centrality values, we rank in the order of appearance; Liao et al., 2017). As shown in the lower part of **Figure 2**, different centrality concepts identified—in a high percentage of subjects—different brain regions (vertices) and functional connections (edges) between them as most important, as expected. Moreover, important edges frequently connected important vertices, and both these observations confirm previous studies (Kuhnert et al., 2012; Geier and Lehnertz, 2017; Bröhl and Lehnertz, 2019). Interestingly though, we could not identify taVNS-induced modifications of most important vertices and edges. Although this might, at first glance, contradict our findings of a large amount of constituents with significant taVNS-induced changes in their centrality values, these globally observed alterations not necessarily affect parts of the importance hierarchy of vertices and edges, such as the most important ones.

Summarizing, we observed characteristics of individual network constituents to be affected by taVNS but without a discernible spatial pattern of specific brain regions or of interactions between brain regions. This observation appears to be in line with the popular view that VNS leads to a rather unspecific, global activation of various brain structures. Interestingly, our findings also indicate that global taVNS-induced effects are constraint by the maintenance of the structure of shortest paths in a majority of subjects between the pre-stimulation phase and the stimulation phase (B1 → S). This—in combination with the changes seen for interaction-strength-based local network characteristic—hints at a possible mechanism describing the effects of taVNS.

3.2. Impact on Global Network Characteristics

We proceed with investigating the impact of taVNS on the global network characteristics global clustering coefficient, average shortest path length, assortativity, and synchronizability. In **Figure 3**, we provide a detailed picture of relative stimulation-related changes of these characteristics for networks transiting between the different phases. We show and report changes that were statistically significant on a per-subject base and note that—depending on the characteristic under investigation—not all of the initially selected 23 subjects presented with significant taVNS-induced alterations. We report the size N_s' of the respective subgroups in the following.

For the global clustering coefficient C, we observed medians to increase from the pre-stimulation baseline B1 to the stimulation phase S by 5.8% ($N_s' = 22$) and to decrease when networks transit back to the post-stimulation baseline (S → B2: −3.6% ($N_s' = 19$). We observed a slight overshoot effect between the pre- and post-stimulation phase [B1 → B2: 5.3% ($N_s' = 17$)]. We can derive similar indications with changes of the average shortest path length L, for which we attained a similar though inverted patterning as with C (which is to be expected given the definition of a path length in a weighted network); B1 → S: −4.9% ($N_s' = 19$); S → B2: 6.8% ($N_s' = 18$), and B1 → B2: −4.7 % ($N_s' = 19$), where the latter would indicate a slight undershoot effect. For assortativity A, we observed an only minor stimulation-induced decrease [B1 → S: −2.1 % ($N_s' = 12$)]. The tendency toward a less assortative network, however, was even increased when networks transited back to the post-stimulation phase [S → B2: −14.2 %($N_s' = 14$)]. As with C, we observed a slight overshoot effect between the pre- and post-stimulation phase [B1 → B2: 6.7% ($N_s' = 11$)]. We note though, that these alterations could be observed in only about half the number of the initially selected 23 subjects. For synchronizability S, we observed relative changes that mostly compared to the ones seen for the average shortest path length L [B1 → S: −4.6% ($N_s' = 17$); S → B2: 8.0% ($N_s' = 15$)]. Between the pre- and post-stimulation phase, we noted a minor overshoot effect [B1 → B2: 2.7% ($N_s' = 14$)].

Summarizing our findings obtained for the global network scale, we conclude that taVNS indeed induced

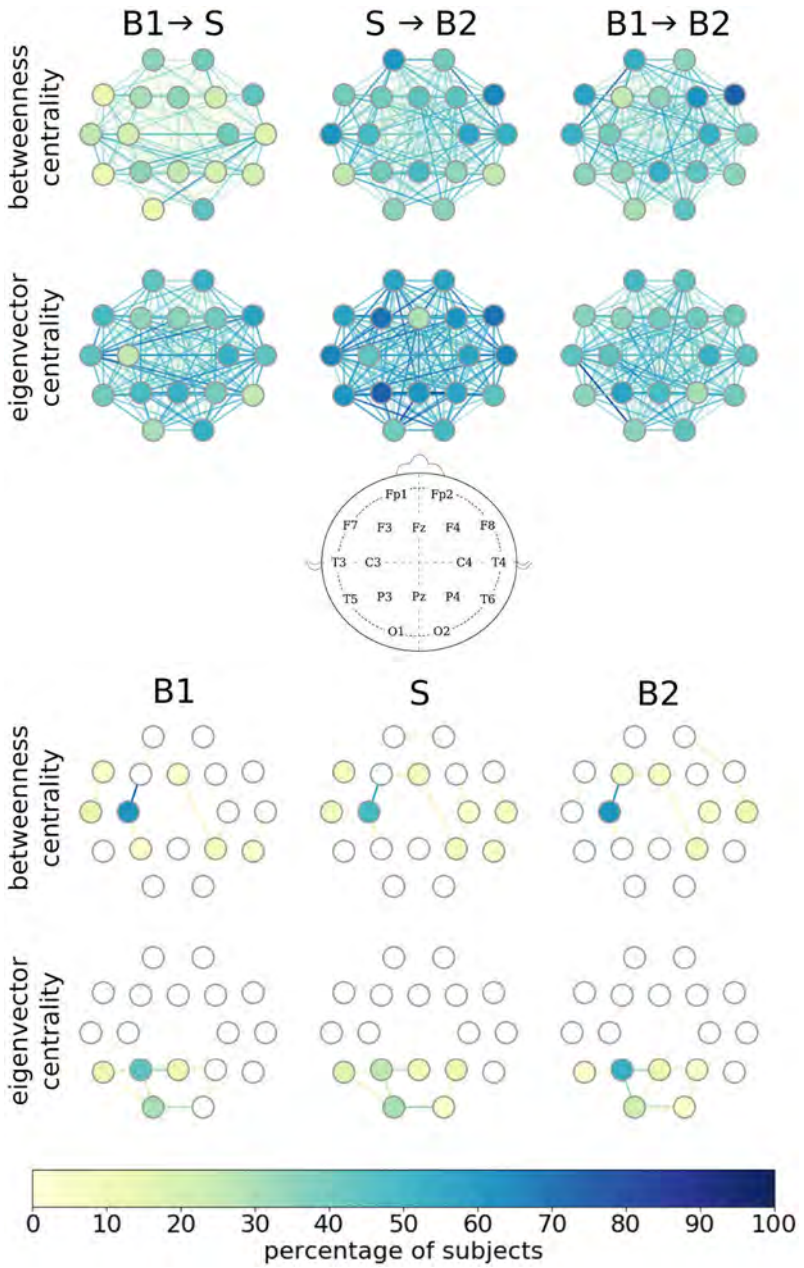

FIGURE 2 | Alterations in local network characteristics when functional brain networks transit from the baseline (B1) to the taVNS stimulation phase (S) and back to the baseline (B2). Network vertices arranged according to the international 10-20 system for EEG recording (middle plot). **(Top)** Vertices and edges with significant stimulation-induced changes of their betweenness/eigenvector centralities. Color coding of individual network constituents according to the percentage of subjects for whom we obtained significant changes in vertex/edge centralities on a per-subject base. **(Bottom)** Most important vertices and edges during phases B1, S, and B2. Importance estimated with vertex/edge betweenness/eigenvector centrality. Color coding of individual network constituents according to the percentage of subjects for which the constituent was most important.

global modifications of evolving functional brain networks in tandem with the local modifications described above. These global modifications were a reorganization of the networks' topologies, where the networks' segregation was reduced during stimulation in comparison to the pre-stimulation phase while the networks' integration was increased. As with the observed local modifications, this indicates a global activation of various brain structures, that—while spatially unspecific on the local scale—modified the topology of evolving functional brain networks and (indirectly) their stability and robustness properties in a discernible pattern.

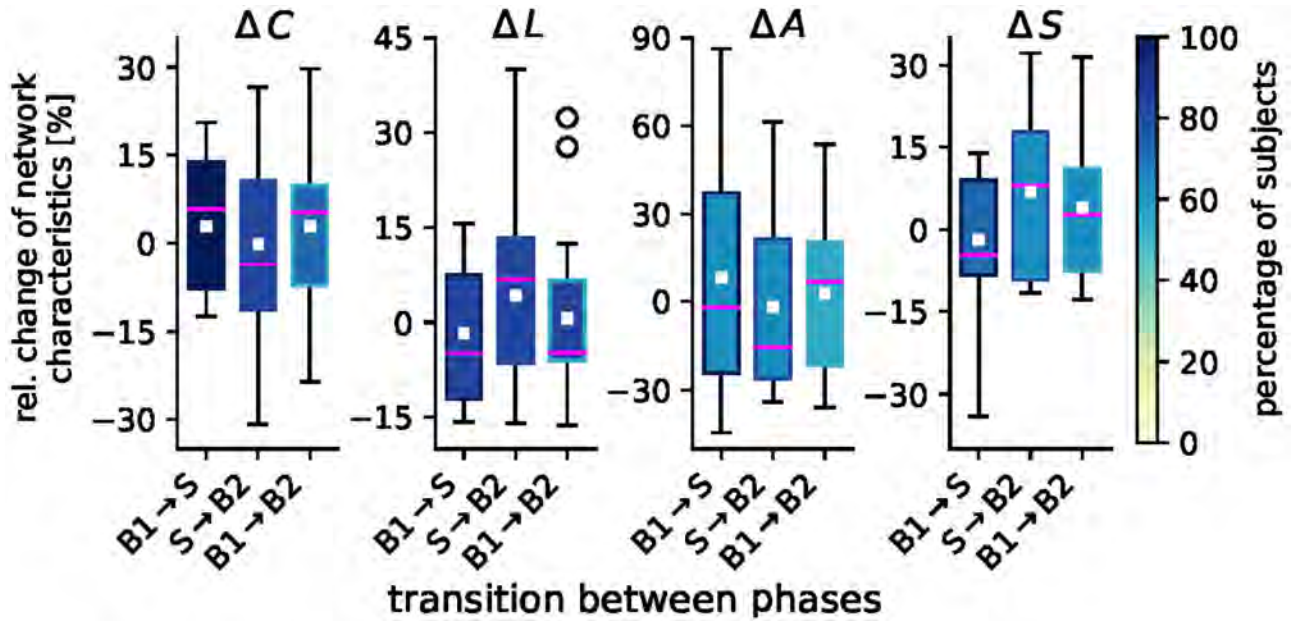

FIGURE 3 | Sample distributions of taVNS-related alterations in global network characteristics (global clustering coefficient C, average shortest path length L, assortativity A, and synchronizability S). Boxplots of significant relative changes in network characteristics $\Delta = (M_l - M_k)/M_k$, where M_k and M_l denote placeholders for the temporal average of the respective characteristics from phase k and phase l. Bottom and top of a box are the first and third quartiles, and the magenta band and the white square are the median and the mean of the distribution. The ends of the whiskers represent the interquartile range of the data. Outliers are marked by a ○ sign. Color coding of boxes according to the percentage of subjects for whom we obtained significant changes in global network characteristics on a per-subject base.

4. DISCUSSION

We investigated whether short-term transcutaneous auricular vagus nerve stimulation (taVNS) induces measurable immediate modifications of evolving functional brain networks, from the local to the global scale. In what follows, we will discuss our findings and relate them to the state of the art.

4.1. Modifications on the Local Network Scale

On the local scale of single vertices and edges, we observed taVNS to induce significant but unspecific modifications of local network characteristics (edge and vertex centralities) throughout the network. As a result, network constituents identified as most important during the pre-stimulation phase remained unaffected during the stimulation and the post-stimulation phase. As regards most important vertices from the pre-stimulation phase, our findings are in line with previous observations that reported left frontocentral brain regions to be most important with betweenness centrality (van den Heuvel and Sporns, 2013; Jin et al., 2014; Makarov et al., 2018) as well as parieto-occipital brain regions to be most important with eigenvector centrality (Lohmann et al., 2010) and closeness centrality (van den Heuvel and Sporns, 2013; Baravalle et al., 2019) during a so-called resting state condition. Together, these findings corroborate the common perspective of different centrality concepts generally identifying different constituents as most important (Lü et al., 2016; Bröhl and Lehnertz, 2019). Our findings are also in line with previous observations of most important edges connecting most important vertices, which has been shown to be rather typical in dense networks (Bröhl and Lehnertz, 2019).

4.2. Modifications on the Global Network Scale

On the global network scale, we observed taVNS to modify topological and stability- and robustness-associated network properties in a majority of subjects. In addition, we found these modifications to be enduring even after the end of the stimulation in a sizable subset of subjects (see scenarios **b** and **c**) corroborating results from a previous study (von Wrede et al., 2021). Topological network characteristics indicated on average a small stimulation-induced immediate increase in integration and a decrease in segregation of the subjects' evolving functional brain networks. After the end of the stimulation, this effect was enduring. This result contrasts findings from the study by von Wrede et al. (2021), where the same enduring effect was observed, but the immediate effect of the stimulation perceived to be reverse (in median a decrease in integration and a increase in segregation). For stability- and robustness-associated network characteristics, we here observed almost no immediate change in assortativity, i.e., in the vulnerability to synchronize. On the other hand,

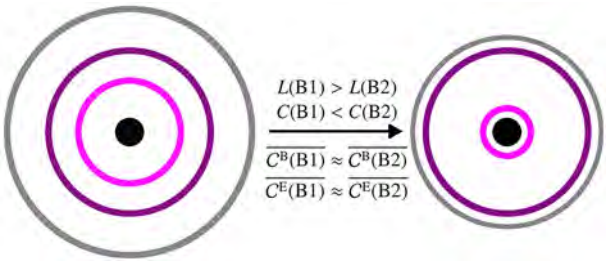

FIGURE 4 | Schematic of a spatial network prior to stimulation (phase B1, **left**) and after stimulation (phase B2, **right**). The network is separated into different areas based on global clustering coefficient *C* and average shortest path length *L*: boundary (gray outermost ring), periphery (purple and pink rings in the middle), core (black innermost ring/circle). The larger the radius of a ring the higher the average shortest path length and the smaller the average clustering coefficient of vertices in this area. The closer two rings the more clustered are the vertices in the two areas relative to each other. Averaged centralities ($\overline{C^B}$ and $\overline{C^E}$) are not affected by the stimulation.

in the post-stimulation phase we observed an enduring effect of an increased robustness against synchronization when compared to the pre-stimulation phase. Changes in synchronizability indicated an immediate increase of the stability of a potential synchronized state of the networks during taVNS and a more easily perturbed synchronized state after the stimulation. Again, the enduring effect on stability- and robustness-associated network characteristics was comparable to findings from von Wrede et al. (2021) and the immediate effect was different. In the previous study, the immediate stimulation-induced effect also indicated a decreased vulnerability to synchronize and a decreased stability of the synchronized state. The discrepancies between the current and the study by von Wrede et al. (2021) are possibly the result of our larger group of subjects (30 vs. 14) and the inclusion of subjects with a broader range of different pathologies and etiologies, which also may assist in explaining the larger inter-subject variability of modifications of global network characteristics observed here. Further studies might shed light on the existence of different subgroups of subjects and the influence of such subgroups on taVNS-induced modifications of topological and stability- and robustness-associated network properties.

4.3. A Model for taVNS-Mediated Modifications of Functional Brain Networks

The differential, taVNS-mediated modifications of local and global topological characteristics of evolving functional brain networks may appear contradictory, at first glance. This inconsistency, however, can be resolved when considering the following model of a stimulation-induced *stretching* and *compression* of the network (see **Figure 4**; cf. Fruengel et al., 2020), which may be due to some nonlinear mechanism. The stimulation-induced increase of global clustering coefficient and decrease of average shortest path length points to an, on average, global compression of the evolving functional brain network. However, since spatially and temporally averaged centrality values remained constant, the compression is compensated by some stretching effect. Even though the functional brain network is a fully connected network and has no actual spatial embedding, we can nevertheless visualize (at least conceptually) the stretching- and compression-mediated network modifications by making use of the definitions of path and strength (i.e., weighted degree) in our network approach. To this end, we consider a spatial network scheme in which we separate the network in different areas based on their different global properties (global clustering coefficient and average shortest path length). In **Figure 4**, these areas—referred to here as core, periphery, and boundary—are represented by rings. The network core contains vertices with highest strength, and we visualize this property as spatial closeness, being the innermost ring with the smallest radius. The network boundary, represented by the outermost ring, contains vertices that are positioned farthest from the core. Eventually, the network periphery (purple and pink rings) comprises everything in between core and boundary. Now, as a result of the stimulation the network periphery does not change homogeneously. The observed increase of global clustering coefficient and decrease of average shortest path length, together with an unaltered average centrality, can be explained by a compression of the network boundary as well as by a partly compression of the network periphery (colored rings). Hence the network boundary gets closer to the network core, leading to the decrease in average shortest path length. The network boundary also gets closer to a part of the network periphery (gray ring gets closer to purple ring), while on the other hand parts of the network periphery get closer to the network core (pink ring gets closer to black innermost ring). This represents the increase in the global clustering coefficient. The network periphery in itself, however, is stretching (purple and pink rings get further apart) compensating for the global compression, while retaining the path structure prior to stimulation. This then results in an unaltered average eigenvector and betweenness centrality as well as an unaltered ranking. We conjecture that these topology-modifying stretching and compression effects also affect assortativity and synchronizability of the network, thereby enhancing its robustness and stability. The implications of these global and local stimulation-induced network modifications for (patho-)physiological brain functioning, however, remain to be shown. Nevertheless, in the future, tracking network characteristics could be utilized for monitoring stimulation-based interventions in diverse CNS disorders (Helmstaedter et al., 2021).

5. CONCLUSION

We demonstrated that short-term taVNS modifies local and global topological properties as well as stability and robustness properties of evolving functional brain networks, which is in line with the prevalent view of a global-acting mode of action of taVNS. This mode of action, being spatially unspecific on a local network scale, can be explained with the here proposed model

of a stimulation-related stretching and compression of functional brain networks.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because they contain information that could compromise the privacy of research participants. Requests to access the datasets should be directed to klaus.lehnertz@ukbonn.de.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committee of the University of Bonn. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors conceived the research project and wrote the paper. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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REFERENCES

Arenas, A., Díaz-Guilera, A., Kurths, J., Moreno, Y., and Zhou, C. (2008). Synchronization in complex networks. *Phys. Rep.* 469, 93–153. doi: 10.1016/j.physrep.2008.09.002

Atay, F. M., Bıyıkoğlu, T., and Jost, J. (2006). Network synchronization: Spectral versus statistical properties. *Phys. D* 224, 35–41. doi: 10.1016/j.physd.2006.09.018

Barahona, M., and Pecora, L. M. (2002). Synchronization in small-world systems. *Phys. Rev. Lett.* 89:54101. doi: 10.1103/PhysRevLett.89.054101

Baravalle, R., Guisande, N., Granado, M., Rosso, O. A., and Montani, F. (2019). Characterization of visuomotor/imaginary movements in EEG: an information theory and complex network approach. *Front. Phys.* 7:115. doi: 10.3389/fphy.2019.00115

Bauer, S., Baier, H., Baumgartner, C., Bohlmann, K., Fauser, S., Graf, W., et al. (2016). Transcutaneous vagus nerve stimulation (tVNS) for treatment of drug-resistant epilepsy: a randomized, double-blind clinical trial (cMPsE02). *Brain Stimul.* 9, 356–363. doi: 10.1016/j.brs.2015.11.003

Bialonski, S., and Lehnertz, K. (2013). Assortative mixing in functional brain networks during epileptic seizures. *Chaos* 23:033139. doi: 10.1063/1.4821915

Bottomley, J. M., LeReun, C., Diamantopoulos, A., Mitchell, S., and Gaynes, B. N. (2020). Vagus nerve stimulation (VNS) therapy in patients with treatment resistant depression: a systematic review and meta-analysis. *Compr. Psychiatry* 98:152156. doi: 10.1016/j.comppsych.2019.152156

Bretherton, B., Atkinson, L., Murray, A., Clancy, J., Deuchars, S., and Deuchars, J. (2019). Effects of transcutaneous vagus nerve stimulation in individuals aged 55 years or above: potential benefits of daily stimulation. *Aging* 11, 4836–4857. doi: 10.18632/aging.102074

Briand, M.-M., Gosseries, O., Staumont, B., Laureys, S., and Thibaut, A. (2020). Transcutaneous auricular vagal nerve stimulation and disorders of consciousness: a hypothesis for mechanisms of action. *Front. Neurol.* 11:933. doi: 10.3389/fneur.2020.00933

Bröhl, T., and Lehnertz, K. (2019). Centrality-based identification of important edges in complex networks. *Chaos* 29:033115. doi: 10.1063/1.5081098

Bröhl, T., and Lehnertz, K. (2020). Identifying edges that facilitate the generation of extreme events in networked dynamical systems. *Chaos* 30:073113. doi: 10.1063/5.0002743

Bullmore, E., and Sporns, O. (2009). Complex brain networks: graph theoretical analysis of structural and functional systems. *Nat. Rev. Neurosci.* 10, 186–198. doi: 10.1038/nrn2575

Colzato, L., and Beste, C. (2020). A literature review on the neurophysiological underpinnings and cognitive effects of transcutaneous vagus nerve stimulation: challenges and future directions. *J. Neurophysiol.* 123, 1739–1755. doi: 10.1152/jn.00057.2020

Dickten, H., Porz, S., Elger, C. E., and Lehnertz, K. (2016). Weighted and directed interactions in evolving large-scale epileptic brain networks. *Sci. Rep.* 6:34824. doi: 10.1038/srep34824

Elliott, R. E., Morsi, A., Kalhorn, S. P., Marcus, J., Sellin, J., Kang, M., et al. (2011). Vagus nerve stimulation in 436 consecutive patients with treatment-resistant epilepsy: long-term outcomes and predictors of response. *Epilepsy Behav.* 20, 57–63. doi: 10.1016/j.yebeh.2010.10.017

Ellrich, J. (2019). Transcutaneous Auricular Vagus Nerve Stimulation. *J. Clin. Neurophysiol.* 36, 437–442. doi: 10.1097/WNP.0000000000000576

Fang, J., Rong, P., Hong, Y., Fan, Y., Liu, J., Wang, H., et al. (2016). Transcutaneous vagus nerve stimulation modulates default mode network in major depressive disorder. *Biol. Psychiatry* 79, 266–273. doi: 10.1016/j.biopsych.2015.03.025

Fruengel, R., Bröhl, T., Rings, T., and Lehnertz, K. (2020). Reconfiguration of human evolving large-scale epileptic brain networks prior to seizures: an evaluation with node centralities. *Sci. Rep.* 10:21921. doi: 10.1038/s41598-020-78899-7

Geier, C., and Lehnertz, K. (2017). Long-term variability of importance of brain regions in evolving epileptic brain networks. *Chaos* 27:043112. doi: 10.1063/1.4979796

Hein, E., Nowak, M., Kiess, O., Biermann, T., Bayerlein, K., Kornhuber, J., et al. (2013). Auricular transcutaneous electrical nerve stimulation in depressed patients: a randomized controlled pilot study. *J. Neural Transm.* 120, 821–827. doi: 10.1007/s00702-012-0908-6

Helmstaedter, C., Rings, T., Buscher, L., Janssen, B., Alaeddin, S., Krause, V., et al. (2021). Assessing improvement of unresponsive wakefulness condition with evolving functional brain networks. Under review.

Jin, S.-H., Jeong, W., Lee, D.-S., Jeon, B. S., and Chung, C. K. (2014). Preserved high-centrality hubs but efficient network reorganization during eyes-open state compared with eyes-closed resting state: an MEG study. *J. Neurophysiol.* 111, 1455–1465. doi: 10.1152/jn.00585.2013

Kaniusas, E., Kampusch, S., Tittgemeyer, M., Panetsos, F., Gines, R. F., Papa, M., et al. (2019). Current directions in the auricular vagus nerve stimulation I–A physiological perspective. *Front. Neurosci.* 13:854. doi: 10.3389/fnins.2019.00772

Kong, J., Fang, J., Park, J., Li, S., and Rong, P. (2018). Treating depression with transcutaneous auricular vagus nerve stimulation: state of the art and future perspectives. *Front. Psychiatry* 9:20. doi: 10.3389/fpsyt.2018.00020

Kuhnert, M.-T., Bialonski, S., Noennig, N., Mai, H., Hinrichs, H., Helmstaedter, C., et al. (2013). Incidental and intentional learning of verbal episodic

material differentially modifies functional brain networks. *PLoS ONE* 8:e80273. doi: 10.1371/journal.pone.0080273

Kuhnert, M.-T., Elger, C. E., and Lehnertz, K. (2010). Long-term variability of global statistical properties of epileptic brain networks. *Chaos* 20:043126. doi: 10.1063/1.3504998

Kuhnert, M.-T., Geier, C., Elger, C. E., and Lehnertz, K. (2012). Identifying important nodes in weighted functional brain networks: a comparison of different centrality approaches. *Chaos* 22:023142. doi: 10.1063/1.4729185

Lehnertz, K., Ansmann, G., Bialonski, S., Dickten, H., Geier, C., and Porz, S. (2014). Evolving networks in the human epileptic brain. *Phys. D* 267, 7–15. doi: 10.1016/j.physd.2013.06.009

Liao, H., Mariani, M. S., M.Medo, Zhang, Y.-C., and Zhou, M.-Y. (2017). Ranking in evolving complex networks. *Phys. Rep.* 689, 1–54. doi: 10.1016/j.physrep.2017.05.001

Liu, A., Song, L., Wang, X., Wang, Y., Gong, L., Li, L., et al. (2018). Efficacy and safety of treatment with transcutaneous vagus nerve stimulation in 17 patients with refractory epilepsy evaluated by electroencephalogram, seizure frequency, and quality of life. *Med. Sci. Monit.* 24, 8439–8448. doi: 10.12659/msm.910689

Lohmann, G., Margulies, D. S., Horstmann, A., Pleger, B., Lepsien, J., Goldhahn, D., et al. (2010). Eigenvector centrality mapping for analyzing connectivity patterns in fMRI data of the human brain. *PLoS ONE* 5:e10232. doi: 10.1371/journal.pone.0010232

Lü, L., Chen, D., Ren, X.-L., Zhang, Q.-M., Zhang, Y.-C., and Zho, T. (2016). Vital nodes identification in complex networks. *Phys. Rep.* 650, 1–63. doi: 10.1016/j.physrep.2016.06.007

Makarov, V. V., Zhuravlev, M. O., Runnova, A. E., Protasov, P., Maksimenko, V. A., Frolov, N. S., et al. (2018). Betweenness centrality in multiplex brain network during mental task evaluation. *Phys. Rev. E* 98:062413. doi: 10.1103/PhysRevE.98.062413

Mormann, F., Lehnertz, K., David, P., and Elger, C. E. (2000). Mean phase coherence as a measure for phase synchronization and its application to the EEG of epilepsy patients. *Phys. D* 144, 358–369. doi: 10.1016/S0167-2789(00)00087-7

Newman, M. (2018). *Networks, 2nd Edn*. Oxford: Oxford University Press.

