Universitat Autònoma de Barcelona

HUMAN AUTONOMIC AND RESPIRATORY RESPONSES TO DIRECT CORTICAL ELECTRICAL STIMULATION

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Programa de Doctorado en Medicina

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To my family

ABBREVIATIONS

ANS: autonomic nervous system

BA 25: Brodmann area 25

BRS: baroreflex sensitivity

BPV: blood pressure variability

CO_2 : carbon dioxide

CNAP: continuous non-invasive arterial pressure

EKG: electrocardiograms

EEG: electroencephalogram

$ETCO_2$: end tidal carbon dioxide

GTCS: generalized tonic-clonic seizure

Hz: Hertz

HRV: heart rate variability

MATLAB: matrix laboratory

mA: milliamperes

µs: microseconds

PGES: post-ictal generalized EEG suppression

SEEG: stereotactic electroencephalogram

SpO_2: Peripheral capillary oxygen saturation

SAP: systolic arterial pressure

DAP: diastolic arterial pressure

SUDEP: sudden unexpected death in epilepsy

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1. SUMMARY

Patients with epilepsy are well known to be at increased risk of sudden unexpected death. The risk of Sudden Unexpected Death in Epilepsy Patients (SUDEP) ranges from 0.35 to 2.3 per 1000 people per year in community-based populations, to 6.3 to 9.3 in epilepsy surgery candidates. SUDEP's precise agonal mechanisms are unknown, although recent evidence from the Mortality in Epilepsy Monitoring Units Study (MORTEMUS) points to combined respiratory and cardiovascular collapse driving the fatal event.

Adverse autonomic nervous system signs are prominent during seizures. Cardiac arrhythmias (bradycardia, asystole, tachyarrhythmias) in approximately 72% of epilepsy patients, post-ictal hypotension, impaired baroreflex sensitivity (potentially compromising cerebral blood flow), enhanced sympathetic outflow, expressed as increased sweating and decreased inter-ictal nocturnal heart rate variability (HRV) are common. Severe alteration of breathing is typically seen in generalized tonic clonic seizures (GTCS). Electroencephalogram (EEG) characteristics, including post-ictal generalized EEG suppression (PGES), are suggestive of high SUDEP-risk, strongly correlate with increased sweating and decreased HRV, and may be accompanied by profound hypotension. Neural mechanisms underlying these patterns need to be defined.

Epilepsy is a prototypic cortical disorder, where most of the symptoms are produced by the activation or inhibition of specific regions in the cortex. Epileptiform discharges involving a specific area in the brain may induce symptoms related with that area's functionality. In a similar manner, electrical brain stimulation can be used to map brain functions.

Although several studies using brain electrical stimulation have suggested the possible role of cortical structures in respiration and autonomic control, reports from some investigators

have indicated mixed findings, such that there is no consensus on the precise areas of cortex concerned.

We aimed to identify cortical sites with roles in respiratory and/or autonomic control and to correlate seizure induced activation or inhibition of these structures to particular peri-ictal autonomic and breathing patterns recognized as potential indices of risk for death. This study describes the role of several limbic/paralimbic structures in respiration and human blood pressure control, and pathomechanisms of breathing and autonomic responses during epileptic seizures, providing insights into mechanisms of failure in SUDEP.

1.1. RESUMEN (Summary in Spanish)

Los pacientes con epilepsia son bien conocidos por tener un mayor riesgo de muerte súbita inesperada. El riesgo de muerte súbita inesperada en pacientes con epilepsia (SUDEP) varía de 0,35 a 2,3 por cada 1000 personas por año en las poblaciones de base comunitaria, a 6,3 a 9,3 en los candidatos a cirugía para la epilepsia. Los mecanismos agónicos precisos que desencadenan SUDEP son desconocidos, aunque la evidencia reciente del estudio de unidades de monitoreo de Epilepsia (MORTEMUS) apunta al colapso combinado respiratorio y cardiovascular que conduce al fatal evento.

Los signos adversos del sistema nervioso autónomo son prominentes durante las convulsiones. Arritmias cardíacas (bradicardia, asistolia, taquiarritmias) en aproximadamente el 72% de los pacientes con epilepsia, hipotensión post ictal, sensibilidad barorrefleja alterada (que puede comprometer el flujo sanguíneo cerebral), incremento del tono simpático, expresado como aumento de la sudoración y disminución de la variabilidad inter-ictal del ritmo cardíaco nocturno (HRV) son comunes. La alteración severa de la respiración se ve típicamente en las convulsiones clónicas tónicas generalizadas (GTCS). Las características del electroencefalograma (EEG), incluida la supresión generalizada post-ictal en el EEG (PGES), sugieren un alto riesgo de SUDEP, se correlacionan fuertemente con un aumento de la sudoración y una disminución de la HRV y pueden ir acompañadas de hipotensión profunda. Los mecanismos neuronales subyacentes a estos patrones necesitan ser definidos.

La epilepsia es un trastorno cortical prototípico, donde la mayoría de los síntomas se producen por la activación o inhibición de regiones específicas en la corteza. Las descargas epileptiformes que involucran un área específica en el cerebro pueden inducir síntomas relacionados con la funcionalidad de ese área. De manera similar, la estimulación eléctrica del cerebro se puede usar para mapear funciones cerebrales.

Aunque varios estudios que usan estimulación eléctrica cerebral han sugerido el posible papel de estructuras corticales en la respiración y el control autonómico, los informes de algunos investigadores han indicado hallazgos mixtos, de tal manera que no hay consenso sobre las áreas precisas de la corteza involucrada.

Nuestro objetivo fue identificar los sitios corticales con funciones en el control respiratorio y/o autonómico y correlacionar la activación inducida por las crisis epilepticas o la inhibición de estas estructuras, con particulares patrones autonómicos y respiratorios peri-

ictales reconocidos como posibles índices de riesgo de muerte. Este estudio describe el papel de varias estructuras límbicas/paralímbicas en la respiración y el control de la presión arterial humana, y los mecanismos patogénicos de la respiración y las respuestas autonómicas durante las crisis epilépticas, proporcionando información sobre los mecanismos que pueden desencadenan la muerte súbita inesperada en los pacientes con epilepsia (SUDEP).

2. INTRODUCTION

2.1. Sudden Unexpected Death in Epilepsy (SUDEP) definition

SUDEP is defined as the sudden, unexpected, witnessed or unwitnessed, non-traumatic, and non-drowning death of patients with epilepsy with or without evidence of a seizure, excluding documented status epilepticus, and in whom post-mortem examination does not reveal a structural or toxicological cause for death (1, 2) .

Cases that fulfil the above definition fall into the category of “definite SUDEP”, and sudden deaths occurring in benign circumstances with no known competing cause for death but without autopsy are classified as “probable SUDEP” (1, 2). Cases in which SUDEP cannot be excluded, either because of limited information about the circumstances of death or because there is plausible competing explanation for death, are classified as “possible SUDEP” (1, 2).

2.2. Incidence and scale of the problem

The risk of sudden unexpected death in patients with epilepsy is 20-40 times higher than the general population (3, 4). The risk of SUDEP varies in different epilepsy populations; it ranges from 0.35 to 2.3 per 1000 people with epilepsy per year in community-based populations (5, 6), 1.1 to 5.9 in epilepsy clinic populations-most with large proportions of patients with refractory seizures (6-12) and 6.3 to 9.3 in epilepsy surgery candidates or patients who continue to have seizures after surgery (13-15).

SUDEP’s precise agonal mechanisms are unknown and therefore, no preventive strategies exist. The suddenness and apparent silence of death suggest seizure-driven autonomic nervous system (ANS) failure, sustained apnea/asystole and some combination of respiratory and cardiovascular collapse. Recent evidence from the Mortality in Epilepsy Monitoring Units Study (MORTEMUS) points to combined respiratory and cardiovascular collapse driving the fatal event (1).

2.3. Risk factors for SUDEP

SUDEP is usually seizure-related (16). Epidemiological studies have consistently pointed to generalized tonic–clonic seizures (GTCS) as the seizure type most commonly associated with SUDEP (17-19). Witnessed, as well as monitored Epilepsy Monitoring Unit (EMU) deaths are noted to occur after GTCS, with frequent breathing difficulties (1, 20, 21). Poor seizure control, frequent and longstanding epilepsy are consistent risk factors (13, 17, 22-26). Deaths are typically un-witnessed, nocturnal events (8, 27) associated with prone position (23, 28, 29), and often have evidence of seizures (bitten tongue, urinary incontinence) (26). Young persons with epilepsy (20–40 years) are 24 times more likely to die suddenly than the general population, although SUDEP can occur at other ages (5, 6, 27, 30).

However, heterogeneity in SUDEP phenomenology is also described, and SUDEP and near SUDEP cases have been reported after partial seizures (1, 23). Three SUDEP cases without preceding seizure have recently been reported in literature (31). Although most of studies have shown consistency of risk factors, most individuals with similar risk profiles do not suffer SUDEP, and better definition of risk has been elusive to determine individualized risk profiles.

2.4. Peri-ictal autonomic and respiratory dysregulation and SUDEP mechanisms

2.4.1. Impact of cardiac autonomic dysregulation

Cardiac arrhythmias appear in ~72% of epilepsy patients (32-35). Ventricular tachycardia and fibrillation resulting in near-SUDEP have been reported (36). Nocturnal heart rate variability (HRV) (an indirect measure of autonomic function) is significantly reduced in epilepsy patients (37, 38) and decreased HRV accompanies increased sudden cardiac death risk (39). Refractory seizures are accompanied by decreased HRV, decreased cardiac sympathetic innervation, and may serve as autonomic SUDEP markers (40, 41). Autonomic dysfunction is marked in Dravet's

syndrome, where SUDEP incidence is high (42). Knockout Dravet's syndrome models show decreased HRV and prolonged atropine-sensitive ictal bradycardia, with tonic phases of GTCS preceding SUDEP (43). Brain, but not cardiac knockout of SCN1A produced SUDEP phenotypes, suggesting a hyperactive parasympathetic role, leading to lethal bradycardia (43).

2.4.2. Impaired baroreflex sensitivity and hypotension in peri-ictal periods

Maintenance of blood pressure and heart rate is partially mediated through medullary baroreflex circuitry, regulated by hypothalamic influences presumed to be modulated by insular, frontal, and cingulate cortices. Baroreflex (the ability to recover from blood pressure perturbations to maintain cardiovascular homeostasis) is altered in epilepsy patients in inter-ictal, non-seizure states (44, 45) and during Valsalva and tilt test blood pressure challenges (44). Hypotension has been described after seizures, and has been suggested as one potential sudden unexpected death in epilepsy (SUDEP) biomarker (46, 47).

2.4.3. Impact of cerebral dysregulation, Postictal Generalized EEG Suppression (PGES) and "Cerebral Shutdown"

Postictal Generalized EEG Suppression (PGES), consists of a background suppression pattern (activity less than 10 microvolts in the EEG) after a seizure, posited to represent complete cessation of cortical function, or "cerebral shutdown". PGES occurs in more than 65% of adult patients with generalized motor seizures (21). It has been suggested that prolonged (>50 seconds) PGES increases the risk of SUDEP (21), it correlates with decreased HRV, and is typically accompanied by profound hypotension. It is been suggested that interventions after GTCS shorten PGES, potentially reducing SUDEP risk.

2.4.4. Respiratory dysregulation.

Breathing dysfunction and hypoxia are typically seen in GTCS and have been suggested as a possible mechanism of Sudden Unexpected Death in Epilepsy (SUDEP) (1). However, oxygen desaturations have been also seen in 127 out of 253 seizures (50.3%) of patients with focal

seizures without generalized convulsions (48), being significantly more likely to be associated with temporal lobe seizures than extratemporal seizures (48). Ictal apnea has also been noted in 44-48 % focal seizures without generalization (48, 49) and has been reported as the main or isolated feature of focal seizures in a few case studies (50, 51). Some SUDEP and near SUDEP cases were preceded by a focal seizure without subsequent convulsive seizure (1) (52), and central apnea has been suggested as a potential mechanism of death or near-death (53, 54). A number of breathing abnormalities can occur during and after non-convulsive seizures. Ictal apnea, post-ictal apnea, and immediate post-ictal tachypnea are of greatest SUDEP interest as far as pathomechanisms are concerned (1, 55, 56). Laryngospasm has been also linked to Sudden Infant Death Syndrome and Sudden Unexplained Death in Childhood, and a recent study in an adult Sprague-Dawley urethane/kainite rat seizure model recorded severe laryngospasm, ST segment elevation, bradycardia and death (57).

The landmark MORTEMUS study showed consistent and previously-unrecognized patterns of tachypnea, profound cardiorespiratory dysfunction, and terminal apnea followed by cardiac arrest in 10 monitored SUDEP patients (1). Some SUDEP and near SUDEP cases have occurred with complex partial seizures without secondarily generalized convulsive seizures (1, 22). The hypoxemia is of special concern, since brain areas mediating hypoxia challenges show injury in SUDEP cases, which may impair recovery (58).

2.5. Cortical regulation for autonomic and respiratory function

2.5.1 Electrical cortical stimulation and brain mapping

Invasive electrical stimulation represents a non-physiological activation of the central nervous system. It does not mimic the elaborate physiological mechanisms that lead to selective excitation and inhibition of specific neurons in the central nervous system. Still, it may mimic some basic effects mediated by a given neuronal circuit. The most important application of

electro-cortical stimulation is mapping of eloquent cortex prior to resective brain surgery, including language, motor, visual and sensory areas. In addition to that, it allows confident identification of additional physiological functions of the cortex.

The following technical parameters influence the effectiveness of invasive electrical stimulation: polarity of the stimulus, current intensity, pulse width, frequency, and train duration (59).

2.5.2. Autonomic and blood pressure cortical regulation

Anterior limbic region stimulation in dogs and monkeys has produced marked falls in arterial blood pressure, as well as occasional rises (60-62). Such falls usually occurred without significant alteration in heart rate (60). Similar responses were seen after subcallosal, postorbital, anterior insular, cingulate gyrus, hippocampal, amygdalar, temporal and motor cortices stimulation (61-65).

In humans, where opportunities to conduct similar experiments are limited, few studies of cortical stimulation targeting blood pressure control structures exist. Stimulation of bilateral rostro-caudal cingulate gyrus (Brodmann areas 9 and 10) was carried out in psychotic patients prior to ablation in twelve cases (66). Blood pressure changes of systolic (SAP) and diastolic (DAP) elevation in eight patients, and a drop in one, were noted. Unilateral stimulation produced no responses at all. Orbitofrontal cortical stimulation in nine patients undergoing frontal lobotomies for psychiatric disease produced inconsistent elevation of SAP in six (67). Only subtle DAP and heart rate changes have been reported after stimulation of insular cortex in five patients with epilepsy undergoing surgery for control of intractable seizures.

2.5.3. Respiratory cortical regulation.

Breathing responses induced have been described with electrical stimulation in cats, dogs, and monkeys in a variety of brain regions including the posterior orbital surface of the frontal lobe

(68, 69), cingulate gyrus (70-72), amygdala (73), temporal polar cortex, uncus, anterior insula, and subcallosal region (68).

Kaada et al. found that stimulation of the parahippocampal gyrus, temporo-polar cortex, insula and anterior cingulate gyrus, had effects on respiratory movements in 8 patients undergoing brain surgery (74). Chapman et al. obtained cessation or decrease in respirations after orbitofrontal cortex stimulation in 7 of 9 patients in whom frontal lobectomy was carried out as a treatment of psychoses (75). Pool and Ransohoff reported increase of respiratory rate in 2 cases during bilateral cingulate gyrus stimulation and decrease of respiratory rate in 2 cases, in both, a period of complete apnea was observed (76). In current literature, there is only one study of human brain stimulation that assesses breathing function, and demonstrated amygdala stimulation-induced apnea in three patients (77).

3. RATIONALE AND OBJECTIVES

Individuals with intractable epilepsy have an approximately 0.5-1% annual risk of Sudden Unexpected Death in Epilepsy. SUDEP's precise agonal mechanisms are unknown and effective preventive strategies are unavailable. Recent evidence from the Mortality in Epilepsy Monitoring Units Study (MORTEMUS) points to combined respiratory and cardiovascular collapse driving the fatal event (1). Severe post-ictal hypotension has been suggested as a potential SUDEP biomarker. Hypoventilation and hypoxemia are typically seen in generalized tonic clonic seizures (GTCS) (1, 48), and severe alteration of breathing patterns after such seizures has been suggested as a possible mechanism of SUDEP (1). Ictal and post-ictal central apnea has been suggested as a potential mechanism in some SUDEP and near-SUDEP cases (54).

We hypothesize that certain focal brain structures have a specific role in autonomic and respiratory control. Identification of this structures may help us to understand autonomic and breathing patterns surrounding ictal discharges and may help us to determine processes underlying SUDEP risk.

The objectives of our project are:

Primary objective. To identify cortical control sites through electrical brain stimulation in epilepsy patients undergoing stereotactic electroencephalogram (SEEG) evaluation for epilepsy surgery. Several suprapontine brain structures will be investigated, including orbitofrontal cortex, cingulate gyrus, subcallosal gyrus, insula, hippocampus, parahippocampal gyrus, amygdala, temporo-polar cortex, lateral temporal cortex, and basal temporal cortex.

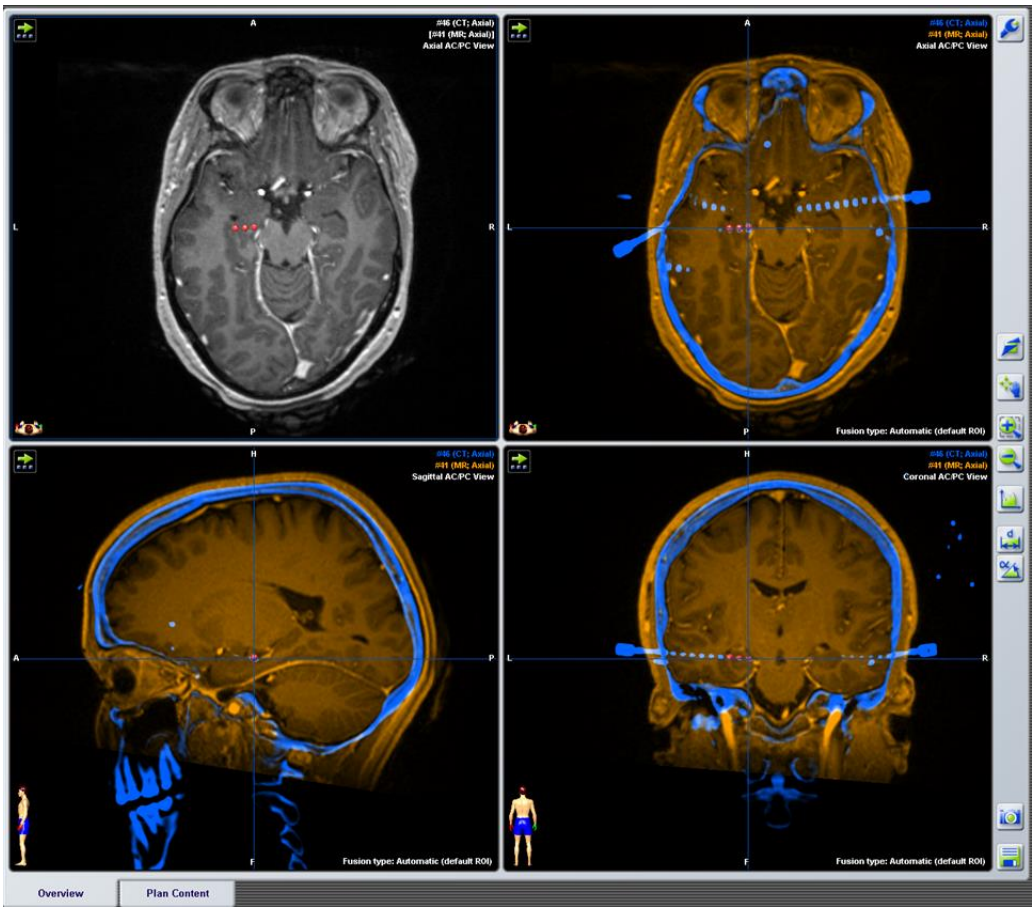
Secondary objective. To define the precise nature of respiratory and autonomic responses to stimulation of identified control sites, using polygraphic recordings of electroencephalography (EEG), oxygen saturation of arterial hemoglobin (SpO_2), end tidal and transcutaneous CO_2: (carbon dioxide), nasal airflow, respiratory rates, electrocardiograms (EKG) and continuous blood pressure monitoring.

4. MATERIALS AND METHODS

4.1. Experimental design. We prospectively studied 15 consecutive patients with medically intractable focal epilepsy undergoing stereotactic electroencephalogram (SEEG) evaluations for epilepsy surgery in the Epilepsy Monitoring Unit at University Hospitals Cleveland Medical Center. Inclusion criteria were patients $\geq$ 18 years of age, who had electrodes implanted in one or more of our brain regions of interest, and in whom direct cortical electrical stimulation was indicated for mapping of ictal onset and/or eloquent cortex regions. The number and locations of depth electrodes were tailored according to the putative epileptogenic zone in each patient, based on clinical history, semiology, neuroimaging, and scalp EEG. The local Institutional

Review Board reviewed and approved the study, and all patients signed informed consent prior to any study procedures. Platinum-iridium depth electrodes measuring 1.1 mm in diameter and 2.5 mm in length, evenly spaced at 5-mm intervals, were implanted stereotactically under general anesthesia. Implantation trajectories were simulated using iplan-stereotaxy 2.6 software (Brainlab, Munich, Germany) based on recent 3T MRI images of the brain. Cranial CT was performed within 24 hours post-surgically. Using iPlan software, postsurgical cranial computed tomography and presurgical brain MRI scans were co-registered for precise localization of single electrode contacts within each subject's pre-surgical MRI (Figure 1).

Figure 1. Preoperative brain magnetic resonance imaging co-registered with postoperative computed tomography scans showing the location of depth electrodes in the left hippocampus (red color) using iplan-stereotaxy 2.6 software (Brainlab, Munich, Germany).



Anatomical electrodes from post-operative CT scans were registered to standard Montreal Neurological Institute template MRI brain images using FLIRT linear registration available in FSL v5.0.9 (78) (https://fsl.fmrib.ox.ac.uk/fsl/). MRI cortical reconstruction and volumetric amygdala segmentations were performed using Freesurfer image analysis suite (http://surfer.nmr.mgh.harvard.edu/) (79). Resulting images were reconstructed in 3D space using 3D Slicer version 4.8 (80).

4.2. Stimulation

Bedside cortical electrical stimulation was carried out using one of the following stimulators: Ojemann (Integra Life Sciences, Plainesboro, NJ), Grass S-88X (Astro-Med, Inc., RI) or Nihon Kohden (MS-120BK-EEG). We used the following parameters:

A) Polarity. Monopolar stimulation refers to usage of a set of two electrodes where one electrode, the reference, is distant from the stimulating electrode, which we expect to produce the desired effect as a cathode or anode. Bipolar stimulation refers to a set of two electrodes in close proximity, in which either of the two electrodes can produce a cortical response. We carried out both bipolar and monopolar biphasic stimulation.

B) Phase. “Monophasic” when the stimulus is either positive or negative and “biphasic”, when positive and negative stimuli are delivered alternately. We exclusively used biphasic stimulation.

C) Frequency indicates the number of stimuli applied every second. We used the following frequencies: 1, 5 and 50 Hertz [Hz].

D) Pulse width is the pulse duration of each delivered stimulus. We used 200 microseconds [μs].

E) Train duration is the duration of each stimulation period. In our study they were from 2 up to 40 seconds.

F) Current intensity is the magnitude of the electric current as measured by the quantity of electricity across a specified area per unit time. We started at 1 milliampere [mA], increased in 1mA increments to a maximum of 10 mA, unless the stimulation induced a seizure.

These parameters were chosen for safety reasons, since these are the same as those used in brain mapping for clinical proposes (81). In the event of stimulation-induced seizures, stimulation was discontinued and testing was aborted. Resuscitation equipment and intravenous Lorazepam were always kept in close proximity to the patient in case of need.

4.3. Breathing, cardiac, blood pressure and EEG monitoring

Peripheral capillary oxygen saturation (SpO_2), and heart rate were monitored using pulse oximetry (Nellcor OxiMax N-600x; Covidien). Beat-to-beat systolic (SAP), diastolic (DAP) and mean arterial blood pressure (MAP) were continuously recorded using continuous noninvasive arterial pressure monitoring (AP Monitor 500 by CN Systems). Nasal airflow was recorded using a nasal thermistor (Thermocouple Airflow Sensor; Pro-Tech). End-tidal carbon dioxide ($ETCO_2$) was monitored using a capnograph (Model 7900; Philips) and transcutaneous CO_2 using a digital transcutaneous CO_2 sensor (Digital SenTec V-Sign™). Chest and abdominal excursions were recorded using inductance plethysmography (Ambu [Ballerup, Denmark] Sleepmate). EEG and ECG were acquired using a diagnostic system (EEG-1200; Nihon Kohden).

CNAP® Monitor 500; CNSystems Medizintechnik AG

Beat-to-beat systolic, diastolic and mean arterial blood pressure, were continuously recorded using a continuous noninvasive arterial pressure monitor (AP Monitor 500 by CN Systems).

The CNAP® Monitor 500 is designed for continuous noninvasive hemodynamic monitoring in a wide range of applications. The system provides real-time systolic, diastolic, mean blood pressure and pulse rate, high-fidelity blood pressure waveforms (pulse pressure variation), allowing for hemodynamic monitoring, automatic calibration to upper arm blood pressure and easy integration with the EEG acquisition system.

Nellcor OxiMax N-600x

Peripheral capillary oxygen saturation (SpO_2), and heart rate were monitored using pulse oximetry (Nellcor OxiMax N-600x).

The Nellcor OxiMax N-600x, Covidien pulse oximeter provides continuous non-invasive monitoring of SpO_2 and pulse rate. It includes a SatSeconds alarm. Advanced digital signal processing technology ensures accurate, reliable SpO_2 and pulse rate measurements even when low perfusion and interference occurs.

Ambu Sleepmate RIPmate®

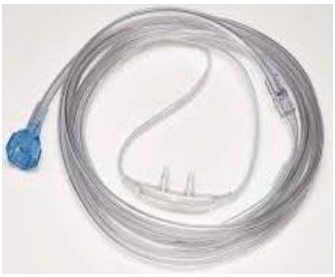
The RIPmate™ Respiratory Effort Sensors collect data for thoracic and abdominal respiratory effort. The system measures inductance changes in the wire of the belt as it expands and contracts with breathing. This change is represented as voltage output from the sensor.

The RIPmate™ system consists of a belt, a respiratory effort sensor that measures inductance changes in the belt and interfaces directly with a bipolar input on the recording device, and a cable that connects the sensor directly to the belt. The Ambu Sleepmate RIPmate™ Inductance Belts are designed for high sensitivity and patient comfort during measurement of chest and abdominal expansion associated with respiratory effort. Problems of signal loss due to lost belt tension and false paradoxical signals are eliminated.

Thermocouple Airflow Sensor; Pro-Tech®

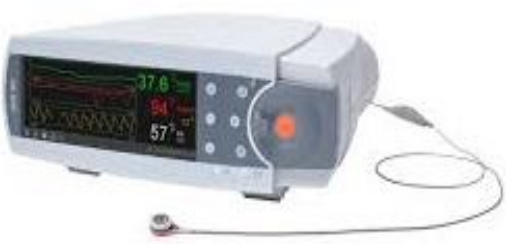
Nasal and oral airflow were recorded using a nasal thermistor (Air flow Sensor; Pro-Tech®). The thermistors are temperature sensors, and detect the temperature difference when the air flows in and out.

Phillips Respironics CAPNOGARD® Capnograph



The CAPNOGARD® provides reliable mainstream measurement and display of end tidal carbon dioxide ($ETCO_2$) and respiratory rate. Data from the Capnograph is acquired in real time by the EEG acquisition systems and downloaded to MATLAB.

Digital SenTec V-Sign™ Sensor CO2



The Digital SenTec V-Sign™ Sensor provides continuous and noninvasive real-time monitoring of transcutaneous CO_2, SpO_2 and pulse rate. It is a Stow-Severinghaus-type $SpCO_2$ sensor combined with reflectance 2-wavelength pulse oximetry. The highly integrated digital sensor head comprises a micro pH-electrode and an optical oximetry unit. The sensor temperature is regulated by two independent temperature sensors. All data is digitized in the sensor head, allowing the transmission of robust, low-noise signals to the monitor. Sensor sensitivity and calibration data is stored in the sensor head during manufacturing and regularly updated during use.

Nihon Kohden EEG-1200

Electroencephalogram (EEG) and electrocardiogram (EKG) were acquired using Nihon Kohden EEG-1200. The EEG acquisition system is a Nihon Kohden (Japan) system which is capable of simultaneously recording up to 192 channels of EEG at 1000 samples/second along with video recordings. In addition to video-EEG recording, 16 analog input signals can be connected to the system. These inputs are used for collection of other physiological measurements such as SpO_2, CO_2, respiratory rate, continuous blood pressure and EKG.

4.4. Breathing, blood pressure, cardiac and autonomic responses

A custom MATLAB (matrix laboratory) program was developed to analyze respiratory, SpO_2, end tidal and transcutaneous CO_2, airflow, thoracic and abdominal respiratory movement, EEG, ECG, beat to beat blood pressure, heart rate variability (HRV) and baroreflex sensitivity (BRS) (Figure 2).

A) Breathing. We defined central apnea as involuntary cessation of breathing (excursions and airflow) where at least one breath was missed, compared to baseline breathing rate, with a drop in peak signal excursion by >90% of pre-event baseline. Apnea onset was measured from the nadir of the preceding breath (that was clearly reduced) to the beginning of the first inspiratory effort that approximated baseline amplitude.

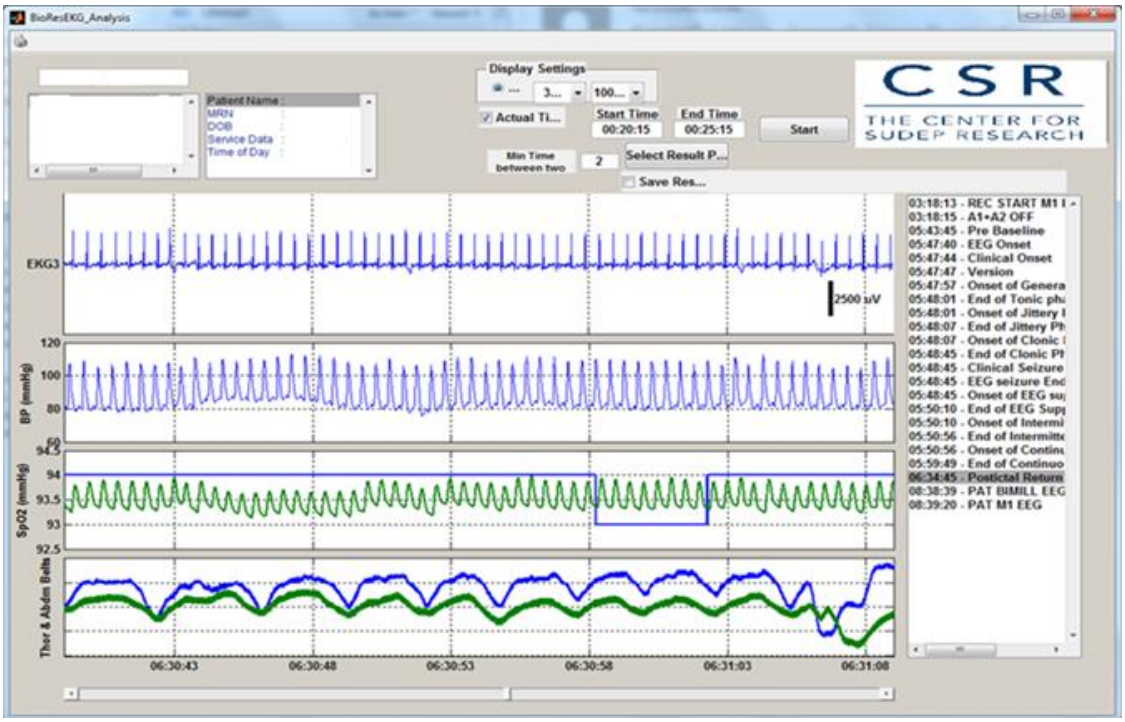
B) Blood pressure. We defined a significant response as a decrease or increase by >5 millimeters of mercury (mmHg) from the baseline mean during the stimulation period. The blood pressure response was only considered positive when there was a subsequent tendency to recover when stimulation was discontinued and when this response was consistently reproduced (during at least 5 sessions). Stimulation was only initiated when SAP was within normal limits (100-125mmHg), and immediately discontinued if it either dropped below 90 mmHg or by more than 25 mmHg from baseline.

