Connectivity in ictal single photon emission computed tomography perfusion: a cortico-cortical evoked potential study

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Subtraction ictal and interictal single photon emission computed tomography can demonstrate complex ictal perfusion patterns. Regions with ictal hyperperfusion are suggested to reflect seizure onset and propagation pathways. The significance of ictal hypoperfusion is not well understood. The aim of this study was to verify whether ictal perfusion changes, both hyper- and hypoperfusion, correspond to electrically connected brain networks. A total of 36 subtraction ictal and interictal perfusion studies were analysed in 31 consecutive medically refractory focal epilepsy patients, evaluated by stereo-electroencephalography that demonstrated a single focal onset. Cortico-cortical evoked potential studies were performed after repetitive electrical stimulation of the ictal onset zone. Evoked responses at electrode contacts outside the stimulation site were used as a measure of connectivity. The evoked responses at these electrodes were compared to ictal perfusion values noted at these locations. In 67% of studies, evoked responses were significantly larger in hyperperfused compared to baseline-perfused areas. The majority of hyperperfused contacts also had significantly increased evoked responses relative to pre-stimulus electroencephalogram. In contrast, baseline-perfused and hypoperfused contacts mainly demonstrated non-significant evoked responses. Finally, positive significant correlations (P < 0.05) were found between perfusion scores and evoked responses in 61% of studies. When the stimulated ictal onset area was hyperperfused, 82% of studies demonstrated positive significant correlations. Following stimulation of hyperperfused areas outside seizure onset, positive significant correlations between perfusion changes and evoked responses could be seen, suggesting bidirectional connectivity. We conclude that strong connectivity was demonstrated between the ictal onset zone and hyperperfused regions, while connectivity was weaker in the direction of baseline-perfused or hypoperfused areas. In trying to understand a patient’s epilepsy, one should consider the contribution of all hyperperfused regions, as these are likely not random, but represent an electrically connected epileptic network.

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Keywords: SPECT; network; epilepsy; cortico-cortical evoked potential; stereo-electroencephalography

Abbreviations: CCEP = cortico-cortical evoked potential; ILAE = International League Against Epilepsy; IOZ = ictal onset zone; RMS = root mean squared; SEEG = stereo-electroencephalography; SISCOM = subtraction ictal SPECT co-registered to MRI; SPECT = single photon emission computed tomography

Received October 25, 2016. Revised March 26, 2017. Accepted April 14, 2017. Advance Access publication June 3, 2017
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Introduction

There is growing evidence that so-called ‘focal’ epilepsies are not confined to focal brain areas, but constitute larger epileptic networks (Laufs, 2012). A brain network is defined as ‘a functionally and anatomically connected, bilaterally represented, set of cortical and subcortical brain structures and regions in which activity in any one part affects activity in all the others’ (Spencer, 2002). A significant percentage of patients with focal epilepsy, refractory to antiepileptic drugs, may be amenable to epilepsy surgery. This suggests that, in this subset of patients, some parts of the epileptic network may be more crucial than others in maintaining the epileptic network (Nair et al., 2004). In order to establish an adequate surgical strategy, insights into the key nodes of the patient-specific epileptic networks are mandatory (Pittau et al., 2014).

Subtraction ictal single photon emission computed tomography (SPECT) co-registered to MRI (SISCOM) shows a snapshot of cerebral perfusion during a seizure compared to brain perfusion in an interictal period. It has been suggested that hyperperfused brain areas include seizure onset and propagation pathways (Van Paesschen, 2004). Whether these widespread brain regions of hyperperfusion reflect an effectively connected ‘network’ is still largely unexplored (Halász, 2010). Also, the relationship between regions of ictal hypoperfusion and hyperperfusion is not well understood.

The study of cortico-cortical evoked potentials (CCEPs) is a relatively recent way to investigate in vivo electrical brain connectivity. In this technique, a cortical brain area is repetitively electrically stimulated using subdural grid or stereotactically placed intracranial EEG (SEEG) electrodes. The presence of evoked responses at a distance from the stimulation site indicates effective connectivity between these regions (Matsumoto et al., 2004; Enatsu et al., 2012; Kunieda et al., 2015). The aim of this study was to assess connectivity, using CCEPs, between the region of seizure onset and areas with increased, decreased and baseline SISCOM perfusion. Our hypothesis was that there is a preferentially strong connectivity between the ictal onset zone, the part of the cortex that generates epileptic seizures (Rosenow and Lüders, 2001), and hyperperfused regions.

Materials and methods

Inclusion criteria

This retrospective study was approved by the institutional review board on human experimentation of the Cleveland Clinic. Included were consecutive patients with refractory focal epilepsy who underwent (i) a SISCOM study as part of a full presurgical evaluation [including neuro(psych)ological examination, video-EEG-monitoring, structural MRI, magnetoencephalography and when available interictal 18F-fluorodeoxyglucose PET]; (ii) a SEEG evaluation between January 2014 and August 2015; (iii) with absence of brain surgery between SISCOM and SEEG. Patients had (iv) a well-defined unifocal ictal onset zone (IOZ) based on this SEEG evaluation (as documented in the clinical SEEG report); and (v) a CCEP study during this SEEG evaluation (done for purposes other than this research) with stimulation of at least one IOZ contact.

