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Research Paper

Epileptic Seizures are Reduced by Autonomic Biofeedback Therapy Through Enhancement of Fronto-limbic Connectivity: A Controlled Trial and Neuroimaging Study

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ABSTRACT

Background: Thirty-percent of patients with epilepsy are drug-resistant, and might benefit from effective noninvasive therapeutic interventions. Evidence is accumulating on the efficacy of autonomic biofeedback therapy using galvanic skin response (GSR; an index of sympathetic arousal) in treating epileptic seizures. This study aimed to extend previous controlled clinical trials of autonomic biofeedback therapy with a larger homogeneous sample of patients with temporal lobe epilepsy. In addition, we used neuroimaging to characterize neural mechanisms of change in seizure frequency following the therapy.

Methods: Forty patients with drug-resistant temporal lobe epilepsy (TLE) (age: 18 to 70 years old), on stable doses of anti-epileptic medication, were recruited into a controlled and parallel-group trial from three screening centers in the UK. Patients were allocated to either an active intervention group, who received therapy with GSR biofeedback, or a control group, who received treatment as usual. Allocation to the group was informed, in part, by whether patients could travel to attend repeated therapy sessions (non-randomized). Measurement of outcomes was undertaken by an assessor blinded to the patients' group membership. Resting-state functional and structural MRI data were acquired before and after one month of therapy in the therapy group, and before and after a one-month interval in the control group. The percentage change of seizure frequency was the primary outcome measure. The analysis employed an intention-to-treat principle. The secondary outcome was the change in default mode network (DMN) and limbic network functional connectivity tested for effects of therapy. The trial was registered with the National Institute for Health Research (NIHR) portfolio (ID 15967).

Findings: Data were acquired between May 2014 and October 2016. Twenty participants were assigned to each group. Two patients in the control group dropped out before the second scan, leaving 18 control participants. There was a significant difference in reduction of seizure frequency between the therapy and control groups ($p < 0.001$: Mann Whitney *U* Test). The seizure frequency in the therapy group was significantly reduced ($p < 0.001$: Wilcoxon Signed Rank Test) following GSR biofeedback, with a mean seizure reduction of 43% ($SD = \pm 32.12$, median = -37.26 , 95% CI -58.02% to -27.96%). No significant seizure reduction was observed in the control group, with a mean increase in seizure frequency of 31% ($SD = \pm 88.27$, median = 0 , 95% CI -12.83% to 74.96%). The effect size of group comparison was 1.14 (95% CI 0.44 to 1.82). 45% of patients in the therapy group showed a seizure reduction of $>50\%$. Neuroimaging analysis revealed that post-therapy seizure reduction was linearly correlated with enhanced functional connectivity between right amygdala and both the orbitofrontal cortex (OFC) and frontal pole (FP).

Interpretation: Our clinical study provides evidence for autonomic biofeedback therapy as an effective and potent behavioral intervention for patients with drug-resistant epilepsy. This approach is non-pharmacological, non-invasive and seemingly side-effect free.

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Abbreviations: GSR, galvanic skin response; DMN, default mode network; SUDEP, sudden unexpected death in epilepsy; MPFC, medial prefrontal cortex; OFC, orbitofrontal cortex; FP, frontal pole; TLE, temporal lobe epilepsy.

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1. Introduction

The autonomic nervous system is crucial for maintenance of homeostatic control of the internal state of the body and interacts closely with central nervous system through interoceptive feedback (Jänig, 2008). In epilepsy, the autonomic nervous system is usually considered in relation to differential diagnoses (e.g. syncope) or in the context of Sudden Unexpected Death (SUDEP). Its contribution, and its central regulation, to the generation and suppression of epileptic seizures are largely overlooked.

Penfield and Jasper (1954) showed that normal and abnormal supratentorial brain activity, including focal epileptic activity, can differentially influence autonomic function. Animal models show that single spike activity can alter the discharge of cardiac autonomic neurons (Schraeder and Celesia, 1977) and that ictal tachycardia significantly modulates both sympathetic and parasympathetic activities (Sevcencu et al., 2016). Patients with epilepsy can manifest autonomic abnormalities even between seizure events, including abnormal measures of sympathetic (galvanic skin response [GSR]) and parasympathetic (heart rate variability [HRV]) function (Drake et al., 1998; Evrengül et al., 2005). Acute autonomic dysfunction originating in epileptic activity is implicated as the likely pre-terminal mechanism for SUDEP (Aiba and Noebels, 2015).

The converse impact of autonomic function on epilepsy should not be disregarded. There is a convergent evidence suggesting that disturbances in autonomic activity influence the generation of seizures. Surveys involving large patient samples identify emotional stress, tiredness, and sleep deprivation, each associated with transient autonomic dysregulation, as the top three triggers of seizures (Pinikahana and Dono, 2009; Nakken et al., 2005). Psychological states can dysregulate autonomic control: for example perseverative cognition (worry and rumination) is associated with sympathetic–parasympathetic imbalance reflected in abnormal HRV (Ottaviani et al., 2015). Visceral afferent feedback of (dysregulated) physiological state influences the activation of ascending neuromodulator pathways (Critchley and Harrison, 2013). Direct stimulation of the vagus nerve can abort ongoing seizure activity, perhaps through re-regulated (regimented) autonomic afferent signaling. Despite both direct and circumstantial evidence indicating that autonomic nervous system function is closely linked to the generation of epileptic seizures, the pathophysiological mechanisms underpinning this association are poorly understood.

We reported (Nagai et al., 2004a; Nagai et al., 2009) investigation into the relationship between peripheral sympathetic activity (measured using GSR) and central cortical excitation (an experimentally-induced slow cortical potential, measured using EEG). GSR reflects activity of sympathetic nervous innervation to skin sweat glands and is a sensitive index of psychophysiological arousal. We demonstrated an inverse relationship between peripheral and central arousal: heightened peripheral sympathetic arousal (increased skin conductance) provoked a decrease in cortical excitation (reduction in slow cortical potential), observed both in healthy participants (Nagai et al., 2004a) and people with epilepsy (Nagai et al., 2009). Crucially, GSR can be modulated non-invasively using biofeedback. GSR has the advantage of being an accessible (hence easy-to-measure) autonomic parameter, which is exclusively coupled to the sympathetic nervous system. In GSR biofeedback, patients are trained to control their physiological responses through visual and auditory feedback (Fig. 1). The first fully randomized controlled trial of structured GSR biofeedback training in patients with drug-resistant epilepsy elicited a reduction in seizure frequency of 50% or more in 60% of patients allocated to the active therapy group (Nagai et al., 2004b). In parallel to our current work, the efficacy of GSR biofeedback is supported by findings of recent independent studies, drawing from our group's earlier published works (Micoulaud-Franchi et al., 2014; Kotwas et al., 2017). The clinical benefit of GSR biofeedback therapy can persist over time: a subset of patients who kept over four years of seizure records demonstrated no apparent re-increase after >50% seizure reduction (Nagai and Trimble, 2014).