Newman, M. E. J. (2003). Mixing patterns in networks. *Phys. Rev. E* 67:026126. doi: 10.1103/PhysRevE.67.026126

Onnela, J. P., Saramäki, J., Kertész, J., and Kaski, K. (2005). Intensity and coherence of motifs in weighted complex networks. *Phys. Rev. E* 71:065103. doi: 10.1103/PhysRevE.71.065103

Osterhage, H., Mormann, F., Staniek, M., and Lehnertz, K. (2007). Measuring synchronization in the epileptic brain: a comparison of different approaches. *Int. J. Bifurcation Chaos Appl. Sci. Eng.* 17, 3539–3544. doi: 10.1142/S0218127407019330

Pecora, L. M., and Carroll, T. L. (1998). Master stability functions for synchronized coupled systems. *Phys. Rev. Lett.* 80, 2109–2112.

Redgrave, J., Day, D., Leung, H., Laud, P., Ali, A., Lindert, R., et al. (2018). Safety and tolerability of transcutaneous vagus nerve stimulation in humans; a systematic review. *Brain Stimul.* 11, 1225–1238. doi: 10.1016/j.brs.2018.08.010

Rings, T., von Wrede, R., and Lehnertz, K. (2019). Precursors of seizures due to specific spatial-temporal modifications of evolving large-scale epileptic brain networks. *Sci. Rep.* 9:10623. doi: 10.1038/s41598-019-47092-w

Stegeman, I., Velde, H., Robe, P., Stokroos, R., and Smit, A. (2021). Tinnitus treatment by vagus nerve stimulation: a systematic review. *PLoS ONE* 16:e0247221. doi: 10.1371/journal.pone.0247221

Straube, A., Ellrich, J., Eren, O., Blum, B., and Ruscheweyh, R. (2015). Treatment of chronic migraine with transcutaneous stimulation of the auricular branch of the vagal nerve (auricular t-VNS): a randomized, monocentric clinical trial. *J. Headache Pain* 16:63. doi: 10.1186/s10194-015-0543-3

Tu, Y., Fang, J., Cao, J., Wang, Z., Park, J., Jorgenson, K., et al. (2018). A distinct biomarker of continuous transcutaneous vagus nerve stimulation treatment in major depressive disorder. *Brain Stimul.* 11, 501–508. doi: 10.1016/j.brs.2018.01.006

van den Heuvel, M. P., and Sporns, O. (2013). Network hubs in the human brain. *Trends Cogn. Sci.* 17, 683–696. doi: 10.1016/j.tics.2013.09.012

von Wrede, R., Moskau-Hartmann, S., Rüber, T., Helmstaedter, C., and Surges, R. (2019). Sustained seizure freedom with transcutaneous vagal nerve stimulation in drug-resistant epilepsy caused by subcortical band heterotopias. *Seizure* 70, 25–26. doi: 10.1016/j.seizure.2019.06.026

von Wrede, R., Rings, T., Schach, S., Helmstaedter, C., and Lehnertz, K. (2021). Transcutaneous auricular vagus nerve stimulation induces stabilizing modifications in large-scale functional brain networks: towards understanding the effects of taVNS in subjects with epilepsy. *Sci. Rep.* 11:7906. doi: 10.1038/s41598-021-87032-1

Wang, Y., Li, S.-Y., Wang, D., Wu, M.-Z., He, J.-K., Zhang, J.-L., et al. (2020). Transcutaneous auricular vagus nerve stimulation: from concept to application. *Neurosci. Bull.* 37, 853–862. doi: 10.1007/s12264-020-00619-y

Wu, X., Zhang, Y., Luo, W.-T., Mai, R.-R., Hou, X.-Y., Xia, Z.-Q., et al. (2021). Brain functional mechanisms determining the efficacy of transcutaneous auricular vagus nerve stimulation in primary insomnia. *Front. Neurosci.* 15:186. doi: 10.3389/fnins.2021.609640

Yap, J. Y. Y., Keatch, C., Lambert, E., Woods, W., Stoddart, P. R., and Kameneva, T. (2020). Critical review of transcutaneous vagus nerve stimulation: challenges for translation to clinical practice. *Front. Neurosci.* 14:284. doi: 10.3389/fnins.2020.00284

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von Wrede R, Bröhl T, Rings T, Pukropski J, Helmstaedter C, Lehnertz K. (2022). Modifications of functional human brain networks by transcutaneous auricular vagus nerve stimulation: impact of time of day. *Brain Sci*. 2022 Apr 26;12(5):546

Zielsetzung der Arbeit: In dieser Arbeit wurde der Einfluss der taVNS auf sich entwickelnde funktionelle Hirnnetzwerke auf lokaler und globaler Netzwerkebene in Abhängigkeit von der Tageszeit untersucht, sowie geprüft, ob ein taVNS-induzierter neuromodulatorischer Effekt auf funktionelle Hirnnetzwerke identifiziert werden kann.

Methoden und Ergebnisse: In Erweiterung der zuvor erläuterten Studien (Rings et al., 2021; von Wrede et al., 2021) wurde bei 15 Proband*innen zweimal täglich eine transkutane aurikuläre Vagusnervstimulation (eine Stunde lang am Vormittag und eine weitere Stunde lang am frühen Nachmittag) durchgeführt, während die Proband*innen eine kontinuierliche Video-EEG-Vielkanal-Aufzeichnung erhielten. Mittels zeitaufgelöster Synchronisationsanalyse der oben genannten EEG-Aufzeichnungen wurden sich entwickelnde, vollständig verbundene, gewichtete und ungerichtete Netzwerke abgeleitet, wichtige globale (C, L, A, S) und lokale Kenngrößen der Netzwerke (C^B, C^E und C^S/C^N) berechnet und ihre Veränderungen im Laufe der Zeit und unter der taVNS verfolgt. Zusätzlich erfolgte die Schätzung der spektralen Leistungsdichte von Zeitverläufen lokaler und globaler Netzwerkkenngrößen, um einen möglichen Einfluss insbesondere ultradianer Rhythmen zu identifizieren, die häufig als Perioden von weniger als 20 h, aber mehr als 1 h definiert werden. Aufgrund der jeweiligen Dauer des Studienprotokolls wurde insbesondere die Periodenlängen zwischen 30 und 180 Minuten berücksichtigt. Daten von drei Proband*innen, bei denen starke Beiträge (Spektraldichte > 20 [a.u.]) bei diesen Periodenlängen festgestellt wurden, wurden bei der weiteren Analyse nicht berücksichtigt. Sowohl die morgendliche als auch die nachmittägliche Stimulation induzierte bei der Mehrheit der Proband*innen sofortige und anhaltende Veränderungen der globalen Netzwerkcharakteristika (taVNS-Responder), jedoch ohne spezifische Auswirkungen auf lokale Netzwerkcharakteristika. Die Stimulation am Nachmittag führte zu homogeneren Auswirkungen als die vormittägliche Stimulation: fast alle Proband*innen wiesen immediat eine weniger segregierte und eine stärker integrierte Netzwerktopologie auf, und die Zunahme der Netzwerkintegration zeigte sich auch anhaltend. Zudem erhöhte taVNS bei fast allen Respondern die Netzwerkstabilität (verringerte Synchronisierbarkeit S; sofortiger und anhaltender Stimulationseffekt). Die durch die

morgendliche Stimulation vermittelten Veränderungen waren eher uneinheitlich, obwohl einige Veränderungen stärker ausgeprägt zu sein schienen als die entsprechenden Veränderungen durch die nachmittägliche Stimulation. Während die zuvor beschriebenen sofortigen und dauerhaften topologieverändernden und stabilitätssteigernden Effekte bei etwa 50 % der Proband*innen beobachtet werden konnten, traten bei weiteren 50 % entgegengesetzte Veränderungen auf.

Nur ein Teil Proband*innen, welche signifikante Änderungen der globalen Netzwerkcharakteristika durch die vormittägliche taVNS zeigten (taVNS1-Responder) zeigten auch durch die nachmittägliche Stimulation ausgelöste unmittelbare Veränderungen der globalen Netzwerkkenngrößen (50 % der taVNS1-*C*-Responder, 78 % der taVNS1-*L*-Responder und 67 % der taVNS1-*S*-Responder). Vergleicht man die Richtung der unmittelbaren Veränderung (Zu- bzw. Abnahme) zwischen Morgen- und Nachmittagsstimulation, so zeigten nur einige Proband*innen unmittelbare Veränderungen mit der gleichen Richtung. Somit konnte die postulierte neuromodulatorische Wirkung auf funktionelle Hirnnetzwerke, die durch zweimal täglich durchgeführte Kurzzeitstimulationen ausgelöst wird, nicht belegt werden, zumindest nicht für die hier betrachteten Zeiträume.

Bei etwa der Hälfte der Proband*innen konnten länger anhaltende Stimulationseffekte (vor der 1. Stimulation → vor der 2. Stimulation) auf die globalen Netzwerkmerkmale beobachtet werden: bei etwa der Hälfte der Proband*innen eine Zunahme und bei der anderen Hälfte eine Abnahme der Netzwerkkenngrößen, was eine Interpretation der Auswirkungen einer Stimulation auf längeren Zeitskalen schwierig macht. Darüber hinaus anhaltende Stimulationseffekte (vor der 1. Stimulation → nach der 2. Stimulation) auf globale Netzwerkcharakteristika konnten nur bei einer kleinen Untergruppe von Proband*innen beobachtet werden. Wie aus den Voruntersuchungen zu erwarten war, konnten keine eindeutigen stimulationsbedingten Änderungen lokaler Netzwerkkenngrößen beobachtet werden und die zentralsten Netzwerkbestandteile blieben unverändert.

Schlussfolgerung: Für die taVNS-induzierten Netzwerkmodifikationen zeigte sich ein nicht zu vernachlässigender Einfluss der Tageszeit, der weitere Untersuchungen erfordert und bei zukünftigen Forschungs- und klinischen Studien zu diesem nicht-pharmazeutischen

Interventionsansatz berücksichtigt werden sollte. Mit o.g. Untersuchungsschema konnte kein potenzieller neuromodulatorischer Effekt durch eine zweimalige taVNS festgestellt werden.

brain sciences

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Article

Modifications of Functional Human Brain Networks by Transcutaneous Auricular Vagus Nerve Stimulation: Impact of Time of Day

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Abstract: Transcutaneous auricular vagus nerve stimulation (taVNS) is a novel non-invasive treatment option for different diseases and symptoms, such as epilepsy or depression. Its mechanism of action, however, is still not fully understood. We investigated short-term taVNS-induced changes of local and global properties of EEG-derived, evolving functional brain networks from eighteen subjects who underwent two 1 h stimulation phases (morning and afternoon) during continuous EEG-recording. In the majority of subjects, taVNS induced measurable modifications of network properties. Network alterations induced by stimulation in the afternoon were clearly more pronounced than those induced by stimulation in the morning. Alterations mostly affected the networks' topology and stability properties. On the local network scale, no clear-cut spatial stimulation-related patterns could be discerned. Our findings indicate that the possible impact of diurnal influences on taVNS-induced network modifications would need to be considered for future research and clinical studies of this non-pharmaceutical intervention approach.

Keywords: epilepsy; transcutaneous auricular vagus nerve stimulation; functional brain networks; biological rhythms

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1. Introduction

Brain stimulation is a rapidly evolving field of research and treatment that involves different invasive and non-invasive techniques. For vagus nerve stimulation (VNS), invasive and non-invasive devices are available. Invasive VNS is an established stimulation treatment that finds application in several diseases, including depression and epilepsy. Non-invasive VNS is a more recent approach that is still under experimental and clinical investigation. Non-invasive transcutaneous vagus nerve stimulation (tVNS) can be performed as transcutaneous cervical VNS (tcVNS), percutaneous auricular VNS (paVNS), and transcutaneous auricular VNS (taVNS). The ease of use—and therefore the possibility of a rapid introduction of therapy and immediate removal of this stimulation device—explains the great interest in taVNS for research and treatment. A broad spectrum of symptoms and diseases such as CNS disorders (e.g., epilepsy [1], migraine [2], disorders of consciousness [3], and cognitive impairment [4]), as well as cardiovascular or digestive system diseases (e.g., [5,6], pain [7], insomnia [8], or COVID [9,10], are targets of taVNS. So far, the mechanism of action of taVNS is not fully understood, but widespread activity in expected vagal projection areas, including nucleus tractus solitarius, locus coeruleus, hypothalamus, thalamus, amygdala, hippocampus, as well as the prefrontal cortex and other widespread areas [11] were reported, though different study protocols and investigated subjects make interpretation difficult. There is a growing

body of evidence of efficacy in several diseases [12,13]; nevertheless, there is currently no final agreement on optimal stimulation parameters. As for clinical use, it might be difficult for some patients to integrate the recommended stimulation time (e.g., for epilepsy treatment, four hours a day continuously, or as blocks of a minimum of one hour stimulation (patients' information, tVNS technology®)) into their daily lives, which may lead to worse adherence to treatment advice [14,15]. Furthermore, there are currently no recommendations that state which time of the day taVNS should preferably be performed. Diurnal variation of vagal activity is a well-known phenomenon [16], and association of medical conditions linked to the vagal tone or vagal activity to certain times of the day have been demonstrated before [16–20]. One approach to elucidate the mechanism of action of taVNS is that it mimics an impaired or lost vagal sensory feedback to the brain [7]. Considering this ansatz and taking into account the diurnal fluctuations of the vagal activity, one can hypothesize a variation of the effect of taVNS depending on time of day.

Previous studies [15,21] demonstrated that the impact of short-term taVNS on brain dynamics can be monitored and characterised with EEG-derived evolving functional brain networks [22,23]. Stimulation-mediated modifications of various network properties indicate that short-term taVNS has a topology-modifying, robust, and stability enhancing effect. Network properties, however, may also be influenced by various biological rhythms [24], and it is not yet clear if, and to what extent, these influences impact taVNS-mediated network modifications. Addressing these issues, here, we extend our previous investigations on short-term taVNS-mediated modifications of evolving functional brain networks, and put forward the following hypotheses:

- modifications of the networks' global (topology, stability, and robustness) and local characteristics (importance of network constituents) depend on the time of day the stimulation was performed; and
- a taVNS-related neuromodulatory effect on functional brain networks (i.e., a repeated stimulation amplifies network modifications induced by the previous stimulation) can be identified using short-term stimulations performed twice a day

2. Materials and Methods

2.1. Subjects

Subjects who were diagnosed and treated between January 2021 to July 2021 as inpatients at the Department of Epileptology, University Hospital Bonn, were screened for suitability for this study. Inclusion criteria were clinical necessity for long-term video-EEG-recording. Exclusion criteria were previous brain surgery, actual or previous neurostimulation such as invasive or non-invasive vagus nerve stimulation or deep brain stimulation, progressive disease, seizures occurring within 24 h before the start of the study or within the study, insufficient German language capability, mental disability, and incabability to follow instructions. All subjects were provided with written information and were given the opportunity to ask further questions. Eighteen subjects volunteered to participate and signed an informed consent form. The study protocol had previously been approved by the ethics committee of the Medical Faculty of the University of Bonn and was performed in accordance with the tenets of the Declaration of Helsinki.

2.2. Transcutaneous Auricular Vagus Nerve Stimulation and Examination Schedule

Extending previous studies [15,21], we applied transcutaneous auricular vagus nerve stimulation twice on the same day: for one hour in the mid-morning and for another hour in early afternoon while the subjects underwent continuous video-EEG-recording (morning (taVNS1): 1 h pre-stimulation phase (pre 1), 1 h taVNS phase (stim 1), and 1 h post-stimulation phase (post 1); afternoon (taVNS2): 1 h pre-stimulation phase (pre 2), 1 h taVNS phase (stim 2), and 1-h post-stimulation phase (post 2), see Figure 1).

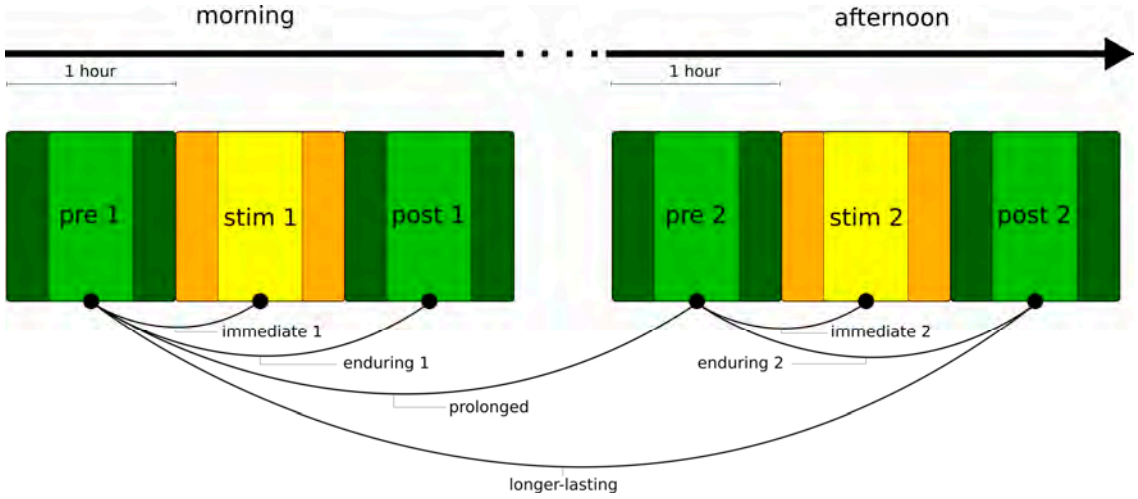

Figure 1. Examination schedule to probe for short and longer-lasting taVNS-induced changes in evolving functional brain networks. The schedule consisted of two 1 h stimulation phases (morning: stim 1 and afternoon: stim 2; yellow-shaded blocks) each phase was preceded and followed by a pre- and post-stimulation phase, each lasting one hour (green-shaded blocks). During each of the 3 h blocks, subjects continued laid-back activities (no other activation methods applied, no eating) and they continued performing daily activities during the break (no other activation methods applied). The whole examination schedule (including the break) was embedded into a continuous video-EEG-recording. In our analyses, we neglected data from the first and last 15 min of each phase (darker colours) in order to remove possible transient effects (e.g., due to movements or expectation effects).

Stimulation was carried out unilaterally in the left cymba conchae with a taVNS device (tVNS Technologies GmbH, Erlangen, Germany) with non-adjustable parameters (biphasic signal form, impulse frequency 25 Hz, impulse duration 20 s, impulse pause 30 s). The intensity of stimulation was increased until the subject noticed a non-painful "tingling". Both stimulation phases were carried out with the same intensity. There was neither alteration of CNS medication (if taking any) nor application of activation methods (such as photo stimulation, hyperventilation, or sleep deprivation) at least 24 h before start of the study.

2.3. EEG Recording and Data Pre-Processing

Electroencephalograms (EEG) were recorded from 19 electrodes according to the 10–20 system (with Cz as physical reference). EEG data were sampled at 256 Hz using a 16 bit analogue-to-digital converter (Micromed, S.p.A., Mogliano Veneto, Italy) and were band-pass filtered offline between 1–45 Hz (4th order Butterworth characteristic). A notch filter (3rd order) was used to suppress contributions at the line frequency (50 Hz). All recordings were visually inspected for strong artefacts (subject movements, amplifier saturation, or stimulation artefacts), and such data were excluded from further analyses.

2.4. Characterising Evolving Functional Brain Networks on Global and Local Scale

Functional networks consist of vertices and edges. When analysing brain dynamics with network-theoretical approaches, vertices are usually associated with brain regions sampled by the EEG electrode contacts and edges with time-varying estimates of the strength of interactions between the vertices' dynamics, regardless of their anatomical connections. Following previous studies [15,21], we derived evolving, fully connected, weighted, and undirected networks from a time-resolved synchronisation analysis of the abovementioned EEG-recording (sliding-window analysis on data windows of 20 s duration each), assessed important global and local characteristics of the networks, and tracked their changes over time (see [15,21] for details).

On the global network scale, we calculated the topological characteristics' average clustering coefficient C and average shortest path length L to assess the networks' functional segregation and integration. The former reflects independent information processes between brain regions, and the latter reflects dependent information [25]. A network's functional segregation can be characterized by the average clustering coefficient C: the lower the C, the more segregated the network. Functional integration can be characterized by the average shortest path length L: the lower the L, the more integrated the network. In order to characterize the networks' stability, we calculated synchronisability S, which assesses the networks' propensity (or vulnerability) to be synchronised by an admissible input activation: the higher the S, the easier it is for the synchronised state to be perturbed. Eventually, we calculated assortativity A, to assess the networks' robustness [26]. Assortativity reflects the tendency of edges to connect vertices with similar or equal properties, here weighted degree [27,28]. If edges preferentially connect vertices with similar properties, such networks are called assortative, and they tend to disintegrate into different groups more strongly than disassortative networks do. Disassortative networks are more vulnerable to perturbations and appear to be easier to synchronize than assortative networks (decreased robustness).

On the local network scale, we employed three opposing centrality concepts to characterize the role networks constituents (vertices and edges) play in the larger network [29–33]. A vertex or edge with a high betweenness centrality index C^B is central if it connects different regions of the network as a bridge. A vertex or edge with a high eigenvector centrality index C^E is central if it is connected to the vertices or edges which are central as well, reflecting the influence of the vertex or edge on the network as a whole. With strength centrality C^S, the larger the sum of weights of a vertice's adjacent edges the more central is the vertex [34,35]. For the edges, we employed the novel nearest neighbor centrality concept; a high nearest neighbor centrality index C^N highlights an edge that is more central the larger its weight, and the more similar and the higher the strengths of the connected vertices [33]. Consequently, C^N is largely independent of the networks' topology as it is solely based on local vertex and edge properties. Thus, an edge with a high C^N value reflects a local bottleneck possible coinciding with global bottlenecks.

2.5. Evaluating the Possible Influence of Biological Rhythms on Time-Dependent Network Characteristics

We estimated the power spectral density (Lomb–Scargle periodogram [36]) of time courses of local and global network characteristics (see Figure 2) to identify a possible influence of ultradian rhythms in particular, which are often defined as having periods shorter than 20 h but longer than 1 h. Given our examination schedule, we concentrated on period lengths between 30 min and 180 min. Data from subjects, for which we encountered strong contributions (spectral density > 20 [a.u.]) at these period lengths, were not taken into account for further analysis.

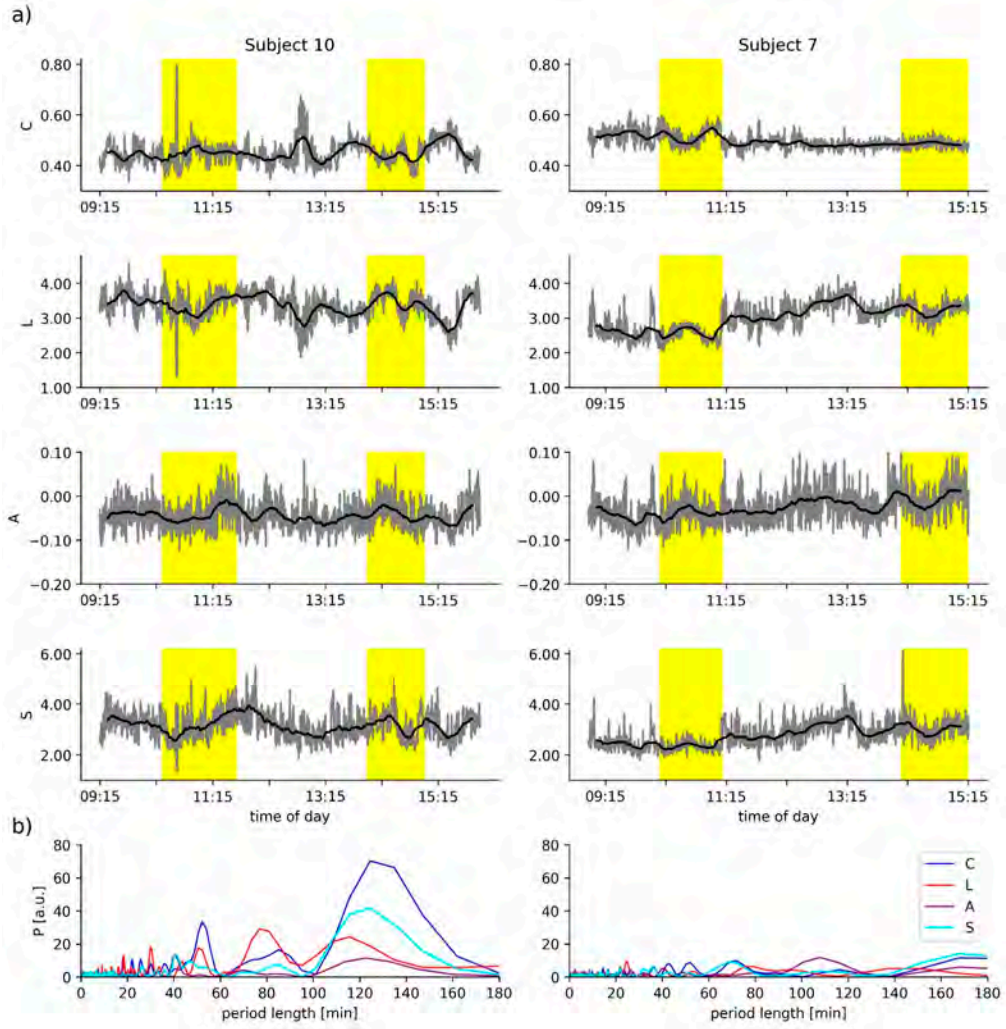

Figure 2. (**a**) Exemplary time courses (grey lines) of average clustering coefficient *C*, average shortest path length *L*, assortativity *A*, and synchronisability *S* of two subjects. Smoothed time courses (moving average over 17 min) are shown as black lines. Yellow-shaded areas mark the two 1 h stimulation phases. (**b**) Power spectral density estimates *P* of the respective time courses.

