Functional connectivity in the human language system: a cortico-cortical evoked potential study

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Summary
A better understanding of the mechanisms involved in human higher cortical functions requires a detailed knowledge of neuronal connectivity between functional cortical regions. Currently no good method for tracking in vivo neuronal connectivity exists. We investigated the inter-areal connections in vivo in the human language system using a new method, which we termed ‘cortico-cortical evoked potentials’ (CCEPs). Eight patients with epilepsy (age 13–42 years) underwent invasive monitoring with subdural electrodes for epilepsy surgery. Six patients had language dominance on the side of grid implantation and two had bilateral language representation by the intracarotid amobarbital test. Conventional cortical electrical stimulation was performed to identify the anterior and posterior language areas. Single pulse electrical stimuli were delivered to the anterior language (eight patients), posterior language (four patients) or face motor (two patients) area, and CCEPs were obtained by averaging electrocorticograms (ECoGs) recorded from the perisylvian and extrasylvian basal temporal language areas time-locked to the stimulus. The subjects were not asked to perform any tasks during the study. Stimulation at the anterior language area elicited CCEPs in the lateral temporo-parietal area (seven of eight patients) in the middle and posterior part of the superior temporal gyrus, the adjacent part of the middle temporal gyrus and the supramarginal gyrus. CCEPs were recorded in 3–21 electrodes per patient. CCEPs occurred at or around the particular electrodes in the posterior language area which, when stimulated, produced speech arrest. Similar early and late CCEPs were obtained from the basal temporal area by stimulating the anterior language area (three of three patients). In contrast, stimulation of the adjacent face motor area did not elicit CCEPs in language areas but rather in the postcentral gyrus. Stimulation of the posterior language area produced CCEPs in the anterior language (three of four patients) as well as in the basal temporal area (one of two patients). These CCEPs were less well defined. These findings suggest that perisylvian and extrasylvian language areas participate in the language system as components of a network by means of feed-forward and feed-back projections. Different from the classical Wernicke–Geschwind model, the present study revealed a bidirectional connection between Broca’s and Wernicke’s areas probably through the arcuate fasciculus and/or the cortico-subcortico-cortical pathway. CCEPs were recorded from a larger area than the posterior language area identified by electrical stimulation. This suggests the existence of a rather broad neuronal network surrounding the previously recognized core region of this area.

Keywords: language areas; functional connectivity; cortical stimulation; evoked potential; epilepsy

Abbreviations: AD = afterdischarge; AL = anterior language area; BA = Brodmann’s area; BT = basal temporal language area; CCEP = cortico-cortical evoked potential; ECoG = electrocorticogram; EPSP = excitatory postsynaptic potential; FLAIR = fluid-attenuated inversion recovery; FrOp = frontal operculum; MI = primary motor cortex; MTG = middle temporal gyrus; PL = posterior language area; SI = primary sensory cortex; SMG = supramarginal gyrus; STG = superior temporal gyrus; TE = anterior region of the inferior temporal cortex; TEO = posterior region of the inferior temporal cortex; TMS = transcranial magnetic stimulation

Introduction

With the advent of PET and functional MRI (fMRI), there have been significant advances in the field of functional neuroimaging techniques for studying the human brain functions (Fox and Raichle, 1986; Kwong et al., 1992; Ogawa et al., 1992). In contrast, little progress has been made in the understanding of the neuronal connectivity of the human brain. Almost all of the research on white matter connectivity such as cortico-cortical and cortico-subcortical connections has been conducted in animals using a variety of invasive tracer techniques (Cowan et al., 1972; Mesulam, 1978; Keizer et al., 1983). The corresponding information available in humans comes from gross dissections (Dejerine, 1895), or clinical pathological correlation of post-mortem brains using a modified reduced silver method (Nauta, 1957). Obviously the latter has limitations due to the inability to control lesion size and location.