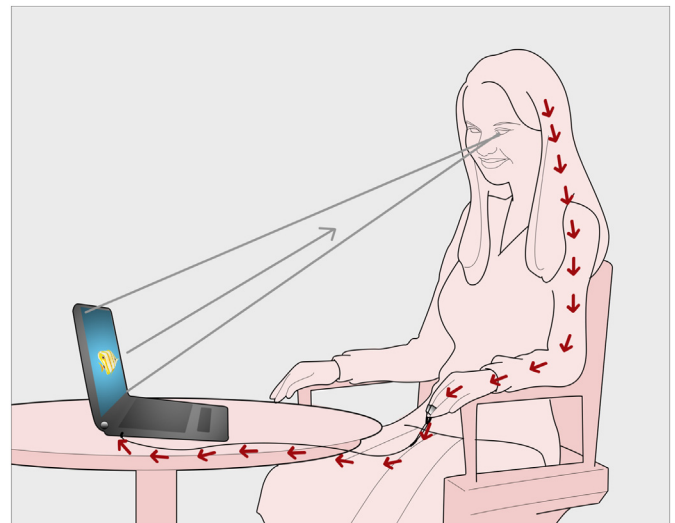


Fig. 1. Biofeedback setting. An animation moves forward with increase in skin conductance and backward with decrease in skin conductance.

Functional neuroimaging, undertaken to identify neural substrates engaged during performance of GSR biofeedback, highlighted an inverse coupling between activity within ventral medial and orbital frontal cortical regions (ventral MPFC/OFC) and GSR measures of peripheral sympathetic tone (Nagai et al., 2004c).

These clinical and neuroimaging observations suggest a potential mechanism through which longer term therapeutic training using autonomic biofeedback might impact on frontolimbic neurocircuitry, supporting both the tonic regulation of internal bodily arousal and the triggering of epileptic seizures within connected mesial temporal centers. In patients with epilepsy, functional neural connectivity is of key relevance to understanding seizure propagation (Lemieux et al., 2011). Correspondingly, 'constitutional' abnormalities in network connectivity, notably impaired coupling within the default mode network, are reported in patients with epilepsy (Kay et al., 2013). Animal models of temporal lobe epilepsy engender disruption of functional brain networks, including dysconnectivity within the default mode network (DMN) and limbic networks (Gill et al., 2017). The DMN, one of the most reproducible networks of functional connectivity, encompasses precuneus, medial prefrontal, inferior parietal and medial temporal cortices (Raichle et al., 2001); activity across these regions is typically greater at rest and during self-referential processing (thinking about oneself), and decreases when an individual engages in an external task. DMN dysfunction is associated with loss of consciousness (Danielson et al., 2011). Given the previous observation of frontal control over sympathetic activity and limbic involvement of pathological and emotional impact on temporal lobe epilepsy, we investigated changes in frontolimbic functional network connectivity which may underlie the therapeutic effects of autonomic biofeedback training to reduce seizures in patients with drug-resistant epilepsy. The current study aimed to address the efficacy of autonomic biofeedback in a larger clinical sample of 40 patients with drug-resistant temporal lobe epilepsy and to understand further, using neuroimaging, neural mechanisms supporting seizure reduction. We tested the prediction that intrinsic resting state network connectivity, particularly between DMN and limbic networks, predicted improvement in seizure frequency in this patient group.

2. Materials and Methods

2.1. Study Design and Participants

We conducted a controlled, single-blinded, single-center trial, recruiting patients from three screening sites in the UK and

advertisement through an epilepsy charity. Forty patients with TLE (either cryptogenic or symptomatic), aged between 18 and 70 years participated in the study. Each patient had a diagnosis and EEG evidence of TLE, a clinical history lasting more than two years, and could keep a seizure diary. Medication was required to be unchanged for more than two months before patient participated. Patients with a history of severe psychiatric illness, drug abuse, major head injury or significant learning disability were excluded. The study was approved by the clinical ethics committee at the Health Research Authority and was registered in the UK clinical research network. All the patients gave written informed consent in advance of their participation in the study. The study followed the Declaration of Helsinki, following ethics regulations and standards of good clinical practice. Data management followed strict policies of the University of Sussex (Supplementary material, p4). The trial was registered with the National Institute for Health Research (NIHR) portfolio (ID 15967).

2.2. Patient Allocation and Masking

Patients were allocated to therapy (active intervention) or to control (treatment as usual) groups initially using a randomization table. Consultant neurologists enrolled the patients, and group assignment was conducted by the PI (the therapist). Allocation was then subsequently informed by the logistics of whether the patient was willing to commit to attending the therapy sessions, which was heavily influenced by geographical location: Patients were required to travel to the south coast of England three times a week consecutively for four weeks. This created a deviation from the randomization by 15%. The ethics committee was notified. Patients in the control group remained on the same medication (or no medication) at a constant dose throughout the study and were not involved in any other therapeutic intervention. Although full double-blinding of behavioral studies is difficult, the assessment of behavioral outcomes (seizure frequency changes) was undertaken by an independent assessor blinded to group membership. To mitigate motivation bias, patients in the control group were informed that they would be offered online version of therapy following the study and all patients accepted an offer.

2.3. Procedure

Patients in the therapy and control groups recorded seizure frequency over three months before the initial neuroimaging session. In the therapy group this was followed by the first biofeedback training session. During biofeedback training, GSR was recorded continuously using dry nickel electrodes attached to the palmar surface of the non-dominant hand of each patient, connected to Inner Tuner biofeedback equipment and a customized version of the software (Ultrasys plc, UK). GSR biofeedback training was performed for 30 min, three times a week, for four weeks. Patients were asked to drive forward a digital animation on a computer display to the best of their ability, by making themselves more alert. Positive visual feedback (animation goes forward) was given as the measured (GSR) sympathetic tone was increased by the patient. Patients were encouraged to practice the acquired skill (to increase sympathetic activity) between therapy sessions by recollection of the therapy and associated sensations. Patients continued to record their seizure frequency for three further months: i.e. in the therapy group, three months before the start of the therapy, during the month of active therapy, and for three months after the last therapy session (and, in the treatment as usual group, over equivalent periods; Supplementary Fig. S1). At the beginning and end of the study, each patient completed standardized self-report questionnaires probing affective symptoms (Spielberger State and Trait Anxiety, Beck Depression Inventory, Perceived Stress Scale).

Neuroimaging experiments were performed using a 1.5T system (Magnetom Avanto, Siemens, Erlangen, Germany), equipped with a maximum gradient strength of 44 mTm^{-1} , and a 32-channel head

coil. All patients underwent two identical sessions: at baseline and at the end of the biofeedback training for the therapy group, and after a month interval for the control group. The acquisitions of each session are described in Supplementary material. Resting-state fMRI data were pre-processed using SPM8 (see Supplementary material p3, 4).