2.6. Classification of Stimulation Effects

Taking into account earlier observations [15,21], here, we define various stimulation effects acting on different timescales if network characteristics differ significantly between two phases of the examination schedule (cf. Figure 1):

- immediate stimulation effect: network characteristics during the pre-stimulation phase and during the stimulation phase (pre → stim) differ significantly (either in the morning or in the afternoon);
- enduring stimulation effect: an immediate stimulation effect can be observed and network characteristics during the pre-stimulation phase and during the post-stimulation phase (pre → post) differ significantly (either in the morning or in the afternoon);
- prolonged stimulation effect: an immediate stimulation effect of the morning stimulation can be observed and network characteristics during the pre-stimulation phase 1 and during the pre-stimulation phase 2 (pre 1 → pre 2) differ significantly;
- longer-lasting stimulation effect: an immediate stimulation effect of the morning stimulation can be observed and network characteristics during the pre-stimulation phase 1 and during the post-stimulation phase 2 (pre 1 → post 2) differ significantly. If immediate effects can be observed for both stimulations (pre 1 → stim 1 and pre 2 → stim 2), we consider the long-lasting effect to be accumulating.

A subject for whom an immediate effect of taVNS could be identified is classified as a taVNS responder (morning stimulation: taVNS1 responder; afternoon stimulation: taVNS2 responder). In addition, we refer to a network characteristic which exhibited a significant difference as annex (e.g., a subject for whom an immediate effect during the morning stimulation can be observed for the average clustering coefficient is classified as taVNS1-*C* responder).

2.7. Statistical Analyses

We investigated differences between network characteristics from the three phases in the morning (pre 1: pre-stimulation; stim 1: during stimulation; post 1: post-stimulation) and in the afternoon (pre 2: pre-stimulation; stim 2: during stimulation; post 2: post-stimulation) on a per-subject level using the Mann–Whitney U-test (pre 1 vs. stim 1; pre 1 vs. post 1; pre 2 vs. stim 2; pre 2 vs. post 2; pre 1 vs. pre 2; pre 1 vs. post 2; $p < 0.05$; Bonferroni correction). In order to remove possible transient effects, we neglected data from the first and last 15 min of each phase. Further downstream analyses were performed for taVNS responder only.

3. Results

Due to the clinical setting on the ward, recruiting participants for longer EEG-recordings without disturbing the clinically necessary work flow was challenging. From the 18 eligible subjects, three subjects had to be excluded (one due to previous seizure, one due to withdrawal of consent, one due to EEG data quality). Data from fifteen subjects (9 females; age 19–75 years, mean 40 years; duration of disease 0.1–36 years, mean 10.5 years) were included in the analyses. The same current intensities were used in both stimulation phases (range: 1.0–5.0 mA, mean 2.48, SD $\pm$ 1.2). No correlation between demographic data (age, duration of disease), as well as current intensity and immediate and enduring significant changes of network characteristics, could be observed (Pearson's ρ $p > 0.05$).

In Figure 2a, we show exemplary time courses of the global network characteristics—average clustering coefficient *C*, average shortest path length *L*, synchronisability *S*, and assortativity *A*—of two subjects. All time courses exhibit both short-time and long-time fluctuations, albeit to varying degrees. Evaluating the possible influence of ultradian rhythms on the time-dependent network characteristics, we observed negligible contributions at period lengths between 30 and 180 min for subject 7 (see periodograms in Figure 2b). In contrast, we identified pronounced contributions at period lengths around 50, 80, and 120 min for subject 10. Similar pronounced contributions were obtained for the time courses of the global network characteristics from subjects 12 and 13. Data from these three subjects were excluded from further analysis, in order to avoid misinterpreting changes of network characteristics related to the waxing and waning of ultradian rhythms as possible taVNS-induced modifications of evolving functional brain networks.

3.1. Morning taVNS-Induced Immediate and Enduring Network Modifications on the Global and Local Scale

We observed in the majority of subjects an immediate stimulation effect (pre 1 $\rightarrow$ stim 1) on all global network characteristics (average clustering coefficient *C*, average shortest path length *L*, assortativity *A*, and synchronisability *S*). Depending on the investigated network characteristic, 42% to 75% of the subjects presented with significant immediate taVNS-induced modifications of their evolving functional brain networks (taVNS1-*C*: 67% (8 subjects); taVNS1-*L*: 75% (9 subjects), taVNS1-*A*: 42% (5 subjects), taVNS1-*S*: 75% (9 subjects)). Similarly, we observed an enduring stimulation effect (pre 1 $\rightarrow$ post 1) in a comparable number of subjects (*C*: 58% (7 subjects); *L*: 67% (8 subjects), A: 17% (2 subjects), *S*: 67% (8 subjects)). A small number of subjects neither responded to the morning nor to the afternoon stimulation (*C*: 2 subjects; *L*: 2 subjects and *A*: 2 subjects; note that these were not the same subjects).

Tracking the taVNS-induced modifications of networks on a single-subject level (Figure 3, left) suggested that the responders can be assigned to two subgroups, those with positive and those with negative significant modifications of their global network characteristics. Five subjects presented with an immediate increase of average clustering coefficient *C* (+8.7%, we report the change of mean values in the following) and three with an immediate decrease (−6.1%). Similarly, three subjects presented with an enduring increase of *C* (+6.2%) and another four with an enduring decrease (−4.2%). The enduring stimulation effect was, in general, less pronounced. We derived similar results for the average shortest path length *L*. Five subjects presented an immediate increase of *L* (+10.8%) and another four presented an immediate decrease (−18.1%). Four subjects presented a more pronounced enduring increase of *L* (+15.4%) and another four presented a less pronounced enduring decrease (−5.2%). For assortativity *A*, we observed an immediate decrease (−44.6%) for five subjects. Only two subjects presented enduring effects (increase in one subject, +15.6%; decrease in one subject, −31.7%). The latter figures have to be interpreted with care, given the anomalous large relative changes. In the following, we therefore refrain from an interpretation in terms of modifications of the networks' robustness. With regard to synchronisability *S*, four subjects presented an immediate increase (+7.3%) and another five presented a more pronounced immediate decrease (−13.0%). Five subjects presented a more pronounced enduring increase of *S* (+11.2%) and another three presented a less pronounced enduring decrease (−6.2%).

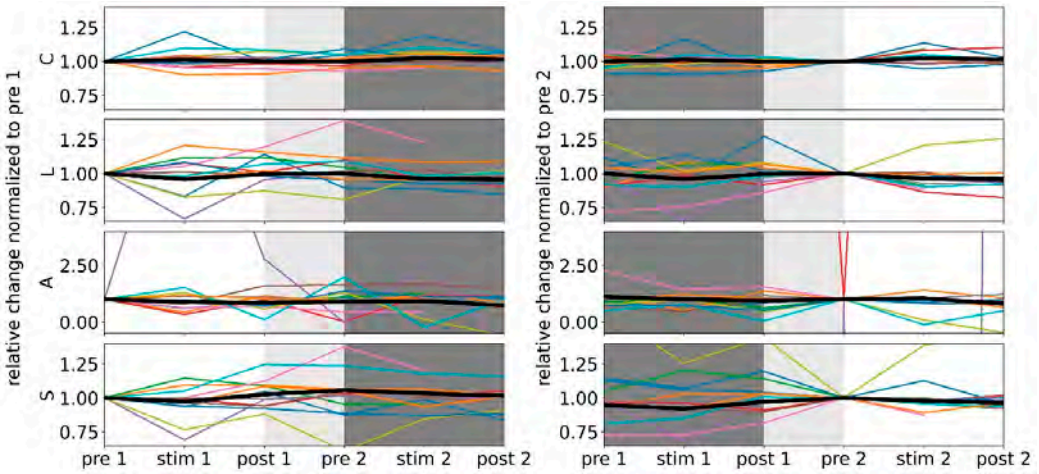

Figure 3. Relative changes of global network characteristics (average clustering coefficient *C*, average shortest path length *L*, assortativity *A*, and synchronisability *S)* of all responders (colour-coded). Group medians are shown in black, and all lines are for eye-guidance only. Data normalized to the respective values from the morning pre-stimulation phase (**left**; white-shaded area) and from the afternoon pre-stimulation phase (**right**; white-shaded area). Light-grey shaded area marks the break between the two 3 h examination phases. The different scaling of the *y*-axis for assortativity is due to the fact that this characteristic only rarely deviated from −0.02 which resulted in anomalous large relative deviations. We note that we obtained for the vast majority of investigated networks, indications for a random topology (their assortativity values were confined to the range of *A* values derived from 1000 random networks with the same number of vertices and edge densities as the evolving functional brain networks).

On the local network scale, different vertex and edge centrality concepts highlighted different brain regions and interactions between brain regions as most central (highest centrality value), as expected. Vertex betweenness centrality highlighted left fronto-centro-temporal brain regions as most central, vertex eigenvector centrality posterior brain regions, and vertex strength centrality left temporo-parietal brain regions. Edge betweenness centrality highlighted edges as most central that connect fronto-central vertices, whereas edge eigenvector centrality as well as nearest neighbor centrality rated edges connecting

left parieto-temporo-occipital vertices as most central. Note that quite often, most central edges connected vertices, one of which is also most central. Despite these distinctions, and in line with previous observations [15,21], taVNS-mediated alterations of vertex or edge centralities were presented without any clear-cut spatial pattern.

3.2. Afternoon taVNS-Induced Immediate and Enduring Network Modifications on the Global and Local Scale

As with the morning taVNS, we observed significant stimulation-related immediate (pre 2 → stim 2) changes to all global network characteristics (average clustering coefficient, average shortest path length, assortativity and synchronisability). Depending on the investigated network characteristic, 33% to 75% of the subjects presented significant, immediate taVNS-induced modifications of their evolving functional brain networks (taVNS2-*C*: 58% (7 subjects); taVNS2-*L*: 67% (8 subjects), taVNS2-*A*: 33% (4 subjects), taVNS2-*S*: 75% (9 subjects)). Additionally, we observed an enduring stimulation effect (pre 2 → post 2) in a comparable number of subjects for average shortest path length and synchronisability (*L*: 50% (6 subjects), *S*: 67% (8 subjects)). Significant stimulation-related enduring changes for the average clustering coefficient were observed only in two subjects, and significant changes in assortativity were observed in another subject.

We proceeded on a single subject level (Figure 3, right) and considered responders with positive and negative significant modifications of their global network characteristics. Six subjects presented with an immediate increase of average clustering coefficient *C* (+5.0%) and another subject with an immediate decrease (−5.5%). For two subjects, an enduring decrease of *C* (−1.1%) was observed. We derived similar results for average shortest path length *L*. Seven subjects presented an immediate decrease of *L* (−8.3%) and another subject an immediate increase (+20.9%). Five subjects presented a similar enduring decrease of *L* (−8.5%) and an enduring increase was observed in one subject (+25.7%, this subject also presented an immediate increase of *L*). For assortativity *A*, three subjects presented an immediate decrease of *A* (−68.2%) and one subject presented an immediate increase (+3.8%). Only one subject displayed an enduring effect (+6.3%). Again, these results have to be interpreted with care. With regard to synchronisability *S*, two subjects presented an immediate increase (+6.3%) and another seven presented a decrease (−5.0%). One subject presented a negligible enduring increase of *S* (+0.03%) and another seven presented an enduring decrease (−4.8%).

On the local network scale, we observe that most central brain regions were highlighted, most central interactions between brain regions remained unaltered, and taVNS-mediated alterations of vertex or edge centralities were again presented without any clear-cut spatial pattern.

Summarizing our findings achieved so far, both the morning and the afternoon stimulation led to immediate and enduring modifications of global network characteristics in the majority of subjects (Figure 4), but they did not specifically affect local network characteristics. These observations corroborate previous studies [15,21]. The afternoon stimulation, however, appeared to have more homogenous effects: almost all responders presented with a less segregated (increased average clustering coefficient *C*) and a more integrated (decreased average shortest path length *L*) network topology (immediate stimulation effect), and the latter (decreased *L*) even persisted into the post-stimulation phase (enduring stimulation effect). Moreover, in almost all responders taVNS increased network stability (decreased synchronisability *S*; immediate and enduring stimulation effect).

Only a subset of taVNS1 responders presented with immediate modifications of global network characteristics induced by the afternoon stimulation (50% of taVNS1-*C* responders (4 subjects), 78% of taVNS1-*L* responders (7 subjects), and 67% of taVNS1-*S* responders (6 subjects)). Comparing the direction of immediate change between morning and afternoon stimulation, only some subjects displayed immediate changes with the same direction (i.e., an increase or a decrease (*C*: 1 of 4, *L*: 2 of 7, and *S*: 3 of 6)). These numbers even decreased when considering enduring changes (*C*: 2 of 4, and *S*: 3 of 5). Thus, the

hypothesized neuromodulatory effect on functional brain networks induced by short-term stimulations performed twice a day could not be identified, at least for the time scales considered here.

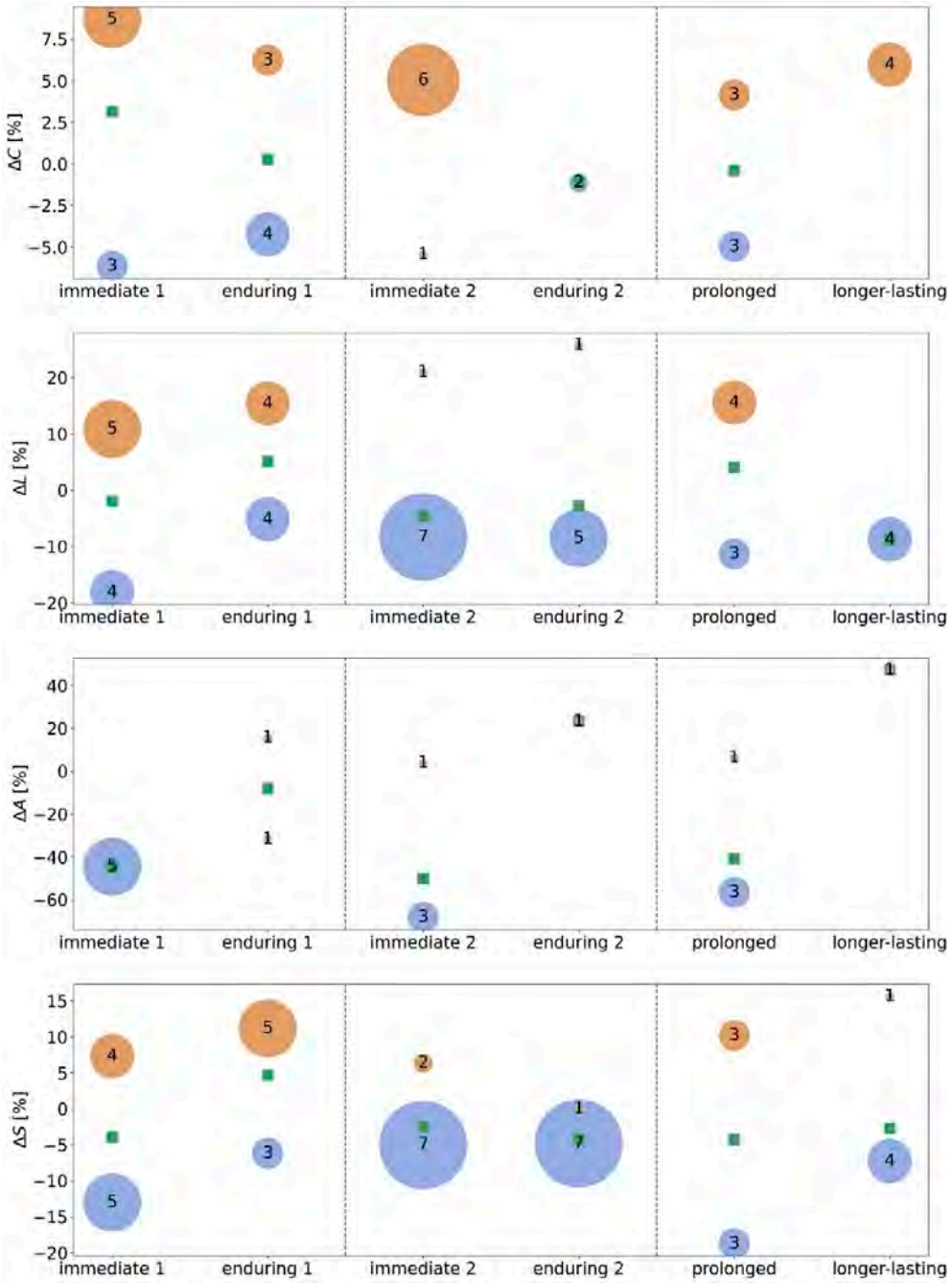

Figure 4. Bubble chart of taVNS-induced immediate, enduring, prolonged, and longer-lasting relative changes Δ of global network characteristics of all responders (average clustering coefficient C, average shortest path length L, assortativity A, and synchronisability S; $\Delta = (M_l - M_k)/M_k$, where M_k and M_l denote placeholders for the temporal average of the respective characteristics from phase k and phase l; cf. Figure 1). Responders are assigned to subgroups according to their direction of change (positive/negative changes are shown in orange/blue). A disk is centred at a subgroup's mean relative change, and the diameter of a disk encodes the number of subjects per subgroup. Mean relative changes from both groups are shown as green squares.

3.3. Prolonged and Longer-Lasting taVNS-Induced Modifications on the Global and Local Network Scale

About half of the subjects presented prolonged stimulation effects with regard to global network characteristics (taVNS1-*C*: 50% (6 subjects); taVNS1-*L*: 58% (7 subjects), taVNS1-*S* 50% (6 subjects). For roughly half of them, we observed network characteristics increasing, and for the other half, they decreased (Figure 4), which renders an interpretation of prolonged stimulation effects rather difficult. Breaking the data down to a single subject level, changes in the same direction were observed in all subjects for *C*, in 4 of 7 for *L*, and in 4 of 6 for *S*.

Longer-lasting stimulation effects on global network characteristics could be observed in only a small subset of subjects. Nevertheless, four of them consistently presented an increase of *C* (+6.0%). Decreased *L* was found in four subjects (*L*: −8.8%), *S* was decreased in four subjects as well (*S*: −7.3%), and increased in one subject (+15.6%). Breaking the data down to a single subject level, changes in the same direction were observed in all subjects for *C*, in 3 of 4 for *L*, and in 4 of 5 for *S*.

As expected from the abovementioned results, prolonged and longer-lasting stimulation effects on local network characteristics presented no clear-cut substructures and the most central network constituents remained unaltered.

4. Discussion

We employed an examination schedule consisting of two short-term transcutaneous auricular vagus nerve stimulations (one taVNS in the morning and one in the afternoon) to investigate whether taVNS-induced modifications of global and local characteristics of evolving human functional brain networks depend on time of day, and whether a neuromodulatory effect (afternoon stimulation amplifies modifications induced by the morning stimulation) can be identified. In the following, we discuss our findings obtained from twelve subjects in the light of the available research results.

4.1. Time-of-Day-Dependence of taVNS-Mediated Network Modifications: From Global to Local

Both the morning and the afternoon stimulation led to measurable immediate and enduring modifications of the global characteristics of the subjects' large-scale evolving brain networks. Modifications, however, presented a clear dependence on time of day, despite our efforts to minimize the potential confounding influence of various ultradian rhythms. Whereas the afternoon-stimulation-mediated, pronounced, immediate, topology-modifying (more integrated and less segregated network), and stability-enhancing effects seen in the majority of responders corroborate previous findings [15,21] (note that in these studies, stimulations were also performed in the afternoon), pre-described enduring effects presented slightly different. This might be explained by the fact that subjects were stimulated twice, whereas in previous studies [15,21], subjects were taVNS-naïve. Interestingly, morning-stimulation-mediated modifications were rather inconsistent, despite the fact that some modifications appeared to be more strongly pronounced than the corresponding ones following the afternoon stimulation. In general, whereas pre-described immediate, enduring, topology-modifying, and stability-enhancing effects [15,21] could be observed in about 50% of responders, another 50% presented opposing modifications. Although this observation, at first glance, appears to put into perspective previous reports on taVNS-mediated modifications of global network properties, it clearly points to non-negligible influences of time of day and needs further investigation.

Contrasting the aforementioned findings, neither the morning nor the afternoon stimulation appeared to impact the most central network constituents, whose role in the larger network we rated with various opposing centrality concepts. As expected, these concepts identified different constituents as most central. In line with previous observations [15,21], our findings indicate short-term taVNS to be spatially unspecific on the local scale, thus supporting the prevalent view of a global-acting mode of action of taVNS.

Taken together, our results indicate an important influence of time of day on taVNS-mediated modifications of various properties of evolving functional brain networks. This emphasizes not only the need to report the time of day of stimulation as recommended previously [37], but also the necessity to consider diurnal variations for the interpretation of research findings and clinical trials, as well as for the formulation of application recommendations. It is, however, conceivable that there are additional confounding factors that affect the results of taVNS stimulation, such as habituation effects.

Thus, at first glance, counter-intuitive observations of unaffected local properties but strongly-affected global ones, which, additionally appear to be dependent on the time of day, can be reconciled well with an extension of the previously suggested model of a stimulation-induced stretching and compression of the functional brain network (see [21] for a detailed description of the model). With this model, taVNS-mediated modifications of the larger networks are characterized by taking into account the changes of the network's path structure (average shortest path length), its tendency to form tightly knit groups of vertices (average clustering coefficient), and the importance of hierarchies of vertices and edges. Here, we observed the dynamics of this model for the vast majority of responders with the afternoon stimulation, and for about half of the responders with the morning stimulation. Since the other half of the responders in the morning stimulation resulted in a reversed pattern, this might point to a sensitive dependence on time of day. With the extension of the model proposed here (Figure 5), we propose that this dependence can be characterized by some rhythmic activities that interfere with the stimulation sequence. These activities may predominantly represent biological rhythms with different period lengths (ultradian and/or circadian rhythms), diurnal fluctuations of the vagal activity, as well as superpositions thereof.

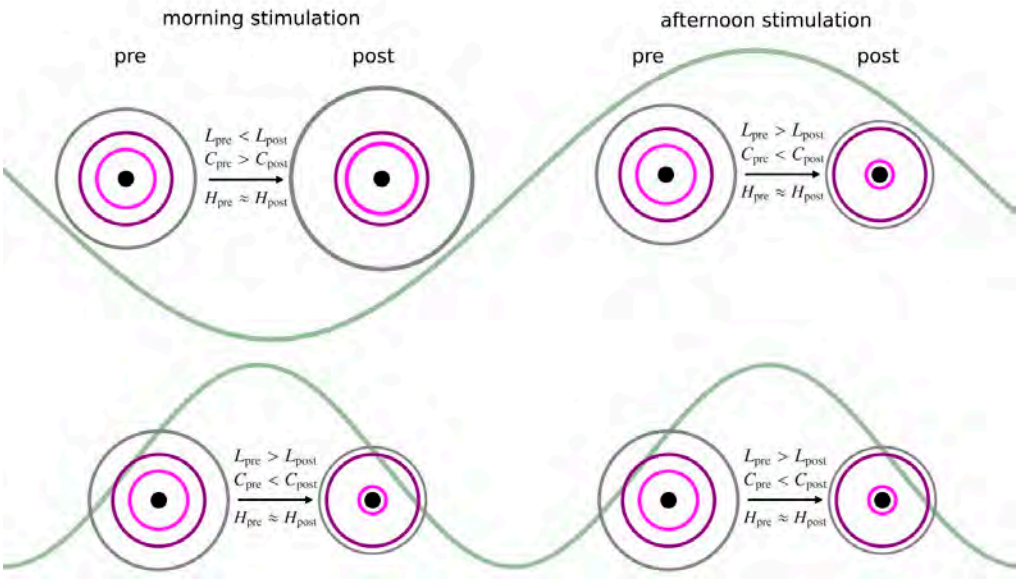

Figure 5. Schematic of spatial networks prior to and after taVNS stimulation in the morning (**left**) and in the afternoon (**right**), with different rhythmic activities (longer period lengths: top, shorter period lengths: bottom; indicated by greenish waves in the background) that interfere with the stimulation-mediated network modification. The network is separated into different areas based on network properties: (average clustering coefficient C and average shortest path length L): boundary (gray outermost ring), periphery (purple and pink rings in the middle), and core (black innermost ring/circle). The larger the radius of a ring, the higher the L and the smaller the C of vertices in this area. The closer two rings, the more clustered the vertices are in the two areas relative to each other. Local importance hierarchies H (assessed with different centrality concepts) of vertices and edges are not affected by the stimulation.

4.2. Prolonged and Longer-Lasting taVNS-Mediated Network Modifications

Our approach allowed us to assess taVNS-mediated network modifications on shorter (immediate and enduring effects) and longer time scales (prolonged and longer-lasting effects). For the latter, we solely observed modifications of global network properties, whereas local ones remained largely unaltered. Prolonged effects resembled, to a large extent, the immediate effects seen for the morning stimulation, and in general, few responders presented inconsistent modifications of their functional brain networks. For an even smaller subset of responders, we observed longer-lasting effects, and these subjects presented network modifications similar to the ones observed as immediate effects following the afternoon stimulation. Nevertheless, given that the majority of subjects did not present longer-lasting effects, potential factors affecting short-term taVNS-mediated network modifications on longer time scales remain to be identified. In this regard, a comparison with previous research findings is only of limited value. Although immediate effects of short-term taVNS on brain dynamics have been reported repeatedly in healthy and different medical conditions, so far information on potential effects acting on time scales that range from hours to years can only be derived from clinical studies in different diseases [12,13,38]. These studies, however, are largely based on repeated daily stimulations over long periods of time, and there might be other potential influencing factors, such as pharmaceutical treatment, that would need to be taken into account when interpreting stimulation-related modifications of brain dynamics. In addition, in these studies, taVNS-mediated effects are assessed only indirectly via the clinical outcome (e.g., seizure frequency, headache scores, depression scores). During the time after the stimulation and over subsequent days, knowledge about taVNS-mediated effects on the human brain is sparse. We expect, however, that ultradian rhythms with period lengths longer than the ones considered here, as well as circadian or even infradian rhythms, would need to be considered as potential confounders when investigating long-lasting taVNS-mediated modifications of evolving functional brain networks.

4.3. Can a Neuromodulatory Effect of Short-Term taVNS Be Identified?

In line with previous studies [15,21], we observed significant immediate short-term modifications of topology- and stability-related network properties in up to three quarters of investigated subjects. A subset of those subjects presented as responders to both stimulations, but the percentage of responders to both stimulations was essentially the same. With our examination schedule (one stimulation in the morning and one in the afternoon, with a heuristically chosen gap between them) and classification of stimulation effects, we could not identify a potential neuromodulatory effect (the second stimulation amplifies network modifications induced by the first stimulation); however, an influence cannot be excluded by our design, since even non-significant changes resulting from the first stimulation might serve as amplifier for the second one. There is experimental (cf. [39]) and clinical evidence (for example, from studies in epilepsy, cf. [13]) for vagal nerve stimulation (VNS) to have a neuromodulatory effect that increases over time. As the vagal nerve itself has a transmitting and not a processing function [39], amplifying effects due to repeated stimulations are assumed to act on the molecular, neurotransmitter, and synaptic level. Though the exact mechanism of action is not fully understood, a growing body of evidence for VNS-induced alterations of different transmitter pathways is available, and to some extent, can be extended to taVNS [7]. It is supposed that a certain—not yet fully determined—threshold must be exceeded to achieve neuromodulatory effects of taVNS. Future studies would need to identify the necessary amount, duration, and period of time of stimulations along with their temporal arrangement that are required to surpass the hypothesized threshold.

