

SPECT imaging of cerebral blood flow changes induced by acute trigeminal nerve stimulation in drug-resistant epilepsy. A pilot study

Questa è la versione Post print del seguente articolo:

Original

SPECT imaging of cerebral blood flow changes induced by acute trigeminal nerve stimulation in drug-resistant epilepsy. A pilot study / Mercante, Beniamina; Nuvoli, Susanna; Sotgiu, Maria A.; Manca, Andrea; Todesco, Sara; Melis, Francesco; Spanu, Angela; Deriu, Franca. - In: CLINICAL NEUROPHYSIOLOGY. - ISSN 1388-2457. - 132:Mar 24(2021), pp. 1274-1282. [10.1016/j.clinph.2021.01.033]

Availability:

This version is available at: 11388/246469 since: 2021-06-04T11:34:57Z

Publisher:

Published

DOI:10.1016/j.clinph.2021.01.033

Terms of use:

Chiunque può accedere liberamente al full text dei lavori resi disponibili come "Open Access".

Publisher copyright

note finali coverpage

(Article begins on next page)

This is the Author's accepted manuscript version of the following contribution:

SPECT imaging of cerebral blood flow changes induced by acute trigeminal nerve stimulation in drug-resistant epilepsy. A pilot study / Mercante, Beniamina; Nuvoli, Susanna; Sotgiu, Maria A.; Manca, Andrea; Todesco, Sara; Melis, Francesco; Spanu, Angela; Deriu, Franca. - In: CLINICAL NEUROPHYSIOLOGY. - ISSN 1388-2457. - 132:Mar 24(2021), pp. 1274-1282. [10.1016/j.clinph.2021.01.033]

The publisher's version is available at:

<https://dx.doi.org/10.1016/j.clinph.2021.01.033>

When citing, please refer to the published version.

Clinical Neurophysiology

SPECT imaging of cerebral blood flow changes induced by acute trigeminal nerve stimulation in drug-resistant epilepsy. A pilot study.

--Manuscript Draft--

Manuscript Number:	CLINPH-D-20-13781R2
Article Type:	Full Length Article
Section/Category:	Epilepsy
Keywords:	cerebral blood flow; Cerebral perfusion; Drug-resistant epilepsy; Brain SPECT; Trigeminal nerve stimulation.
Corresponding Author:	Franca Deriu, M.D., Ph.D. Dept Biomedical Sciences University of Sassari Sassari, ITALY
First Author:	Beniamina Mercante, PhD
Order of Authors:	Beniamina Mercante, PhD Susanna Nuvoli Maria A. Sotgiu Andrea Manca Sara Todesco Francesco Melis Angela Spanu Franca Deriu, M.D., Ph.D.
Abstract:	<p>OBJECTIVE: To explore the cortical areas targeted by acute transcutaneous trigeminal nerve stimulation (TNS) in patients with drug-resistant epilepsy (DRE) using single photon emission computed tomography (SPECT).</p> <p>METHODS: Ten patients with DRE underwent brain SPECT at baseline and immediately after a 20-minute TNS (0.25 ms; 120 Hz; 30 s ON and 30 s OFF) applied bilaterally to the infraorbital nerve. The French Color Standard International Scale was used for qualitative analyses and Z-scores were used to calculate the Odds Ratio (OR).</p> <p>RESULTS: At baseline global hypoperfusion (mainly in temporo-mesial, temporo-parietal and fronto-temporal and temporo-occipital areas) was detected in all patients. Following TNS, a global increase in cortical tracer uptake and a significant decrease in median hypoperfusion score were observed. A significant effect favoring a general TNS-induced increase in cortical perfusion (OR=4.96; p=0.0005) was detected in 70% of cases, with significant effects in the limbic (p=0.003) and temporal (p=0.003) lobes. Quantitative analyses of z-scores confirmed significant TNS-induced increases in perfusion in the temporal (+0.59 SDs; p=0.001), and limbic (+0.43 SDs; p=0.03) lobes.</p> <p>CONCLUSION: Short-term TNS is followed a global increase in cortical perfusion, namely in the temporal and limbic lobes.</p> <p>SIGNIFICANCE: The TNS-induced perfusion increase may reflect neurons' activity changes in cortical areas implicated in the epilepsy network.</p>

Abstract

OBJECTIVE: To explore the cortical areas targeted by acute transcutaneous trigeminal nerve stimulation (TNS) in patients with drug-resistant epilepsy (DRE) using single photon emission computed tomography (SPECT).

METHODS: Ten patients with DRE underwent brain SPECT at baseline and immediately after a 20-minute TNS (0.25 ms; 120 Hz; 30 s ON and 30 s OFF) applied bilaterally to the infraorbital nerve. The French Color Standard International Scale was used for qualitative analyses and *z*-scores were used to calculate the Odds Ratio (OR).

RESULTS: At baseline global hypoperfusion (mainly in temporo-mesial, temporo-parietal and fronto-temporal and temporo-occipital areas) was detected in all patients. Following TNS, a global increase in cortical tracer uptake and a significant decrease in median hypoperfusion score were observed. A significant effect favoring a general TNS-induced increase in cortical perfusion (OR=4.96; $p=0.0005$) was detected in 70% of cases, with significant effects in the limbic ($p=0.003$) and temporal ($p=0.003$) lobes. Quantitative analyses of *z*-scores confirmed significant TNS-induced increases in perfusion in the temporal (+0.59 SDs; $p=0.001$), and limbic (+0.43 SDs; $p=0.03$) lobes.

CONCLUSION: Short-term TNS is followed a global increase in cortical perfusion, namely in the temporal and limbic lobes.

SIGNIFICANCE: The TNS-induced perfusion increase may reflect neurons' activity changes in cortical areas implicated in the epilepsy network.

1 **Title**

2 **SPECT imaging of cerebral blood flow changes induced by acute trigeminal nerve stimulation**
3 **in drug-resistant epilepsy. A pilot study.**

4 **Author**

5 Beniamina Mercante^{1*}, Susanna Nuvoli^{2*}, Maria A. Sotgiu^{1*}, Andrea Manca^{1*}, Sara Todesco³,
6 Francesco Melis¹, Angela Spanu² and Franca Deriu¹✉

7
8 **Affiliations**

9 ¹Department of Biomedical Sciences, University of Sassari, Sassari, Italy; ²Unit of Nuclear
10 Medicine, Department of Medical, Surgical and Experimental Science, University of Sassari,
11 Sassari, Italy; ³Neurology Unit, «A. Segni» Hospital, ASL n. 1, Sassari, Italy

12 *These authors contributed equally to this work.

13
14 ✉ **Corresponding Author:**

15 Franca Deriu
16 Department of Biomedical Sciences
17 University of Sassari,
18 Viale San Pietro 43/b, 07100 Sassari, Italy
19 Telephone number: +39 079228289
20 Email: deriuf@uniss.it
21 ORCID: 0000-0001-8235-6091

22
23 **Acknowledgments**

24 The Authors acknowledge Drs. Chiara Fois and Vittoria Porcella for their contribution in patients'
25 recruitment.

26 **Declaration of Competing Interest**

27 None of the authors has potential conflicts of interest to be disclosed.

28 **Abstract**

29

30 **OBJECTIVE:** To explore the cortical areas targeted by acute transcutaneous trigeminal nerve
31 stimulation (TNS) in patients with drug-resistant epilepsy (DRE) using single photon emission
32 computed tomography (SPECT).

33 **METHODS:** Ten patients with DRE underwent brain SPECT at baseline and immediately after a
34 20-minute TNS (0.25 ms; 120 Hz; 30 s ON and 30 s OFF) applied bilaterally to the infraorbital
35 nerve. The French Color Standard International Scale was used for qualitative analyses and z-scores
36 were used to calculate the Odds Ratio (OR).

37 **RESULTS:** At baseline global hypoperfusion (mainly in temporo-mesial, temporo-parietal and
38 fronto-temporal and temporo-occipital areas) was detected in all patients. Following TNS, a global
39 increase in cortical tracer uptake and a significant decrease in median hypoperfusion score were
40 observed. A significant effect favoring a general TNS-induced increase in cortical perfusion
41 (OR=4.96; p=0.0005) was detected in 70% of cases, with significant effects in the limbic (p=0.003)
42 and temporal (p=0.003) lobes. Quantitative analyses of z-scores confirmed significant TNS-induced
43 increases in perfusion in the temporal (+0.59 SDs; p=0.001), and limbic (+0.43 SDs; p=0.03) lobes.

44 **CONCLUSION:** Short-term TNS is followed a global increase in cortical perfusion, namely in the
45 temporal and limbic lobes.

46 **SIGNIFICANCE:** The TNS-induced perfusion increase may reflect neurons' activity changes in
47 cortical areas implicated in the epilepsy network.

48

49 **Highlights:**

50
51

- 52 • Patients with drug-resistant epilepsy showed global hypoperfusion at brain SPECT.
- 53 • After trigeminal nerve stimulation perfusion increased in the temporal and limbic lobes.
- 54 • TNS effects may reflect activity changes in cortical areas associated to epilepsy.

55

56

57 **Keywords:** Cerebral blood flow; Cerebral perfusion; Drug-resistant epilepsy; Brain SPECT;
58 Trigeminal nerve stimulation.

59

60 **Funding:** This research was funded by the Regione Autonoma della Sardegna (LR n7 07-08-07).

61

62 **1. Introduction**

63 It is estimated that up to one third of patients with epilepsy are drug-resistant (DRE) (Kwan, 2004,
64 2010; Löscher and Schmidt, 2011). Most of them are not suitable candidates for surgery due to
65 difficult location of the epileptogenic focus, identification of the seizure focus within eloquent
66 areas, presence of multiple foci or refusal for surgical treatment by patients (Schulze-Bonhage,
67 2019).