2.4. Outcomes

The primary outcome of the study was the percentage change in seizure frequency after a month of therapy course, compared to the baseline seizure frequency. The baseline monthly seizure frequency was calculated as an average of three months' seizure frequency prior to the therapy. The post-therapy seizure frequency was the average of three months' seizure frequency following the last therapy and neuroimaging session. In the control group the equivalent measure was the average of the last three months of seven months of seizure recording, following the second neuroimaging session (see Supplementary Fig. S1). These data were acquired from patients by an independent assessor who was blinded to group membership. The sample size was determined by our previous study. We had observed a mean decrease in seizure frequency of 49.3% ($SD \pm 41.6\%$) in the biofeedback active group compared to an increase of 24.6% ($SD \pm 45.6\%$) in the sham control group. Here we expected no change in seizure frequency in the treatment as usual group. With power and threshold of significance set at 90% and 5% respectively, 15 patients were necessary in each group (study size = 30) to detect an effect size of 1.2 (difference between group = 50, $SD = 42$). The number of patients recruited in the present study was therefore large enough to detect this anticipated treatment effect.

A secondary outcome was the change in functional connectivity putatively induced by therapy focusing on the two primary networks of interest, the DMN and the limbic network. In the neuroimaging context (with functional magnetic resonance imaging; fMRI), functional connectivity is defined as the association of neuronal activity patterns between anatomically separated brain regions. One method to quantify functional connectivity from fMRI datasets is seed-based analysis, in which connectivity between regions is estimated from the correlation between fluctuations of the hemodynamic signal (reflecting neural activation) within a given region (seed) and fluctuations occurring in the rest of the brain. Typically, connectivity analyses are performed on fMRI data acquired at rest. We undertook this seed-based analysis on task-free, resting state datasets. We selected an a priori region-of-interest (ROI) for target networks (medial prefrontal cortex for the DMN and amygdala for the limbic network, separately examining left and right amygdala). We quantified functional connectivity between each ROI and the rest of the brain for the first and second session (i.e. before and after the intervention for the therapy group; details in Supplementary material).

2.5. Statistical Analysis

For the behavioral data, first we checked the distribution of the percentage change in seizure frequency and confirmed its non-normality. A Wilcoxon Signed Rank Test was used to analyze the patients' mean seizure frequency before and after therapy in the active therapy and control groups. A Mann-Witney *U* test was used to explore differences in percentage seizure frequency change between the therapy and control groups.

For the neuroimaging data, all statistical analyses were performed using SPM8. For each network (DMN, left amygdala network, and right amygdala network), a flexible factorial model was used to model the effects of the group (therapy vs. control) and of time (before vs. after intervention), and their interaction (see Supplementary material, Seed based analysis, Figs. S3 and S4). Separate models were then used to investigate correlations between changes in functional connectivity and changes in seizure frequency and in psychological variables (STAI,

STAT, BDI, Stress score). Results were corrected for multiple comparisons using the family-wise error (FWE) method at cluster level, forming clusters at uncorrected level using $p < 0.001$. Thus, significance is individually first assessed independently at each voxel, using a threshold of $p < 0.001$, followed by correction for multiple comparisons, using an FWE corrected threshold significance of $p < 0.05$.

2.6. Role of Funding Source

The funding source was not involved in data collection and analysis, interpretation of the results, nor decision to submit the manuscript for publication.

3. Results

This clinical trial was conducted between May 2014 and October 2016. 168 patients were invited as eligible candidates from the National Hospital for Neurology and Neurosurgery (Tertiary care center), London, the Brighton and Sussex University Hospital, Brighton (secondary care center), and the St George's University Hospital (secondary care center), London (Fig. 2): twenty-four patients agreed to take part in the study. In addition, sixteen patients were recruited from advertisement. Patients were allocated to either therapy + two scanning, or control + two scanning groups. There was no significant difference between the groups for age (years \pm S.D.; control: 43.25 ± 11.90 ; therapy, 44.80 ± 15.55 ; $p = 0.73$, independent t -test), age of onset (years \pm S.D.; control: 17.60 ± 15.13 ; therapy, 24.10 ± 15.14 ; $p = 0.18$), duration of epilepsy (years \pm S.D.; control: 26.40 ± 17.63 ; therapy, 20.70

± 15.70 ; $p = 0.29$) or baseline seizure frequency (seizures/month; median 10.84 for control, 5.36 for therapy; $p = 0.09$). However, we report that the study deviated from the planned full randomization. Patients who were not geographically close to the institution were mostly unable to travel to the institution three times a week for four weeks and consequently could not commit to participating in the therapy group. Thus, full randomization was not possible. Six patients in control group were influenced by geographical restriction and nine patients in biofeedback group had to travel far away up to 3 h. The demographic characteristics of the patients at baseline are described in Table 1. All twenty patients in the therapy group completed the training course, indicating good compliance; however one patient dropped out in the follow-up period (due to reluctance to maintain a detailed seizure record). All 20 control group patients completed the first scanning session; two patients dropped out due to medication changes after the first scanning session and one further patient chose to leave during the follow-up period (due to medication change). Data were analyzed on an intention-to-treat basis.

All patients in the therapy group completed autonomic biofeedback successfully, achieving significant increase in skin conductance (Supplementary Fig. S2). There was a significant between-group difference in the percentage seizure reduction ($p < 0.001$: Mann-Witney U Test). The therapy group had a significant reduction in seizure frequency after one month of intervention ($p < 0.001$: Wilcoxon Signed Rank Test) with a mean seizure reduction of 43.0% (SD = ± 32.12 , median = 37.26, 95% CI -58.02% to -27.96%, IQR -74.67% to -13.75%). In 9/20 patients, there was >50% seizure reduction, representing a 45% response rate. One patient became seizure-free. In contrast, overall

