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Increase in 20–50 Hz (gamma frequencies) power spectrum and synchronization after chronic vagal nerve stimulation

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Abstract

Objective: Though vagus nerve stimulation (VNS) is an important option in pharmacoresistant epilepsy, its mechanism of action remains unclear. The observation that VNS desynchronised the EEG activity in animals suggested that this mechanism could be involved in VNS antiepileptic effects in humans. Indeed VNS decreases spiking bursts, whereas its effects on the EEG background remain uncertain. The objective of the present study is to investigate how VNS affects local and inter regional syncronization in different frequencies in pharmacoresistent partial epilepsy.

Methods: Digital recordings acquired in 11 epileptic subjects 1 year and 1 week before VNS surgery were compared with that obtained 1 month and 1 year after VNS activation. Power spectrum and synchronization were then analyzed and compared with an epileptic group of 10 patients treated with AEDs only and with 9 non-epileptic patients.

Results: VNS decreases the synchronization of theta frequencies ($P<0.01$), whereas it increases gamma power spectrum and synchronization (<0.001 and 0.01, respectively).

Conclusions: The reduction of theta frequencies and the increase in power spectrum and synchronization of gamma bands can be related to VNS anticonvulsant mechanism. In addition, gamma modulation could also play a seizure-independent role in improving attentional performances.

Significance: These results suggest that some antiepileptic mechanisms affected by VNS can be modulated by or be the reflection of EEG changes.

Keywords: Vagus nerve stimulation (VNS); Partial epilepsy; Digital EEG; Power spectrum frequency analysis; Intra–inter hemispheric synchronization analysis; Gamma activity

1. Introduction

The use of long-term vagus nerve stimulation (VNS) with intermittent electrical current was introduced for the treatment of refractory epilepsy first in adults (Binnie, 2000; Handforth et al., 1988; Labar et al., 1998; Morris and Mueller, 1999; Salinsky, 1995) and subsequently in children (Hornig et al., 1997; Patwardhan et al., 2000) and in older individuals (Sirven et al., 2000). VNS reduces seizure frequency by 50% in 30–40% of patients with severe epilepsy (Ben-Menachem, 2002) and represents the only non-pharmacological, non-surgical option for epilepsy treatment (Binnie, 2000). The mechanism of the therapeutic action of VNS remains unclear, however. Although its efficacy has been suggested to result from complex interactions of biochemical and electrical events (Beckstead and Norgren, 1979; Kalia and Sullivan, 1982; Rutecki, 1990), few studies have examined the effects of VNS on the EEG.

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Early investigations showed that VNS at a frequency of 24–50 Hz induced rapid desynchronized activity in the orbitofrontal cortex and cerebellum both in intact and in 'encephale isolè' cats (Bremer and Bonnet, 1951; Zanchetti et al., 1952). Further studies found that vagal stimulation at 30 Hz induced EEG desynchronization in cats (Magnes et al., 1961) in a manner dependent on the histological composition of the nerve fibers receiving the electrode impulses. This effect is similar to that observed after electrical stimulation of peripheral nerves (Pompeiano and Swett, 1962). The pattern and target of stimulation are important determinants of the effect observed, however, given that desynchronization results from high-frequency stimulation of fibers with a low level of myelination, whereas synchronization occurs in response to low-frequency stimulation of highly myelinated fibers (Chase et al., 1967).

These experimental observations spurred several studies that attempted to elucidate whether VNS induces similar bioelectrical effects in humans. The first such study failed to demonstrate substantial differences in recordings obtained during wakefulness, sleep, or anaesthesia in epileptic individuals examined before and after VNS activation (Hammond et al., 1993). Another study detected a stable rate of interictal spiking activity after the delivery of trains of electrical impulses by VNS (Salinsky and Burchiel, 1993). However, more recent studies of the effects of chronic VNS with long-term EEG monitoring have revealed a progressive reduction in the frequency and duration of sharp waves as well as a substantial decrease in interictal spiking (Koo, 2002; Kuba et al., 2002)

These studies thus suggest that VNS modifies interictal activity, although it has remained unclear whether modification of the EEG pattern by long-term VNS plays a role in the observed therapeutic action. In particular, it remains scarcely investigated whether VNS modulates the spectrum of EEG frequency and if such activity can be related with variations of spiking and seizure episodes. In addition, it is of interest to investigate possible relations between the inter and intrahemisferic synchronization of the EEG frequency bands, by assessing the coherences of the EEG signals over the recording areas. As the procedure of 'generalized synchronization' (Rulkov et al., 1995) studies coupled identical systems to coupled systems with different parameters, these investigations are expected particularly useful in assessing coupled EEG signals. Generalized synchronization occurs between two dynamical systems **X** and **Y** (a driver and a response) when the state of response system is a generalized function of the state of the driver [i.e. $\mathbf{Y}=\Psi(\mathbf{X})$; in Appendix A for more details]. This method might allow for relevant information on possible similar features among EEG signals in different cortical areas.

As such observations might contribute not only to investigate cortical rhythms in relation to the epileptic discharges, but also can improve some basic knowledge of general VNS mechanism, we have now examined the effect of VNS on the EEG frequency profile by comparing the average of the power spectra recorded 1 year and 1 week before VNS activation with that obtained, respectively, 1 month and 1 year after VNS therapeutic activation. A group of epileptic subjects affected by partial seizures and treated with AEDs only and another group of non-epileptic patients served as control.

