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Title: DBS of thalamic centromedian nucleus for Lennox-Gastaut syndrome (ESTEL trial)

Running Head: Deep brain stimulation in Lennox-Gastaut syndrome

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SUMMARY FOR SOCIAL MEDIA

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What is the current knowledge on the topic? Deep brain stimulation (DBS) is an emerging treatment for patients with Lennox-Gastaut syndrome (LGS), with increasing off-label use based on small, open-label studies targeting the centromedian nucleus of thalamus (CM; also called centre médian nucleus). Only two randomized control trials (RCTs) have evaluated CM-DBS; these negative trials were small (*n=20* in total), heterogenous (*n=10* LGS participants) and were with significant methodological limitations.

What question did this study addresses? Does CM-DBS reduce seizures in patients with Lennox-Gastaut syndrome? Is CM-DBS treatment safe in patients with Lennox-Gastaut syndrome?

What does this study add to our knowledge? – ESTEL (Electrical Stimulation of the Thalamus in Epilepsy of the Lennox-Gastaut phenotype) is the first randomized, double-blind, controlled study to evaluate the efficacy and safety of CM-DBS in a carefully characterised cohort of young adults with LGS. We found almost 60% of stimulated participants had a ≥50% reduction in electrographic seizures compared with none of the controls, indicating a therapeutic effect of CM-DBS in patients with LGS. Overall, median diary-recorded seizure reduction at the end of this study was 47% and no adverse effects on cognition were seen.

How might this potentially impact on the practice of neurology? Our results support the current evidence that CM-DBS can reduce seizures in patients with LGS. These results guide clinicians on how to counsel patients undergoing CM-DBS for likely magnitude of benefit and potential complications.

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Abstract:

Objective

Prior uncontrolled studies have reported seizure reductions following Deep Brain Stimulation (DBS) in patients with Lennox-Gastaut syndrome (LGS), but evidence from randomized controlled studies is lacking. We aimed to formally assess the efficacy and safety of DBS to the centromedian thalamic nucleus (CM) for treatment of LGS.

Methods

Prospective, double-blind, randomized study of continuous, cycling stimulation of CM-DBS, in patients with LGS. Following pre- and post-implantation periods, half received three-months stimulation (blinded phase), then all received three-months stimulation (unblinded phase). The primary outcome was the proportion of participants with ≥50% reduction in diary-recorded seizures in stimulated versus control participants, measured at the end of the blinded phase. A secondary outcome was the proportion of participants with a ≥50% reduction in electrographic seizures on 24-hour ambulatory EEG at the end of blinded phase.

Results

Between November 2017-December 2019, 20 young adults with LGS (17-37 years;13 females) underwent bilateral CM-DBS at a single centre in Australia, with 19 randomized (treatment, n=10; control, n=9). 50% of the stimulation group achieved ≥50% seizure reduction, compared with 22% of controls (OR3.1; 95%CI 0.44-21.45; p=0.25). For electrographic seizures, 59% of the stimulation group had ≥50% reduction at the end of the blinded phase, compared with none of the controls (OR23.25; 95%CI 1.0-538.4; p=0.05). Across all patients, median seizure reduction (baseline vs study exit) was 46.7% (IQR 28-67%) for diary-recorded seizures and 53.8% (IQR 27-73%) for electrographic seizures.

Interpretation

CM-DBS in patients with LGS reduced electrographic rather than diary-recorded seizures, after three-months of stimulation. 50% of all participants had diary-recorded seizures reduced by half at study exit, providing supporting evidence of treatment effect.

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Introduction

Lennox-Gastaut syndrome (LGS) is a treatment-resistant form of childhood-onset epilepsy, defined by multiple seizure types, including tonic seizures, specific EEG abnormalities and cognitive impairment. Reported prevalence is 1-2% of all patients with epilepsy[1]. LGS is a prototypical developmental and epileptic encephalopathy, and one of the most complex epileptic disorders to manage, with a high rate of morbidity and mortality. The combination of frequent seizures, intellectual disability, and behavioral co-morbidities create major challenges and place significant carer burden on families. Anti-seizure medication (ASM) side effects including drowsiness are common. New treatment strategies are required.

Deep brain stimulation (DBS) is an emerging treatment for drug-resistant epilepsies where resective neurosurgery is not indicated. In focal epilepsy, randomized controlled trials have demonstrated seizure reduction from chronic stimulation of anterior thalamic nucleus (AnT)[2] and hippocampus[3]. Unblinded studies of DBS to the centromedian nucleus of thalamus (CM; also termed centre médian nucleus) have reported dramatic reductions in generalized seizures following implantation and stimulation[4, 5]. A small number of DBS studies in LGS provide preliminary evidence that CM stimulation is likely to be beneficial, with one uncontrolled study reporting an 80% seizure reduction in 13 patients[6]. Minimal cognitive side-effects of CM stimulation have been reported in epilepsy studies to date, although neuropsychological data have not been systematically collected[6, 7]. Randomized-controlled trials of CM-DBS are required to evaluate benefits and side-effects.

We report efficacy and safety findings from the first randomized, double-blind, placebo-controlled clinical trial of CM-DBS in adult patients with LGS (trial name "ESTEL": Electrical

Stimulation of Thalamus for Epilepsy of Lennox-Gastaut phenotype). We followed the CONSORT 2010 recommendations for reporting randomized trials.

Methods

Study design

Our trial utilised a prospective, randomized, double blind, parallel group design (Figure 1B; Australian New Zealand Clinical Trials Registry number ACTRN12621001233819). There were four phases, each lasting three-months, with each month defined as 28 days: i) '*baseline*', denoting three-months before DBS implantation (months '-3', '-2', '-1'); ii) '*pre-stimulation'*, denoting three-months post-implantation but prior to randomisation/stimulation (months '1', '2', '3'); iii) '*blinded*', denoting the three-month blinded phase in which participants were randomized to either the stimulated (treatment) or non-stimulated (control) group (months '4', '5', '6'); and iv) '*unblinded*', denoting the final three-months of the study in which all participants received stimulation (months '7', '8', '9'). Participants in the control group received stimulation for the first time during the unblinded phase, and those who first received stimulation in the blinded phase continued to receive stimulation at the same dose until study exit.