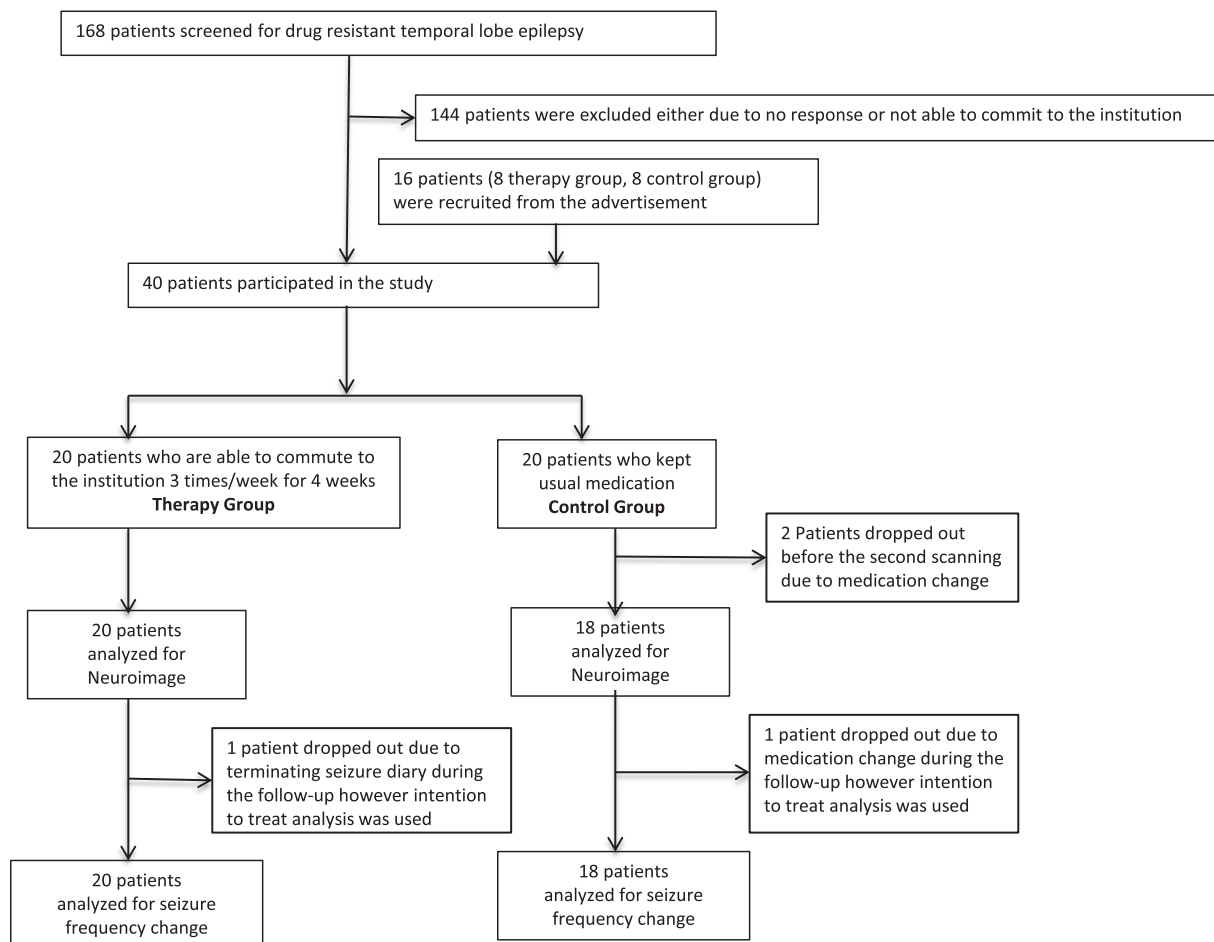


Fig. 2. CONSRT diagram.

Table 1
Demographics and baseline clinical characteristics.

	Therapy group (n = 20)	TAU group (n = 20)
Sex		
Male	10/20 (50%)	6/20 (30%)
Female	10/20 (50%)	14/20 (70%)
Age	44.80 (\pm 15.55) years old	43.25 (\pm 11.90) years old
Age of onset	24.10 (\pm 15.14) years old	17.60 (\pm 15.13) years old
Duration of epilepsy	20.70 (\pm 15.70) years	26.40 (\pm 17.63) years
Medication	Carbamazepine 5/20 (25%) Clobazam 6/20 (30%) Levetiracetam 7/20 (35%) Lamotrigine 5/20 (25%) Lacosamide 1/20 (5%) Oxcarbazepine 1/20 (5%) Perampnel 5/20 (25%) Phenytoine 2/20 (10%) Pregavarine 6/20 (30%) Sodium valproate 6/20 (30%)	Carbamazepine 5/20 (25%) Clobazam 3/20 (15%) Clonazepam 1/20 (5%) Levetiracetam 5/20 (40%) Lamotrigine 8/20 (40%) Oxcarbazepine 2/20 (10%) Perampnel 2/20 (10%) Prevabalin 1/20 (5%) Topiramate 1/20 (5%) Sodium valproate 2/20 (10%) Zonisamide 2/20 (10%)
Affected side		
Right	10/20 (50%)	6/20 (30%)
Left	8/20 (40%)	9/20 (45%)
Not identifiable	2/20 (10%)	4/20 (20%)
Bilateral	0/20 (00%)	1/20 (5%)
Seizure frequency per month	31.58 (\pm 10.67); 10.84/month	21.12 (\pm 12.23); 5.86/month
Mean (\pm SE): Median BEFORE		
Seizure frequency per month	20.28 (\pm 9.12); 6.67/month	62.33 (\pm 51.16); 7.0/month
Mean (\pm SE): Median AFTER		
Marital state	Single 12/20 (60%) Married 8/20 (40%)	Single 12/20 (60%) Married 8/20 (40%)
Education	Primary education 3/20 (15%) High school education 5/20 (25%) College education 3/20 (15%) Bachelor degree 6/20 (30%) Post doctoral education 1/20 (5%) Not disclosed 2/20 (10%)	Primary education 3/20 (15%) High school education 5/20 (25%) College education 1/20 (5%) Bachelor degree 7/20 (35%) Post doctoral education 3/20 (15%) Not disclosed 1/20 (5%)
Employment	Not working 12/20 (60%) Part time 5/20 (25%) Full time 3/20 (15%)	Not working 11/20 (55%) Part time 6/20 (30%) Full time 3/20 (15%)

seizure frequency increased in the control group by 31.1% (SD = \pm 88.27, median = 0, 95% CI – 12.83% to 74.96%, IQR – 17.42% to 41.56%) (Fig. 3). The observed effect size [(Therapy group mean – Control group mean)/sample SD] was 1.14 (95% CI 0.44 to 1.82). There was no adverse events or side effects reported through this behavioral therapy.

We observed increases in functional connectivity compared to baseline between MPFC (as a seed) and bilateral middle/superior frontal gyrus, anterior cingulate cortex, right angular gyrus and left caudate nucleus in the therapy group (Fig. 4A). There were no apparent increases in MPFC functional connectivity changes in the control group. We observed also a significant decrease in functional connectivity of MPFC to a boundary region of insula and frontal operculum in the therapy group. There were no apparent decreases in MPFC functional connectivity in the control group (Fig. 4B).

Secondly, using seizure frequency changes as a regressor within neuroimaging analyses, we tested how and where changes of MPFC functional connectivity related to therapy-evoked changes in seizure frequency. We observed increased functional connectivity between MPFC and amygdala/bilateral temporal pole complex that correlated significantly with seizure reduction (Fig. 4C).

This result links the reduction in seizure frequency in patients with temporal lobe epilepsy to the enhancement of functional connectivity between MPFC and amygdala complex. For the limbic networks, seeds were placed in the left and right amygdala separately (Supplementary Fig. S4). Across all participants (main effect), the amygdala seeds showed strong functional connectivity with each other, with striatal regions, and with the anterior cingulate ($p < 0.01$ FWE). The left and right network connectivity was symmetrical at visual inspection (Supplementary Fig. S5).