C) Heart rate responses were defined as a heart rate change of >50% during stimulation period, when concomitant seizures were excluded in that period.

D) Blood pressure (BPV) and heart rate variability (HRV) and baroreflex sensitivity (BRS). To calculate autonomic responses before and during the stimulation, ECG R-Waves, SAP, and DAP values, as the maximum and minimum points between two consecutive R-Peaks were calculated. A series of four 5-minute consecutive epochs of artifact free awake state rest recordings were identified as baseline. Twenty minutes of frequency domain baroreflex sensitivity (BRS), blood pressure variability (BPV), and heart rate variability (HRV) values were averaged to calculate baseline values. Stimulation values were calculated from initiation of

stimulus until heart rate and blood pressure returned to baseline levels. Frequency-domain BRS was calculated as the average of the magnitude of the transfer function between oscillations of SAP and RR-Interval. Low frequency (LF) range was defined between 0.04-0.15 Hz and high frequency (HF) range was defined between 0.15-0.40 Hz. The LF/HF Ratio was used as a measure of sympatho-vagal balance. Total power (TP) for BRS 15 and HRV 10 was calculated as the sum of the LF and HF Bands (TP=LF+HF) and was used to normalize frequency domain values to correct for overall drops in total autonomic power. These normalized values were calculated by dividing the HF or LF band by TP and is reported as a percentage. The quantitative measure of the BRS was also provided by the slope of the fitted line, commonly expressed as the change in RR interval in milliseconds per millimeter of mercury change in SAP (ms/mmHg).

Figure 2. Analytics tool for physiological signal analysis



MATLAB program. Research analytics tool for physiological signal analysis including as pictured SpO_2 and abdominal and thoracic excursions, ECG and beat by beat blood pressure.

4.5. Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Science (SPSS, version 24; IBM Corp, Armonk, NY, USA). Summary statistics were reported as mean + standard deviation (median, range). Chi square tests were used to assess the association between several stimulation parameters and breathing response characteristics. The strength of the linear association (correlation) between the non-parametric variable apnea duration, with other variables, was measured using the Spearman's Rho correlation coefficient *r*. A paired samples t-test was conducted to compare autonomic responses to baseline values. The strength of the correlation between spontaneous SAP and RR intervals (baroreflex) was assessed by Pearson's correlation coefficient *r* and only those data sequences with r>0.7 were analyzed further. Significance was set at 2-sided $p<0.05$.

5. COPY OF THE PUBLICATIONS

5.1. AMYGDALA AND HIPPOCAMPUS ARE SYMPTOMATOGENIC ZONES FOR CENTRAL APNEIC SEIZURES. *Neurology* 2017; 88: 1-5.

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Amygdala and hippocampus are symptomatogenic zones for central apneic seizures

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ABSTRACT

Objective: To identify limbic sites of respiratory control in the human brain, and by extension, the symptomatogenic zone for central apnea.

Methods: We used direct stimulation of anatomically, precisely placed stereotactic EEG electrodes to analyze breathing responses. We prospectively studied 3 patients who were explored with stereotactically implanted depth electrodes. The amygdala and hippocampus, as well as extralimbic sites (orbitofrontal, temporal tip, and temporal neocortex), were investigated.

Results: Individual stimulation of the amygdala and hippocampal head consistently elicited central apnea in the expiratory phase, as did exquisitely focal hippocampal seizures.

Conclusions: These findings confirm that hippocampus and amygdala are limbic breathing control sites in humans, as well as the symptomatogenic zone for central apneic seizures. ***Neurology*®** **2017;88:1–5**

GLOSSARY

SUDEP = sudden unexpected death in epilepsy.

Video-monitored sudden unexpected death in epilepsy (SUDEP) cases suggest breathing dysfunction following generalized tonic-clonic seizures is involved in mechanisms of death, and terminal apnea precedes asystole.[1] Some SUDEP and near SUDEP cases have occurred with complex partial seizures without secondarily generalized tonic-clonic seizures.[1] Apnea was seen in 16 of 35 (48%) complex partial seizures[2] and seizure-associated O_2 desaturations were seen in 127 out of 253 (50.3%) nongeneralized focal seizures (temporal > extratemporal).[3] Apnea and O_2 desaturation mechanisms during partial seizures, and their relation to SUDEP, is not understood.[4–6] The role of cortical breathing control sites, through seizure-induced, disordered, or inhibited function, may be the key. Although several animal studies identify respiratory cortical control structures, human studies of cortical breathing are few. Invasive studies of brains of patients with refractory epilepsy undergoing assessment for epilepsy surgery provide opportunities for mapping cortical functions. Their identification may help us understand why people die after an epileptic seizure.

METHODS Patients and clinical setting. Patients aged >18 years with medically intractable epilepsy were undergoing stereotactic EEG evaluations for epilepsy surgery in the epilepsy monitoring unit at University Hospital Cleveland Medical Center. We prospectively studied 3 such consecutive patients. All patients were consented participants in a University Hospitals Case Medical Center institutional review board–approved research project evaluating the role of cortical structures in human respiratory and autonomic function.

Procedure. Platinum-iridium depth electrodes measuring 1.1 mm in diameter and 2.5 mm in length, evenly spaced at 5-mm intervals, were implanted stereotactically in the hippocampus, amygdala, and lateral temporal, orbitofrontal, temporal tip, and posterior cingulate neocortex under general anesthesia. Implantation trajectories were simulated using iplan-stereotaxy 2.6 software (Brainlab, Munich, Germany) based on recent 3T MRI of the brain. X-ray and cranial CT were performed within 24 hours postsurgically. Using iplan-stereotaxy 2.6 software, postsurgical cranial CT and presurgical brain MRI were superimposed for precise localization of single electrode contacts within the patient's presurgical MRI.

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Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

Stimulation. Bedside cortical electrical stimulation was carried out using the same parameters (bipolar stimulation, 50 Hz, and pulse width 0.2 ms) as for clinical brain mapping, with train durations of up to 30 seconds, using a Grass S-88X Stimulator (Astro-Med, Inc., West Warwick, RI). Current intensity started at 1 mA, and increased in 1 mA increments to a maximum of 10 mA. In case a seizure was induced, stimulation was discontinued and IV lorazepam (Ativan) was given to stop the seizure. Resuscitation equipment was always kept in intimate proximity to the patient in case of need.

Cardiorespiratory and EEG monitoring. Nasal airflow was recorded using a nasal thermistor (Pro-Tech airflow sensor). Chest and abdominal excursions were recorded using inductance plethysmography (Ambu [Ballerup, Denmark] Sleepmate). Arterial oxygen saturation (SpO_2) and heart rate were monitored using pulse oximetry (Nellcor OxiMax N600x; Medtronic, Langhorne, PA) and end tidal carbon dioxide ($ETCO_2$) using a capnograph (model 7900; Philips, Best, the Netherlands). EEG and ECG were acquired using a Nihon Kohden (Tokyo, Japan) EEG-1200 with 256-channel amplifier. We defined apnea as involuntary cessation of breathing where at least 2 breaths were missed, compared to baseline breathing rate, with a drop in peak signal excursion by ≥90% of pre-event baseline. Apnea onset was measured from the nadir of the preceding breath (that was clearly reduced) to the beginning of the first breath that approximated baseline amplitude. During stimulation, all patients had nasal thermal sensors to confirm presence and type of apnea. EEG was then analyzed for seizures and afterdischarges during the stimulation.

RESULTS Patient characteristics are shown in table 1. Stimulation of the amygdala and hippocampal head elicited consistent (reproduction of the same response at the same or higher stimulation intensity at the same site) apnea in all 3 patients (table 2); no breathing responses were seen from the temporal tip, lateral temporal, or orbitofrontal neocortex. At stimulation onset, immediate inhibition of respiration was clearly evident on belt/plethysmography signal and video with the thorax assuming the expiratory position. Patients were able to breathe out once but unable to breathe in again. Apnea periods ended before stimulation ended in all cases. When amygdalohippocampal seizures were induced, apnea duration was prolonged, but always ended before seizures ended. Slight increases in $ETCO_2$ were noted, but with no clear relationship to onset of rebreathing. Patients were always agnostic of apnea. In subsequent experiments, patient 3 was instructed to breathe during hippocampal stimulation–induced apnea and was immediately able to do so. Heart rate decreased or remained constant during apnea periods but significantly increased when clinical seizures were induced (table 2). In patient 2, whereas amygdala stimulation at 10 mA induced apnea, intensities of 6, 7, and 8 mA produced clear and instant disordered breathing pattern, more evident in the initial phase of stimulation, without fulfilling our definition of apnea (figures 1 and 2).

Table 1 Patient characteristics

Case	Age, y	Sex	Epilepsy duration	Handedness	Epileptogenic zone	Sz semiology	Sz frequency	Etiology	Brain MRI	Comorbidities
1	43	F	12 mo	Right	Left temporal[a]	Apneic sz → aura → dialeptic sz → GTCS	4/wk	Unknown	Normal	None
2	39	M	5 y	Right	Left temporal	Automotor sz → GTCS	3/wk	Unknown	Normal	None
3	39	M	10 y	Right	Left temporal	Dialeptic sz → GTCS	2/mo	Unknown	Normal	None

Abbreviations: Sz = seizure; GTCS = generalized tonic-clonic seizure.
[a]All patients were left temporal, since dominant hemisphere patients are more likely to undergo invasive evaluations.

Table 2 Characteristics of stimulation-induced apnea

Case	Location	Discharges before apnea	Induced discharges (time after apnea), s	Clinical symptome	Minimal stimulation intensity, mA	Time after apnea onset, s	Apnea duration, s	Prestimulation pO_2, %	Minimal pO_2, %	Prestimulation $ETCO_2$, mm Hg	Maximal $ETCO_2$, mm Hg	HR mean 10 seconds before stimulation, bpm	Maximum HR during stimulation, bpm
1	Amygdala (medial)	No	Yes (+2 s)	Yes	4	1.5	56	98	91	32	42	84	126
2	Amygdala (lateral)	No	No	No	10	2	8	95	92	38	43	60	60
2	Hippocampus head	No	Yes (+3 s)	No	3	1	14	95	95	38	40	60	54
3	Amygdala (lateral)	No	No	No	6	2	9	92	92	27	29	84	72
3	Hippocampus head	No	No	No	3	2	6	92	92	27	32	84	72
3	Hippocampus head	No	Yes (+8 s)	Yes	4	2	40	92	92	27	28	84	126

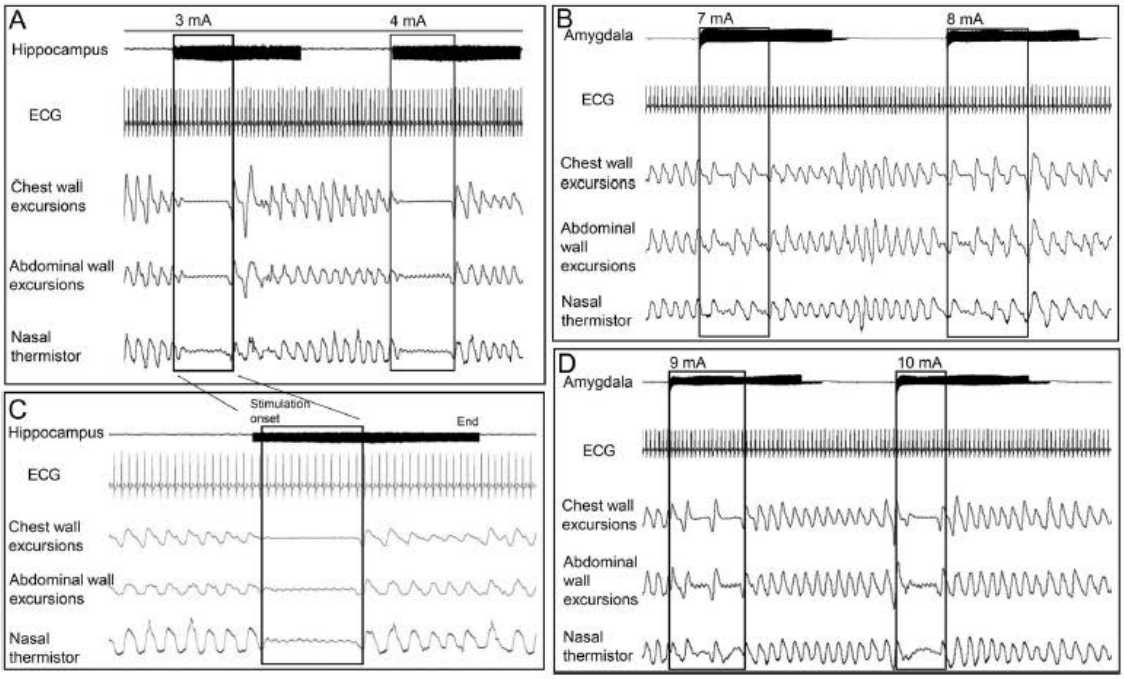
DISCUSSION Breathing responses have been described with electrical stimulation in cats, dogs, and monkeys in a variety of brain regions including amygdala, hippocampus, anterior cingulate, and anterior insular cortices. In humans, where opportunities for similar experiments are limited, few studies of cortical stimulation targeting breathing control structures exist. To our knowledge, there are only 2 studies in the literature describing amygdalar stimulation–induced apnea[7,8] and none of hippocampal stimulation–induced apnea in the modern era.

Our study, through electrical stimulation, definitively establishes the role of major limbic structures, confirming the amygdala stimulation results of Dlouhy et al.[8] and confirming the role of the hippocampus in breathing control. Inspiration was preferentially affected, and as an active process, is likely immediately inhibited, whereas expiration is allowed to occur as a passive phenomenon. The most likely mechanism for immediate apnea is stimulation-induced inhibition or disruption of brainstem inspiratory neuronal function. Inspiratory breathing rhythm is thought to be generated in the pre-Bötzinger complex, a group of neurons in the ventrolateral medulla of the brainstem. Perturbation of neural function in and around this area severely disrupts breathing rhythm.[9] The different types of breathing responses (apnea and disordered, irregular breathing) seen in our studies likely represent varying degrees of magnitude of response, depending on stimulus intensity.

In all stimulation periods producing apnea, patients started rebreathing before stimulation ended. Similar findings have been described in electrical stimulation studies in animals. Brain adaptation may explain this phenomenon. Several mechanisms for the overriding of apnea are possible. A previous study assessing breathing responses after amygdala stimulation postulated increased pCO_2 as an override mechanism that compelled rebreathing. It is indeed well-known that small changes in CO_2/pH affect breathing. Another plausible explanation lies in a lessening of seizure discharge intensity. In our patients, there was distinct devolution in discharge frequency or amplitude coincident with rebreathing.

The central apnea induced by amygdalohippocampal stimulation is of particular interest given its potential role as a SUDEP pathomechanism. It seems plausible that some apneic seizures produce prolonged, potentially fatal apnea. Asphyxia in animal models results in decline of heart rate and cardiac arrest within 5 minutes.[10] Based on our study and the most current observations, patients are able

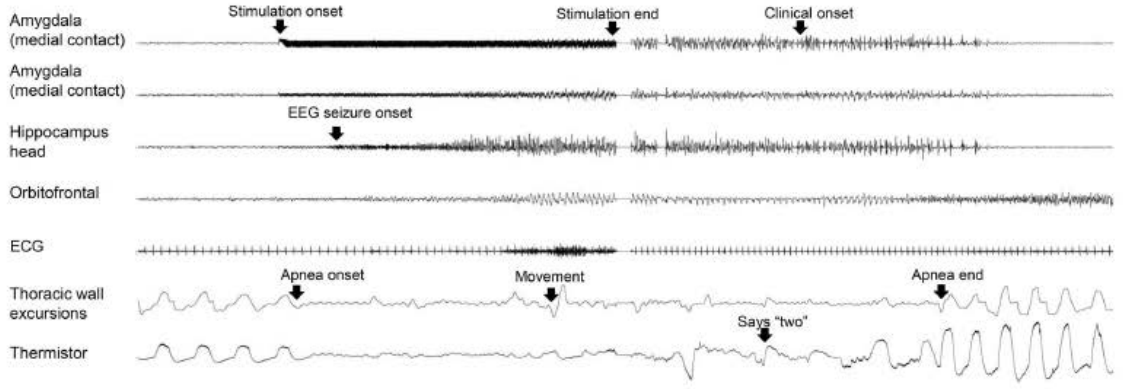
Figure 1 Apnea after hippocampus and amygdala stimulation



(A) Hippocampus head stimulation at intensities of 3 and 4 mA induced instant central apnea in patient 2. Apnea periods ended before stimulation was discontinued. (C) Apnea with 3 mA intensity stimulation of the hippocampus head in patient 2. (B, D) Stimulation in patient 2 at 6, 7, and 8 mA current intensities induced immediate change in respiratory rhythm. Central apnea was elicited at 10 mA current intensity in the same patient.

to override ictal apnea when instructed to talk, and asking patients with preserved consciousness to breathe may be helpful. However, since patients frequently lose consciousness or comprehension during seizures, active attempts to abort seizures producing apnea may be more crucial. It is worth emphasizing that respiratory monitoring of seizures in the epilepsy monitoring unit is an important facet to safety and the understanding of seizure, near-SUDEP, and SUDEP phenomena.

Figure 2 Apnea induced by amygdala stimulation and hippocampal seizure



Amygdala stimulation induced an immediate apnea in patient 1. Three seconds after apnea onset, a hippocampal seizure was seen. Breathing resumed when the seizure ended in the hippocampus and amygdala, but continued in the orbitofrontal cortex for a few seconds.

One of the limitations of our study is the small number of patients and the few structures that were stimulated. Involuntary suprapontine control of respiration may not be limited to the amygdala and hippocampus. Further studies including other brain regions are necessary for complete understanding of suprapontine breathing control.

AUTHOR CONTRIBUTIONS

Dr. Lhatoo: study supervision and critical revision of the manuscript for important intellectual content. Dr. Lacuey: study concept and design, analysis and interpretation. Dr. Zonjy: acquisition of data. Dr. Londono: acquisition of data.

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DISCLOSURE

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5.2. CORTICAL STRUCTURES ASSOCIATED WITH HUMAN BLOOD PRESSURE CONTROL. ***JAMA Neurology, 2018 Feb 1;75 (2):194-202***

JAMA Neurology | Original Investigation

Cortical Structures Associated With Human Blood Pressure Control

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IMPORTANCE A better understanding of the role of cortical structures in blood pressure control may help us understand cardiovascular collapse that may lead to sudden unexpected death in epilepsy (SUDEP).

OBJECTIVE To identify cortical control sites for human blood pressure regulation.

DESIGN, SETTING, AND PARTICIPANTS Patients with intractable epilepsy undergoing intracranial electrode implantation as a prelude to epilepsy surgery in the Epilepsy Monitoring Unit at University Hospitals Cleveland Medical Center were potential candidates for this study. Inclusion criteria were patients 18 years or older who had electrodes implanted in one or more of the regions of interest and in whom deep brain electrical stimulation was indicated for mapping of ictal onset or eloquent cortex as a part of the presurgical evaluation. Twelve consecutive patients were included in this prospective case series from June 1, 2015, to February 28, 2017.

MAIN OUTCOMES AND MEASURES Changes in continuous, noninvasive, beat-by-beat blood pressure parameter responses from amygdala, hippocampal, insular, orbitofrontal, temporal, cingulate, and subcallosal stimulation. Electrocardiogram, arterial oxygen saturation, end-tidal carbon dioxide, nasal airflow, and abdominal and thoracic plethysmography were monitored.

RESULTS Among 12 patients (7 female; mean [SD] age, 44.25 [12.55] years), 9 electrodes (7 left and 2 right) all in Brodmann area 25 (subcallosal neocortex) in 4 patients produced striking systolic hypotensive changes. Well-maintained diastolic arterial blood pressure and narrowed pulse pressure indicated stimulation-induced reduction in sympathetic drive and consequent probable reduction in cardiac output rather than bradycardia or peripheral vasodilation–induced hypotension. Frequency-domain analysis of heart rate and blood pressure variability showed a mixed picture. No other stimulated structure produced significant blood pressure changes.

CONCLUSIONS AND RELEVANCE These findings suggest that Brodmann area 25 has a role in lowering systolic blood pressure in humans. It is a potential symptomatogenic zone for peri-ictal hypotension in patients with epilepsy.

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Video

Supplemental content

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Peri-ictal autonomic dysregulation can occur during and after epileptic seizures, including significant blood pressure changes.[1,2] Hypotension has been described after seizures and has been suggested as a potential sudden unexpected death in epilepsy (SUDEP) biomarker.[3,4] The role of cortical blood pressure control sites, through seizure-induced, disordered, or inhibited function, may be key. Although some animal studies[5,6] describe cortical blood pressure control structures, only downstream, mainly brainstem regions have been convincingly identified in humans.[7] Invasive investigations of brains of patients with refractory epilepsy undergoing assessment for epilepsy surgery provide unique opportunities for mapping such potential cortical sites.[8] Their identification and electroclinical seizure characterization may yield clues to SUDEP pathomechanisms, as well as provide therapeutic targets in intractable essential hypertension.

Key Points

Question What cortical structures in the human brain are associated with blood pressure control?

Findings In this case series of 12 patients with intractable epilepsy who underwent deep brain electrical stimulation, 4 patients had electrodes placed in Brodmann area 25 (rostral subcallosal neocortex). Stimulation of 9 such electrodes (7 left and 2 right) in this area in these patients induced significant, consistent decreases in systolic blood pressure.

Meaning Brodmann area 25 may have a role in lowering systolic blood pressure in humans.

Methods

Rationale

Several suprapontine brain structures governing blood pressure function have been identified, albeit inconsistently, in animals (orbitofrontal, cingulate, subcallosal, insular, hippocampal, amygdalar, temporal, and motor cortices)[5,9-13] and in humans (orbitofrontal, insula, and anterior cingulate cortices).[4,14,15] Therefore, these were our regions of interest for this study. Using modern stereotactic techniques in patients undergoing invasive electroencephalogram (EEG) studies as a prelude to epilepsy surgery, we aimed to identify the role of these structures in human blood pressure control. All patients provided written informed consent as participants in a University Hospitals Cleveland Medical Center Institutional Review Board-approved research project evaluating the role of cortical structures in human respiratory and autonomic function.

Patients and Clinical Setting

From June 1, 2015, to February 28, 2017, we prospectively studied 12 consecutive patients with medically intractable epilepsy undergoing stereotactic EEG evaluations for epilepsy surgery in the Epilepsy Monitoring Unit at University Hospitals Cleveland Medical Center. Inclusion criteria were patients 18 years or older who had electrodes implanted in one or more of the above-mentioned brain regions of interest and in whom direct cortical electrical stimulation was indicated for mapping of ictal onset or eloquent cortex regions. Electrodes surrounded by radiologically visible hemorrhage were excluded from study. The number and locations of depth electrodes were tailored according to the suspected location of the epileptogenic zone in each patient based on clinical history, semiology, neuroimaging, and noninvasive EEG.

Procedure

Platinum-iridium depth electrodes measuring 1.1 mm in diameter and 2.5 mm in length, evenly spaced at 5-mm intervals, were implanted stereotactically using general anesthesia. Implantation trajectories were simulated using a software package (iPlan-Stereotaxy, version 2.6; Brainlab) based on recent 3-T magnetic resonance imaging (MRI) of the brain. Cranial computed tomography was performed within 24 hours after surgery. Using the iPlan-Stereotaxy software, postsurgical cranial computed tomography and presurgical brain MRI scans were superimposed for precise localization of single-electrode contacts within the patient's presurgical MRI.

Stimulation

Bedside cortical electrical stimulation was carried out using a stimulator (Ojemann; Integra Life Sciences) (bipolar and monopolar stimulation, 50 Hz and 0.2 milliseconds pulse width, with train durations of up to 30 seconds).[16,17] Current intensity started at 1 mA to a maximum of 10 mA. These parameters were chosen for safety reasons because they are identical to those used for brain mapping for clinical purposes. If a seizure was induced, stimulation was discontinued. Resuscitation equipment was always kept in intimate proximity to the patient in case of need.

Blood Pressure, Cardiac, Respiration, and EEG Monitoring

Beat-to-beat systolic (SAP), diastolic (DAP), and mean arterial pressure (MAP) were recorded using a continuous noninvasive arterial pressure monitor (Monitor 500; CNSystems Medizintechnik AG). Nasal airflow was recorded using a nasal thermistor (Thermocouple Airflow Sensor; Pro-Tech). Arterial oxygen saturation and heart rate were monitored using pulse oximetry (Nellcor OxiMax N-600x; Covidien) and end-tidal carbon dioxide using a capnograph (Model 7900; Philips). Electroencephalogram and electrocardiogram were acquired using a diagnostic system (EEG-1200; Nihon Kohden) with a 256-channel amplifier. We arbitrarily defined significant blood pressure response as a decrease or increase by more than 5 mm Hg from the baseline mean during the stimulation period. The blood pressure response was only considered positive if there was a subsequent tendency to recover when stimulation was discontinued and when this response was consistently reproduced (during ≥5 sessions). Stimulation was initiated when SAP was within normal limits (100-125 mm Hg) and was immediately discontinued if it dropped either below 90 mm Hg or by more than 25 mm Hg from baseline. The EEG was closely scrutinized for stimulation-induced seizures and after-discharges during stimulation; if after-discharges were induced, that stimulation period was excluded from analysis.

Table. Patient and Epilepsy Characteristics and Blood Pressure Responses

Patient No./ Sex/Age, y	Seizure Duration, y	Handedness	Epileptogenic Zone	Seizure Semiology	Seizure Frequency	GTCS Frequency	Etiology	Brain MRI	Hypotensive Responses
1/F/43	2	Right	Left temporal	Aura→dialeptic seizure→GTCS	4/wk	1/y	Cryptogenic	Normal	No
2/F/36	4	Right	Left temporal	Automotor seizure→right versive seizure→GTCS	1/wk	1/y	Cryptogenic	Normal	No
3/M/48	3	Right	Left temporal	Aura→dialeptic seizure	2/d	None	Cryptogenic	Normal	No
4/M/39	10	Right	Left temporal	Automotor seizure→GTCS	3/wk	2/mo	Cryptogenic	Normal	No
5/M/40	5	Right	Left temporal	Dialeptic seizure→GTCS	1-2/mo	1/y	Cryptogenic	Normal	No
6/M/66	30	Right	Left temporal	Automotor seizure→GTCS	2-3/wk	1/y	Cryptogenic	Normal	No
7/F/20	14	Right	Left hemisphere	Apnea seizure→right versive seizure→GTCS	1/d	1/d	Cryptogenic	Normal	Yes
8/F/32	25	Right	Left hemisphere	Hypnopompic seizure→right versive seizure→GTCS	1/mo	1/mo	Heterotopia	Right frontal heterotopia	Yes
9/M/26	8	Right	Right temporal	Automotor seizure	1/mo	None	Cryptogenic	Normal	Yes
10/F/49	3	Left	Left temporal	Automotor seizure	2/d	1/y	Cryptogenic	Normal	No
11/F/69	44	Right	Right temporal	Abdominal aura→automotor seizure→GTCS	1/mo	Once	Cryptogenic	Normal	No
12/F/63	50	Right	Left frontal	Asymmetric tonic seizure→GTCS	1/d	Twice	Cryptogenic	Normal	Yes

Abbreviations: GTCS, generalized tonic-clonic seizure; MRI, magnetic resonance imaging.

Data Analysis

A custom-developed graphical user interface (Matlab; MathWorks Inc) that included both signal processing and computational tools was used to automatically detect electrocardiogram R-wave, SAP, and DAP values as the maximum and minimum points between 2 consecutive R peaks. A series of four 5-minute consecutive epochs of artifact-free awake-state rest recordings were identified as baseline. Twenty minutes of frequency-domain baroreflex sensitivity (BRS), blood pressure variability (BPV), and heart rate variability (HRV) values were averaged to calculate baseline values. Stimulation values were calculated from initiation of stimulus until heart rate and blood pressure returned to baseline levels.

Frequency-domain BRS was calculated as the average of the magnitude of the transfer function between oscillations of SAP and RR interval. Low-frequency (LF) range was defined between 0.04 and 0.15 Hz, and high-frequency (HF) range was defined between 0.15 and 0.40 Hz. The ratio of LF to HF was used as a measure of sympathovagal balance. Total power (TP) for BRS[18] and HRV[13] was calculated as the sum of the LF and HF bands (TP = LF + HF) and was used to normalize frequency-domain values to correct for overall drops in total autonomic power. These normalized values were calculated by dividing the LF or HF band by TP and are reported as a percentage using the following equation: Normalized Unit Value for HF = [HF / (LF + HF)] × 100.

The evaluation of BRS is an established tool to assess autonomic control of the cardiovascular system.[18] Changes in the characteristics of baroreflex function reflect alterations in autonomic control of the cardiovascular system.[19] The quantitative measure of BRS is provided by the slope of the fitted line, and it is commonly expressed as the change in RR interval in ms per mm Hg change in SAP (ms/mm Hg). Calculated slope in sleep and awake states in healthy individuals is 9 to 12 ms/mm Hg.[20] An increase in SAP accompanied by a limited change in RR interval with a calculated slope lower than 3 ms/mm Hg identifies a pathological weak autonomic response.

Statistical Analysis

All values are expressed as the mean (SEM). A paired-samples *t* test was conducted to compare stimulation averages with baseline values. Significance was set at 2-sided $P < .05$. The strength of the linear association (correlation) between spontaneous SAP and RR intervals was assessed by Pearson product moment correlation coefficient *r*, and only those data sequences with *r* exceeding 0.7 were analyzed further.

Results

Demographics and characteristics of the 12 patients (7 female; mean [SD] age, 44.25 [12.55] years) are summarized in the **Table**. Patient 12 had essential hypertension treated with amlodipine besylate (5 mg/d) that was continued during the evaluation. The rest of the patients did not have any cardiorespiratory comorbidity and were not taking any cardiovascular medications. At the time of stimulation, patient 1 was taking his regular dosage of lacosamide (200 mg/d) and topiramate

(400 mg/d), patient 5 was taking clonazepam (0.25 mg/d), and patient 12 was taking levetiracetam (4500 mg/d), vimpat (600 mg/d), and clobazam (30 mg/d). The remaining patients were off antiepileptic medications as a part of the clinical protocol aimed at capturing seizures to localize the epileptogenic zone. In total, 1084 electrode contacts were implanted based on the surgical hypothesis in each case. Of these, 544 electrodes were implanted in our regions of interest, including 43 amygdala, 87 hippocampus head and body, 16 insular, 31 orbitofrontal, 31 temporopolar, 296 lateral temporal, 4 basal temporal, 13 anterior cingulate, 9 subcallosal (Brodmann area 25), and 14 posterior cingulate neocortex. Of 544 electrodes, 126 were stimulated according to the study protocol, including 23 amygdala, 17 hippocampus head, 8 anterior insula, 16 orbitofrontal, 12 temporopolar, 24 lateral temporal, 2 basal temporal, 13 anterior cingulate, 9 subcallosal neocortex (Brodmann area 25), and 2 posterior cingulate (eTable in the Supplement). The rest of the electrodes (540 of 1084) were placed either in white matter or in gray matter outside our regions of interest.