Following a three-month pre-implantation baseline, participants proceeded to DBS surgery. To minimise potential DBS implantation effects on seizures[8] which could confound the benefit of stimulation, all participants waited a minimum of three-months before being randomized to blinded/unblinded phases. ASMs remained stable throughout the trial, however rescue doses of benzodiazepines were permitted.

Participants

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Eligible participants were aged 15-65 years, with confirmation of LGS diagnosis made by two neurologists (LJD, JSA). Inclusion criteria were: i) an electroclinical diagnosis of LGS; ii) generalized paroxysmal fast activity (GPFA) and slow spike-and-wave (SSW) on interictal EEG; and iii) generalized tonic seizures documented on prior video-EEG monitoring or clearly described by a reliable eyewitness. Additional but not essential seizure types included generalized tonic-clonic seizures, atonic seizures, spasms, myoclonic seizures, and focal impaired awareness seizures. Atypical absence seizures were common but were not an inclusion criterion due to difficulty with reliable detection[9]. Participants with clusters of seizures were allowed, provided seizures within the cluster were able to be counted. A pre-defined seizure frequency per cluster was estimated by the carer responsible for maintaining the seizure diary; for example, if the carer estimated 4 spasms in a 10 second cluster, then 'spasms for 50 seconds' on the seizure diary equated to 20 seizures. Required minimum seizure frequency (all seizure types combined) was ≥4 per month (28 days), with no maximum frequency stipulated. Participants were required to be on existing stable doses of ≥2 ASMs, and previously failed at least three different ASMs. Prior vagal nerve stimulator (VNS) insertion and/or neurosurgery (e.g., corpus callosotomy) was permitted. VNS devices remained on their existing programmed settings throughout the duration of the study. All participants had documented intellectual disability, ranging from moderate (independent for some activities of daily living) to profound (non-verbal, non-ambulant). A consistent and reliable parent/carer was required to maintain an accurate seizure diary for twelve months.

Exclusion criteria were: i) participants with elevated risks for bleeding; ii) cerebral anatomical variations precluding safe CM-DBS implantation; iii) predominant seizure type being focal impaired awareness seizures; iv) current or prior psychogenic non-epileptic seizures.

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Investigator LJD identified potential participants (Figure 1A) and reviewed data with JSA to determine whether the participant was eligible for study inclusion. Parents or a responsible guardian provided written informed consent before any study-specific procedures commenced. The trial protocol received institutional approval from Austin Health Human Research Ethics Committee prior to trial commencement (approval number HREC/16/Austin/139).

Randomization and masking

Randomization occurred after implantation, prior to the stimulation phase, to either immediate stimulation (i.e., starting three-months after implantation) or delayed-stimulation/control (i.e., starting six-months after implantation) in a 1:1 ratio, stratified by age (below vs above 30 years of age)[10]. Randomisation and stimulation adjustments were conducted by a single, unblinded programmer (AR; not responsible for study assessments), while clinical assessments and data collation were performed by separate clinicians (LJD, JSA), blinded to treatment group and voltage settings. Excluding the unblinded programmer, all study personnel, participants and carers remained blinded to treatment group until all data collation (all participants) was completed. Data remained locked until the unblinded programmer revealed which group each participant was assigned.

To maintain the double-blind study design, the unblinded programmer spent equal time programming each participant in the stimulation and control groups. This was done immediately after randomisation at the beginning of the blinded phase, with testing for stimulation side-effects up to 3V, prior to reducing to the planned stimulator setting (e.g., zero or 2.5V). The study was initially designed to deliver stimulation up to 5V, but side-effect testing for the first two participants (randomized on the same day) resulted in paresthesia and speech disturbance, thus prompting adoption of lower voltages throughout the trial. For

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subsequent participants, where tolerability issues/side-effects were identified, the unblinded programmer had the liberty of increasing voltages more slowly (i.e., over 2 to 4 weeks) and/or choosing the adjacent contact within CM as the cathode (bipolar stimulation) when the participant was scheduled to receive stimulation. For all participants, the supplied controller (used by participants to monitor battery life) was reprogrammed to 'ON' at the beginning of the blinded phase and voltage levels removed from the dashboard, thereby removing identification of the participant's voltage setting.

EEG was acquired and reviewed with standard settings: sampling rate 256Hz, 70Hz low-pass filter, 0.5Hz high-pass filter. DBS stimulation, although delivered at 145Hz, consists of a train of 90 microsecond impulses, meaning the actual frequency of each impulse is 11,000Hz, which is well outside the sampling range of our EEG. Therefore, DBS stimulation artifact was not present on ambulatory EEG, alloing blinded assessments of EEG data.

Procedures

Monthly seizure diaries, documenting daily seizure counts for each seizure type, were collected for each month of the study at each study visit. Throughout the study, four overnight ambulatory (24 hour) EEG recordings were obtained for each participant: during the baseline phase (i.e., Baseline), at the end of pre-stimulation phase before randomisation (i.e., Week 12), at the end of the blinded phase (i.e., Week 24), and at the end of the unblinded phase/study exit (i.e., Week 36) (Figure 1B). These were used to determine: i) the number of electrographic seizures per 24 hour recording, and ii) the burden of interictal GPFA, a marker of abnormal epileptic networks in LGS[11]. Cognitive assessments were performed during baseline, at the end of the blinded phase (Week 24) and at the end of the unblinded phase/study exit (Week 36). These included the Global Assessments of Epilepsy Severity (GASE)[12, 13] , Global

Assessments of Disability (GADS)[14] and the Adaptive Behavior Assessment System – Third Edition (ABAS-III)[15], used to monitor epilepsy severity, disability and adaptive functioning, respectively.

Bilateral CM-DBS electrode insertion was performed using our previously described neurosurgical targeting approach [16]. Briefly, Medtronic model 3389 leads (4 x 1.5-mm contacts per lead, 0.5-mm intercontact distance; Medtronic, Minneapolis, MN, U.S.A) (Figure 2), were implanted under stereotactic guidance by a single neurosurgeon (KJB) at Austin Health, Melbourne, Australia. Leads were connected to a dual-channel Activa PC® neurostimulator (Medtronic) via Low Profile Extensions (Medtronic) connectors, tunnelled subcutaneously. The CM was identified preoperatively on patients' 3T MRI scans using a magnetisation-prepared two-rapid-gradient-echoes (MP2RAGE) sequence that was post-processed using Sobel filtering to highlight intrathalamic borders, together with the three-dimensional Krauth/Morel thalamic atlas[17] that was nonlinearly spatially warped to each patient's brain[16, 18]. All surgeries were performed under a modified anesthetic regimen combining intravenous remifentanil (0.1-0.3 μg/kg/min) with inhalational isoflurane (0.5%-0.7%), permitting intraoperative simultaneous EEG recordings from thalamus and scalp[19]. Up to one day postoperatively, a brain CT scan was acquired to confirm accurate DBS electrode positioning. DBS lead contact positions were established by coregistering postoperative CT to preoperative MRI scans (Figure 2).