To conclude, our findings point to an important influence of time of day on taVNS-mediated modifications of various properties of evolving functional brain networks. Future studies should address the influence with a double-blind approach (stimulation versus sham stimulation) and interpersonal work-up (morning stimulation versus afternoon stimulation group) to further corroborate our findings. Future studies should also investigate a possible relationship between taVNS-induced modifications of functional brain networks

and clinical efficacy, which is needed to translate experimental results into clinical decision making. Importantly though, future clinical trials should take into account the potential diurnal influence. Considering the time of day when vagal stimulating is supposed to be most effective, and might not only increase the clinical outcome, but might also allow the stimulation time to be reduced, which could increase adherence. Investigating taVNS-mediated modifications of evolving human functional brain networks on longer time scales is necessary to better understand the mechanism of action in taVNS, as well as to establish meaningful protocols for research and treatment trials.

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References

1. Lampros, M.; Vlachos, N.; Zigouris, A.; Voulgaris, S.; A Alexiou, G. Transcutaneous Vagus Nerve Stimulation (t-VNS) and epilepsy: A systematic review of the literature. *Seizure* **2021**, *91*, 40–48. [CrossRef] [PubMed]
2. Straube, A.; Ellrich, J.; Eren, O.; Blum, B.; Ruscheweyh, R. Treatment of chronic migraine with transcutaneous stimulation of the auricular branch of the vagal nerve (auricular t-VNS): A randomized, monocentric clinical trial. *J. Headache Pain* **2015**, *16*, 63. [CrossRef] [PubMed]
3. Briand, M.-M.; Gosseries, O.; Staumont, B.; Laureys, S.; Thibaut, A. Transcutaneous Auricular Vagal Nerve Stimulation and Disorders of Consciousness: A Hypothesis for Mechanisms of Action. *Front. Neurol.* **2020**, *11*, 993. [CrossRef]
4. Colzato, L.; Beste, C. A literature review on the neurophysiological underpinnings and cognitive effects of transcutaneous vagus nerve stimulation: Challenges and future directions. *J. Neurophysiol.* **2020**, *123*, 1739–1755. [CrossRef]
5. Carandina, A.; Rodrigues, G.D.; Di Francesco, P.; Filtz, A.; Bellocchi, C.; Furlan, L.; Carugo, S.; Montano, N.; Tobaldini, E. Effects of transcutaneous auricular vagus nerve stimulation on cardiovascular autonomic control in health and disease. *Auton. Neurosci.* **2021**, *236*, 102893. [CrossRef] [PubMed]
6. van Beekum, C.J.; Willis, M.A.; von Websky, M.W.; Sommer, N.P.; Kalff, J.C.; Wehner, S.; Vilz, T.O. Electrical vagus nerve stimulation as a prophylaxis for SIRS and postoperative ileus. *Auton. Neurosci.* **2021**, *235*, 102857. [CrossRef]
7. Kaniusas, E.; Kampusch, S.; Tittgemeyer, M.; Panetsos, F.; Gines, R.F.; Papa, M.; Kiss, A.; Podesser, B.; Cassara, A.M.; Tanghe, E.; et al. Current Directions in the Auricular Vagus Nerve Stimulation I–A Physiological Perspective. *Front. Neurosci.* **2019**, *13*, 854. [CrossRef]
8. Jiao, Y.; Guo, X.; Luo, M.; Li, S.; Liu, A.; Zhao, Y.; Zhao, B.; Wang, D.; Li, Z.; Zheng, X.; et al. Effect of Transcutaneous Vagus Nerve Stimulation at Auricular Concha for Insomnia: A Randomized Clinical Trial. *Evidence-Based Complement. Altern. Med.* **2020**, *2020*, 6049891. [CrossRef]
9. Kaniusas, E.; Szeles, J.C.; Kampusch, S.; Alfageme-Lopez, N.; Yucumá, D.; Li, X.; Mayol, J.; Neumayer, C.; Papa, M.; Panetsos, F. Non-invasive Auricular Vagus Nerve Stimulation as a Potential Treatment for Covid19-Originated Acute Respiratory Distress Syndrome. *Front. Physiol.* **2020**, *11*, 890. [CrossRef]
10. Azabou, E.; Bao, G.; Bounab, R.; Heming, N.; Annane, D. Vagus Nerve Stimulation: A Potential Adjunct Therapy for COVID-19. *Front. Med.* **2021**, *8*, 625836. [CrossRef]

11. Yap, J.Y.Y.; Keatch, C.; Lambert, E.; Woods, W.; Stoddart, P.R.; Kameneva, T. Critical Review of Transcutaneous Vagus Nerve Stimulation: Challenges for Translation to Clinical Practice. *Front. Neurosci.* **2020**, *14*, 284. [CrossRef]
12. Straube, A.; Eren, O. tVNS in the management of headache and pain. *Auton. Neurosci.* **2021**, *236*, 102875. [CrossRef] [PubMed]
13. von Wrede, R.; Surges, R. Transcutaneous vagus nerve stimulation in the treatment of drug-resistant epilepsy. *Auton. Neurosci.* **2021**, *235*, 102840. [CrossRef] [PubMed]
14. Sabers, A.; Aumüller-Wagner, S.; Christensen, L.R.; Henning, O.; Kostov, K.; Lossius, M.; Majoie, M.; Mertens, A.; Nielsen, L.; Vonck, K.; et al. Feasibility of transcutaneous auricular vagus nerve stimulation in treatment of drug resistant epilepsy: A multicenter prospective study. *Epilepsy Res.* **2021**, *177*, 106776. [CrossRef]
15. von Wrede, R.; Rings, T.; Schach, S.; Helmstaedter, C.; Lehnertz, K. Transcutaneous auricular vagus nerve stimulation induces stabilizing modifications in large-scale functional brain networks: Towards understanding the effects of taVNS in subjects with epilepsy. *Sci. Rep.* **2021**, *11*, 7906. [CrossRef] [PubMed]
16. Huikuri, H.V.; Kessler, K.M.; Terracall, E.; Castellanos, A.; Linnaluoto, M.K.; Myerburg, R.J. Reproducibility and circadian rhythm of heart rate variability in healthy subjects. *Am. J. Cardiol.* **1990**, *65*, 391–393. [CrossRef]
17. Van Dijk, N.; Boer, M.C.; De Santo, T.; Grovale, N.; Aerts, A.J.J.; Boersma, L.; Wieling, W. Daily, weekly, monthly, and seasonal patterns in the occurrence of vasovagal syncope in an older population. *Europace* **2007**, *9*, 823–828. [CrossRef]
18. Jarczok, M.N.; Aguilar-Raab, C.; Koenig, J.; Kaess, M.; Borniger, J.C.; Nelson, R.J.; Hall, M.; Ditzen, B.; Thayer, J.F.; Fischer, J.E. The Heart´s rhythm 'n' blues: Sex differences in circadian variation patterns of vagal activity vary by depressive symptoms in predominantly healthy employees. *Chronobiol. Int.* **2018**, *35*, 896–909. [CrossRef]
19. Chow, E.; Bernjak, A.; Williams, S.; Fawdry, R.A.; Hibbert, S.; Freeman, J.; Sheridan, P.J.; Heller, S.R. Risk of Cardiac Arrhythmias During Hypoglycemia in Patients With Type 2 Diabetes and Cardiovascular Risk. *Diabetes* **2014**, *63*, 1738–1747. [CrossRef]
20. Morris, C.J.; Yang, J.N.; Scheer, F.A. The impact of the circadian timing system on cardiovascular and metabolic function. *Prog. Brain Res.* **2012**, *199*, 337–358. [CrossRef]
21. Rings, T.; von Wrede, R.; Bröhl, T.; Schach, S.; Helmstaedter, C.; Lehnertz, K. Impact of Transcutaneous Auricular Vagus Nerve Stimulation on Large-Scale Functional Brain Networks: From Local to Global. *Front. Physiol.* **2021**, *12*, 1328. [CrossRef]
22. Bullmore, E.T.; Sporns, O. Complex brain networks: Graph theoretical analysis of structural and functional systems. *Nat. Rev. Neurosci.* **2009**, *10*, 186–198. [CrossRef] [PubMed]
23. Lehnertz, K.; Ansmann, G.; Bialonski, S.; Dickten, H.; Geier, C.; Porz, S. Evolving networks in the human epileptic brain. *Phys. D Nonlinear Phenom.* **2013**, *267*, 7–15. [CrossRef]
24. Lehnertz, K.; Rings, T.; Bröhl, T. Time in Brain: How Biological Rhythms Impact on EEG Signals and on EEG-Derived Brain Networks. *Front. Netw. Physiol.* **2021**, *1*, 755016. [CrossRef]
25. Tononi, G.; Sporns, O.; Edelman, G.M. A measure for brain complexity: Relating functional segregation and integration in the nervous system. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 5033–5037. [CrossRef] [PubMed]
26. Newman, M. *Networks*, 2nd ed.; Oxford University Press: Oxford, UK, 2018.
27. Newman, M.E.J. Assortative Mixing in Networks. *Phys. Rev. Lett.* **2002**, *89*, 208701. [CrossRef]
28. Bialonski, S.; Lehnertz, K. Assortative mixing in functional brain networks during epileptic seizures. *Chaos Interdiscip. J. Nonlinear Sci.* **2013**, *23*, 33139. [CrossRef]
29. Bröhl, T.; Lehnertz, K. Centrality-based identification of important edges in complex networks. *Chaos Interdiscip. J. Nonlinear Sci.* **2019**, *29*, 033115. [CrossRef]
30. Fruengel, R.; Bröhl, T.; Rings, T.; Lehnertz, K. Reconfiguration of human evolving large-scale epileptic brain networks prior to seizures: An evaluation with node centralities. *Sci. Rep.* **2020**, *10*, 21921. [CrossRef]
31. Geier, C.; Lehnertz, K. Long-term variability of importance of brain regions in evolving epileptic brain networks. *Chaos Interdiscip. J. Nonlinear Sci.* **2017**, *27*, 043112. [CrossRef]
32. Kuhnert, M.-T.; Geier, C.; Elger, C.E.; Lehnertz, K. Identifying important nodes in weighted functional brain networks: A comparison of different centrality approaches. *Chaos Interdiscip. J. Nonlinear Sci.* **2012**, *22*, 023142. [CrossRef] [PubMed]
33. Bröhl, T.; Lehnertz, K. A straightforward edge centrality concept derived from generalizing degree and strength. *Sci. Rep.* **2022**, *12*, 4407. [CrossRef] [PubMed]
34. Yook, S.H.; Jeong, H.; Barabási, A.-L.; Tu, Y. Weighted Evolving Networks. *Phys. Rev. Lett.* **2001**, *86*, 5835–5838. [CrossRef]
35. Barrat, A.; Barthelemy, M.; Pastor-Satorras, R.; Vespignani, A. The architecture of complex weighted networks. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 3747–3752. [CrossRef] [PubMed]
36. Press, W.H.; Rybicki, G.B. Fast Algorithm for Spectral Analysis of Unevenly Sampled Data. *Astrophys. J.* **1989**, *338*, 277–280. [CrossRef]
37. Farmer, A.D.; Strzelczyk, A.; Finisguerra, A.; Gourine, A.V.; Gharabaghi, A.; Hasan, A.; Burger, A.M.; Jaramillo, A.M.; Mertens, A.; Majid, A.; et al. International Consensus Based Review and Recommendations for Minimum Reporting Standards in Research on Transcutaneous Vagus Nerve Stimulation (Version 2020). *Front. Hum. Neurosci.* **2021**, *14*, 409. [CrossRef] [PubMed]
38. Rong, P.; Liu, J.; Wang, L.; Liu, R.; Fang, J.; Zhao, J.; Zhao, Y.; Wang, H.; Vangel, M.; Sun, S.; et al. Effect of transcutaneous auricular vagus nerve stimulation on major depressive disorder: A nonrandomized controlled pilot study. *J. Affect. Disord.* **2016**, *195*, 172–179. [CrossRef]
39. Beekwilder, J.P.; Beems, T. Overview of the Clinical Applications of Vagus Nerve Stimulation. *J. Clin. Neurophysiol.* **2010**, *27*, 130–138. [CrossRef]

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Zielsetzung der Arbeit: In dieser Arbeit wurde der Einfluss der taVNS auf sich entwickelnde funktionelle Hirnnetzwerke auf lokaler und globaler Netzwerkebene bei Proband*innen mit verschiedenen Epilepsietypen und solchen ohne Epilepsien untersucht.

Methoden und Ergebnisse: Es wurden 30 Proband*innen entsprechend ihres Epilepsietyps in drei Gruppen eingeteilt (Gruppe mit fokaler Epilepsie (G1), Gruppe mit generalisierter Epilepsie (G2) und Gruppe ohne Epilepsie oder epileptische Anfälle (G3)) und durchliefen ein Studienprotokoll mit einer kontinuierlichen mindestens 3-stündigen EEG-Vielkanal-Aufzeichnung, welche von einer standardisierten neuropsychologischen Untersuchung begleitet wurde. Aus der zeitaufgelösten Synchronisationsanalyse der EEG-Aufzeichnung wurden sich entwickelnde, vollständig verbundene und gewichtete Netzwerke abgeleitet und verschiedene lokale (C^B, C^E) und globale (C, L, A, S) Kenngrößen erhoben. Auf der globalen Netzwerkskala wiesen die Gruppen G1 und G3 in allen Phasen der Studie vergleichbare topologische Netzwerkcharakteristika auf. Bereits vor als auch während der Stimulation zeigten sich die Netzwerke der Gruppe G2 signifikant weniger segregiert und stärker integriert als die Netzwerke der Gruppen G1 und G3. Die verschwindenden Unterschiede zwischen den Gruppen nach der Stimulation verweisen möglicherweise auf einen taVNS-vermittelten topologieverändernden Effekt in G3. Hinsichtlich der Stabilitäts- und Robustheitsmerkmale der Netzwerke zeigten die drei Gruppen in allen drei Studienphasen vergleichbare Ergebnisse. Sowohl die immediaten als auch die anhaltenden taVNS-induzierten Veränderungen der Netzwerkcharakteristika unterschieden sich zwischen den untersuchten Gruppen: für G1 und G3 fand sich ein unmittelbarer Stimulationseffekt, der diese Netze stärker segregiert (Abnahme von C) und, zumindest für G1, weniger integriert (Anstieg von L) machte. Als anhaltender Effekt zeigten sich die Netzwerke von G1 und G3 weniger segregiert und stärker integriert. Netzwerke der Gruppe mit generalisierter Epilepsie (G2) hingegen wiesen ein gegenläufiges Muster der stimulationsvermittelten Veränderungen auf (immediater Stimulationseffekt von weniger segregierten/stärker integrierten Netzwerken und anhaltenden Effektes von stärker segregierten/weniger integrierten Netzwerken). TaVNS übte in allen Gruppen eine unmittelbare robustheitssteigernde Wirkung auf die Netzwerke aus.

Diese robustheitssteigernde Wirkung war bei G1 und G3 anhaltend, während sich bei G2 ein die Robustheit vermindernder anhaltender Effekt zeigte. In Bezug auf die Netzwerkstabilität induzierte die taVNS eine immediate Verringerung der Anfälligkeit der Netzwerke für Störungen des synchronisierten Zustands bei G2 und G3, während dieser immediate Effekt bei G1 vernachlässigbar war. Die anhaltenden Effekte führten bei G1 zu einer Verringerung und bei G2 zu einer Erhöhung der Vulnerabilität der Netzwerke.

Auf der lokalen Netzwerkskala erhielten wir je nach dem verwendeten Konzept der Vertex-Zentralität, wie zu erwarten, unterschiedliche Ergebnisse: mit der Betweenness-Zentralität C^B erschienen die mit den linken fronto-centro-temporalen Hirnregionen assoziierten Vertices in allen Proband*innengruppen als besonders wichtig. Im Gegensatz dazu zeigte die Eigenvektor-Zentralität C^E einen posterioren-anterioren Gradienten der Vertexwichtigkeit. Die wichtigsten Vertices unterschieden sich weder zwischen den Gruppen noch zwischen den Phasen signifikant. Hinsichtlich der Wichtigkeit von Kanten, d.h. von Interaktionen zwischen Hirnregionen, zeigte keines der verwendeten Konzepte der Kantenzentralität ein klares räumliches Muster von Unterschieden, weder zwischen Gruppen noch zwischen Phasen. Die meisten Proband*innen wiesen signifikante taVNS-vermittelte Veränderungen ihrer lokalen Netzwerkeigenschaften auf, hierbei interessanterweise am häufigsten in G2, allerdings ohne ein erkennbares räumliches Muster. In G1 zeigten sich die taVNS-vermittelten Veränderungen der Wichtigkeit (mit C^B bestimmt) vor allem bei Vertices, die mit fronto-temporalen Hirnarealen verbunden waren. Andere taVNS-vermittelte Veränderungen der Knoten und Kantenzentralitäten zeigten sich diffus und ohne klares räumliches Muster.

Schlussfolgerungen: Im Ruhezustand unterschieden sich die topologischen Eigenschaften der funktionellen Hirnnetzwerke von Proband*innen bei fokalen und generalisierten Epilepsien. Ebenso zeigten sich bei beiden Gruppen unterschiedliche taVNS-induzierte sofortige und dauerhafte Reorganisationen der globalen Netzwerkeigenschaften ohne ein erkennbares räumliches Muster auf der lokalen Netzwerkskala.

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Transcutaneous Auricular Vagus Nerve Stimulation Differently Modifies Functional Brain Networks of Subjects With Different Epilepsy Types

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Epilepsy types differ by pathophysiology and prognosis. Transcutaneous auricular vagus nerve stimulation (taVNS) is a non-invasive treatment option in epilepsy. Nevertheless, its mode of action and impact on different types of epilepsy are still unknown. We investigated whether short-term taVNS differently affects local and global characteristics of EEG-derived functional brain networks in different types of epilepsy. Thirty subjects (nine with focal epilepsy, 11 with generalized epilepsy, and 10 without epilepsy or seizures) underwent a 3-h continuous EEG-recording (1 h pre-stimulation, 1 h taVNS stimulation, 1 h post-stimulation) from which we derived evolving functional brain networks. We assessed—in a time-resolved manner—important global (topological, robustness, and stability properties) and local (centralities of vertices and edges) network characteristics. Compared to the subjects with focal epilepsies and without epilepsy, those with generalized epilepsies clearly presented with different topological properties of their functional brain network already at rest. Furthermore, subjects with focal and generalized epilepsies reacted differently to the stimulation, expressed as different taVNS-induced immediate and enduring reorganization of global network characteristics. On the local network scale, no discernible spatial pattern could be detected, which points to a rather unspecific and generalized modification of brain activity. Assessing functional brain network characteristics can provide additional information for differentiating between focal and generalized epilepsy. TaVNS-related modifications of global network characteristics clearly differ between epilepsy types. Impact of such a non–pharmaceutical intervention on clinical decision-making in the treatment of different epilepsy types needs to be assessed in future studies.

Keywords: epileptic brain networks, epilepsy, epilepsy type, transcutaneous vagal nerve stimulation (TVNS), functional networks

INTRODUCTION

Epilepsy is one of the most common neurological disorders with a prevalence of 0.5–1% and about 50 million affected subjects (people with epilepsy; PWE) worldwide (GBD 2016 Epilepsy Collaborators, 2019; Hauser and Hesdorffer, 2019; World Health Organization [WHO], 2019). According to the recent proposal of the International League against Epilepsy (ILAE), this disorder is a disease of the brain with at least two unprovoked (or reflex) seizures, or one unprovoked (or reflex) seizure and a probability of at least 60% for further seizures to occur over the next 10 years, or diagnosis of an epilepsy syndrome (Fisher et al., 2014). The most recent ILAE classification of epilepsy provides a very sophisticated schedule for seizure type, epilepsy type and, at each stage of classification, potential etiology of epilepsy (Scheffer et al., 2017). Therefore, the actual classified epilepsy might change over time in some PWE. Nevertheless, classification of epilepsies is substantial for clinical decisions, for clinical and basic epilepsy research as well as for the evaluation and development of new treatment options. Obviously, PWE with structural focal epilepsies might be candidates for epilepsy surgery, PWE with genetic epilepsies due to Glut-1 deficiency are candidates for ketogenic diet, and PWE with limbic encephalitis might profit from immunomodulation. What is more, studies on antiseizure medication (ASM) provide information on efficacy in different epilepsy types, thus providing useful and indispensable information for clinical consultation (Marson et al., 2007a,b, 2021).

The human brain can be understood as a complex network and epilepsy as a network disorder (Bullmore and Sporns, 2009; Berg and Scheffer, 2011). The study of network dynamics can be carried out in spatial as well as in temporal dimensions using different approaches. Electroencephalography is a non-invasive and easy-to-use method in terms of spatial and temporal scales. Tracking network characteristics over time can help to identify intervention-related alterations of brain activity as already been shown by the so-called "pharmaco-EEG" which has provided relevant insights in treatment response, ASM side effects and prediction of those (Höller et al., 2018). Namely, by using an analysis approach that investigates EEG-derived evolving functional brain networks, different global and local network characteristics can be assessed. It is conceivable that different epilepsy types display differences in network characteristics that might provide additional information for differentiating epilepsy types to support clinical evaluation.

ASM is the basis of any epilepsy treatment, but unfortunately for one third of PWE extensive pharmacotherapy attempts have to be undertaken for an at least acceptable seizure situation (Kwan and Brodie, 2000); even the newly developed ASM have not changed this situation significantly (Chen et al., 2018). Pharmacotherapy-resistance is a great burden for PWE and their caregivers. Thus, there is a strong need for alternative or complementary non-pharmaceutical treatment options. Brain stimulation techniques are well established in the treatment of epilepsy. Invasive vagus nerve stimulation (iVNS) is used for decades with more than 100,000 implanted systems (Fisher et al., 2020), and efficacy and safety are well documented over the years with responder (PWE in whom seizure frequency is reduced by more than 50%) rates of up to 50% (Elliott et al., 2011; Morris et al., 2013). Though generally well tolerated and even having a positive impact on mood, risk of anesthesia and surgery have to be considered with an overall complication rate of up to 12%, and surgical complication rate amounts up to 8.6% (Révész et al., 2016). Transcutaneous auricular vagus nerve stimulation (taVNS), the non-invasive external stimulation of the auricular branch of the vagus nerve, is an alternative worth of investigation. Good efficacy, tolerability and usability was previously shown for taVNS (Stefan et al., 2012; Bauer et al., 2016; Barbella et al., 2018; Liu et al., 2018; von Wrede et al., 2019). Most clinical trials have been conducted with PWE with focal epilepsy or in groups consisting of subjects with focal or generalized epilepsy. However, a thorough work up on differences in terms of efficacy in different epilepsy types is missing (Lampros et al., 2021). As the number of participants in above mentioned studies is quite low and only few data from randomized controlled trials is available, a final assessment of the efficacy is not yet available.

To date, the mode of action of vagus nerve stimulation is not fully understood, but may involve alterations of different metabolic pathways (for an overview see Farmer et al., 2021). Hence, it is supposed that VNS leads to a rather unspecific, global activation of various brain structures [including thalamus, limbic system, insular cortex (Rutecki, 1990; Ben-Menachem, 2002)]. Recently, modifications of brain network topology as well as modification of network stability and robustness were shown in a larger group of subjects with and without central nervous system diseases corroborating the idea of an unspecific global activation (Rings et al., 2021; von Wrede et al., 2021). As epilepsy types differ clinically and pathophysiologically, we hypothesized that effects of non-pharmaceutical interventions on functional brain networks in different epilepsy types differ as well. To test this hypothesis, we investigated short-term taVNS-induced immediate and enduring modifications of global and local characteristics of evolving functional brain networks in subjects with different types of epilepsy and non-epilepsy subjects.

MATERIALS AND METHODS

Subjects

Subjects who were admitted to our ward from March 2020 to February 2021 were screened for suitability for this study. Inclusion criteria were clinical necessity (differential diagnosis or electrophysiological follow-up) for long-term video-EEG-recording and age 18 years and older. Exclusion criteria were previous brain surgery, actual or previous neurostimulation such as invasive or non-invasive vagus nerve stimulation or deep brain stimulation, progressive disease, seizures occurring within 24 h before the start of the study, insufficient German language capability, mental disability and incompetence to follow instructions. Demographic data were derived from patient reports, and epilepsy type was classified according to Scheffer et al. (2017). Subjects were assigned to three different groups: focal epilepsy group (G1), generalized epilepsy group (G2), and non-epilepsy group (G3). After being provided with written

information and being given the opportunity to ask further questions, 35 subjects volunteered to participate and signed informed consent.

Transcutaneous Auricular Vagus Nerve Stimulation and Examination Schedule

Following previous studies (Rings et al., 2021; von Wrede et al., 2021), we applied transcutaneous auricular vagus nerve stimulation for 1 h in the early afternoon while the subjects underwent a 3 h continuous video-EEG-recording [1 h pre-stimulation baseline 1 (B1), 1 h taVNS (S) and 1 h post-stimulation baseline 2 (B2)]. During this 3-h block, subjects continued laid-back activities (awake, no other activation methods applied). Stimulation was carried out unilaterally (left cymba conchae) using two hemispheric titanium electrodes of a taVNS device (tVNS Technologies GmbH, Erlangen, Germany) with a set of non-adjustable parameters (biphasic signal form, impulse frequency 25 Hz, impulse duration 20 s, impulse pause 30 s) and individually adjusted intensity of stimulation until the subject experienced a "tingling," but no painful sensations. All subjects were under stable CNS medication (if taking any) and no activation methods (such as hyperventilation or sleep deprivation) were applied at least 24 h before start of the examination. In order to track possible changes of cognition and behavior, a standardized neuropsychological assessment [EpiTrack® and a modified version of the Adverse Events Profile (AEP)] preceded and followed the EEG-recording. After stimulation the subjects answered a questionnaire on the evaluation of the device usability and tolerability (for details of tests see **Supplementary Appendix A**).

Electroencephalogram Recordings and Data Pre-processing

We recorded electroencephalograms (EEG) from 19 electrodes (18 electrode sites according to the 10–20 system and Cz served as physical reference). EEG data were sampled at 256 Hz using a 16 bit analog-to-digital converter and were band-pass filtered offline between 1 and 45 Hz (4th order Butterworth characteristic). To suppress contributions at the line frequency (50 Hz) a notch filter (3rd order) was applied. All recordings were visually inspected for strong artifacts (subject movements, amplifier saturation, or stimulation artifacts) and such data were excluded from further analyses.