Stimulation in all electrodes placed in Brodmann area 25 in patients 7, 8, 9, and 12 (in whom 9 electrodes [7 left and 2 right] produced striking systolic hypotensive changes) resulted in rapid and consistently reproducible decreases in SAP, with a mean (SEM) drop of 15 (10-42) mm Hg (**Figures 1**, **2**, **3**, and **4** and **Video 1** and **Video 2**). The SAP decreases appeared after a mean (SEM) latency of 8.5 (1-14) seconds. At times, the fall in SAP was preceded by a slight rise (Figure 2). Once stimulation was discontinued, SAP began to increase within a mean (SEM) of 12 (1-47) seconds (Figures 1, 2, and 3 and the eTable in the Supplement). The DAP did not change concurrently with SAP, resulting in a consistent narrowing of pulse pressure in all patients (Figures 1, 2, and 3 and the eFigure in the Supplement). Heart rate responses differed. In patient 8, heart rate increased accordingly with SAP. On the other hand, in patients 7, 9, and 12, heart rate did not significantly change (Figures 1, 2, and 3 and the eFigure in the Supplement). Arterial oxygen saturation and end-tidal carbon dioxide did not change at any time during or after stimulation. Frequency-domain analysis of BRS, BPV, and HRV comparing baseline with the stimulation period and baroreflex slope was performed in patients 7, 8, and 9 and showed a mixed picture (Figures 1, 2, and 3).

During some of the Brodmann area 25 stimulation sessions, brief after-discharges were induced, although there were no differences in blood pressure responses when the after-discharges were induced or not. However, we excluded stimulations with after-discharges to ensure that blood pressure responses were being produced exclusively by Brodmann area 25 stimulation and not by after-discharges in other brain areas.

We analyzed recorded seizures in those patients in whom we found hypotensive responses to specifically look for spontaneous peri-ictal hypotensive changes and for correlation of seizure discharges in Brodmann area 25 with hypotension. Patient 7 did not have hypotensive changes with the single seizure that was recorded; the Brodmann area 25 electrode was involved in the seizure, although the seizure discharge was widespread at that point. Patient 8 had no blood pressure recordings during seizures. Patient 9 had no seizures recorded during intracranial EEG monitoring with blood pressure recordings. However, he previously had 3 complex partial seizures with oral automatisms recorded with surface EEG and continuous blood pressure monitoring. Two of these had ictal and post-ictal hypotension (Figure 4). Patient 12 had asymmetric tonic seizures lasting for less than 10 seconds in which blood pressure did not change and where the seizure did not involve Brodmann area 25.

No significant blood pressure responses were noted after stimulation of amygdala, hippocampus, and insular, orbitofrontal, temporopolar, lateral temporal, basal temporal, anterior cingulate, and posterior cingulate neocortex. Central apnea induced by stimulation was observed in temporal lobe structures and reported separately.[21] Apnea was not associated with blood pressure responses.

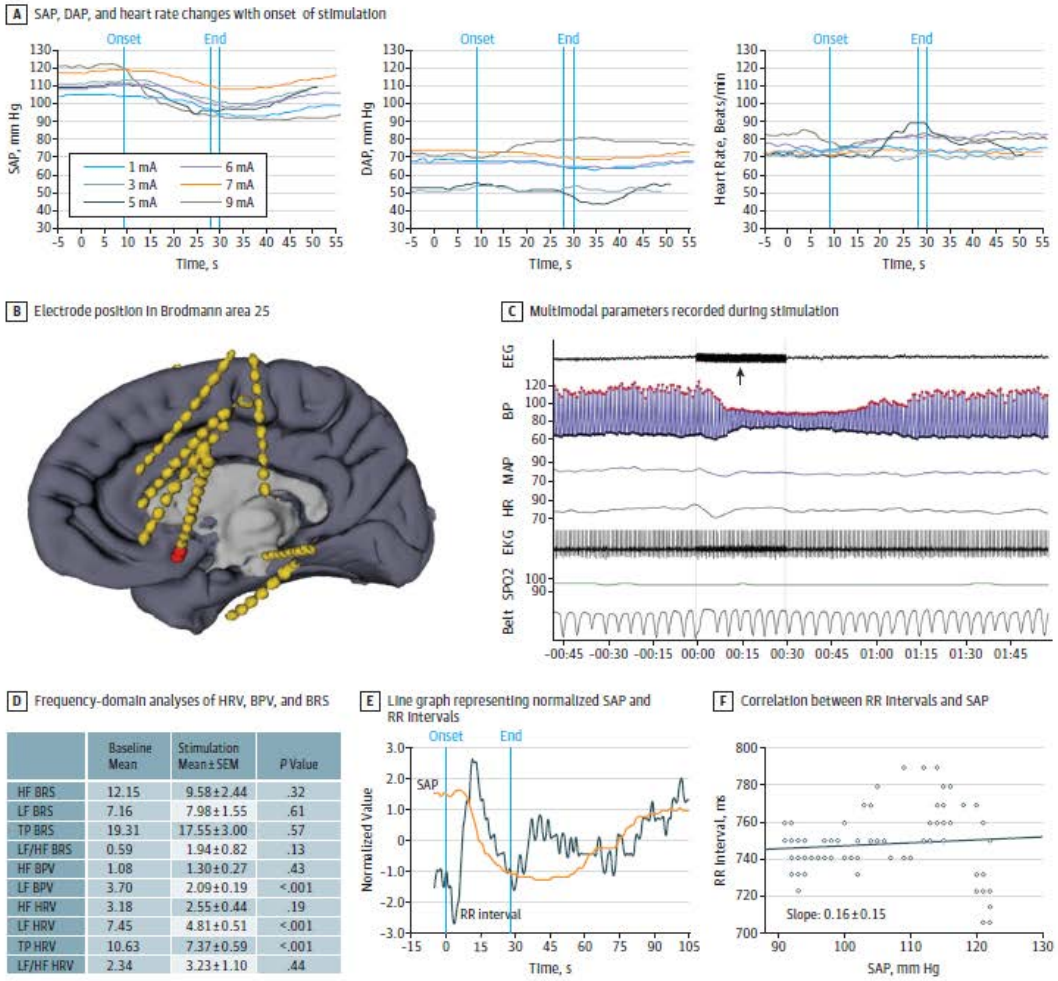
Discussion

The results of this study suggest that Brodmann area 25 has a role in lowering systolic blood pressure in humans and is a likely symptomatogenic site for peri-ictal hypotension. However, these data need to be reproduced in a larger sample of patients. This region is infrequently studied as a part of orbitofrontal and anterior cingulate invasive EEG explorations in refractory focal epilepsy, hence the small sample size in our study for which implantations were driven by the surgical rather than study hypothesis. Brodmann area 25 is also a site that has been reported to produce hypotensive changes in animals.[5] In humans, although the role of cortical structures in blood pressure control is inferred, this has hitherto not been conclusively established, and no single brain region has been universally accepted as a control site. Anterior limbic region stimulation in dogs and monkeys has produced marked falls in arterial blood pressure, as well as occasional rises.[5,9,10] Such falls usually occurred without significant alteration in heart rate.[9]

Similar responses were seen after subcallosal, postorbital, anterior insular, cingulate gyrus, hippocampal, amygdalar, temporal, and motor cortices stimulation.[5,10-12,22] In humans, for whom opportunities to conduct similar experiments are limited, few studies of cortical stimulation targeting blood pressure control structures exist. In one study,[23] stimulation of bilateral rostrocaudal cingulate gyrus (Brodmann areas 9 and 10) was carried out among patients with psychosis before ablation in 12 cases. Blood pressure changes of SAP and DAP elevation in 8 patients and a drop in 1 patient were noted. Unilateral stimulation produced no responses at all. In another study,[24] orbitofrontal cortical stimulation in 9 patients undergoing frontal lobotomies for psychiatric disease produced inconsistent elevation of SAP in 6 of them. In a third study,[25] only subtle DAP and heart rate changes were reported after stimulation of insular cortex in 5 patients with epilepsy undergoing surgery for control of intractable seizures. Therefore, the present investigation is the first report to date of a dramatic, consistently reproducible blood pressure influence in all patients who had the same, restricted, cortical site stimulated, namely, the subcallosal region of Brodmann area 25.

Our use of stereotactic EEG provides unprecedented depth electrode placement precision in deep cortical structures.[17] This

Figure 1. Stimulation-Induced Cardiovascular Changes in Patient 7

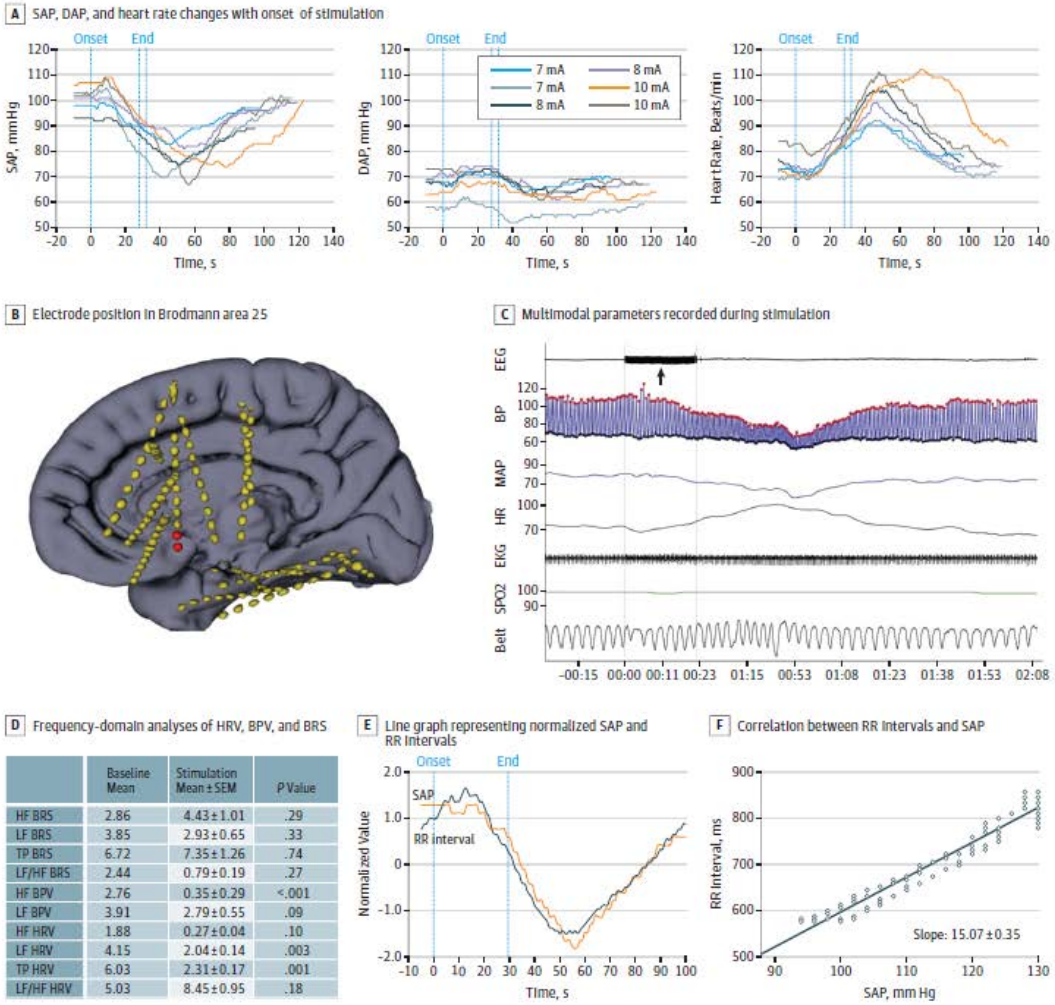


	Baseline Mean	Stimulation Mean±SEM	*P* Value
HF BRS	12.15	9.58±2.44	.32
LF BRS	7.16	7.98±1.55	.61
TP BRS	19.31	17.55±3.00	.57
LF/HF BRS	0.59	1.94±0.82	.13
HF BPV	1.08	1.30±0.27	.43
LF BPV	3.70	2.09±0.19	<.001
HF HRV	3.18	2.55±0.44	.19
LF HRV	7.45	4.81±0.51	<.001
TP HRV	10.63	7.37±0.59	<.001
LF/HF HRV	2.34	3.23±1.10	.44

Stimulation parameters used were 50 Hz, 0.2 milliseconds, and 1 to 9 mA. A, Systolic (SAP), diastolic (DAP), and heart rate (HR) changes with onset of stimulation. B, Electrode position in Brodmann area 25 (in red). C, Multimodal parameters recorded during stimulation (black arrow) demonstrating a clear reduction in SAP and a narrowing of pulse pressure, with no changes in oxygenation or breathing. Red dots in the blood pressure (BP) tracing represent SAP, and black dots represent DAP. D, Frequency-domain analyses of heart rate variability (HRV), blood pressure variability (BPV), and baroreflex sensitivity (BRS) showing significant decrease of low-frequency (LF) BPV and LF HRV. E, Line graph representing normalized SAP and RR intervals. F, Correlation between RR intervals and SAP, and the corresponding RR milliseconds/mm Hg slope of 0.2, indicating reduced BRS. EEG indicates electroencephalogram; EKG, electrocardiogram; HF, high frequency; MAP, mean arterial pressure; SPO_2, arterial oxygen saturation; and TP, total power.

not only localizes putative epileptogenic zones but also allows unique anatomical resolution for direct electrical stimulation and subsequent, confident identification of eloquent cortical structures. Stimulation parameters identical to those used in routine clinical cortical mapping suffice for these purposes.[16,17] Repeated stimulation of 7 electrodes in the immediately adjacent anterior cingulate cortex of patients 10 and 11 herein produced no such responses. In patient 9, blood pressure responses were definitely present but were less impressive, most likely due to their significantly more anterior location close to the border between Brodmann areas 24 and 25 (Figures 1, 2, and 3). This suggests that blood pressure influences are limited to Brodmann area 25. It is also consistent with previous observations of no blood pressure responses with unilateral anterior cingulate stimulation.[23] Similarly, albeit with the limited number of patients in our study, orbitofrontal, insula (anterior part), amygdalar, hippocampal head, posterior cingulate, temporopolar, and temporal neocortex stimulations did not produce blood pressure responses; therefore, we could not confirm the role of these structures in human

Figure 2. Stimulation-Induced Cardiovascular Changes in Patient 8



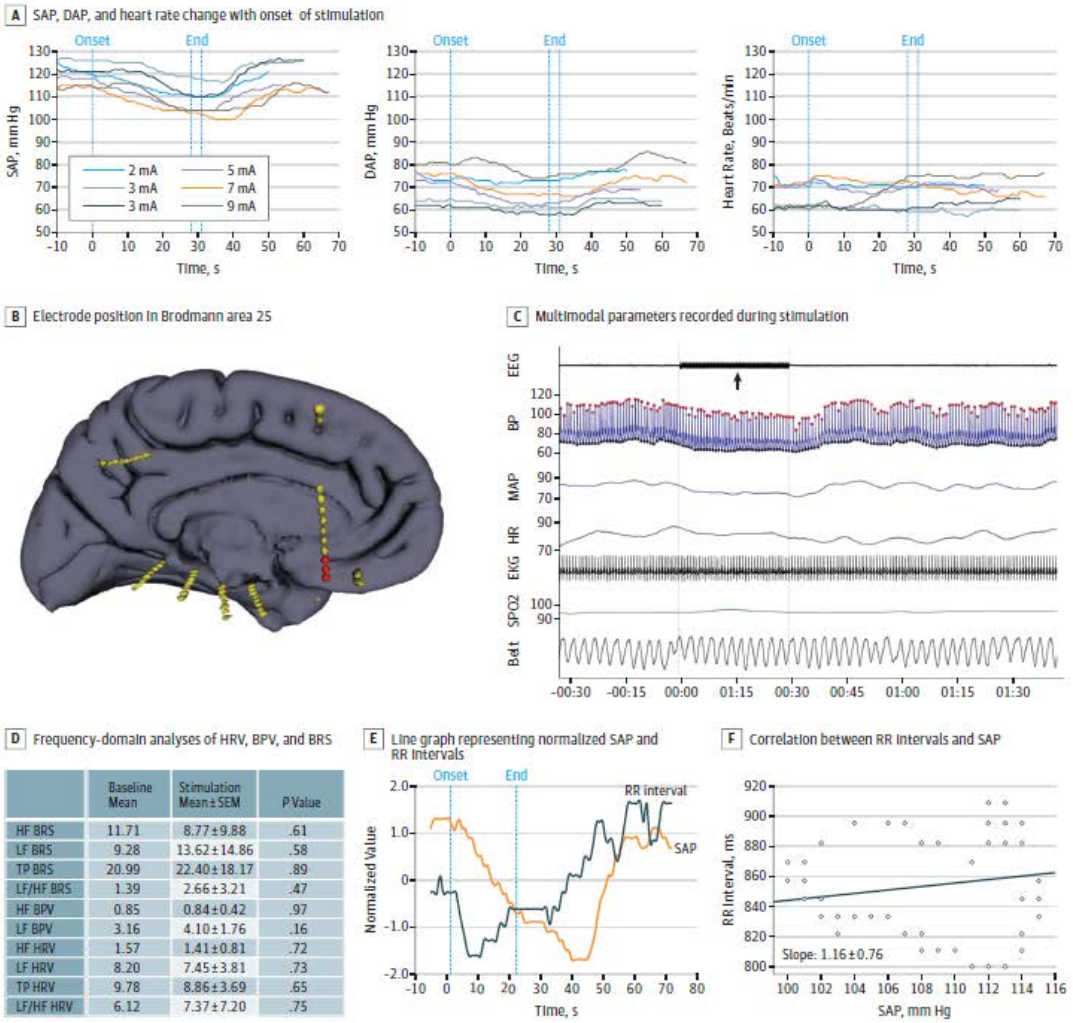
	Baseline Mean	Stimulation Mean ± SEM	*P* Value
HF BRS	2.86	4.43 ± 1.01	.29
LF BRS	3.85	2.93 ± 0.65	.33
TP BRS	6.72	7.35 ± 1.26	.74
LF/HF BRS	2.44	0.79 ± 0.19	.27
HF BPV	2.76	0.35 ± 0.29	<.001
LF BPV	3.91	2.79 ± 0.55	.09
HF HRV	1.88	0.27 ± 0.04	.10
LF HRV	4.15	2.04 ± 0.14	.003
TP HRV	6.03	2.31 ± 0.17	.001
LF/HF HRV	5.03	8.45 ± 0.95	.18

Stimulating parameters used were 50 Hz, 0.2 milliseconds, and 1 to 10 mA. A, Systolic (SAP), diastolic (DAP), and heart rate (HR) changes with onset of stimulation. Red dots represent SAP, and black dots represent DAP. B, Electrode position in Brodmann area 25 (in red). C, Multimodal parameters recorded during stimulation (black arrow) demonstrating a clear reduction in SAP and a narrowing of pulse pressure, with no changes in oxygenation or breathing. D, Frequency-domain analyses of heart rate variability (HRV), blood pressure variability (BPV), and baroreflex sensitivity (BRS). E, Line graph representing normalized SAP and RR intervals. F, Correlation between RR intervals and SAP, and the corresponding RR milliseconds/mm Hg slope of 15, indicating intact BRS. EEG indicates electroencephalogram; EKG, electrocardiogram; HF, high frequency; MAP, mean arterial pressure; SPO_2, arterial oxygen saturation; and TP, total power.

autonomic control of blood pressure. Several reasons are possible. Human studies that have reported blood pressure changes with stimulation of the cingulate and insula regions have used stimulation parameters with substantially greater stimulus intensity than in our study. For example, Pool and Ransohoff[23] used up to 120-Hz frequency and 60-second train durations in their study, possibly accounting for their positive findings in these brain structures. Patients 7, 8, and 12 had insula stimulation in our study, both of whom only had anterior insular stimulation.

The mechanism of such striking falls in SAP without concurrent falls in DAP and heart rate is likely due to a cardioinhibitory reduction in myocardial contractility and a reduction in left ventricular stroke volume, as indicated by the narrowing of pulse pressure in all of our patients. The lack of significant changes in DAP (a product of resting transmural

Figure 3. Stimulation-Induced Cardiovascular Changes in Patient 9 With Impaired Autonomic Responses



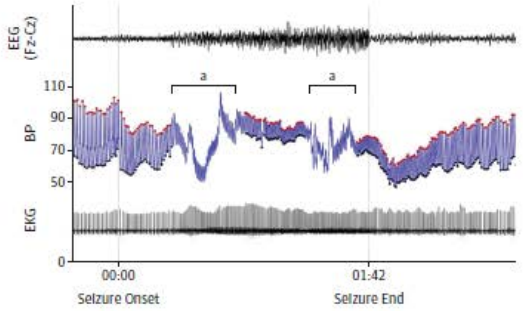
	Baseline Mean	Stimulation Mean±SEM	*P* Value
HF BRS	11.71	8.77±9.88	.61
LF BRS	9.28	13.62±14.86	.58
TP BRS	20.99	22.40±18.17	.89
LF/HF BRS	1.39	2.66±3.21	.47
HF BPV	0.85	0.84±0.42	.97
LF BPV	3.16	4.10±1.76	.16
HF HRV	1.57	1.41±0.81	.72
LF HRV	8.20	7.45±3.81	.73
TP HRV	9.78	8.86±3.69	.65
LF/HF HRV	6.12	7.37±7.20	.75

Stimulating parameters used were 50 Hz, 0.2 milliseconds, and 1 to 9 mA. A, Systolic (SAP), diastolic (DAP), and heart rate (HR) changes with onset of stimulation. B, Electrode position in Brodmann area 25 (in red). C, Multimodal parameters recorded during stimulation (black arrow). Red dots represent SAP, and black dots represent DAP. D, Frequency-domain analyses of heart rate variability (HRV), blood pressure variability (BPV), and baroreflex sensitivity (BRS). E, Line graph representing normalized SAP and RR intervals. F, Correlation between RR intervals and SAP, and the corresponding RR milliseconds/mm Hg slope. EEG indicates electroencephalogram; EKG, electrocardiogram; HF, high frequency; MAP, mean arterial pressure; SPO_2, arterial oxygen saturation; and TP, total power.

force blood volume exerted against vascular walls) during the stimulation period excludes peripheral vasodilatation as a cause. Stimulation of Brodmann area 25 likely produces downstream influences in or distal to the lateral hypothalamic nuclei or ventral periventricular or periaqueductal gray areas, brainstem regions known to produce blood pressure effects.[26-28] Rich connections with Brodmann area 25 are found in these regions.[29] Downstream candidate brainstem structures include the nucleus of the rostral and ventrolateral medulla, medullary raphe, and the A5 noradrenergic group of the pons.[14,30] The ultimate influence is likely to be a reduction in sympathetic outflow in the efferent arm of the baroreflex emanating from the rostral ventrolateral medulla.

The LF component of systolic BPV and HRV represents a good marker of sympathetic activity. Increase in HF HRV is thought to reflect an increase in vagal tone.[13] The baroreflex response represents the autonomic response to changes in blood pressure and can be quantified by a slope (9-12 ms/mm Hg in

Figure 4. Hypotension During and After a Seizure



Shown is ictal and postictal hypotension during a complex partial seizure with oral automatisms in patient 9 recorded with surface electroencephalogram (EEG) and continuous blood pressure (BP) monitoring. Red dots represent systolic arterial pressure, and black dots represent diastolic arterial pressure. EKG indicates electrocardiogram.
[a]Two periods during ictus when movement artifact of the blood pressure–cuffed limb renders acquisition unreliable.

healthy individuals).[20] In patient 7 herein, LF BPV and LF HRV were both significantly lower (−43.51% and −35.44%) during stimulation compared with baseline values ($P < .001$) without significant changes in HF HRV. This finding suggests a decrease in sympathetic tone. In patient 8, frequency-domain analyses showed mixed results. Both LF and HF BPV and HRV were decreased, suggesting a combination of sympathetic and parasympathetic influences. That could reflect the combination of stimulation cardiovascular influences and a subsequent baroreflex response. The calculated baroreflex slope was 15 ms/mm Hg in this patient, pointing to an excellent baroreceptor response characterized by a decrease in vagal efferent neural traffic, allowing a compensatory increase in heart rate. On the other hand, in patient 7, the calculated baroreflex slope was 0.2 ms/mm Hg, suggesting a poor baroreflex response. In patient 9, frequency-domain BRS analysis showed no significant changes before and during stimulation, although the responses were significantly weaker (Figure 3).

The differences in BRS between patients suggest that patients 7 and 9 have impaired compensatory responses to SAP decreases. The reasons for this were not explained by any phenotypic features, including seizure type, presence of generalized tonic-clonic seizures, seizure frequency, epileptogenic zone, or duration of epilepsy, and much larger study samples may be required to discern their relevance.

Whether these findings can be extrapolated to the population with epilepsy or to healthy individuals at large is questionable given our sample size. However, autonomic dysregulation is well described in epilepsy.[31,32] Impaired baroreflex function has been observed in temporal lobe epilepsy.[15] Whereas such autonomic dysregulation may have a number of implications, a major unknown in the understanding of SUDEP pathomechanisms is the role of profound peri-ictal hypotension.[3] None of the observed SUDEP and near-SUDEP cases reported in literature has had blood pressures recorded.[33] It is possible that some persons with epilepsy accrue greater tendencies to autonomic dysfunction than others and thus become prone to peri-ictal, potentially fatal, hypotension due to impaired homeostatic mechanisms. Certainly, patient 9 herein demonstrated hypotension during and immediately after partial seizures, although its significance with regard to the patient's SUDEP risk is unknown. We could not use the SUDEP-7 risk inventory score[34] for our study because there was no nonhypotensive group with which to make SUDEP-7 score comparisons. However, this is planned as an important aspect of a larger study as we continue to accrue patients.

Our findings may have therapeutic implications. Stimulation of Brodmann area 25 in patient 12, with chronic hypertension, induced similar reduction in systolic blood pressure (Figure 4 and Video 2). Stimulation of the ventral periventricular and periaqueductal gray areas, used as a treatment for chronic pain, has been proposed as sites for deep brain stimulation for the treatment of intractable hypertension.[26-28,35,36] Variability in blood pressure responses at the same locations[37] and the potential for complications in the brainstem render the periaqueductal and periventricular gray regions as unattractive targets for that purpose. On the other hand, Brodmann area 25 is an easily accessible and safe target used in deep brain stimulation for the treatment of refractory depression.[38-40] Investigations of Brodmann area 25 have not reported symptomatic hypotension and may reflect differences in stimulation parameters (typically 3.5 mA) used or brain adaptation to continuous stimulation.[41] Further studies are warranted to determine potential effective parameters of long-term electrical stimulation of Brodmann area 25 in intractable essential hypertension.

Limitations

Some limitations of our study need to be considered. First of all, the sample size was small, and we included only patients with medically refractory focal epilepsy. These data need to be reproduced in a larger cohort of patients to elucidate if our findings can be extrapolated to the general epilepsy population or to individuals without epilepsy.

Conclusions

The findings in this study suggest that Brodmann area 25 has a role in lowering systolic blood pressure in humans. It is a potential symptomatogenic zone for peri-ictal hypotension in patients with epilepsy.

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responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Miller, Lhatoo.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Lacuey, Hampson, Zonjy, Vithala, Hupp, Loparo, Miller, Lhatoo.
Critical revision of the manuscript for important intellectual content: Theeranaew, Miller, Lhatoo.
Statistical analysis: Lacuey, Hampson, Vithala.
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Study supervision: Loparo, Lhatoo.

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6. SUMMARY OF THE RESULTS

Subjects and clinical setting. Between June 2015 and February 2018, 15 subjects were recruited into the study after informed consent (eight female, seven male; mean age 42 [20-69] years old). Only one subject had a lesion visible on MRI. All but one were right handed. Patient demographics and characteristics are shown in Table 1. Putative epileptogenic zones, were temporal in 11 (eight left, three right), left frontal in two and non-localizable left hemispheric in two. Apart from essential hypertension treated with a calcium channel blocker (5 mg Amlodipine) in subject 12, none had cardiorespiratory comorbidity, and none were on any medications other than anti-seizure agents. At the time of stimulation, subject 1 was on habitual doses of Lacosamide (200 mg/day) and Topiramate (400 mg a day); subject 5 (Clonazepam 0.25 mg a day) and 12 (Levetiracetam 1500 mg three times a day, Lacosamide 200 mg three times a day and Clobazam 15 mg twice a day) were on reduced medication. The remaining subjects were off seizure medications as a part of the clinical protocol aimed at capturing seizures to localize the epileptogenic zone.

Stimulating electrodes.1410 electrodes were implanted, using SEEG techniques, of which 633 were placed in regions of interest (56 amygdala, 100 hippocampus, 24 insular, 41 orbitofrontal, 41 temporopolar, two parahippocampal gyrus, 323 lateral temporal, four basal temporal, 19 anterior cingulate, nine subcallosal, and 14 posterior cingulate neocortices). Of 633 electrodes, 185 were stimulated according to the study protocol (33 amygdala, 27 hippocampus, two parahippocampal gyrus, 10 anterior insula, 20 orbitofrontal, 26 temporopolar, 35 lateral temporal, two basal temporal, 19 anterior cingulate, nine subcallosal and two posterior cingulate neocortices (Figure 4 and Table 2). The remaining electrodes (777/1410) were outside our regions of interest.

Table 1. Patient and epilepsy characteristics

Case	Age	Sex	Seizure Duration (years)	Handedness	Epileptogenic Zone	Seizure semiology	Seizure frequency	GTCS frequency	Brain MRI	Pathology
1	43	F	2	R	L mesial temporal	Apneic sz→Aura→ Dialeptic sz→ GTCS	4/week	1/year	Normal	Gliosis
2	36	F	4	R	L temporal	Automotor sz→ R versive sz→ GTCS	1/week	1/year	Normal	N/A
3	48	M	3	R	L mesial temporal	Aura → Dialeptic sz	2/day	None	Normal	N/A
4	39	M	10	R	L temporal	Automotor sz→ GTCS	3/week	2/month	Normal	Gliosis
5	39	M	5	R	L lateral temporal	Dialeptic sz→ GTCS	1-2/month	1/year	Normal	Neuronal Heterotopia
6	66	M	30	R	L hippocampus	Automotor sz→ GTCS	2-3/week	1/year	Normal	N/A
7	20	F	14	R	L hemisphere	Apnea sz→ R versive sz→ GTCS	1/day	1/day	Normal	N/A
8	32	F	25	R	L hemisphere	Hypnopompic sz→ R versive sz→ GTCS	1/month	1/month	Heterotopia	N/A
9	26	M	8	R	R amygdala	Automotor sz	1/month	None	Normal	N/A
10	49	F	3	L	L mesial temporal	Apneic sz→ Automotor sz	2/day	1/year	Normal	Gliosis
11	69	F	44	R	R mesial temporal	Abdominal aura→ Automotor sz→ GTCS	1/month	Once	Normal	Gliosis/ Astrocytosis
12	63	F	50	R	L mesial frontal	Asymmetric tonic sz→GTCS	1/day	Twice	Normal	FCD I
13	38	M	2	R	L orbito-frontal	Tonic sz→ Automotor sz	2/week	None	Normal	FCD II
14	33	M	10	R	R temporal	Apneic sz→Aura→Automotor sz→GTCS	1-2/week	1 / 2 months	Normal	Neuronal loss with reactive astrocytosis
15	27	F	7	R	L temporal	Apneic sz→Automotor sz	1-2/month	Twice	Normal	N/A

Legend: F: female, M: male, R: right, L: left, GTCS: generalized tonic-clonic seizure, sz: seizure, FCD: focal cortical dysplasia, N/A: not available.