The most centrally located contact within each CM was selected as the site for cathodal referential stimulation with the stimulator case as anode (monopolar stimulation)[16]. Initial stimulation for those in the stimulation group during the blinded phase was 1V; after 2 weeks, this was increased to 2.5V bilaterally. Voltage, rather than current output, was chosen to reflect

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settings used in the SANTE trial[2]. Other stimulation settings also reflected those used in the SANTE trial: 90μs pulse width, 145Hz, cycling at "ON" one minute and "OFF" five minutes. Participants in the control group received no stimulation during the blinded phase (i.e., DBS was "turned on" and set to 0V), and then were later stimulated at 1V/2.5V during the unblinded phase (Figure 1B). Patient-specific voltage and current parameters are shown in Table 2.

Outcomes

The primary outcome was the proportion of responders (participants with ≥50% reduction in seizures, recorded in seizure diaries) for the last month of the blinded period compared to the three-month baseline period in treatment (stimulated) versus control groups. A secondary efficacy outcome was the proportion of participants with ≥50% reduction in clinical electrographic seizures in 24-hour EEG at the end of the blinded period compared to the baseline 24-hour EEG in treatment (stimulated) versus control groups. Other secondary endpoints were longitudinal comparison of seizure frequency (diary-recorded and electrographic seizures) during each study month/phase (absolute and relative to baseline) and GPFA burden at the end of each study phase (relative to baseline). Exploratory outcomes compared baseline GASE/GADS/ABAS-III scores to those at week 24 (end of blinded phase) and 36 (end of study); GASE/GADS (single-item, 7-point global ratings assessed by parents/caregivers) outcomes were based on scores being "better", "worse" or "same", and ABAS-III scores reported absolute improvement, as a percentage of the maximum cumulative raw score.

Diary-recorded seizures were expressed as a monthly (28 day) average. Where participants had a seizure-free day, the parent/carer was asked to record a '0' for that diary entry. On days where no record of seizures was kept (e.g., due to a temporary participant stay in respite), the number

of days in the month was adjusted (i.e., if two days were missed in one month, the total number of seizures were divided over a 26-day month, then multiplied by 28).

EEG data were manually evaluated, blinded to study phase *and* treatment group. For each participant, pre-study video-EEG monitoring that captured typical clinical seizures was reviewed. This informed the study clinician of the electrographic signature that corresponded to the participant's seizure so that it could be identified on EEG as an electrographic seizure. Other identified electrographic seizures were defined by the following criteria: (i) a sustained run of generalized, fast epileptiform activity with evolution, causing a change in the background rhythm, and (ii) a duration of ≥5 sec. The total number of electrographic seizures was measured over the 24-hour EEG recording. GPFA was defined as bursts of generalized fast epileptic activity lasting longer than 250msec, but <5 sec. Cumulative duration of GPFA events (sec) was calculated during a two-hour period of sleep-EEG, between midnight to 2am. This period was chosen due to the higher likelihood of interictal abnormalities occurring during sleep in LGS patients[20]. Where participants had more than ten arousals or were awake >5 mins during this period, the two-hour window of analysis commenced 30 minutes later.

All adverse events (AE) were collected from receipt of informed consent to trial completion, as reported to study personnel. Serious adverse events (SAE) were defined as adverse events requiring hospital admission. These were monitored by an independent data and safety monitoring board.

Statistical analysis

The power calculation for this study was based on the assumption of at least a 70% seizure reduction with stimulation[6]. The positive response was defined as reduction of at least 50% [21].

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Ten patients per group yielded 80% power to detect a statistically significant difference of 0.6 or higher in proportions of responders between two groups (0.7 in stimulated, 0.1 controls), assuming two-sided alpha of 0.05.

A statistical analysis plan (SAP) document was formulated and finalised prior to the study database lock. The analysis was conducted on intention to treat basis. Data are summarised as median with IQR for continuous variables and as counts (proportions) for categorical variables. The effect size for the primary outcome was presented as baseline seizure frequency adjusted odds ratios with respective 95% confidence intervals (95% CIs). This was estimated by Firth logistic regression model, using presence or absence of response as the dependent variable, treatment group (stimulated vs control) as the independent variable, and the mean within-participant baseline seizure frequency (measured over three-months) as a covariate. Due to the nature of the underlying distributions, longitudinal secondary endpoints were assessed with clustered median regression modelling with individual patients as clusters. Both GASE and GADS outcomes were investigated using Fisher's exact test. ABAS-III outcomes were investigated using median regression with the ABAS-III value at the end of the blinded period as the outcome and the treatment group as independent variable, adjusted for ABAS-III baseline score. Safety outcomes were reported descriptively with standard summary statistics. Data was made available to an independent safety and data monitoring committee. Calculations were performed using Stata software (Version 16 IC; Stata Corp, College Station, TX, USA).

Results

Of the 25 enrolled participants with LGS, 20 (mean age ± SD = 25 ± 6.29; 13 females) underwent bilateral CM-DBS at Austin Health, Australia, between November 2017 and December 2019 (Figure 1A). Following implantation, one participant was excluded from the

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study due to device removal; see *Safety* below. Randomisation assigned ten participants to the stimulation group and nine to the control group (Figure 1B), with comparable baseline demographic and seizure characteristics (Table 1). Due to COVID-19 mandated lock-down, only 17/19 participants had all four EEGs performed throughout the study.