SISCOM acquisition and processing

Technetium-99 m-ethyl cysteinate dimer was used as tracer (20–40 mCi). Epilepsy-trained nurses performed an ictal SPECT injection during a typical seizure, recorded in the video-EEG monitoring unit. Seizure onset time was retrospectively defined on clinical or EEG grounds, whichever was first. Injection of the tracer for the interictal SPECT was performed during scalp video-EEG monitoring. SPECT imaging commenced within 2 h of tracer injection. Images were acquired using a Siemens Symbia dual-head camera (SPECT: 15 s per stop × 60 stops, 128 × 128 matrix, iterative reconstruction with attenuation correction, six iterations, eight subsets).

Analysis was carried out using statistical parametric mapping (SPM) software (Wellcome Department of Imaging Neuroscience, University College London, UK; available from http://www.fil.ion.ucl.ac.uk/spm/) in MATLAB (MathWorks, Natick, MA, USA).

The ictal and interictal SPECT scans from each subject were co-registered using maximal mutual information and the interictal image was subtracted from the ictal after normalization for global brain counts. The subtracted image was smoothed using a (3D) Gaussian smoothing kernel (full-width at half-maximum = 12 mm) and transformed into a z-score using the mean and the standard deviation (SD) of the differences in all brain voxels. The interictal image was used for co-registration with the preoperative structural MRI and the same transformation was applied to the z-map. Structural data included a high-resolution 3D T1-weighted magnetization prepared rapid gradient echo scan. If more than one ictal SPECT was available in a patient, each SISCOM study contributed to the subsequent analyses. As seizures might show different spread patterns, we did not want to discard the results of one of the two studies.

Localization of SEEG electrode contacts and co-registration with SISCOM

Implantation was performed using our standard SEEG protocol based on the available presurgical investigations; the implantation took into account the SISCOM localization but did not specifically target the hyperperfused areas (Gonzalez-Martinez et al., 2013). Post-implantation electrode localization was performed after fusing the postoperative thin-sliced CT scan with the preoperative structural MRI using automatic full-volume maximal-mutual-information registration algorithms in Curry software (Compumedics, NeuroScan Laboratories, Charlotte, NC, USA). Electrode contacts were manually marked. The 3D coordinates and SISCOM z-score at the voxel location were extracted for each electrode contact.
in MATLAB. Only electrode contacts located within the SISCOM mask were used for analysis.

CCEP acquisition and processing

Cortico-cortical evoked responses were obtained using our previously described method (Matsumoto et al., 2004; Lega et al., 2015). Data were collected towards the end of the invasive evaluation after antiepileptic drugs were restarted. The localization of the ictal onset zone, determined during the SEEG registration, guided the stimulation procedure. Stimulation was performed in and outside the IOZ with a Grass S88 stimulator using an automated interface in Nihon-Kohden software (Tokyo, Japan). Stimulation was conducted at 1 Hz with 300 μs square wave pulses of alternating polarity using adjacent electrodes. Trials consisted of 60 pulses (two consecutive trials of 30 pulses) at 8 mA. If after-discharges appeared at 8 mA, a 60-pulse trial at a lower stimulation threshold (1, 2, 4 or 6 mA) was done. EEG data were extracted in MATLAB, sampled at 1000 Hz, notch-filtered at 60 Hz and bandpass filtered from 1 to 300 Hz. Synchronization pulses were captured in the Nihon-Kohden software to detect the precise time at which stimulation events occurred.

Evoked responses were quantified in each contact in two different ways: (i) the root mean square (RMS) value of the averaged response in the (20–400 ms) time window following 60 stimulations (‘CCEP RMS-value’); and (ii) the t-value of a paired two-sample t-test between the 60 RMS-values of individual non-averaged (20–400 ms) post-stimulation time windows and the 60 RMS-values of individual non-averaged (−200 to −20 ms) prestimulation time windows (‘CCEP t-value’). The CCEP t-value expresses how evoked responses compare to baseline prestimulus EEG.

Stimulation of the ictal onset zone

CCEP responses following the stimulation of IOZ contacts were compared to SISCOM z-values for each contact. If multiple IOZ contact pairs were stimulated, the stimulation at the pair with the highest average z-score was used for analysis. CCEP RMS and CCEP t-values obtained in stimulation site contacts were not included in the analyses.

Comparison between SISCOM z-values and CCEP root mean square values

Electrode contacts were classified based on their respective SISCOM z-values as: (i) hyperperfused (z ≥ 1.5); (ii) baseline-perfused (−1.5 < z < 1.5); or (iii) hypoperfused (z ≤ −1.5). The z-threshold of 1.5 was chosen as it was shown to be a highly sensitive and specific threshold for localizing the epileptogenic zone (Newey et al., 2013). The CCEP RMS values were compared between these three perfusion groups using a two-sided Kolmogorov-Smirnov test (minimum 10 electrode contacts in the groups were required for this comparison). In subsequent analyses, there were no restrictions on the number of required contacts and complete datasets could be used.