As it relates to language functions, studies performed in non-human primates are largely not relevant. Although the cortical electrical stimulation studies (Lüders et al., 1991) and non-invasive neuroimaging studies (for reviews, see Chertkow and Murtha, 1997; Grabowski and Damasio, 2000; Price, 2000) have expanded the functional language domains (e.g. basal temporal and prefrontal regions) outside the aphasia-related regions in the perisylvian area, knowledge about cortico-cortical connections in the human language system has not gone beyond the classical concepts of Meynert in the 1870s that a deeply situated white matter tract connects Wernicke’s to Broca’s area (Bastian, 1887). From this observation, Wernicke and then later Geschwind postulated that lesions of this tract, named the arcuate fasciculus, would produce conduction aphasia (Geschwind, 1970). Anatomical dissections of these white matter tracts have not proven this hypothesis, since the exact termination of these fibre tracts could not be identified. Diffusion tensor MRI promises to shed new light on the understanding of white matter pathways by its ability to image fibre trajectories (Makris et al., 1997; Mori et al., 2000). Catani et al. (2002) employed this diffusion tensor MR tractography to visualize ‘in vivo’ dissections’ of association fibres, and confirmed the presence of major white matter fasciculi in the living human brain. The findings were in favour of a direct connection via the arcuate fasciculus between Wernicke’s and Broca’s areas. This technique, however, cannot provide details of functional aspects of the brain, i.e. cortical functions and functional connectivity among cortical regions. It would be optimal if both functional cortical regions and their white matter connections could be mapped within the same subject, so as to be able to track exact neuronal connections.

The opportunity to perform such mapping is the basis of this report. In this study, we enrolled patients undergoing invasive monitoring with subdural electrodes for presurgical evaluation of intractable partial epilepsy. These patients underwent standard cortical electrical stimulation to map cortical language areas. Several attempts have been made to track neuronal connectivity by applying electrical stimuli to the brain, e.g. stimulation of depth electrodes for recording responses from the functionally connected cortex (Wilson et al., 1990; Brugge et al., 2003). Reported in this study is a method we developed for stimulating and recording from subdural grids to track the cortico-cortical connections in vivo in humans, and we termed this method cortico-cortical evoked potentials (CCEPs). The methodology is akin to the previous attempts to look at local evoked potentials, namely ‘direct cortical responses’, in the vicinity of the site of cortical surface stimulation (Adrian, 1936; Purpura et al., 1957). The methodology employed here is a variation of this technique in that we apply an electrical stimulus to look at the potentials that emanate from a distant region of the cortex. In this manner, we are attempting to analyse inter-areal connectivity of the two distant cortical regions. By electrically stimulating one language area and recording neuronal responses from others, we studied for the first time the inter-areal or cortico-cortical connections within the perisylvian language area as well as between the perisylvian and extrasylvian language areas.

Methods

Subjects

Eight patients who underwent chronic subdural electrode placement for the presurgical evaluation of medically intractable partial epilepsy were studied (Table 1). Language dominance was evaluated using the intracarotid amobarbital test. Six patients had subdural electrodes placed in the hemisphere dominant for language, and two (patients 5 and 7) had bilateral language representation. The implanted electrodes were made of platinum measuring 3.97 mm in diameter with a centre–centre interelectrode distance of 1 cm (custom-made in the Cleveland Clinic, OH). The invasive recordings were performed to identify the epileptogenic area by recording