68 For this subset of DRE patients, in 1997 vagal nerve stimulation (VNS) was approved by the U.S.
69 Food and Drug Administration as an alternative and/or adjunctive treatment option (Schulze-
70 Bonhage, 2019).

71 Although VNS is generally safe and well tolerated, this therapy is not risk-free owing to the
72 implantation-related invasiveness and costs (Ben-Menachem et al. 2015) and, mainly, to the
73 vegetative side effects associated to VNS (Howland, 2014). These drawbacks, along with a 49.4%
74 rate of non-responders (Englot et al., 2011; Gschwind and Seeck, 2016), have led researchers to
75 propose the transcutaneous stimulation of the trigeminal nerve (TNS) as a valid alternative to VNS
76 for the treatment of DRE (DeGiorgio et al., 2003, 2006, 2009; Fanselow et al., 2000). Subsequent
77 studies have shown that TNS is a safe and promising treatment for DRE in the long term
78 (DeGiorgio et al., 2013; Pop et al., 2011; Soss et al. 2015; Olivié et al., 2019; Gil-López et al.,
79 2020), with additional positive effects on sleep quality, mood and quality of life (Slaght and Nashef,
80 2017; Olivié et al., 2019).

81 The sites of the antiepileptic effects of TNS in the central nervous system (CNS) and its
82 mechanisms of action are yet to be elucidated. Studies carried out in animal models evidenced that
83 trigeminal afferents activated by short-term TNS protocols target the nucleus of the solitary tract,
84 the locus coeruleus and the dorsal raphe nucleus (Mercante et al., 2017). These nuclei are crucial to
85 establish ascending connections with other brainstem and forebrain structures (i.e. parabrachial
86 nucleus, cerebellum, reticular formation, hypothalamus, thalamus, amygdala, hippocampus,
87 entorhinal cortex, endopyriform gyrus), that are thought to be implicated in the inhibitory control of

88 seizures (Bari and Pouratian, 2012; George et al., 2000; Magdaleno-Madrigal et al., 2002; Mercante
89 et al., 2017; Moseley and DeGiorgio, 2014; Rutecki, 1990; Wang et al., 2016). In line with
90 anatomical evidences (Mercante et al., 2017), physiological studies carried out in humans indicate
91 the brainstem as the structure mainly targeted by TNS with a long-term depression-like mechanism
92 (Mercante et al. 2015; Pilurzi et al. 2016). In addition, short-term TNS has been shown to influence
93 EEG activity both in healthy and DRE subjects, suggesting that the cerebral cortex is eventually
94 involved by TNS (Ginatempo et al., 2018, 2019).

95 Despite the increasing therapeutic use of TNS in DRE and in a number of other neuropsychiatric
96 conditions (Shiozawa et al., 2014), no neuroimaging procedures have been undertaken to
97 investigate the anatomo-functional correlates of TNS.

98 Therefore, the main purpose of this study was to explore which cortical areas are targeted by acute
99 TNS in DRE patients using functional imaging modalities commonly used in the evaluation and
100 diagnosis of epilepsy. To this aim, we used brain perfusion single photon emission computed
101 tomography (SPECT), which represents the most used functional procedure in ictal and interictal
102 epilepsy evaluation (Buch et al., 2008; Mielke et al., 1994).

103

104 **2. Material and Methods**

105

106 **2.1 Patients**

107 Ten patients (44 ± 9.0 years old; 7 males) referring to the Center for Diagnosis and Treatment of
108 Epilepsy, Unit of Neurology, University of Sassari, and diagnosed with DRE in accordance with the
109 criteria of the International League Against Epilepsy (Kwan et al. 2010), participated in this study.
110 Exclusion criteria were: history of migraine, diabetes, trigeminal neuralgia, psychiatric disorders,
111 cardiovascular diseases, history or signs of neurological disorders other than DRE, pregnancy and
112 seizure attack in the 24 hours preceding the SPECT scan. All patients underwent EEG examination
113 and magnetic resonance imaging (MRI).

114 The study was approved by the local Ethics Committee (Prot 982/2/L ASL n. 1 Sassari, 17/11/2011)
115 and written informed consent was obtained from all participants.

116

117 **2.2 TNS**

118 TNS was delivered bilaterally to the infraorbital nerve (ION) through an electrical neurostimulator
119 (Winner, Fisioline Biomedical Instrumentation, Verduno, CN, IT) connected to 26-mm disposables,
120 hypoallergenic, silver-gel self-adhesive stimulating electrodes (Globus, Domino s.r.l., Codognè,
121 TV, IT) with the cathode placed over each infraorbital foramen and the anode 2 cm lateral, to
122 stimulate V2 branches of trigeminal nerve. In each subject, perceptual and pain thresholds were
123 bilaterally calculated and stimulation intensity was set, for each ION, to the maximal intensity that
124 the subject could sustain comfortably, and always below the pain threshold. The stimulus consisted
125 of trains of a symmetric biphasic square wave pulse (duration 0.25 ms, frequency 120 Hz),
126 delivered in a cyclic modality (30 s ON and 30 s OFF) for 20 min, according to protocols described
127 in previous works (Ginatempo et al., 2019).

128

129 **2.3 Brain SPECT**

130 Brain SPECT was performed according to International guidelines (Kapucu et al., 2009) at baseline
131 (T0) and after a 20-minute TNS session (T1), which were carried out two weeks apart.

132 Patients were asked to avoid eating, smoking and the intake of CNS stimulants (such as caffeine,
133 cola, energy drinks), alcohol, and any drugs known to affect cardiovascular function in the 12 hours
134 preceding the SPECT scan. They were positioned in a quiet, dimly-lit room in a supine comfortable
135 position with an eye-mask and were instructed not to speak, read, or move from at least 5 min
136 before to 5 min after the injection of the radiopharmaceutical tracer (^{99m}Tc HM-PAO) through an
137 intravenous cannula inserted before TNS administration. The ^{99m}Tc HM-PAO complexes are
138 lipophilic and of small size, thus they circulate with the blood to the brain and pass the blood-brain-
139 barrier. Intracellularly, the tracer is rapidly metabolized and retained unmodified for a relatively

140 long time (4-6 hours) since a steric hydrophilic transformation of the ^{99m}Tc HM-PAO occurs by the
141 intracellular glutathione reductase (Asenbaum et al.,1998). The tracer retention in the brain is very
142 stable when the early back-diffusion has ceased, and only a small loss of tracer (approximately
143 0.4%/hour) is observed in most human cases during the following 24 hours (Andersen, 1989).
144 Hence, the steady-state ^{99m}Tc -HMPAO of the distribution images, obtained from 10 min to 2-3
145 hours after the injection of the tracer, agrees more closely with regional cortical blood flow (rCBF)
146 and can be used to evaluate the functional status of the brain (Zerarka et al., 2001). The ^{99m}Tc
147 HM-PAO was prepared using pertechnetate coming from a generator eluted in the previous 24
148 hours, which eluted was not older than 2 hours. Radiochemical purity (>80%) was determined on
149 each vial prior to injection. A dose of 740 Mega-Becquerel of tracer was injected intravenously
150 within 30 min since radioligand reconstitution and one minute before the end of the TNS procedure.
151 Images were acquired within 30-60 minutes from injection using a dedicate dual-head gamma
152 camera equipped with fan-beam collimators (rotation: 180° , matrix size: 128x128, zoom factor: 1,
153 frame time: 30 seconds, angular step: 3 degrees).

154

155 **2.3.1. Image processing**

156 Unprocessed projection data and sinogram forms were evaluated in a cinematic display prior to a
157 filtering process to assess the presence of motion or other potential artefacts, then the filtered back-
158 projection reconstruction was applied.

159 Transaxial slices were reformatted into at least three orthogonal planes. Transverse sections were
160 generated parallel to a given anatomic orientation (e.g. anterior commissure-posterior commissure
161 line) ensuring a high degree of standardization in the plane orientation. Subsequent coronal and
162 sagittal sections were created on the basis of transaxial slices. Finally, the set of transaxial, sagittal
163 and coronal slices were produced in an automated standard color scale with a section thickness of
164 2.1 mm. Finally, the cortical uptake of ^{99m}Tc -HMPAO was normalized and expressed as a
165 percentage of the maximum uptake observed in the cerebellum.

166

167 **2.3.2 SPECT Data analysis**

168 A qualitative evaluation of images was performed separately by two experienced nuclear medicine
169 physicians (SN and AS) by visual interpretation of tracer uptake in cortical regions based on the
170 French Color Standard International Scale. Each patient's dataset was normalized individually to the
171 mean cerebellar pixel values.

172 Brain SPECT perfusion deficits were scored by visual qualitative analysis with respect to
173 localization (frontal, parietal, temporal and occipital cortex), lateralization (left or right hemisphere)
174 and severity of hypoperfusion. A consensus reading was reached in case of discordant evaluations.
175 The scores were subdivided into four grades, as follows: grade 1 = normal (80-100%); grade 2 =
176 slight (60%-80%); grade 3 = moderate (60%-50%); grade 4 = severe (<50%).

177 Quantitative assessment was performed using the NeuroGam® software (GE Medical System,
178 Segami Corp., Columbia, MD, USA) to evaluate any statistically significant TNS-induced changes
179 in the five cerebral lobes of both hemispheres. To accurately locate abnormal rCBF, the areas of
180 cerebral cortex were analyzed in the Brodmann areas (BAs) (Fu et al. 2015, 2018).

181 The mean rCBF for each region was obtained through a predefined algorithm which converts the
182 mean pixel value in each corresponding region of interest into a percentage of the maximum pixel
183 value in the entire volume of brain. This software uses an affine anatomical coregistration by blocks
184 of data defined in the Talairach space. Patient images were normalized to determine the z-scores
185 which were calculated automatically by the system for each patient, as the number of standard
186 deviations from normative data stored in the NeuroGam® software package, obtained from age-
187 and gender-matched healthy control subjects.

188 A surface mapping was done according to color scale depending on the number of standard
189 deviations from normal values.