Post-operative scanning confirmed accuracy of CM-DBS electrodes for all participants, with none requiring re-do surgery. Participants received a total of 346 weeks of active stimulation throughout all phases (treatment=238 weeks vs control=108 weeks; Table 2). Due to side-effects (paresthesia), two participants in the treatment group were not able to be stimulated at the intended maximum voltage; one participant spent ten weeks at 2V before being increased to 2.5V for the remaining 12 weeks, and the other was stimulated at 2V for 12 weeks before increasing to 2.5V for the remaining ten weeks. In the treatment group, 80% (8/10) of participants received bilateral monopolar stimulation; one participant required bilateral bipolar stimulation; and one required mixed stimulation (i.e., bipolar on the left and monopolar on the right). Mean (±1 SD) current output on the left and right, respectively, were 2.45±0.16mA and 2.25±0.48mA, in the treatment group. In the control group, all participants were stimulated in the final unblinded phase using bilateral monopolar settings, receiving mean (±1 SD) current outputs of 2.27±0.38mA and 2.18±0.47mA on the left and right, respectively; one participant was unable to be stimulated on the maximum voltage of 2.5V, requiring voltages of 1.5-2V for seven weeks, then 2.5V for the final three-weeks.

Efficacy

Five (50%) patients in the treatment group had a ≥50% reduction in diary-recorded seizures, compared with two (22%) in the control group (adjusted OR=3.1; 95% CI=0.44 to 21.45; p=0.25). Clustered median regression analysis identified a significant time by treatment

interaction for absolute seizure reduction (p=0.025), and a potential signal for relative seizure reduction (p=0.063) (Table 3A and 3B). Median (relative-to-baseline) difference in diary-reported seizure reduction in the last month of the blinded phase was 36.3% (95% CI=-83.6 to 11.09; p=0.124) between early stimulated and control groups (Figure 3A).

24-hour ambulatory EEGs at key timepoints provided an additional, objective marker of seizure frequency. In the blinded phase, eight patients (89%) in the treatment group had a ≥50% reduction in electrographic seizures, compared with none in the control group (adjusted OR 23.25; 95% CI 1.0 to 538.4; p=0.05). Median (relative-to-baseline) change in electrographic seizures in the last month of the blinded phase (i.e., week 24 EEG) was 57% (95% CI=-1.15 to -0.08·; p=0.027) between early stimulated and control groups (Figure 3B).

Overall, the median reduction in diary-recorded seizure frequency at study exit compared to baseline, for all participants irrespective of group, was 46.7% (IQR 28% to 67%) (Figure 4C), while the median reduction in electrographic seizures across all participants was 53.8% (IQR 27% to 73%) (Figure 4D).

GPFA burden (total duration [sec] in 2h window) reduced following implantation and stimulation. However, the pattern of change was not significantly different between groups receiving early or late stimulation (p=0·52) (Table 3C). In all participants, median relative reduction in GPFA at study exit was 45.1% (IQR 16% to 64%).

No statistically significant changes were observed in GASE or GADS scores over the course of the study, and no significant differences between groups were observed, after either the blinded or unblinded periods. After three-months of stimulation, the median ABAS-III

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improvement was 0.2% (IQR -0.7% to 1.85%), with no significant difference in scores between groups.

Safety

SAE occurred in seven (35%) participants (Table 4). Cerebral *Staphylococcus aureas* infection necessitated DBS hardware removal in one participant, 58 days post-operatively. One participant required emergency evacuation of a subdural haematoma following a seizure/fall 41 days post-operatively, prior to the device being switched on. DBS hardware remained in-situ and imaging confirmed no lead movement or disruption. Two participants had prolonged seizures/status epilepticus requiring hospital admission. One participant had a drop seizure leading to facial laceration, requiring stitches under sedation. Median hospital length-of-stay post-operatively was three days (range=1 to 17 days). Post-operative seizures were observed in 70% (14/20) of participants during the hospital admission, consistent with the high seizure burden experienced by participants pre-implantation.

Twelve participants (60%) had transient post-operative drowsiness, ranging 24 hours to 13 days. Three of these had more profound drowsiness, prolonging bed-stay and requiring temporary nasogastric feeding to maintain nutrition, with acute imaging showing quite marked cerebral edema (i.e., mega-edema) along the course of DBS electrodes. One of these participants was the same participant who developed intracerebral infection (see above); treatment of this participant's edema was with steroids, with subsequent infection development requiring removal of the device and leads. In the two other subjects with mega-edema, drowsiness and imaging evidence of lead edema settled spontaneously over 1-2 weeks and neither steroids nor antibiotics were used.

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Other AE, reported by participants and their carers are displayed in Table 4. Thirty AE were reported across all participants, from implantation to week 36 post-operatively. Except for food aversion/change in appetite, none of the listed AE persisted throughout the entirety of the study and were often short-lived. Swallowing difficulty, fatigue, headache, pain and chest box discomfort were more likely to be reported in the first two months post-implantation (i.e., prior to stimulation). AEs in the blinded phase were reported by three participants only. All were assigned to the treatment group, reporting paraesthesia (*n=3*) and fatigue (*n=1*).

Anecdotally, parents/carers of 18/19 participants reported "improved alertness" following CM-DBS, ranging from 4-34 weeks post-implantation, and not specifically reported during the stimulation period. At study completion, parents/carers were asked whether there was a perceived overall difference (responses = "better", "same", "worse"). 74% (14/19) reported "better", with three (16%) reporting "same" and one (5%) reporting "worse". Interestingly, despite one participant's diary revealing a 77.4% reduction in seizures at the end of the blinded phase (treatment group), the parent deemed the participant as "same" following completion of the trial. This was attributed to the ever-present risk of injuries associated with drop seizures, meaning that a similar degree of observation was required with one *vs* ten daily drop seizures, despite overall seizure reduction. Additionally, "same" and "worse" responses were recorded despite those participants having an overall mean reduction in their electrographic and diary-reported seizures.

Discussion

This prospective, double-blind, randomized study of continuous, cycling stimulation to the bilateral CM in patients with LGS did not find a significant difference in the proportion of patients with a 50% reduction in diary recorded seizures after three-months of stimulation.

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However, several secondary outcomes were positive. We did find a significant reduction in electrographic seizures measured objectively from intermittent 24-hour ambulatory EEGs, possibly reflecting the greater precision of objective measures of seizures. In addition, seizure diaries showed a significantly greater reduction in absolute seizure counts at the end of three-months of blinded stimulation, and also confirmed an overall reduction of seizures by 46.7% on study completion, compared to pre-implantation. On balance, the trend towards seizure reduction (measured from seizure diaries), combined with significant reductions in seizure frequency (measured from ambulatory EEG), suggests there is therapeutic benefit of CM-DBS in LGS.