<table>
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<th>Patient</th>
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<td>37F R</td>
<td>13M R</td>
<td>20M R</td>
<td>18F L</td>
<td>22F R</td>
<td>29M R</td>
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<td>Implanted hemisphere</td>
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<td>HS</td>
<td>HS + CD</td>
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Language dominance was evaluated by intracarotid amobarbital test. HS = hippocampal sclerosis, CD = focal cortical dysplasia.
seizures and to delineate the regions of cortical function in and around the epileptogenic zone by cortical electrical stimulation and evoked potentials (Lüders et al., 1987). In six subjects, the epileptogenic focus was located in the mesial or basal temporal area (see Table 1 for aetiology). Patient 6 had cortical dysplasia and the epileptogenic focus was located in the orbitofrontal region. This patient had already had a previous invasive evaluation with subdural electrodes and had a partial resection of the left orbitofrontal region. Patient 8 had a severe closed lip schizencephaly in the bilateral perisylvian areas, and the epileptogenic focus could not be localized well. In patients 5 and 7, the MRI revealed cortical dysplasia involving the lateral temporal area. In patient 7, the fluid-attenuated inversion recovery (FLAIR) image showed a well-demarcated lesion in the left middle superior temporal gyrus. In patient 4, neuroimaging showed evidence of encephalomalacia in the left superior anterior parietal region, and in patient 3, atrophy and signal abnormality consistent with calcification in the left temporoparieto-occipital area were found. The neurological examinations were normal except for patient 3 who had a right homonymous hemianopsia, patient 4 who had a mild right hemiparesis with a right lower quadrantanopsia, and patient 8 who had orofacial apraxia due to an opercular syndrome.

The present study was approved by the Institutional Review Board Committee at The Cleveland Clinic Foundation, and informed consent was obtained from all subjects (IRB #4513).

Functional mapping of the cortical areas

Standard cortical stimulation was performed for functional mapping in all subjects as a part of the presurgical evaluation. Repetitive square wave electric currents of alternating polarity with a pulse width of 0.3 ms and a frequency of 50 Hz were delivered for 2–5 s (Grass S-88 and SUI-7, Astro-Med Inc., RI).

The subdural electrodes covering areas of functional interest were studied using cortical stimulation. Details of the methodology for cortical stimulation and the subsequent cortical mapping have been described elsewhere (Lüders et al., 1987). Cortical areas were defined as language areas when stimulation produced an interruption of the ability to read aloud a sentence in the absence of the following: (i) positive tongue motor response (e.g. tonic tongue contraction); (ii) negative tongue motor response (e.g. impairment of rapid alternating movements); and (iii) afterdischarges (ADs) (Schaffler et al., 1996). Stimulation was performed in the periolandic and perisylvian language areas. The basal temporal area was not studied using standard stimulation parameters.

As the language impairment can be elicited within the perisylvian language area outside the classical location of Broca’s and Wernicke’s areas, such as in the supramarginal gyrus (Penfield and Roberts, 1959; Schaffler et al., 1996), we use the term anterior and posterior language areas, originally coined by Penfield and Roberts (1959), to describe these regions. We will use the terms Broca’s and Wernicke’s areas specifically to refer to their classical anatomic locations in Brodmann’s area (BA) 44/45 and the posterior section of BA 22, respectively. Furthermore, as stimulation of the anatomically defined language area does not always elicit a language deficit, we refer to the site of the electrode which produced language impairment on electrical stimulation as the ‘language electrode’ herein.

Stimulus condition and data acquisition of CCEP

The electrical stimulus used for this purpose consisted of a constant-current square wave pulse of 0.3 ms duration, which was given at a frequency of 1 Hz. Two adjacent electrodes were stimulated in a bipolar fashion to achieve more localized current flow in the cortex beneath the electrodes (Nathan et al., 1993). Stimulation polarity was alternated to (i) reduce the stimulus artefacts; (ii) to avoid electric charges building up at the cortex (a safety consideration); and (iii) to avoid polarization of platinum electrodes which can decrease the current density over time (Ikeda et al., 2000). The current was given at 80% of the intensity that produced clinical signs or ADs during the standard cortical stimulation. The intensity was set at 10–12 mA if no clinical sign or ADs were present at 15 mA. In cases in which excessive artefact obscured the recordings, the intensity was lowered stepwise by 1 mA until artefacts became small enough to visualize the evoked responses.