2. Methods

2.1. Patient selection

The patients for the study were selected from 1420 individuals who attended the Epilepsy Diagnostic and Treatment Centre of Cagliari (Italy). From 16 patients affected by drug-resistant partial epilepsy who were recently implanted with a VNS device, we selected eleven subjects (six men, five women) ranging in age from 26 to 44 years (mean, 33.5 years) affected by non-lesional epilepsy ruling out five subjects affected by lesional epilepsy. Although the number of the patients enrolled in the study was relatively small, the group was homogeneous in that the age, the treatment, and the general characteristics of the seizures were similar for all individuals. The right hemisphere was considered to be the most likely site of the epileptic focus in seven subjects while the left side was primarily involved in four patients on the basis of several EEG recordings and reports from the patients themselves or from family members who witnessed ictal events. We also obtained ictal video-EEG recordings for nine patients. In addition, the focal activity was confirmed by a 24 h Holter EEG recording for the other patients.

Each of the patients had been monitored for several years (mean, 5.2 years) by the outpatient service before the decision to implant a VNS device was taken. The main selection criteria for inclusion in the study were: a relative stability of clinical features related to interictal EEG activity, the resistance to classical first- and second-line antiepileptic drugs (AEDs) assessed monthly for optimal range, the normal findings of neurological and psychiatric evaluations, and the lack of abnormalities of cerebral structure as revealed by a recent MRI scan. The possibility and priority of treatment with a VNS device were discussed with the patients, family members, and the institutional ethical ad hoc committee. Informed consent was obtained from the patients and their relatives after the nature of the procedure had been fully explained and approved by institutional review.

The characteristics of the selected patients are summarized in Table 1. The change in seizure frequency was calculated as: [(number of seizures after VNS implant per trimester)−(number of seizures before implant per trimester)]/(number of seizures before implant per trimester) (Labar et al., 1998). Epileptogenic activity was

Table 1
Characteristics of epileptic patients and effect of VNS on seizure frequency

Patient	Age (years)	Sex	Age at seizure onset (years)	AEDs	Estimated site of focal IEDs	Pre-VNS seizure frequency (/trimester)	Post-VNS seizure frequency (/trimester)	VNS-induced change in seizure frequency (% after 1 year)
1	44	F	14	CBZ+PRI	Right FT	164	27	(+83)
2	32	M	7	CBZ+FELB	Left F	139	37	(+75)
3	26	M	19	CBZ+VA	Right F	102	18	(+83)
4	28	M	18	CBZ+LMT	Right FT	120	75	(+37)
5	37	M	7	CBZ+LMT	Right FT	184	122	(+33)
6	29	F	8	CBZ+LMT	Left F	108	89	(+17)
7	33	M	17	CBZ+VA	Right FT	202	141	(+30)
8	34	F	3	CBZ	Right F	228	198	(+13)
9	44	M	6	CBZ+VA	Right FT	196	212	(−0.8)
10	33	F	10	VA+TOP	Left TP	98	61	(+37)
11	29	M	7	CBZ+LMT	Left FT	122	85	(+30)

Pre-VNS seizure frequency is the mean of the value obtained in the trimester immediately before implantation of the VNS device and that obtained 1 year previously. Post-VNS seizure frequency is the mean of the value obtained in the last trimester after 1 year from VNS implantation.VNS-induced change in seizure frequency (% after 1 year) indicates the percentage of seizure decrease (negative number) or increase (positive number) after 1 year of vagus stimulation. CBZ, carbamazepine; PRI, primidone; FELB, felbamate; VA, Valproic Acid; LMT, lamotrigine; TOP, topiramate. FT, fronto-temporal lobe; TP, temporal-parietal lobe; F, frontal lobe.

defined by the number of interictal epileptiform discharges (IEDs), including isolated spikes, spikes and slow waves, spikes-waves, and polyspikes-waves during 40 min recording interpreted by a neurophysiologist blinded of the experimental design (MM). The approved protocol included no changes in anticonvulsant treatment during the trial, unless serious side effects were manifested.

In order to detect possible early modifications in power spectra after VNS, we calculated the data obtained 1 month after the implant and 2 months after surgery when VNS was switched at therapeutic values. Moreover, given that the studies reporting real therapeutic advantage for different parameters of VNS are still limited and not systematic, no further attempt to modify the general settings (e.g. shift to 'rapid cycling') has been done during the entire trial.

To take into account possible variations in EEG pattern related to an efficacious AEDs effect during 1 year period, we examined a separate group of 10 epileptic subjects affected by partial epilepsy (mean age, 34 years) who were admitted to the Regional Centre Against Epileptic Disorders for an adjustment of the treatment. Though these patients differed from VNS group in that they showed a less severe form of epilepsy, most of the drugs administered, either de novo or adjusted for the optimal range, were similar to VNS group (Tables 1 and 2). In particular, carbamazepine (CBZ) which represents by far the most used AED, was administered at doses between 1000 and 1600 mg/day, Valproic Acid (VPA) was administered at daily doses between 800 and 1500 mg following the indications obtained from seric concentrations. Lamotrigine (LMT)