Table 2. Stimulating electrode contacts location in each patient

	Numbers of electrodes in each cortical site (L=left hemisphere, R=right hemisphere)										
Case	**AMY**	**H**	**PH**	**Insula (anterior)**	**OF**	**T Pole**	**Lateral T**	**Basal T**	**AC**	**Subcallosal**	**PC**
1	2 (L)	0	0	0	4 (L)	0	4(L)	0	0	0	0
2	2 (L)	0	0	0	4 (L)	0	0	0	0	0	2 (L)
3	3 (L)	0	0	0	4 (L)	0	0	0	0	0	0
4	3(L)	4 (L)	0	0	0	2 (L)	4(L)	0	0	0	0
5	4 (L)	4 (L)	0	0	0	0	0	0	0	0	0
6	3 (L)	0	0	0	0	0	2 (L)	0	0	0	0
7	2 (L)	4 (L)	0	3 (L)	0	0	4 (L)	0	4 (L)	2 (L)	0
8	2 (R)	0	2(R)	2(L)	0	2(L)	2(L)	2(L)	2 (L)	2 (L)	0
9	2(R)	2(R)	0	0	0	3(R)	0	0	0	2 (R)	0
10	0	2 (L)	0	0	0	2 (L)	4(L)	0	3 (L)	0	0
11	2 (R)	3 (R)	0	0	4 (R)	4 (R)	4 (R)	0	2 (R)	0	0
12	0	0	0	3(L)	0	0	0	0	2 (L)	3 (L)	0
13	2(R)	2(L)	0	2(R)	2(R)	3(R) 3(L)	0	0	2(R) 2(L)	0	0
14	2 (R)	2(R)	0	0	0	3 (R)	5(R)	0	2 (R)	0	0
15	4(L)	4(L)	0	0	2(L)	2(L)/2(L)	6(L)	0	0	0	0

Legend: AMY: amygdala; H: Hippocampus; PH: Parahippocampal gyrus; OF: orbitofrontal, T: temporal; AC: anterior cingulate gyrus; PC: posterior cingulate gyrus.

6.1. Respiratory responses to cortical electrical stimulation

Table 3 summarizes stimulated electrode contact locations that induced breathing alterations. In each case, the breathing response was cessation of both thoracic wall movements and airflow, consistent with central apnea. Precise anatomical locations of electrodes are shown in Figure 4. Unilateral stimulation of limbic regions (amygdala, hippocampus, anterior parahippocampal gyrus), and the paralimbic region of the mesial temporo-polar cortex in both hemispheres independently elicited central apnea in 12/15 cases. In the remaining 3/15 subjects, precise assessments on apnea could not be made because of recording artifact (two cases); one additional patient only had electrodes in the insula, anterior cingulate and rostral subcallosal gyri, regions that did not elicit apnea with stimulation in this and any other patient. During apnea periods, there were no significant changes in blood pressure or heart rate.

Stimulation of temporal structures

Amygdala

Amygdala stimulation of 26 electrodes (18 left, eight right [Figure 4A and 5 and Table 3]) induced apnea in 10 of 13 subjects. In the remaining three subjects, no apnea was seen, as breathing was either obscured by movement artifact in the plethysmographic breathing belt signal (Subjects 2 and 3 [Table 3]), or testing was aborted because of stimulation-induced clinical seizures (Subject 11 [Table 3]).

In subject 1, amygdala stimulation induced a habitual seizure with ictal central apnea (ICA) at seizure onset that lasted for 56 seconds. The patient had left mesial temporal lobe epilepsy, and ICA during habitual seizures. An example of ICA during one spontaneous seizure is shown in figure 6. In subject 10, hippocampus stimulation induced a seizure with ICA as the only clinical manifestation at seizure onset that lasted for 12 seconds (Figure 7). The patient had left mesial temporal lobe epilepsy, and ICA as the first clinical manifestation during habitual

spontaneous seizures (Figure 8). Subject 6 had an amygdala stimulation-induced seizure and ICA lasting 34 seconds.

Mean apnea durations during periods uncontaminated by seizures or afterdischarges, was 10.6± 2.5 (10; 7-16) seconds. We found breathing responses in all major amygdalar nuclei, including lateral, basal and central nuclei (Figure 5 and Table 4). However, there were some differences in stimulus parameters used. Low frequency stimulation (1 and 5 Hz) of the mesial part of the amygdala, including basal (Subjects 8, 9 and 14) and central nuclei (Subject 13) was sufficient to induce apnea. However, the lateral amygdala nucleus required higher current intensity and stimulation frequency to induce apnea (Subject 5 and 15 [Table 4]). An example of amygdala stimulation-induced apnea is shown in figure 9A.

Hippocampus

Hippocampal head and body stimulation induced apnea in seven of nine subjects, after stimulation of 20 electrode contacts (16 left, four right [Table 3 and Figure 4B]). In the remaining two subjects, no apnea comment could be made because of stimulation-induced seizures and consequently aborted testing. In two subjects, stimulation produced apneic seizures; apnea persisted beyond stimulation end, with no other clinical signs.

Mean apnea duration during periods uncontaminated by seizures or afterdischarges was 10.2+ 2.5 (10; 5-16) seconds. Apnea induced by stimulation of the hippocampus in subject 4 is shown in figure 10.

Parahippocampal gyrus: (anterior parahippocampal gyrus [Brodmann Area 35]) Parahippocampal stimulation was carried out in subject eight only (Figure 4E and 9D). The longest non-seizure, stimulation-induced apnea in our study was seen with parahippocampal gyrus stimulation in this case (23 seconds). Mean apnea duration was 19.3+ 2.9 (20; 15-23)

seconds. An example of parahippocampal gyrus stimulation-induced apnea is shown in figure 9D.

Temporo-polar cortex (Brodmann Area 38)

Temporo-polar stimulation induced apnea in five of eight subjects with electrodes in this structure during stimulation of 13 electrodes (seven right, six left [Table 3]). All stimulated electrode contacts that induced apnea were located in the mesial part of the temporo-polar region (Figure 4C). An example of mesial temporo-polar cortex stimulation-induced apnea is shown in figure 11. Temporal tip and lateral temporo-polar electrodes in four cases (four, nine, 13 and 15) failed to induce breathing responses. Mean apnea duration was 8.4± 2.6 (8; 4-13) seconds.

Lateral temporal and basal temporal neocortices

A total of 35 electrodes were stimulated in the superior and middle temporal gyri and two in the basal temporal; none produced breathing changes.

Stimulation of extra-temporal structures

Anterior, posterior cingulate and rostral subcallosal gyri stimulation (Brodmann Area 32, 24 and 25 respectively) was investigated in 30 sites in nine subjects [Figure 4H]. In subject 13, additional bilateral anterior cingulate stimulation was carried out. Neither unilateral nor bilateral stimulation (in subject 13) induced breathing changes.

Orbitofrontal (20), and anterior insula (10) gyri were also investigated in both hemispheres, without breathing changes. Stimulation of these areas did not induce any seizures during stimulation.

Table 3. Stimulating electrode contacts location in each patient

	Numbers of electrodes in each cortical site (L=left hemisphere, R=right hemisphere)										
Case	**AMY**	**H**	**PH**	**Insula (anterior)**	**OF**	**T Pole**	**Lateral T**	**Basal T**	**AC**	**Subcallosal**	**PC**
1	2 (L)	0	0	0	4 (L)	0	4(L)	0	0	0	0
2	2 (L)*	0	0	0	4 (L)	0	0	0	0	0	2 (L)
3	3 (L)*	0	0	0	4 (L)	0	0	0	0	0	0
4	**3(L)**	4 (L)	0	0	0	2 (L)	4(L)	0	0	0	0
5	**4 (L)**	**4 (L)**	0	0	0	0	0	0	0	0	0
6	3 (L)	0	0	0	0	0	2 (L)	0	0	0	0
7	**2 (L)**	**4 (L)**	0	3 (L)	0	0	4 (L)	0	4 (L)	2 (L)	0
8	**2 (R)**	0	**2(R)**	2(L)	0	**2(L)**	2(L)	2(L)	2 (L)	2 (L)	0
9	**2(R)**	**2(R)**	0	0	0	3(R)	0	0	0	2 (R)	0
10	0	2 (L)	0	0	0	**2 (L)**	4(L)	0	3 (L)	0	0
11	2 (R)**	3 (R)**	0	0	4 (R)	**4 (R)**	4 (R)	0	2 (R)	0	0
12	0	0	0	3(L)	0	0	0	0	2 (L)	3 (L)	0
13	**2(R)**	**2(L)**	0	2(R)	2(R)	3(R) 3(L)	0	0	2(R) 2(L)	0	0
14	**2 (R)**	**2(R)**	0	0	0	**3 (R)**	5(R)	0	2 (R)	0	0
15	**4(L)**	4(L)**	0	0	2(L)	**2(L)**/2(L)	6(L)	0	0	0	0

In red, sites with stimulation-induced apnea (without induced seizures).

* Breathing not assessable due to movement or electrode artifact.

** Testing aborted because of induced clinical seizure.

▭ Stimulation sites where low current intensity induced both seizure and apnea.

AMY: amygdala; H: Hippocampus; PH: Parahippocampal gyrus; OF: orbitofrontal, T: temporal; AC: anterior cingulate gyrus; PC: posterior cingulate gyrus.

Characteristics of Stimulation-induced Central Apnea.

Effect of stimulation on breathing cycle.

Apnea always occurred following completion of an expiratory cycle. Stimulation in the inspiratory phase of the breathing cycle, produced inhibition of inspiratory effort at any point of that phase, evident on respiratory belt signal and video, with the thorax then assuming a resting position (i.e., no active inspiration or expiration). If stimulation was initiated at any point in the expiratory phase of the breathing cycle, expiration was continued to completion.

Perception of breathlessness from apnea during stimulation.

Patients were always agnostic of apnea and thus upon questioning, none were aware of any breathing difficulties or dyspnea during the apnea period. When reiteratively asked "did you feel anything", the answer was always "no". Processes for voluntary air movement involving vocalization superseded stimulation effects; when patients were instructed to voluntarily breathe, and then, stimulation was initiated, we were unable to produce apnea, despite using identical stimulation parameters.

Effect of stimulus current intensity and frequency on breathing.

High current intensity ($\geq$5mA) was significantly associated with longer apnea duration ($p=0.04$) compared to low intensity (<5mA) (Figure 12B). On the other hand, high versus low stimulation frequency (50 Hz versus 1 or 5 Hz) did not affect apnea duration ($p=0.6$). High frequency stimulation (50Hz) was followed by immediate apneic responses, whereas low frequency stimulations (1 or 5 Hz) resulted in delayed apnea onset (apnea began 1-2 breaths after stimulation onset) ($p<0.001$) (Figure 12C). An example of delayed apnea after low frequency stimulation (at 5 Hz) is shown in figure 9D.

Although breathing responses were more easily elicitable using high current intensity and high stimulus frequency, we found that doing so with amygdala and hippocampus stimulations often resulted in electroclinical seizures, where seizure manifestations prevented conclusions on the pure effect of stimulations.

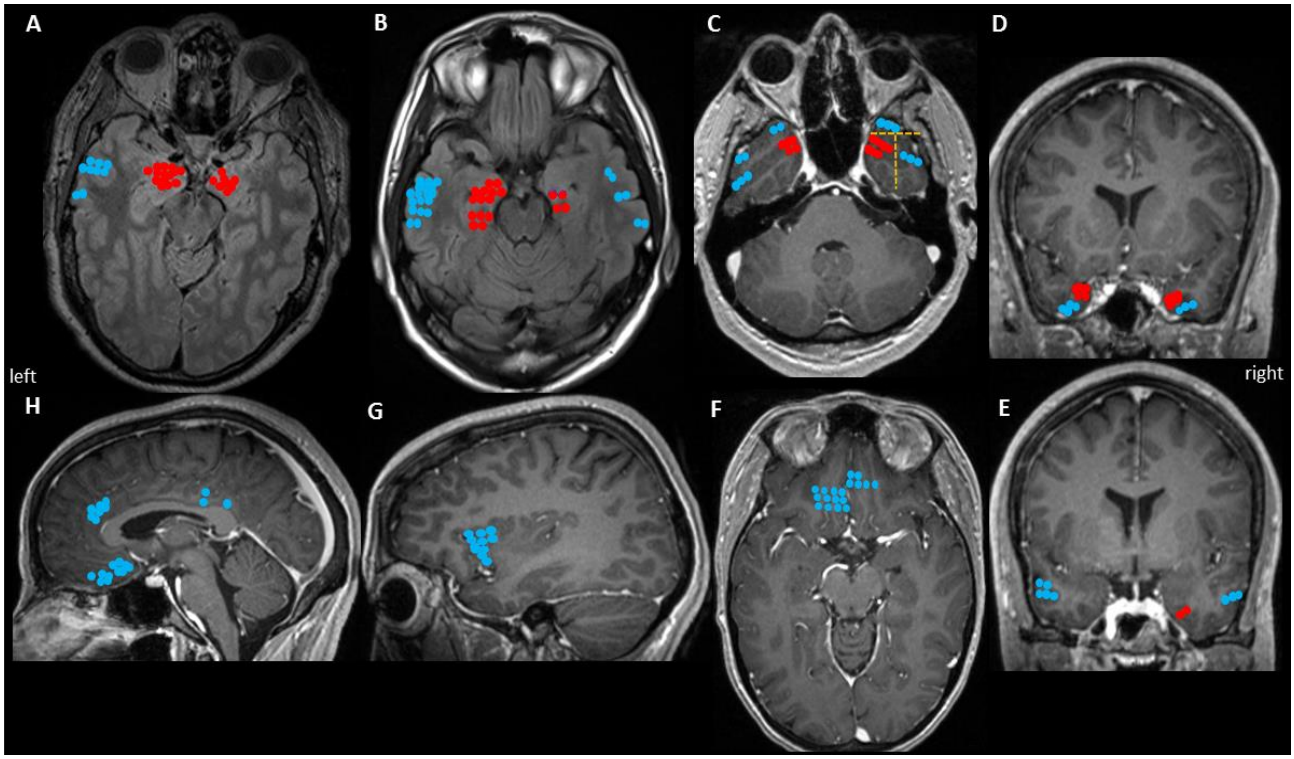
Effect of stimulation duration on respiration.

Stimulation duration was significantly associated with apnea duration ($p<0.01$) (Figure 12A). Mean (all structures) apnea duration was 11± 10 (median 10; range 4-23) seconds without induced seizures or afterdischarges. When an apneic seizure was induced, the apnea period lasted longer (up to 56 seconds) (Table 4).

Little or no change in SpO_2 and CO_2 was noted when patients began re-breathing. We found no causal relationship between apnea duration, and low SpO_2/high CO_2 levels, as possible apnea overriding mechanisms. Resumption of breathing usually occurred before any changes in SpO_2 or CO_2 (Table 4). However, there were two notable exceptions in subject 8, during parahippocampal gyrus stimulation. Apnea durations were the longest noted in our series, 21 and 23 seconds respectively, and associated with significant increases in $ETCO_2$ (from 43 to 48 mmHg) and drops in SpO_2 (from 99 to 94%) (Figure 13).

In 51/66 (78%) of stimulations, patients resumed breathing (after the apnea period), before or at stimulation end. In 15/66 (22%), apnea continued for 1-10 seconds after stimulation was discontinued. An example of apnea continuing after stimulation was discontinued is shown in figure 11 (lower panel) during short (5-6 seconds) stimulation durations.

Figure 4. Locations of electrode contacts investigated for structures that produce stimulation-induced apnea.



Legend: This composite figure shows electrode locations of stimulated electrodes. Locations that produced apnea are shown in red: amygdala **(A)**, hippocampus head and body **(B)**, mesial temporo-polar cortex **(C** and **D)** and parahippocampal gyrus **(E)**. Locations that did not produce apnea are shown in blue: temporal tip [anterior] and lateral temporo-polar cortices **(C** and **D),** lateral temporal **(A, B, C** and **E)**, orbitofrontal cortex **[F** and **H]**, anterior insular **[G]**, anterior and posterior cingulate and subcallosal gyri **[H])**. The temporo-polar cortex subregions are marked with a yellow dashed line **(C)**.

Figure 5. Locations of stimulated electrode contacts in amygdala nuclei that produce stimulation-induced apnea.

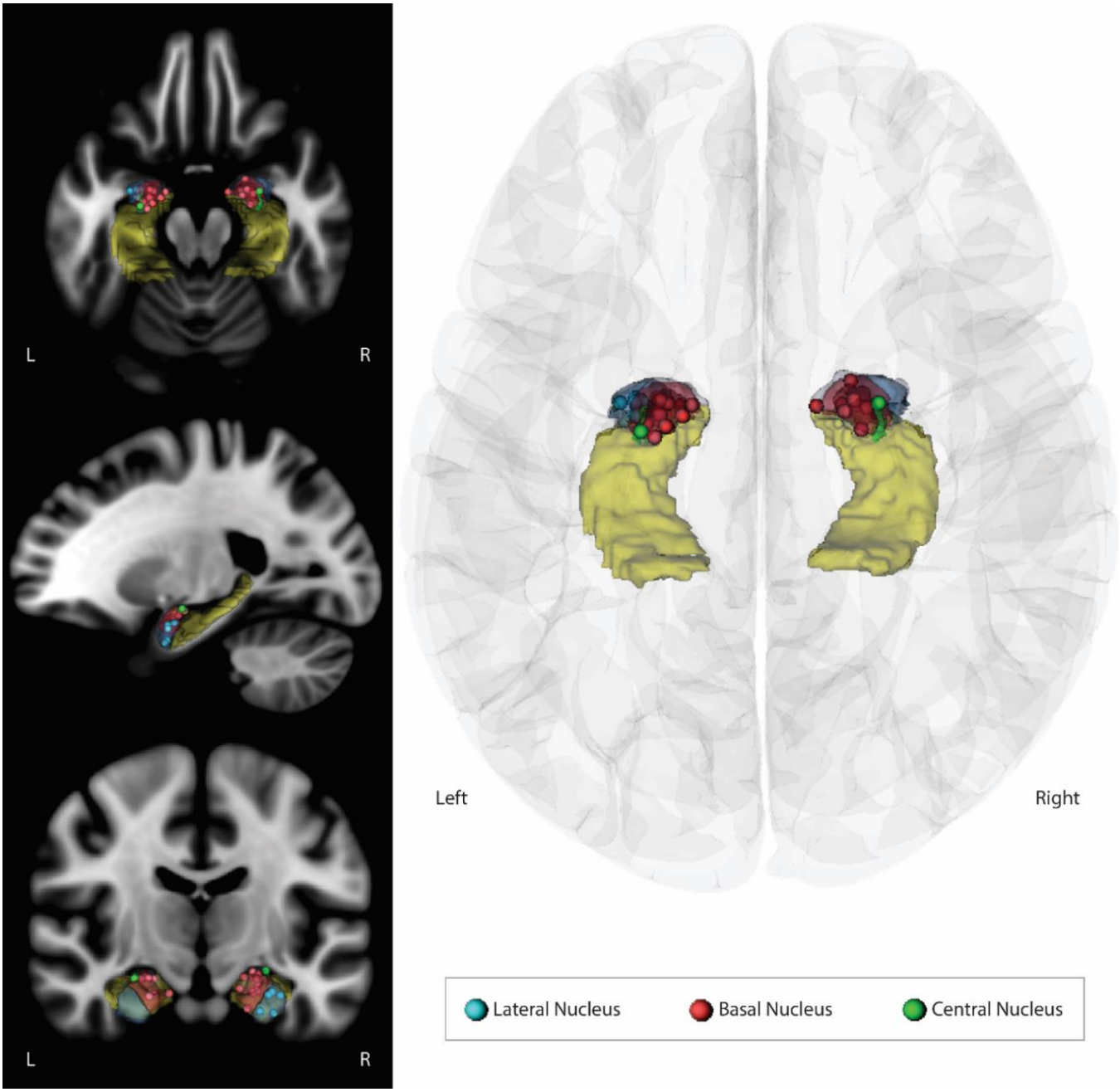


Table 4. Characteristics of stimulation and stimulation-induced apnea

Case	Stim. Site	Side	Stim. Frequency	Stim. Intensity	Stim. Duration	Apnea Duration	Breaths before apnea	Apnea duration in relation to stim. end (- = before, + = after stim. end)	SpO_2 (%) Baseline/ At resumption of breathing	$ETCO_2$ (mmHg) Baseline/ At resumption of breathing
1**	AMY(b)	L	50Hz	4mA	15s	56s	n/a	n/a	98/92	32/40
4	AMY(l)	L	50Hz	10mA	15s	8 s	0	-20	95/93	38/39
4	H	L	50Hz	3mA	15s	14s	0	-16	95/95	38/38
4**	H	L	50Hz	4mA	15s	14s	n/a	n/a	95/95	38/38
5	AMY(l)	L	50Hz	6mA	30s	9s	0	-19	92/92	27/29
5	H	L	50Hz	3mA	30s	6s	0	-23	92/92	27/29
5**	H	L	50Hz	4mA	30s	40s	n/a	n/a	92/92	27/28
6**	AMY (l)	L	50Hz	9mA	33s	34s	n/a	n/a	92/89	-
7	AMY(b)	L	50Hz	8mA	16s	9s	0	-7s	98/98	42/42
7	AMY(b)	L	50Hz	9mA	16s	10s	0	-5s	98/98	41/41
7	AMY(b)	L	50Hz	10mA	16s	12s	0	-5s	99/42	97/43
7	AMY(b)	L	50Hz	10mA	6s	9s	0	+4s	98/97	40/40
7	AMY(b)	L	50Hz	10mA	6s	9s	0	+4s	98/97	40/40
7	AMY(b)	L	50Hz	10mA	6s	9s	0	+3s	98/97	40/40
7	AMY(b)	L	50Hz	10mA	6s	8s	0	+4s	98/98	40/40
7	H	L	50Hz	3mA	10s	11s	0	+1s	98/98	38/38
7	H	L	50Hz	3mA	16s	12s	0	-3s	98/98	39/39
7	H	L	50Hz	4mA	20s	9s	0	-10s	98/98	39/39
7	H	L	50Hz	7mA	15s	6s	0	-7s	99/99	40/40
7	H	L	50Hz	8mA	16s	6s	0	-11s	99/99	40/40
7	H	L	50Hz	9mA	16s	8s	0	-4s	99/99	41/41
7	H	L	50Hz	10mA	16s	8s	0	-7s	99/99	40/40
8	PH	R	5Hz	6mA	22s	20s	2	+4s	99/98	42/42
8	PH	R	5Hz	6mA	22s	20s	2	-8s	98/98	43/43
8	PH	R	5Hz	6.5mA	32s	15s	2	-11s	98/98	43/43
8	PH	R	5Hz	7mA	31s	17s	2	-7s	98/99	43/43
8	PH	R	5Hz	8mA	34s	23s	2	-6s	98/94	43/48
8	PH	R	5Hz	8mA	40s	21s	2	-15s	99/94	43/48
8	AMY(b)	R	5Hz	2mA	13s	12s	0	-1s	99/99	42/42
8	AMY(b)	R	5Hz	8mA	16s	14s	2	+7s	97/97	-
8	AMY(b)	R	5Hz	9mA	16s	13s	2	+3s	98/98	-
8	AMY(b)	R	5Hz	10mA	16s	16s	2	+4s	99/99	-

8	TP	L	50Hz	5mA	15s	8s	1	-3s	99/99	-
9	AMY(b)	R	1Hz	10mA	25s	11s	0	-3s	97/97	-
9	AMY(b)	R	50Hz	1.4mA	10s	9s	0	-4	90/90	-
9	AMY(b)	R	50Hz	1.6mA	10s	7s	0	-3	90/90	-
9	H	R	50Hz	4mA	15s	14s	0	0	96/96	-
9	H	R	50Hz	5mA	15s	7s	0	-7s	96/95	-
9	H	R	50Hz	5mA	30s	9s	0	-9s	96/96	-
9	H	R	50Hz	5mA	6s	5s	0	0	96/94	-
10**	H	L	50Hz	2mA	5s	12s	n/a	n/a	99/99	35/35
10	TP	L	50Hz	5mA	15s	8s	0	-4s	99/99	35/35
11	TP	R	50Hz	3mA	16s	10s	0	-4s	96/94	37.8/37.8(*)
11	TP	R	50Hz	3mA	11s	8s	0	-3s	95/95	37.6/37.8(*)
11	TP	R	50Hz	4mA	5s	8s	0	+4s	94/94	38/38(*)
11	TP	R	50Hz	4mA	6s	8s	0	+3s	94/94	38.1/38.1(*)
11	TP	R	50Hz	4ma	5s	7s	0	+4s	93/93	38.1/38.1(*)
11	TP	R	50Hz	4mA	2s	6s	0	+2s	93/93	38.2/38.2(*)
11	TP	R	50Hz	4mA	9s	6s	0	+1s	94/93	38.3/38.4(*)
11	TP	R	50Hz	4mA	9s	4s	0	+3s	94/93	38.5/38.7(*)
11	TP	R	50Hz	5mA	19s	13s	0	-4s	94/94	39.2/39.2(*)
13	AMY(c)	R	1Hz	9mA	19s	10s	2	-4s	93/93	-
13	AMY(c)	R	50Hz	5mA	13s	10s	0	-3s	89/89	47/47.2(*)
13	H	L	1Hz	3mA	21s	9s	2	-6s	95/95	-
13	H	L	1Hz	10mA	22s	9s	2	-6s	95/95	-
13	H	L	50Hz	3mA	16s	12s	0	-2s	90/88	47.5/47.5(*)
13	H	L	50Hz	5mA	13	10s	0	-2s	89/88	47.1/47.2(*)
14	AMY (b)	R	1Hz	6mA	23s	9s	0	-11s	95/95	-
14	AMY (b)	R	1Hz	7mA	21s	12s	0	-9.5s	95/95	-
14	AMY(b)	R	1Hz	10mA	20s	16s	0	-10s	95/95	-
14	H	R	1Hz	1mA	21s	14s	1	-2.5s	99/99	-
14	H	R	1Hz	5mA	17s	16s	0	-4s	95/95	41/41(*)
14	H	R	1Hz	8mA	21s	12s	1	-3s	95/95	41/41(*)
14	H	R	1Hz	10mA	21s	15s	2	0s	95/95	41/41(*)
14	H	R	50Hz	1mA	15s	10s	0	-3s	99/99	-
14	H	R	50Hz	2mA	17s	13s	0	-2s	95/95	41/41(*)
14	TP	R	1Hz	5mA	21s	12s	1	-7s	96/96	-
14	TP	R	50Hz	2mA	15s	12s	1	-2s	99/99	42/42(*)
14	TP	R	50Hz	3mA	14s	12s	0	-2s	94/95	42.5/42.6(*)
14	TP	R	50Hz	6mA	13s	7s	0	-2s	95/95	41/41(*)
14	TP	R	50Hz	8mA	11s	6s	0	-3s	95/95	41/41(*)
15	TP	L	20Hz	4mA	19s	11s	0	-8s	99/99	-

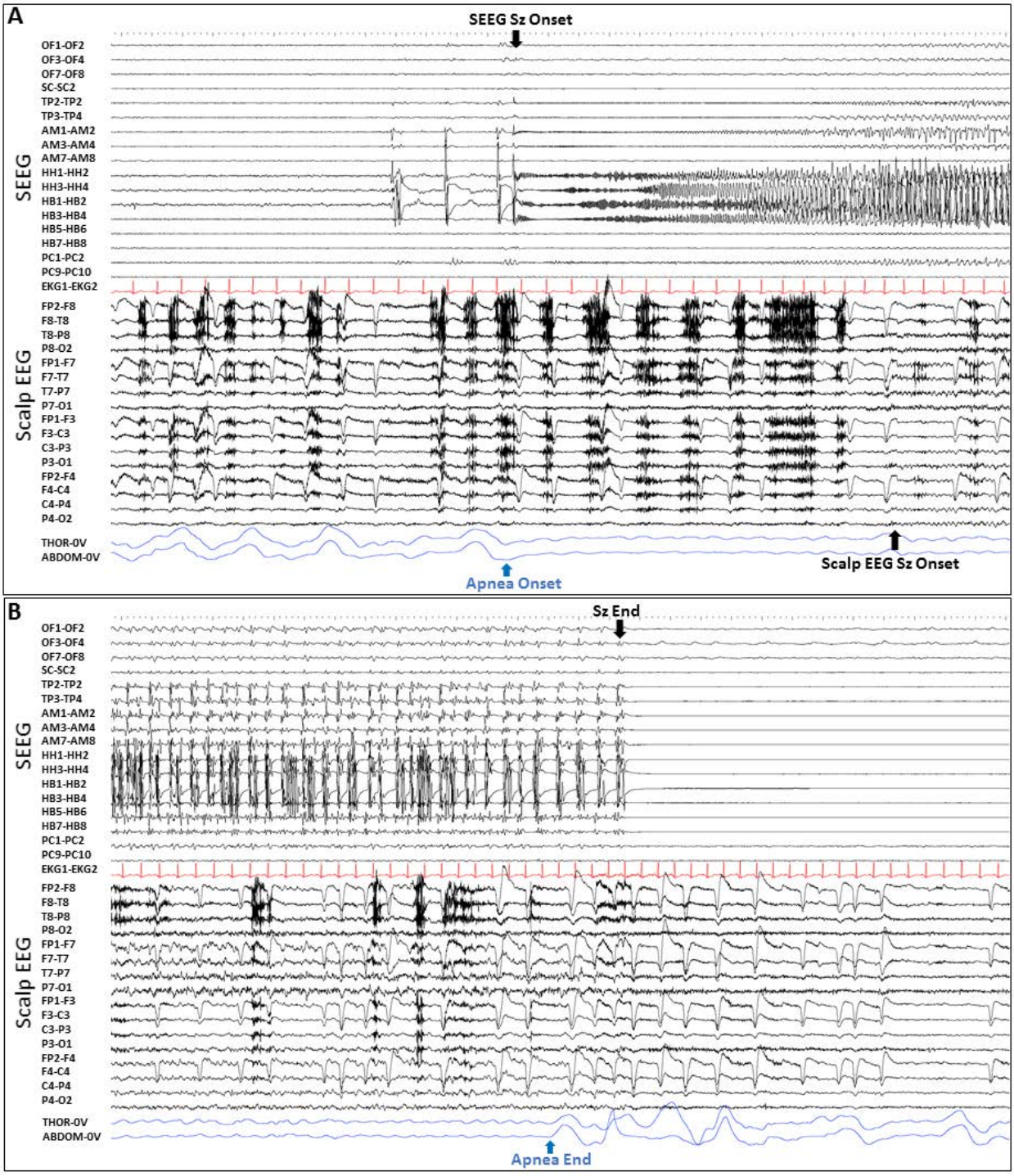
15	TP	L	50Hz	2mA	17s	7s	1	-3s	99/99	-
15	TP	L	50Hz	3mA	17s	12s	0	-3s	99/99	-
15	TP	L	50Hz	4mA	20s	9s	0	-10s	99/99	-
15	TP	L	50Hz	5mA	12s	10s	0	-3s	99/99	-
15	TP	L	50Hz	6mA	6s	9s	0	+2s	99/99	-
15	AMY(l)	L	50Hz	3mA	11s	7s	0	-4s	99/99	-
15	AMY(l)	L	50Hz	4mA	13s	8s	0	-5s	99/99	-
15	AMY(l)	L	50Hz	4mA	16s	10s	0	-4s	99/99	-
15	AMY(b)	L	20Hz	3mA	21s	12s	0	-8s	98/98	-
15	AMY(b)	L	20Hz	3mA	9s	13s	+2s	0	99/99	-
15	AMY(b)	L	20Hz	4mA	9s	14s	+6s	0	99/99	-

*Transcutaneous CO_2 values (mmHg)

**Stimulation induced seizure/afterdischarges

AMY: amygdala; HH: hippocampus head; HB: hippocampus body; TP: temporo-polar cortex; PH: parahippocampal gyrus; Hz: hertz; mA: milliampers; R: right; L: left; s: seconds; stim: stimulation; SpO_2: peripheral capillary oxygen saturation; $ETCO_2$: end-tidal carbon dioxide; n/a: not applicable; b: basal nucleus; l: lateral nucleus; c: central nucleus.