ESTEL included objective measures of cognition and adaptive behavior. These showed no deterioration in cognitive function following CM-DBS stimulation. In the ESTEL cohort, 35% (7/20) of participants were non-verbal, and a further 30% (6/20) had severely limited verbal output of less than 3–5-word sentences. Despite this, subjective reports from parents/carers were of increased alertness in 95% (18/19), and overall benefit was reported by 79% (15/19). Specific improvements reported included allowing cutting of fingernails, counting to 50, adopting more assertive behavior (e.g., devising strategies to avoid participation in swimming class), walking unsupervised and reduced time during the day spent sleeping. Interestingly, with reduction in seizure frequency, some participants with autism showed exacerbation of autistic traits, such as increased periods of perseveration.

Although patient and carer-reported seizures often form the primary outcome measure in clinical trials, there is a well-described phenomenon of underreporting[22, 23] and poor correlation between seizure-diary counts and objective seizure frequencies[24]. In ESTEL, an objective measure of seizure activity (seizure counts from 24-hour ambulatory EEGs at key time points),

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found a significant effect of stimulation, in contrast to seizure diaries. Median diary-recorded seizures per month in the baseline period across all subjects was 79 (i.e., ~three seizures/day), compared with 13 electrographic seizures per hour on baseline EEG (i.e., ~300 seizures/day), a 100-fold difference. This implies diaries are capturing only a fraction of electrographic seizures. Patients with LGS are usually unable to reliably report seizures, and despite the best efforts of carers, it is very difficult to accurately document subtle tonic and nocturnal seizures from observation alone. Also, there can be additional rotating carers, with varied levels of experience tasked with documenting seizures, likely contributing to unreliable seizure recording. Psychiatric comorbidities, including autistic traits and oppositional behaviors, are often a greater management challenge than seizures themselves[25], deflecting the focus from seizure recording. Together, these characteristics are important limitations when performing efficacy trials in patients with LGS and highlight the need for including objective markers of seizure frequency.

Despite the limitation of diary-based assessments of seizure frequency, ESTEL participants had an overall median seizure reduction of 46.7% during the last month of the trial. This is in line with the varying degrees of seizure reductions reported in unblinded CM-DBS studies[5, 6, 26, 27] of LGS patients. In addition, there are anecdotal reports of improved quality-of-life and cognitive abilities following CM-DBS[6], further adding to its appeal as a therapeutic option. Recognising there are few effective treatment options for this cohort[28], clinicians are increasingly turning to CM-DBS despite no robust and controlled studies having been performed before the present trial.

We found that transient post-operative drowsiness was very common after CM-DBS (12/20 patients) and thus future treating clinicians should expect to see varying degrees of this.

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Although it was quite significant in three cases, it usually resolved with no specific treatment over days to weeks; the one patient we treated with steroids subsequently developed device infection. Potential explanations for post-operative drowsiness include a 'stun effect' on the CM or bi-frontal cerebral edema along the electrode tract. Lead edema is a recognised issue in Parkinson's disease DBS[29], but the extent of drowsiness we observed seemed more prominent. This may reflect increased vasoreactivity in our younger population or reduced cognitive reserve.

Our infection rate of 5% (1/20 participants) was similar to prospective studies of DBS for Parkinson's disease (9.9%)[30] and focal drug-refractory epilepsy (12.7%)[2]. There were no deaths or symptomatic spontaneous hemorrhage. Two participants required hospitalisation for status-epilepticus in the post-operative periods and therefore not because of stimulation. In fact, numbers of stimulation-related AE in the blinded phase were low, with the most common being transient ipsilateral hand/face/lip paresthesia which usually resolved over hours to days. The first two implanted participants received stimulation of 5V during tolerability testing at the beginning of the blinded phase; intolerability of this (ipsilateral face pain and speech disturbance) determined the maximum voltage of 2.5V for participants, which may explain the low numbers of stimulation-related AE documented.

Given the negative primary outcome but multiple positive secondary outcomes, the ESTEL study may have been underpowered. In study design planning, power and sample size calculations were based on previous uncontrolled studies documenting an 80% reduction in seizures in patients with LGS following CM-DBS.[6] At the end of the three-month blinded phase in ESTEL, only one stimulated participant had an >80% reduction in diary-recorded seizures, and we found an overall seizure reduction of ~50%. This apparent reduction in

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treatment effect appears to be a similar phenomenon to that described when comparing blinded to observational studies of anti-seizure medications[21]. Another factor considered in determining sample size for device studies is limiting potential harm from an invasive procedure where there is the possibility of no treatment effect.

Much work remains in deciphering the optimal stimulation paradigms for DBS in LGS, and for patients with epilepsy more broadly. We based our stimulation parameters on that of the successful SANTE study[2], but it is important to note that our DBS stimulation/targeting and patient cohort differed. Maximum stimulation varied between the studies (2.5V in ESTEL *vs* 5-10V in SANTE) to maintain adequate blinding in ESTEL. There may have been more sizeable seizure reductions seen in ESTEL if higher stimulation was delivered. CM, rather than AnT, was chosen due to its widespread connections with the striatum, brainstem, and diffuse frontal areas, theoretically making it a good target for generalized epilepsies[4-6]. Additionally, our intraoperative depth electrode recordings from the beginning of tonic seizures demonstrated sustained burst firing in CM[31, 32], confirming its involvement in this characteristic seizure type. However, other thalamic nuclei may be similarly or more effective. Furthermore, different stimulation parameters produce specific neuronal activity patterns[33]. Future studies are required to evaluate optimal frequency, amplitude, mode (i.e., controlled current vs voltage), and stimulation duration parameters.

A progressive longer-term benefit has been suggested in DBS studies for other epilepsy types[34, 35], and thus continued follow-up of our ESTEL cohort will be crucial in assessment of CM-DBS efficacy. This is thought to occur due to effects on pathological brain circuits which show cellular, molecular and neuroplastic changes over time[36]. Given the high frequency and long seizure history in these ESTEL participants before CM-DBS, pathological networks

responsible for seizure generation may need more time before such progressive benefits are seen.

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Author contributions

JSA, AELW, WT, KJB, AR and LC contributed to conception and design of the study. LJD, AELW, KB, AR, LC and JSA contributed to acquisition and analysis of data. LJD, AELW, LC and JSA contributed to the drafting of text or preparing of figures.