190

191 **2.4. Statistical analysis**

192 All statistical analyses were conducted using the SPSS statistical software (version 20.0, IBM, New
193 York, USA). Odds ratio (OR) calculations were performed with RevMan 5.4 software (The
194 Cochrane Collaboration). Analyses of individual data were carried out employing the NeuroGam[®]
195 software to create a z-score map, which calculates z-scores using the following equation: $z\text{-score} =$
196 $(\text{control mean} - \text{individual value}) / \text{control SD}$.

197 This is equivalent to presenting the individual data in terms of standard deviations (SDs) from the
198 NeuroGam[®] normative database obtained from matched healthy controls, which distribution is set
199 as follows: $z = -1.5$ to $z = +1.5$.

200 Values outside that distribution in either direction were considered abnormal. In particular, z-scores
201 < -1.5 were interpreted as “decreased uptake” whilst z scores $> +1.5$ were interpreted as “increased
202 uptake” in comparison to normative data.

203 To test for any differences in tracer uptake between patients’ z-scores in basal conditions and
204 following TNS, a three-fold approach was followed: qualitative, quantitative/dichotomous (i.e.
205 changed Vs. unchanged), and quantitative/scalar (i.e. by comparing z-scores before and after TNS).
206 Scores deriving from the French Color Standard International Scale were analyzed by the non-
207 parametric Wilcoxon Signed-Rank Test. Quantitative analyses were carried out on dichotomous
208 data. Data were analyzed by lobes and BAs, and corrections (Bonferroni) for multiple comparisons
209 applied. OR and their 95% confidence interval (95% CI) were calculated by comparing the number
210 of events showing increased tracer uptake (responders) *versus* those showing no change (non-
211 responders) following TNS.

212 To test for within-subjects’ differences in perfusion following TNS delivery, the standardized mean
213 differences (SMD) of the pre-to-post change were calculated. According to Cohen (1988), these
214 effect sizes were categorized as follows: trivial (< 0.2), small (< 0.5), moderate ($0.5 \leq d < 0.8$) and large
215 (≥ 0.8).

216 To study and weigh the influence of the history of epilepsy in years on the findings, bivariate
217 correlations with baseline (pre), post-TNS and pre-to-post data were carried out by calculating the
218 Pearson's interclass correlation coefficients.

219 For all the comparisons and analyses, significance was set at the 0.05 level.

220

221

222 **3. Results**

223 **3.1 Patients characteristics**

224 The demographic and clinical characteristics of the participants are detailed in Table 1. Patients had
225 an average seizure frequency of 3.0 seizures per month. None of our patients exhibited a picture of
226 epileptogenic encephalopathy but focal slow wave activity, within a normal background. Only in
227 P09 and P10, who had undergone pre-surgery evaluation, the EEG recording of seizure activity
228 allowed to localize a temporal epileptogenic zone and define a multifocality in the former and a
229 stable unilateral epileptogenic focus in the latter. In particular, in P09 seizure started at different
230 temporal EEG electrodes including both temporal lobes. The remaining patients (P01-P08) had
231 interictal unspecific or epileptiform EEG abnormalities with constant-side regional localization and
232 a tendency to spread contralaterally, with the exception of P03, who showed independent and
233 asynchronous activity on both sides. Consequently, in patients P01-P08 the epileptogenic zone
234 could only be hypothesized from the site of the structural lesion, when present, from clinical ictal
235 semiology of localizing value, and/or from interictal EEG data. When linking symptomatology to
236 the EEG findings, epileptic foci were localized in the temporal lobe (60% of cases), frontal lobe
237 (20%), fronto-temporal lobe (10%) and temporo-occipital lobe (10%). As evidenced by the MRI,
238 the most common etiology was cryptogenic (70% of cases), with only 30% of patients (P01, P03
239 and P05) exhibiting an anatomical lesion with probable epileptogenic meaning (but often without a
240 clear functional correlate, e.g. P01).

241 The intensity of ION stimulation ranged from 6.0–12.0 mA. During the SPECT and TNS
242 procedures, no epileptic seizures were clinically detected neither perceived by the patients.

243

244 **3.2. Qualitative and quantitative analyses of SPECT data**

245 A representation of SPECT images used for qualitative and quantitative analysis is provided in
246 Figure 1 for a representative subject (P04) of whom an EEG trace is also shown (Figure 2). Table 2
247 summarizes the results of the qualitative analysis. All patients presented a certain degree of cerebral
248 hypoperfusion at baseline, which involved focal areas only in 30% of them and both hemispheres in
249 70% of cases. The cortical areas that were more frequently found hypoperfused were the temporo-
250 mesial (70%), temporo-parietal (60%) and temporal (20%) areas. After TNS, 70% of patients
251 exhibited a decrease in the French Color Standard International Scale hypoperfusion score, and a
252 global increase in the cortical tracer uptake. In particular, the Wilcoxon Signed-Rank test revealed a
253 significant reduction in the median hypoperfusion score after TNS (baseline, 3; 95% CI 2.64 to
254 3.36; after TNS, 1.5; 95% CI 0.91 to 2.09; $p=0.015$).

255 The calculation of the OR, which was run by comparing over 5 lobes on both hemispheres the
256 number of events showing increased tracer uptake Vs. events showing no change following TNS,
257 evidenced an overall significant general effect favoring a TNS-induced increase in rCBF (OR =
258 4.96; 95% CI 2.02 to 12.18; $p=0.0005$), showing that the increase in rCBF was 4.96 times more
259 likely to occur than no change. When data were analyzed by lobe, Bonferroni-corrected multiple
260 comparisons revealed significant differences only in the temporal (OR = 16.00; $p=0.003$) and limbic
261 (OR = 16.00; $p=0.003$) and lobes (Figure 3a), **the latter including the area surrounding the corpus**
262 **callosum, the cingulate cortex and the parahippocampal gyri.**

263 When correcting by lobe and by side (Figure 3b), the calculation of the OR evidenced that,
264 following TNS, patients showing a bilaterally increased tracer uptake in the limbic (left and right:
265 $p=0.02$) and temporal lobe (left and right: $p=0.02$) were significantly more than those who
266 displayed no change (Figure 3b).

267 Temporal and limbic lobes were, therefore, further inspected by calculating the OR for the specific
268 BAs of each lobe. Within the temporal lobe, following TNS, patients with increased uptake were
269 significantly more than those displaying no change in left BA 21 (OR= 45.00; p=0.02), right BA 22
270 (OR= 71.40; p=0.007), and left BA 38 (OR= 13.50; p=0.04). After correcting for multiple
271 comparisons over 6 areas, a significant difference emerged only for right BA 22 (p=0.04).
272 Regarding the limbic lobe, after correcting for multiple comparisons over 5 areas, a significant
273 difference was detected in right BA 23 (OR= 36.00; p=0.04).
274 The comparison of the z-scores of the change in perfusion following TNS revealed a significant
275 increase in tracer uptake by 0.59 SD (95% CI 0.27 to 0.90; baseline: -2.41 ± 0.59 z-scores; post-TNS:
276 -1.98 z-scores; p=0.001) in the temporal lobe, and by 0.43 SD (95% CI 0.06 to 0.81; baseline: $-$
277 2.28 ± 0.94 z-scores; post-TNS: -1.69 z-scores; p=0.03) in the limbic lobe.

278 Bivariate correlations revealed no significant relationship between the history of epilepsy in years
279 and the perfusion at baseline (r=0.46; p=0.18), after TNS (r=0.34; p=0.33) and the pre-to-post
280 change in perfusion (r=0.41; p=0.22), as assessed quantitatively by z-scores. Likewise, no
281 relationship emerged when correlating the history of epilepsy with the qualitative estimates of
282 perfusion (as assessed with the hypoperfusion score through subjective visual inspection via the
283 French Color Standard International Scale) at baseline (r=0.50; p=0.14), at the post (r=0.11;
284 p=0.77), and pre-to-post change (r=0.30; p=0.40).

285

286 **4. Discussion**

287 The present study investigated the responses of cortical brain regions to acute TNS administration
288 in DRE patients. To this purpose, the variations in rCBF were studied using ^{99m}Tc HM-PAO brain
289 perfusion SPECT. Data provided first evidence that, in these selected patients, short-term TNS was

290 associated to a consistent increase of rCBF in the temporal and limbic lobes, within the bounds of a
291 global increase in cortical perfusion.

292 The length since epilepsy diagnosis in our cohort was found not to influence baseline cerebral
293 perfusion, post-TNS perfusion as well as the pre-to-post data, assessed either qualitatively or
294 quantitatively. The influence exerted by the early onset and duration of epilepsy on SPECT imaging
295 remains controversial, since these features were positively correlated to the extent of hypoperfusion
296 in the intercritical SPECT by some studies (Duncan, 1992; Duncan et al., 1992; Avery et al., 2001)
297 but not by others (Andersen et al., 1996). Although the spread in illness duration exhibited by our
298 sample was found uncorrelated with SPECT data, the role played by the clinical history in the
299 observed changes of perfusion following TNS delivery needs cautious interpretation.