Comparison between SISCOM z-values and CCEP t-values

The number of contacts across all studies with positive significant (t ≥ 2), not significant (−2 < t < 2) and negative significant (t ≤ −2) CCEP t-values (corresponding to P < 0.05) was determined in hyperperfused, baseline-perfused and hypoperfused areas. The number of contacts was compared within each perfusion group using a two-sided Kolmogorov-Smirnov test.

Correlation between unthresholded SISCOM z-values and CCEP t-values

For each individual study, Pearson’s correlation coefficient between SISCOM z-values and CCEP t-values across all contacts was calculated after stimulating the IOZ (SISCOM/CCEP correlation). To assess statistical significance, a non-parametric permutation test was applied.

Subgroup analysis

Subgroup analyses were performed to assess possible associations between specific features and the proportion of studies with positive significant, negative significant and non-significant SISCOM/CCEP correlations (chi-square test). The subgroups were based on the number of contacts classified as IOZ (≤5 or > 5), post-surgical seizure outcome (seizure freedom or recurrence), IOZ location (temporal lobe or extratemporal lobe epilepsy), ictal SPECT injection time (< or > median injection time), ictal SPECT seizure type (non-generalized or secondary generalized), structural MRI findings (lesional or non-lesional) and average SISCOM z-score at the stimulated IOZ contact pairs (z ≥ 1.5 or z < 1.5).

Distance effect

To investigate the effect of electrode contact distance from the stimulated IOZ pair on SISCOM/CCEP correlations, the correlation analysis was repeated within three electrode contact groups of increasing Euclidean distance from the stimulated IOZ contact pair (matched for the number of contacts). Possible associations between the proportion of positive significant, negative significant and non-significant SISCOM/CCEP correlations (P < 0.05) and distance were evaluated (chi-square test).

Stimulations outside the ictal onset zone

For each individual study, Pearson’s correlation coefficients were calculated between unthresholded SISCOM z-values and unthresholded CCEP t-values in all contacts following each available stimulation, in and outside the IOZ (non-parametric permutation test).

Statistical analysis

The significance level was set at P < 0.05. False discovery rate was used to correct for multiple comparisons.
Results

Study population

Thirty-one consecutive patients [age: 24 years (range: 11–69), age at epilepsy onset: 10 years (range: 1–50), 14 female] met the inclusion criteria (Table 1).

Five patients underwent two presurgical ictal SPECT scans and both SISCOM studies were analysed separately. All ictal SPECT injections were performed during the ictal phase [median time of initiation of ictal SPECT tracer injection: 16.5 s (range: 3–74)]. The mean SISCOM z-score over the IOZ labelled contacts ranged from −1 up to 3.2, mean 1.4 (Supplementary material). Twenty-four SISCOMs were based on focal seizures without secondary generalization (one aura), whereas 12 had secondary generalization. Comparing the relative volume of SISCOM hyperperfusion (z ≥ 1.5) following seizures with or without secondary generalization did not reveal statistically significant differences (P = 0.60).

Twelve patients underwent a unilateral (five left-sided, seven right-sided) and 19 patients a bilateral SEEG electrode implantation. Median 13 electrodes (range: 6–18) were implanted per patient. A median 150 (range: 82–220) electrode contacts were used for SEEG analysis. The number of contacts classified as ictal onset zone ranged from 2 to 15 with a median of 5, distributed over median one (range: 1–5 adjacent) electrode. A median 3 (range: 1–12) of these IOZ contacts (60%) was stimulated for CCEPS. Outside the IOZ, a median 16 (range: 6–38) of 143 (range: 78–216) electrode contacts (11%) had been stimulated for CCEPS.

Eighteen patients (58%) had temporal lobe epilepsy, while seizure onset was extratemporal in 13 patients (42%). Sixteen of 31 patients (52%) had a non-lesional structural MRI and 15 of 31 patients (48%) had lesions. Following SEEG evaluation, 23 patients (74%) underwent resective epilepsy surgery, four patients (13%) laser-induced thermotherapy and one patient (3%) underwent responsive neural stimulation. Three patients (10%) did not undergo surgical treatment (one awaits surgery, one declined surgery, one had high risk for postsurgical functional decline). The median postsurgical follow-up period was 13 months (range: 2–21). Seventeen of 28 patients (61%) were completely seizure-free [International League Against Epilepsy (ILAE) class 1], one patient (4%) had only auras (ILAE class 2), three patients (11%) had one to three seizure days per year (ILAE class 3), five patients (18%) had four seizure days per year up to a 50% reduction in baseline number of seizure days (ILAE class 4) and two patients (7%) had <50% reduction in baseline number of seizure days up to 100% increase in baseline number of seizure days (ILAE class 5) (Wieser et al., 2001).

Comparison between SISCOM z-values and CCEP root mean square values

Twenty-seven studies had sufficient electrode sampling (≥10 contacts) in both hyperperfusion and baseline-perfusion to allow a reliable comparison of RMS values (Fig. 1). The majority of studies (67%) had significantly higher RMS values in hyperperfused compared to baseline-perfused contacts, 26% had similar RMS values and 7% had significantly lower RMS values (Fig. 2). The only study that had adequate SEEG sampling in both hyper- and hypoperfusion, had significantly higher RMS values in hyperperfused contacts. Finally, the two studies, with at least 10 electrode contacts in baseline- and hypoperfusion, had similar RMS values in these contacts.