Table 2
Characteristics of epileptic patients treated with AEDs only

Patient	Age (years)	Sex	Age at seizure onset (years)	AEDs	Estimated site of focal IEDs	Pre-AEDs adjustment seizure frequency (/trimester)	Post-AEDs adjustment seizure frequency (/trimester)	AEDs-adjustment induced change in seizure frequency (% after 1 year)
1	35	F	24	CBZ+VA	Left T	15	4	(+73)
2	39	M	18	CBZ+LMT	Right PT	21	6	(+71)
3	46	M	28	CBZ	Left FT	16	2	(+87.5)
4	23	M	15	CBZ	Right F	8	0	(+100)
5	34	M	11	TOP	Right PT	6	2	(+66)
6	42	F	32	LMT+LEV	Right FT	22	4	(+82)
7	27	M	20	CBZ	Left FT	11	3	(+73)
8	33	F	26	CBZ+LMT	Right FP	7	0	(+100)
9	31	M	14	CBZ	Left F	14	6	(+57)
10	28	F	17	VA+LEV	Left TP	13	7	(+46)

Pre-AEDs adjustment seizure frequency is the mean of the value obtained in the trimester immediately before the adjustment of the AEDs treatment and that obtained 1 year previously. Post-AEDs adjustment seizure frequency is the mean of the value obtained in the last trimester after 1 year from AEDs adjustment AEDs-induced change in seizure frequency (% after 1 year) indicates the percentage of seizure decrease after 1 year AEDs adjustment. CBZ, carbamazepine; PRI, primidone; LEV, levetiracetam; VA, valproic acid; LMT, lamotrigine; TOP, topiramate; T, temporal lobe FT, fronto-temporal lobe; TP, temporal-parietal lobe; F, frontal lobe.

administration in both epileptic groups varied from 200 to 400 mg. Less used drugs were Topiramate (TOP), utilized at 250 mg a day, primidone (PRI) administered at 200 mg a day, levetiracetam (LEV) used at 3000 mg a day and felbamate given at 2400 mg a day.

2.2. Implantation of the VNS device

A vagus nerve stimulator (model 100 NCP Pulse Generator; Cyberonics, Houston, TX), comprising a pulse generator programmable by telemetry, was implanted in the upper left side of the chest of each subject by a neurosurgeon. A lead terminating in a double-coiled electrode was positioned on the cervical portion of the left vagus nerve. The stimulator was tested before implantation by serial connection to an IBM-compatible laptop computer. The position of the stimulator was checked after surgery by a routine chest X-ray. The device was switched on at an initial current of 0.25 mA after 1 week. The current was increased by 0.25 mA each week until the value of 2 mA was achieved. Stimulation of patients 2 and 9 was subsequently reduced to 1.75 mA because of a painful sensation that developed in the throat region after delivery of a current of 2 mA. The other parameters conformed to the 'high stimulation' criteria (Binnie, 2000); the stimulation cycle was 30 s on followed by 5 min off, the pulse duration was 500 ms, and the signal frequency was 30 Hz. Once set, these parameters were maintained throughout the duration of the study.

2.3. EEG analysis

EEG data were recorded from the scalp with a 19 non-polarizable Ag–AgCl electrode-cap using the BQS 98 System Micromed (Mogliano, Veneto, Italy). The impedance was <5 kΩ, sampling frequency 256 Hz, 16 bit resolution. All electrophysiological signals were transduced by BQS98 System Micromed alternating current (A/C) amplifiers and an amplifier sensitivity of 5 was used for EEG (50 μV, 0.5 s duration calibration) corresponding to a gain of 50,000 with half-amp low and high bandpass filters set at 0.03 and 70 Hz, respectively. Monopolar left and right electroculograms (EOG) and bipolar chin-check electromyograms (EMG) were also recorded in order to detect possible sleepiness and the 10/20-systems was used (1986). The reference electrode was placed on the nose and the ground electrode on the forehead.. The signals were stored on the hard disk for off-line analysis. Five samples corresponding to 75 s per trial were randomly selected for each patient from a 40 min EEG recording. These samples were screened for eye blinks, horizontal–vertical eye movements, muscle artefacts and possible sleepiness by visual inspection and were analyzed by averaging the results obtained in each trial. The EEG signals were filtered with elliptical filter banks to obtain the optimal resolution of broadband parameters for delta (0.5–3 Hz), theta (4–7.5 Hz), alpha (8–12 Hz), beta (13–20 Hz), and gamma (20–50 Hz) frequencies. Given that synchronization analysis requires a zero-phase filtering distortion, we performed a further procedure of forward–backward filtering (Gustafsson, 1996).

Given that the EEG records are based on the 10/20 system with 19 electrodes, the power spectrum was analyzed at each electrode location for each of the 5 frequency bands selected for the experimental design. The mean of the squared Fourier amplitude coefficients was determined. Coherence, defined as the cross spectral density function normalized by individual auto-spectral density functions (Nunez et al., 1997) and a robust nonlinear interdependence estimator (N) were calculated in order to assess the generalized synchronization among all channels (Quian Quiroga et al., 2000).

For calculation of /N/, a reconstruction of state spaces of each signal was performed with an embedding dimension (/m/) related to the frequency band to be studied. We used the method of Liangyue (Liangyue, 1997) to find the minimum embedded dimension for each frequency band and we set $m=10$ for lower bands (delta, theta and alpha) and $m=6$ for beta and gamma band. A time lag (τ) of 2 was chosen in order to measure frequencies of <128 Hz.