300 All patients exhibited a global hypoperfusion at baseline, which is compatible with their clinical
301 condition characterized by a long-term intake of pharmacological polytherapy (Spanaki et al. 1999;
302 Joo et al. 2008). Qualitative analysis evidenced that, compared to baseline, 70% of patients
303 exhibited a global increase of cortical tracer uptake after 20 minutes of TNS. In particular, the
304 specific cortical areas that appeared hypoperfused at baseline (mainly temporo-mesial, temporo-
305 parietal and fronto-temporal and temporo-occipital areas), showed a significant decrease of the
306 hypoperfusion score, as assessed with the French Color Standard International Scale. It is
307 noteworthy that both the localization of the hypoperfusion and its reduction after short-term TNS
308 converge with the epileptic focus (temporal in 80% of cases), which was clearly determined in P09
309 and P10 and supposed on the basis of electro-clinic signs in the remaining patients. Although this
310 concordance corroborates the idea that SPECT reflects those changes in cortical perfusion that are
311 associated to functional changes in neuronal activity, namely the epileptic focus, it has to be
312 interpreted with due caution since interictal SPECT has been associated to limited diagnostic
313 accuracy and variable correlation with MRI data (Lee et al., 2000; Siegel et al., 2002; Thadani et al.,
314 2004; Tepmongkol et al., 2015). In addition, our sample was small and heterogeneous, with

315 epileptogenic lesions of diverse nature and interictal specific or aspecific focality (P01-P08),
316 contrasting EEG and clinical data (e.g. P07). Furthermore, EEG lateralization was uncertain (e.g.
317 P03) in all but two patients (P09 and P10), which do not allow mapping an electric network and
318 thus making any correlation of the EEG information with the SPECT signal. Moreover, the lack of
319 EEG co-registration in our study does not allow proving that the hypoperfusion observed in the
320 interictal SPECT might be the result of subclinical electric seizures (Lee et al., 2000).

321 The quantitative analysis confirmed the observations made following the visual inspection of
322 SPECT scans. In particular, a significant increase in rCBF was found in the temporal and limbic
323 lobes of both hemispheres. More specifically, the calculation of the OR evidenced that, after TNS,
324 patients showing increased tracer uptake in the limbic (80%) and temporal (80%) lobes were
325 significantly more than those displaying no change. Furthermore, the quantitative analyses of z-
326 scores to assess the magnitude of the changes in perfusion from baseline to post-TNS, confirmed
327 significant TNS-induced increases in perfusion only in the above said lobes.

328 Taken together, these data indicate that, in DRE patients, short-term TNS is able to produce a global
329 increase in cortical perfusion and, within this frame, the temporal and limbic lobes of both sides
330 were particularly responsive to TNS. Since a close relationship exists between perfusion
331 distribution and the metabolism of ^{99m}Tc HM-PAO (Silverman, 2004), changes in the bio-
332 molecular structure of neurons as well as in the neurophysiopathological substrate are believed to
333 subtend the information obtained with ^{99m}Tc HM-PAO brain SPECT (Palumbo et al., 2014; Nuvoli
334 et al., 2016). In this light, we offer that TNS-induced changes in cortical blood perfusion may result
335 from a change in the functional activity of cortical neurons, which may have occurred after TNS, in
336 line with two recent studies showing EEG changes both in healthy individuals and in DRE patients
337 following short-term TNS (Ginatempo et al., 2018, 2019).

338 Neurophysiological studies performed in healthy subjects suggested that TNS action is exerted
339 mainly at the brainstem than cortical level (Mercante et al., 2015), with a long-term depression-like

340 mechanism (Pilurzi et al., 2016). The connections of the trigeminal nerve within the CNS are
341 considered neuroanatomically strategic in influencing several cortical structures, among which
342 those involved in cognitive, affective and hyper-arousal symptoms (Bathla and Hegde, 2013; Cook
343 et al., 2016). In fact, trigeminal afferents projecting to the spinal trigeminal nucleus reach not only
344 the somatosensory cortex, via the lemniscal pathway, but also the nucleus of the solitary tract, the
345 locus coeruleus and the dorsal raphe nucleus of the ipsilateral side (Mercante et al., 2017). The
346 nucleus of the solitary tract is considered the relay station mediating the TNS-induced changes in
347 several areas of the brain, such as the amigdala, the entorhinal cortex, the structures of the limbic
348 system and the hippocampus, (Fanselow, 2012; Magdaleno-Madrigal et al., 2010; Mercante et al.,
349 2017). The latter also receives a dense bilateral projection from the locus coeruleus (Loy et al.,
350 1980), which regulates hippocampal and cortical functions through its noradrenergic projections
351 (Loy et al., 1980; Hansen and Manahan-Vaughan, 2015). In addition, the nucleus of the solitary
352 tract and the locus coeruleus are potential relay stations mediating the neuromodulatory influence
353 exerted by trigeminal afferents on limbic structures (Sasa and Takaori, 1973). These experimental
354 data support an indirect action of TNS on cortical structures through a main action exerted at the
355 brainstem level from where trigeminal afferents are widely distributed to the above cerebral
356 structures, which have been associated to temporal lobe epilepsy (Bone et al., 2012), are considered
357 as highly epileptogenic (Bertram et al., 1998; O'Shea et al., 2000; Sato et al., 1998; Vismar et al.,
358 2015) and major contributors to the spread of epileptic activity to other brain areas (Majak et al.,
359 2002; Stevens et al., 1988). In light of these experimental data, the mechanisms by which TNS
360 exerts its antiepileptic effects have been mainly attributed to its regulatory action on the
361 abovementioned brainstem and forebrain structures, which play a pivotal role in the
362 physiopathology of the epilepsy (Wang et al., 2016; Mercante et al., 2017). In this regard, TNS may
363 act within the frame of an epileptic network, a system composed of cortical and subcortical regions
364 that are anatomically and functionally connected, where changes in activity in any part of it can
365 influence the others (Spencer, 2002; Kramer et al., 2012). In this context, a TNS action exerted

366 mainly at brainstem level may spread to diverse cortical areas, such as temporal and limbic ones.
367 The convergence between SPECT signal (which evidenced significant effects of TNS in the
368 temporal and limbic regions) and the anatomo-electro-clinical signs indicating a picture of temporal
369 lobe epilepsy in 80% of cases, might allow considering also a possible direct action of TNS on these
370 regions.

371 Even though the present data do not allow claiming that the cortical rCBF changes are causally
372 related with the antiepileptic action of TNS, it stands to reason that such modifications of cerebral
373 perfusion could indirectly influence the neuronal activity or rather represent the result of a TNS-
374 induced change in neuronal activity. In this regard, a correlation between rCBF redistribution and
375 epileptogenicity has been previously reported, with a rCBF increase in the temporal lobe leading to
376 a reduction of epileptic seizures in a linear way (Weinand et al., 1997).

377 **4.1 Study limitations**

378 Some limitations to this pilot study need to be acknowledged. One relates to the heterogeneous and
379 small sample size. However, DRE is a rare condition and samples of this magnitude are common in
380 studies conducted in this category of epileptic patients. Moreover, given that PET and SPECT
381 imaging studies using TNS are not available, those using VNS enrolled samples from 3 to 12
382 patients (Ko et al., 1996; Ring et al., 2000; Vonck et al., 2000; Sucholeiki et al., 2002; Barnes et al.,
383 2003; Henry et al., 2004).

384 Another limitation of the study is the lack of a control group, which may raise concerns on the
385 specificity of the TNS effects for epilepsy. Unfortunately, due to the invasiveness of the SPECT
386 procedure, the institutional ethics committee did not approve the study in healthy controls, which
387 prevented the enrolment of a control group. Possibly for the same ethical concerns, none of the PET
388 and SPECT studies using VNS as peripheral neuromodulation method in epilepsy had a healthy

389 control group (Ko et al., 1996; Vonck et al., 2000; Ring et al., 2000; Sucholeiki et al., 2002; Barnes
390 et al., 2003; Henry et al., 2004).

391 Finally, the lack of a sham TNS session is another limitation which leaves it open whether any
392 change in imaging parameters is due to the intervention or reflects unspecific effects related to
393 repeated measuring or placebo effects. A sham TNS had been planned in patients but, again, was
394 listed among the ethical concerns due to the need to perform multiple administrations of radioactive
395 tracer. To partially compensate for this limitation, we chose to renounce a sham TNS SPECT and
396 rather perform a baseline and a real TNS SPECT to be able to compare the baseline with our
397 normative dataset and the real TNS SPECT with baseline, so that each patient could serve as her/his
398 own control.

399 Although these limitations may reduce the impact of this pilot study, its preliminary results offer
400 useful cues for the interpretation of clinical works describing the antiepileptic effect of TNS but
401 lacking mechanistic explanations, and should be viewed as a starting point for further research over
402 a larger sample, integrated by a control condition and a control group.

403

404 **4.2. Conclusions**

405 This study provides first evidence that short-term TNS induces a consistent increase in cortical
406 perfusion of the temporal and limbic lobes. However, the main issue about the network involved by
407 TNS within different brain regions, remains unresolved and further large controlled studies are
408 needed to confirm the efficacy of TNS in epilepsy, as it is the case of attention-deficit/hyperactivity
409 disorder, for which TNS has received the approval of the Food and Drug Administration (FDA
410 News Release, 2019).

411 Epilepsy is a network disease process involving multiple regions of the brain in the genesis of
412 seizures or in the maintenance of epileptogenicity. Therefore, further studies are needed to improve

413 the anatomical knowledge of the cortical areas involved by trigeminal nerve stimulation. In this
414 regard, the clinical benefits of TNS vs sham have been documented in DRE only with chronic
415 protocols of stimulation (De Giorgio et al. 2013) and, more recently, in temporal DRE in
416 comparison with medical therapy (Gil-López et al., 2020). Therefore future research should
417 investigate the effects of chronic TNS on neurophysiological and neuroimaging outcomes and
418 correlate the findings with the clinical effects of TNS in epileptic patients.