Comparison between thresholded SISCOM z-values and CCEP t-values

Across all studies, the mean CCEP t-value in hyperperfused contacts was 10.5, compared to 4.7 in baseline-perfused and 4.1 in hypoperfused contacts. Within areas of ictal hyperperfusion, the number of contacts with positive significant CCEP t-values (mean = 12.6) was significantly higher compared to contacts with non-significant (mean = 6.4) and negative significant (mean = 0) CCEP responses (P < 0.05) (Fig. 3C).

In baseline-perfused and hypoperfused brain areas (Fig. 3A and B), the majority of contacts had non-significant CCEP responses. The few contacts with negative significant CCEP t-values were only located in baseline-perfused regions.

Correlation between unthresholded SISCOM z-values and CCEP t-values

The median correlation coefficient was 0.20 (range: −0.12–0.69). The correlation was positive significant (P < 0.05) in 22/36 studies (61%), negative significant in 0/36 studies (0%) and not significant in 14/36 studies (39%) (Fig. 4A and Table 1).

Subgroup analysis

Of 36 SISCOM studies, 22 (61%) showed hyperperfusion (z ≥ 1.5) at a stimulated IOZ contact pair. A significantly larger proportion of studies (82% versus 29%) had positive significant SISCOM/CCEP correlations when the stimulated IOZ contact pair was hyperperfused (z ≥ 1.5) compared to non-hyperperfused (z < 1.5) (Fig. 4H).

Although differences did not achieve statistical significance, larger proportions of positive significant SISCOM/CCEP correlations were seen when (i) ≤5 electrode contacts were classified as IOZ; (ii) patients were seizure-free
Table 1  Study population

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender/age (y)/age at seizure onset (y)</th>
<th>Structural MRI</th>
<th>Ictal onset zone (number of IOZ labelled contacts)</th>
<th>Ictal SPECT injection time (s)/seizure continuation after injection (s)</th>
<th>SPECT seizure type</th>
<th>IOZ contact pair hyperperfused (SISCOM z ≥ 1.5)</th>
<th>Treatment</th>
<th>ILAE outcome (follow-up time, months)</th>
<th>SISCOM/CCEP correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F/19/7</td>
<td>Non-lesional</td>
<td>L precentral sulcus/gyrus (3)</td>
<td>7/71</td>
<td>Gen</td>
<td>Yes</td>
<td>Resection</td>
<td>I (12)</td>
<td>p.s.</td>
</tr>
<tr>
<td>2</td>
<td>M/43/12</td>
<td>Non-lesional</td>
<td>R collateral sulcus (3)</td>
<td>7/50</td>
<td>Non-Gen</td>
<td>No</td>
<td>Resection</td>
<td>V (20)</td>
<td>n.s.</td>
</tr>
<tr>
<td>3</td>
<td>F/20/14</td>
<td>L temporal atrophy</td>
<td>L middle temporal gyrus (3)</td>
<td>6/10</td>
<td>Gen</td>
<td>Yes</td>
<td>Resection</td>
<td>I (17)</td>
<td>n.s.</td>
</tr>
<tr>
<td>4</td>
<td>M/21/12</td>
<td>R occipital abnormal sulcation</td>
<td>R lateral occipital gyrus/transverse sulcus/collateral sulcus (7)</td>
<td>15/14</td>
<td>Non-Gen</td>
<td>No</td>
<td>Resection</td>
<td>III (19)</td>
<td>n.s.</td>
</tr>
<tr>
<td>5</td>
<td>F/33/14</td>
<td>R occipital postsurgical changes</td>
<td>R hippocampus (4)</td>
<td>14/44</td>
<td>Non-Gen</td>
<td>Yes</td>
<td>Resection</td>
<td>I (21)</td>
<td>p.s.</td>
</tr>
<tr>
<td>6</td>
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<td>L middle frontal gyrus/superior frontal sulcus/superior frontal gyrus (5)</td>
<td>33/28</td>
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<td>Yes</td>
<td>Resection</td>
<td>I (16)</td>
<td>p.s.</td>
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<tr>
<td>7</td>
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<td>R basal temporal cavernoma</td>
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<td>Yes</td>
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<td>p.s.</td>
</tr>
<tr>
<td>8</td>
<td>M/24/8</td>
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<td>R parahippocampal gyrus (5)</td>
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<td>No</td>
<td>Resection</td>
<td>I (16)</td>
<td>n.s.</td>
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<tr>
<td>9</td>
<td>F/24/2</td>
<td>R temporal postsurgical changes</td>
<td>R posterior insula (3)</td>
<td>26/46</td>
<td>Gen</td>
<td>Yes</td>
<td>Resection</td>
<td>II (16)</td>
<td>p.s.</td>
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<tr>
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<td>16/97</td>
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<td>Yes</td>
<td>Resection</td>
<td>I (19)</td>
<td>p.s.</td>
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<tr>
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<td>27/105</td>
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<td>Yes</td>
<td>RNS</td>
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<td>L posterior insula (3)</td>
<td>56/51</td>
<td>Non-Gen</td>
<td>Yes</td>
<td>No surgical therapy</td>
<td>n.a.</td>
<td>p.s.</td>
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<tr>
<td>13</td>
<td>M/31/2</td>
<td>Non-lesional</td>
<td>R amygdala (3)</td>
<td>7/53</td>
<td>Gen</td>
<td>No</td>
<td>Resection</td>
<td>IV (19)</td>
<td>p.s.</td>
</tr>
<tr>
<td>14</td>
<td>F/24/15</td>
<td>Non-lesional</td>
<td>R lateral temporo-occipital sulcus (2)</td>
<td>63/24</td>
<td>Non-Gen</td>
<td>Yes</td>
<td>Resection</td>
<td>I (11)</td>
<td>p.s.</td>
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<td>46/18</td>
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<td>No</td>
<td>Resection</td>
<td>I (16)</td>
<td>n.s.</td>
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<tr>
<td>16</td>
<td>F/69/14</td>
<td>R HS</td>
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<td>9/46</td>
<td>Gen</td>
<td>Yes</td>
<td>Resection</td>
<td>IV (13)</td>
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<td>31/81</td>
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<td>Yes</td>
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<tr>
<td>18</td>
<td>F/45/5</td>
<td>Bilateral hippocampal signal changes</td>
<td>R hippocampus/amygdala (10)</td>
<td>8/53</td>
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<td>No</td>
<td>Resection</td>
<td>I (14)</td>
<td>p.s.</td>
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<tr>
<td>19</td>
<td>F/27/7</td>
<td>R frontal intraventricular tumor</td>
<td>R lateral orbitofrontal (6)</td>
<td>27/52</td>
<td>Non-Gen</td>
<td>No</td>
<td>Resection</td>
<td>IV (10)</td>
<td>n.s.</td>
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<tr>
<td>20</td>
<td>M/20/1</td>
<td>Non-lesional</td>
<td>R insula (3)</td>
<td>5/19</td>
<td>Non-Gen</td>
<td>Yes</td>
<td>Laser-induced thermotherapy</td>
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<td>p.s.</td>
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<tr>
<td>21</td>
<td>F/14/4</td>
<td>Non-lesional</td>
<td>R hippocampal tail/parahippocampal gyrus/inferior temporal gyrus (8)</td>
<td>6/70</td>
<td>Gen</td>
<td>No</td>
<td>Resection</td>
<td>III (16)</td>
<td>p.s.</td>
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<tr>
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<td>F/40/17</td>
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<td>L amygdala/hippocampus/temporal pole (14)</td>
<td>9/22</td>
<td>Non-Gen</td>
<td>Yes</td>
<td>Planned surgery</td>
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</tbody>
</table>