Characterizing Functional Brain Networks on Global and Local Scale

Functional networks consist of vertices and edges. We here associated network vertices with brain regions sampled by the EEG electrode contacts and network edges with time-varying estimates of the strength of interactions between the dynamics of pairs of those brain regions, regardless of their anatomical connections. Following previous studies, we derived evolving, fully connected and weighted networks from a time-resolved synchronization analysis of the above mentioned 3-h EEG-recording, assessed important global and local characteristics of the networks, and tracked their changes over time (for details see **Supplementary Appendix B**).

On the global network scale, we assessed the topological characteristics average clustering coefficient C and average shortest path length L. The average clustering coefficient C characterizes the network's functional segregation; the lower C, the more segregated is the weighted fully connected network. The average shortest path length L characterizes the network's functional integration; the lower L, the more integrated is the weighted fully connected network. In this model, functional segregation (integration) reflects independent (dependent) information processes between brain regions (Tononi et al., 1994).

Furthermore, we assessed the network's robustness and stability characteristics. Assortativity A reflects the tendency of edges to connect vertices with similar or equal properties. If edges preferentially connect vertices with dissimilar properties, such networks are called disassortative. Disassortative networks are more vulnerable to perturbations and appear to be easier to synchronize than assortative networks. Synchronisability S assesses the network's propensity (or vulnerability) to get synchronized by an admissible input activation: the higher S, the more easily can the synchronized state be perturbed.

On the local network scale, we assessed importance of vertices and edges using two different and opposing centrality concepts: a path-based and an interaction-strength-based one. Both of them provide non-redundant information about the role vertices and edges play in the larger network. As path-based centrality index, we employed betweenness centrality C^B. A vertex/edge with high C^B is central since it connects different regions of the network as a bridge. As interaction-strength-based centrality index, we employed eigenvector centrality C^E. A vertex/edge with high C^E is central since the vertices/edges connected to it are central as well, therefore it reflects the influence of the vertex/edge on the network as a whole (for details see **Supplementary Appendix C**).

Statistical Analyses

For each phase of the examination schedule (B1, S, and B2), we investigated whether the three subject groups (G1, G2, and G3) presented with different global and local network characteristics (Mann-Whitney U-test; $p < 0.05$). For each subject group, we investigated whether global and local network characteristics differed between the phases of the examination schedule (Mann-Whitney U-test; $p < 0.05$). In addition, and in order to distinguish cases that responded to the stimulation from non-responding cases, we repeated the latter analysis on a single subject level. All p-values were corrected for multiple comparisons using the Bonferroni method. Differences in taVNS intensities were investigated in the three subject groups (Kruskal-Wallis test; $p < 0.05$). Eventually, we tested for differences between neuropsychological variables assessed prior to and after the EEG-recording [repeated measures ANOVA; within-subject factor: EpiTrack® pre/post score; between-subject factor: group (G1, G2, and G3); $p < 0.05$]. Furthermore, we investigated whether the assessment of usability of the device differs between the three

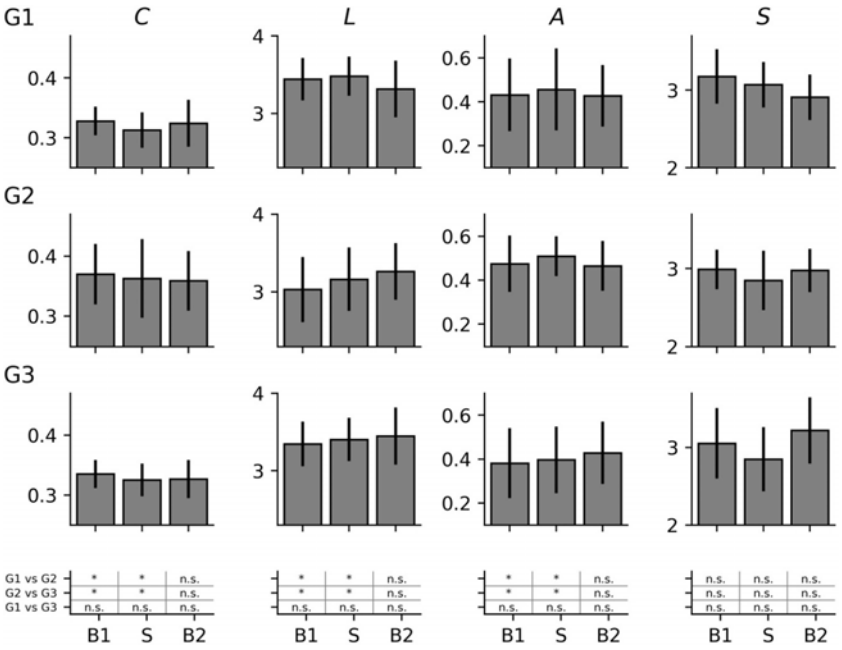

FIGURE 1 | Global network characteristics (average clustering coefficient *C*, average shortest path length *L*, synchronisability *S*, and assortativity *A*) of the investigated groups (G1 = focal epilepsy group, G2 = generalized epilepsy group, G3 = non-epilepsy group) in the three phases of the study (B1 = pre-stimulation baseline 1, S = stimulation, B2 = post-stimulation baseline 2). Mean values and standard deviation. *significant (Mann-Whitney *U*-test, $p < 0.05$), n.s. = non-significant.

subject groups (G1, G2, and G3) (Mann-Whitney *U*-test; $p < 0.05$).

RESULTS

From the thirty-five eligible subjects, five subjects had to be excluded due to EEG data quality. Data from thirty subjects (20 females; age 18–55 years, median 26.5 years) were included in the analyses. Twenty subjects suffered from epilepsy, 9 subjects from focal (G1: 5 females; age 18–55 years, median 26 years) and 11 subjects from generalized epilepsy (G2: 7 females; age 18–54 years, median 22 years). Fifteen of those 20 PWE (75%) had to be considered as drug-resistant according to the definition of the ILAE (Kwan et al., 2010), with 6 PWE with focal epilepsy and 9 PWE with generalized epilepsy. Ten subjects did not suffer from epilepsy and had never experienced seizures before (G3: 8 females; age 19–42 years, median 27.5 years). TaVNS stimulation intensities did not differ significantly between subject groups (G1: range: 0.9–3.5 mA, mean 2.5, *SD* ± 0.8; G2: range: 0.5–3.2 mA, mean 1.6, *SD* ± 0.9; G3: range: 1.0–5.0 mA, mean 2.3, *SD* ± 1.2).

Global Network Characteristics in Different Epilepsy Groups (G1 and G2) and Non-epilepsy Group (G3)

On the global network scale (see **Figure 1**), the focal epilepsy group (G1) and the non-epilepsy group (G3) presented with comparable topological network characteristics (average clustering coefficient *C* and average shortest path length *L*) during all phases of the examination schedule. Contrary to this, we observed the group of subjects with generalized epilepsies (G2) to possess topological characteristics that differed significantly from the characteristics seen in both the focal epilepsy group and the non-epilepsy group. Already before (phase B1) but also during stimulation (phase S), the networks of group G2 were less segregated (higher average clustering coefficient *C*) and more integrated (lower average shortest path length *L*) than the networks of groups G1 and G3. Interestingly, the vanishing differences seen after the stimulation (phase B2) possibly point to a taVNS-mediated topology-modifying effect in the group of subjects with generalized epilepsies. As regards the networks' stability and robustness characteristics (synchronisability *S* and assortativity *A*), the three subject groups presented with comparable findings during all phases of the examination schedule.

Testing for differences between network characteristics from each phase led to non-significant results in each subject group (data not shown). On this population sample level, taVNS thus appeared to not immediately affect the investigated global network characteristics. Nevertheless, since not all subjects may display taVNS-mediated changes of their functional brain network (Rings et al., 2021; von Wrede et al., 2021), we specifically investigated those subjects for whom we identified significant changes of their network characteristics (see **Figure 2**) and observed the subject groups to present with a different pattern of change. When the networks of both the focal epilepsy group (G1) and the non-epilepsy group (G3) transited from phase B1 to phase S, their average

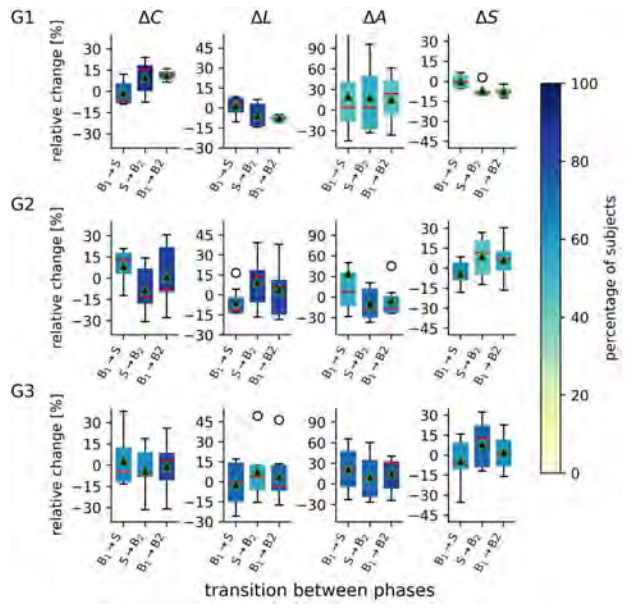

FIGURE 2 | Distributions of taVNS-related alterations in global network characteristics of the three investigated groups (G1 = focal epilepsy group, G2 = generalized epilepsy group, G3 = non-epilepsy group) between the three investigated phases (B1 = pre-stimulation baseline 1, S = stimulation, B2 = post-stimulation baseline 2). Boxplots of relative changes in network characteristics (average clustering coefficient *C*, average shortest path length *L*, synchronisability *S*, and assortativity *A*). Bottom and top of a box are the first and third quartiles, and the red band and the black triangle are the median and the mean of the distribution. The ends of the whiskers represent the interquartile range of the data. Outliers are marked by an o-sign. Boxes are color coded according to the percentage of subjects for whom significant changes in global network characteristics were observed on per subject base.

clustering coefficient *C* decreased (relative change of median values in G1: −6.5%; G3: −4.7%) while the average shortest path length *L* of G1 increased (+ 3.9%) and changes were negligible for G3 (−0.4%). This points to an immediate stimulation effect that renders these networks more segregated and, at least for G1 less integrated. When comparing network characteristics from the phases prior to (B1) and after the stimulation (B2), we could identify an enduring effect that rendered network less segregated (*C* increased; G1: + 11.4%, G3: + 3.6%) and more integrated (*L* decreased; G1: −7.8%; G3: −3.5%). Interestingly, for the networks of the generalized epilepsy group (G2), we observed these stimulation-mediated changes to present with an inverted pattern: the immediate stimulation effect resulted in less segregated (*C* increased by + 12.8%) and more integrated networks (*L* decreased by −11.3%), while the enduring effect presented with more segregated (*C* decreased; −7.6%) and less integrated networks (*L* increased; + 5.7%).

TaVNS exerted an immediate robustness-enhancing effect over the networks in all groups (changes in assortativity *A*; G1: + 4.9%; G2: + 8.4%; G3: + 21.8%). On the longer term (comparing phases B1 and B2), we observed a strong robustness-enhancing enduring effect for the focal epilepsy group and the non-epilepsy group (G1: + 24.4%; G3: 31.4%). In contrast, in the generalized epilepsy group appeared to have a robustness-decreasing enduring effect (G2: -17.4%).

As regards network stability, we observed taVNS to decrease the networks' vulnerability of the synchronized state to get perturbed when transiting from phase B1 to phase S in the generalized epilepsy group and the non-epilepsy groups (changes in synchronisability *S*: G2: −6.4%; G3: −7.3%) while this immediate effect in the focal epilepsy group was negligible (G1: −0.4%). Interestingly, in the focal epilepsy group this minor reduction increased into the post-stimulation phase (G1: −7.6%), while taVNS had an enduring vulnerability-enhancing effect on the networks in the generalized group (G2: + 7.4) and a negligible effect in the non-epilepsy group (G3: + 0.3%).

Local Network Characteristics in Different Epilepsy Groups (G1 and G2) and Non-epilepsy Group (G3)

On the local network scale (see **Figure 3**), we obtained different results on the population sample level depending on the employed vertex centrality concept. Betweenness centrality highlighted vertices associated with left fronto-centro-temporal brain regions as most important (high C^B values) in all subject groups. In contrast, eigenvector centrality highlighted a posterior-anterior gradient of vertex importance with the most important (high C^E values) vertices associated with posterior brain regions in all subject groups. Most important vertices differed significantly neither between groups nor between phases, apart from some few, locally mostly unspecific differences seen particularly for the generalized and non-epilepsy group. As regards the importance of edges, i.e., of interactions between brain regions, none of the employed edge centrality concepts highlighted a clear-cut spatial pattern of differences, neither between groups nor between phases. On the population sample level, taVNS thus appeared to have an only minor (if at all) immediate impact on the investigated local network characteristics.

Proceeding as above and investigating solely those subjects that presented with significant taVNS-mediated changes of their local network characteristics, we observed that most subjects displayed such changes (see **Figure 4**). Interestingly, the highest proportion of subjects showing significant changes was seen in the generalized epilepsy group, however, with no discernible spatial pattern of change. In contrast, for most subjects from the focal epilepsy group, taVNS-mediated changes of vertex importance (assessed with betweenness centrality) were confined to vertices associated with fronto-temporal brain areas. Other taVNS-mediated alterations of vertex or edge centralities presented as diffuse with no clear-cut spatial pattern.

Stimulation-Related Change of EpiTrack® Score and Subjective Measures

An improvement in attentional-executive functioning as measured with EpiTrack® from pre- to post-assessment (main effect time: $F = 28.97$, $p < 0.001$), but no interaction effect of time and group ($F = 1.31$, $p = 0.29$) was observed.

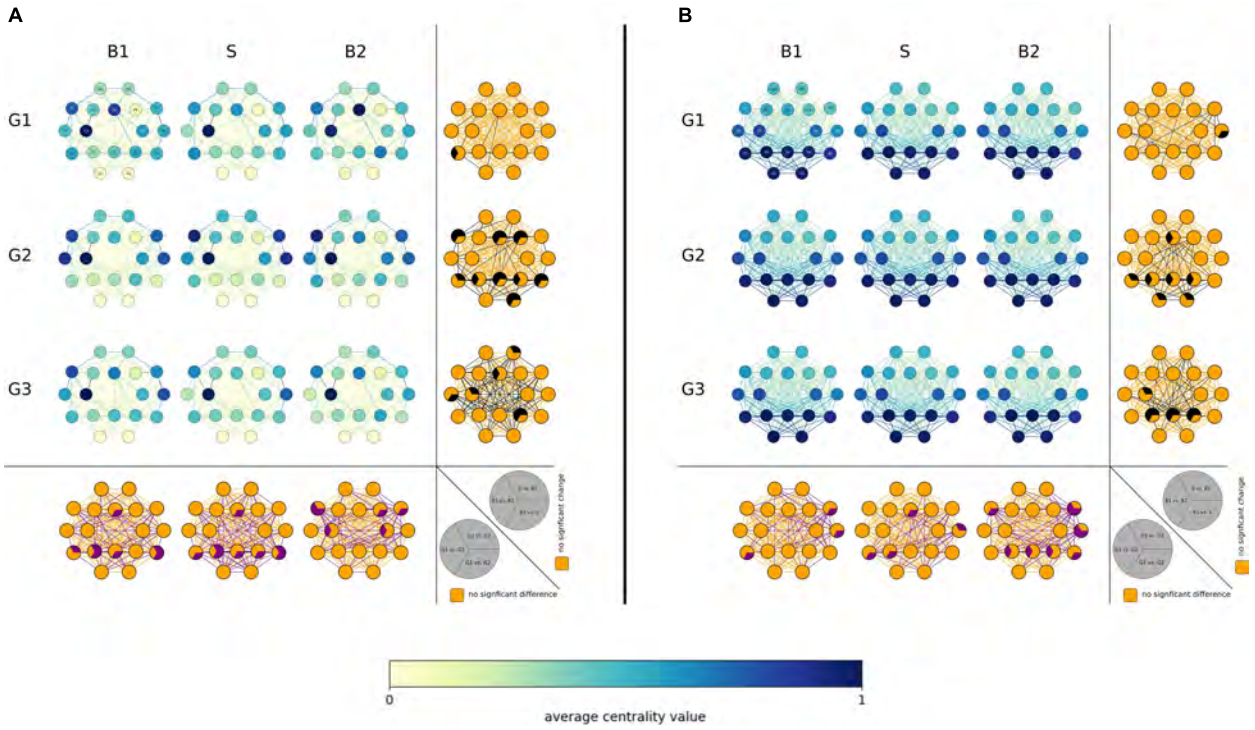

FIGURE 3 | Local network characteristics. [**(A)** Betweenness centrality C^B and **(B)** eigenvector centrality C^E] of the three investigated groups (G1 = focal epilepsy group, G2 = generalized epilepsy group, G3 = non-epilepsy group) in the three investigated phases (B1 = pre-stimulation baseline 1, S = stimulation, B2 = post-stimulation baseline 2). Network vertices arranged according the international 10–20 system for EEG-recording (electrode naming see first plot). Color coding of vertices and edges according to the average centrality values. Bottom: Difference between groups (G1, G2, G3) for local network characteristics in the three investigated phases. Orange: no significance, purple: significant difference ($p < 0.05$). Right side each plot: Differences between phases (B1, S, B2) for local network characteristics in the three investigated groups. Orange: no significance, black: significant change ($p < 0.05$).

Seven subjects (23.3%; 2 in G1, 1 in G2, 4 in G3) showed a significant intraindividual improvement (EpiTrack®; $\geq$ 4 points). None of the subjects worsened significantly. No significant self-perceived changes were observed regarding the total scores in the cognitive, behavioral, and physiological domains of the modified Adverse Events Profile ($p > 0.05$).

Usability, Tolerability and Side Effects of Transcutaneous Auricular Vagus Nerve Stimulation

Usability data were analyzed across all subjects as there were no differences between groups ($p > 0.05$). Handling of the device was rated as good or very good by all subjects. 93.1% felt that the continuation of their activities was not affected by the stimulation. Wearing comfort was rated as good or very good by 83.3% of the subjects. Most subjects stated that the device is well or very well suited for long-term use during the day (80%) or repeated use within 1 day (86.6%). Side effects were neither reported nor clinically observed.

DISCUSSION

In this study, we investigated whether global and local characteristics of functional brain networks differ between different types of epilepsy and non-epilepsy subjects and whether short-term taVNS differently modifies their global and local network characteristics. In the following, we discuss our findings in the light of the available research results.

Global and Local Network Characteristics Differ Between Different Epilepsy Types During Rest Phase

We observed significant differences between global characteristics (average clustering coefficient and average shortest path length) of networks from subjects with generalized epilepsies, focal epilepsies and from non-epilepsy subjects, which corroborates previous studies (Niso et al., 2015; Drenthen et al., 2020). Here, subjects with generalized epilepsies presented with less segregated and more integrated functional brain networks. These findings are in line with earlier studies (Chavez et al., 2010; Chowdhury et al., 2014), though contrast with another study (Zhang et al., 2011). Network studies in epilepsy and especially epilepsy syndromes is an evolving research field, and although results and knowledge are published at a tremendous pace, the applied methods differ and results are not easy to reconcile and might therefore explain opposing results. On the local scale and in par with previous studies (Lohmann et al.,

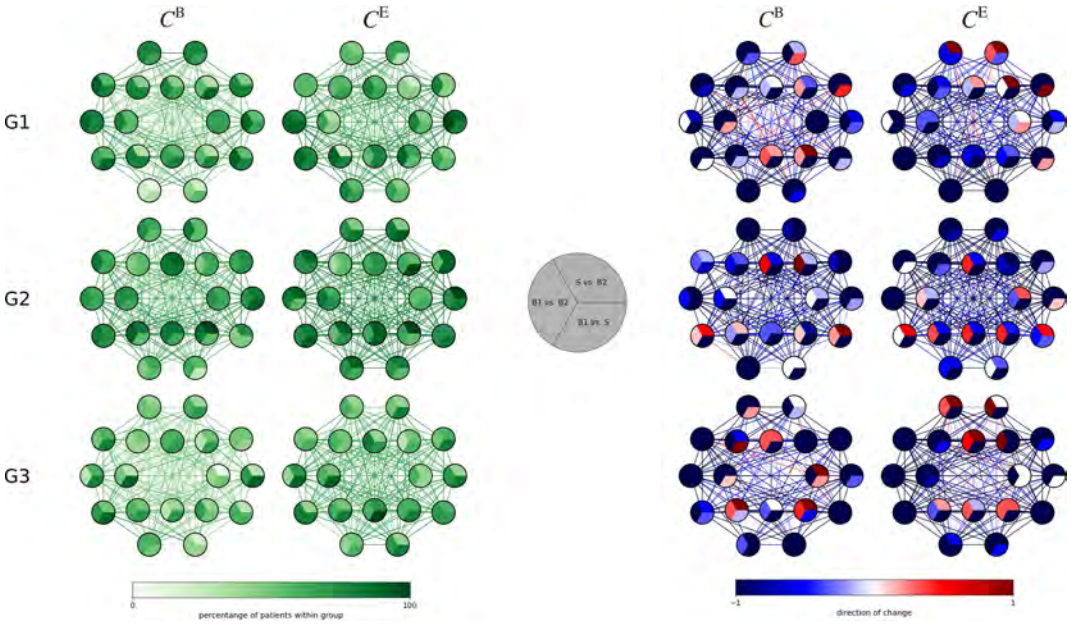

FIGURE 4 | (Left) Distributions of taVNS-related alterations in local network characteristics (betweenness centrality C^B and eigenvector centrality C^E) of the three investigated groups (G1 = focal epilepsy group, G2 = generalized epilepsy group, G3 = non-epilepsy group) between the three investigated phases (B1 = pre-stimulation baseline 1, S = stimulation, B2 = post-stimulation baseline 2). Network vertices arranged according the international 10–20 system for EEG-recording. Color coding of vertices and edges according to the percentage of subjects for whom significant changes in local network characteristics were observed on per subject base. **(Right)** Differences between phases (B1, S B2) for local network characteristics in the three investigated groups. Color coding of vertices and edges according to the direction of change of importance for vertices and edges (per subject base).

TABLE 1 | Synopsis of taVNS-induced immediate and enduring modifications of global and local characteristics of weighted fully connected functional brain networks in different epilepsy types.

		Focal epilepsy group	Generalized epilepsy group	Non-epilepsy group
Global network scale				
Topology	Immediate effect	Segregation ↑ integration ↓	Segregation ↓ integration ↑	Segregation ↑ integration ↔
	Enduring effect	Segregation ↓ integration ↑	Segregation ↑ integration ↓	Segregation ↓ integration ↑
Robustness	Immediate effect	↑	↑	↑↑
	Enduring effect	↑↑	↓↓	↑↑
Stability of the synchronized state	Immediate effect	↔	↑	↑
	Enduring effect	↑	↓	↔
Local network scale				
Path-based centrality index	Vertices	Diffuse	Diffuse	Diffuse
	Edges	Diffuse	Diffuse	Diffuse
Interaction-strength-based centrality index	Vertices	Diffuse	Diffuse	Diffuse
	Edges	Diffuse	Diffuse	Diffuse

↑, *increase;* ↑↑, *strong increase;* ↓, *decrease,* ↓↓, *strong decrease;* ↔, *negligible change.*

2010; van den Heuvel and Sporns, 2013; Rings et al., 2021), different brain regions were highlighted as important with the different centrality concepts. Our findings for the three subject groups, namely left fronto-central brain regions are characterized as most important with betweenness centrality and parieto-occipital regions with eigenvector centrality, are in line with previous observations (Lohmann et al., 2010; van den Heuvel and Sporns, 2013). Differences in functional connections between brain regions were negligible as no clear-cut spatial differences were observed between subjects with generalized epilepsies, focal epilepsies and non-epilepsy subjects.

Summarizing these findings, we could show that already during rest global but not local characteristics of functional

brain networks are different in generalized epilepsies compared to focal epilepsies and the non-epilepsy group. Results derived from brain network analyses might thus provide additional information for differentiating between different types of epilepsy, and thereby supporting a thorough work-up for classification of epilepsy type which is indispensable for optimal patients' care.

Transcutaneous Auricular Vagus Nerve Stimulation Differently Modifies Global and Local Network Characteristics in Different Epilepsy Types

As in previous studies (Redgrave et al., 2018; von Wrede et al., 2021), taVNS was easy to use, well tolerated and without negative impact on attention and executive function; in some subjects these even improved.

On the global network scale, short-term taVNS induced modifications of topology-, robustness-, and stability-associated network characteristics in the majority of investigated subjects as it was observed in previous studies (Rings et al., 2021; von Wrede et al., 2021). A taVNS-related enduring topological reorganization of functional brain networks in focal epilepsies in terms of a more integrated and less segregated network structure was shown recently (von Wrede et al., 2021). Extending this finding, we here observed modifications of functional brain network organization to differ between different epilepsy types. We found an inverted pattern of reorganization between focal and generalized epilepsies, with the latter displaying an immediate reorganization toward a more integrated/less segregated and an enduring reorganization toward a more segregated/less integrated network. The taVNS-mediated topological reorganization of functional brain networks in the non-epilepsy subjects resembled those of the focal epilepsy group though being less pronounced. These epilepsy-type-related findings might explain the differing results for immediate modifications of brain network reorganization by taVNS reported previously (Rings et al., 2021).

TaVNS induced a comparable immediate robustness-enhancing modification of the functional brain networks of subjects with focal and generalized epilepsies as well as non-epilepsy subjects. The enduring effect, however, clearly differed between epilepsy types: robustness increased in the focal epilepsy group (which is in par with a previous study (von Wrede et al., 2021), but decreased in the generalized epilepsy group. What is more, taVNS induced an enduring higher vulnerability for perturbation in generalized epilepsies and a lower one in focal epilepsies, leading to different network stability.

Interestingly, on a local network scale, more subjects with generalized epilepsy than with focal epilepsy displayed taVNS-induced modifications of importance of brain regions and functional connections. We hypothesize that in focal epilepsies important brain regions are more susceptible for modifications, whereas in generalized epilepsy the pattern of modified brain regions is more diffuse. No clear-cut spatial pattern could be observed for the importance of functional connections.

Summarizing these findings (see **Table 1**), we could provide first evidence that in subjects with generalized or focal epilepsy, short-term taVNS differently modified global characteristics of their functional brain networks. Local network characteristics remained largely unaffected as already reported on previously (Rings et al., 2021).