The number of nearest neighbours (k) was set to 10 and a Theiler correction for temporal correlation (w) was set to 20. These settings have been used in order to increase the sensitivity for possible underlying synchronization according to non-linearity studies applied to EEG signals (Quian Quiroga et al., 2002).

Both power spectrum and synchronization data were calculated for non-overlapping epochs (1 s at 256 samples). To reduce the amount of data, we considered short- and long-range synchronization according to the location of the electrodes in the same hemisphere or in the contra lateral areas (Fig. 1). The data obtained were spatially arranged following the EEG montage. To characterize regional differences, we defined five region-based groups of electrode-pair combinations and the absolute band power

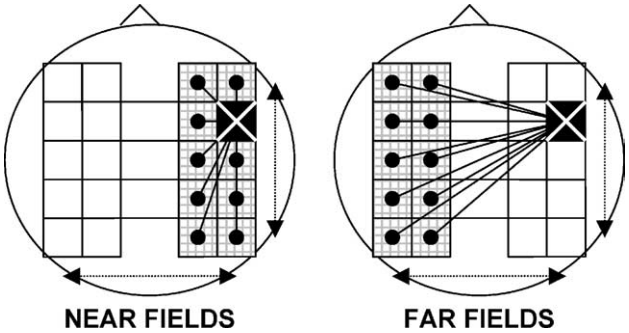

Fig. 1. Procedure for calculation of intra- and interhemispheric synchronization. The ipsilateral or contralateral synchronization index was obtained by comparison of EEG data recorded by the test electrode (×) with those recorded by electrodes positioned on the same or contralateral side. The arrows indicate that the same procedure was applied for 19 electrode positions.

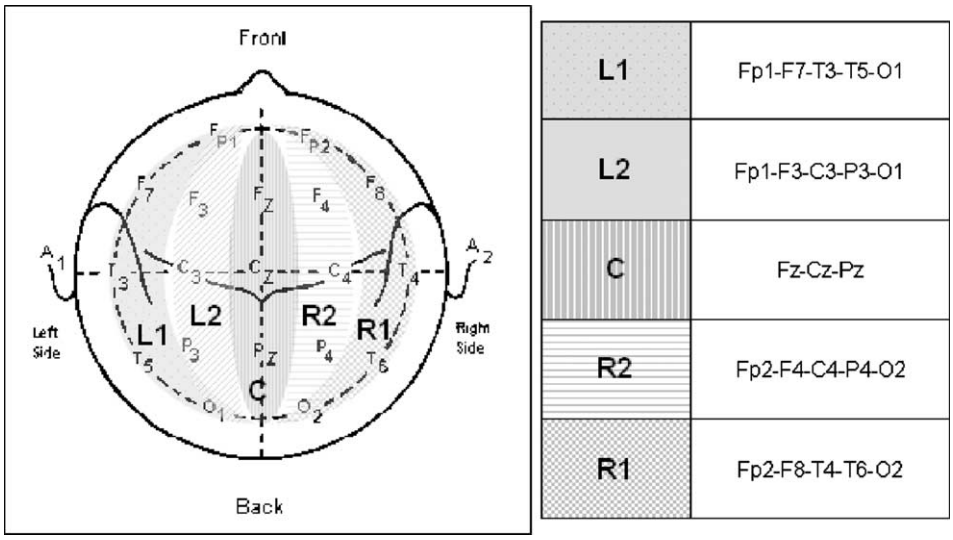

Fig. 2. Regions of interest for synchronization analysis. L1: left Fp1-F7, F7-T3, T3-T5, T5-O1. L2: left Fp1-F3, F3-C3, C3-P3, P3-O1. C: central Fz-Cz, Cz-Pz, Pz-Oz. R1: right Fp2-F8, F8-T4, T4-T6, T6-O2. R2: right Fp2-F4, F4-C4, C4-P4, P4-O2.

for each band of these combinations was used as the dependent vector for comparisons (Fig. 2). The power spectra obtained after 1 year of VNS stimulation were compared with the average of those obtained 1 year and 1 week before implantation of the VNS device.

2.4. Data analysis

Analysis of variance (ANOVA) for repeated measures with the Huynh–Feldt correction where appropriate and the post hoc Bonferroni–Duhn test were used for comparisons of power spectra and synchronization within and between groups. Because of spatial correlation between the 5 zones, /*P*/ values have been corrected for multiple testing with false discovery rate (FDR) method (Benjamini and Hochberg, 1995) instead of using the too conservative Bonferroni correction, which is better suitable for assessing global variations. Alpha level was set to 0.05. A normal distribution of power spectra and of N was indicated by application of Lilliefors modified Kolmogorov–Smirnov test (Dallal and Wilkinson, 1986). Coherence data were subjected to Fisher's transformation, yielding z-coherences with an approximately normal distribution. All calculations were performed with The Matlab toolboxes (The Mathworks, Natick, MA, USA).

The relation between the modification of percentage changes in seizure frequency and in power spectra and synchronization distribution was assessed from a bivariate scattergram plot and Fisher's R to Z two-tailed test (StatView Software, Abacus Concepts, Berkeley, CA, USA). A P value of <0.05 was considered statistically significant.