(continued)
Table 1 Continued

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender/age (y)/age at seizure onset (y)</th>
<th>Structural MRI</th>
<th>Ictal onset zone (number of IOZ labelled contacts)</th>
<th>Ictal SPECT injection time (s)/seizure continuation after injection (s)</th>
<th>SPECT seizure type</th>
<th>IOZ contact pair hyperperfused (SISCOM z ≥ 1.5)</th>
<th>Treatment</th>
<th>ILAE outcome (follow-up time, months)</th>
<th>SISSOM/CCEP correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>M/30/18</td>
<td>R temporo-occipital band heterotopia</td>
<td>R parahippocampal gyrus/ fusiform gyrus (4)</td>
<td>20/82</td>
<td>Gen</td>
<td>No</td>
<td>Resection</td>
<td>I (5)</td>
<td>n.s.</td>
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<tr>
<td>24</td>
<td>F/24/21</td>
<td>R MCD, periventricular nodular heterotopia</td>
<td>R planum temporale/Heschl's gyrus/superior temporal gyrus (1)</td>
<td>23/90</td>
<td>Gen</td>
<td>Yes</td>
<td>Resection</td>
<td>I (2)</td>
<td>p.s.</td>
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<tr>
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<td>M/17/9</td>
<td>Non-lesional</td>
<td>L superior frontal gyrus (5)</td>
<td>30/16</td>
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<td>Yes</td>
<td>Laser-induced thermo therapy</td>
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<td>p.s.</td>
</tr>
<tr>
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<td>M/57/41</td>
<td>Non-lesional</td>
<td>L intraparietal sulcus (2)</td>
<td>3/50</td>
<td>Gen</td>
<td>No</td>
<td>No surgical therapy</td>
<td>n.a.</td>
<td>n.s.</td>
</tr>
<tr>
<td>27</td>
<td>M/18/3</td>
<td>Non-lesional</td>
<td>R posterior insula/parietal operculum (9)</td>
<td>10/47</td>
<td>Non-Gen</td>
<td>No</td>
<td>Laser-induced thermo therapy</td>
<td>I (7)</td>
<td>n.s.</td>
</tr>
<tr>
<td>28</td>
<td>M/41/35</td>
<td>Non-lesional</td>
<td>R postcentral gyrus/central sulcus/precentral sulcus (4)</td>
<td>6/18</td>
<td>Non-Gen</td>
<td>Yes</td>
<td>Laser-induced thermo therapy</td>
<td>V (4)</td>
<td>p.s.</td>
</tr>
<tr>
<td>29</td>
<td>F/11/4</td>
<td>R fronto-parietal postsurgical changes</td>
<td>R posterior insula/parietal operculum (9)</td>
<td>17/57</td>
<td>Non-Gen</td>
<td>Yes</td>
<td>Resection</td>
<td>I (3)</td>
<td>p.s.</td>
</tr>
<tr>
<td>30</td>
<td>M/20/4</td>
<td>L temporal postsurgical changes</td>
<td>L frontopolar gyrus/superior frontal gyrus (6)</td>
<td>31/40</td>
<td>Non-Gen</td>
<td>No</td>
<td>Resection</td>
<td>III (8)</td>
<td>n.s.</td>
</tr>
<tr>
<td>31</td>
<td>M/24/10</td>
<td>R temporo-occipital signal changes</td>
<td>R middle temporal gyrus/inferior temporal gyrus/angular gyrus (13)</td>
<td>20/23</td>
<td>Non-Gen</td>
<td>Yes</td>
<td>Resection</td>
<td>I (5)</td>
<td>p.s.</td>
</tr>
</tbody>
</table>