Continuous electrocorticogram (ECoG) was monitored with a digital EEG (Vangard, Cleveland, OH) to detect EEG seizures or ADs. All the subdural electrodes were referenced to a scalp electrode placed on the skin at the mastoid process contralateral to the side of implantation. An evoked potential machine equipped with a low noise and high common mode rejection ratio amplifier (Axon Epoch 2000 Neurological Workstation, Axon Systems Inc., NY) was used simultaneously for the recording of CCEPs. The bandpass filter for data acquisition was set to 1–800 (patients 1, 3 and 5–8) or 1000 Hz (patients 2 and 4) with a sampling rate of 2000 or 2500 Hz for each channel. Responses were averaged using the stimulus onset as the trigger. In each session, at least two trials of 20–100 responses were averaged separately to confirm the reproducibility of the responses. During the recording of CCEPs, the subjects were either lying or sitting on a bed and were allowed to continue their physical activity such as talking or eating. The subjects were not requested to perform a specific task. The details of this methodology have been described elsewhere (Matsumoto et al., 2004).

Language areas investigated

In the cases where electrodes associated with speech arrest were identified in the anterior language area by the conventional stimulation study, the stimulus was delivered to these language electrodes in an attempt to investigate cortico-cortical connections from the anterior language area to the posterior or basal temporal language area. In patient 4 in whom the anterior language area was not identified by standard cortical stimulation, a pair of electrodes over the anatomical Broca’s area was stimulated instead. In all subjects, evoked potentials were recorded from electrodes placed in the lateral temporal-parietal area (16–44 electrodes) including the language electrodes, and in three subjects (patients 1, 4 and 6) also from the basal temporal area (11–15 electrodes). In two subjects (patients 6 and 7), CCEPs were also recorded by stimulating the face motor area to compare with the anterior language area. In four subjects (patients 1, 2, 6 and 7), after recording CCEPs to stimulation of the anterior language area, the posterior language area was stimulated; the language electrodes (patients 1 and 6) and/or the electrodes where CCEPs were obtained by stimulation of the anterior language area (patients 1, 2 and 7). Two to eight pairs of electrodes were stimulated per subject. CCEPs were recorded from the frontal region covering the anterior language area (8–15 electrodes) in four subjects (patients 1, 2, 6 and 7) and from the basal temporal area (11 electrodes) in two (patients 1 and 6). In order to simplify the description of the results and discussion, we will use the following convention when discussing the regions which were stimulated and the region from which the CCEP responses were recorded. A descriptor of “CCEPX1–X2” will be used to indicate that CCEP is recorded from region “Y” in response to
stimulation of region ‘X’. For example, CCEP\textsubscript{AL---PL} means CCEP recorded from the posterior language area in response to stimulation of the anterior language area.

**Display and measurement of waveform components**

Preliminary results showed that CCEPs consist of an early (N1) and a late (N2) negative potential. The N1 peak was visually identified as a first negative deflection that was clearly distinguishable from the stimulus artefact. The conventional measurement of the N1 amplitude from either a predetermined baseline or from peak to trough was usually not possible due to frequent occurrence of a stimulus artefact preceding the N1 deflection. Therefore, in order to standardize the N1 amplitude measurement, the following methodology was adopted. A line was drawn from the onset to the offset of the N1 potential for each data point, and the N1 amplitude was measured as the height of a vertical line drawn from the negative peak of N1 to the intersection of the vertical line with the above-described line (Fig. 1). The amplitude of N2 was measured from the preceding positive peak. In order to illustrate the distribution of each activity over the cortex, a circle map was employed based on the amplitude percentage distribution, in which the diameter of the circle at each electrode represented the percentile to the maximal amplitude of that particular activity.

**Co-registration of subdural electrodes with 3D MRI**

T1-weighted MRIs were acquired and combined to form a single three-dimensional volume (1.5 T, MP-RAGE sequence). Volumes were processed to remove background elements (Otsu, 1979) and to segment the brain (Brunner et al., 1993) automatically. The location of each electrode on a subdural grid was identified on the 2D MRIs using its signal void due to the property of the platinum alloy of each electrode on a subdural grid was identified on the 2D MRIs. The surface of the brain was reconstructed using an in-house computer program that interactively renders the brain volume and surface locations of the subdural electrodes (Cabral et al., 1994), working on a Silicon Graphics computer (Mountain View, CA). To display the electrodes, a 2 mm diameter sphere was surface-rendered at the centre of each identified electrode and overlaid on the volume-rendered image. To compensate for the artefacts arising from subdural grids on the surface of the brain, the volume-rendered MRI was chamfered by 4–5 mm from the cortical surface to locate the sulci beneath the grids. To superimpose lesions detected by FLAIR sequence, FLAIR scans were co-registered to the 3D MP-RAGE MRI scans using in-house software based on the method of maximization of mutual information (Maes et al., 1997). The details of the analysis have been described elsewhere (Hadar et al., 2002; Marusic et al., 2002).