3. Results

Comparison of the power spectrum of each frequency band in the regions of interest for the EEGs recorded 1 year and 1 week, respectively, before implantation of the VNS device as well as 1 month after VNS activation at values of 1.25 mA, revealed neither quantitative nor qualitative differences (not shown). Furthermore, whereas the power spectra for the delta, theta, alpha, and beta frequency bands were not significantly affected by VNS in the epileptic patients, that for the gamma band was increased in both hemispheres after VNS, with this effect being more pronounced in the right hemisphere (Fig. 3). In contrast, after 1 year the epileptic control group failed to show modifications in the power spectra profile of all bands though a small increase in gamma power spectrum at R1 and R2 was observed (Table 3).

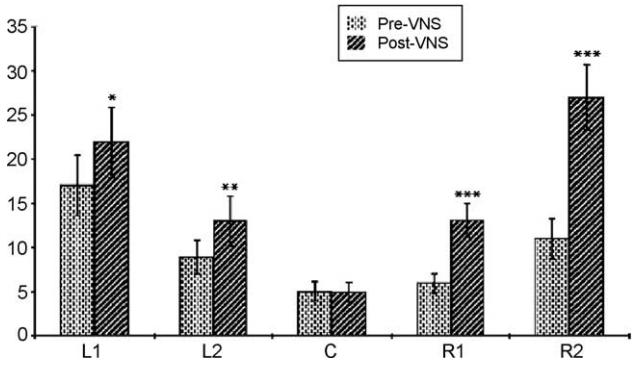

Fig. 3. power spectra of gamma bands in the regions of interest for epileptic patients before and after VNS. Power spectrum ($\mu V^2/s \pm SD$) are reported in the x axis, the regions assessed L1, L2, C, R1 and R2 are reported in the y axis. Statistical significance: $*P<0.05$, $**P<0.01$, $***P<0.001$.

Table 3
Power spectra of frequency bands in the regions of interest for epileptic patients before and after VNS and for control subjects before and after AEDs change

Band	Region	EP AEDs only Pre	EP AEDs only Post	Epileptic patients pre-VNS	Epileptic patients post-VNS
Delta	L1	73.0±11.9	87.9±5.0	90.0±10.6	84.0±7.2
	L2	75.7±11.4	66.0±8.9	86.0±14.8	76.1±8.8
	C	43.6±7.7	38.9±3.4	49.0±8.2	33.4±3.7
	R2	81.9±5.7	72.2±14.4	80.0±7.2	94.2±13.3
	R1	91.0±11.3	80.8±13.6	96.0±10.1	123.1±15.1
Theta	L1	54.2±12.5	52.5±11.8	101.0±19.6	72.0±10.9
	L2	56.8±12.2	50.8±11.9	90.0±13.8	81.7±11.9
	C	46.9±11.0	36.9±10.9	79.0±14.8	50.4±7.4
	R2	61.7±12.0	53.6±12.6	100.8±16.7	89.3±10.9
	R1	67.9±11.7	64.3±12.3	107.0±17.2	82.4±11.9
Alpha	L1	70.0±13.3	44.7±10.1	67.0±13.5	43.8±9.0
	L2	61.4±12.0	61.7±9.3	59.0±11.7	52.7±11.1
	C	38.6±6.6	34.6±9.0	32.0±8.5	31.5±8.0
	R2	58.4±12.7	59.9±9.8	58.0±11.9	44.5±10.6
	R1	56.1±13.0	44.8±12.8	61.0±11.4	41.7±9.5
Beta	L1	17.6±3.1	18.9±2.8	21.0±3.7	22.1±3.2
	L2	16.9±5.6	12.8±2.6	17.0±3.7	21.0±2.9
	C	11.3±2.8	19.7±3.3	13.0±3.2	12.1±1.9
	R2	18.5±5.2	20.0±4.6	17.0±4.0	20.0±2.7
	R1	18.6±4.5	18.9±3.5	19.0±3.2	24.0±3.2
Gamma	L1	9.7±4.0	11.5±3.6	17.0±3.4	21.9±4.0[a]
	L2	6.5±2.0	7.9±2.8	8.9±1.9	13.0±2.9[b]
	C	4.6±1.1	5.0±1.3	5.0±1.1	4.9±1.1
	R2	7.3±1.4	7.9±2.4	5.9±1.1	13.1±1.9[c]
	R1	10.7±3.0	12.2±3.4	11.0±2.3	27.0±3.7[c]

EP-AEDs Pre and EP-AEDs-Post, power spectra values in the control group treated with AEDs only (basal values) and the same group assessed 1 year after AEDs switch. Values are calculated as mean between basal (EP-AEDs-Pre) and after 1 year from the switch (AEDs-Post). EP Pre-VNS values for epileptic patients as mean average of the power spectra determination 1 year and 1 week before VNS implant. EP Post-VNS spectra values obtained after 1 year from the switch of >1.25 mA (following therapeutic values of VNS). Values expressed as $\mu V^2/s \pm SD$ values are assessed with analysis of variance (ANOVA): a, $P<0.05$; b, $P<0.01$; c, $P<0.001$.

Moreover, after 1 year of VNS activation, in addition to the general increase in gamma power spectrum, VNS-treated group showed an increased coherence for gamma signals at the R1 region of the right hemisphere, confirmed by statistical analysis based on the interdependence factor *N* (Table 4). Although VNS failed to decrease theta power spectrum (Table 2), this group showed a significant reduction in the inter- and intrahemispheric coherence for this band in the central (C) region (Table 4). Again, the VNS-induced decrease in synchronization for the theta band in the C region was confirmed by analysis of N.