We observed a significant therapy-related (group \times session interaction, $p < 0.05$ FWE) increase in left amygdala functional connectivity with left insula, primary motor cortex (M1) bilaterally, precuneus, and right angular gyrus (Supplementary Fig. S6, A), with larger increases in connectivity in the therapy group than in the controls. We tested for similar therapy-related changes in the connectivity of the right amygdala (Supplementary Fig. S6, B) There were significant increases in the functional connectivity of right amygdala with precuneus, left primary motor cortex (M1), and lateral occipital cortex bilaterally in the therapy group. The control group showed no change.

When looking at the association between changes in functional connectivity and the magnitude of reductions in seizure frequency, significant results were only found for the right amygdala network. In particular, patients who showed the greatest seizure frequency reduction also showed the greatest increase in right amygdala functional connectivity with the orbitofrontal cortex (OFC: 18, 28, –20 MNI coordinate) and the frontal pole (FP: –4, 56, 10) (Fig. 5A). This effect was only present in the therapy group (Fig. 6B lower panel). Interestingly, the OFC region expressing this significant correlation was encompassed within the region described in a previous study (Nagai et al., 2004c) in which a negative linear correlation was found between neural activity and skin conductance level (Fig. 5B upper panel). This result links mechanistically the modulation of sympathetic tone using GSR biofeedback training to sustained reduction of seizure frequency in patients with drug-resistant TLE. Changes in right amygdala functional connectivity to OFC and FP predicted reduction in seizure frequency changes, but not changes in anxiety score or other psychological variables recorded (Fig. 6A–C). Seizure frequency was uncorrelated with change with these reported psychological symptoms.

4. Discussion

Autonomic biofeedback as a behavioral therapy for epilepsy is not yet widely recognized. The present clinical trial demonstrates a significant beneficial effect of this therapy in reducing the seizure frequency in patients with drug-resistant TLE. Our neuroimaging analysis identified response-related changes in fronto-limbic connectivity, providing insight into likely neural mechanisms underlying the efficacy of this therapy.

These results are encouraging. However, the study also had some limitations. Firstly, one might anticipate that seeing a therapist three times a week for four weeks may evoke a placebo effect that in itself reduces seizures, and engender, in parallel, improvement-related changes in functional neural connectivity. This argument could have been addressed by comparison with an active sham control group, rather than a treatment as usual group. Nevertheless, our previous experience of RCT using an active sham control for the same GSR biofeedback therapy protocol demonstrated no reduction in seizure frequency within the sham control group (in fact an increase) (Nagai et al., 2004b). This strongly suggests that non-specific interaction with the therapist could not alone account for the observed seizure reduction in the current study. Our neuroimaging findings also consistent with therapy-evoked changes in epilepsy-relevant neural circuitry associated with autonomic modulation, rather than a generic placebo response. Notably, the observed changes in functional connectivity between OFC and medial temporal lobe (amygdala), build on previously recognized tight modulation of OFC activity by GSR biofeedback (Nagai et al., 2004c).

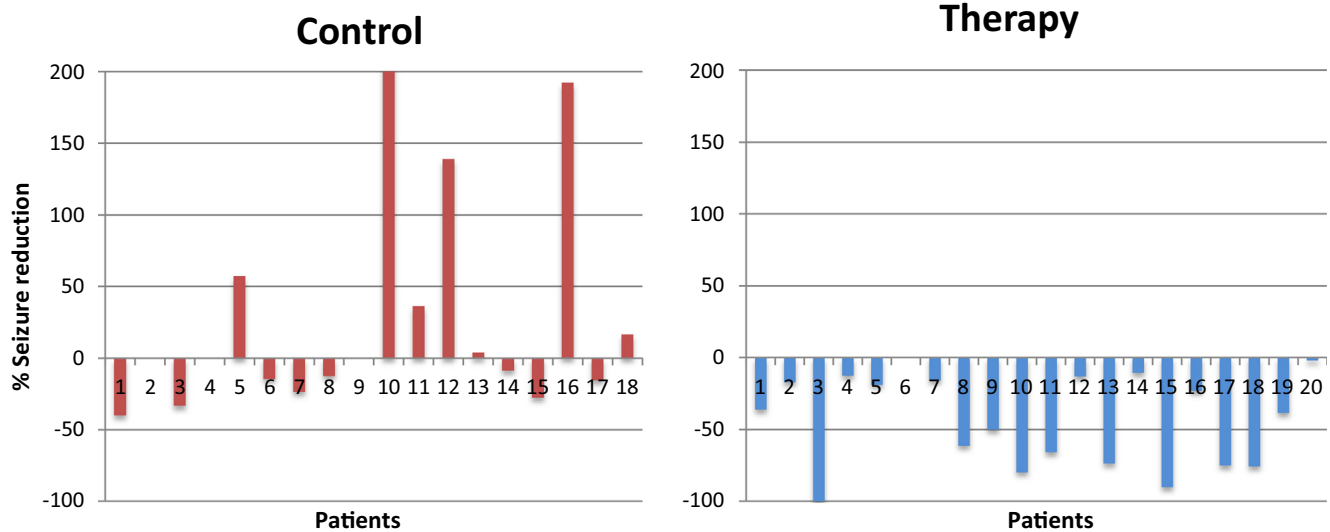


Fig. 3. Percentage seizure frequency changes. % Seizure frequency changes after GSR biofeedback therapy in control and biofeedback groups. Percentage seizure frequency change = $[(\text{Post averaged seizure frequency} - \text{baseline averaged seizure frequency}) / \text{baseline averaged seizure frequency}] \times 100$. The averaged seizure frequency change of Control group = 31.10% (SD = \pm 88.27, median = 0), Therapy group = 43.0% (SD = \pm 32.12, median = 37.26).

The patients who displayed the strongest enhancement of OFC-amygdala connectivity demonstrated the most seizure reduction. Thus even without a sham intervention, the involvement of autonomic control centers, and the correlation with seizure reduction, suggest that the brain changes observed in the therapy group are more attributable to GSR biofeedback training than to non-specific therapeutic ‘placebo’ effects. Although the study faced these limitations, our report sheds light on a relatively neglected area of behavioral therapy and the role of autonomic function in epilepsy.

A second important limitation of this study is the pragmatic deviation from complete randomization to the therapy and control groups. As described, ‘geographical bias’ occurred on account of the heavy commitment required to attend behavioral therapy sessions. Nevertheless, the groups were well matched on demographic and clinical measures. It is therefore very unlikely that the reported therapy effects on seizure frequency or brain connectivity reflected this allocation bias.

An average seizure reduction of 43% was observed across the therapy group of patients with drug-resistant TLE. After a month of autonomic biofeedback therapy, 45% of these patients reported seizure reduction of >50%. These results are similar to those of the previous single-blinded sham-controlled RCT (Nagai et al., 2004b) and independent studies with open label (Micoulaud-Franchi et al., 2014) and case control study (Kotwas et al., 2017).