Figure 6. Central apnea in relation to intracranial and scalp EEG seizure onset.

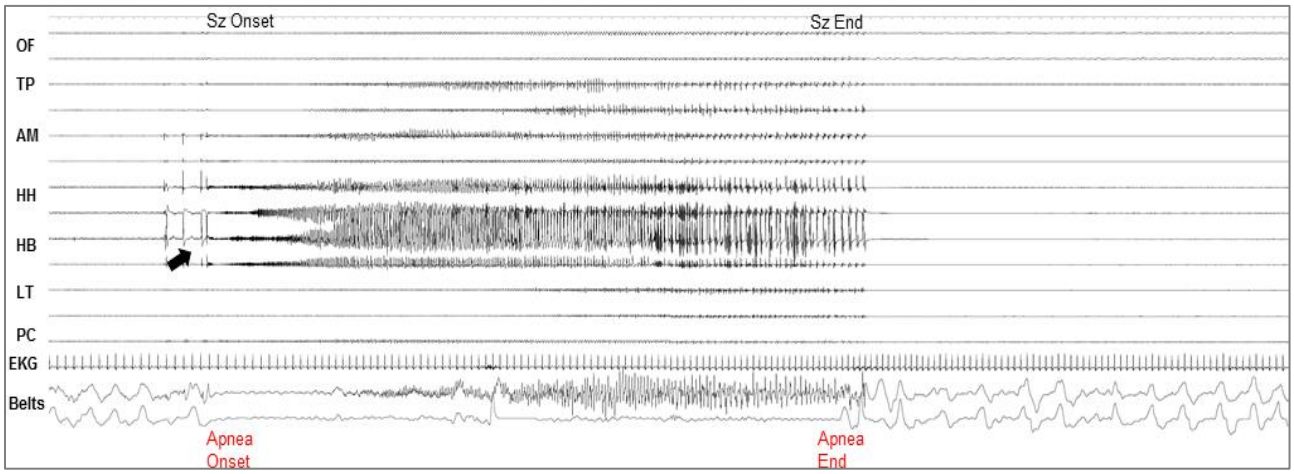


Legend: **A)** Stereo-electroencephalographic (SEEG) recording of spontaneous hippocampal seizure onset in subject 1 after repetitive inter-ictal spiking, with concurrent thoraco-abdominal breathing signal cessation, which is replaced by a pulse artifact, and indicative of ictal central

apnea. Twelve seconds later, surface EEG seizure onset appears. **B)** Resumption of breathing occurred two seconds before seizure end.

OF: orbitofrontal, SC: subcallosal, TP: temporal pole, AM: amygdala, HH: hippocampus head, HB: hippocampus body, PC: posterior cingulate.

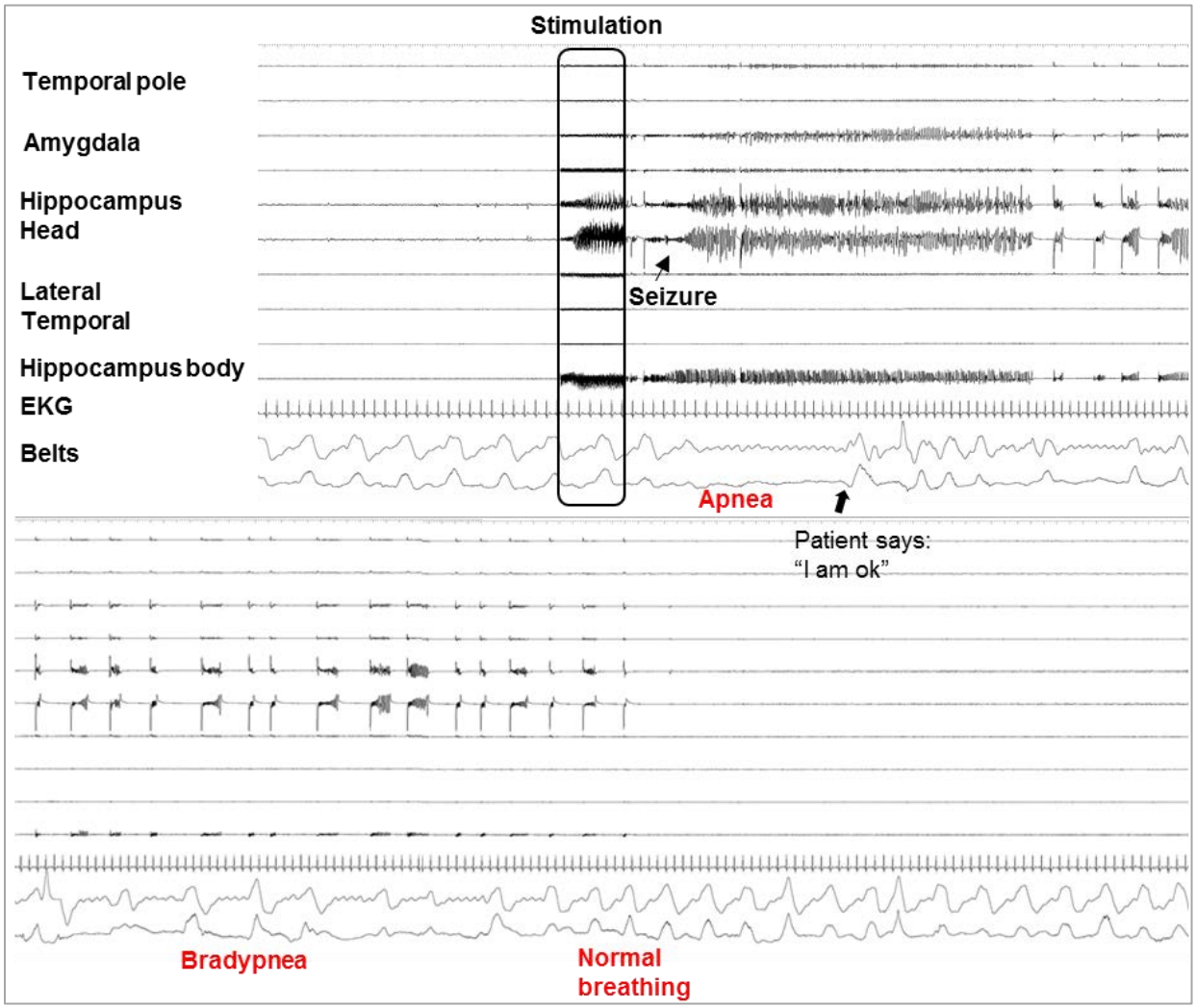
Figure 7. Spontaneous seizure-induced central apnea.



Legend: A spontaneous left hippocampus and amygdala EEG seizure in subject 10 induced a 60 second apnea. The patient had no aura. When the nurses came into the room, she was unresponsive and had mouth automatisms. She did not talk during the seizure. The apnea ended at seizure end. SpO_2 and $ETCO_2$ were not available during this seizure.

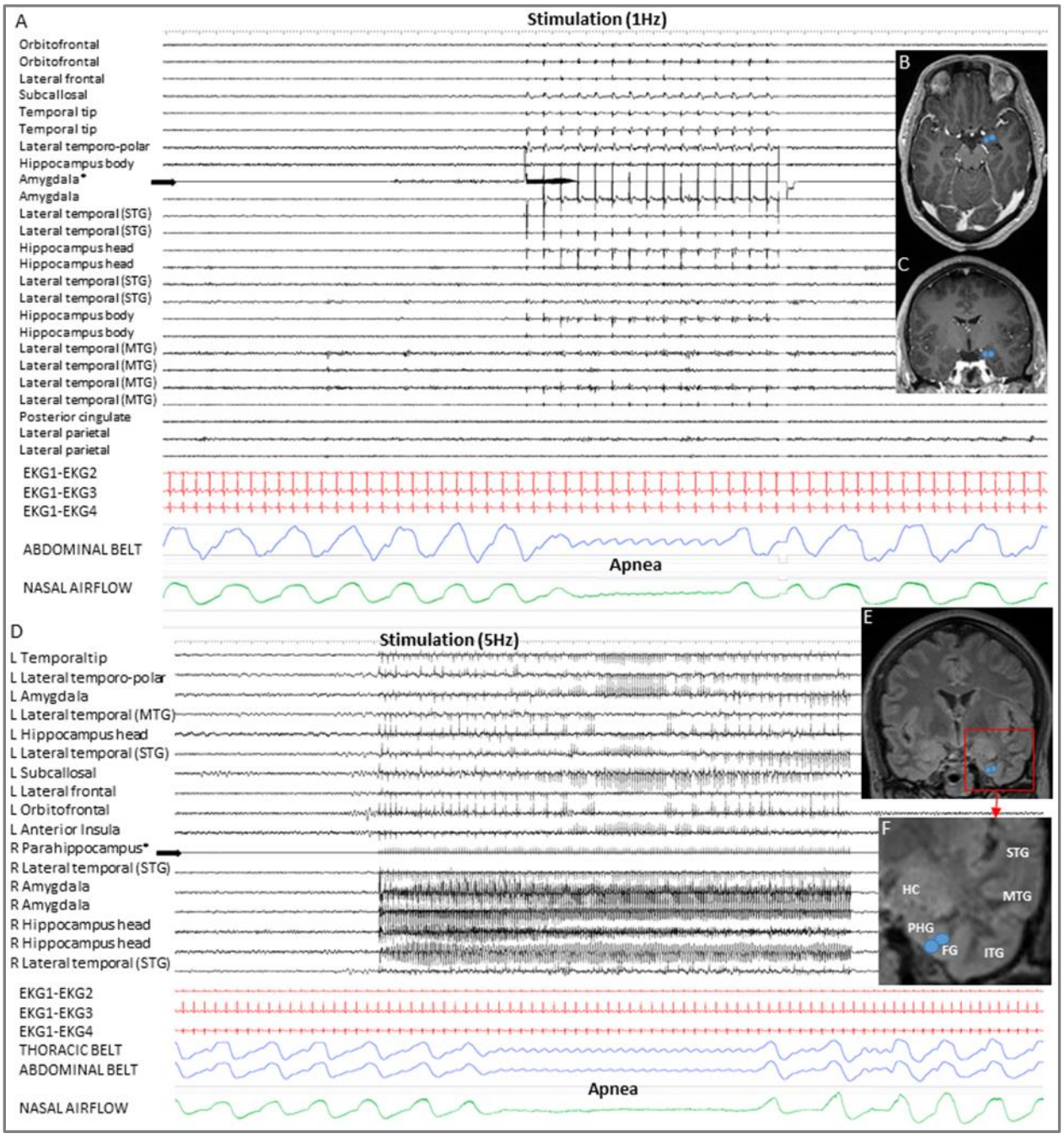
Sz: seizure, OF: orbitofrontal cortex, TP: temporo-polar cortex, AM: amygdala, HH: hippocampus head, HB: hippocampus body, LT: lateral temporal cortex, PC: posterior cingulate gyrus.

Figure 8. Stimulation-induced seizure accompanied by central apnea followed by after-discharges and bradypnea.



Legend: Left hippocampus body stimulation in subject 10 at 1 mA induced a seizure arising from the hippocampus. Belts showed respiratory arrest at seizure onset. Patient was agnostic of apnea and had no other symptoms associated. When the patient started talking and answering questions, apnea ended. When the seizure ended, afterdischarges persisted with maximum amplitude in the hippocampus. The patient was able to breathe, but at an irregular, slower rhythm, compared to baseline. Once the afterdischarges ended, the patient's breathing rhythm went back to baseline.

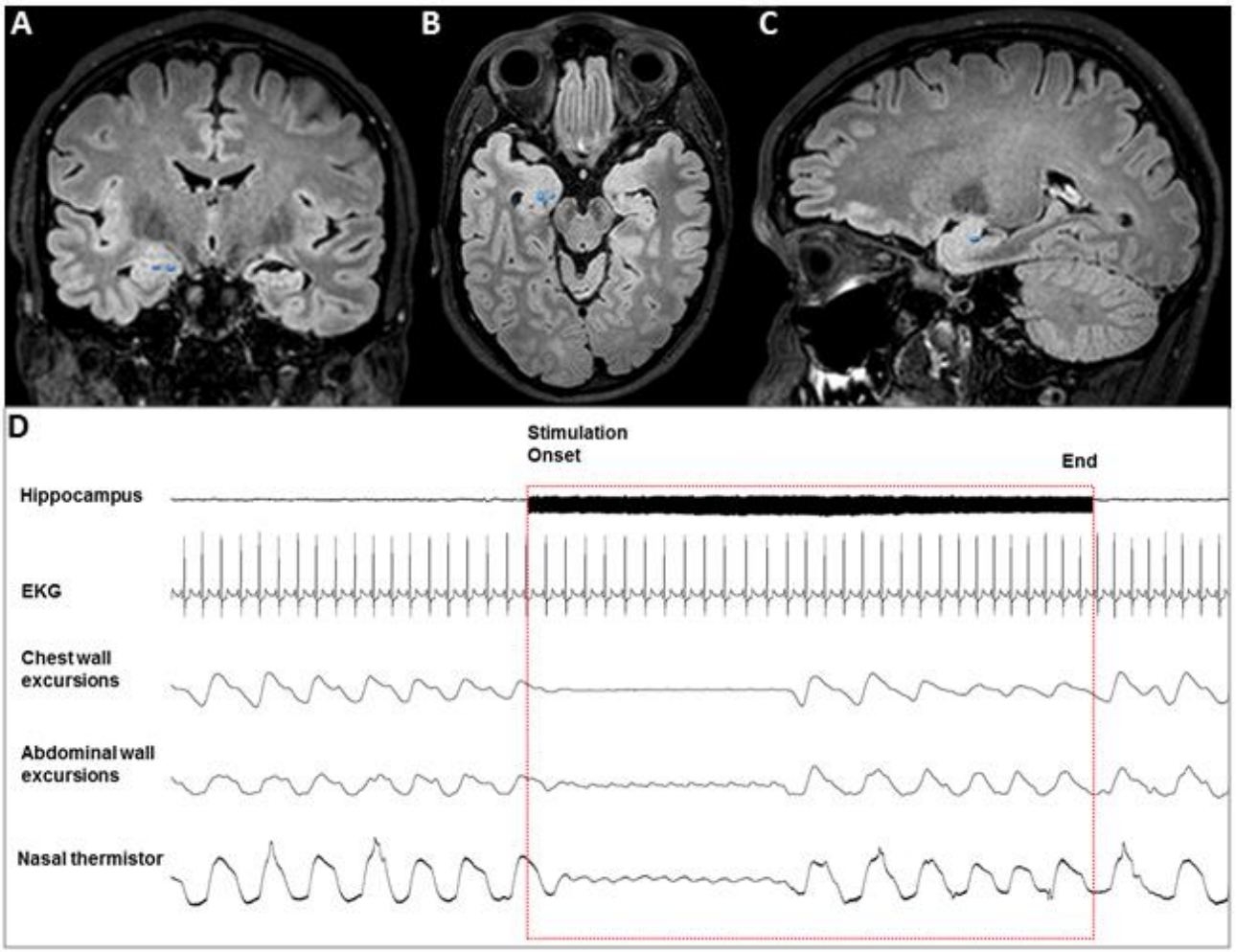
Figure 9. Amygdala and parahippocampal gyrus stimulation-induced apnea with low frequency (1 Hertz and 5 Hertz) stimulation.



Legend: A) Central apnea appears in abdominal and nasal airflow channels, with 1 Hertz right amygdala stimulation in subject nine. All implanted electrodes shown are in the right hemisphere grey matter. Stimulated electrode contacts are seen in axial (B) and coronal (C) MRI sections. No after-discharges or seizures are seen. D) Central apnea appears in abdominal

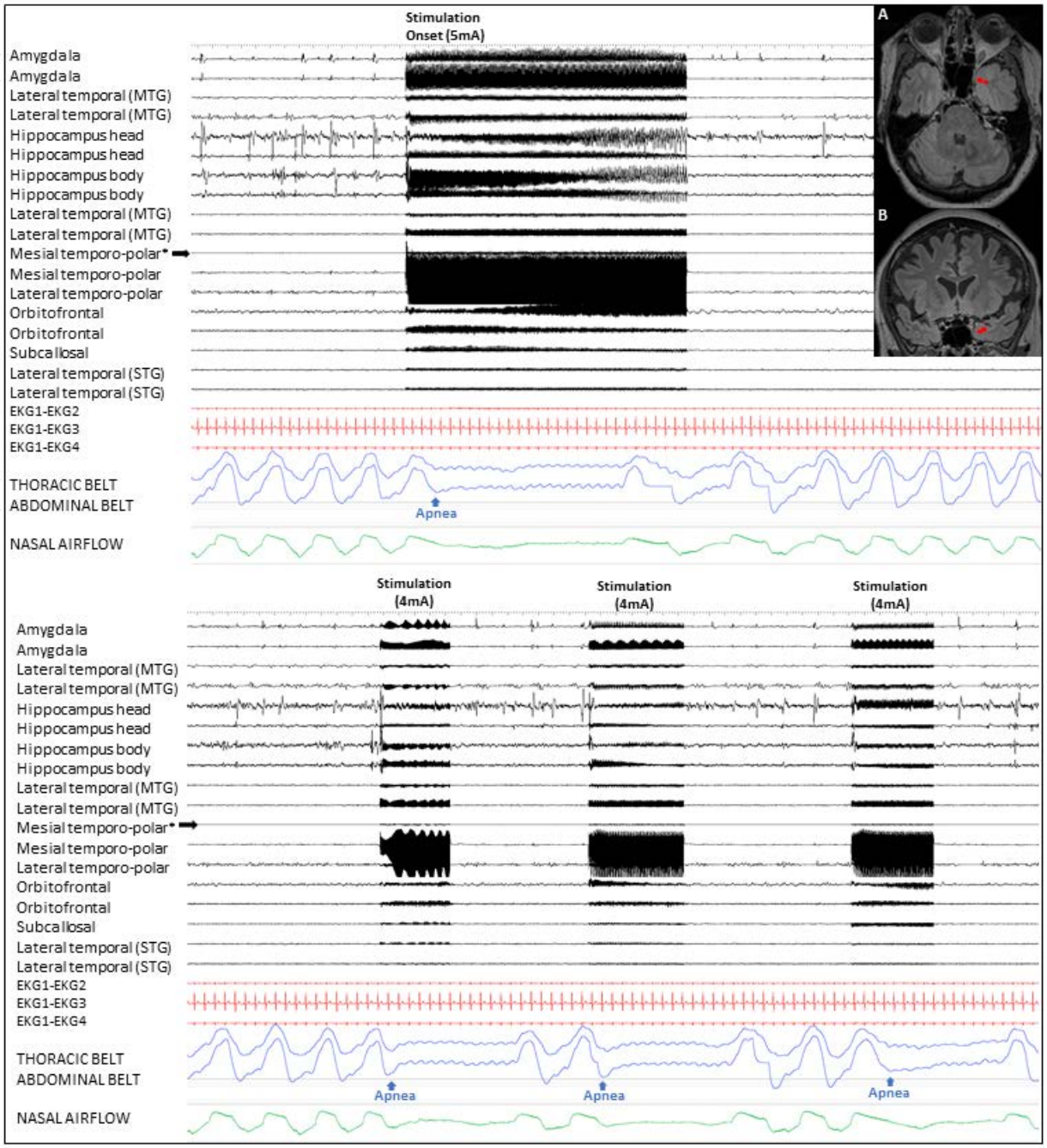
and nasal airflow channels, with low frequency (5 Hertz) right parahippocampus gyrus stimulation in subject eight. The implanted electrodes shown are in the left (L) and right (R) hemispheres. Stimulated electrode contacts are seen in coronal (E and F) MRI sections. No after-discharges or seizures are seen.

Figure 10. Hippocampus stimulation-induced central apnea.



Legend: The upper panel shows the stimulating hippocampal electrode position in subject 4. Preoperative brain MRI co-registered with postoperative CT scans showing the location of the stimulated electrodes in the left hippocampus in blue color in coronal **(A)**, axial **(B)** and sagittal **(C)** cuts. The lower panel (**D)** represents hippocampus stimulation-induced, instantaneous central apnea at 3 milliamperes (mA) of current intensity, 50 Hz frequency and 0.2 ms pulsewidth in subject 4. The subject was able to breathe out once after stimulation was started but unable to resume breathing for 15 seconds. At resumption, breathing rate was similar to baseline.

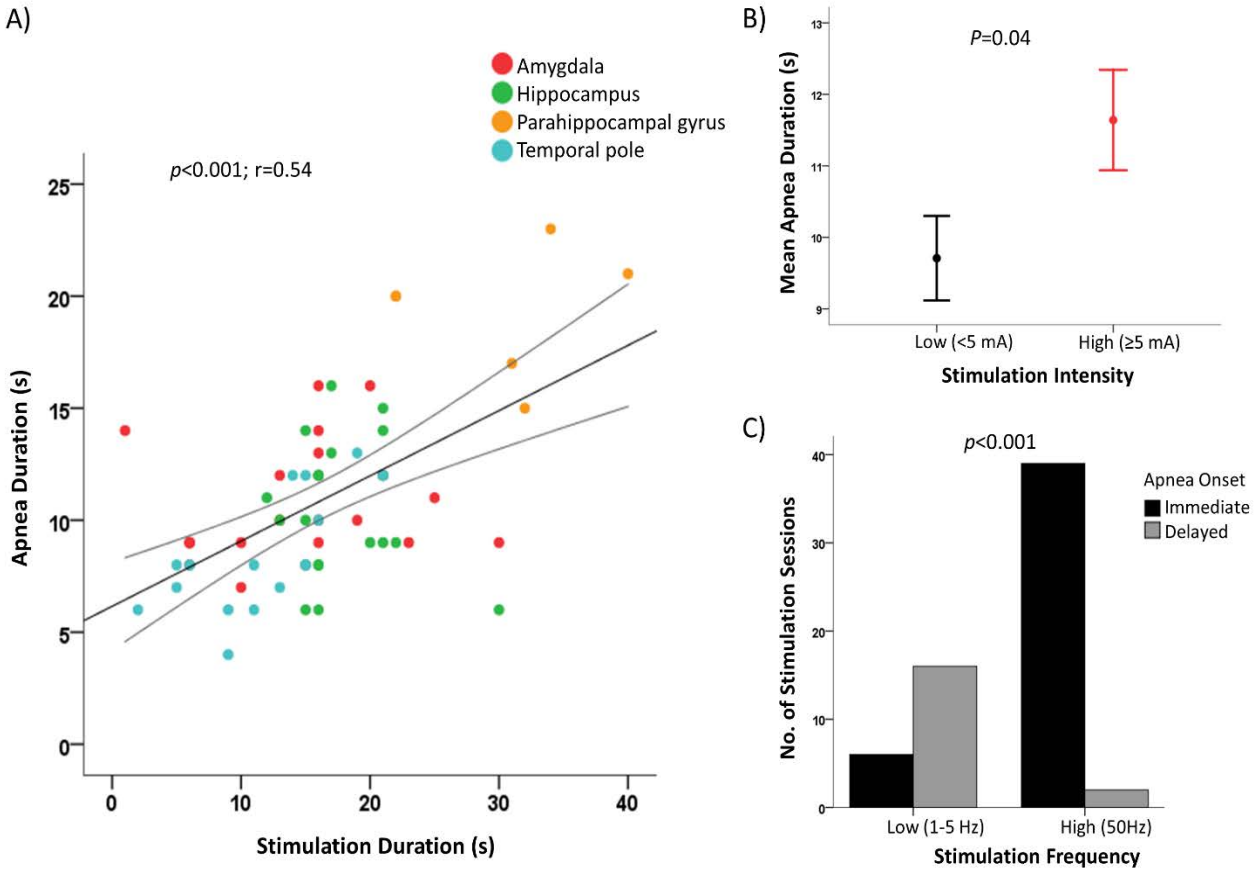
Figure 11. Mesial temporal polar cortex stimulation-induced apnea in subject 11.



Legend: Right mesial temporo-polar stimulation in case 11. The upper panel represents a 19 second stimulation train (50Hz, 0.2 ms pulsewidth, 5mA), which induced immediate cessation of thoracic and abdominal excursions following the end of expiration (blue signal) in addition to airflow cessation (green). The apnea period lasted for 13 seconds. SpO2 and transcutaneous CO2 were 94% and 39.2mmHg, respectively, and remained steady during the apnea period.

The locations of stimulation electrodes are shown in axial (A) and coronal (B) FLAIR MRI sections. The lower panel represents short stimulation sessions inducing immediate apnea, with persistence of apnea into the post-stimulation period. STG: superior temporal gyrus, MTG: middle temporal gyrus. *Stimulated electrodes.

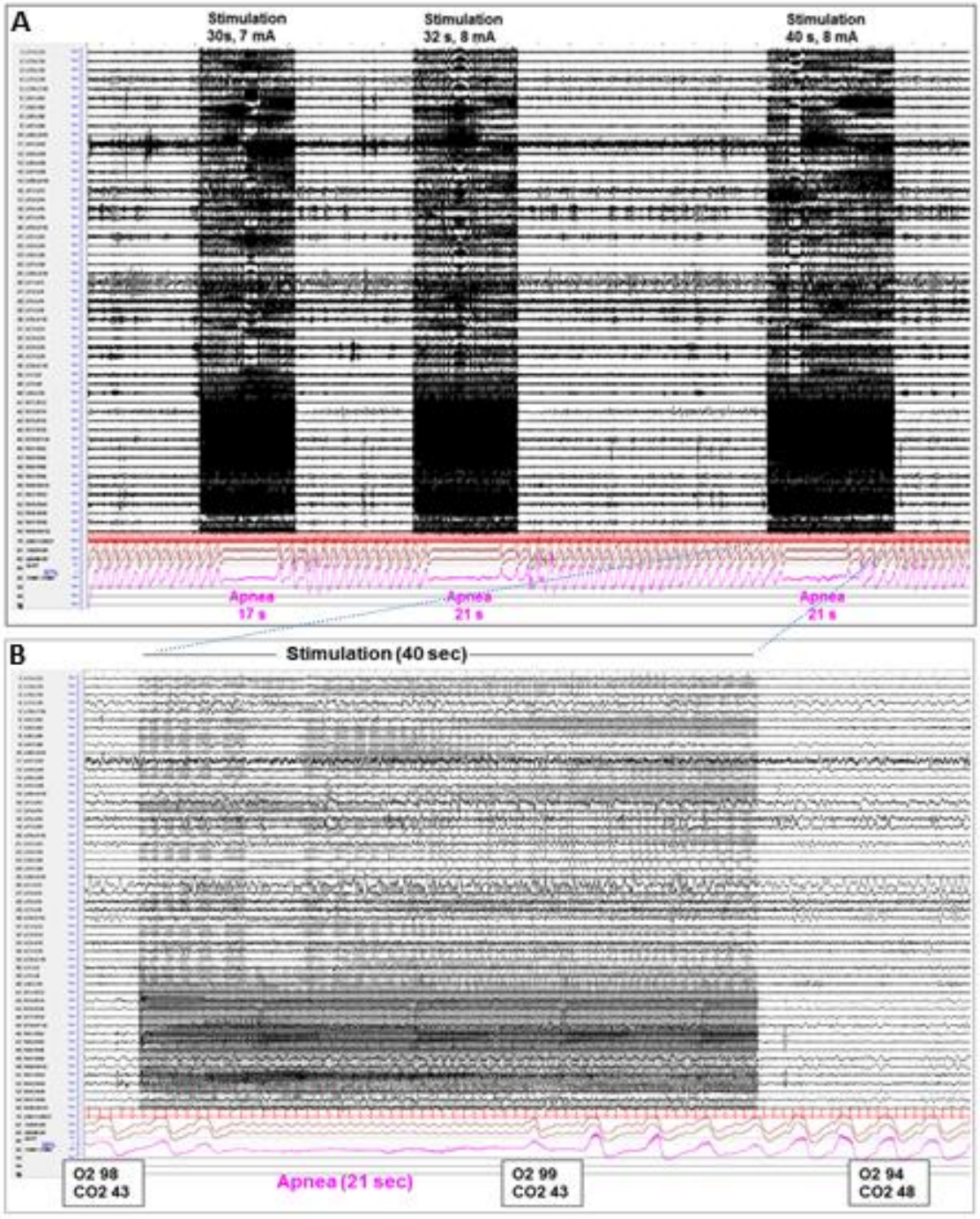
Figure 12. Association between stimulation duration, intensity and frequency, with apnea features.



Legend: A) Apnea duration (s) vs stimulation duration (s). The abscissa is stimulation duration (in seconds) and the ordinate is apnea duration. Each data point represents the cortical site of each stimulation. The simple linear regression line and 95% confidence intervals are shown. B) Plot with error bars of stimulation current intensity, divided into low (<5) and high (>5 milliamperes) current intensity, and mean apnea duration (in seconds), showing that high current intensity (>5mA) was significantly associated with longer apnea duration (p=0.04), compared to low intensity (<5mA). C) Bar graph shows that high frequency stimulation (50 Hz)

was statistically significantly associated ($p<0.001$) with immediate apnea onset with stimulation. Immediate apnea onset = apnea that occurred following completion of an expiratory cycle; delayed = apnea began 1-2 completed breaths after stimulation onset.

Figure 13. Mesial temporo-polar cortex stimulation-induced central apnea in subject 11.



Legend: Right mesial temporal pole stimulation in subject 8 at 50 Hz. **A)** The temporal relationship between stimulation period and apnea is shown. Apnea ended before stimulation was discontinued. **B)** The panel shows one of right mesial temporal pole stimulation session at 8 mA. The stimulation period lasted 40 seconds. Apnea lasted for 21 seconds and ended before stimulation was discontinued. There was no apparent relationship between pO_2 or CO_2 levels and resumption of breathing.

6.2. Blood pressure responses to cortical electrical stimulation

Stimulation in all electrodes placed in Brodmann area 25 (BA25) in cases 7, 8, 9 and 12 produced rapid and consistently reproducible decreases in SAP (Table 5). The mean drop was 15 [10-42] mmHg. SAP decreases appeared after a mean latency of 8.5 [1-14] seconds. An example of stimulation induced decreased of blood pressure is shown is figure 15.

Figure 14. Brodmann area 25 (BA25) stimulating electrode positions that induced blood pressure responses (drop of systolic blood pressure).