Potential conflicts of interest

LJD , AELW, LC and AR report no conflicts of interest relevant to this study. JSA has received honoraria from Medtronic. KJB and WT are co-founders and hold shares and option in DBS Technologies Pty Ltd. KJB and WT are also named inventors on related patents, which are assigned to DBS Technologies Pty Ltd. WT has received honoraria from Medtronic and Boston Scientific. Medtronic and Boston Scientific are manufacturers of DBS equipment.

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Figure legends

Figure 1: **Trial design. (A)** The ESTEL population consisted of 20 participants (ten assigned to the early treatment group, nine assigned to the control [delayed-treatment] group; one participant developed infection, leading to device removal, *prior* to randomisation at week 12. Eligible participants were identified from the Austin Health Comprehensive Epilepsy Program database or referred by treating epileptologists. **(B)** Study timeline depicting each 3-month phase of the trial and key timepoints relative to CM-DBS implantation (week 0). Randomisation occurred at week 12 in 19 participants. The blue line depicts the time-period in which participants received stimulation.

Abbreviations: CNS=central nervous system; DBS=deep brain stimulation; PNES=psychogenic non-epileptic seizures.

Figure 2: **Localisation of DBS electrodes implanted into the bilateral thalamic centromedian nucleus (CM) for all nineteen randomized ESTEL participants.** *Left:* Coronal view of Krauth/Morel[12] thalamic atlas showing location of centromedian nucleus (CM; also called centre médian nucleus) and surrounding nuclei (PF=parafascicular; CL=central lateral; MD=mediodorsal; VPM=ventral posterior medial; VM=ventral medial; VLpv=ventral lateral posterior ventral; VLpd=ventral lateral posterior dorsal; VPL=ventral posterior lateral; LD=lateral dorsal) (R=right direction; L=left direction; S=superior direction; I=inferior direction). *Middle:* Bilateral quadripolar DBS electrode leads (Medtronic 3389) reconstructions based on postoperative CT scans for all participants (n=19), shown separately for the left and right CM, indicated by the shapes in yellow[17]. 3D positions are shown in a coronal orientation, as per the 2D view in the left panel. Lead reconstructions were performed using lead-DBS software[18]. *Right:* Diagram depicting bilateral DBS leads width, contact size, and inter-contact distance for the Medtronic 3389 model. Each lead has four stimulation contacts (Left contacts: C0, C1, C2, and C3; Right contacts: C8, C9, C10 and C11) spaced 0.5 mm apart.

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***Figure 3*: Relative change in seizures after three-months of stimulation. (A)** Following three-months of stimulation (blue box-plot on right), the median relative decline in diary-recorded seizures was not significant (p=0.124). **(B)** In contrast, the median relative change in electrographic seizures was different between treatment (blue; right) and control (grey; left) groups for the median (p=0.027), 25th percentile (p=0.031) and 75th percentile (0.037). The median reduction in electrographic seizures after three-months of stimulation was 57%. EEG measures of seizure frequency show less variance likely facilitating detection of significant change.

***Figure 4*: Changes in clinical and electrographic seizures following stimulation. (A)** Median seizure reduction in diary-recorded seizures, relative to baseline seizure frequency plotted for the early stimulation (blue line; n=10) and delayed treatment (grey line; n=9) groups. At the end of a 3-month baseline period, all participants underwent DBS-insertion, with reductions in median seizures (%) recorded for each of the 9-months following surgery. In the first three-months following surgery, both groups had a reduction in seizures (stimulator *not* on), possibly related to the well-described 'implantation effect'. In the blinded phase (months 3-6; dark pink shaded box), participants who received stimulation had a greater reduction in seizures compared with those not receiving stimulation, although this difference was not significant. In the unblinded phase (months 6-9; light pink shaded box) those in the delayed treatment arm received stimulation for the first time with a subsequent reduction in diary-recorded seizures. **(B)** Median electrographic seizure reduction, relative to baseline frequency plotted for the early stimulation (blue line; n=9) and delayed treatment (grey line; n=8) groups. Electrographic seizures were determined from 24h-ambulatory EEGs performed during baseline, 3-months after implantation (but before stimulation) and prior to randomization, at the end of the blinded period and at study exit. **(C)/(D)** At the end of the ESTEL trial, across all participants (n=19) there was a median reduction in diary-recorded seizures of 46.7%, represented by the red dotted line on the left (Fig. 4C). Median reduction in clinical electrographic seizures across all participants (n=17) at the end of the trial was 53.8% (red dotted line on Fig. 4D). Each blue bar represents a single participant's % reduction in electrographic seizures at the end of the trial, relative to baseline seizure count. Participant numbers (1-19) depicted along the horizontal axis of Figure 4C do not correspond to participant

numbers in Figure 4D. Two participants were unable to have their study exit-EEG due to COVID-19 lockdown restrictions in place at the time. Each blue bar represents a single patient's % reduction in electrographic seizures at the end of the trial, relative to baseline seizure count.

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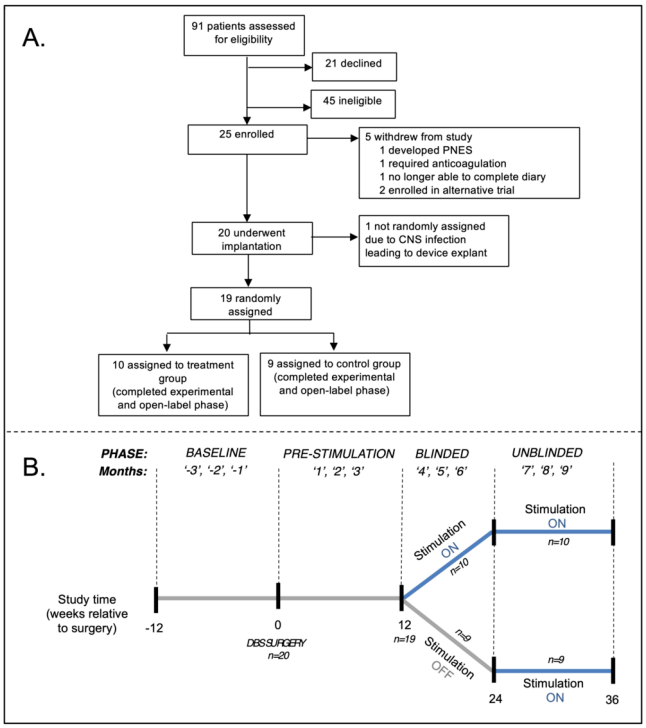