In the AEDs group the synchronization analysis showed a decrease in both coherences and *N* after 1 year for delta, theta and gamma bands (Table 5). The correlation between the percentage changes in seizure frequency pattern distribution and the power spectra and synchronization

Table 4
Effects of VNS on intra- and interhemispheric synchronization of EEG activity for the regions of interest

Region		Coherence			Interdependence measure (*N*)		
		Band	VNS-induced change (%)	*P*	Band	VNS-induced change (%)	*P*
L1							
L2	Inter						
	Intra				Beta	−1.3	0.027
C	Inter	Theta	−4.4	0.041	Theta	−4.8	0.021
					Beta	−2.1	0.035
	Intra	Theta	−6.2	0.026	Theta	−5.3	0.013
					Beta	−2.2	0.043
R2	Inter				Beta	−1.6	0.037
R1	Inter				Gamma	3.2	0.039
	Intra	Gamma	4.9	0.028	Gamma	4.3	0.01

The change in coherence or interdependence (*N*) obtained 1 year after VNS switch (>1.25 mA) is compared with mean baseline values between 1 year and 1 week before surgery $P<0.05$ respect baseline.

Table 5
AEDs-group: effects of AEDs on intra- and interhemispheric synchronization of EEG activity for the regions of interest of epileptic controls

Region		Coherence			Interdependence measure (*N*)		
		Band	AEDs-induced change (%)	*P*	Band	AEDs-induced change (%)	*P*
L1	Inter	Gamma	−0.29	0.043	Gamma	−0.54	0.018
	Intra						
L2	Inter						
	Intra				Delta	−1.1	0.042
					Theta	−0.9	0.034
C							
R2	Inter						
	Intra	Delta	−1.4	0.031	Delta	−1.3	0.028
R1							

The change in coherence or interdependence (*N*) obtained 1 year after AEDs change is compared with the basal values$P < 0.05$ respect basal values.

distribution was not significant in both groups, thus suggesting that the improvement in seizure frequency was not proportionally related with these parameters (not shown). No variations were observed in spectra profiles and coherence in the group of non-epileptic subjects after 1 year (Table 2). Furthermore, the number of IEDs was not significantly modified by VNS.

4. Discussion

We have shown that VNS increases the power spectrum as well as the intra- and interhemispheric synchronization of EEG frequencies between 20 and 50 Hz (gamma band) (Fig. 3), whereas it reduces the synchronization of frequencies under 20 Hz without substantially affecting their power spectra. At variance, the control group showed similar power spectra after 1 year of AEDs adjustment (Table 3).

Though VNS failed to show acute modifications following on and off periods of activation (Salinski and Burchiel, 1993), experimental evidences have suggested that electrical stimulation induced modification of brain rhythms via the nucleus tractus solitarius (NTS), which is the main site of visceral afferent complex termination and is regulated by cholinergic inputs (Beckstead and Norgren, 1979; Kalia and Sullivan, 1982; Rutecki, 1990; Schacther and Saper, 1998). However, the extended network of NTS connections (Saper, 1995) might mediate the biochemical and electrical effects of VNS through several mechanisms. Given that the NTS does not directly innervate cortical areas, chronic VNS-induced EEG changes are likely mediated by modulation of pathways indirectly involved in the genesis of cortical rhythms. Indeed, the parabrachial nucleus, which receives NTS efferents (Quattrocchi et al., 1998) projects to several thalamic nuclei that contribute to EEG activity and receives an important input from the locus coeruleus (LC). In addition, the integrity of the LC is important for the antiepileptic, desynchronizing, and arousal-promoting effects of VNS (Krahl et al., 1998). Moreover, experimental stimulation of vagal components of the LC by injection of the cholinergic agonist bethanechol into the LC was found to increase cortical desynchronization and to reduce the contribution of slow frequencies to the EEG (Berridge and Foote, 1991). Although such studies cannot be readily replicated in humans, in addition to its antiepileptic effect VNS affects EEG desynchronization through the entire sleep-waking cycle by increasing the proportion of rapid eye movements (REM) sleep and decreasing daytime sleepiness (Galli et al., 2003; Malow et al., 2001). Our present results confirm that chronic VNS increases intra-interhemispheric desynchronization of cortical rhythms at frequencies of <20 Hz. These results are in accordance with previous experimental and clinical data. It has been shown, for instance, that VNS suppress sleep spindling in cats whereas it attenuates synchronized activities (Chase et al., 1967l; Zanchetti et al., 1952). Though in initial investigations EEG background seemed unaffected by VNS (Hammond et al., 1992; Salinski and Burchiel, 1993) in more recent studies it has been suggested a 'cortical activation among the effects of VNS' as it promotes REM sleep and increases alertness without any change in overnight sleep architecture (Malow et al., 2001).

The VNS-induced desynchronization described in cats after VNS, (Magnes et al., 1961), represents a finding which apparently seems to contradict the increased synchronization of gamma frequency bands in humans. However, it is difficult to compare these experimental settings given the methodological differences in stimulus parameters and in species population and the different acquisition and processing of EEG signals. Together, the existence of a single mechanism responsible both for the decreased synchronization of frequency bands under 20 Hz and for the marked increase in the power spectrum and synchronization of the gamma frequency band appears difficult to council.

Gamma activity has previously been found to be increased in experimental models of epilepsy and in epileptic patients (Mackenzie et al., 2002; Willoughby et al., 2003). Consistent with these findings, the epileptic

patients in the present study showed an increased power spectrum of the gamma frequency band compared with that observed in non-epileptic subjects. Moreover, the VNS group manifested a further increase in this parameter after 1 year of stimulation.