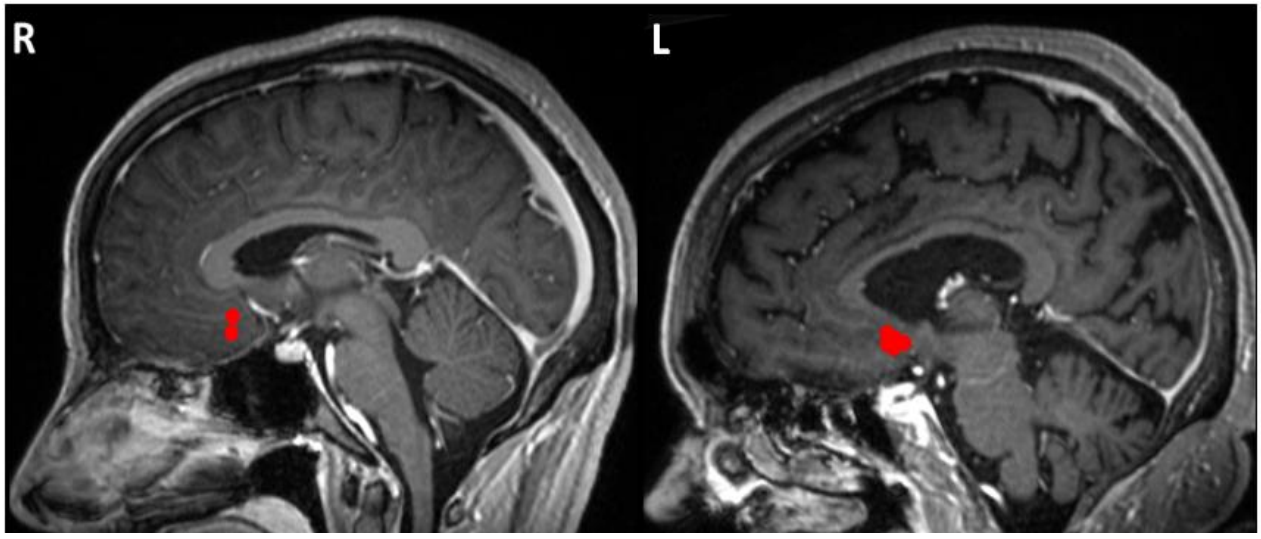


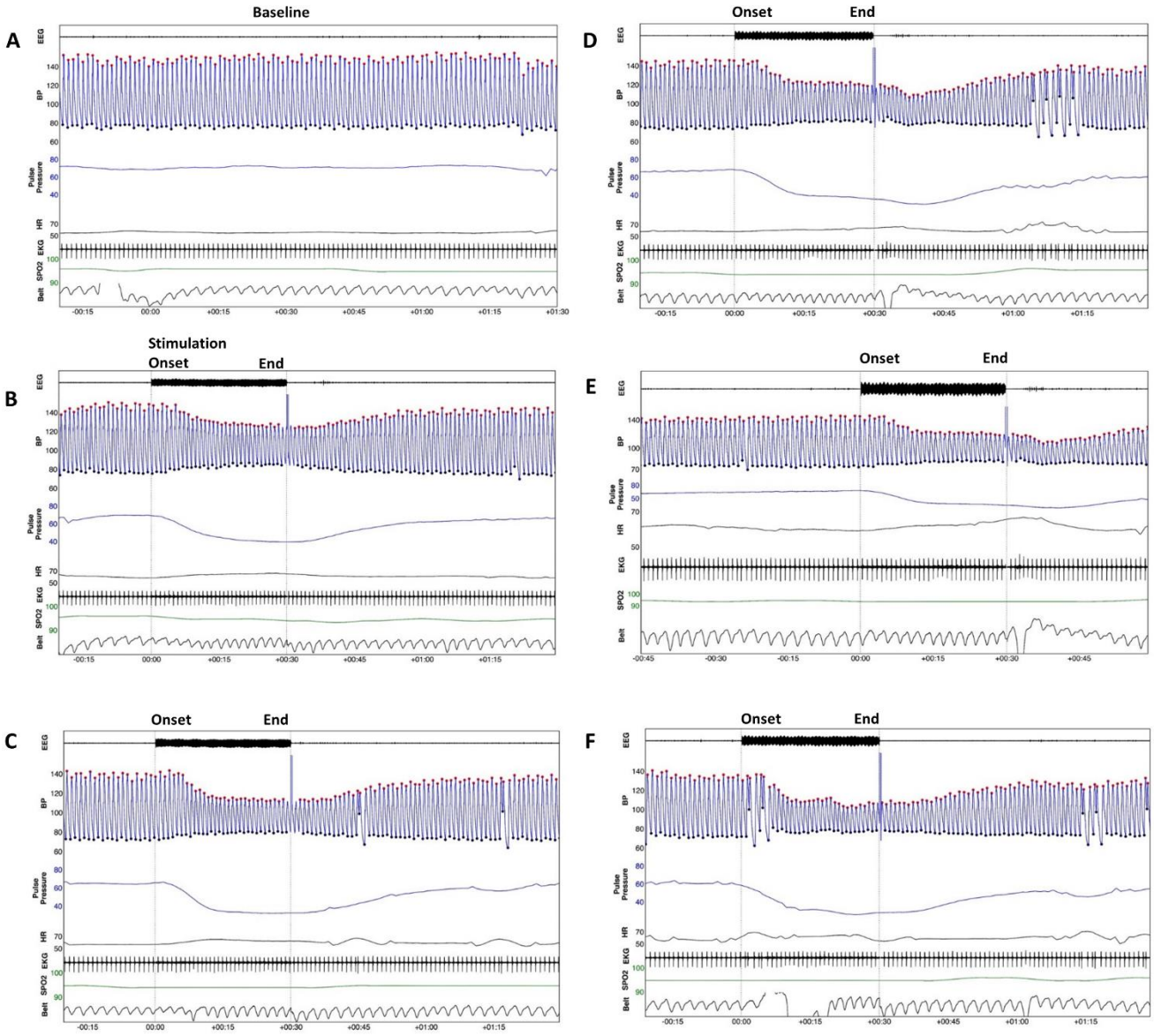
Table 5. Stimulating electrode contacts location in each patient

	Numbers of electrodes in each cortical site (L=left hemisphere, R=right hemisphere)										
Case	AMY	H	PH	Insula (anterior)	OF	T Pole	Lateral T	Basal T	AC	Subcallosal	PC
1	2 (L)	0	0	0	4 (L)	0	4(L)	0	0	0	0
2	2 (L)	0	0	0	4 (L)	0	0	0	0	0	2 (L)
3	3 (L)	0	0	0	4 (L)	0	0	0	0	0	0
4	3(L)	4 (L)	0	0	0	2 (L)	4(L)	0	0	0	0
5	4 (L)	4 (L)	0	0	0	0	0	0	0	0	0
6	3 (L)	0	0	0	0	0	2 (L)	0	0	0	0
7	2 (L)	4 (L)	0	3 (L)	0	0	4 (L)	0	4 (L)	2 (L)	0
8	2 (R)	0	2(R)	2(L)	0	2(L)	2(L)	2(L)	2 (L)	2 (L)	0
9	2(R)	2(R)	0	0	0	3(R)	0	0	0	2 (R)	0
10	0	2 (L)	0	0	0	2 (L)	4(L)	0	3 (L)	0	0
11	2 (R)	3 (R)	0	0	4 (R)	4 (R)	4 (R)	0	2 (R)	0	0
12	0	0	0	3(L)	0	0	0	0	2 (L)	3 (L)	0
13	2(R)	2(L)	0	2(R)	2(R)	3(R) 3(L)	0	0	2(R) 2(L)	0	0
14	2 (R)	2(R)	0	0	0	3 (R)	5(R)	0	2 (R)	0	0
15	4(L)	4(L)	0	0	2(L)	2(L)/2(L)	6(L)	0	0	0	0

In red, the structures were stimulation induced blood pressure responses

AMY: amygdala; H: Hippocampus; OF: orbitofrontal, T: temporal, AC: anterior cingulate, PC: posterior cingulate.

Figure 15. Stimulation of Brodmann Area 25 (BA25) in patient 12 induced decrease of systolic blood pressure and pulse pressure at different current intensities.



Legend. **A)** Cardiovascular features in the resting state before stimulation session (baseline). Cardiovascular responses after stimulation with **B)** 5 mA, **C)** 7 mA, **D-E)** 6 mA and **F)** 8 mA.

Red dots represent systolic arterial pressure (SAP) and black dots diastolic arterial pressure (DAP).

At times, the fall in SAP was preceded by a slight rise. Once stimulation was discontinued, SAP began to increase within 12 [1-47] seconds. DAP did not change concurrently with SAP, resulting in a consistent narrowing of pulse pressure in all patients.

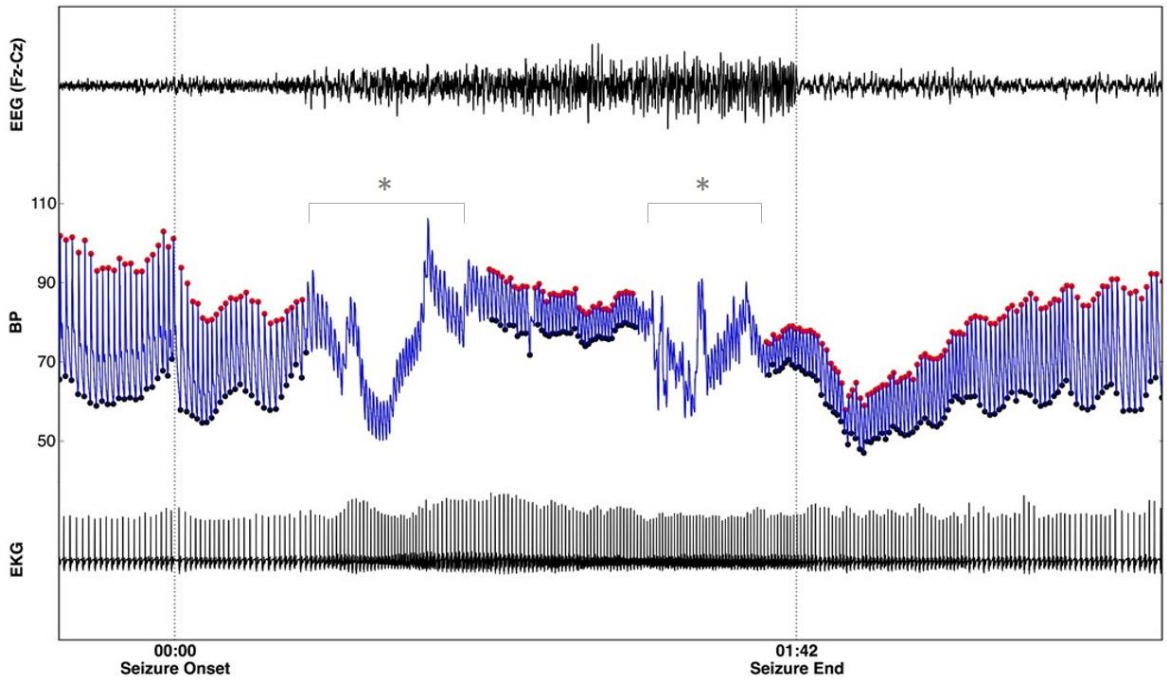
Heart rate responses differed. In case 8, heart rate increased accordingly with SAP. On the other hand, in subjects seven, nine and 12, heart rate did not significantly change. SpO_2 and $ETCO_2$ did not change at any time during or after stimulation. Frequency domain analysis of HRV, BPV and BRS comparing baseline with the stimulation period and baroreflex slope were calculated in subjects seven, eight and nine and showed mixed pictures.

During some of the BA 25 stimulation sessions, brief afterdischarges were induced, although there were no differences in blood pressure responses in either situation. However, we excluded stimulations with afterdischarges to ensure that the blood pressure responses were being produced exclusively by BA25 stimulation, and not by afterdischarges in other brain areas.

We analyzed recorded seizures in those patients in whom we found hypotensive responses to specifically look for spontaneous peri-ictal hypotensive changes, and for correlation of seizure discharges in BA25 with hypotension. Subject 7 did not have hypotensive changes with the single seizure that was recorded; the BA25 electrode was involved in the seizure although the seizure discharge was widespread at that point. Subject 8 had no blood pressure recordings during seizures. Subject 9 had no seizures recorded during intracranial EEG monitoring with blood pressure recordings. However, he previously had three complex partial seizures with oral automatisms, recorded with surface EEG and continuous blood pressure monitoring. Two of these had ictal and post-ictal hypotension (Figure 16). Subject 12 had asymmetric tonic seizures lasting for less than 10 seconds, in which blood pressure did not change, and where the seizure did not involve Brodmann area 25.

No significant blood pressure responses were noted after stimulation of amygdala, hippocampus, and insular, orbitofrontal, temporopolar, lateral temporal, basal temporal, anterior cingulate and posterior cingulate neocortex.

Figure 16. Ictal and postictal hypotension during a complex partial seizure with oral automatism in subject 9 recorded with surface EEG and continuous blood pressure (BP) monitoring.



Legend: Red dots represent systolic arterial pressure and black dots represent diastolic arterial pressure. EKG indicates electrocardiogram.

*Two periods during ictus when movement artifact of the blood pressure-cuffed limb renders acquisition unreliable.

6.3. Cardiac responses to cortical electrical stimulation

Neither significant changes in heart rate nor arrhythmias were induced by electrical cortical stimulation. Increase of heart rate was only seen when induced seizures, but they were excluded in the analysis.

7. DISCUSSION

7.1. Cortical control of respiration

We prospectively evaluated limbic and paralimbic structures in both hemispheres for central apnea using direct cortical electrical stimulation, while controlling for after-discharges in potentially symptomatogenic sites.

We confirmed amygdalohippocampal complex influences on breathing, and also found evidence of additional structures located in the parahippocampal gyrus and mesial temporo-polar regions that influence respiratory patterning. Further, we were able to localize amygdalar apneic responses to basal, central and lateral nuclei stimulation.

Our study, as well as recent reports meticulously assessing for seizure and afterdischarge influence (to prevent false positive results from seizure spread to other apnea-producing structures), provide evidence of a role for the amygdala and hippocampus to influence breathing, findings which are consistent with other recent studies (77, 82, 83).

We reproducibly elicited central apnea with amygdala and hippocampus stimulation.

In the amygdala, we confirmed the appearance of apneic responses with basal and central amygdalar nuclei, and, with higher stimulation parameters, the lateral nucleus (Figure 5 and Table 4). An earlier study reported breathing responses after stimulation of only lateral and basal nuclei (77); whereas a more recent study found breathing responses exclusively from the central amygdalar nucleus (82). Our findings are likely robust, since careful localization of electrodes (Figure 5) demonstrates that low frequency stimulation elicited amygdalar apnea in

the mesial part of the amygdala, including basal (Subjects 8, 9 and 14) and central nuclei (Subject 13) nuclei. However, the lateral part of the amygdala required higher frequency and current intensity stimulation to induce apnea (Subject 5 and 15 [Table 4]). This finding could represent a response to stimulation-effect-spread to nearby structures (basal or central nuclei) rather than true symptomatogenicity in the stimulated lateral nucleus). Amygdala nuclei projections may underlie these findings; the central nucleus of the amygdala has major projections to phase-switching areas of the parabrachial pons (Hopkins and Holstege, 1978) as well as brainstem respiratory areas implicated in resting respiratory rhythm (84). One of these regions, the periaqueductal gray (PAG), modulates pre-inspiratory neurons in the pre-Botzinger complex, the kernel for eupneic rhythm generation. However, the lateral nucleus does not directly project to the brainstem, but sends substantial projections to the hippocampus, thalamus (85), mesial temporo-polar cortex (86), and other cortical areas. All amygdaloid nuclei are intrinsically connected (87), and the lateral amygdala apnea influences may be exerted through those intrinsic connections to mesial amygdala nuclei or through hippocampal and thalamic projections.

Both animal and human studies show cortical influences on breathing from structures outside the amygdalo-hippocampal complex; these sites include the anterior insula, anterior and posterior cingulate, subcallosal gyrus, orbitofrontal and temporo-polar cortex (68, 70, 74-76, 86, 88). Modern image co-registration techniques allow extremely precise localization of stimulated electrodes in these regions; additional extensive scalp and intracranial EEG recordings during stimulation sessions monitor after-discharges and seizures that could contaminate results. Thus, we set out to exhaustively sample all these structures previously implicated in influencing breathing.

In addition to the amygdala and hippocampus, we found that stimulation of the parahippocampal gyrus and temporo-polar cortex also induced apnea. These findings are

consistent with Kaada et al's animal, and human observations of the same area (68, 74). They described central apnea with human temporo-polar cortex and hippocampal gyrus stimulation in patients undergoing brain surgery. Of interest, in our study, temporo-polar apneic responses were exclusive to the paralimbic, mesial subregion of the temporal pole; neither temporal tip (anterior) nor lateral temporo-polar stimulation produced apnea. Cytoarchitectural differences may explain this observation (89), since the mesial temporo-polar area is considered a paralimbic cortical region that consists of agranular cortex, is distinct from the temporal tip and lateral temporo-polar region (dysgranular regions), and is phylogenetically related to hippocampus and parahippocampal allocortex, structures we also found to produce apnea on stimulation (90).

In addition to identification of breathing influences from parahippocampal and mesial temporo-polar cortex stimulation, anterior insula, orbitofrontal and anterior and posterior cingulate regions were examined. These areas were previously posited by Chapman, Pool and Kaada (74-76) in their stimulation experiments. In our study, exploration of these regions did not produce any change in breathing patterns. There are two possible explanations for this discrepancy. First, previous studies did not distinguish between stimulation-induced apnea and stimulation-induced seizures producing apnea. Second, stimulated electrodes may not have been placed with the image-guided anatomical precision achievable today, such as in insula or anterior cingulate gyrus. The descriptions of stimulation-induced apnea (74) included patient DF reporting a "funny feeling like before an attack", patient RSu developing habitual aura and drowsiness, and patient MR having a habitual seizure ("Oh, now I'm having an attack"). Thus, reported apnea may have been symptomatic of seizure spread to other apnea-producing structures. These reports of affect changes are of particular relevance to brain regions that we consistently found to be non-apnea producing. We stimulated the anterior cingulate in 19 electrode sites in seven patients, without finding breathing responses in any. Neither unilateral

nor bilateral stimulation (in case 13, similar to Pool experiments), induced breathing changes. It is again possible that this outcome may result from electrode placements different from ours; however, our anterior cingulate electrode positions were similar to those that produced apnea in Kaada's and Pool's study. A notable exception was coverage of the anterior- most region, just anterior to the callosal genu, which was not examined in any of our patients. This part of the anterior cingulate cannot therefore be excluded as a site which influences breathing, based on our data. Similarly, the posterior cingulate region cannot be entirely excluded, since the single instance of that site producing stimulation-induced apnea occurred with stimulation at 120 Hz (76), a frequency beyond that permitted by our study protocol. The anterior insular (10 electrodes in four subjects), and orbitofrontal (20 electrodes in six subjects) regions were similarly silent despite repeated stimulation trials. In common with previous reports, lateral temporal neocortical stimulation (35 electrodes in nine subjects) did not induce apnea in any subjects.

Our results point to a set of mesial temporal structures which can influence breathing patterns. Electrical stimulation of these sites modify resting breathing rate. This set of structures likely represents the anatomical substrate of limbic/paralimbic breathing modulation in a well-described emotional motor system (EMS) (91) (92). Although various nuclei in the pons and medulla contribute to normal, unlabored (eupneic) respiratory rhythm (93-95), eupnea is continuously adjusted by several rostral brain structures, including the EMS to suit changing environmental circumstances, including emotional reactions (laughter, crying or fear), but also other basic behaviors such as coughing, vomiting or voluntary vocalization (92). The amygdalar component of the EMS (involved in various aspects of emotional processing) maintains extensive efferent and afferent pathways with the hypothalamus and bed nucleus of the stria terminalis (86). The central nucleus of the amygdala, together with the lateral bed nucleus of the stria terminalis, has strong projections to the periaqueductal gray (PAG), a significant

component within the EMS. The PAG modulates pre-inspiratory neurons in the pre-Botzinger complex region, the kernel for eupneic rhythm generation. For example, during apnea, the pre-inspiratory neurons are inhibited. Perturbation of neural function in and around this area severely disrupts breathing rhythm (94). Stimulation in the most caudal portions of the ventrolateral PAG generates apnea (84). However, since the PAG has no direct connections with any somatic or preganglionic parasympathetic motoneuronal cell groups in brainstem and spinal cord, in the context of respiratory control, the PAG uses its projections to the parabrachial pons, Kolliker-Fuse nuclei, and the medullary ventrolateral tegmental field to modulate respiratory reflexes. The cardiorespiratory modulation induced by PAG is mediated by neurons of the dorsomedial hypothalamus (96). Upstream, the amygdala also has rich inter-nodal connectivity with the temporo-polar cortex through the fasciculus amygdalo-temporalis, which connects the lateral amygdala to mesial temporo-polar cortex (86). The temporo-polar cortex has heavy projection to the hippocampus; its afferents synapse with entorhinal cortex to project to the subiculum, hippocampus and the dentate gyrus. Conversely, the temporal pole receives hippocampal afferents via the subiculum (86). Both amygdala and temporo-polar cortex are connected with the midbrain tegmentum through the temporopontine tract (74). Thus, there is rich connectivity between all the mesial temporal network nodes identified through stimulation.

Mesial temporal modulation of breathing is evidenced by hippocampal activity increases before apnea termination in cats (97). Some hippocampal and amygdalar neurons phase-lock with the respiratory cycle in humans, suggesting that these structures are intimately involved in breathing regulation (98, 99). Single-pulse stimulation of the amygdala central nucleus in cats pace the inspiratory cycle during waking; that relationship disappears during quiet sleep (100).

This abundant anatomical and functional connectivity between temporo-limbic, paralimbic, and brainstem structures is likely to explain the functional role of these nodes in a respiratory network, mainly archicortex and paleocortex that have a primitive phylogenetic

hierarchy. However, in the suprapontine limbic and paralimbic network, what mesial temporal nodes assume preeminence cannot be determined from our study. Apart from case eight, in whom the longest apnea periods were seen after parahippocampal gyrus stimulation, there were no significant differences in apnea durations produced by individual structures, nor in the stimulus intensities that produced these apneas. In all subjects, low current intensity stimulation (<5mA) elicited apnea.

Breathing responses and stimulation parameters

Because apnea occurred with cessation of inspiratory efforts rather than termination of expiration in all stimulation sessions, it is likely that the "next" inspiration was inhibited by the stimulation. These observation suggests that the most likely downstream driver for apnea is stimulus-induced inhibition or disruption of brainstem inspiratory neuronal action. Similar findings have been reported in previous simulation studies and also during ictal central apnea in focal seizures (77, 82, 83).

The observation of apnea agnosia, also made by previous studies, is consistent with functional imaging studies aiming to delineate the time-course of air hunger or dyspnea perception (101). Cognitive awareness of breathing occurs when ventilation is obstructed, stimulated, challenged or attended to, and mesial temporal electrical stimulation appears to interfere with this reflex. Prominent activation of the insula, anterior cingulate and prefrontal cortices, amygdala and the peri-amygdaloid striae terminalis occurs with air hunger (101-103), and stimulation induced, functional deafferentation of these structures may block brainstem inputs. Apnea agnosia (during stimulation and/or seizures) may also explain why ICA has largely gone unrecognized until most recently, apart from the fact that plethysmography is not commonly used in the epilepsy monitoring units. In this study, no scalp EEG was available to determine the precise sleep stage during each stimulation session, and therefore, we could not

investigate if breathing responses disappeared during non-REM sleep, as in a previous amygdala stimulation study done in cats (100).

Efficient apnea induction depended on stimulus settings that provided higher current and frequency parameters; 50 Hz stimulation was more likely to produce immediate apnea, whereas lower frequency parameters were significantly associated with apnea onset delay by one or two breaths. That higher current intensity (>5mA) was significantly accompanied by apneas is not surprising, since it is the most influential parameter in electrical stimulation. Apnea duration positively correlated significantly with stimulation duration, suggesting that both apnea onset and termination are stimulus-related. As in previous studies, (83), patients resumed breathing before termination of stimulation in the majority of the trials (78%). Adaptation of neural processes may underlie these phenomena, rather than increased pCO2 as an override mechanism that compels resumption of breathing (77). Indeed, we found no relationship between CO2 or SpO2 changes and apnea termination. On two occasions, apnea lasted beyond 20 seconds with significant CO2 and SpO2 changes when breathing was restored, although it is unclear that these findings represents a causal relationship. In animal models for example, ICAs were not reversed by increasing CO2 as an impetus for ventilatory drive (104). In 22% of our stimulations trials, apnea persisted beyond stimulation termination. This finding was typically with short stimulation periods (always <10 seconds), and produced apnea periods 1-10 seconds after stimulation cessation. Such post-stimulation apneas might indicate stimulation- induced refractoriness in respiration control in the brainstem that prevents resumption of breathing. Subject eight was an exception, where apnea persisted 1-7 seconds beyond prolonged (>10 seconds) amygdala and parahippocampal gyrus stimulations (Table 4).

7.2. Cortical control of blood pressure

The results of this study suggests that Brodmann area 25 (BA25) has a role in lowering systolic blood pressure in humans and it is likely symptomatogenic site for peri-ictal hypotension.

However, these data need to be reproduced in a larger sample of patients. This region is infrequently studied as part of orbitofrontal and anterior cingulate invasive EEG explorations in refractory focal epilepsy, hence the small sample size in our study where implantations were driven by the surgical rather than study hypothesis. BA25 is also a site that has been reported to produce hypotensive changes in animals (68). In humans, although the role of cortical structures in blood pressure control is inferred, this has hitherto neither been conclusively established nor any single brain region universally accepted as a control site. Anterior limbic region stimulation in dogs and monkeys has produced marked falls in arterial blood pressure, as well as occasional rises (60, 62, 68). Such falls usually occurred without significant alteration in heart rate (60).

Similar responses were seen after subcallosal, postorbital, anterior insular, cingulate gyrus, hippocampal, amygdalar, temporal and motor cortices stimulation (62-65, 68). In humans, where opportunities to conduct similar experiments are limited, few studies of cortical stimulation targeting blood pressure control structures, exist. In one study (66), stimulation of bilateral rostro-caudal cingulate gyrus (BA 9 and 10) was carried out among patients with psychosis before ablation in 12 cases. Blood pressure changes of systolic (SAP) and diastolic arterial pressure (DAP) elevation in 8 patients, and a drop in one, were noted. Unilateral stimulation produced no responses at all. In another study (67), orbitofrontal cortical stimulation in 9 patients undergoing frontal lobotomies for psychiatric disease produced inconsistent elevation of SAP in 6 of them. In a third study (105), only subtle DAP and heart rate changes have been reported after stimulation of insular cortex in 5 patients with epilepsy undergoing surgery for control of intractable seizures. Therefore, the present investigation is the first report of a dramatic, consistently reproducible blood pressure effect in all patients who had the same, restricted, cortical site stimulated, namely the subcallosal region of BA25.

Similarly, albeit with the limited number of patients in our study, orbitofrontal, insula (anterior), amygdalar, hippocampal head, posterior cingulate, temporopolar, basal temporal and temporal neocortex stimulations did not produce blood pressure responses; we therefore could not confirm the role of these structures in human autonomic control of blood pressure. Several reasons are possible. Human studies that have reported blood pressure changes with stimulation of the cingulate and insula regions, have used stimulation parameters with substantially greater stimulus intensity than in our study. For example, Pool and Ransohoff used up to 120 Hz frequency and 60 second train durations in their study, possibly accounting for their positive findings in these brain structures. Only four patients had insula stimulation in our study, all of whom only had anterior insular stimulation.

The mechanism of such striking falls in SAP, without concurrent falls in DAP and heart rate, is likely to be due to a cardioinhibitory reduction in myocardial contractility and a reduction in left ventricular stroke volume as indicated by the narrowing of pulse pressure in all our patients. The lack of significant changes in DAP (a product of resting transmural force blood volume exerted against vascular walls), during the stimulation period, excludes peripheral vasodilatation as a cause. Stimulation of BA25 likely produces downstream effects in or distal to the lateral hypothalamic nuclei or ventral periventricular/periaqueductal grey areas, brainstem regions known to produce blood pressure effects (106-108). These regions have rich connections with area 25 (109). Downstream candidate brainstem structures include the nucleus of the rostral and ventrolateral medulla, medullary raphe, and the A5 noradrenergic group of the pons (110, 111). The ultimate effect is likely to be a reduction in sympathetic outflow in the efferent arm of the baroreflex emanating from the rostral ventrolateral medulla.

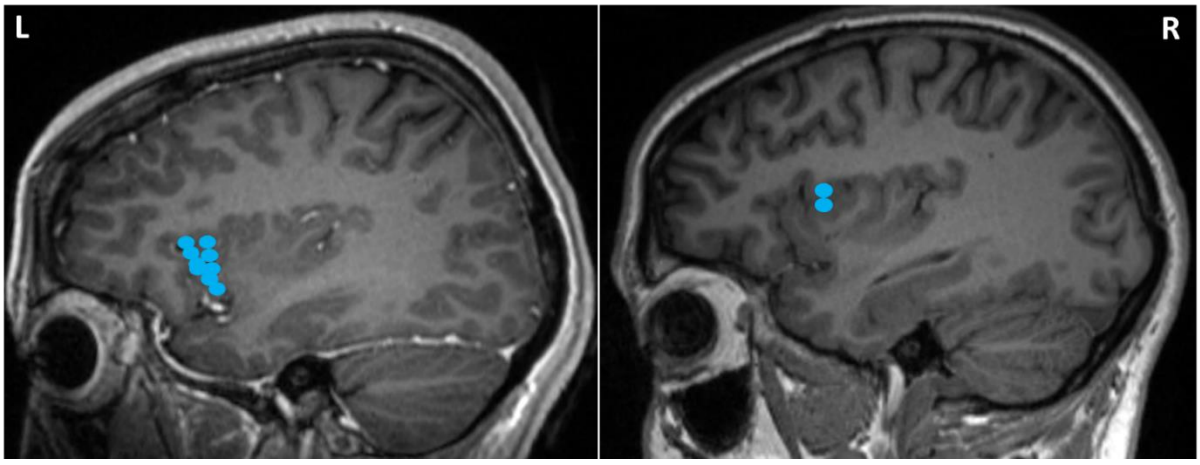
7.3. Cortical control of cardiac rhythm

Ictal tachycardia is frequently seen during seizures. On the other hand, ictal bradycardia and asystole (IA) are infrequently reported. IA, defined as sinus arrest triggered by an epileptic

seizure, is a rare event observed in 0.3 to 0.4% of patients undergoing long-term video-EEG monitoring in Epilepsy Monitoring Units (112, 113).

IA has mainly been observed during temporal lobe seizures (112, 114, 115) but also in frontal epilepsy and in several cases of lesional insular epilepsy (112, 116). IA has been significantly associated with left hemispheric focal epilepsy (112). Such lateralization is consistent with insular cortex stimulation studies demonstrating left insular control of parasympathetic cardiovascular tone (105). Electrical stimulation of the human insula has been reported to produce cardiac chronotropic and blood pressure responses in 5 patients (3 right hemisphere, 2 left side) (105). Bradycardia and depressor responses (diastolic blood pressure) were significantly more frequently encountered with stimulation of the left insular cortex, mainly from posterior regions. In our study, stimulation of insular cortex in 4 subjects (3 left, 1 right) did not induce significant and/or consistent heart rate changes after stimulation of 10 electrode contacts in the right (2 electrode contracts) and left (8) insular cortices. This could be explained by lack of coverage, mainly of the posterior regions (Figure 17). Autonomic function (sympathetic/parasympathetic cardiovascular tone) in our study could not be systematically assessed during stimulation due to short stimulation period duration (less than 40 seconds).