F = female; M = male; L = left; R = right; HS = hippocampal sclerosis; MCD = malformation of cortical development; Gen = secondary generalized seizure; Non-Gen = non-generalized seizure; RNS = responsive neural stimulation; n.a. = not applicable; p.s. = positive significant; n.s. = not significant (significance level of $P < 0.05$).
after surgery; (iii) seizure onset was in the temporal lobe; (iv) ictal SPECT injections occurred within 16 s after seizure onset; (v) ictal SPECT seizures were secondarily generalized; and (vi) structural brain imaging was lesional (Fig. 4B–G).

### Effect of distance

Across all 36 studies, CCEP \( t \)-values were negatively correlated with Euclidean distance from the stimulated IOZ contact pair \( (r = -0.36, P < 0.05) \). A negative correlation was also found between SISCOM z-scores and distance from the stimulated IOZ \( (r = -0.32, P < 0.05) \).

To ensure that the relationship between SISCOM and CCEP \( t \)-values was not driven by distance alone, we calculated correlations between SISCOM z-scores and CCEP \( t \)-values at electrode contacts within similar distance ranges from the stimulated IOZ. We did not find a significant difference \( (P = 0.25) \) in the proportion of studies with positive significant, non-significant and negative significant SISCOM/CCEP correlations for contacts located close by, more distant and far away from the stimulation site.

### Stimulations outside the ictal onset zone

Positive significant SISCOM/CCEP correlations were present following stimulation of hyperperfused electrode contact pairs inside, but also after stimulation of electrode contacts in hyperperfusion outside the IOZ (Fig. 6). Negative significant SISCOM/CCEP correlations could be found for stimulation sites in ictal hypoperfusion. For similar SISCOM z-scores, there was a tendency of higher SISCOM/CCEP correlations when the IOZ was stimulated.

Seven of the 17 patients with successful surgical outcome had statistically significant correlation coefficients in at least one hyperperfused \( (z > 1.5) \) non-IOZ contact pair. A postoperative brain MRI was present in five of these seven patients. Interestingly, in four of these five patients, these contacts pairs were located outside the resected brain region.

### Discussion

Ictal and interictal SPECT imaging is a valuable non-invasive tool for localizing the ictal onset zone in the presurgical evaluation for refractory focal epilepsy. Interpretation of different perfusion patterns is, however, not straightforward (Dupont et al., 2006). It is hypothesized that regions of ictal hyperperfusion may represent a network of seizure onset...
and spread. A prerequisite for propagation within such network is neuronal connectivity. This connectivity between seizure onset and ictal perfusion changes is not well studied. The presence of evoked responses by direct electrical brain stimulation (CCEPs), in patients undergoing SEEG, indicates effective brain connectivity. Our hypothesis was to find strong preferential connectivity between the ictal onset zone and ictally hyperperfused brain regions.

Main findings

Hyperperfusion

In the majority of studies, when the ictal onset zone was stimulated, significantly higher evoked responses were found in hyperperfused compared to baseline-perfused areas. Across all studies, the mean CCEP t-value in hyperperfused contacts was >2-fold that in baseline-perfused or hypoperfused contacts. Hyperperfused brain regions had significantly more contacts with increased than baseline or decreased CCEP responses relative to prestimulus EEG. Finally, positive significant correlations between ictal perfusion changes and evoked responses were present in 61% of cases. All these findings corroborate the idea that SISCOM hyperperfusion patterns represent strongly connected networks of ictal onset and propagation pathways.