It has been shown that a reduction in synchronization of the gamma band has been detected immediately before an epileptic seizure (Mormann et al., 2003). In addition, experimental data suggest that an antisynchronizing gamma-mediated mechanism may antagonize ictal and interictal epileptogenic spiking activity (Medvedev, 2002) as these figures are proportionally inverse. Accordingly, the increases in the power spectrum of the gamma band observed in response to VNS in the present study might be hypothesized as a kind of protective effect. The performance of standard EEG, recorded for only 40 min in the present study,was not optimally suited to detect significant variations in spiking activity. However, previous studies with longer periods of EEG monitoring have shown that long-term VNS induces a delay in interspiking activity and a reduction in the frequency of epileptic interictal spikes (Koo, 2001; Kuba et al., 2002; Olejniczak et al., 2001), though a recent study which evaluated in children whether spike rates are useful as an outcome parameter following VNS yielded contrasting results (Ebus et al., 2004).

Chronic VNS has been found to enhance recognition memory and memory storage in humans (Clark et al., 1999) and to increase cortical inhibition by up-regulating the cortical density of γ-aminobutyric acid type A ($GABA_A$) receptors, as assessed by [^{123}I] iomazenil SPECT, in individuals with drug-resistant partial epilepsy (Marrosu et al., 2003). These results suggest that VNS may modulate neuronal plasticity. Moreover, given that modifications of $GABA_A$ receptors affect brain excitability, it seems likely that this receptor modulation might also be involved in changes of bioelectrical activity.

Recent studies have suggested that local gamma rhythms are dependent on the activation of $GABA_A$ receptors in perisomatic neural networks whose synaptic inputs synchronize the interneuronal activity in the gamma range (Jefferys et al., 1996; Traub et al., 2003). Though these studies are performed on neuronal models represented by hippocampal slices, the possibility that the GABAergic inhibition in a subset of interneurons into pyramidal cells modulates gamma activity (Wendling et al., 2002) can be hypothesized also in other brain areas that share high density $GABA_A$ receptors. Given that VNS increases the metabolic rate in the thalamus (Henry et al., 1998, 1999, 2004; Ko et al., 1996) the long-term administration of such stimulation might affect thalamocortical regulation of brain circuitry involved in the modulation of EEG rhythms. It is likely that this effect is part of the antiepileptic mechanism, and that it is induced after a medium-long delay from VNS activation, since both clinical activity and power spectrum assessed 1 month after settling the device at 1.25 mA failed to show significant differences in comparison with these calculated for the pre-implant periods (not shown).

The modulation of the $GABA_A$ receptors by VNS might be relevant for the regulation of cortical rhythms, given that the nucleus reticularis thalami contains a large proportion of $GABA_A$ergic neurons and acts as a pacemaker of thalamocortical volleys (Gibbs et al., 1996). Though VNS activates both thalami and this mechanism represents the most likely candidate to the regulation of oscillatory brain activities, our data show an asymmetric prevalence of gamma bands over the right hemisphere, regardless the putative epileptogenic side. Although the lateralized gamma power spectrum can be interpreted as a casual finding, nonetheless the peculiar pattern of gamma profiles detected in the VNS group might suggest a more direct mechanism. Indeed, it has been observed that VNS induces bilateral thalamo-cortical blood flow increase both acutely and chronically (Henry et al., 1998, 1999; Ko et al., 1996), and that the left position of VNS implant enhances an additional chronic increase in the right inferior postcentral gyrus (Henry et al., 2004). Though it is difficult to directly correlate minute variations in blood flow with EEG changes, the chronic blood flow asymmetry could play a role in modifying synaptic plasticity in these areas. However, the role of the AEDs in determining the EEG changes deserves some comment. Indeed, it cannot be completely ruled out that the epileptic groups receiving the same AEDs may show different power spectra for complex AEDs–VNS interactions (e.g. VNS acts by enhancing AEDs–GABA related mechanisms) rather than by a VNS 'mechanistic' effect, though the group treated with AEDs only failed to show significant changes in frequency profiles (Tables 3 and 5).

In addition, several studies reported a time-dependent improvement of the quality of life and mood among the patients treated with VNS (Ben-Menachem, 2002; Cramer, 2001; Elger et al., 2000; Harden et al., 2000 for a review) and recent investigations have suggested a potential effect of VNS in the treatment of depression (Kosel and Schlaepfer, 2003). Given that the modulation of gamma bands plays a role in linking different brain areas involved in object representation as well as in unifying coherent percepts and in focusing the top-down flow of attentional mechanisms (Bertrand and Tallon-Baudry, 2000) it could be suggested that, perhaps independently by the antiepileptic mechanism, these effects might contribute to the improvement of the quality of life in epileptic subjects as well as in depressed patients.

Though it is not currently possible to hypothesize the exact role played by the modifications of the frequency power spectrum and synchronization in VNS antiepileptic effects and the small sample of the patients selected for the present report does not allow for sufficient statistical analysis, these preliminary results suggest that a time-dependent VNS-mediated mechanism can modulate the expression of several brain rhythms possibly involved in more than the seizure control. Larger prospective

and randomised multicentric studies are needed in order to set a critical investigation of these aspects of VNS treatment.