419

420

421 **References**

- 422 Andersen AR. 99mTc-D,L-hexamethylene-propyleneamine oxime (99mTc-HMPAO): basic kinetic
423 studies of a tracer of cerebral blood flow. *Cerebrovasc Brain Metab Rev.* 1989;1(4):288-318.
- 424 Andersen AR, Hansen BA, Høgenhaven H, Herning M, Paulson O, Dam M. Interictal SPECT of
425 rCBF is of clinical utility in the preoperative evaluation of patients with partial epilepsy. *Epilepsy*
426 *Res.* 1996;25(1):41-50. doi:10.1016/0920-1211(96)00018-6.
- 427 Asenbaum S, Brücke T, Pirker W, Pietrzyk U, Podreka I. Imaging of cerebral blood flow with
428 technetium-99m-HMPAO and technetium-99m-ECD: a comparison. *J Nucl Med.* 1998;39(4):613-
429 8.
- 430 Avery RA, Zubal IG, Studholme C, Slawski J, Corsi M, Spencer DD, Spencer SS. Interictal
431 99mTc-HMPAO SPECT in temporal lobe epilepsy: relation to clinical variables. *Epilepsia.*
432 2001;42(7):869-74. doi: 10.1046/j.1528-1157.2001.042007869.x.
- 433 Barnes A, Duncan R, Chisholm JA, Lindsay K, Patterson J, Wyper D. Investigation into the
434 mechanisms of vagus nerve stimulation for the treatment of intractable epilepsy, using 99mTc-
435 HMPAO SPET brain images. *Eur J Nucl Med Mol Imaging.* 2003;30(2):301-5. doi:
436 10.1007/s00259-002-1026-8.
- 437 Bari AA, Pouratian N. Brain imaging correlates of peripheral nerve stimulation. *Surg Neurol Int.*
438 2012;3(Suppl 4):S260-8. doi: 10.4103/2152-7806.103016.
- 439 Bathla G, Hegde AN. The trigeminal nerve: an illustrated review of its imaging anatomy and
440 pathology. *Clin Radiol.* 2013;68(2):203-213.
- 441 Ben-Menachem E, Revesz D, Simon BJ, Silberstein S. Surgically implanted and non-invasive
442 vagus nerve stimulation: a review of efficacy, safety and tolerability. *Eur J Neurol.* 2015;
443 22(9):1260-8. doi: 10.1111/ene.12629.

- 444 Bertram EH, Zhang DX, Mangan P, Fountain N, Rempe D. Functional anatomy of limbic epilepsy:
445 a proposal for central synchronization of a diffusely hyperexcitable network. *Epilepsy*
446 *Res.* 1998;32(1-2):194-205.
- 447 Bone B, Fogarasi A, Schulz R, Gyimesi C, Kalmar Z, Kovacs N, Ebner A, Janszky J. Secondarily
448 generalized seizures in temporal lobe epilepsy. *Epilepsia.* 2012;53(5):817-824.
- 449 Buch K, Blumenfeld H, Spencer S, Novotny E, Zubal IG. Evaluating the accuracy of
450 perfusion/metabolism (SPET/PET) ratio in seizure localization. *Eur J Nucl Med Mol Imaging.*
451 2008;35(3):579-588.
- 452 Cohen J. *Statistical Power Analysis for the Behavioral Sciences.* 2nd ed. Hillsdale, NJ: L. Erlbaum
453 Associates, 1988.
- 454 Cook IA, Abrams M, Leuchter AF. Trigeminal Nerve Stimulation for Comorbid Posttraumatic
455 Stress Disorder and Major Depressive Disorder. *Neuromodulation.* 2016;19(3):299-305. doi:
456 10.1111/ner.12399.
- 457 DeGiorgio CM, Shewmon DA, Whitehurst T. Trigeminal nerve stimulation for epilepsy.
458 *Neurology.* 2003;61(3):421-422.
- 459 DeGiorgio CM, Shewmon A, Murray D, Whitehurst T. Pilot study of trigeminal nerve stimulation
460 (TNS) for epilepsy: a proof-of-concept trial. *Epilepsia* 2006;47(7):1213-1215.
- 461 DeGiorgio CM, Murray D, Markovic D, Whitehurst T. Trigeminal nerve stimulation for epilepsy:
462 long-term feasibility and efficacy. *Neurology.* 2009;72(10):936-938. doi:
463 10.1212/01.wnl.0000344181.97126.b4.
- 464 DeGiorgio CM, Soss J, Cook IA, Markovic D, Gornbein J, Murray D, Oviedo S, Gordon S,
465 Corralle-Leyva G, Kealey CP, Heck CN. Randomized controlled trial of trigeminal nerve
466 stimulation for drug-resistant epilepsy. *Neurology.* 2013;80(9):786-791. doi:
467 10.1212/WNL.0b013e318285c11a.
- 468 Duncan R. Epilepsy, cerebral blood flow, and cerebral metabolic rate. *Cerebrovasc Brain Metab*
469 *Rev.* 1992;4(2):105-21.
- 470 Duncan S, Gillen G, Adams FG, Duncan R, Brodie MJ. Interictal HM-PAO SPECT: a routine
471 investigation in patients with medically intractable complex partial seizures? *Epilepsy Res.*
472 1992;13(1):83-7. doi: 10.1016/0920-1211(92)90010-q.
- 473 Englot DJ, Chang EF, Auguste KI. Efficacy of vagus nerve stimulation for epilepsy by patient
474 age, epilepsy duration, and seizure type. *Neurosurg Clin N Am.* 2011;22(4):443-8, v. doi:
475 10.1016/j.nec.2011.07.002.
- 476 **FDA News Release, 2019.** [https://www.fda.gov/news-events/press-announcements/fda-permits-](https://www.fda.gov/news-events/press-announcements/fda-permits-marketing-first-medical-device-treatment-adhd)
477 [marketing-first-medical-device-treatment-adhd](https://www.fda.gov/news-events/press-announcements/fda-permits-marketing-first-medical-device-treatment-adhd) (accessed 12 November 2020).
- 478 Fanselow EE, Nicolelis MA. Reduction of pentylenetetrazole-induced seizure activity in awake rats
479 by seizure-triggered trigeminal nerve stimulation. *J Neurosci.* 2000;20(21):8160-8.

- 480 Fanselow E. Central mechanisms of cranial nerve stimulation for epilepsy. *Surg Neurol Int.*
481 2012;2(3):S247-254. doi: 10.1227/00006123-201203000-00024.
- 482 Fu P, Zhang F, Gao J, Jing J, Pan L, Li D, Wei L. NeuroGam Software Analysis in Epilepsy
483 Diagnosis Using 99mTc-ECD Brain Perfusion SPECT Imaging. *Med Sci Monit.* 2015;21:2801-
484 2808. doi: 10.12659/MSM.894566.
- 485 Fu P, Wei L, Zhang F, Gao J, Jing J, Wu W, Liu H. Added Value of NeuroGam Software Analysis
486 in Single Photon Emission Computed Tomography Localization Diagnosis of Epilepsy in Interictal
487 Stage. *Med Sci Monit.* 2018;24:1494-1501.
- 488 George MS, Sackeim HA, Rush AJ, Marangell LB, Nahas Z, Husain MM, Lisanby S, Burt T,
489 Goldman J, Ballenger JC. Vagus nerve stimulation: a new tool for brain research and therapy. *Biol*
490 *Psychiatry.* 2000;47(4):287-295.
- 491 Gil-López F, Boget T, Manzanares I, Donaire A, Conde-Blanco E, Baillés E, Pintor L, Setoain X,
492 Bargalló N, Navarro J, Casanova J, Valls J, Roldán P, Rumià J, Casanovas G, Domenech G, Torres
493 F, Carreño M. External trigeminal nerve stimulation for drug resistant epilepsy: A randomized
494 controlled trial. *Brain Stimul.* 2020;13(5):1245-1253. doi: 10.1016/j.brs.2020.06.005
- 495 Ginatempo F, De Carli F, Todesco S, Mercante B, Sechi GP, Deriu F. Effects of acute trigeminal
496 nerve stimulation on rest EEG activity in healthy adults. *Exp Brain Res.* 2018;236(11):2839-2845.
497 doi: 10.1007/s00221-018-5338-8.
- 498 Ginatempo F, Fois C, De Carli F, Todesco S, Mercante B, Sechi G, Deriu F. Effect of short-term
499 transcutaneous trigeminal nerve stimulation on EEG activity in drug-resistant epilepsy. *J Neurol*
500 *Sci.* 2019;400:90-96. doi: 10.1016/j.jns.2019.03.004.
- 501 Gschwind M, Seeck M. Modern management of seizures and epilepsy. *Swiss Med Wkly.*
502 2016;146:w14310. doi: 10.4414/smw.2016.14310.
- 503 Hansen N, Manahan-Vaughan D. Locus Coeruleus Stimulation Facilitates Long-Term Depression
504 in the Dentate Gyrus That Requires Activation of β -Adrenergic Receptors. *Cereb Cortex.*
505 2015;25(7):1889-1896. doi: 10.1093/cercor/bht429.
- 506 Henry TR, Bakay RA, Pennell PB, Epstein CM, Votaw JR. Brain blood-flow alterations induced by
507 therapeutic vagus nerve stimulation in partial epilepsy: II. prolonged effects at high and low levels
508 of stimulation. *Epilepsia.* 2004;45(9):1064-70. doi: 10.1111/j.0013-9580.2004.03104.x.
- 509 Howland RH. Vagus nerve stimulation. *Curr Behav Neurosci Rep.* 2014;1(2):64-73.
- 510 **Joo EY, Tae WS, Hong SB. Cerebral blood flow abnormality in patients with idiopathic generalized**
511 **epilepsy. *J Neurol.* 2008 Apr;255(4):520-5. doi:10.1007/s00415-008-0727-8.**
- 512 Kapucu OL, Nobili F, Varrone A, Booij J, Vander Borght T, Någren K, Darcourt J, Tatsch K, Van
513 Laere KJ. EANM procedure guideline for brain perfusion SPECT using 99mTc-labelled
514 radiopharmaceuticals, version 2. *Eur J Nucl Med Mol Imaging.* 2009;36(12):2093-2102. doi:
515 10.1007/s00259-009-1266-y.