Evidence from other independent modalities indeed suggest that large-scale SISCOM perfusion changes are not random. Patterns of ictal hyperperfusion and semiological seizure progression were congruent (Shin et al., 2002). Ictal scalp (Kim et al., 2007) and intracranial (Spanaki et al., 1999; Kaminska et al., 2003; Jacobs et al., 2008) EEG changes were seen in hyperperfused regions distant from ictal discharge onset. A high rate of concordance between SISCOM and interictal PET abnormalities has been described (Bouilleret et al., 2002; Nelissen et al., 2006). Finally, haemodynamic changes in spike-related EEG–functional MRI studies varied with the same sign and within a common network as SISCOM perfusion changes (Tousseyn et al., 2015). In a supplementary analysis, a quantitative comparison of ictal activities during an SEEG seizure and SISCOM perfusion changes was made (Supplementary material). The principal finding was that hyperperfused and hypoperfused areas have different power distribution in higher frequency bands during seizures.

Interestingly, we found positive significant correlations between SISCOM perfusion changes and evoked responses following stimulation of hyperperfused contacts distant from the ictal onset zone. Exclusion of these distant interconnected areas from resection did not preclude successful surgical outcome in four patients. Bidirectional interactions of the early responses measured in the evoked potential

Figure 3 Comparison between thresholded SISCOM z-values and CCEP t-values. Bar graph of the (mean ± standard error) number of contacts with negative significant (t ≤ −2, blue), non-significant (−2 < t < 2, red) and positive significant (t ≥ 2, yellow) CCEP responses, in SISCOM (A) hypoperfused (z ≤ −1.5), (B) baseline-perfused (−1.5 < z < 1.5) and (C) hyperperfused (z ≥ 1.5) areas. *Significance level of P < 0.05. Note that within SISCOM hyperperfusion, the number of contacts with positive significant CCEP responses is significantly higher compared to those with non-significant or negative significant CCEP responses.
were found to be prevalent in the epileptogenic zone in another study (Boido et al., 2014) evaluating single pulse stimulation in SEEG patients and was a reliable indicator of pathological tissue in those locations. However the degree of bidirectionality was not significantly higher in the early propagation of the ictal discharge compared to healthy tissue.

**Hypoperfusion**

The significance of ictal hypoperfusion in SISCOM is not well understood. In our study, few cases had adequate SEEG sampling (≥10 contacts) in regions with ictal hypoperfusion, making large comparisons of CCEP RMS values between hypoperfused and baseline- or hyperperfused regions difficult. In two of our cases, CCEP RMS values were similar in hypoperfused compared to baseline-perfused contacts and in one case, CCEP RMS values were higher in hyperperfused compared to hypoperfused contacts. Following stimulation of the IOZ, the majority of evoked potentials in hypoperfused and baseline-perfused contacts were not significantly different from prestimulus EEG. A lack of significant responses in hypoperfused regions following the stimulation of the ictal onset zone could indicate that seizures have an indirect modulating effect on these regions, instead of directly propagating there. In line with this hypothesis, neocortical slow waves and decreased cerebral blood flow were found in focal seizures induced by rat hippocampal stimulation, whereas neuronal activity and blood flow increased during propagated seizures (Englot et al., 2008). This indirect modulation of brain activity in hypoperfused regions may also explain discrepancies, described by Enatsu et al. (2012), between CCEP responses and ictal propagation on human intracranial EEG recordings. They found areas that were not connected to the ictal onset zone, but did show ictal EEG modifications after seizure onset, explained as secondary propagation from areas outside the IOZ.

Following stimulation of hypoperfused contacts outside the ictal onset zone, negative significant SISCOM/CCEP correlations could be found, suggesting that hypoperfused regions are strongly interconnected, but again separated from hyperperfused areas. This prompts the idea that hypoperfusion is not random either, but may be embedded in preformed physiological networks. Ictal hypoperfusion during focal temporal lobe seizures (Van Paesschen et al., 2003) and secondary generalized seizures (Blumenfeld et al., 2009) has been reported in specific brain constellations, potentially incorporated in physiological networks of normal ‘resting brain’ (Blumenfeld et al., 2009).
Methodological considerations

Ictal onset zone

This study being retrospective, we adopted the IOZ that was determined by the clinical expert at the time of the SEEG evaluation. The limited coverage of brain by SEEG, however, inherently precludes an exact delineation of the margins of the IOZ. Positive significant CCEP/SISCOM correlations tended to be present more frequently (although not statistically significant) when the number of electrode contacts classified as IOZ was limited to five or less or when patients were seizure-free after epilepsy surgery. It could be speculated that less IOZ contacts and good surgical outcome are related to a more exact localization of the seizure onset, thus allowing more efficient stimulation of the epileptic network. Alternatively, an extended IOZ may be more difficult to evaluate using electrical excitation of only a single contact pair (Enatsu et al., 2012).