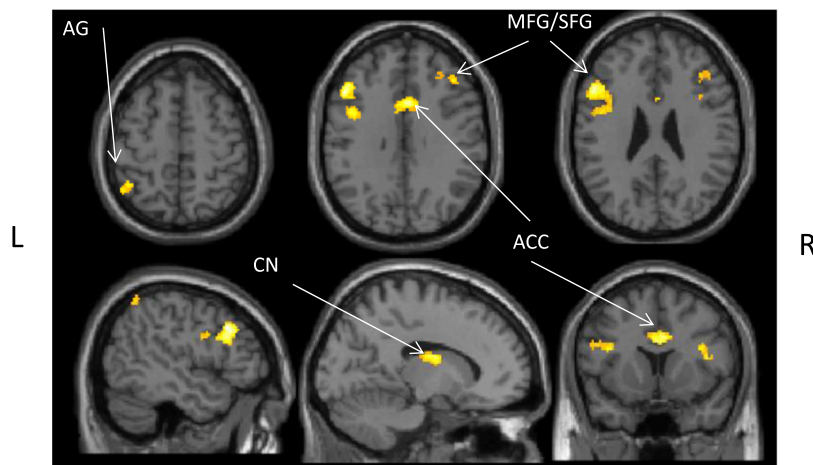
We observed a trend towards an overall increase in seizure frequency in the control group. Three patients reported >100% increase during the follow up period. However, the median seizure frequency change in the control group was 0% and there were no patients who showed >50% seizure reduction. Thus there was no evidence to suggest an impact of study participation on seizure frequency in the control group. The mean seizure frequency increased in this control group (+31%) by a similar magnitude to increases observed following sham biofeedback (+25%) (Nagai et al., 2004b). Overall, in comparison with other treatments, the observed effects of GSR biofeedback in reducing seizure frequency are favorable (45%–66% response rates across existing trials). This compares to a 21–47% response rate for new anti-epileptic drugs (Cramer et al., 2001), a 30–70% response rate reported for Vagus Nerve Stimulation (Englot et al., 2011) and a 30–55% response rate for the ketogenic diet (Neal et al., 2008). However, the greatest advantage of autonomic biofeedback therapy is that the approach is primarily behavioral and the effects can persist, once necessary skills are acquired after a month of therapy training, without a device (Nagai and

Trimble, 2014). Patients continued to apply the skills they had learned to increase sympathetic activity in their daily lives as a counter-measure to seizures. No side effects are recorded for this autonomic biofeedback therapy over the last 20 years, suggesting that this non-invasive behavioral intervention to enable patients with drug-resistant epilepsy to control their seizures is also very well tolerated. The present study reinforces motivation for a much larger multi-central clinical trial to further consolidate the efficacy of this new intervention.

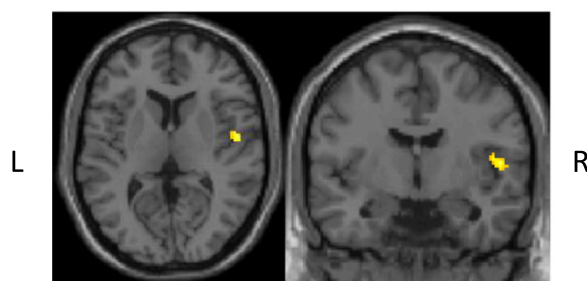
In this study, we provide neuroscientific insight of how this therapy works. Establishing the biological validity of behavioral therapies through a comprehensive neuroscientific account is generally challenging, even for well-established methodologies proven through clinical trials, such as cognitive behavioral therapy. The current study aimed to describe the core neural mechanisms that might account for the efficacy of GSR biofeedback therapy. We demonstrated changes in fore-brain neural functional connectivity following one month of active GSR biofeedback therapy. Theory-driven studies have led to converging empirical evidence linking the propensity for epileptic seizures to abnormalities in resting-state functional connectivity (Lemieux et al., 2011; Tracy and Doucet, 2015). We tested for functional connectivity changes with emphasis on dynamic coupling with MPFC, predicated upon established neuroimaging evidence of close links between neural activity in this brain region and tonic level of sympathetic arousal, indexed by GSR (Nagai et al., 2004c). Observed changes in network connectivity were specific to the therapy group, with no significant changes observed in the control group. A month of training using GSR biofeedback engendered enhancement of functional connectivity between ventral MPFC/OFC and brain regions implicated in executive control and attentional regulation, notably medial/superior frontal gyrus, angular gyrus, and anterior cingulate cortex (also implicated in autonomic control) (Critchley and Harrison, 2013).

A striking finding was the observed linear correlation between seizure frequency reduction and enhancement of functional connectivity between right amygdala and prefrontal cortex (orbitofrontal cortex, OFC and frontal pole). This part of OFC was encompassed within the area identified as a brain region where the activity inversely coupled to GSR level (Nagai et al., 2004c). The finding is consistent with the notion that autonomic biofeedback training with GSR, ultimately enabling the volitional modulation of sympathetic tone, also consolidate a neural network between OFC and amygdala. Although we observed a change in functional connectivity, our finding has relevance to the structural

A) Increased connectivity to VMPFC



B) Decreased connectivity to VMPFC



C) Increased neural connectivity associated with seizure reduction

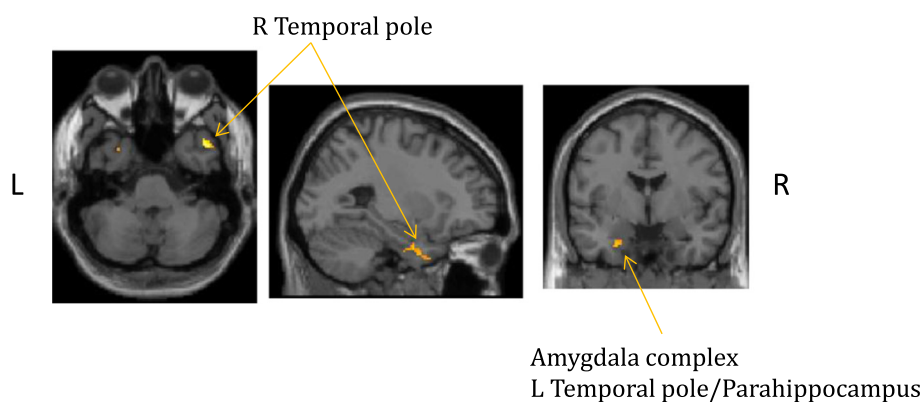
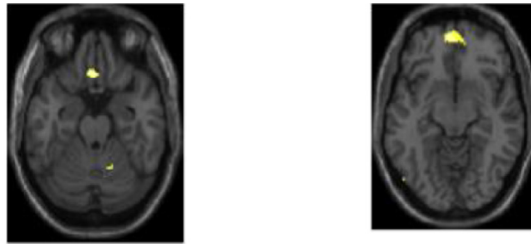