- 516 Ko D, Heck C, Grafton S, Apuzzo ML, Couldwell WT, Chen T, Day JD, Zelman V, Smith T,
517 DeGiorgio CM. Vagus nerve stimulation activates central nervous system structures in epileptic
518 patients during PET H₂(15)O blood flow imaging. *Neurosurgery*. 1996;39(2):426-30; discussion
519 430-1. doi: 10.1097/00006123-199608000-00061.
- 520 Kramer MA, Cash SS. Epilepsy as a disorder of cortical network organization. *Neuroscientist*.
521 2012;18(4):360-72. doi: 10.1177/1073858411422754.
- 522 Kwan P, Sander JW. The natural history of epilepsy: an epidemiological view. *Neurol Neurosurg*
523 *Psychiatry*. 2004;75(10):1376-1381.
- 524 Kwan P, Arzimanoglou A, Berg AT, Brodie MJ, Allen Hauser W, Mathern G, Moshé SL, Perucca
525 E, Wiebe S, French J. Definition of drug resistant epilepsy: consensus proposal by the ad hoc Task
526 Force of the ILAE Commission on Therapeutic Strategies. *Epilepsia*. 2010;51(6):1069-1077. doi:
527 10.1111/j.1528-1167.2009.02397.
- 528 Lee HW, Hong SB, Tae WS. Opposite ictal perfusion patterns of subtracted SPECT.
529 Hyperperfusion and hypoperfusion. *Brain*. 2000;123 (Pt 10):2150-9. doi:
530 10.1093/brain/123.10.2150.
- 531 Loy R, Cosell DA, Lindsey JD, Moore RY. Noradrenergic innervation of the adult rat hippocampal
532 formation. *J Comp Neurol*. 1980;189:699-710.
- 533 Löscher W., Schmidt D. Modern antiepileptic drug development has failed to deliver: Ways out of
534 the current dilemma. *Epilepsia*. 2011;52:657-678. doi: 10.1111/j.1528.
- 535 Magdaleno-Madrigal VM, Valdés-Cruz A, Martínez-Vargas D, Martínez A, Almazán S, Fernández-
536 Mas R, Fernández-Guardiola A. Effect of electrical stimulation of the nucleus of the solitary tract
537 on the development of electrical amygdaloid kindling in the cat. *Epilepsia*. 2002;43(9):964-969.
- 538 Magdaleno-Madrigal VM, Martínez-Vargas D, Valdés-Cruz A, Almazán-Alvarado S, Fernández-
539 Mas R. Preemptive effect of nucleus of the solitary tract stimulation on amygdaloid kindling in
540 freely moving cats. *Epilepsia*. 2010;51(3):438-444. doi: 10.1111/j.1528-1167.2009.02337.
- 541 Majak K, Pikkarainen M, Kempainen S, Jolkkonen E, Pitkänen A. Projections from the
542 amygdaloid complex to the claustrum and the endopiriform nucleus: a Phaseolus vulgaris
543 leucoagglutinin study in the rat. *J Comp Neurol*. 2002;451(3):236-249.
- 544 Mercante B, Pilurzi G, Ginatempo F, Manca A, Follesa P, Tolu E, Deriu F. Trigeminal nerve
545 stimulation modulates brainstem more than cortical excitability in healthy humans. *Exp Brain Res*.
546 2015;233(11):3301-3311. doi: 10.1007/s00221-015-4398-2.
- 547 Mercante B, Enrico P, Floris G, Quartu M, Boi M, Serra MP, Follesa P, Deriu F. Trigeminal nerve
548 stimulation induces Fos immunoreactivity in selected brain regions, increases hippocampal cell
549 proliferation and reduces seizure severity in rats. *Neuroscience*. 2017;361:69-80. doi:
550 10.1016/j.neuroscience.
- 551 Mielke R, Pietrzyk U, Jacobs A, Fink GR, Ichimiya A, Kessler J, Herholz K, Heiss WD. HMPAO
552 SPET and FDG PET in Alzheimer's disease and vascular dementia: comparison of perfusion and
553 metabolic pattern. *Eur J Nucl Med*. 1994;21(10):1052-1060.

- 554 Moseley BD, DeGiorgio CM. Refractory status epilepticus treated with trigeminal nerve
555 stimulation. *Epilepsy Res.* 2014;108(3):600-603. doi: 10.1016/j.epilepsyres.2013.12.010.
- 556 Nuvoli S, Spanu A, Madeddu G. Brain SPECT with Perfusion Radiopharmaceuticals and
557 Dopaminergic System Radiocompounds in Dementia Disorders. *Curr Alzheimer Res.*
558 2017;14(2):143-153.
- 559 Olivie L, Giraldez BG, Sierra-Marcos A, Díaz-Gómez E, Serratosa JM. External trigeminal nerve
560 stimulation: A long term follow up study. *Seizure.* 2019; 69:218-220.
- 561 O'Shea MS, Rosen JB, Post RM, Weiss SR. Specific amygdaloid nuclei are involved in suppression
562 or propagation of epileptiform activity during transition stage between oral automatisms and
563 generalized clonic seizures. *Brain Res.* 2000; 873:1-17.
- 564 Palumbo B, Buresta T, Nuvoli S, Spanu A, Schillaci O, Fravolini ML, Palumbo I. SPECT and PET
565 serve as molecular imaging techniques and in vivo biomarkers for brain metastases. *Int J Mol Sci.*
566 2014;15(6):9878-9893.
- 567 Pilurzi G, Mercante B, Ginatempo F, Follesa P, Tolu E, Deriu F. Transcutaneous trigeminal nerve
568 stimulation induces a long-term depression-like plasticity of the human blink reflex. *Exp Brain Res.*
569 2016;234(2):453-461. doi: 10.1007/s00221-015-4477-4.
- 570 Pop J, Murray D, Markovic D, DeGiorgio CM. Acute and long-term safety of external trigeminal
571 nerve stimulation for drug-resistant epilepsy. *Epilepsy Behav.* 2011;22(3):574-576. doi:
572 10.1016/j.yebeh.2011.06.024.
- 573 Ring HA, White S, Costa DC, Pottinger R, Dick JP, Koeze T, Sutcliffe J. A SPECT study of the
574 effect of vagal nerve stimulation on thalamic activity in patients with epilepsy. *Seizure.*
575 2000;9(6):380-4. doi: 10.1053/seiz.2000.0438.
- 576 Rutecki P. Anatomical, physiological, and theoretical basis for the antiepileptic effect of vagus
577 nerve stimulation. *Epilepsia.* 1990;31 Suppl 2:S1-6.
- 578 Sasa M, Takaori S. Influence of the locus coeruleus on transmission in the spinal trigeminal nucleus
579 neurons. *Brain Res.* 1973;55(1):203-8.
- 580 Sato T, Yamada N, Morimoto K, Uemura S, Kuroda S. A behavioral and immunohistochemical
581 study on the development of perirhinal cortical kindling: a comparison with other types of limbic
582 kindling. *Brain Res.* 1998; 811:122-132.
- 583 Schulze-Bonhage A. Long-term outcome in neurostimulation of epilepsy. *Epilepsy Behav.* 2019;
584 91:25-29. doi: 10.1016/j.yebeh.2018.06.011.
- 585 Shiozawa P, Silva ME, Carvalho TC, Cordeiro Q, Brunoni AR, Fregni F. Transcutaneous vagus and
586 trigeminal nerve stimulation for neuropsychiatric disorders: a systematic review. *Arq*
587 *Neuropsiquiatr.* 2014;72(7):542-547.
- 588 Siegel A, Lewis P, Siegel AM. The value of interictal brain SPECT in epilepsy patients without
589 mesial-temporal sclerosis. *Clin Nucl Med.* 2002;27(10):716-20. doi: 10.1097/00003072-
590 200210000-00007.

- 591 Silverman DH. Brain 18F-FDG PET in the diagnosis of neurodegenerative dementias: comparison
592 with perfusion SPECT and with clinical evaluations lacking nuclear imaging. *J Nucl Med.* 2004;
593 45(4):594-607.
- 594 Slaght SJ, Nashef L. An audit of external trigeminal nerve stimulation (eTNS) in epilepsy. *Seizure.*
595 2017;52:60-62. doi: 10.1016/j.seizure.2017.09.004.
- 596 Soss J, Heck C, Murray D, Markovic D, Oviedo S, Corrale-Leyva G, Gordon S, Kealey C,
597 DeGiorgio C. A prospective long-term study of external trigeminal nerve stimulation for drug-
598 resistant epilepsy. *Epilepsy Behav.* 2015; 42:44-47. doi:10.1016/j.yebeh.2014.10.029.
- 599 **Spanaki MV, Siegel H, Kopylev L, Fazilat S, Dean A, Liow K, Ben-Menachem E, Gaillard WD,**
600 **Theodore WH. The effect of vigabatrin (gamma-vinyl GABA) on cerebral blood flow and**
601 **metabolism. *Neurology.* 1999 Oct 22;53(7):1518-22. doi:10.1212/wnl.53.7.1518.**
- 602 Spencer SS. Neural networks in human epilepsy: evidence of and implications for treatment.
603 *Epilepsia.* 2002;43(3):219-27. doi:10.1046/j.1528-1157.2002.26901.x.
- 604 Stevens JR, Phillips I, Freed WJ, Poltorak M. Cerebral transplants for seizures: preliminary results.
605 *Epilepsia.* 1988;29(6):731-737.
- 606 Sucholeiki R, Alsaadi TM, Morris GL 3rd, Ulmer JL, Biswal B, Mueller WM. fMRI in patients
607 implanted with a vagal nerve stimulator. *Seizure.* 2002;11(3):157-62. doi: 10.1053/seiz.2001.0601.
- 608 Thadani VM, Siegel A, Lewis P, Siegel AM, Jobst BC, Gilbert KL, Darcey TM, Roberts DW,
609 Williamson PD. Validation of ictal single photon emission computed tomography with depth
610 encephalography and epilepsy surgery. *Neurosurg Rev.* 2004;27(1):27-33. doi: 10.1007/s10143-
611 003-0289-2.
- 612 Tepmongkol S, Tangtrairattanakul K, Lerdlum S, Desudchit T. Comparison of brain perfusion
613 SPECT parameters accuracy for seizure localization in extratemporal lobe epilepsy with discordant
614 pre-surgical data. *Ann Nucl Med.* 2015;29(1):21-8. doi: 10.1007/s12149-014-0905-y.
- 615 Vismar MS, Forcelli PA, Skopin MD, Gale K, Koubeissi MZ. The piriform, perirhinal, and
616 entorhinal cortex in seizure generation. *Front Neural Circuits.* 2015;9:27. doi:
617 10.3389/fncir.2015.00027.
- 618 Vonck K, Boon P, Van Laere K, D'Havé M, Vandekerckhove T, O'Connor S, Brans B, Dierckx R,
619 De Reuck J. Acute single photon emission computed tomographic study of vagus nerve stimulation
620 in refractory epilepsy. *Epilepsia.* 2000;41(5):601-9. doi: 10.1111/j.1528-1157.2000.tb00215.x.
- 621 Wang QQ, Zhu LJ, Wang XH, Zuo J, He HY, Tian MM, Wang L, Liang GL, Wang Y. Chronic
622 Trigeminal Nerve Stimulation Protects Against Seizures, Cognitive Impairments, Hippocampal
623 Apoptosis, and Inflammatory Responses in Epileptic Rats. *J Mol Neurosci.* 2016;59(1):78-89. doi:
624 10.1007/s12031-016-0736-5.
- 625 Weinand ME, Carter LP, el-Saadany WF, Sioutos PJ, Labiner DM, Oommen KJ. Cerebral blood
626 flow and temporal lobe epileptogenicity. *J Neurosurg.* 1997;86(2):226-232.