Subtraction ictal SPECT

The primary aim of SISCOM is to localize the IOZ. However, SISCOM provides a snapshot of perfusion in a time period following seizure onset compared to that in an interictal period. As seizures may spread quickly, the technique’s low temporal resolution can complicate the interpretation of perfusion patterns (Dupont et al., 2006). Sixty-one per cent of our SISCOM studies had hyperperfusion in at least one of the stimulated IOZ contact pairs, using a z-score threshold of 1.5. This SISCOM threshold was recommended for detecting the IOZ (Newey et al., 2013); however, it may not necessarily reflect the optimal threshold for delineating the whole ‘epileptic network’ (Van Paesschen, 2004). When the stimulated IOZ was part of this hyperperfused network, the proportion of positive significant SISCOM/CCEP correlations was significantly higher than when this was not the case (82% versus 29%). Therefore, direct access to the hyperperfused regions seems essential to demonstrate connectivity within the network. Absence of hyperperfusion at the IOZ could have been caused by a failure to identify the actual IOZ by SISCOM or SEEG.

SISCOM studies have been more localizable with early ictal SPECT tracer injections (Lee et al., 2011), SPECT seizures without secondary generalization (Varghese et al., 2009; von Oertzen et al., 2011) and lesional MRIs (von...
Oertzen et al., 2011). In our subgroup analysis, none of these factors substantially influenced the proportion of studies with positive significant SISCOM/CCEP correlations, probably because our analysis was not focused on localizing the IOZ, but on detecting connectivity at a larger scale. There was also no significant difference in SISCOM/CCEP correlations between temporal and extratemporal lobe epilepsies.

CCEPs

‘Effective connectivity refers explicitly to the influence that one neural system exerts over another, either at a synaptic or population level’ (Friston, 2011). For CCEP studies, it is unclear whether these interactions differ within physiological and pathological networks. In other words, it is unknown whether the increased responses in the hyperperfused network only represent the effect of neuronal connectivity, or if there is an additional effect of remote epileptic responses. Accentuated CCEPs following stimulation of IOZ contacts compared to non-IOZ contacts, have been described close to the stimulation site (Iwasaki et al., 2010). Cortico-cortical evoked potentials and post-stimulation changes in gamma band activity differentiated early versus late seizure spread sites (Lega et al., 2015). Future studies directly comparing aspects of individual evoked responses (e.g. frequency, morphology, latency) in established physiological and pathological brain networks, could help to elucidate this question.

Structural and functional connectivity studies are typically confounded by a distance bias: connectivity measures decrease with increasing distance (Honey et al., 2009). Parallel to this, a drop in evoked responses with distance from stimulation was found in our CCEP study. However, if different perfusion levels would have been equally distributed and sampled in space, distance would not affect our correlation analysis between connectivity and perfusion. Nevertheless, hyperperfusion tended to be concentrated around the (stimulated) IOZ and hypoperfusion in more remote contacts. In an attempt to account for this distance-related fall-off, electrode contacts were matched for distance from stimulation site. We did not find a significant influence of different distance ranges on the proportion of significant SISCOM/CCEP correlations, supporting a true relationship between perfusion and connectivity.

Figure 6 Scatter plot across all studies of SISCOM z-score at each stimulation site (average over contact pair) and corresponding SISCOM/CCEP correlations, for all available stimulations inside (red) and outside (black) the ictal onset zone. Filled circles = significant correlations ($P < 0.05$), open circles = non-significant (n.s.) correlations ($P \geq 0.05$). The regression line, corresponding to stimulated non-IOZ contacts (black line), is parallel but shifted by 0.041 to lower SISCOM/CCEP correlations compared to the regression line of stimulated IOZ contacts (red line).
Implications

From a clinical perspective, this study may have practical implications for the use of SISCOM and CCEPs in the presurgical evaluation of refractory epilepsy. Patients with seizure-free outcome after surgery more likely showed positive significant SISCOM/CCEP correlations following stimulation of the IOZ compared to non-seizure-free patients (75% versus 55%). Nevertheless, high SISCOM/CCEP correlations did not necessarily mean that the IOZ was stimulated. Also, the presence of low CCEP t-values in electrodes at a distance from stimulation did not necessarily exclude connectivity with the stimulation site.

Prospective CCEP studies with rational electrode coverage of various SISCOM perfusion levels and a comprehensive stimulation protocol could further explore the role of both techniques in understanding epileptic networks. High SISCOM/CCEP correlations may unexpectedly disclose relevant epileptic nodes despite subthreshold ictal perfusion changes at the stimulation site. Future research combining SISCOM and CCEPs with other modalities like functional MRI (Jones et al., 2014) or diffusion tractography (Hamandi et al., 2008) could improve our insight of pathways used by epileptic activity.

Conclusion

Our cortico-cortical evoked potential study was able to demonstrate that SISCOM perfusion changes are not random but supported by underlying neuronal connectivity. Increased knowledge of patient-specific epileptic networks is important in the selection of candidates for (disconnective) epilepsy surgery or connectivity-based neuromodulation.

Acknowledgements

I would like to express my gratitude to the clinical staff at the Epilepsy Center of the Cleveland Clinic Foundation. We thank Dr Ammar Kheder for help in data collection.

Funding

This study was supported by grants from the Belgian American Educational Foundation (S.T.), NIH NIBIB R01 NS089212 (R.M.L.) and NIH NINDS R01-NS074980 (Z.I.W.).

The authors have no conflicts of interest.

Supplementary material

Supplementary material is available at Brain online.

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