There are some limitations of our study; due to the special setting on the ward and the necessity of the longer EEG-recording as well as due to drop outs, the number of investigated subjects was rather low. What is more, though matched between groups, the span in age and epilepsy duration was rather high, which might have influenced our findings. Further studies in larger groups are thus necessary.

Using non–pharmaceutical interventions in epilepsy treatment often starts rather late in the course of treatment, especially since most of the current non–pharmaceutical interventions, such as epilepsy surgery or invasive stimulation methods, are accompanied by clearly defined risks. The non-invasive stimulation-based treatment is still in its infancy. The search for candidates who might profit from taVNS-based treatment should thus be extended, as it is common for epilepsy surgery and also ASM. Our experimental findings suggest, to our knowledge for the first time, different stimulation-mediated modifications of functional brain networks in different epilepsy types and point at potentially different responses of epileptic brain networks to taVNS in focal and generalized epilepsies. Further studies that investigate possible relationships between taVNS-induced modifications of functional brain networks and clinical efficacy are necessary to translate these experimental findings into clinical decision-making. The search for predictors of successful vagus nerve stimulation is a major challenge, for which first interesting insights have already been presented for iVNS (Workewych et al., 2020), but it is of importance to proceed and to install standardized protocols for experimental VNS research (Farmer et al., 2021) and also for future clinical applications.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because they contain information that could comprise the privacy of the participants. Requests to access the datastes should be directed to RW, randi.von.wrede@ukbonn.de.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the University of Bonn.

The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

RW, CH, and KL: conceptualization. RW and KL: methodology, writing—original draft preparation, writing—review, editing, and supervision. RW, TR, TB, JP, SS, CH, and KL: validation, formal analysis, and data curation. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnhum.2022.867563/full#supplementary-material

REFERENCES

Barbella, G., Cocco, I., Freri, E., Marotta, G., Visani, E., Franceschetti, S., et al. (2018). Transcutaneous vagal nerve stimulatio (t-VNS): an adjunctive treatment option for refractory epilepsy. *Seizure* 60, 115–119. doi: 10.1016/j.seizure.2018.06.016

Bauer, S., Baier, H., Baumgartner, C., Bohlmann, K., Fauser, S., Graf, W., et al. (2016). Transcutaneous Vagus Nerve Stimulation (tVNS) for Treatment of Drug-Resistant Epilepsy: a Randomized, Double-Blind Clinical Trial (cMPsE02). *Brain Stimul.* 9, 356–363. doi: 10.1016/J.BRS.2015.11.003

Ben-Menachem, E. (2002). Vagus-nerve stimulation for the treatment of epilepsy. *Lancet Neurol.* 1, 477–482. doi: 10.1016/s1474-4422(02)00220-x

Berg, A. T., and Scheffer, I. E. (2011). New concepts in classification of the epilepsies: entering the 21st century. *Epilepsia* 52, 1058–1062. doi: 10.1111/j.1528-1167.2011.03101.x

Bullmore, E., and Sporns, O. (2009). Complex brain networks: graph theoretical analysis of structural and functional systems. *Nat. Rev. Neurosci.* 10, 186–198. doi: 10.1038/nrn2575

Chavez, M., Valencia, M., Navarro, V., Latora, V., and Martinerie, J. (2010). Functional modularity of background activities in normal and epileptic brain networks. *Phys. Rev. Lett.* 104:118701. doi: 10.1103/PhysRevLett.104.118701

Chen, Z., Brodie, M. J., Liew, D., and Kwan, P. (2018). Treatment outcomes in patients with newly diagnosed epilepsy treated with established and new antiepileptic drugs a 30-year longitudinal cohort study. *JAMA Neurol.* 75, 279–286. doi: 10.1001/jamaneurol.2017.3949

Chowdhury, F. A., Woldman, W., FitzGerald, T. H. B., Elwes, R. D. C., Nashef, L., Terry, J. R., et al. (2014). Revealing a brain network endophenotype in families with idiopathic generalised epilepsy. *PLoS One* 9:e110136. doi: 10.1371/journal.pone.0110136

Drenthen, G. S., Fasen, F., Fonseca Wald, E. L. A., Backes, W. H., Aldenkamp, A. P., Vermeulen, R. J., et al. (2020). Functional brain network characteristics are associated with epilepsy severity in childhood absence epilepsy. *NeuroImage Clin.* 27:102264. doi: 10.1016/j.nicl.2020.102264

Elliott, R. E., Morsi, A., Kalhorn, S. P., Marcus, J., Sellin, J., Kang, M., et al. (2011). Vagus nerve stimulation in 436 consecutive patients with treatment-resistant epilepsy: long-term outcomes and predictors of response. *Epilepsy Behav.* 20, 57–63. doi: 10.1016/j.yebeh.2010.10.017

Farmer, A. D., Strzelczyk, A., Finisguerra, A., Gourine, A. V., Gharabaghi, A., Hasan, A., et al. (2021). International Consensus Based Review and Recommendations for Minimum Reporting Standards in Research on Transcutaneous Vagus Nerve Stimulation (Version 2020). *Front. Hum. Neurosci.* 14:568051. doi: 10.3389/fnhum.2020.568051

Fisher, B., DesMarteau, J. A., Koontz, E. H., Wilks, S. J., and Melamed, S. E. (2020). Responsive Vagus Nerve Stimulation for Drug Resistant Epilepsy: a Review of New Features and Practical Guidance for Advanced Practice Providers. *Front. Neurol.* 11:610379. doi: 10.3389/fneur.2020.610379

Fisher, R. S., Acevedo, C., Arzimanoglou, A., Bogacz, A., Cross, J. H., Elger, C. E., et al. (2014). ILAE Official Report: a practical clinical definition of epilepsy. *Epilepsia* 55, 475–482. doi: 10.1111/epi.12550

GBD 2016 Epilepsy Collaborators (2019). Global, regional, and national burden of epilepsy, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol.* 18, 357–375. doi: 10.1016/S1474-4422(18)30454-X

Hauser, W., and Hesdorffer, D. (1991). "Epidemiology in Epilepsy," in *Neurepidemiology*, eds D. W. Anderson and D. G. Schoenberg (Boca Raton, FL: CRC Press).

Höller, Y., Helmstaedter, C., and Lehnertz, K. (2018). Quantitative Pharmaco-Electroencephalography in Antiepileptic Drug Research. *CNS Drugs* 32, 839–848. doi: 10.1007/s40263-018-0557-x

Kwan, P., and Brodie, M. J. (2000). Early Identification of Refractory Epilepsy. *N. Engl. J. Med.* 342, 314–319. doi: 10.1056/NEJM200002033420503

Kwan, P., Arzimanoglou, A., Berg, A. T., Brodie, M. J., Allen Hauser, W., Mathern, G., et al. (2010). Definition of drug resistant epilepsy: consensus proposal by the ad hoc Task Force of the ILAE Commission on Therapeutic Strategies. *Epilepsia* 51, 1069–1077. doi: 10.1111/j.1528-1167.2009.02397.x

Lampros, M., Vlachos, N., Zigouris, A., Voulgaris, S., and Alexiou, G. A. (2021). Transcutaneous Vagus Nerve Stimulation (t-VNS) and epilepsy: a systematic review of the literature. *Seizure* 91, 40–48. doi: 10.1016/j.seizure.2021.05.017

Liu, A., Song, L., Wang, X., Wang, Y., Gong, L., Li, L., et al. (2018). Efficacy and Safety of Treatment with Transcutaneous Vagus Nerve Stimulation in 17 Patients with Refractory Epilepsy Evaluated by Electroencephalogram, Seizure Frequency, and Quality of Life. *Med. Sci. Monit.* 24, 8439–8448. doi: 10.12659/msm.910689

Lohmann, G., Margulies, D., Horstmann, A., Pleger, B., Lepsien, J., Goldhahn, D., et al. (2010). Eigenvector Centrality Mapping for Analyzing Connectivity Patterns in fMRI Data of the Human Brain. *PLoS One* 5:e10232. doi: 10.1371/journal.pone.0010232

Marson, A. G., Al-Kharusi, A. M., Alwaidh, M., Appleton, R., Baker, G. A., Chadwick, D. W., et al. (2007a). The SANAD study of effectiveness of carbamazepine, gabapentin, lamotrigine, oxcarbazepine, or topiramate for treatment of partial epilepsy: an unblinded randomised controlled trial. *Lancet* 369, 1000–1015. doi: 10.1016/S0140-6736(07)60460-7

Marson, A. G., Al-Kharusi, A. M., Alwaidh, M., Appleton, R., Baker, G. A., Chadwick, D. W., et al. (2007b). The SANAD study of effectiveness of valproate, lamotrigine, or topiramate for generalised and unclassifiable epilepsy: an unblinded randomised controlled trial. *Lancet* 369, 1016–1026. doi: 10.1016/S0140-6736(07)60461-9

Marson, A., Burnside, G., Appleton, R., Smith, D., Leach, J. P., Sills, G., et al. (2021). The SANAD II study of the effectiveness and cost-effectiveness of levetiracetam, zonisamide, or lamotrigine for newly diagnosed focal epilepsy: an open-label, non-inferiority, multicentre, phase 4, randomised controlled trial. *Lancet* 397, 1363–1374. doi: 10.1016/S0140-6736(21)00247-6

Morris, G. L. III, Gloss, D., Buchhalter, J., Mack, K. J., Nickels, K., and Harden, C. (2013). Evidence-based guideline update: vagus nerve stimulation for the treatment of epilepsy: report of the Guideline Development Subcommittee of the American Academy of Neurology. *Neurology* 81, 1453–1459. doi: 10.1212/WNL.0b013e3182a393d1

Niso, G., Carrasco, S., Gudín, M., Maestú, F., Del-Pozo, F., and Pereda, E. (2015). What graph theory actually tells us about resting state interictal MEG epileptic activity. *NeuroImage Clin.* 8, 503–515. doi: 10.1016/j.nicl.2015.05.008

Redgrave, J., Day, D., Leung, H., Laud, P. J., Ali, A., Lindert, R., et al. (2018). Safety and tolerability of Transcutaneous Vagus Nerve stimulation in humans; a systematic review. *Brain Stimul.* 11, 1225–1238. doi: 10.1016/j.brs.2018.08.010

Révész, D., Rydenhag, B., and Ben-Menachem, E. (2016). Complications and safety of vagus nerve stimulation: 25 years of experience at a single center. *J. Neurosurg. Pediatr.* 18, 97–104. doi: 10.3171/2016.1.PEDS15534

Rings, T., von Wrede, R., Bröhl, T., Schach, S., Helmstaedter, C., and Lehnertz, K. (2021). Impact of Transcutaneous Auricular Vagus Nerve Stimulation on Large-Scale Functional Brain Networks: from Local to Global. *Front. Physiol.* 12:700261. doi: 10.3389/fphys.2021.700261

Rutecki, P. (1990). Anatomical, physiological, and theoretical basis for the antiepileptic effect of vagus nerve stimulation. *Epilepsia* 31, S1–S6. doi: 10.1111/j.1528-1157.1990.tb05843.x

Scheffer, I. E., Berkovic, S., Capovilla, G., Connolly, M. B., French, J., Guilhoto, L., et al. (2017). ILAE classification of the epilepsies: position paper of the ILAE Commission for Classification and Terminology. *Epilepsia* 58, 512–521. doi: 10.1111/epi.13709

Stefan, H., Kreiselmeyer, G., Kerling, F., Kurzbuch, K., Rauch, C., Heers, M., et al. (2012). Transcutaneous vagus nerve stimulation (t-VNS) in pharmacoresistant epilepsies: a proof of concept trial. *Epilepsia* 53, e115–e118. doi: 10.1111/j.1528-1167.2012.03492.x

Tononi, G., Sporns, O., and Edelman, G. M. (1994). A measure for brain complexity: relating functional segregation and integration in the nervous system. *Proc. Natl. Acad. Sci. U. S. A.* 91, 5033–5037. doi: 10.1073/pnas.91.11.5033

van den Heuvel, M. P., and Sporns, O. (2013). Network hubs in the human brain. *Trends Cogn. Sci.* 17, 683–696. doi: 10.1016/j.tics.2013.09.012

von Wrede, R., Moskau-Hartmann, S., Rüber, T., Helmstaedter, C., and Surges, R. (2019). Sustained seizure freedom with transcutaneous vagal nerve stimulation in drug-resistant epilepsy caused by subcortical band heterotopias. *Seizure* 70, 25–26. doi: 10.1016/j.seizure.2019.06.026

von Wrede, R., Rings, T., Schach, S., Helmstaedter, C., and Lehnertz, K. (2021). Transcutaneous auricular vagus nerve stimulation induces stabilizing modifications in large-scale functional brain networks: towards understanding the effects of taVNS in subjects with epilepsy. *Sci. Rep.* 11:7906. doi: 10.1038/s41598-021-87032-1

Workewych, A. M., Arski, O. N., Mithani, K., and Ibrahim, G. M. (2020). Biomarkers of seizure response to vagus nerve stimulation: a scoping review. *Epilepsia* 61, 2069–2085. doi: 10.1111/epi.16661

World Health Organization [WHO] (2019). *Epilepsy*. Geneva: World Health Organization.

Zhang, Z., Liao, W., Chen, H., Mantini, D., Ding, J.-R., Xu, Q., et al. (2011). Altered functional-structural coupling of large-scale brain networks in idiopathic generalized epilepsy. *Brain* 134, 2912–2928. doi: 10.1093/brain/awr223

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4. Diskussion

Die vorgelegten Arbeiten untersuchten den Einfluss der transkutanen aurikulären Vagusnerv-Stimulation (taVNS) auf funktionelle Hirnnetzwerke bei Proband*innen mit und ohne Epilepsien.

Einfluss der taVNS auf globale Netzwerkcharakteristika

Hierfür wurde zunächst ein Studienprotoll entwickelt, welches erlaubt, die Untersuchungen in den Klinikalltag der Proband*innen zu integrieren, ohne den klinisch diagnostischen und therapeutischen Ablauf zu stören oder zu verlangsamen. Das Protokoll ist durch sehr strenge Ein- und Ausschlusskriterien sowie Durchführungsregeln gekennzeichnet, was zwar zu Rekrutierungsherausforderungen, jedoch zu einer Datenlage mit deutlich reduzierten Störfaktoren führte. In der Proof-of-Concept Studie (von Wrede et al., 2021) konnte gezeigt werden, dass eine kurzzeitige taVNS eine immediate Topologie- und robustheitsverändernde und stabilitätssteigernde Wirkung auf funktionelle epileptische Hirnnetzwerke hat. Die stimulationsbedingten anhaltenden Veränderungen der untersuchten Netzwerkeigenschaften verweisen auf eine taVNS-induzierte Änderung der topologischen Organisation des Netzwerks, was sich in einem stärker integrierten und weniger segregierten Netzwerk widerspiegelt. Ähnliche Ergebnisse konnten erst kürzlich auch bei der invasiven Langzeitstimulation des Vagusnervs erzielt werden (Wang et al., 2020). Zusätzlich zu den Veränderungen der Netzwerktopologie kann eine kurzzeitige taVNS die Stabilität und Robustheit funktioneller epileptischer Hirnnetzwerke beeinflussen. Hierbei zeigt sich nicht nur eine anhaltende stabilisierende Wirkung von taVNS, sondern auch stärkste Hinweise auf eine präventive Wirkung der taVNS durch eine Zunahme der Assortativität der Netzwerke. Bereits vor der Stimulation waren epileptische Hirnnetzwerke assortativ, was frühere Beobachtungen bestätigt (Geier et al., 2015; Chiosa et al., 2019), und die taVNS führte zu einer weiteren Zunahme der Assortativität.

Einfluss der taVNS auf lokale Netzwerkcharakteristika

Die erfolgreiche Proof-of-Concept Studie eröffnete die Möglichkeit, zusätzlich lokale Netzwerkcharakteristika und ihre Modulation durch taVNS zu untersuchen (Rings et al., 2021). Auf der lokalen Skala einzelner Knoten und Kanten konnte zwar bei einem Großteil der

untersuchten Personen taVNS-induzierte Veränderungen der lokalen Netzwerkcharakteristika (Kanten- und Knotenzentralitäten) beobachtet werden; Knoten oder Kanten jedoch, die in der Phase vor der Stimulation als besonders wichtig identifiziert wurden, blieben während der Stimulation und in der Phase nach der Stimulation unverändert, so dass von unspezifischen Veränderungen der lokalen Netzwerkcharakteristika im gesamten Netzwerk ausgegangen werden muss. Was die wichtigsten Knoten aus der Phase vor der Stimulation anbelangt, so stehen die hier vorgelegten Ergebnisse im Einklang mit früheren Beobachtungen, wonach während eines sogenannten Ruhezustands links fronto-zentrale Hirnregionen bei der Betweenness-Zentralität (van den Heuvel and Sporns, 2013; Jin et al., 2014; Makarov et al., 2018) sowie parieto-okzipitale Hirnregionen bei der Eigenvektor-Zentralität (Lohmann et al., 2010) am wichtigsten sind. Zusammengenommen bestätigen diese Befunde die gängige Sichtweise, dass verschiedene Zentralitätskonzepte, welche unterschiedliche Netzwerkeigenschaften beschrieben, unterschiedliche Netzwerkanteile (Knoten/Kanten) als am wichtigsten identifizieren (Lü et al., 2016; Bröhl and Lehnertz, 2019). Die Ergebnisse sind gut vereinbar mit früheren Beobachtungen, nach denen die wichtigsten Kanten die wichtigsten Knoten verbinden, wie es typisch für dichte Netzwerke ist (Bröhl and Lehnertz, 2019).

Für die topologischen Netzwerkcharakteristika konnten die anhaltenden Veränderungen eines stärker integrierten und weniger segregierten Netzwerks reproduziert werden, während sich für die immediaten Veränderungen, welche sich in der Proof-of-Concept Studie als eine Abnahme der Integration und Zunahme der Segregation darstellen, inverse Veränderungen ergaben. Auch für die stabilitäts- und robustheitsbezogenen Netzwerkmerkmalen war der anhaltende Effekt auf stabilitäts- und robustheitsassoziierte Netzwerkeigenschaften vergleichbar mit den Ergebnissen der Vorstudie, während die unmittelbaren Effekte sich unterschieden. Die Diskrepanzen zwischen den beiden Studien (von Wrede et al., 2021; Rings et al., 2021) sind möglicherweise das Ergebnis der unterschiedlichen Proband*innengruppen (Gruppenstärke, Spektrum unterschiedlicher Pathologien und Ätiologie). Die o.g. taVNS-vermittelten Modifikationen lokaler und globaler topologischer Eigenschaften funktioneller Hirnnetzwerke mögen zunächst widersprüchlich erscheinen. Diese Widersprüchlichkeit lässt sich jedoch durch das folgende Modell (siehe Abbildung 3) einer stimulationsinduzierten Dehnung und Komprimierung des Netzwerks (Fruengel et al., 2020), welches auf einen

nichtlinearen Mechanismus zurückzuführen sein könnte, erklären. Die stimulationsbedingte Zunahme des durchschnittlichen Clustering-Koeffizienten und die Abnahme der durchschnittlichen kürzesten Weglänge deuten auf eine im Durchschnitt globale „Komprimierung“ des funktionellen Hirnnetzwerks hin. Da jedoch die Zentralitätswerte unverändert blieben, wird die Komprimierung durch einen gewissen „Dehnungseffekt“ des Netzwerkes kompensiert. Auch wenn das funktionelle Hirnnetzwerk ein vollständig verbundenes Netzwerk ist und keine tatsächliche räumliche Einbettung aufweist, kann man dennoch (zumindest konzeptionell) die durch „Dehnung“ und „Komprimierung“ vermittelten Netzwerkveränderungen visualisieren, indem die Definitionen von Pfad und Stärke (d. h. gewichtetem Grad) in diesem Netzwerkansatz verwendet werden. Der „Kern“ des Netzwerks enthält Knoten mit der höchsten Stärke (dargestellt als räumliche Nähe). Der „Netzwerkrand“ enthält Knoten, die am weitesten vom Kern entfernt sind. Die „Netzwerkperipherie“ umfasst schließlich alles, was sich zwischen Kern und Rand befindet.

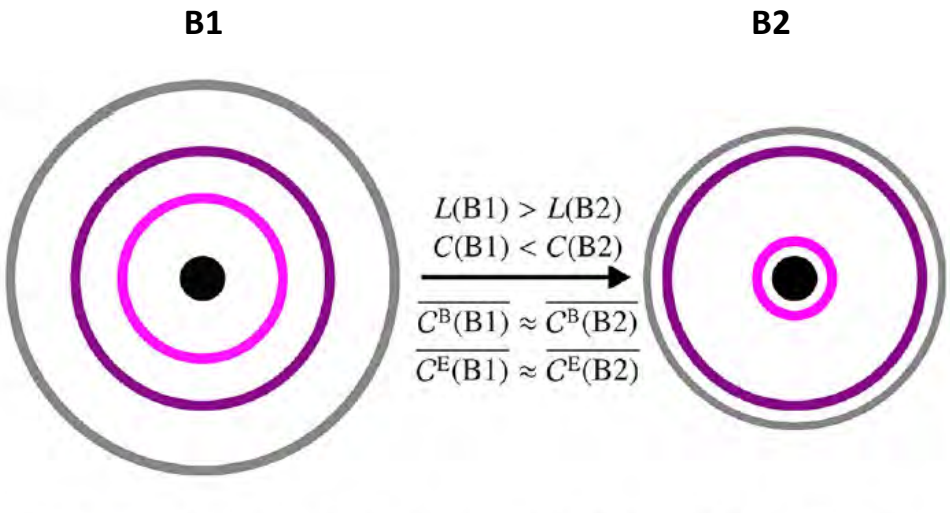

Abb. 4: Schematische Darstellung eines räumlichen Netzwerks vor der Stimulation (B1, links) **und nach der Stimulation** (B2, rechts)**.** Das Netzwerk wird anhand des durchschnittlichen Clustering-Koeffizienten *C* und der durchschnittlichen kürzesten Weglänge *L* in verschiedene Bereiche unterteilt: Rand (grauer äußerster Ring), Peripherie (lila und rosa Ringe in der Mitte), Kern (schwarzer innerster Ring/Kreis). Je größer der Radius eines Rings ist, desto größer ist die durchschnittliche Länge des kürzesten Weges und desto kleiner ist der durchschnittliche Clustering-Koeffizient der Knoten in diesem Bereich. Je näher zwei Ringe beieinanderliegen, desto stärker sind die Vertices in den beiden Bereichen zueinander geclustert. Zeitlich gemittelte Zentralitäten der Noten und Kanten ($\overline{C^B}$) und ($\overline{C^E}$) werden durch die Stimulation nicht beeinflusst.

Durch die Stimulation verändert sich die Peripherie des Netzes nicht gleichförmig. Die beobachtete Zunahme des durchschnittlichen Clustering-Koeffizienten und die Abnahme der durchschnittlichen kürzesten Pfadlänge, zusammen mit einer unveränderten durchschnittlichen Zentralität von Knoten und Kanten, lassen sich durch eine Komprimierung des Netzwerkrandes sowie durch eine teilweise Komprimierung der Netzwerkperipherie (farbige Ringe) erklären. Die stimulationsbedingte Komprimierung des Netzwerkrandes führt zur Annäherung an den Kern und damit zu einer Abnahme der durchschnittlichen kürzesten Pfadlänge. Darüber hinaus nähert sich der Netzwerkrand aber auch einem Teil der Peripherie und ein Teil der Peripherie dem Kern, woraus sich eine Zunahme des durchschnittlichen Clustering-Koeffizienten ergibt. Die Netzwerkperipherie selbst dehnt sich somit aus und kompensiert so die globale Komprimierung bei Beibehalten der Pfadstruktur (unveränderte durchschnittliche Eigenvektor- und Betweenness-Zentralität der Noten und Kanten). Es bleibt zu vermuten, dass diese die Topologie modifizierenden Dehnungs- und Komprimierungseffekte auch die Assortativität und Synchronisierbarkeit des Netzwerks beeinflussen und dadurch seine Robustheit und Stabilität erhöhen. Die physiologische Interpretation des Modells steht noch aus.

Bedeutung der Tageszeit der transkutanen Vagusnerv Stimulation

Netzwerkeigenschaften können durch verschiedene biologische Rhythmen beeinflusst werden (Lehnertz et al., 2021), ob und in welchem Ausmaß sich diese Einflüsse auf taVNS-vermittelte Netzwerkmodifikationen auswirken, ist jedoch bislang nicht untersucht. Unter Berücksichtigung dieser Einflüsse und der bekannten tageszeitlichen Schwankungen der vagalen Aktivität ließ sich eine tageszeitabhängige Variation der Wirkung von taVNS vermuten. Dies konnte in der dritten Arbeit (von Wrede et al., 2022a) bestätigt werden: sowohl die morgendliche als auch die nachmittägliche Stimulation führten zu messbaren sofortigen und dauerhaften Veränderungen globaler Merkmale der funktionellen Hirnnetzwerke der Proband*innen. Diese Veränderungen zeigten eine deutliche Abhängigkeit von der Tageszeit, trotz der Bemühungen, den potenziell störenden Einfluss verschiedener ultradianer Rhythmen zu minimieren. Während die durch die nachmittägliche Stimulation vermittelten ausgeprägten unmittelbaren Topologie-verändernden (integrierteres und weniger segregiertes Netzwerk) und stabilitätssteigernden Effekte, die bei der Mehrheit der Proband*innen beobachtet wurden, frühere Ergebnisse bestätigen (Rings et al., 2021; von

Wrede et al., 2021), zeigten sich die zuvor beschriebenen dauerhaften Effekte hierzu diskrepant. Dies könnte darauf zurückzuführen sein, dass die Proband*innen in der aktuellen Untersuchung zweimal stimuliert wurden, während in früheren Studien (Rings et al., 2021; von Wrede et al., 2021) die Proband*innen taVNS-naiv waren. Die durch die morgendliche Stimulation induzierten Veränderungen zeigten sich eher uneinheitlich, wenngleich teils stärker ausgeprägt als die entsprechenden Veränderungen nach der nachmittäglichen Stimulation. Im Allgemeinen konnten bei etwa 50 % der Proband*innen die zuvor beschriebenen sofortigen und dauerhaften topologieverändernden und stabilitätssteigernden Effekte beobachtet werden, während bei weiteren 50 % der Proband*innen entgegengesetzte Veränderungen auftraten. In Übereinstimmung mit früheren Beobachtungen deuten die Ergebnisse darauf hin, dass kurzzeitige taVNS auf lokaler Ebene, auch unter Anwendung weiterer Zentralitätskonzepte, räumlich unspezifisch sind, was die vorherrschende Ansicht einer global wirkenden Wirkungsweise von taVNS unterstützt.
Zusammengenommen weisen die Ergebnisse eindeutig auf einen wichtigen Einfluss der Tageszeit auf die durch taVNS vermittelten Veränderungen verschiedener Eigenschaften sich entwickelnder funktioneller Hirnnetzwerke hin. Dies unterstreicht nicht nur die Sinnhaftigkeit der Dokumentation der Tageszeit der taVNS, wie es bereits empfohlen wurde (Farmer et al., 2021), sondern auch die Notwendigkeit, tageszeitliche Schwankungen bei der Interpretation von Forschungsergebnissen und klinischen Studien sowie bei der Formulierung von Anwendungsempfehlungen zu berücksichtigen. Die Berücksichtigung der Tageszeit, zu der die vagale Stimulation am wirksamsten sein soll, könnte nicht nur die klinischen Ergebnisse verbessern, sondern auch eine Verkürzung der Stimulationszeit ermöglichen, was wiederum zu einer Zunahme der Adhärenz führen könnte.