Fig. 4. Changes in neural connectivity to VMPFC after a month of therapy. A) Increased neural connectivity to ventromedial prefrontal Cortex (VMPFC) was found in left angular gyrus, bilateral medial and superior frontal gyrus, anterior cingulate, caudate nucleus in the therapy group. B) Decreased connectivity to VMPFC was found in right insular however significance was marginal. There was no significant neural connectivity changes found in control group. C) The seizure reduction was correlated with increased functional neural connectivity between VMPFC and amygdala complex, left temporal pole/parahippocampus. All results are significant a $p < 0.05$, after FWE correction at cluster level.

connections underpinning this network to epilepsy. The fiber tract coupling OFC has also an anatomical connection to amygdala, which is termed as uncinate fasciculus (Von Der Heide et al., 2013). The integrity of this white matter tract has established relevance to seizure propagation in TLE (Diehl et al., 2008; Lin et al., 2008; Von Der Heide et al., 2013). We speculate that increased functional connectivity between OFC and amygdala corresponding to efficient information flow through the uncinate fasciculus, could have been achieved by GSR biofeedback

training through the conjoint roles of amygdala and OFC in autonomic regulation. Anatomically, the uncinate fasciculus splits into two branches within frontal lobe, where the larger branch terminates within OFC and the smaller branch terminates within frontal pole (de Schotten et al., 2012). Correspondingly, we also observed that a strengthening connectivity between amygdala and the frontal pole was associated with seizure reduction following the autonomic biofeedback therapy. Resection of a specific white matter connection (uncinate fasciculus)

A) Two distinctive functional connectivity changes to Right Amygdala



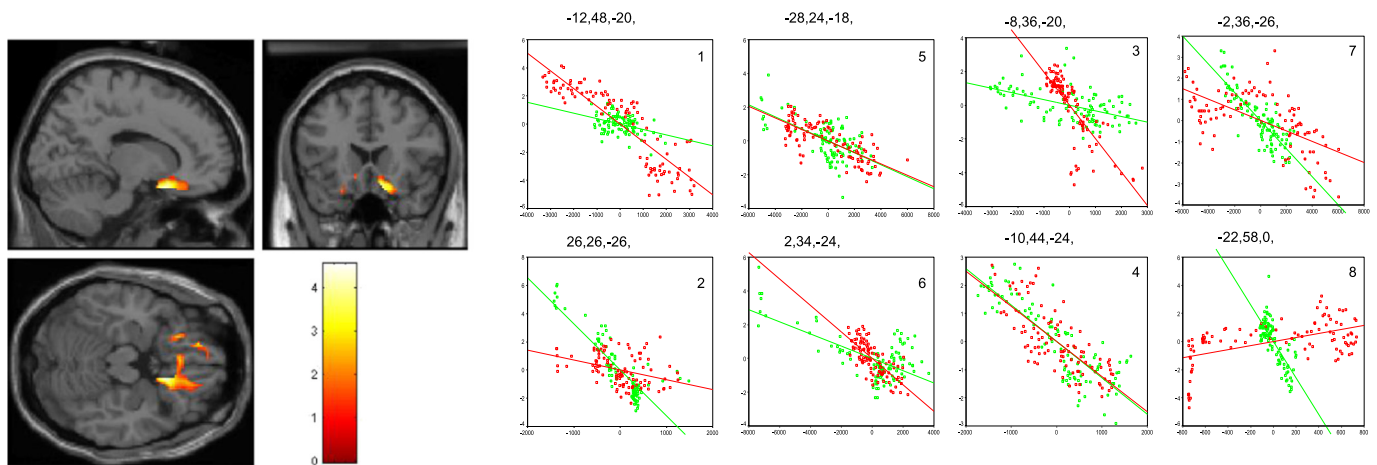
Orbitofrontal Cortex (OFC)

Frontal Pole (FP)

B) Linking OFC activity, skin conductance change, OFC to Right Amygdala connectivity and seizure reduction

OFC activity during GSR biofeedback

Correlation: OFC activity and skin conductance level



(Nagai et al., Neuroimage 2004 : Permission obtained)

OFC –r Amygdala connectivity increased after therapy

Correlation: OFC cluster and seizure reduction

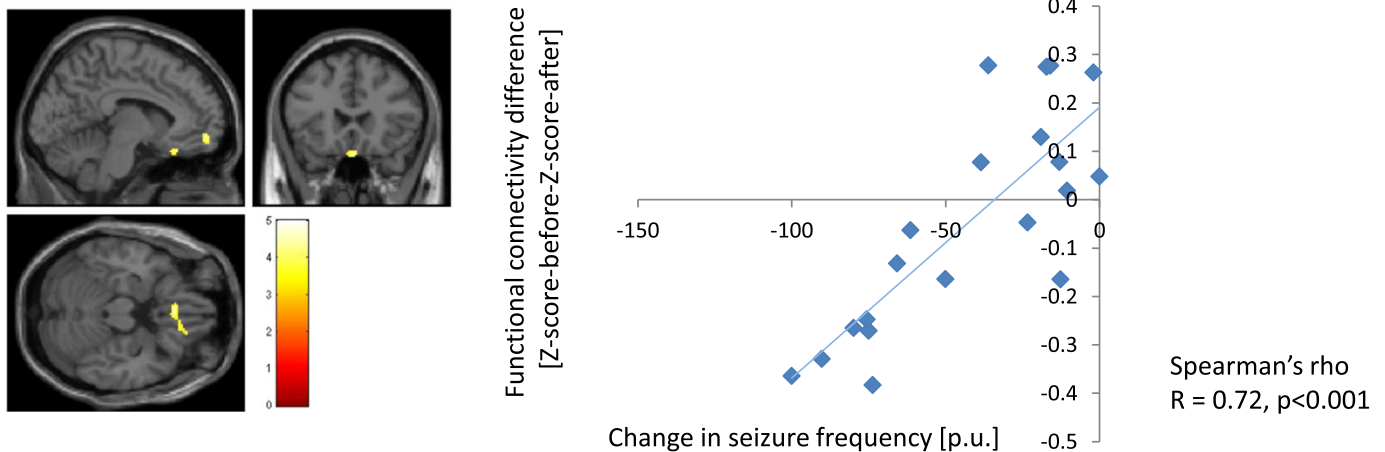
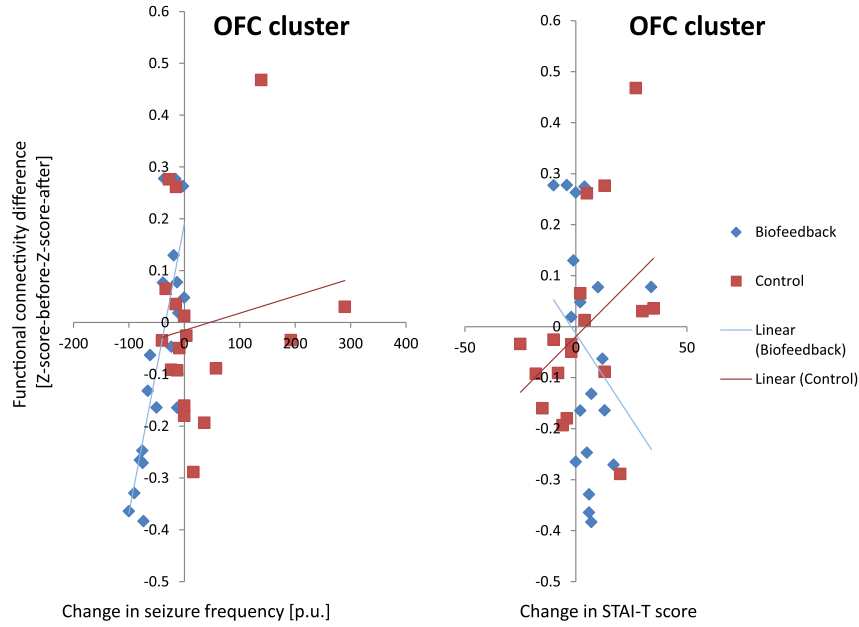