5. Uncited reference

American Electroencephalographic Society Guidelines in EEG and Evoked Potentials (1986).

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Appendix A

Embedded synchronization in EEG signals is performed by adapting the non-linear synchronization measure introduced by Quian Quiroga et al. (2002) from the work of Arnhold et al. (1999). Unlike coherence, cross-correlation and mutual information, this measure is non-symmetric and yields more detailed information about the 'direction' of interdependence. Briefly, in order to detect generalized synchronization we synthesize the general principles.

Let $\mathbf{x}=(x_1,\ldots,x_N)$ and $\mathbf{y}=(y_1,\ldots,y_N)$ two different simultaneously observed EEG time sequences opportunely sampled related to dynamical systems $\mathbf{X}$ and $\mathbf{Y}$.

Time-delay embedding in a m-dimensional phase-space leads to vectors $\mathbf{x_n}=(x_n,\ldots,x_{n-(m-1)\tau})$ and $\mathbf{y_n}=(y_n,\ldots,y_{n-(m-1)\tau})$ where τ indicates the time delay.

Let $r_{n,j}$ and $S_{n,j}$, $j=1,\ldots,k$ the time indices of the k nearest neighbours of $\mathbf{x_n}$ and $\mathbf{y_n}$, respectively. Thus, the first neighbour distances from $\mathbf{x_n}$ are $d(\mathbf{X})_n^{(1)}\equiv||\mathbf{x_n}-\mathbf{x_{r_{n,1}}}||=\min_q||\mathbf{x_n}-\mathbf{x_q}||$, $d(\mathbf{X})_n^{(2)}\equiv||\mathbf{x_n}-\mathbf{x_{r_{n,2}}}||=\min_{q\neq r_{n,1}}||\mathbf{x_n}-\mathbf{x_q}||$, etc., where $||\mathbf{x}-\mathbf{x}'||$ is the 'Euclidean distance' in delay space. For each $\mathbf{x_n}$ and $\mathbf{y_n}$, the squared mean Euclidean distance to its k closest neighbours is defined as

$$R_n^{(k)}(\mathbf{X})=\frac{1}{k}\sum_{j=1}^{k}||\mathbf{x_n}-\mathbf{x_{r_{n,j}}}|| \tag{1}$$

and the conditional mean square Euclidean distance, conditioned on the closest neighbour times in the time series $\mathbf{Y}$, is

$$R_n^{(k)}(\mathbf{X}|\mathbf{Y})=\frac{1}{k}\sum_{j=1}^{k}||\mathbf{x_n}-\mathbf{x_{s_{n,j}}}|| \tag{2}$$

Note that in (2), instead of summing over nearest neighbours as in (1), we sum over points whose equal time partners are nearest neighbours of $\mathbf{y_n}$. The same can be done symmetrically for $\mathbf{y_n}$ obtaining

$$R_n^{(k)}(\mathbf{Y})=\frac{1}{k}\sum_{j=1}^{k}||\mathbf{y_n}-\mathbf{y_{s_{n,j}}}|| \tag{3}$$

and

$$R_n^{(k)}(\mathbf{Y}|\mathbf{X})=\frac{1}{k}\sum_{j=1}^{k}||\mathbf{y_n}-\mathbf{y_{r_{n,j}}}||. \tag{4}$$

Introducing the average distance between the reference vector and all other vectors in the data set $\{\mathbf{X_n}\}$

$$R_n^{(N-1)}(\mathbf{X})\equiv R_n(\mathbf{X})=\frac{1}{N-1}\sum_{m=1}^{N-1}||\mathbf{x_n}-\mathbf{x_m}||, \tag{5}$$

we can observe that, if $\mathbf{X}$ and $\mathbf{Y}$ are strongly coupled we have $R_n^{(k)}(\mathbf{X}|\mathbf{Y})\approx R_n^{(k)}(\mathbf{X})\ll R_n(\mathbf{X})$ while if they are independent $R_n^{(k)}(\mathbf{X}|\mathbf{Y})\approx R_n(\mathbf{X})\gg R_n^{(k)}(\mathbf{X})$.

Thus, we define local and global interdependence measure, respectively as

$$N_n^{(k)}(\mathbf{X}|\mathbf{Y})=\frac{R_n(\mathbf{X})-R_n^{(k)}(\mathbf{X}|\mathbf{Y})}{R_n(\mathbf{X})} \tag{6}$$

and

$$N^{(k)}(\mathbf{X}|\mathbf{Y})\equiv\frac{1}{N}\sum_{n=1}^{N}N_n^{(k)}(\mathbf{X}|\mathbf{Y})$$

$$=\frac{1}{N}\sum_{n=1}^{N}\frac{R_n(\mathbf{X})-R_n^{(k)}(\mathbf{X}|\mathbf{Y})}{R_n(\mathbf{X})} \tag{7}$$

This measure is normalized in the sense that $N^{(k)}(\mathbf{X}|\mathbf{Y})=0$ in case of uncorrelated signal and $N^{(k)}(\mathbf{X}|\mathbf{Y})\approx 1$ in the case of perfect coupling (in general $R_n^{(k)}(\mathbf{X}|\mathbf{Y})$ will not go exactly to zero so this measure will not reach 1 even in case of perfect synchronization).

We used this interdependence measure to compare non-overlapping epochs for each couple of channels. Results have been averaged on regions reported in Fig. 1.

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