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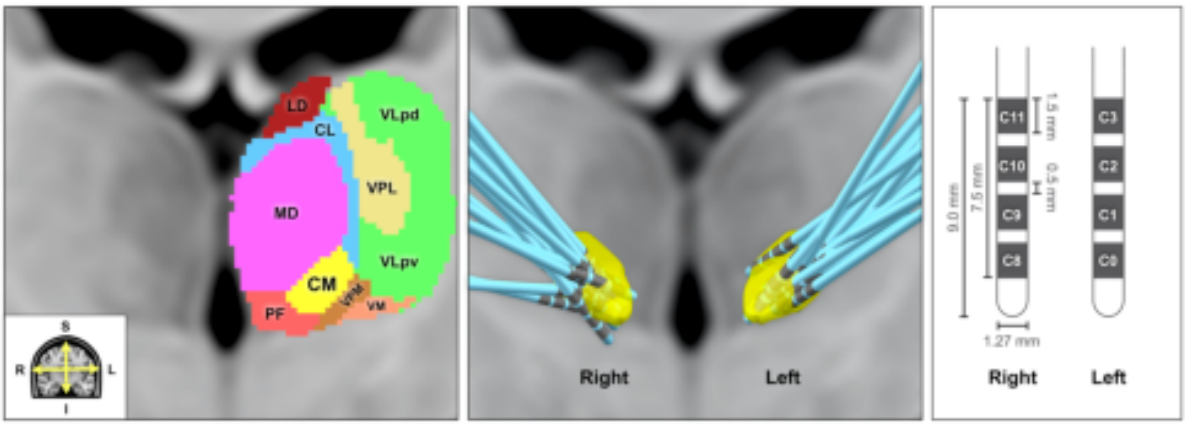

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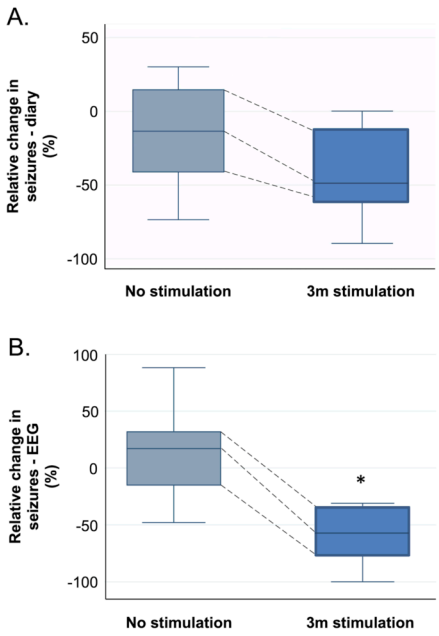

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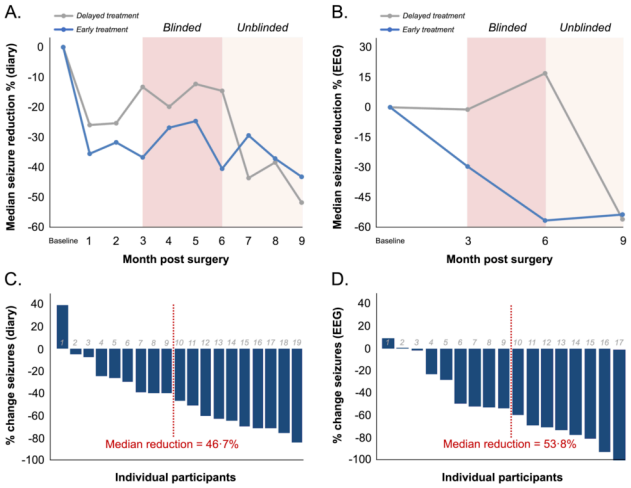

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Table 1. *Baseline characteristics of implanted ESTEL participants*

Characteristics	***Baseline:*** ***All patients (N = 20)***	***Treatment (N=10)***	***Control (N=9)***
Participant characteristics			
Female sex [no. (%)]	13 (65%)	7 (70%)	6 (66.6%)
Mean epilepsy duration [years], SD	21.7 ± 7.4	19.77 ± 8.21	22.85 ± 6.24
Mean age at implantation [years], SD	25 ± 6.3	24.4 ± 6.92	25 ± 5.94
Cause of LGS [(no (%)]			
Unknown	8 (40%)	3 (30%)	5 (55.6%)
Structural	5 (25%)	2 (20%)	3 (33.3%)
Genetic	3 (15%)	2 (20%)	1 (11.1%)
Structural/genetic	4 (20%)	3 (30%)	0 (0%)
Seizure types [no (%)]			
Tonic	20 (100%)	10 (100%)	9 (100%)
Generalized tonic-clonic	18 (90%)	9 (90%)	8 (88.9%)
Drop attacks*	16 (80%)	9 (90%)	6 (66.6%)
Other[#]	16 (80%)	5 (50%)	3 (33.3%)
Treatments prior to surgery			
Number of epilepsy medications at baseline [no. (%)]			
Two	2 (10%)	2 (20%)	0 (0%)
Three	9 (45%)	4 (40%)	4 (44.5%)
Four	6 (30%)	4 (40%)	2 (22.2%)
Five	2 (10%)	0 (0%)	2 (22.2%)
Six	1 (5%)	0 (0%)	1 (11.1%)
Surgical categories [no. (%)]			
No prior neurosurgery	10 (50%)	7 (70%)	3 (33.3%)
VNS implanted (turned on)	4 (20%)	1 (10%)	2 (22.3%)
VNS implanted (turned on) + CC	1 (5%)	0 (0%)	1 (11.1%)
VNS implant (turned off/removed)	2 (10%)	1 (10%)	1 (11.1%)
VNS implant (turned off/removed) + CC	2 (10%)	1 (10%)	1 (11.1%)
CC alone	1 (5%)	0 (0%)	1 (11.1%)
Seizures and electrographic characteristics			
Mean monthly seizure count in baseline period [median (IQR)]	79 (38-151)	70 (40-375)	85 (55-111)
No. electrographic seizures/hr [median (IQR)]	13 (6-16)	12 (6-15)	13 (6-19)
PFA duration in 2h (sec) [median (IQR)]	404 (192-716)	361 (182-600)	714 (314-798)
Cognitive scores			
Median GASE score	6	6	7
Median GADS score	7	7	7
ABAS-III score (%)[median (IQR)]	14.3 (4.1-37.3)	18.5 (5.7-35.9)	15 (9.3-37.2)

Abbreviations: ABAS-III, Adaptive Behavior Assessment System – Third Edition; CC, corpus callosotomy; GADS, Global Assessments of Disability; GASE, Global Assessments of Epilepsy Severity; GPFA, generalized paroxysmal fast activity; IQR, interquartile range; VNS, vagal nerve stimulator

*seizures associated with a sudden fall, typically atonic seizures

[#]focal impaired awareness seizures, myoclonic seizures, spasms.