Fig. 5. Changes in neural connectivity to Right Amygdala associated to reduction in seizure frequency and alteration in subjective feeling of anxiety. A) Two distinctive functional connectivity changes to Right Amygdala was observed in Orbitofrontal Cortex (OFC) and Frontal Pole (FP). B) The identified OFC area is linked to the regulatory center of skin conductance which was previously discovered. The amygdala-OFC connection linearly correlated with degree of seizure reduction. All results are significant a $p < 0.05$, after FWE correction at cluster level.

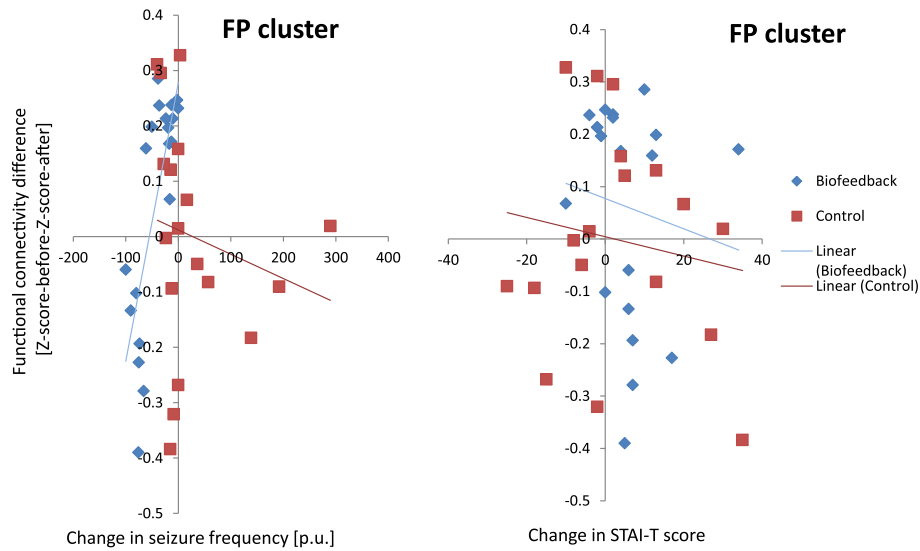
influences the outcome of surgery in patients with TLE, suggesting this white matter tract to be epileptogenic (Keller et al., 2016). In TLE, functional integrity of the uncinate fasciculus, as the anatomical conduit between frontal lobe and limbic system, is likely to underpin the

expression of neuropsychiatric features of the disorder. Impairment and dysfunction of the uncinate fasciculus is associated with the expression of psychiatric disorders, including schizophrenia, psychopathy, anxiety and antisocial personality disorders (Von Der Heide et al.,

A) Correlation between OFC cluster, seizure frequency change and STAI score changes



B) Correlation between FP cluster, seizure frequency change and STAI score changes



C) Bivariate correlations: Spearman correlation coefficient

	Percentage change in seizure frequency	
	Therapy	Control
FC change in OFC	R=0.72, p<0.001	R=-0.18, p=0.48
FC change in FP	R=0.71, p<0.001	R=-0.27, p=0.279

Fig. 6. Correlation between seizure reduction, neural connectivity changes and STAI score changes. Correlation of seizure frequency change and State and Trait Anxiety Inventory (STAI) score was separately plotted with OFC cluster (A) and FP cluster (B). Both OFC cluster and PF cluster was correlated only with seizure frequency changes but not with changes in psychological questionnaire (STAI score). C) The degree of correlation between seizure reduction and both OFC and FC was presented for both therapy and control group.

2013). Although speculative, the psychiatric co-morbidity observed in many patients with TLE may originate in changes in uncinate fasciculus with recurrent epileptic seizures. An implication of the current

behavioral study is that there is plasticity in this functional connectivity even in patients with drug-resistant epilepsy that may represent a treatment target for other interventions. Nevertheless, in this study we did

not observe a strong association between changes in amygdala functional connectivity with OFC/Frontal pole and self-reported anxiety symptoms, although such a relationship might be predicted (Makovac et al., 2016; Kim and Whalen, 2009). Our analysis tested for linear associations, therefore we could not exclude a more complex relationship between functional connectivity and anxiety exists.

Our findings reinforce the accumulating independent evidence supporting the effectiveness of autonomic biofeedback therapy in reducing the frequency of epileptic seizures (Nagai et al., 2004b; Nagai and Trimble, 2014; Micoulaud-Franchi et al., 2014; Kotwas et al., 2017). Moreover, our study extends the theoretical and neuroscientific understanding of body-brain interactions relevant to epilepsy management, by providing fresh empirical data regarding the neural mechanisms through which autonomic biofeedback training may promote seizure reduction. These findings have practical implications. For the therapy to attain broad impact, there is a need for greater patient access, proof of cost-effectiveness and population-level evaluation of its efficacy. One solution under development is the production of a digital online (yet supervised) version of the therapy. Once available, this new therapy platform can be assessed using clinical trial methodology (double-blinded randomized controlled) that more closely replicate sample sizes and statistical approaches used in drug studies. Finally the strategies developed by the therapist to enable the patient to transfer skills learned through GSR biofeedback can apply as countermeasures for seizure control in daily life. Autonomic biofeedback training using GSR offers a promising and potentially potent adjunctive non-pharmacological means of reducing seizures in patients with drug-resistant epilepsy. Moreover, we are defining neural mechanisms that may have wider relevance as biomarkers and treatment targets.

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Disclosure

YN is an inventor of a patent Treatment of Epilepsy granted, and has two national and international patents Treatment Device pending. YN is a director of social enterprise Biofeedback Global.

Authors Contributions

YN, JA, MK, LL, SS, MC participated in the planning and design of the study. YN collected the data and JA, MK, MM, SS contributed to the recruitment of the patients. Data analysis was conducted by YN, HC and MC. All authors contributed to interpretation of the data and preparation, critical revision of the manuscript.

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Appendix A. Supplementary Data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ebiom.2017.12.012>.

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