Figure 17. Stimulating electrode contact positions in the left (L) anterior insula (short gyrus) and



the right (R) anterior insula (middle short gyrus). Stimulation did not induce any heart rate change.

8. CONCLUSIONS

A) Clinical significance of precise localization of limbic/paralimbic structures influencing respiratory patterning.

These findings provide robust evidence that sites within limbic/paralimbic (amygdala, hippocampus, parahippocampal gyrus and mesial temporo-polar region) structures have the potential to suppress breathing and may provide the symptomatogenic substrate for ICA.

Direct electrical stimulation of mesial temporal structures may simulate focal seizures in these structures; hence, apnea thus produced is highly likely to represent ICA. Accurate identification of apnea-inducing sites has important implications in the unraveling of the semiological impact of ICA. ICA is a frequent seizure sign, seen in 37-44% of focal epileptic seizures (48) (Appendix 10.1) (54% of temporal lobe seizures), and can occur as long as 29 seconds before unequivocal scalp EEG onset (8+4.9 [1-29] seconds) in 54% of focal seizures, and up to 50 seconds (12.3+9.7 [1-50]) before any other clinical signs in 69% of focal seizures (Appendix 10.1).

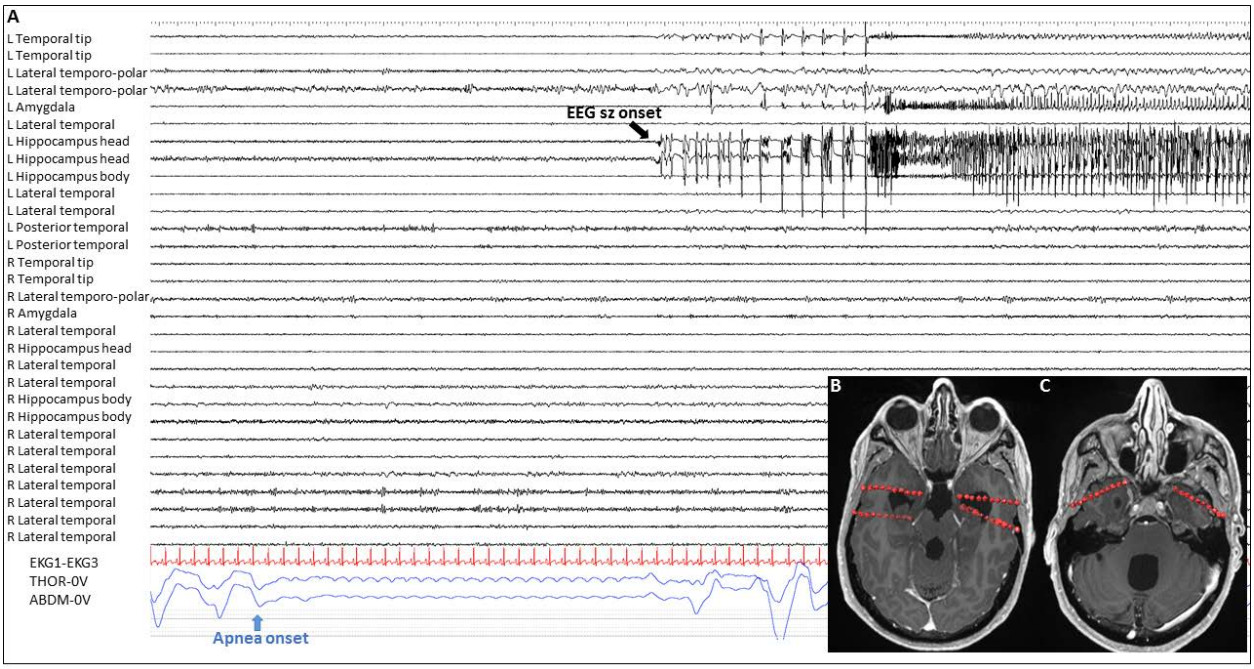
The apnea-inducing phenomenon can indicate a highly focal amygdalo-hippocampal seizure intracranially, sufficient to inhibit breathing rhythm or inspiration, and drive ICA, but so exquisitely localized as to cause no surface EEG change.

An example of an SEEG recording of spontaneous hippocampal seizure onset in subject 1, and ictal central apnea is shown in Figure 6 preceding a surface EEG seizure 12 seconds later. Our findings may have significant impact in the epilepsy surgery domain. Precise localization and depth electrode targeting of apnea producing structures may help improve

SEEG evaluations through additional analysis of ictal onset zones, based on ICA as a clinical symptom. ICA's potential impact was evident in the post-hoc analysis of one patient with suspected mesial temporal lobe (MRI negative) epilepsy, whose hippocampal head, body, amygdala and temporal tip were implanted bilaterally, but without coverage of the parahippocampal gyrus or mesial temporo-polar cortex (Figure 18). ICA was the first clinical sign (similar to habitual seizures) and preceded intracranial left hippocampal seizure onset by up to 21 seconds (Figure 18).

In this case, the unequivocal demonstration of ICA at clinical seizure onset, in the absence of seizure discharge on invasive electrodes, indicated that important extra-amygdalo-hippocampal cortical structures critical to generation of ICA, were not covered in the invasive evaluation. The subject continued to have seizures after left hippocampal transection as a memory sparing procedure, and re-evaluation is planned.

Figure 18. Ictal central apnea (ICA) onset preceding intracranial seizure onset in a patient with left temporal epilepsy.



Legend: Patient with focal seizures consistent with apneic seizures followed by impaired consciousness and oral automatisms, who underwent bilateral temporal intracranial depth electrode implantation (including amygdala, hippocampus head and body, lateral temporal, temporal tip and lateral temporo-polar cortex in both hemispheres). **A)** During a video monitored habitual seizure, a central apnea was seen 20 seconds before unequivocal intracranial EEG seizure onset arising from the left hippocampal body. Electrode contact locations targeting amygdala and hippocampus head **(B)** and **(C)** temporal tip in both hemispheres, are shown. The patient did not become seizure free after a selective left hippocampal surgical procedure, suggesting that the symptomatogenic zone for the patient's apnea was not sampled by the implanted electrodes.

The majority of patients stimulated had a brief, mean apnea duration of 11 seconds with no, or small changes in CO2 and/or SpO2 after the apnea period. However, subject eight provokes major interest. She had longstanding epilepsy (25 years), with onset at age 7 years, and had high generalized tonic clonic seizure frequency (1/month), all characteristics that are associated with substantially increased risk of SUDEP (19). Stimulation-induced apnea periods were consistently longest in this subject (parahippocampal gyrus stimulation), with persistence of apnea beyond termination of stimulation (parahippocampal gyrus and amygdalar stimulation) (Table 4). It is possible that these observations are related to her biological susceptibility to prolonged ICA and susceptibility to SUDEP, although such conclusions cannot be based on a single case.

In this study, we continued to observe how patients are able to override apnea when they are instructed to breathe or talk after apnea onset. This could be explained by activation of the voluntary motor breathing network in the motor cortex (92). Thus, auditory and/or tactile stimulation of ictal apneic patients and asking them to breathe, where possible, may be important to recovery. However, since patients frequently lose consciousness or comprehension during seizures, active attempts to abort seizures producing apnea may be more successful.

B) Clinical significance of the identification of BA 25 influencing blood pressure.

In our study we found that electrical stimulation of Brodmann area 25, in either hemisphere, can induce systolic hypotension in the human brain.

Direct electrical stimulation of Brodmann area 25 may simulate focal seizures in this structure; hence, falls in systolic blood pressure thus produced are highly likely to represent ictal hypotension.

Ictal hypotension can occur during focal seizures and could be due to seizure discharge spread to BA25. Ictal hypotension in patients with autonomic damage, and thus, a weak baroreflex response, may be at especial risk of SUDEP. Profound postictal hypotension can be closely correlated to post-ictal generalized EEG suppression (PGES) duration (38). PGES has been described in ten video monitored SUDEP cases before terminal apnea or cardiac asystole (1). None of the observed SUDEP and near-SUDEP cases reported in literature had blood pressures recorded (95), and thus its role is still uncertain. The role of BA25 in per-ictal hypotension, and the contribution of such hypotension to SUDEP, remains to be elucidated.

Our findings may have therapeutic implications. Stimulation of Brodmann area 25 in patient 12, with chronic hypertension, induced similar reduction in systolic blood pressure. Stimulation of the ventral periventricular and periaqueductal grey, used as a treatment for chronic pain, have been proposed as sites for deep brain stimulation for the treatment of intractable hypertension (99-101, 107, 108). Variability in blood pressure responses at the same locations (109), and the potential for complications in the brainstem, render the periaqueductal/periventricular gray regions as relatively unattractive targets for the purpose. On the other hand, Brodmann area 25 is an easily accessible and relatively safe target used in deep brain stimulation for the treatment of refractory depression (110-112). Studies of the latter have not reported symptomatic hypotension, except in one case, where acute, stimulation (120 Hz, 0.09 ms and 3 volts) induced orthostatic hypotension was observed (117). Further studies

are warranted to determine therapeutic potential, including effective parameters for long term usage in intractable essential hypertension.

C) Clinical significance of insular related autonomic dysfunction and SUDEP (Appendix 10.2)

In our study, electrical stimulation did not elicit significant chronotropic responses, including with insular cortex stimulation. However, we reported autonomic changes in two SUDEP cases and suggested, for the first time, that the presence of insular damage might be an additional risk factor for SUDEP in patients with refractory epilepsy (Appendix 10.2).

9. LIMITATIONS OF OUR STUDY

Some limitations of our study need to be considered. Although this study is the largest case series of breathing and blood pressure responses to human direct cortical electrical stimulation, our conclusions are still based on a relatively small number of patients.

All subjects had refractory epilepsy, and sampling of stimulation areas was dependent on the surgical hypothesis for implantation; limited numbers of electrodes were implanted and/or stimulated in the cortex. These data (both positive and negative findings) require reproduction in a larger cohort of patients.

Our study suggests that BA25 and mesial temporal lobe structures may be involved in autonomic and respiratory cortical control in the human brain, and suggest that seizure spread to these structures may induce blood pressure and breathing dysfunction. However, their precise role in SUDEP mechanisms is still uncertain. Further investigations are necessary to understand the precise pathomechanisms leading to SUDEP.

10. FUTURE

1. We aim to investigate the mechanism of Brodmann area 25 (BA25) stimulation-induced blood pressure (BP) changes and its possible therapeutic application in individuals who have medically refractory hypertension. We will include more subjects and, in addition to brain stimulation, carry out additional real time and post-processed non-invasive echocardiography to estimate cardiac indices (stroke volume, cardiac output, left ventricular ejection fraction, diastolic function grade, and myocardial contractility). We will define differences in baseline and stimulation period BP and cardiac indices, to target optimal therapeutic strategies for deep brain stimulation in intractable hypertension.

2. We are prospectively recording continuous blood pressure in patients undergoing video EEG evaluation in the epilepsy monitoring unit, with the purpose of characterizing blood pressure changes during seizures and to look for a correlation between spontaneous peri-ictal hypotensive changes and seizures discharges in BA 25, in patients undergoing stereotactic electroencephalogram (SEEG) as a prelude to epilepsy surgery.

3. We will investigate the correlation between ictal central apnea (ICA) and seizures discharges and evaluate if ICA helps localization of seizure onset in mesial temporal epilepsy. In addition to focal seizures, we will study breathing in generalized tonic-clonic seizures (GTCS), with the objective of characterizing breathing dysfunction after GTCS and its possible relationship to SUDEP pathomechanisms.

11. APPENDIX

11.1. THE INDICENCE AND SIGNIFICANCE OF PERI-ICTAL APNEA IN EPILEPTIC SEIZURES.

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FULL-LENGTH ORIGINAL RESEARCH

Epilepsia

The incidence and significance of periictal apnea in epileptic seizures

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Summary

Objective: The aim of this study was to investigate periictal central apnea as a seizure semiological feature, its localizing value, and possible relationship with sudden unexpected death in epilepsy (SUDEP) pathomechanisms.

Methods: We prospectively studied polygraphic physiological responses, including inductance plethysmography, peripheral capillary oxygen saturation (SpO_2), electrocardiography, and video electroencephalography (VEEG) in 473 patients in a multicenter study of SUDEP. Seizures were classified according to the International League Against Epilepsy (ILAE) 2017 seizure classification based on the most prominent clinical signs during VEEG. The putative epileptogenic zone was defined based on clinical history, seizure semiology, neuroimaging, and EEG.

Results: Complete datasets were available in 126 patients in 312 seizures. Ictal central apnea (ICA) occurred exclusively in focal epilepsy (51/109 patients [47%] and 103/312 seizures [36.5%]) ($P < .001$). ICA was the only clinical manifestation in 16/103 (16.5%) seizures, and preceded EEG seizure onset by 8 ± 4.9 s, in 56/103 (54.3%) seizures. ICA ≥60 s was associated with severe hypoxemia (SpO_2 <75%). Focal onset impaired awareness (FOIA) motor onset with automatisms and FOA nonmotor onset semiologies were associated with ICA presence ($P < .001$), ICA duration ($P = .002$), and moderate/severe hypoxemia ($P = .04$). Temporal lobe epilepsy was highly associated with ICA in comparison to extratemporal epilepsy ($P = .001$) and frontal lobe epilepsy ($P = .001$). Isolated postictal central apnea was not seen; in 3/103 seizures (3%), ICA persisted into the postictal period.

Significance: ICA is a frequent, self-limiting semiological feature of focal epilepsy, often starting before surface EEG onset, and may be the only clinical manifestation of focal seizures. However, prolonged ICA (≥60 s) is associated with severe hypoxemia and may be a potential SUDEP biomarker. ICA is more frequently seen in temporal than extratemporal seizures, and in typical temporal seizure semiologies. ICA rarely persists after seizure end. ICA agnosia is typical,

and thus it may remain unrecognized without polygraphic measurements that include breathing parameters.

KEYWORDS
apnea, breathing, seizures, sudden unexpected death in epilepsy, temporal epilepsy

1 | INTRODUCTION

Hypoventilation and hypoxemia are typically seen in focal to bilateral tonic–clonic seizures (FBTCS) and generalized tonic–clonic seizures (GTCS),[1–3] and severe alteration of breathing patterns after such seizures has been suggested as a possible mechanism of sudden unexpected death in epilepsy (SUDEP).[1] However, oxygen desaturations are also found in 30-60% of focal seizures without generalized convulsions.[2] Desaturations are more commonly seen with temporal lobe than with extratemporal seizures.[2,4] Electrical stimulation of mesial temporal structures consistently elicits central apnea, potentially explaining this observation.[5,6] Ictal apnea has also been noted in 44-48% of nongeneralizing focal seizures,[2,4,7,8] and has been reported as the main manifestation of focal seizures in a few case reports.[9,10] Ictal and postictal central apnea has been suggested as a potential mechanism in some SUDEP[11] and near-SUDEP[12] cases. However, the role of ictal and postictal central apnea in SUDEP remains to be definitively demonstrated. We set out to examine the phenomenology, localizing value, and impact of ictal and postictal central apnea in patients with intractable epilepsy, in the epilepsy monitoring unit setting.

Key points

- Ictal central apnea (ICA) is frequent in focal seizures and may be their first clinical sign
- Patients are apnea agnostic, and hence polygraphic monitoring of respiration during seizures is necessary for diagnosis
- ICA is 10 times more often seen in temporal than extratemporal seizures
- Prolonged ICA (>60 s) is associated with severe hypoxemia

2 | METHODS

2.1 | Patients and clinical settings

All patients were prospectively consented and recruited participants in the NINDS Center for SUDEP Research's Autonomic and Imaging Biomarkers of SUDEP project (U01-NS090407). Patients with epilepsy aged ≥16 years who were undergoing video-electroencephalography (VEEG) evaluation were studied in the epilepsy monitoring units (EMUs) of University Hospitals Cleveland Medical Center, University of Iowa, Northwestern University, New York University, Thomas Jefferson University, University of California at Los Angeles, University College London, and Columbia University. Inclusion criteria were patients in whom inductance plethysmography (abdominal and/or thoracic belts) and VEEG recording were carried out during the evaluation in the epilepsy unit. Exclusion criteria were movement or electrical artifacts obscuring plethysmographic signal, or obstructed or unavailable video.

2.2 | Cardiorespiratory monitoring and VEEG monitoring

All patients had prolonged surface VEEG monitoring using the 10-20 International Electrode System. EEG and electrocardiography (ECG) were acquired using the Nihon Kohden (Tokyo, Japan), Micromed (Modigliani Veneto, Italy), and Xltek (Natus, Pleasanton, CA, USA) acquisition platforms. Peripheral capillary oxygen saturation (SpO_2) and heart rate were monitored using pulse oximetry (Nellcor OxiMax N-600x [Convidien, Minneapolis, MN, USA], Masimo Radical-7 [Masimo, Irvine, CA, USA] and SenTec Digital Monitoring System [Therwil, Switzerland]). Chest and abdominal excursions were recorded using inductance plethysmography (Ambu, Ballerup, Denmark] Sleepmate and Perfect Fit 2 [Dymedix, St. Paul, MN, USA]). Oxygen desaturations were classified as mild (SpO_2 of 90-94%), moderate (75-89%) and severe (<75%). We defined central apnea as cessation of breathing movements lasting for ≥10 s in the absence of generalized tonic or clonic movements, since such movements invariably produced movement artifact in breathing channels. Tachycardia and bradycardia were defined as heart rate >100 beats per minute and <60 beats per minute respectively, or a >20% deviation from baseline. Seizures were classified according to the International League Against Epilepsy (ILAE) 2017 seizure classification[13] based on the most prominent clinical signs: focal onset impaired awareness (FOIA) motor onset with automatisms, FOIA nonmotor onset (dialepsis), FOIA motor onset with hyperkinesis, focal onset aware (FOA)

motor onset tonic and/or clonic, focal onset to bilateral tonic–clonic seizures (FBTCS), and FOA nonmotor onset (auras). Cognitive seizures, where the main clinical manifestation was aphasia, were further classified as aphasic seizures. Electrographic seizures were defined as seizures where the sole clinical manifestation was ictal central apnea. Generalized onset non-motor typical seizures were classified as absence seizures, and generalized onset tonic–clonic seizures of primary generalized epilepsy were classified as GTCS (or GTCS, diagnosis based on history and EEG findings). The putative epileptogenic zone was defined based on clinical history, seizure semiology, neuroimaging, and scalp EEG.

2.3 | Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Science (SPSS, version 24; IBM Corp, Armonk, NY, USA). Summary statistics were reported as mean ± standard deviation (SD; median, range). Chi-square test and binary logistic regressions were used to assess the association between dichotomous variable apnea (yes/no), with other variables and combinations. Because the 103 apneic seizures were not normally distributed, nonparametric testing (Kruskal-Wallis test) was used to assess apnea duration with other variables.

3 | RESULTS

A total of 473 patients underwent polygraphic study of seizures. Reliable inductance plethysmography recordings and unobstructed seizure videos for the assessment of breathing responses were available in 312 seizures in 126 patients (77 female). Mean age was 40.09 ± 14.71 years (median 38.5; range 16-77). One hundred nine patients had focal epilepsy and 17 patients had primary generalized epilepsy. Mean epilepsy duration was 17.8 ± 13.5 years (median 17; range 0-52).

3.1 | A) Ictal central apnea (ICA) incidence and duration

Ictal central apnea (ICA) was found in 103/312 (36.5%) seizures in 51/126 (40.5%) patients (29 female). Mean ICA duration was 28 ± 18.8 (median 22; range 10-97) seconds (s). Oxygen saturation data were available for 227/312 seizures overall, and in 79/103 seizures with ICA. In the remaining seizures, data were rendered unreliable because of dislodged sensors and movement. Prolonged ICA (≥60 s) occurred in 8 patients and was associated with severe hypoxemia (SpO_2 <75%) in 6. Seizure and epilepsy details of patients with and without ICA are shown in Table 1.

3.2 | Influence of type and duration of epilepsy, age, and gender on apnea

ICA was seen exclusively in focal epilepsy (36.5% of all partial seizures; $P < .001$); none of the 17 primary generalized epilepsy patients (15 with primary GTCS and 7 with absence seizures), had ICA. None of the 15 GTCS were preceded by central apnea. In the focal epilepsy group, 10/22 FBTCS had preceding ICA; ICA was the sole clinical manifestation in all 10 before the onset of tonic–clonic activity. We found that older age was significantly associated with ICA presence ($P < .001$), but not with ICA duration or hypoxemia severity ($P = .6$). There were no gender differences in ICA incidence ($P = .2$). Although longer duration of epilepsy was not significantly associated with the presence of ICA ($P = .3$), it was associated with longer ICA duration ($P < .001$). Examples of presence or absence of ICA are shown in Figures 1 and 2.

3.3 | Epileptogenic and ictal-onset zones and seizure semiology

Temporal lobe epilepsy was highly associated with ICA presence in comparison with extratemporal epilepsy (odds ratio [OR] 10.1, 95% confidence interval [CI] 5.5-18.5; $P = .001$) and to frontal lobe epilepsy (OR 8.3, 95% CI 4-17.3; $P = .001$) (Figure 3). Temporal lobe ictal-onset zone was accordingly significantly associated with ICA ($P < .001$). We then assessed whether the first ictal discharges at or after ICA onset involved one or both hemispheres. The ictal discharge was unilateral in 85/103 apneic seizures (87%), nonlateralizable in 7/103 (7%) and obscured by artifact at ICA onset in 11/103 (11%). ICA was significantly more likely to be associated with unilateral (left or right) ictal discharge at apnea onset compared to nonlateralizable EEG seizure onset ($P < .001$). Temporal lobe epilepsy ($P < .001$) and unilateral temporal lobe EEG ictal onset ($P = .001$) were both significantly associated with longer ICA duration. There was a higher incidence of ICA in FOIA motor onset with automatisms (71.4%) and FOIA nonmotor onset (dialepsis) (55.9%) compared to other seizure types (Figure 3). Both were highly associated with ICA presence ($P < .001$), as well as ICA duration ($P = .002$) and severe hypoxemia ($P = .04$).

3.4 | Awake and sleep states

Mean duration of ICA in seizures during the awake state was 27.87 ± 19.38 s and 28.13 ± 18.43 s during the non-REM (rapid-eye movement) sleep state; the awake/sleep states at seizure onset did not significantly impact either ICA presence or ICA duration ($P = .6$). No seizures arose during REM sleep.

TABLE 1 Seizure and epilepsy characteristics in 126 patients, with and without apnea

	Total number	Apnea	No apnea
Total of seizures	312	103	209
Type of seizures			
Focal	285	103*	182
Generalized	27	0	27
State at the seizure onset			
Awake	146	50	96
Sleep	166	53	113
Epileptogenic zone			
Temporal	156	84*	72
Frontal	79	10	69
Parietal	11	0	11
Occipital	24	4	20
Insula	2	2	0
Generalized	30	0	30
Unknown	10	3	7
Brain MRI (number of patients)	312	103	209
Negative (84)	197	72	125
Mesial temporal sclerosis (8)	17	3	14
Hippocampus atrophy (2)	6	5	1
Focal cortical dysplasia (6)	28	2	26
Gray matter heterotopia (3)	6	1	5
Tumor (11)	38	7	31
Encephalomalacia (3)	5	3	2
Schizencephaly (1)	1	1	0
Encephalocele (1)	3	3	0
Arterial venous malformation (2)	3	2	1
Corpus callosal dysgenesis (1)	2	2	0
MRI unavailable/normal CT head (4)	6	2	4
Seizure semiology[a]			
FOIA motor onset with automatisms	63	45*	18
FOIA nonmotor onset (dialepsis)	34	19*	15
FOA nonmotor onset (auras)	28	3	25
FOIA motor onset hyperkinetic or FOA motor onset tonic/clonic	99	10	89
Aphasic	17	0	17
FBTCS	22	10	12
Absence	7	0	7
GTCS	15	0	15
Electrographic seizures	27	16	11
EEG seizure onset			
Temporal	132	71*	61
Frontal	51	10	41
Parietal	6	0	6
Occipital	14	2	12
Nonlateralizable	64	6	58
Obscured by artifact	45	14	31
EEG seizure discharges at or after ICA onset			
Unilateral right	94	34*	60
Unilateral left	120	51*	69
Nonlateralizable	59	7	52
Obscured by artifact	39	11	28

FOIA, focal onset impaired awareness; FOA, focal onset aware; FBTCS, focal to bilateral tonic–clonic seizures (of focal epilepsy); GTCS, generalized tonic–clonic seizures (of primary generalized epilepsy); ICA, ictal central apnea.
[a]According to ILAE seizure classification.[13]
*Significant values ($P < .05$).

3.5 | Apnea-induced oxygen desaturation of hemoglobin (SpO_2)

Ictal hypoxemia was present in 56/79 (70.8%); desaturation was mild in 26/56 (46%) of seizures (mean 92.5 ± 1.2 [93; 90-94]), moderate in 22/56 (39%) (mean 81.5 ± 4.0 [82.5; 75-89]) and severe in 8/56 (14%) (mean 64.7 ± 9.3 [69.5; 46-72]). Mean oxygen desaturation nadir was 87.7 ± 10.3 (92; 46-98). Duration of ICA was significantly negatively correlated with SpO_2 nadir ($r = -0.89$; $P < .001$) (Figure 4). Fifty-three percent of FOIA motor onset with automatism seizures had moderate or severe hypoxemia compared to all other semiologies combined ($P = .04$). Temporal lobe seizures were more likely to have moderate or severe ictal hypoxemia compared to other epileptogenic zones ($P = .03$).

3.6 | Ictal apnea characteristics and relationship with EEG onset/clinical onset

In 16/103 (16.5%) of seizures, ICA was the only clinical manifestation during the entirety of the seizure. In 56/103 (54.3%) seizures, ICA occurred before unambiguous EEG seizure onset (mean 8 ± 4.9 [7.7; 1-29] seconds). In 15/103 (14.5%), EEG onset and ICA onset were simultaneous, and in 32/103 (31%) EEG seizure onset preceded ICA (mean 7.7 ± 7.9 [7.7; 1-28] seconds). In 61/103 seizures (68.5%), ICA onset occurred before clinical onset (mean 12.3 ± 9.7 [10; 1-50] seconds). These ICA onsets were simultaneous in 15/103 (16.8%), and in 13 seizures (14.6%), clinical onset preceded ICA onset (mean 13.6 ± 9.6 [12; 1-33] seconds). ICA always occurred in the expiratory phase and all patients were agnostic to their apneas, confirmed by questioning.

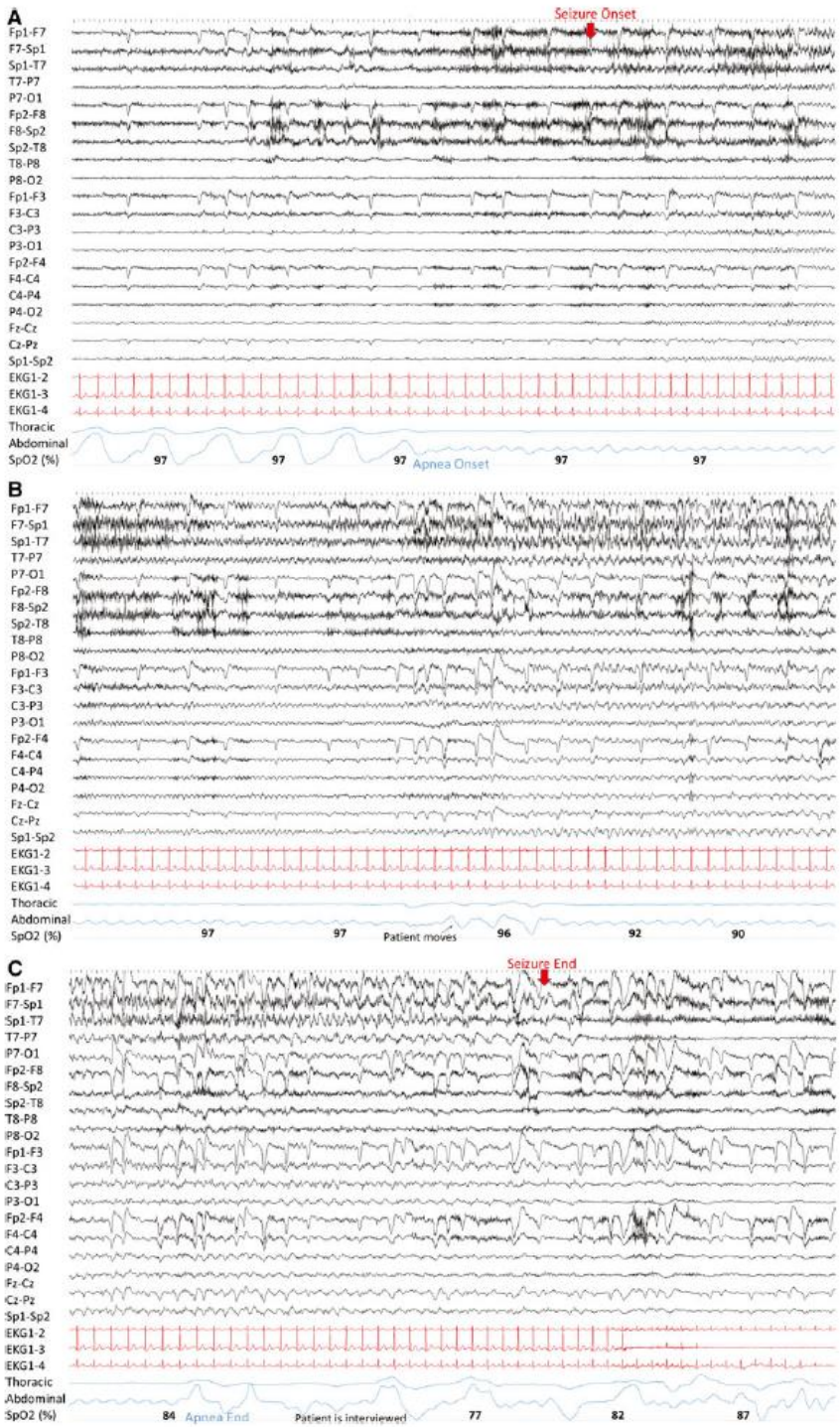

FIGURE 1 A left temporal lobe seizure is shown in 3 consecutive 30 s pages, in polygraphic detail. In A, the patient is awake before seizure onset. Cessation of breathing movements was noted 6 s before epileptiform discharges began. In B, during a 50-s apnea period, complete absence of breathing movement is seen, along with oxygen desaturation, with only pulse artifacts identifiable in the plethysmography signal. In C, the patient restarts breathing 15 s before seizure end, when he is interviewed by nurses. The patient was apnea agnostic

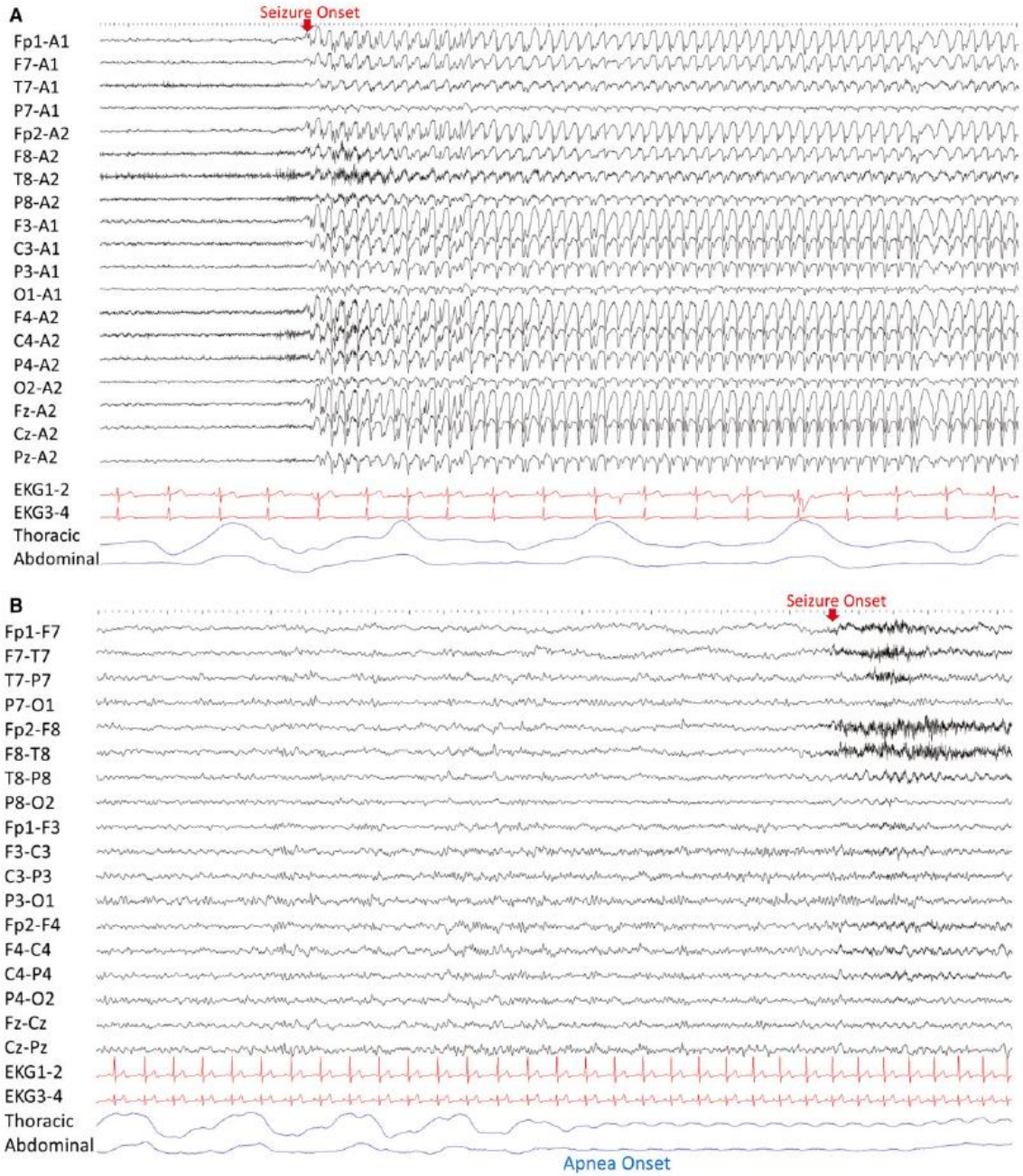

FIGURE 2 Differences in polygraphy studies are represented in a typical (A), generalized seizure with 3 Hz spike and wave's discharges where no apnea is observed and (B), focal epilepsy and right temporal lobe seizure where central apnea is clearly seen

3.7 | Apnea and bradycardia

During ICA periods, heart rate increased in all 103 seizures; it was always seen at or after EEG seizure onset, rather than with ICA onset. Periictal bradycardia was not observed in any seizure.