Table 2: *Stimulation settings of participants during ESTEL trial*

Assigned group	***Participant***	***Stimulation type***	***No. weeks on 1V***	***No. weeks on 2.5V***	***No. weeks (setting) on other stimulation parameter***	***Left / right current outputs for longest duration during trial (mA)***
Early stimulation (treatment; n=10)	E04	Monopolar	0	**24**	0	2.0 / 2.1
	E09	Monopolar	2	**22**	0	2.7 / 2.7
	E10	Monopolar	2	**12**	10 (2V week 16-26)	2.1 / 2.5
	E13	Monopolar & Bipolar	2	10	**12** (2V week 14-26)	0.9 / 2.0
	E14	Monopolar	2	**22**	0	2.3 / 2.0
	E19	Bipolar	2	**20**	2 (1.5V week 14-16)	1.5 / 1.3
	E17	Monopolar	2	**22**	0	2.4 / 2.7
	E25	Monopolar	2	**20**	2 (1.8V at week 14-16)	2.7 / 2.6
	E24	Monopolar	2	**22**	0	2.5 / 2.8
	E23	Monopolar	2	**22**	0	2.5 / 2.0
Delayed stimulation (control; n=9)	E05	Monopolar	2	**10**	0	2.5 / 2.3
	E03	Monopolar	2	**10**	0	2.6 / 2.2
	E08	Monopolar	2	3	**7** (L=2V, R=1.5V at week 26-33)	1.6 / 1.2
	E12	Monopolar	2	**10**	0	2.1 / 2.2
	E15	Monopolar	2	**10**	0	2.6 / 2.9
	E18	Monopolar	2	**10**	0	2.4 / 2.4
	E21	Monopolar	2	**10**	0	2.0 / 1.0
	E22	Monopolar	2	**10**	0	2.7 / 2.4
	E07	Monopolar	2	**10**	0	1.9 / 2.1

Data for participants in the early stimulation group (treatment group) assigned to grey shaded cells. Bold text specifies the longest duration (number of weeks) at corresponding voltage (V) output. For all but two participants (E13 and E08), this voltage was 2.5V bilaterally.

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Table 3: *Longitudinal analyses for diary-recorded seizures and GPFA*

A - Diary-recorded seizures: **absolute reduction in median seizure counts compared to pre-implantation baseline**			
Study phase	**Early-stimulation group (n=10)**	**Delayed-stimulation group (n=9)**	**p-value for interaction**
	Coefficient (95% CI)	**Coefficient (95% CI)**	
Pre-stimulation	-14 (-30.3 to 2.3)	-10.08 (-30.3 to 10.2)	
Blinded	-38.64 (-73.2 to -4.1)	-12.04 (-29.7 to 5.6)	*p=0.025*
Unblinded	-45.92 (-79.3 to -12.6)	-12.04 (-26.8 to 2.7)	
B - Diary recorded seizures: **% relative change in median seizure count compared to pre-implantation baseline**			
Study phase	**Early-stimulation group (n=10)**	**Delayed-stimulation group (n=9)**	**p-value for interaction**
	Coefficient (95% CI)	**Coefficient (95% CI)**	
Pre-stimulation	-34.2 (-49.8 to -18.6)	-17.6% (-37.2% to 2.0%)	
Blinded	-35.2 (-50.8 to -19.6)	-16.9 (-36.5 to 2.8)	*p=0.063*
Unblinded	-39.0 (-54.7 to -23.4)	-40.7 (-60.3 to -21.1)	
C – GPFA: **change in median number of seconds over 2 hour period of sleep EEG compared to pre-implantation baseline**			
Study phase	**Early-stimulation group (n=10)**	**Delayed-stimulation group (n=9)**	**p-value for interaction**
	Coefficient (95% CI)	**Coefficient (95% CI)**	
Pre-stimulation	-153.3 (-436.7 to -130.2)	-249.6 (-690.7 to 191.5)	
Blinded	-134.4 (-439.9 to -171.1)	-259.5 (-700.56 to 181.7)	*p=0.52*
Unblinded	-190.6 (-414.3 to -33.1)	-425.2 (-860.9 to 10.5)	

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Table 4. *Serious adverse events (SAE) and adverse events (AE) for all ESTEL participants*

	SAE		*AE - type*		
Participant	*Type*	*Phase*	*Pre-stim*	*Blinded*	*Unblinded*
Early stimulation					
E04	-	-	-	Paraesthesia	-
E09	-	-	-	Paraesthesia, drooling	-
E10	-	-	Headache	-	-
E13	Seizure-related injury	Pre-stim	Headache/pain over chest box	Paraesthesia, fatigue	Change in food preference
E14	-	-	-	-	-
E19	-	-	-	-	Headache
E17	-	-	Pain over chest box	-	Headache
E25	-	-	Weight loss	-	-
E24	-	-	Coughing with liquids	-	-
E23	-	-	Drooling	-	-
Delayed stimulation					
E05	-	-	-	-	Tongue/throat pain
E03	SE	Pre-stim	Weight loss	-	-
E08	SDH	Pre-stim	-	-	Paraesthesia
E12	Mega-edema	Pre-stim	Reduced appetite	-	-
E15	-	-	-	-	Mouth contraction
E18	Mega-edema	Pre-stim	Dribbling	-	-
E21	-	-	-	-	Drooling, speech disturbance, paraesthesia
E22	-	-	-	-	Paraesthesia
E07	SE	Blinded	-	-	Paraesthesia
Not randomized					
E16	Mega-edema & CNS infection	Pre-stim			

NB. Grey shaded periods denote the 3-months in which participants *first* received stimulation.
Abbreviations: CNS, central nervous system; Pre-stim, pre-stimulation phase of trial (i.e, after implantation but before randomisation/stimulation); SDH, sub-dural haematoma, requiring surgical evacuation; SE, status epilepticus.

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