Die Beobachtungen tageszeitabhängiger unbeeinflusster lokaler, aber stark beeinflusster globaler Netzwerkeigenschaften lassen sich gut mit einer Erweiterung des o.g. Modells einer stimulationsinduzierten Dehnung und Komprimierung des funktionellen Hirnnetzwerks vereinbaren (siehe Abbildung 4). Die vorbeschriebene Dynamik dieses Modells konnte für die überwiegende Mehrheit der Responder*innen mit der Nachmittagsstimulation und für etwa die Hälfte der Responder*innen mit der Morgenstimulation beobachtet werden. Da die morgendliche Stimulation bei der anderen Hälfte der Responder*innen zu einem umgekehrten Muster führte, könnte dies auf eine tageszeitliche Abhängigkeit hindeuten.

Diese tageszeitliche Abhängigkeit könnte durch verschiedene rhythmische Aktivitäten (biologische Rhythmen mit unterschiedlichen Periodenlängen (ultradiane und/oder zirkadiane Rhythmen), tageszeitliche Schwankungen der vagalen Aktivität sowie deren Überlagerungen) charakterisiert werden, welche mit der Stimulationssequenz interferieren könnten.

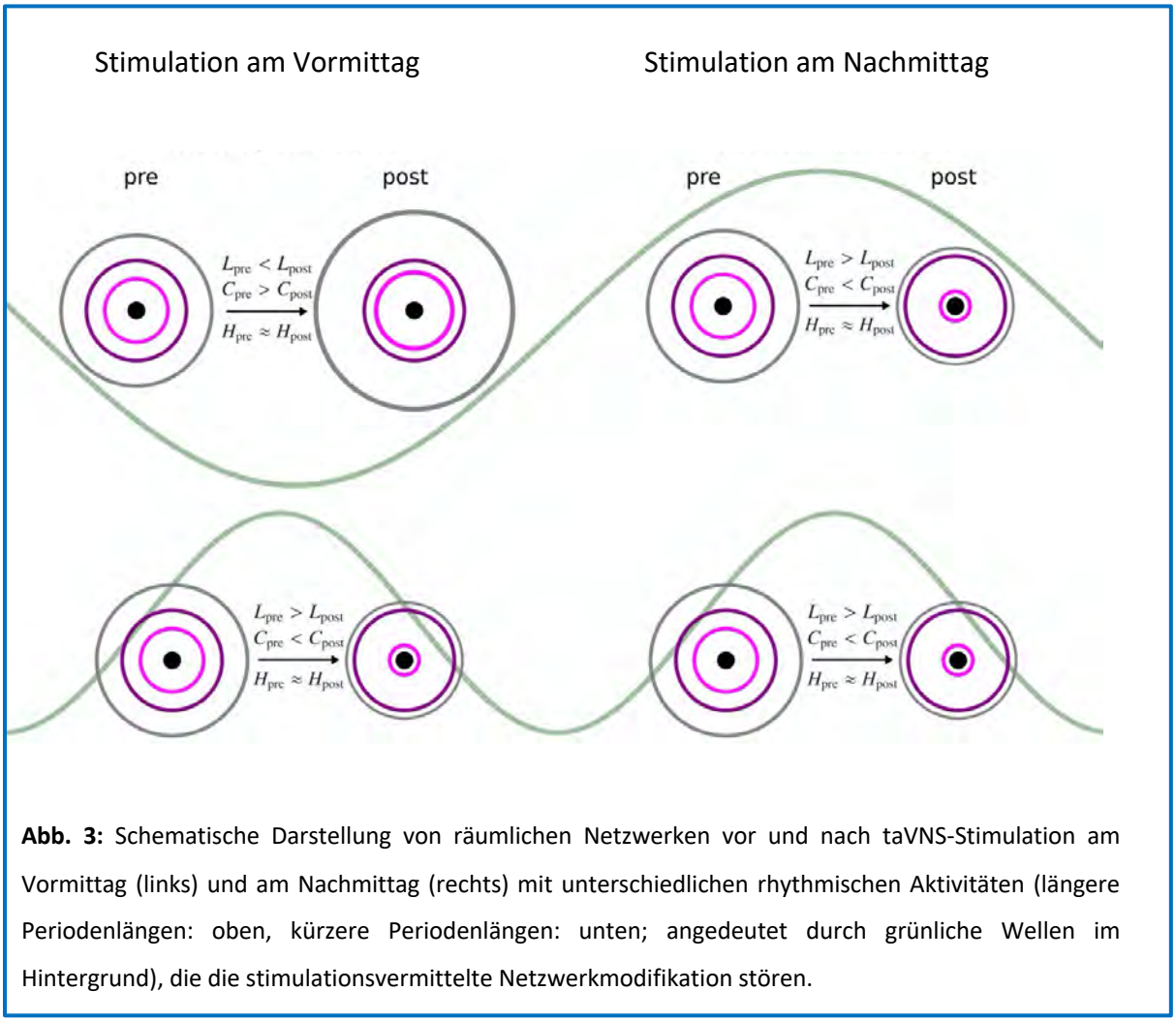

Abb. 3: Schematische Darstellung von räumlichen Netzwerken vor und nach taVNS-Stimulation am Vormittag (links) und am Nachmittag (rechts) mit unterschiedlichen rhythmischen Aktivitäten (längere Periodenlängen: oben, kürzere Periodenlängen: unten; angedeutet durch grünliche Wellen im Hintergrund), die die stimulationsvermittelte Netzwerkmodifikation stören.

Der Ansatz der dritten Arbeit ermöglichte weiterhin, taVNS-vermittelte Netzwerkveränderungen auf längeren Zeitskalen (verlängerte und länger anhaltende Effekte) zu bewerten. Verlängerte Effekte konnten bei einigen Proband*innen gefunden werden und ähnelten weitgehend den unmittelbaren Effekten durch die morgendliche Stimulation, bei einer noch kleineren Gruppe konnten länger anhaltende Effekte beobachtet werden. Da die Mehrheit der Proband*innen jedoch keine länger anhaltenden Effekte zeigte, müssen mögliche Faktoren, die taVNS-vermittelte Netzwerkmodifikationen auf längeren Zeitskalen beeinflussen, noch identifiziert werden. In dieser Hinsicht ist ein Vergleich mit früheren Forschungsergebnissen nur von begrenztem Wert. Während unmittelbare Auswirkungen

kurzfristiger taVNS auf die Hirndynamik bei Gesunden und bei verschiedenen Erkrankungen wiederholt berichtet wurden, können Informationen über mögliche Auswirkungen auf langfristigen Zeitskalen (Monate bis Jahre) bisher vorwiegend aus klinischen Studien bei verschiedenen Erkrankungen abgeleitet werden (Straube and Eren, 2021; von Wrede and Surges, 2021; Rong et al., 2016), welche jedoch auf wiederholten täglichen Stimulationen beruhen und ggf. anderweitigen Einflussfaktoren (wie z. B. eine medikamentöse Behandlung) unterliegen, die bei der Interpretation stimulationsbedingter Veränderungen der Hirndynamik berücksichtigt werden müssten. Darüber hinaus werden in diesen Studien taVNS-vermittelte Effekte im Wesentlichen indirekt über das klinische Ergebnis (z. B. Anfallshäufigkeit, Kopfschmerzscores, Depressionsscores) bewertet. Für die Zeit nach der Stimulation und in den nächsten Tagen ist das Wissen über taVNS-vermittelte Wirkungen auf das menschliche Gehirn daher gering. Es ist zu vermuten, dass spätere Effekte auf molekulare, zelluläre und humorale Veränderungen im Gehirn zurückzuführen sind, die zumindest bei menschlichen Proband*innen für die Zeit nach der Stimulation nicht einfach zu erfassen sind. Weiterführende Untersuchungen von taVNS-vermittelten Veränderungen der sich zeitlich entwickelnden funktionellen Netzwerke des menschlichen Gehirns auf längeren Zeitskalen sind somit sinnvoll, um den Wirkmechanismus von taVNS besser zu verstehen und sinnvolle Protokolle für Forschungs- und Behandlungsstudien zu erstellen.

Mit dem verwendeten Untersuchungsschema (eine Stimulation am Morgen und eine am Nachmittag mit einem heuristisch gewählten Abstand zwischen den Stimulationen) konnte kein potenziell neuromodulatorischer Effekt, im Sinne einer durch die zweite Stimulation erreichten Verstärkung der durch die erste Stimulation induzierten Netzwerkveränderungen) gezeigt werden. Es gibt jedoch experimentelle (vgl. (Beekwilder and Beems, 2010)) und klinische Belege (z. B. aus Studien bei Epilepsie, (vgl. (von Wrede and Surges, 2021)) dafür, dass die Stimulation des Vagusnervs eine neuromodulatorische Wirkung hat, die sich mit der Zeit verstärkt. Da der Vagusnerv selbst eine Übertragungs- und keine Verarbeitungsfunktion hat (Beekwilder and Beems, 2010), wird davon ausgegangen, dass verstärkende Effekte durch wiederholte Stimulationen auf molekularer, Neurotransmitter- und synaptischer Ebene wirken. Obwohl der genaue Wirkmechanismus noch nicht vollständig geklärt ist, gibt es immer mehr Belege für VNS-induzierte Veränderungen verschiedener Transmitterwege, die bis zu einem gewissen Grad auch auf taVNS übertragen werden können (Kaniusas et al., 2019). Es ist

anzunehmen, dass ein bestimmter Schwellenwert überschritten werden muss, um neuromodulatorische Effekte durch die taVNS zu erzielen.

TaVNS-induzierte Beeinflussung funktioneller Hirnnetzwerke bei verschiedenen Epilepsietypen

In der vierten Arbeit wurden die taVNS induzierten erkrankungsspezifischen Veränderungen der Hirnnetzwerkcharakteristika untersucht (von Wrede et al., 2022b): bereits in der Ruhephase zeigten sich signifikante Unterschiede zwischen den globalen Merkmalen (durchschnittlicher Clustering-Koeffizient und durchschnittliche kürzeste Pfadlänge) von Netzwerken von Proband*innen mit generalisierten Epilepsien, fokalen Epilepsien und von Proband*innen ohne Epilepsie, was frühere Studien bestätigt (Niso et al., 2015; Drenthen et al., 2020). In der vorgelegten Arbeit zeigten sich bei Proband*innen mit generalisierten Epilepsien weniger segregierte und stärker integrierte funktionelle Hirnnetzwerke im Vergleich zu Proband*innen mit fokalen Epilepsien und von Proband*innen ohne Epilepsie. Diese Ergebnisse stehen im Einklang mit früheren Studien (Chavez et al., 2010; Chowdhury et al., 2014), jedoch im Gegensatz zu einer anderen Studie (Zhang et al., 2011). Netzwerkstudien zu Epilepsien und insbesondere zu Epilepsiesyndromen sind ein sich entwickelndes Forschungsgebiet, die angewandten Methoden (funktionelle Magnetresonanztomografie, Elektroenzefalografie) und Protokolle unterschieden sich jedoch teils deutlich, sodass es aktuell noch schwierig ist, die Ergebnisse miteinander in Einklang zu bringen. In Erweiterung früherer Ergebnisse (von Wrede et al., 2021; Rings et al., 2021) konnte hier gezeigt werden, dass die transkutane aurikuläre Vagusnervstimulation zu unterschiedlichen Reorganisationen der funktionellen Hirnnetzwerke bei verschiedenen Epilepsietypen führt. Hierbei zeigten generalisierte Epilepsien eine immediate Reorganisation hin zu einem stärker integrierten/weniger segregierten und eine anhaltende Reorganisation hin zu einem stärker segregierten/weniger integrierten Netzwerk. Fokale Epilepsien zeigten ein invertiertes Muster. TaVNS induzierte eine vergleichbare unmittelbare robustheitssteigernde Modifikation der funktionellen Hirnnetzwerke aller Gruppen; dieser Effekt war für die Gruppe der fokalen Epilepsie und die Nicht-Epilepsiegruppe anhaltend, während die Robustheit in der Gruppe der generalisierten Epilepsie abnahm. Darüber hinaus induzierte taVNS eine dauerhaft höhere Anfälligkeit für Störungen des synchronisierten Zustands bei generalisierten Epilepsien und eine geringere bei fokalen Epilepsien, was zu einer unterschiedlichen

Netzwerkstabilität führte. Interessanterweise zeigten mehr Proband*innen mit generalisierter Epilepsie als mit fokaler Epilepsie taVNS-induzierte Veränderungen der Wichtigkeit von Hirnregionen und funktionellen Verbindungen auf lokaler Netzwerkebene. Es konnte daher die Hypothese aufgestellt werden, dass bei fokalen Epilepsien wichtige Hirnregionen anfälliger für Veränderungen sind, während bei generalisierter Epilepsie das Muster der veränderten Hirnregionen eher diffus ist. Für die Wichtigkeit der funktionellen Verbindungen konnte kein eindeutiges räumliches Muster festgestellt werden. Zusammengefasst konnte somit gezeigt werden, dass kurzzeitige taVNS bei Proband*innen mit generalisierter oder fokaler Epilepsie die globalen Eigenschaften ihrer funktionellen Hirnnetzwerke unterschiedlich verändert. Lokale Netzwerkcharakteristika blieben jedoch, wie bereits berichtet, weitgehend unbeeinflusst.

TaVNS-induzierte Veränderungen von Kognition und Verhalten

Bei zwei der Arbeiten (von Wrede et al., 2021; von Wrede et al., 2022b) wurde die taVNS durch neuropsychologische Untersuchungen begleitet. Dabei konnte gezeigt werden, dass bei den Proband*innen die taVNS ohne negative Auswirkungen auf Aufmerksamkeit und Exekutivfunktionen war. Ähnliche Beobachtungen wurden kürzlich auch bei gesunden Proband*innen gemacht, bei denen in der Phase der Gedächtniskonsolidierung eine taVNS durchgeführt wurde (Mertens et al., 2020). Für den iVNS konnte in früheren Studien gezeigt werden, dass eine iVNS, in Abhängigkeit von der Stimulationsintensität, bei Epilepsiepatient*innen das Rekognitionsgedächtnis im Vergleich zur Scheinstimulation verbessern kann (Clark et al., 1999). Nachteilige Auswirkungen wurden bei einer akuten hochintensiven iVNS auf das figurale Gedächtnis, nicht aber auf das verbale Gedächtnis bei Epilepsiepatient*innen festgestellt (Helmstaedter et al., 2001). Ein signifikanter Einfluss der iVNS auf Exekutivfunktionen, Kreativität und kognitive Flexibilität wurde nicht gefunden (Übersicht in (Adair et al., 2020). Für die taVNS sind modulierende Effekte auf die Kognition beschrieben (Ridgewell et al., 2021). Es liegen Beobachtungen für eine Verbesserung der Assoziativgedächtnisses, des motorischen Lernens, der Exekutivfunktionen und der kognitiven Kontrolle vor (Übersicht in (Adair et al., 2020)). Die bislang vorliegenden Ergebnisse zu kurz- und langfristigen Auswirkungen von taVNS auf Kognition und Verhalten sind erfolgversprechend, bedürfen jedoch weiterführender Untersuchungen (Colzato and Beste, 2020), bevor Aussagen für den klinischen Einsatz getroffen werden können.

In den Untersuchungen der vorgelegten Arbeiten wurde die kurzzeitige taVNS von den Proband*innen gut vertragen, und es traten keine lokalen (an der Ohrmuschel) oder systemischen Nebenwirkungen durch die Stimulation auf. Diese Ergebnisse entsprechen den Ergebnissen zur Verträglichkeit in Langzeitstudien (Übersicht in (von Wrede and Surges, 2021)). Die Benutzerfreundlichkeit des Geräts wurde in Bezug auf das Management, die Handhabung, den Komfort und die Möglichkeit der Fortführung der eigenen Aktivitäten als gut und sehr gut bewertet. Die Bewertung der Eignung für eine langfristige oder wiederholte Anwendung wurde jedoch von einigen Proband*innen kritisch gesehen. Beschwerden über die Dauer einer täglichen Stimulation von 4 Stunden wurden von Proband*innen mit Epilepsie in früheren Behandlungssituationen geäußert, dies führte zu Studienprotokollen mit einer kürzeren Stimulationsdauer (Barbella et al., 2018) sowie zu einer schlechteren Einhaltung der Behandlungsempfehlungen (Sabers et al., 2021).

In den vorgelegten Arbeiten konnte somit gezeigt werden, dass bei guter Verträglichkeit und Nutzbarkeit die kurzzeitige taVNS eine topologie, robustheits- und stabilitätsverändernde Auswirkung auf großskalige epileptische Hirnnetzwerke ohne nachteilige Auswirkungen auf Kognition und Verhalten hat.

Das entwickelte Studienprotokoll ermöglichte die Untersuchung der Beeinflussung menschlicher funktioneller Hirnnetzwerke durch den taVNS im klinischen Setting. Die Anwendung von auf der Graphentheorie basierenden Konzepten der Hirnnetzwerk-Charakterisierung aus Langzeit-EEG-Registrierungen erlaubte die Untersuchung der taVNS auf funktionelle Hirnnetzwerke in sehr hoher zeitlicher und guter räumlicher Auflösung. Dieser Ansatz konnte auch auf anderweitige nicht-pharmazeutischer Interventionen, z.B. Biofeedback-Verfahren (Schach et al., 2022) ausgeweitet werden.

Limitierungen der Studien

Es gibt einige Einschränkungen der vorgelegten prospektiven Untersuchungen. Da möglichst viele Beeinflussungsfaktoren auf das EEG vermieden werden sollten (z.B. durch Aktivierungsmethoden, Veränderung der ASM, Anfälle vor der Studie), wurde eine hohe Ausschlussrate generiert und somit war die Rekrutierung der Teilnehmer*innen erschwert. Dies führte zu einer geringen Anzahl und teils höheren Heterogenität der untersuchten

Teilnehmer*innen (z.B. hinsichtlich Lebensalter, Erkrankungsdauer). Zukünftige Studien sollten den Einfluss mit einem doppelblinden Ansatz (Stimulation versus Scheinstimulation) und interpersonellem Work-up (z.B. verschiedene Stimulationsparameter und/oder Stimulationstageszeiten) untersuchen, um die vorliegenden Ergebnisse weiter zu untermauern.

Ausblick

Die vorgelegten Arbeiten eröffnen ein breites Spektrum weiterer Untersuchungsansätze, um den Wirkmechanismus der taVNS besser zu verstehen und langfristig die Stimulationsparameter und damit Effektivität und Adhärenz zu steigern. Künftige Studien sollten die Auswirkungen der Stimulation auf lokale und/oder mittelskalige Eigenschaften epileptischer Hirnnetzwerke (wie Kerne, Motive oder Gemeinschaftsstrukturen) untersuchen, um die taVNS-induzierten Veränderungen funktioneller Hirnnetzwerke umfassender zu charakterisieren. Das hier für die taVNS verwendete Gerät verfügt über nicht einstellbare Stimulationsparameter. Bei der iVNS ist eine individuelle Anpassung der Parameter jedoch nicht nur für eine wirksame Behandlung der Epilepsie entscheidend (Musselman et al., 2019), sondern könnte sich auch auf die topologischen und Robustheitseigenschaften der epileptischen Hirnnetzwerke auswirken. Die Bewertung der Auswirkungen unterschiedlicher Stimulationsparameter könnte zum Verständnis des Wirkungsmechanismus der taVNS beitragen und langfristig helfen, ihren klinischen Einsatz zu optimieren. Ein Vergleich der Wirkungen von iVNS und taVNS unter Verwendung desselben Studiendesigns könnte Gemeinsamkeiten und Unterschiede dieser Stimulationsansätze im Hinblick auf epileptische Netzwerke aufzeigen. Die Begleitung von klinischen Studien unter Nutzung von Netzwerkanalysen, um mögliche Beziehungen zwischen taVNS-induzierten Veränderungen funktioneller Hirnnetzwerke und klinischer Wirksamkeit zu untersuchen, ermöglichen ggf. eine Erweiterung der zuvor für Medikamente beschriebenen Methode des Pharmako-EEGs. Hierunter versteht man die Beschreibung und quantitative Analyse der Wirkungen von Substanzen auf das Zentralnervensystem unter Nutzung neurophysiologischer und elektrophysiologischer Methoden, die im Rahmen der klinischen und experimentellen Pharmakologie, der Neurotoxikologie, der therapeutischen Forschung und verwandter Disziplinen eingesetzt werden (Jobert et al., 2012). Für Antikonvulsiva (ASM) konnten unter Nutzung der Methodik bereits relevante Erkenntnisse über das Ansprechen auf die

Behandlung, den Nachweis neurotoxischer Wirkungen von ASM und die Vorhersage des ASM-Behandlungsansprechens oder der Nebenwirkungen geliefert werden (Höller et al., 2018). Es kann daher postuliert werden, dass diese Ansätze der auf der Graphentheorie basierenden mathematischen EEG-Analyse auf die Auswirkungen nicht-pharmazeutischer Interventionen auf Hirnnetzwerke erweitert werden können.

5. Zusammenfassung

In den vorgelegten vier Arbeiten konnte gezeigt werden, dass eine kurzzeitige taVNS die lokalen und globalen topologischen Eigenschaften sowie die Stabilität und Robustheit von sich entwickelnden funktionellen Hirnnetzwerken sowohl immediat als auch längerfristig verändert, was mit der vorherrschenden Ansicht einer global wirkenden Wirkungsweise von taVNS übereinstimmt. Diese Veränderungen scheinen abhängig vom Epilepsietyp sowie von der Stimulationstageszeit zu sein. Da die Modifikationen auf lokaler Netzwerkebene räumlich unspezifisch erscheinen, könnten die Veränderungen mit dem hier vorgeschlagenen Modell einer stimulationsbedingten Dehnung und Komprimierung funktioneller Hirnnetzwerke erklärt werden. Die Anwendung von auf der Graphentheorie basierenden Konzepten der Hirnnetzwerk-Charakterisierung aus Langzeit-EEG-Registrierungen erlaubte die Untersuchung von nicht-pharmazeutischer Interventionen auf funktionelle Hirnnetzwerke in sehr hoher zeitlicher und guter räumlicher Auflösung. Unser Ansatz eröffnet somit neue Perspektiven für ein besseres Verständnis der Dynamik epileptischer Hirnnetzwerke und kann zum besseren Verständnis des Wirkmechanismus von taVNS beitragen. Andere Untersuchungen, die diese Methoden nutzten, um den Einfluss der taVNS auf epileptische Hirnnetzwerke zu untersuchen, sind zum Zeitpunkt des Abfassens dieser Schrift nicht bekannt. Weitere Studien, die mögliche Beziehungen zwischen taVNS-induzierten globalen und lokalen Veränderungen funktioneller Hirnnetzwerke und klinischer Wirksamkeit untersuchen, sind notwendig, um diese experimentellen Ergebnisse in klinische Entscheidungen umzusetzen. Die Suche nach Prädiktoren für eine wirksame Vagusnervstimulation ist eine große Herausforderung, für die bereits erste interessante Erkenntnisse für die iVNS vorgelegt wurden (Workewych et al., 2020). Mit aus der Graphentheorie abgeleiteten Netzwerkanalysen elektrophysiologischer Daten liegt eine standardisierbare, objektivierbare und reproduzierbare Methode vor, um die Beeinflussung der Hirnnetzwerke durch die transkutane aurikuläre Vagusnervstimulation zu charakterisieren, welche perspektivisch im Rahmen von Studien und langfristig in der Individualbehandlung eingesetzt werden könnte.

6. Überlappung durch geteilte Autorenschaften

Die vorliegende Habilitationsschrift hat vier publizierte Originalarbeiten zur Grundlage. Drei der Arbeiten habe ich als alleinige Erstautorin veröffentlicht. Eine Arbeit habe ich mit Herrn Thorsten Rings, M. Sc., und Herrn Timo Bröhl, M. Sc., als geteilte Erstautor*innenschaft zusammen veröffentlicht. Herr Rings und Herr Bröhl sind Doktoranden in der Arbeitsgruppe von Prof. Dr. rer. nat. Klaus Lehnertz. In dieser Veröffentlichung war ich für das Studiendesign und die Datenerhebung verantwortlich, die mathematisch-physikalischen Berechnungen der Daten zu den globalen Hirnnetzwerken hat Herr Rings, die mathematisch-physikalischen Berechnungen der Daten zu den lokalen Hirnnetzwerken hat Herr Bröhl durchgeführt. Die Ergebnisse wurden gemeinsam mit dem Letztautor Prof. Dr. rer. nat. Klaus Lehnertz ausgewertet, interpretiert und visualisiert, das Manuskripts wurde gemeinsam geschrieben.

Eine Überlappung mit anderen Habilitationsschriften ist nicht gegeben.

7. Bibliographie

Adair, D., Truong, D., Esmaeilpour, Z., Gebodh, N., Borges, H., Ho, L., Bremner, J.D., Badran, B.W., Napadow, V., Clark, V.P., et al. (2020). Electrical stimulation of cranial nerves in cognition and disease. Brain Stimul. *13*, 717–750.

Aihua, L., Lu, S., Liping, L., Xiuru, W., Hua, L., and Yuping, W. (2014). A controlled trial of transcutaneous vagus nerve stimulation for the treatment of pharmacoresistant epilepsy. Epilepsy Behav. *39*, 105–110.

Atay, F.M., Bıyıkoğlu, T., and Jost, J. (2006). Network synchronization: Spectral versus statistical properties. Phys. D Nonlinear Phenom. *224*, 35–41.

Badran, B.W., Dowdle, L.T., Mithoefer, O.J., LaBate, N.T., Coatsworth, J., Brown, J.C., DeVries, W.H., Austelle, C.W., McTeague, L.M., and George, M.S. (2018). Neurophysiologic effects of transcutaneous auricular vagus nerve stimulation (taVNS) via electrical stimulation of the tragus: A concurrent taVNS/fMRI study and review. Brain Stimul. *11*, 492–500.