627 Zerarka S, Pellerin L, Slosman D, Magistretti PJ. Astrocytes as a predominant cellular site of
628 (99m)Tc-HMPAO retention. *J Cereb Blood Flow Metab.* 2001;21(4):456-68. doi:
629 10.1097/00004647-200104000-00014.
630

631 **Figure legends**

632

633 **Figure 1. Brain perfusion SPECT of a representative patient (P04) with drug-resistant**
634 **epilepsy.**

635 A) The qualitative transaxial SPECT slices at baseline, compared with B) those obtained after 20
636 minutes of transcutaneous trigeminal nerve stimulation (TNS) evidenced a global increase in the
637 cortical tracer uptake. C) The quantitative analysis performed with the NeuroGam® software
638 showed, at baseline, areas of decreased cerebral blood flow in the left limbic lobe (z-score = -1.8),
639 in the left temporal lobe (z-score =-2.1) and in parietal lobe bilaterally (z-score = left lobe: -3.4:
640 right lobe: -2.7). D) Following TNS, an overall increase in cerebral perfusion was associated with a
641 reduction of the hypoperfusion in the left limbic lobe (z-score =-1.4), left temporal lobe (z-score = -
642 0.7) and in the parietal lobe bilaterally (z-score = left lobe: -1.2: right lobe: -0.6).

643

644 **Fig. 2. EEG trace recorded from the representative subject (P04) whose brain perfusion**
645 **SPECT is shown in Figure 1.**

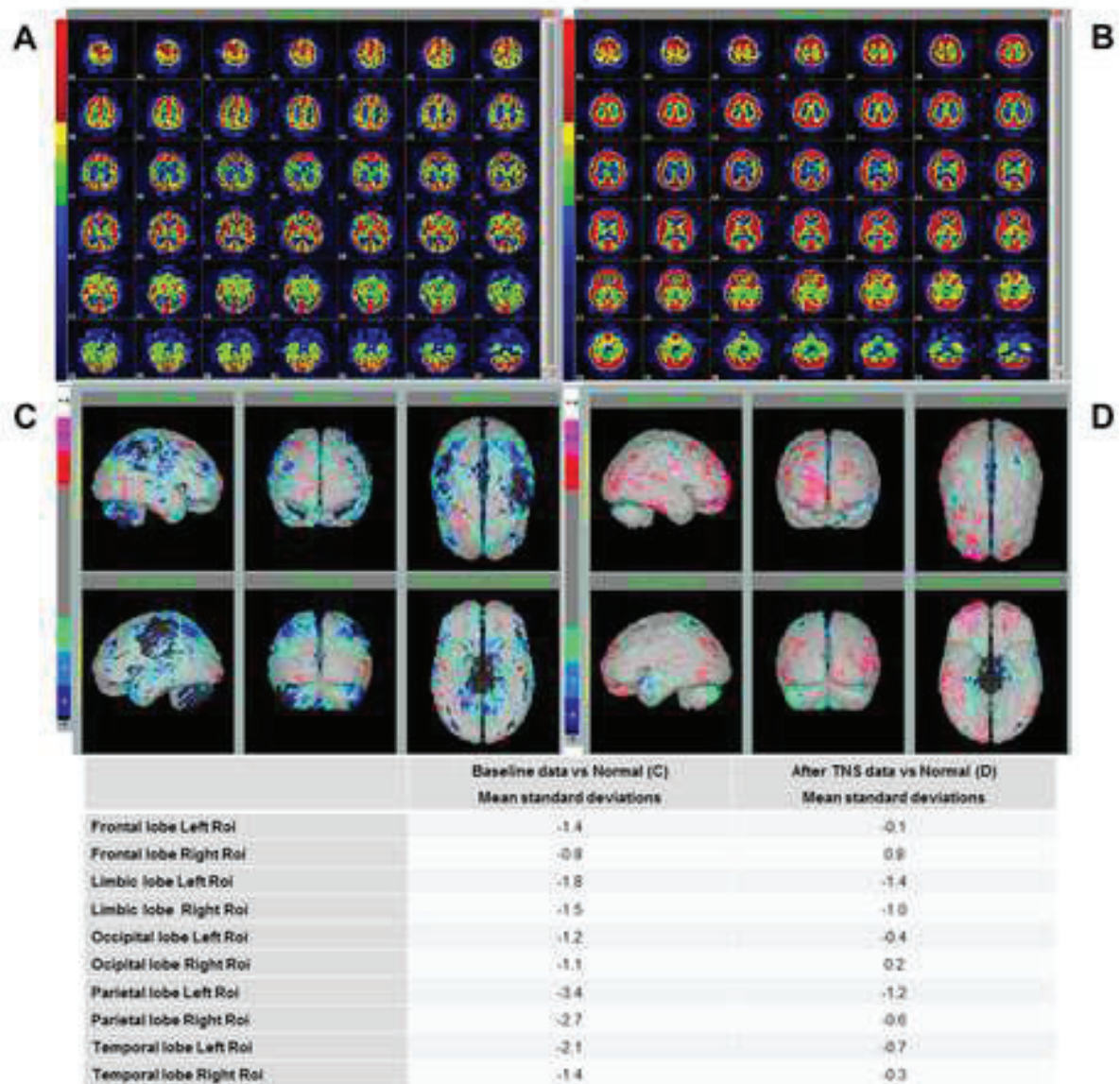
646 Awake scalp EEG showing a posteriorly dominant symmetrical and reactive alpha rhythm with
647 brief trains of interictal and repetitive spike and waves complexes in the right anterior temporal
648 region, spikes with phase reversal at F8 and synchronous sharp waves and spike waves in the right
649 posterior temporal region.

650

651 **Fig. 3. Calculation of the Odds Ratio over five lobes of both cerebral hemispheres**

652 A) Comparing the number of events showing increase in tracer uptake/events showing no change
653 between the trigeminal nerve stimulation (TNS) and baseline conditions in 5 lobes on both

654 hemispheres, the calculation of the odds ratio evidenced an overall significant effect favoring a
655 TNS-induced increase in rCBF (70% of cases; $p=0.0005$). B) The analysis of the odds ratio by lobe
656 and by side evidenced a significant bilateral increase of tracer uptake in the limbic (left and right:
657 $p=0.02$) and temporal (left and right: $p=0.02$) lobes following TNS.