3.8 | B) Postictal central apnea

Spontaneous restoration of breathing before seizure end was seen in 100/103 (97%). In 3 FOIA motor onset with automatism seizures (3%), in 2/126 patients (2%), apnea persisted into the postictal period for 16-22 s (total

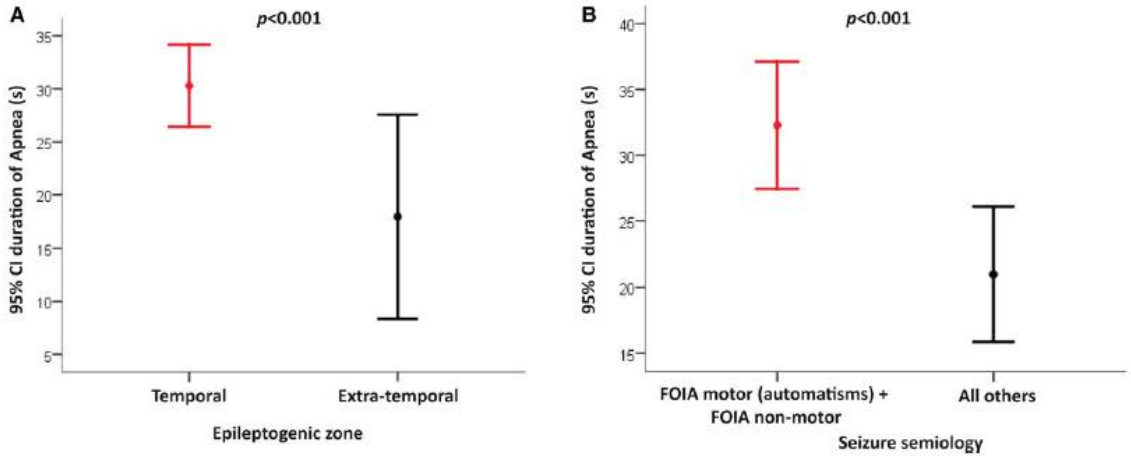

FIGURE 3 Plot with error bars of ictal central apnea duration in seconds by epileptogenic zone (A) and seizure semiology (B), showing 95% confident intervals (CIs) for temporal compared to extra-temporal ($P < .001$) (A) and FOIA motor onset with automatisms and FOIA non-motor (dialepsis), compared to other seizure semiologies ($P < .001$) (B). FOIA = focal onset impaired awareness

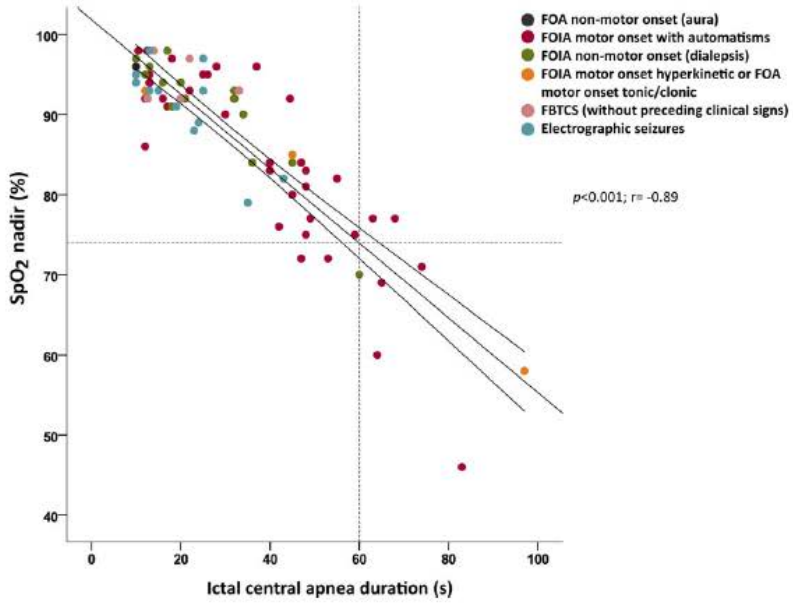

FIGURE 4 Peripheral capillary oxygen saturation (SpO_2) nadir and ictal central apnea (ICA) duration. The abscissa is apnea duration (in seconds) and the ordinate is the SpO_2 at apnea end. The robust simple linear regression line and 95% confidence intervals are shown. Dashed lines show that apnea duration of 60 seconds approximately correlates with SpO2 <75%. FOA = focal onset aware, FOIA = focal onset impaired awareness, FBTCS = focal to bilateral tonic–clonic seizure, s = seconds

periictal [ictal plus postictal] apnea periods were 46-97 s). None had apnea beginning exclusively in the postictal period.

4 | DISCUSSION

This study suggests that ICA is a semiological feature exclusive to focal epilepsy, that it most commonly starts before unambiguous surface EEG onset, and can be the only clinical manifestation of focal seizures. Temporal lobe epilepsy and frontal lobe epilepsy accounted for most focal epilepsies associated with apnea; temporal lobe epilepsy not only had an 8-fold greater association with apnea than frontal lobe epilepsy, it was also significantly more likely to be associated with longer apneas and more severe hypoxemia. FOIA motor onset with automatisms and FOIA

nonmotor onset seizure semiologies, typical of temporal lobe epilepsy, were similarly much more likely to produce ICA, and longer ICA durations. Thus ICA presence and ICA duration may not only help distinguish focal epilepsies, they may also enhance localization to the temporal lobe. Temporal lobe symptomatogenicity for ICA is in concordance with direct electrical cortical stimulation studies in humans that point to highly reproducible apneic responses with[14,15] low intensity, unilateral (left or right) amygdalar and hippocampal stimulation.[5,6,16] Although seizure spread to bilateral temporal structures has been considered necessary to produce ICA, these unilateral stimulation experiments, and the focal, unilateral ictal discharges at the time of ICA in many of our patients, suggest that such spread is not always the case.

It is likely that seizure discharges impair involuntary suprapontine (amygdalohippocampal) breathing control, resulting in ICA. Because ICA occurred after expiration in all our patients, it is likely that inspiration is immediately inhibited by seizure discharge, whereas expiration is mostly passive and allowed to occur to completion. The most likely downstream driver for ICA is seizure-induced inhibition or disruption of brainstem inspiratory neuronal function. Descending amygdala projections to the parabrachial structures, which exert critical roles in phase switching from expiration to inspiration have been described in cats single pulse amygdala stimulation triggers inspiratory onset.[17] Hippocampal activity increases before apnea termination in cats[18] and some hippocampal and amygdalar neurons phase-lock with the respiratory cycle in humans, suggesting that these structures are intimately involved in breathing regulation.[19,20] Thus the relatively frequent occurrence of ICA in patients with temporal lobe epilepsy is unsurprising. Whether ICA in patients with extratemporal epilepsy reflects spread to amygdalohippocampal structures (anterior cingulate, orbitofrontal, and anterior insular regions are extratemporal sites with amygdalar connections that have also been implicated in cortical breathing control[16]), or whether this implies involvement of symptomatogenic extratemporal breathing control structures is uncertain; shorter duration of apnea in these patients may indicate the involvement of breathing network nodes that are distinct from those involved in temporal lobe seizures.

The apnea agnosia described in stimulation studies[5,6] appeared to be true of ICA in our patients, and cessation of apnea in partial seizures was not followed by breathing distress, air hunger, or dyspnea despite significant oxygen desaturations. This may explain why ICA has largely gone unrecognized, aside from a few case series and case reports.[10,21] An additional explanation is that plethysmography is not commonly used in EMUs. Breathing resumption in ICA patients, prior to seizure end, was the rule (97%) with few exceptions (3%). Lack of ICA awareness may be dangerous only in prolonged apnea. We observed that ICA cessation did not appear to be driven by hypoxemia. Although hypercarbia cannot be commented upon here, since carbon dioxide was not measured, ictal central apneas are not reversed by augmentations in ventilatory drive from increasing carbon dioxide in previous human[2] and animal[22] studies, and similar observations have been made in stimulation experiments.[5] ICA durations were highly varied; therefore, changes in seizure discharge intensity in breathing control structures are a more likely explanation.

In our patients, the complete absence of ictal bradycardia with ICA, reported in a minority of seizures in one series,[7] is surprising because bradycardia is a normal response to hypoventilation. Asphyxia in animal models results in heart rate decline and cardiac arrest in approximately 5 min.[23] Consistent with the literature,[12,24] even in the rare, prolonged ICA epochs (up to 97 s), no bradycardia was observed in our study. Seizure-driven tachycardia is common,[25] and may have overcome any physiological tendency to bradycardia in these patients. Combined periictal apnea and bradycardia, therefore, appears rare, but when it does occur, may comprise a potentially deleterious, high vagal tone phenotype in seizure patients in the SUDEP context; the observed tachycardia apnea combination in this study may reflect a more benign, self-limiting seizure manifestation.

Is ictal apnea a SUDEP biomarker? Most patients had a brief, self-limiting apnea with mild or moderate hypoxemia, suggesting that ICA poses no danger in most cases. Mean and median ICA durations in this cohort were 28 and 22 s, respectively; the sheer frequency of ICA in this cohort, their short durations, and cessation before seizure end, suggest that in most cases ICA is self-limiting and unlikely to be a SUDEP concern. However, prolonged ICA (≥60 s) was associated with severe hypoxemia (SpO_2 <75%) (Figure 4), and hence this combination may prove to be a biomarker of SUDEP that deserves prospective study. Indeed, 2 nonfatal ICA durations of 57 and 58 s, with SpO_2 of 68% and 62%, respectively, were recorded in a previously reported patient who subsequently died of SUDEP at home.[12] Longer duration of epilepsy is a known SUDEP risk factor.[26] Our observation of a positive correlation between duration of epilepsy and ICA duration is intriguing, and suggests potential plasticity in respiratory circuitry that may render relatively benign, short duration ICA into potentially lethal, longer duration ICA that may predispose to SUDEP. For example, functional neuroplasticity in the nucleus of the tractus solitarius, as evidenced by long-term changes in glutamate release and γ-aminobutyric acid (GABA)ergic neuronal activity, has been shown to occur with epileptogenesis in mice with acquired temporal lobe epilepsy.[27]

Postictal apnea appears to be a rare phenomenon. Only 3% of ICA persisted (for 16-22 s) beyond electroclinical

seizure end in this study. None had isolated postictal central apnea beginning exclusively after seizure end. Duration of apnea continuance beyond seizure end was short (16-22 s) with total periictal apnea periods between 46 and 97 s. Postictal apneic bradycardia, frequently reported in the Postictal, agonal phases of MORTEMUS SUDEP cases, and near-SUDEP cases, after a partial seizure[11] or GTCS,[28] did not occur in any of our patients. The persistent apnea observed into the postictal period may not have been truly postictal, as epileptiform discharges can persist in deep regions, such as amygdala or hippocampus, and not be seen on scalp EEG. However, persistent apnea could also represent a phenomenon like Todd's paralysis or postictal aphasia, due to dysfunction or "exhaustion" in the major breathing control sites in in the human brainstem.

Some limitations of our study need to be considered. Our conclusions are based on a relatively small number of seizures in the primary generalized epilepsy group. In addition, by considering GTCS or FBTCS onset as ICA end, we may have underestimated ICA duration, since central apnea may conceivably commence in or continue into the tonic–clonic phase. The invariable loss of plethysmographic breathing signal due to movement artifact and the contribution of respiratory muscle spasm to hypoxia render comment on ICA difficult. The exclusion of data that are contaminated by artifact, may also underestimate ICA in extratemporal seizures, since these may be more likely to induce vigorous movements (for example, hypermotor movements) than temporal seizures.

5 | CONCLUSION

ICA is a frequent, self-limiting semiological feature in focal epilepsy and can be its only clinical manifestation. However, prolonged ICA and severe hypoxemia together may comprise a potential biomarker of SUDEP. ICA is seen 10 times more frequently at the beginning of temporal than extratemporal seizures, and in typical temporal (FOIA motor onset with automatisms and FOIA nonmotor onset) seizure semiologies. The apnea is frequently seen before unambiguous scalp EEG or clinical seizure onset. ICA rarely persists after seizure end. Without polygraphic monitoring, including pulse oximetry and breathing plethysmography during VEEG, ICA may go unrecognized, since patients are agnostic to the apnea.

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DISCLOSURE OF CONFLICT OF INTEREST

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Left-insular damage, autonomic instability, and sudden unexpected death in epilepsy

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ABSTRACT

We analyzed the only two sudden unexpected death in epilepsy (SUDEP) cases from 320 prospectively recruited patients in the three-year Prevention and Risk Identification of SUDEP Mortality (PRISM) project. Both patients had surgically refractory epilepsy, evidence of left insular damage following previous temporal/temporo–insular resections, and progressive changes in heart rate variability (HRV) in monitored evaluations prior to death. Insular damage is known to cause autonomic dysfunction and increased mortality in acute stroke. This report suggests a possible role for the insula in the pathogenesis of SUDEP. The presence of intrinsic insular lesions or acquired insular damage in patients with refractory epilepsy may be an additional risk factor for SUDEP.

1. Introduction

Sudden unexpected death in epilepsy (SUDEP) incidence ranges from 0.9–2.3 per 1000 person-years in the general population with epilepsy to 1.1–5.9 per 1000 person-years in those with chronic refractory epilepsy [1]. Evidence points to fatal seizure related phenomena [2] although precise agonal pathophysiological pathways are yet to be elucidated. A variety of heart rate variability (HRV), cardiac conduction, and rhythm abnormalities occur in patients with refractory epilepsy [3], but very few near-SUDEP [4] or SUDEP cases [5] are attributable to these. The combination of bradycardia/asystole and apnea/hypopnea has been more frequently observed [2]. The role of cortical autonomic and respiratory control centers in driving such dysfunction is unknown. The insula, one potential such center, is implicated in cardiac autonomic control [6] as well as stroke mortality [7]. We report two SUDEP cases with acquired insular damage from the NINDS Prevention and Risk Identification of SUDEP Mortality (PRISM) project. Sequential EEG and EKG monitoring records were analyzed before and after insular damage with subsequent progressive cardiac conduction and rhythm disturbances. Progressive cardiac changes in these patients indicate a causal cardiac mechanism for death, possibly driven by insular dysfunction.

2. Patients and methods

All patients with intractable epilepsy aged >18 years were enrolled into the NINDS Prevention and Risk Identification of SUDEP Mortality (PRISM) project, a SUDEP study tasked with establishing infrastructure and feasibility for a SUDEP Center Without Walls. Two hundred forty-six patients were recruited at University Hospitals Case Medical Center in Cleveland, Ohio for prospective follow-up. Detailed phenotypic and electroclinical seizure data were collected in the Epilepsy Monitoring Unit as part of the study. We retrospectively analyzed HRV during interictal periods in different video-EEG admissions using an in-house MATLab software program.

2.1. Case 1

A 28-year-old, left-handed male with medically intractable seizures, putatively from left temporal epilepsy (due to anoxic brain damage in early childhood) of 26-year duration, was referred for presurgical evaluation after a failed left temporal resection 11 years previously. He had

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habitual, daily, automotor seizures, followed by right mouth twitching, altered awareness, and rare secondary generalization. At evaluation in 2012, he was on valproic acid (2000 mg/day), lacosamide (400 mg/day), and lorazepam (2 mg/day). He had severe learning difficulties but otherwise no physical neurological abnormalities. A MRI brain scan showed gliosis surrounding the left anterior temporal resection cavity, extending into the anterior inferior insula (previous preoperative MRI scan of the brain showed a small left hippocampus without abnormal signal and no insular abnormality) (Fig. 1A). He had undergone several video-EEG assessments (because of failure to capture seizures when admitted in three of these) after failed surgery and then unexpectedly died 21 months after his last assessment. Autopsy failed to show any significant pathologic findings other than sclerosis in the left hippocampal remnant, confirming definite SUDEP. There was no family history of sudden death. Serial video-EEG studies were retrospectively scrutinized for any clues indicating predisposition to sudden death. The HRV was analyzed at similar interictal periods (both during the afternoon, with the patient awake lying in bed, and with no seizures) in the v-EEG evaluations in 2009 and 2012. At evaluation in 2009, the patient was on lacosamide (200 mg/day) and valproate (1000/day).

2.1.1. Analysis

We retrospectively analyzed HRV during interictal periods in different video-EEG admissions while the patient was awake and lying in bed in successive video-EEG evaluations when no seizures were recorded. Several time-domain parameters were calculated including MNN (mean of normal to normal heart beats), RMSSD (root mean square of successive differences), SDNN (standard deviation of normal to normal heart beats), and SDSD (standard deviation of successive differences). In addition, frequency-domain parameters were calculated including normalized low frequency (LF) power (0.04–0.15 Hz) and normalized HF power (.15–.4 Hz) and LF/HF power ratio. These were calculated over 5-minute periods during 30-min interictal periods.

Generalized estimating equations (GEEs) were used to compare heart rhythm and HRV measures between interictal periods. A model was fit for each measure, based on data from a single subject. These models include an intercept and a factor variable denoting the two time periods. An autoregressive, AR(1), working correlation matrix was used to account for within-subject correlation of the observations across each of the 5-minute periods. The p-values given in the tables are associated with testing the null hypothesis that there are no period effects. Bonferroni correction for multiple comparisons was adopted, so that p-values less than 0.0056 (.05 divided by 9) are considered as significant.

2.1.1.1. Interictal phase (baseline). Heart rate variability was compared over 5-minute epochs in 30-minute interictal awake state records between most recent (2012) and earliest (2009) available EMU evaluations (Fig.1C). An increase of MNN (7.28%), SDNN (352.84%), RMSSD (196.03%), and HF (271.64%) and decreased LF (18.63%) and LF/HF ratio (80.26%) indicated a statically significant increased HRV in the most current evaluation in comparison with the previous one (Fig. 1 B).

2.1.1.2. Ictal phase. Three habitual partial seizures, each of ~1-minute duration, were recorded. In one seizure, the habitual ictal sinus tachycardia (115 bpm) was followed by an immediate and abrupt sinus bradycardia (59 bpm) for 13 s, after which HR returned to baseline (Fig. 1C).

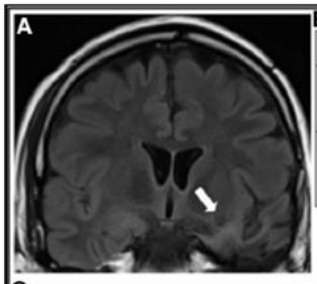
2.2. Case 2

A 33-year-old, right-handed male with medically intractable left hemisphere epilepsy of 6-year duration was referred for repeat presurgical evaluation in 2011. He had previously undergone an unsuccessful left posterior insula and periopercular resection following an invasive evaluation in 2007 (Fig. 2A). Neuropathology showed focal cortical dysplasia (FCD) Palmini type II A. His habitual seizures remained unchanged. At evaluation, he was on phenytoin (400 mg/day), levetiracetam (4000 mg/day), oxcarbazepine (900 mg/day), and pregabalin (1200 mg/day). He had 2–3 seizures/week, characterized by right or left face somatosensory aura, right version, followed by clonic secondary generalization. A habitual seizure was captured on surface EEG with left temporo–parietal onset. The patient subsequently suddenly and unexpectedly died at home. Autopsy failed to show any significant causative pathological findings, fulfilling criteria for definite SUDEP. There was no family history of sudden death. At evaluation in 2006, the patient was on phenytoin (500 mg/day), oxcarbazepine (300/day), and levetiracetam (2000/day).

2.2.1. Analysis

Ictal and interictal heart rhythms and HRV during 2006 (presurgical) and 2011 (postoperative) EMU admissions were analyzed using exactly the same methodology as in Patient 1.

2.2.1.1. Interictal phase. A statistically significant decrease of NNM (13.75%), SDNN (21.14%), RMSSD (48.96%), and HF (21.35%) and an increase in LF (63.65%) and LF/HF ratio (307.95%) were noted (Fig. 2C).



B

Data	Statistics	NNM	SDNN	RMSSD	SDSD	HF	LF	LF/HF
2009	Mean	87.93036	5.3446	0.031215	0.031244	0.960063	8.671267	10.65279
	STD	2.95299	2.661009	0.006677	0.006677	0.360942	2.875559	5.580896
2012	Mean	94.32982	24.20242	0.092405	0.092505	3.567933	7.056219	2.102736
	STD	2.307785	5.728499	0.021509	0.021533	0.567232	1.715551	0.858236
	P-value	0.0000	0.0000	0.0000	0.0000	0.0000	0.0600	0.0000

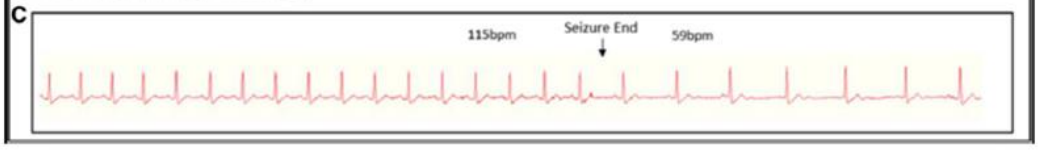
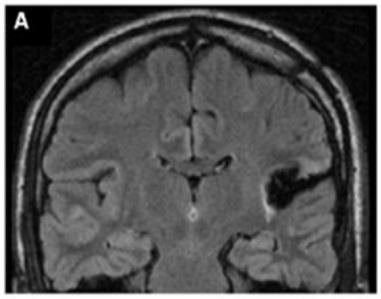
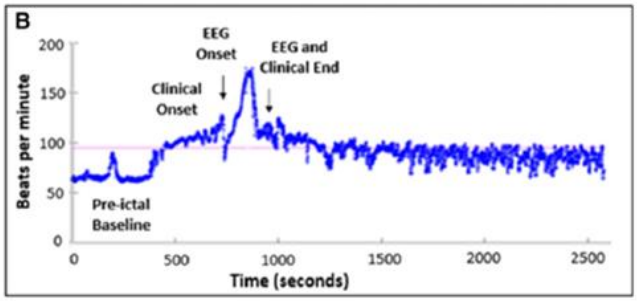


Fig. 1. (A) Flair coronal MRI showing evidence of left inferior temporal resection with surrounding gliosis and left anterior inferior insular gliosis and cavitation (arrow). (B) Heart rate time and frequency-domain parameters calculated during 2009 and 2012 evaluations and results from GEE analysis. (C) Continuous EKG recording showing tachycardia during the seizure and sudden resolution with abrupt onset of sinus bradycardia lasting for 13 s. Legend: MNN (mean of normal to normal heart beats), SDNN (standard deviation of normal to normal heart beats), RMSSD (root mean square of successive differences), SDSD (standard deviation of successive differences), HF (high frequency), LF (low frequency), and STD (standard deviation).





C

Data	Statistics	NNM	SDNN	RMSSD	SDSD	HF	LF	LF/HF
2006	Mean	92.37229	9.7204	0.056725	0.056813	1.918063	5.191156	4.044979
	STD	8.401523	3.202838	0.01478	0.014777	0.851716	3.903904	4.872046
2011	Mean	79.6692	7.66567	0.02895	0.029	1.5085	8.49555	16.5013
	STD	0.51948	3.061114	0.013316	0.013331	1.319259	2.102615	13.55803
	P-value	**0.0033**	**0.0656**	**0.0026**	**0.0026**	**0.3274**	**0.0779**	**0.0208**

Fig. 2. (A) Flair coronal postoperative MRI showed evidence of a left posterior temporo–insular resection cavity with surrounding gliosis. (B) Heart rate plots show ictal sinus tachycardia, followed by sustained absolute and relative postictal sinus tachycardia lasting at least 25 min after a nonfatal secondary generalized clonic seizure. (C) Heart rate time and frequency domain parameters calculated during the pre- (2006) and postsurgery (2011) EMU evaluations and the results from GEE analysis. Legend: MNN (mean of normal to normal heart beats), SDNN (standard deviation of normal to normal heart beats), RMSSD (root mean square of successive differences), SDSD (standard deviation of successive differences), HF (high frequency), LF (low frequency), and STD (standard deviation).

2.2.1.2. Ictal phase. In the postsurgical EMU evaluation in 2011, preictal bradycardia (HR 55–60/min) was noted. He had a secondary generalized clonic seizure of left temporo–parietal onset of ~2-minute duration. Ictal sinus tachycardia was followed by sustained absolute and relative postictal sinus tachycardia lasting at least 25 min, beyond which point the file had been clipped for archiving (Fig. 2B).

3. Discussion

Precise SUDEP pathomechanisms are as yet unknown. Death appears to follow a variable pattern of cardiac autonomic and respiratory dysfunction [2]. Observations from monitored SUDEP and near-SUDEP cases suggest some phenomenological heterogeneity [2,4,8]. The role of autonomic cortical control structures in SUDEP is an intriguing unknown, especially since several such structures (insula, amygdala, hippocampus, orbito–frontal cortex) are often either primary epileptogenic zones or part of putative seizure networks. Electrical stimulation of the human insula produced cardiac chronotropic and blood pressure responses in 5 epilepsy patients [7]; left-sided insular dominance for parasympathetic cardiovascular effects was concluded [7]. The insula has been directly or indirectly implicated in both cardiac autonomic control and mortality in stroke patients. Prolonged direct electrical stimulation of the rat insular cortex produces lethal cardiac arrhythmia and sudden death [6]. Insular damage in patients with cerebral infarction is associated with increased sympathetic activity, cardiac arrhythmias [9], conduction blocks, and mortality, including sudden death [10,11]. Nonfatal ictal bradycardia and asystole have been reported in insular epilepsy [12,13]. Insular seizures have been implicated in just one previous case of SUDEP [14].

In our patients, pre- and postoperative MRI scans indicated acquired insular damage from planned insular resection in Patient 2, as well as from inevitable periresective area gliosis in Patient 1 extending into the anterior inferior insula. Both deaths were unwitnessed, but the possibility of a cardiac contribution is significant. The HRV changes were demonstrably progressive over time. Patient 1 demonstrated an unequivocal increase in vagal tone as manifested by increased HRV and HF during the most current assessment, postictal period and a consequent postictal bradycardia. Neither patient was on medication that could have influenced autonomic tone (lacosamide-induced bradycardia has been reported; although, in Patient 1, this is unlikely as his dosage was stable for several years [15]). In Patient 1, increased vagal tone was progressive in comparisons between two consecutive, both postoperative studies, suggesting that neuronal responses to injury and autonomic alterations thus produced took place over years rather than immediately after surgery. This is strikingly similar to a report of a patient with left hemisphere epilepsy who developed a progressive, seven-month increase in HRV and vagal tone leading to an in-hospital monitored SUDEP [16]. Patient 2 conversely demonstrated increased sympathetic tone and abnormal, prolonged recovery from postictal tachycardia. The patient was on a lower dose of oxcarbazepine (from 900 mg to 300 mg) and a higher dose of phenytoin (from 400 mg to 500 mg) by the time of the second evaluation, and although HRV may have been affected by these changes, we view this as relatively unlikely to be solely responsible for all the changes noted. A previous study documented very similar changes in HRV in 18 patients with intractable temporal lobe epilepsy in comparison with 18 patients with well controlled seizures over a mean follow-up period of 6.1 years [17]. Another report of SUDEP in a patient with bitemporal epilepsy similarly described a progressive decrease in HRV (a 42.4% decrease in SDNN) over approximately 9 months. This evidence may represent a significant predisposition to fatal ventricular arrhythmia, although this is speculative [18]. The HRV is a standard measure of cardiac autonomic function which noninvasively reflects sympathetic and parasympathetic balances using EKG recordings. A close relationship is known to exist between increased sympathetic activity and decreased parasympathetic activity with a consequent tendency to fatal arrhythmia [19]. The high frequency (HF) domain is a marker of parasympathetic activity whereas the low frequency (LH) domain is thought to represent both sympathetic and parasympathetic activities. Nocturnal HRV is significantly reduced in epilepsy, and decreased HRV accompanies increased sudden cardiac death risk [20]. The role of excitation produced by seizure

discharge in a damaged left insula (Patient 1), as opposed to loss of homeostatic correction due to a mostly absent left insula (Patient 2), may explain the divergent cardiac responses to seizures seen in the two cases. Alternatively or additionally, differing handedness and hemispheric dominance may have played a role since lateralization of insula function is well described [21].

The two SUDEP cases reported here present reasonable circumstantial evidence, for the first time, of an insular contribution to SUDEP. The presence of intrinsic insular lesions or acquired insular damage in patients with refractory epilepsy may be an additional risk factor for SUDEP.

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Conflict of interest

The authors have no conflicts of interest to disclose.

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