Barahona, M., and Pecora, L.M. (2002). Synchronization in small-world systems. Phys. Rev. Lett. *89*, 54101.

Barbella, G., Cocco, I., Freri, E., Marotta, G., Visani, E., Franceschetti, S., and Casazza, M. (2018). Transcutaneous vagal nerve stimulatio (t-VNS): An adjunctive treatment option for refractory epilepsy. Seizure *60*, 115–119.

Barrat, A., Barthélemy, M., Pastor-Satorras, R., and Vespignani, A. (2004). The architecture of complex weighted networks. Proc. Natl. Acad. Sci. U. S. A. *101*, 3747–3752.

Baud, M.O., Perneger, T., Rácz, A., Pensel, M.C., Elger, C., Rydenhag, B., Malmgren, K., Cross, J.H., McKenna, G., Tisdall, M., et al. (2018). European trends in epilepsy surgery. Neurology *91*, e96–e106.

Bauer, S., Baier, H., Baumgartner, C., Bohlmann, K., Fauser, S., Graf, W., Hillenbrand, B., Hirsch, M., Last, C., Lerche, H., et al. (2016). Transcutaneous Vagus Nerve Stimulation (tVNS) for Treatment of Drug-Resistant Epilepsy: A Randomized, Double-Blind Clinical Trial (cMPsE02). Brain Stimul. *9*, 356–363.

Beekwilder, J.P., and Beems, T. (2010). Overview of the clinical applications of vagus nerve stimulation. J. Clin. Neurophysiol. Off. Publ. Am. Electroencephalogr. Soc. *27*, 130–138.

Boon, P., De Cock, E., Mertens, A., and Trinka, E. (2018). Neurostimulation for drug-resistant epilepsy: a systematic review of clinical evidence for efficacy, safety, contraindications and predictors for response. Curr. Opin. Neurol. *31* (2), 198-210.

Borniger, C., Nelson, R.J., Hall, M., Ditzen, B., Thayer, J.F., Jarczok, M.N., Aguilar-Raab, C., Koenig, J., and Kaess, M. (2018). Chronobiology International The Heart ´ s rhythm ' n ' blues : Sex differences in circadian variation patterns of vagal activity vary by depressive symptoms in predominantly healthy employees. Chronobiol Int. *3*5 (7). pp. 896-909.

Bröhl, T., and Lehnertz, K. (2019). Centrality-based identification of important edges in complex networks. Chaos *29*, 33115.

Bröhl, T., and Lehnertz, K. (2022). A straightforward edge centrality concept derived from generalizing degree and strength. Sci. Rep. *12*, 4407.

Bröhl, T., Rings, T., and Lehnertz, K. (2020). Von Interaktionen zu Interaktionsnetzwerken: Zeitabhängige funktionelle Netzwerke am Beispiel der Epilepsie. Klin. Neurophysiol. *51*, 132–143.

Bullmore, E., and Sporns, O. (2009). Complex brain networks: graph theoretical analysis of structural and functional systems. Nat. Rev. Neurosci. *10*, 186–198.

Butt, M.F., Albusoda, A., Farmer, A.D., and Aziz, Q. (2020). The anatomical basis for transcutaneous auricular vagus nerve stimulation. J. Anat. *236*, 588–611.

Chavez, M., Valencia, M., Navarro, V., Latora, V., and Martinerie, J. (2010). Functional modularity of background activities in normal and epileptic brain networks. Phys. Rev. Lett. *104*, 118701.

Chen, Z., Brodie, M.J., Liew, D., and Kwan, P. (2018). Treatment outcomes in patients with newly diagnosed epilepsy treated with established and new antiepileptic drugs a 30-year longitudinal cohort study. JAMA Neurol. *75*, 279–286.

Chiosa, V., Ciolac, D., Groppa, S., Koirala, N., Pintea, B., Vataman, A., Winter, Y., Gonzalez-Escamilla, G., Muthuraman, M., and Groppa, S. (2019). Large-scale network architecture and associated structural cortico-subcortical abnormalities in patients with sleep/awake-related seizures. Sleep *42(4)*, zsz006.

Chow, E., Bernjak, A., Williams, S., Fawdry, R.A., Hibbert, S., Freeman, J., Sheridan, P.J., and

Heller, S.R. (2014). Risk of cardiac arrhythmias during hypoglycemia in patients with type 2 diabetes and cardiovascular risk. Diabetes *63*, 1738–1747.

Chowdhury, F.A., Woldman, W., FitzGerald, T.H.B., Elwes, R.D.C., Nashef, L., Terry, J.R., and Richardson, M.P. (2014). Revealing a brain network endophenotype in families with idiopathic generalised epilepsy. PLoS One *9*, e110136.

Clark, K.B., Naritoku, D.K., Smith, D.C., Browning, R.A., and Jensen, R.A. (1999). Enhanced recognition memory following vagus nerve stimulation in human subjects. Nat. Neurosci. *2*, 94–98.

Colzato, L., and Beste, C. (2020). A literature review on the neurophysiological underpinnings and cognitive effects of transcutaneous vagus nerve stimulation: challenges and future directions. J. Neurophysiol. *123*, 1739–1755.

Cook, M.J., O'Brien, T.J., Berkovic, S.F., Murphy, M., Morokoff, A., Fabinyi, G., D'Souza, W., Yerra, R., Archer, J., Litewka, L., et al. (2013). Prediction of seizure likelihood with a long-term, implanted seizure advisory system in patients with drug-resistant epilepsy: a first-in-man study. Lancet. Neurol. *12*, 563–571.

Cramer, J.A., Perrine, K., Devinsky, O., Bryant-Comstock, L., Meador, K., and Hermann, B. (1998). Development and cross-cultural translations of a 31-item quality of life in epilepsy inventory. Epilepsia *39*, 81–88.

Dickten, H., Porz, S., Elger, C.E., and Lehnertz, K. (2016). Weighted and directed interactions in evolving large-scale epileptic brain networks. Sci. Rep. *6*, 34824.

van Dijk, N., Boer, M.C., De Santo, T., Grovale, N., Aerts, A.J.J., Boersma, L., and Wieling, W. (2007). Daily, weekly, monthly, and seasonal patterns in the occurrence of vasovagal syncope in an older population. Eur. Eur. Pacing, Arrhythmias, Card. Electrophysiol. J. Work. Groups Card. Pacing, Arrhythmias, Card. Cell. Electrophysiol. Eur. Soc. Cardiol. *9*, 823–828.

Dijkstra, E.W. (1959). A note on two problems in connexion with graphs. Numer. Math. *1*, 269–271.

Drenthen, G.S., Fasen, F., Fonseca Wald, E.L.A., Backes, W.H., Aldenkamp, A.P., Vermeulen, R.J., Debeij-van Hall, M., Hendriksen, J., Klinkenberg, S., and Jansen, J.F.A. (2020). Functional

brain network characteristics are associated with epilepsy severity in childhood absence epilepsy. NeuroImage. Clin. *27*, 102264.

Elliott, R.E., Morsi, A., Kalhorn, S.P., Marcus, J., Sellin, J., Kang, M., Silverberg, A., Rivera, E., Geller, E., Carlson, C., et al. (2011). Vagus nerve stimulation in 436 consecutive patients with treatment-resistant epilepsy: long-term outcomes and predictors of response. Epilepsy Behav. *20*, 57–63.

Ellrich, J. (2019). Transcutaneous Auricular Vagus Nerve Stimulation. J. Clin. Neurophysiol. Off. Publ. Am. Electroencephalogr. Soc. *36*, 437–442.

Englot, D.J., Rolston, J.D., Wright, C.W., Hassnain, K.H., and Chang, E.F. (2016). Rates and Predictors of Seizure Freedom With Vagus Nerve Stimulation for Intractable Epilepsy. Neurosurgery *79*, 345–353.

Farmer, A.D., Strzelczyk, A., Finisguerra, A., Gourine, A. V, Gharabaghi, A., Hasan, A., Burger, A.M., Jaramillo, A.M., Mertens, A., Majid, A., et al. (2021). International Consensus Based Review and Recommendations for Minimum Reporting Standards in Research on Transcutaneous Vagus Nerve Stimulation (Version 2020). Front. Hum. Neurosci. *14*, 568051.

Fisher, B., DesMarteau, J.A., Koontz, E.H., Wilks, S.J., and Melamed, S.E. (2020). Responsive Vagus Nerve Stimulation for Drug Resistant Epilepsy: A Review of New Features and Practical Guidance for Advanced Practice Providers. Front. Neurol. *11*, 610379.

Fisher, R., Salanova, V., Witt, T., Worth, R., Henry, T., Gross, R., Oommen, K., Osorio, I., Nazzaro, J., Labar, D., et al. (2010). Electrical stimulation of the anterior nucleus of thalamus for treatment of refractory epilepsy. Epilepsia *51*, 899–908.

Fisher, R.S., van Emde Boas, W., Blume, W., Elger, C., Genton, P., Lee, P., and Engel, J.J. (2005). Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). Epilepsia *46*, 470–472.

Fisher, R.S., Acevedo, C., Arzimanoglou, A., Bogacz, A., Cross, J.H., Elger, C.E., Engel Jr, J., Forsgren, L., French, J.A., Glynn, M., et al. (2014). ILAE Official Report: A practical clinical definition of epilepsy. Epilepsia *55*, 475–482.

Fruengel, R., Bröhl, T., Rings, T., and Lehnertz, K. (2020). Reconfiguration of human evolving

large-scale epileptic brain networks prior to seizures: an evaluation with node centralities. Sci. Rep. *10*, 21921.

GBD 2016 Neurology Collaborators. (2019). Global, regional, and national burden of epilepsy, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet. Neurol. *18*, 357–375.

Geier, C., Lehnertz, K., and Bialonski, S. (2015). Time-dependent degree-degree correlations in epileptic brain networks: from assortative to dissortative mixing. Front. Hum. Neurosci. *9*, 462.

Goldenholz, D.M., Goldenholz, S.R., Moss, R., French, J., Lowenstein, D., Kuzniecky, R., Haut, S., Cristofaro, S., Detyniecki, K., Hixson, J., et al. (2017). Does accounting for seizure frequency variability increase clinical trial power? Epilepsy Res. *137*, 145–151.

Helmstaedter, C., Hoppe, C., and Elger, C.E. (2001). Memory alterations during acute high-intensity vagus nerve stimulation. Epilepsy Res. *47*, 37–42.

van den Heuvel, M.P., and Sporns, O. (2013). Network hubs in the human brain. Trends Cogn. Sci. *17*, 683–696.

Höller, Y., Helmstaedter, C., and Lehnertz, K. (2018). Quantitative Pharmaco-Electroencephalography in Antiepileptic Drug Research. CNS Drugs *32*, 839–848.

Hoppe, C., Poepel, A., and Elger, C.E. (2007). Epilepsy: accuracy of patient seizure counts. Arch. Neurol. *64*, 1595–1599.

Huikuri, H. V, Kessler, K.M., Terracall, E., Castellanos, A., Linnaluoto, M.K., and Myerburg, R.J. (1990). Reproducibility and circadian rhythm of heart rate variability in healthy subjects. Am. J. Cardiol. *65*, 391–393.

International Neuromodulation Society (2022). www.neuromodulation.com/aufgerufen 23.06.2022

Jarosiewicz, B., and Morrell, M. (2020). The RNS System: brain-responsive neurostimulation for the treatment of epilepsy. Expert Rev. Med. Devices. *18(2*), 129-138.

Jin, S.-H., Jeong, W., Lee, D.-S., Jeon, B.S., and Chung, C.K. (2014). Preserved high-centrality hubs but efficient network reorganization during eyes-open state compared with eyes-closed resting state: an MEG study. J. Neurophysiol. *111*, 1455–1465.

Jobert, M., Wilson, F.J., Ruigt, G.S.F., Brunovsky, M., Prichep, L.S., and Drinkenburg, W.H.I.M. (2012). Guidelines for the recording and evaluation of pharmaco-EEG data in man: the International Pharmaco-EEG Society (IPEG). Neuropsychobiology *66*, 201–220.

Kaniusas, E., Kampusch, S., Tittgemeyer, M., Panetsos, F., Gines, R.F., Papa, M., Kiss, A., Podesser, B., Cassara, A.M., Tanghe, E., et al. (2019). Current Directions in the Auricular Vagus Nerve Stimulation I - A Physiological Perspective. Front. Neurosci. *13*, 854.

Karoly, P., Goldenholz, D.M., and Cook, M. (2018). Are the days of counting seizures numbered? Curr. Opin. Neurol. *31*, 162–168.

Karoly, P.J., Romero, J., Cook, M.J., Freestone, D.R., and Goldenholz, D.M. (2019). When can we trust responders? Serious concerns when using 50% response rate to assess clinical trials. Epilepsia *60*, e99–e103.

Koo, B. (2001). EEG changes with vagus nerve stimulation. J. Clin. Neurophysiol. Off. Publ. Am. Electroencephalogr. Soc. *18*, 434–441.

Kuhnert, M.-T., Elger, C.E., and Lehnertz, K. (2010). Long-term variability of global statistical properties of epileptic brain networks. Chaos *20*, 43126.

Kwan, P., and Brodie, M.J. (2000). Early Identification of Refractory Epilepsy. N. Engl. J. Med. *342*, 314–319.

Kwan, P., Arzimanoglou, A., Berg, A.T., Brodie, M.J., Allen Hauser, W., Mathern, G., Moshé, S.L., Perucca, E., Wiebe, S., and French, J. (2010). Definition of drug resistant epilepsy: consensus proposal by the ad hoc Task Force of the ILAE Commission on Therapeutic Strategies. Epilepsia *51*, 1069–1077.

Lehnertz, K., Ansmann, G., Bialonski, S., Dickten, H., Geier, C., and Porz, S. (2014). Evolving networks in the human epileptic brain. Phys. D Nonlinear Phenom. *267*, 7–15.

Lehnertz, K., Rings, T., and Bröhl, T. (2021). Time in Brain: How Biological Rhythms Impact on EEG Signals and on EEG-Derived Brain Networks. Front. Netw. Physiol. *1*, 1:755016.

Liu, A., Song, L., Wang, X., Wang, Y., Gong, L., Li, L., and Rong, P. (2018). Efficacy and Safety of Treatment with Transcutaneous Vagus Nerve Stimulation in 17 Patients with Refractory Epilepsy Evaluated by Electroencephalogram, Seizure Frequency, and Quality of Life. Med. Sci. Monit. *24*, 8439–8448.

Lohmann, G., Margulies, D., Horstmann, A., Pleger, B., Lepsien, J., Goldhahn, D., Schloegl, H., Stumvoll, M., Villringer, A., and Turner, R. (2010). Eigenvector Centrality Mapping for Analyzing Connectivity Patterns in fMRI Data of the Human Brain. PLoS One *5*, e10232.

Lü, L., Chen, D., Ren, X.-L., Zhang, Q.-M., Zhang, Y.-C., and Zhou, T. (2016). Vital nodes identification in complex networks. Phys. Rep. *650*, 1–63.

Makarov, V. V, Zhuravlev, M.O., Runnova, A.E., Protasov, P., Maksimenko, V.A., Frolov, N.S., Pisarchik, A.N., and Hramov, A.E. (2018). Betweenness centrality in multiplex brain network during mental task evaluation. Phys. Rev. E *98*, 62413.

Mao, Y., Chen, C., Falahpour, M., MacNiven, K.H., Heit, G., Sharma, V., Alataris, K., and Liu, T.T. (2022). Effects of Sub-threshold Transcutaneous Auricular Vagus Nerve Stimulation on Cingulate Cortex and Insula Resting-state Functional Connectivity. Front. Hum. Neurosci. *16*, 862443.

Mertens, A., Naert, L., Miatton, M., Poppa, T., Carrette, E., Gadeyne, S., Raedt, R., Boon, P., and Vonck, K. (2020). Transcutaneous Vagus Nerve Stimulation Does Not Affect Verbal Memory Performance in Healthy Volunteers. Front. Psychol. *11*, 551.

Mormann, F., Lehnertz, K., David, P., and Elger, C.E. (2000). Mean phase coherence as a measure for phase synchronization and its application to the EEG of epilepsy patients. Phys. D Nonlinear Phenom. *144*, 358–369.

Morris, C.J., Yang, J.N., and Scheer, F.A.J.L. (2012). The impact of the circadian timing system on cardiovascular and metabolic function. Prog. Brain Res. *199*, 337–358.

Morris, G.L. 3rd, Gloss, D., Buchhalter, J., Mack, K.J., Nickels, K., and Harden, C. (2013). Evidence-based guideline update: vagus nerve stimulation for the treatment of epilepsy: report of the Guideline Development Subcommittee of the American Academy of Neurology. Neurology *81*, 1453–1459.

Musselman, E.D., Pelot, N.A., and Grill, W.M. (2019). Empirically Based Guidelines for Selecting Vagus Nerve Stimulation Parameters in Epilepsy and Heart Failure. Cold Spring Harb. Perspect. Med. 9(7); a034264.

Nair, D.R., Laxer, K.D., Weber, P.B., Murro, A.M., Park, Y.D., Barkley, G.L., Smith, B.J., Gwinn, R.P., Doherty, M.J., Noe, K.H., et al. (2020). Nine-year prospective efficacy and safety of

brain-responsive neurostimulation for focal epilepsy. Neurology *95*, e1244–e1256.

Niso, G., Carrasco, S., Gudín, M., Maestú, F., Del-Pozo, F., and Pereda, E. (2015). What graph theory actually tells us about resting state interictal MEG epileptic activity. NeuroImage. Clin. *8*, 503–515.

Pecora, L.M., and Carroll, T.L. (1998). Master Stability Function for Globally Synchronized Systems. Phys Rev Lett *80*, 2109–2112.

Révész, D., Rydenhag, B., and Ben-Menachem, E. (2016). Complications and safety of vagus nerve stimulation: 25 years of experience at a single center. J. Neurosurg. Pediatr. *18*, 97–104.

Ridgewell, C., Heaton, K.J., Hildebrandt, A., Couse, J., Leeder, T., and Neumeier, W.H. (2021). The effects of transcutaneous auricular vagal nerve stimulation on cognition in healthy individuals: A meta-analysis. Neuropsychology *35*, 352–365.

Rings, T., von Wrede, R., and Lehnertz, K. (2019). Precursors of seizures due to specific spatial-temporal modifications of evolving large-scale epileptic brain networks. Sci. Rep. *9*, 10623.

Rings, T., von Wrede, R., Bröhl, T., Schach, S., Helmstaedter, C., and Lehnertz, K. (2021). Impact of Transcutaneous Auricular Vagus Nerve Stimulation on Large-Scale Functional Brain Networks: From Local to Global. Front. Physiol. *12*, 1–10.

Rong, P., Liu, A., Zhang, J., Wang, Y., He, W., Yang, A., Li, L., Ben, H., Li, L., Liu, H., et al. (2014a). Transcutaneous vagus nerve stimulation for refractory epilepsy: a randomized controlled trial. Clin. Sci. (Lond). 2013051

Rong, P., Liu, A., Zhang, J., Wang, Y., Yang, A., Li, L., Ben, H., Li, L., Liu, R., He, W., et al. (2014b). An alternative therapy for drug-resistant epilepsy: transcutaneous auricular vagus nerve stimulation. Chin. Med. J. (Engl). *127*, 300–304.

Rong, P., Liu, J., Wang, L., Liu, R., Fang, J., Zhao, J., Zhao, Y., Wang, H., Vangel, M., Sun, S., et al. (2016). Effect of transcutaneous auricular vagus nerve stimulation on major depressive disorder: A nonrandomized controlled pilot study. J. Affect. Disord. *195*, 172–179.

Rutecki, P. (1990). Anatomical, physiological, and theoretical basis for the antiepileptic effect of vagus nerve stimulation. Epilepsia *31 Suppl 2*, S1-6.

Sabers, A., Aumüller-Wagner, S., Christensen, L.R., Henning, O., Kostov, K., Lossius, M., Majoie, M., Mertens, A., Nielsen, L., Vonck, K., et al. (2021). Feasibility of transcutaneous auricular vagus nerve stimulation in treatment of drug resistant epilepsy: A multicenter prospective study. Epilepsy Res. *177*, 106776.

Schach, S., Rings, T., Bregulla, M., Witt, J.-A., Bröhl, T., Surges, R., von Wrede, R., Lehnertz, K., and Helmstaedter, C. (2022). Electrodermal Activity Biofeedback Alters Evolving Functional Brain Networks in People With Epilepsy, but in a Non-specific Manner. Front. Neurosci. *16*, 828283.

Schuerman, W.L., Nourski, K. V, Rhone, A.E., Howard, M.A., Chang, E.F., and Leonard, M.K. (2021). Human intracranial recordings reveal distinct cortical activity patterns during invasive and non-invasive vagus nerve stimulation. Sci. Rep. *11*, 22780.

Song, G.-F., Wang, H.-Y., Wu, C.-J., Li, X., and Yang, F.-Y. (2018). A retrospective study of transcutaneous vagus nerve stimulation for poststroke epilepsy. Medicine (Baltimore). *97*, e11625.

Stefan, H., Kreiselmeyer, G., Kerling, F., Kurzbuch, K., Rauch, C., Heers, M., Kasper, B.S., Hammen, T., Rzonsa, M., Pauli, E., et al. (2012). Transcutaneous vagus nerve stimulation (t-VNS) in pharmacoresistant epilepsies: a proof of concept trial. Epilepsia *53*, e115-8.

Straube, A., and Eren, O. (2021). tVNS in the management of headache and pain. Auton. Neurosci. *236*, 102875.

Tononi, G., Sporns, O., and Edelman, G.M. (1994). A measure for brain complexity: relating functional segregation and integration in the nervous system. Proc. Natl. Acad. Sci. U. S. A. *91*, 5033–5037.

Vonck, K., Raedt, R., Naulaerts, J., De Vogelaere, F., Thiery, E., Van Roost, D., Aldenkamp, B., Miatton, M., and Boon, P. (2014). Vagus nerve stimulation…25 years later! What do we know about the effects on cognition? Neurosci. Biobehav. Rev. *45*, 63–71.

de Vos, C.C., Melching, L., van Schoonhoven, J., Ardesch, J.J., de Weerd, A.W., van Lambalgen, H.C.E., and van Putten, M.J.A.M. (2011). Predicting success of vagus nerve stimulation (VNS) from interictal EEG. Seizure *20*, 541–545.

Wang, Z.-J., Kim, E.-S., Noh, B.H., Liang, J.-G., Lee, D., Hur, Y.J., Kim, N.-Y., and Kim, H.-D.

(2020). Alteration in brain connectivity in patients with Dravet syndrome after vagus nerve stimulation (VNS): exploration of its effectiveness using graph theory analysis with electroencephalography. J. Neural Eng. *17*, 36014.

Workewych, A.M., Arski, O.N., Mithani, K., and Ibrahim, G.M. (2020). Biomarkers of seizure response to vagus nerve stimulation: A scoping review. Epilepsia *61*, 2069–2085.

World Health Organization (2019). https://www.who.int/news-room/fact-sheets/detail/epilepsy.

World Health Organizsation (2003). Adherence to long-term therapies : evidence for action / [edited by Eduardo Sabaté] apps.who.int/iris/bitstream/handle/10665/42682/9241545992.pdf?sequence=1&isAllowed=y (aufgerufen 23.06.2022)

von Wrede, R., and Surges, R. (2021). Transcutaneous vagus nerve stimulation in the treatment of drug-resistant epilepsy. Auton. Neurosci. *235*, 102840.

von Wrede, R., Rings, T., Schach, S., Helmstaedter, C., and Lehnertz, K. (2021). Transcutaneous auricular vagus nerve stimulation induces stabilizing modifications in large-scale functional brain networks: towards understanding the effects of taVNS in subjects with epilepsy. Sci. Rep. *11*, 7906.

von Wrede, R., Bröhl, T., Rings, T., Pukropski, J., Helmstaedter, C., and Lehnertz, K. (2022a). Modifications of Functional Human Brain Networks by Transcutaneous Auricular Vagus Nerve Stimulation: Impact of Time of Day. Brain Sci. 2022 Apr 26;12(5):546

von Wrede, R., Rings, T., Bröhl, T., Pukropski, J., Schach, S., Helmstaedter, C., and Lehnertz, K. (2022b). Transcutaneous Auricular Vagus Nerve Stimulation Differently Modifies Functional Brain Networks of Subjects With Different Epilepsy Types Front Human Neurosc. 16, 867563.

Wu, X., Zhang, Y., Luo, W.-T., Mai, R.-R., Hou, X.-Y., Xia, Z.-Q., Xu, B.-Y., and Liu, B. (2021). Brain Functional Mechanisms Determining the Efficacy of Transcutaneous Auricular Vagus Nerve Stimulation in Primary Insomnia. Front. Neurosci. *15*, 609640.

Yook, S.H., Jeong, H., Barabási, A.L., and Tu, Y. (2001). Weighted evolving networks. Phys. Rev. Lett. *86*, 5835–5838.

Zhang, S., He, J.-K., Meng, H., Zhao, B., Zhao, Y.-N., Wang, Y., Li, S.-Y., Wang, L., Wu, M.-Z.,

Chen, Y., et al. (2021). Effects of transcutaneous auricular vagus nerve stimulation on brain functional connectivity of medial prefrontal cortex in patients with primary insomnia. Anat. Rec. (Hoboken). *304*, 2426–2435.

Zhang, S., He, J.-K., Zhao, Y.-N., Wang, Y., Zhao, B., Chen, Y., Fang, J.-L., and Rong, P.-J. (2022). [Modulation of transcutaneous auricular vagus nerve stimulation on default mode network in patients with primary insomnia]. Zhongguo Zhen Jiu *42*, 363–368.

Zhang, Z., Liao, W., Chen, H., Mantini, D., Ding, J.-R., Xu, Q., Wang, Z., Yuan, C., Chen, G., Jiao, Q., et al. (2011). Altered functional-structural coupling of large-scale brain networks in idiopathic generalized epilepsy. Brain *134*, 2912–2928.

8. Danksagung

9. Wissenschaftlicher Lebenslauf

Persönliche Daten

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10. Erklärung

Hiermit bestätige ich, dass ich die Richtlinien zur guten wissenschaftlichen Praxis der Universität Bonn, laut Habil-Ordnung, zur Kenntnis genommen habe und ich versichere, dass ich sie beim Verfassen der Habilitationsschrift beachtet habe. Insbesondere versichere ich, dass ich alle in der Habilitationsschrift benutzten Quellen und Hilfsmittel angegeben habe.

Bonn, den 23.06.2022

Dr. med. Randi von Wrede