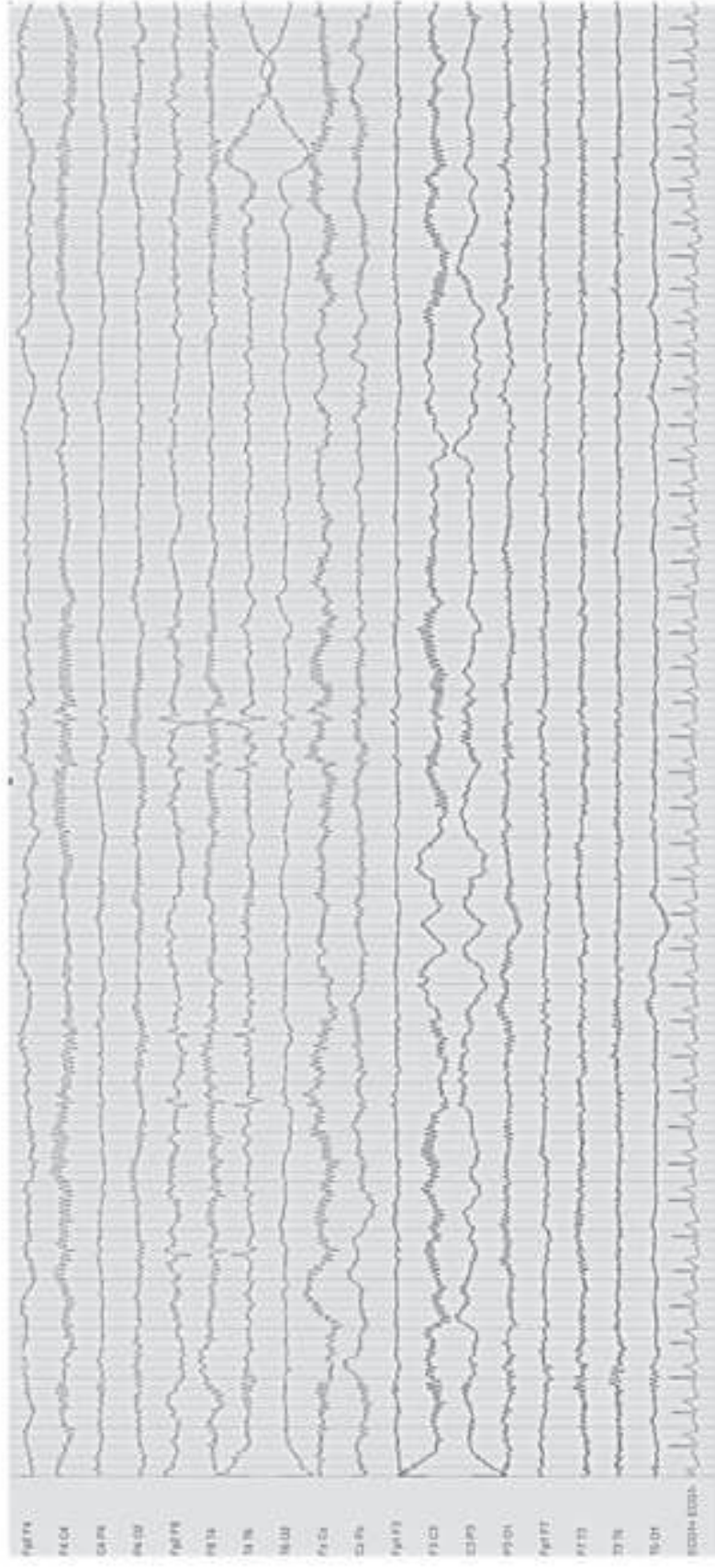
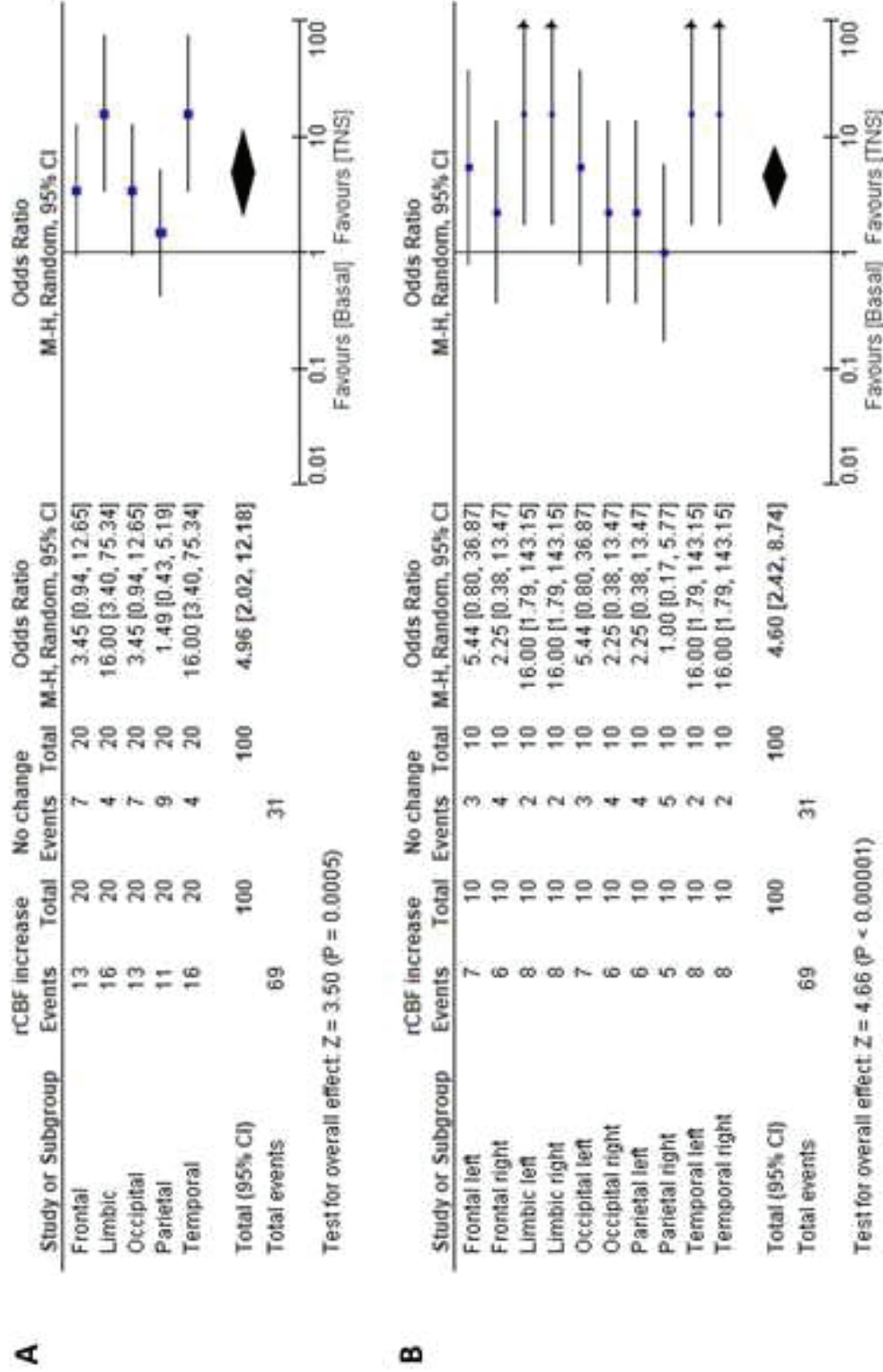


Figure 3



A

B

Table 1. Clinical and demographic features of patients with drug resistant epilepsy

PATIENT ID	SEX	AGE (years)	SIMPTOMATOLOGY	EPILEPTIC FOCUS	MRI	AEDs	NUMBER OF SEIZURE S (/month)	DURATION OF EPILEPSY (years)
P01	M	46	Alteration in consciousness and loss of motor control, contralateral eyes deviation, rare generalized tonic-clonic seizures	Left Temporal-Occipital	Left Occipital Gliosis	LTG+LEV+PER	3	18
P02	M	44	Loss of contact, behavioral automatism, generalized tonic-clonic seizures	Left Fronto-Temporal	Cryptogenic	LEV+LAC	2	42
P03	F	38	confusion and loss of contact (psychomotor state), rare generalized tonic-clonic seizures	Bilateral Temporal	Bilateral Peritrigonal Nodular Heterotopia	LTG+ LEV	3	18
P04	M	29	Psychomotor state, generalized tonic-clonic seizures	Right Temporal	Cryptogenic	LEV+LAC	4	2
P05	M	51	Left clonic seizures and secondarily generalized tonic-clonic seizures	Right Frontal	Right Frontal Focal Cortical Dysplasia	LEV+CBZ	2	3
P06	F	32	Right facial clonus without alteration of consciousness, rare generalized tonic-clonic seizures	Left Frontal	Cryptogenic	VPT+LEV	2	6
P07	M	54	Auditory aura, psychical hallucinations (dreamlike experience and elation) Alteration of consciousness, rare generalized tonic-clonic seizures	Left Temporal (EEG: Bilateral Frontal)	Cryptogenic	PRM+TPM	4	33
P08	F	38	Auditory and labyrinthine auras and loss of contact , rare generalized tonic-clonic seizures	Left Temporal	Cryptogenic	LTG+LEV+PER	4	15
P09	M	58	Automotor seizures with distal automatism and limb dystonia, loss of contact and postictal drowsiness	Bilateral Temporal	Cryptogenic	VPT+LEV	2	9
P10	M	49	Cephalic aura, unresponsiveness, masticatory movements, automatism involving right hand, loss of postural tone and fall, post-ictal aphasia	Right Temporal	Cryptogenic	CBZ+PRM+TPM	3	30

F, female; M, male; EEG, Electroencephalogram; MRI, Magnetic Resonance Imaging; AEDs, Antiepileptic drugs; LTG, lamotrigine; LEV, levetiracetam; LAC, lacosamide; CBZ, carbamazepin; VPT, valproate; PRM, primidone; PER, perampanel; TPM, topiramate; Epileptogenic focus defined by pre-surgery video EEG in P09 and P10; presumed on the basis of anatomo-electro-clinical features in P05, P06 and P08; Expressed on the basis of the symptomatogenic zone and localizatory symptom (in contrast with intercritic EEG) in P07; hypothetical in P01, P02, P03, P04, due to aspecific semiology and intercritic EEG with no localizatory and lateralization value.

Table 2. Data obtained from the qualitative analysis of SPECT imaging of patients with drug-resistant epilepsy at baseline and after 20 minutes of transcutaneous bilateral stimulation of the infraorbital branch of the trigeminal nerve (baseline and TNS sessions separated by two weeks).

Patient ID	Extension of hypoperfusion	Localization	Lateralization	baseline Hyp_score	TNS Hyp_score	Cortical tracer uptake after TNS
P01	FA	TM and FT	Left	3	1	-
P02	WA	T	Bilateral	3	1	Global increase
P03	WA	TM, P T	Bilateral Left	2	2	Global increase
P04	FA	TP	Bilateral	2	1	Global increase
P05	WA	FP, TP, TM	Bilateral	3	3	-
P06	FA	TP TM	Bilateral Left	2	1	Global increase
P07	WA	T, TM F	Bilateral Left	3	3	-
P08	WA	TP, TO	Bilateral	3	1	Global increase
P09	WA	TP, TM, O	Bilateral	3	2	Global increase
P10	WA	TP, TM	Bilateral	4	2	Global increase
Group median data P1 -P10				3	1.5*	

TNS, trigeminal nerve stimulation; Hyp, hypoperfusion; **FA**, focal area; **WA**, wide area; **T**, temporal; **M**, mesial; **TM**, Temporo-mesial; **F**, frontal; **P**, parietal; **O**, occipital. *Significant for $p < 0.05$ (calculated by Wilcoxon Signed Rank Test).