**Results**

**Cortico-cortical connection within the perisylvian language area**

Single pulse electrical stimulation at the anterior language area elicited two separate fields of CCEPs; one at the posterior language area (CCEP\textsubscript{AL---PL}) and the other at the frontal operculum (CCEP\textsubscript{AL---FrOp}). CCEPs consisted of two major negative potentials, N1 and N2, and were recorded in all subjects except patient 8 who had a severely malformed schizencephaly in the perisylvian area.

Waveforms obtained from a representative case for CCEP\textsubscript{AL---PL} (patient 1) are shown in Fig. 2 and circle maps for amplitude percentage distribution obtained from other subjects are shown in Figs 3 and 4 (the colour version of Fig. 2 is available on-line as supplementary data). N1 and N2 peaks were recorded from the middle and posterior portions of the superior temporal gyrus (STG) and the adjacent portion of the middle temporal gyrus (MTG) as well as in the supramarginal gyrus (SMG). The peak latency of N1 ranged from 22 to 36 ms (mean 27.9 ms) and that of N2 from 113 to 164 ms (mean 144.6 ms). The distribution of N2 (3–21 electrodes) was larger than that of N1 (1–20 electrodes) ($P < 0.01$, two tailed paired $t$ test). The maximum of N1 was seen in the inferior parietal lobe in three subjects, i.e. at the SMG in two subjects (patients 2 and 4) and at the adjacent ventrocaudal portion of the postcentral gyrus in one (patient 7); and in the temporal lobe in four subjects, i.e. at the STG in three (patients 3, 5 and 6) and at the adjacent portion of MTG in one (patient 1). In relation to the language electrodes identified in the temporoparietal area in five subjects (patients 1, 3, 4, 5 and 6), the language electrode was located within the area of N1 and N2, except for patient 3, in whom the language electrode was within the distribution of N2 but outside that of N1. The maximum of N1 and N2, however, was not always observed at the language electrode; the maximum was next to the language electrode (patients 1 and 4) or separated by 2–3 cm (N1 of patient 3).

In patients 4 and 6 in whom the anterior margin of recording electrodes covered the anterior opercular region, CCEP\textsubscript{AL---FrOp} were recorded adjacent to the stimulated electrode at the anterior language area (see the circle ‘a’ in patient 4, Fig. 3 and patient 6, Fig. 4). The N1 peak of CCEP\textsubscript{AL---FrOp} was earlier than that of CCEP\textsubscript{AL---PL} (11 versus 26 ms and 19 versus 25 ms in patients 4 and 6, respectively). The maximum response was observed ventral to the tongue motor area at the most ventral portion of the precentral
gyrus (patient 6) or at the adjacent temporal opercular region (patient 4). As CCEPs of short latency were recorded across the sylvian fissure in each patient, these CCEPs seem to be generated as a radial dipole at the most ventral portion of the precentral gyrus or the deeply situated anterior insula.

In contrast to stimulation of the anterior language area, stimulation of the face motor area elicited evoked responses at the more rostral parietal region at and around the primary sensory cortex (SI) (CCEP\textsubscript{FaceMI-SI}) in patients 6 and 7 (Fig. 4). CCEP\textsubscript{FaceMI-SI} were distributed at the ventral half of the postcentral gyrus and the adjacent portion of the anterior inferior parietal area. The N1 peak latency of CCEP\textsubscript{FaceMI-SI} was shorter than that of CCEP\textsubscript{AL-PL} (13 versus 25 ms and 24 versus 32 ms in patients 6 and 7, respectively).

CCEP\textsubscript{PL-AL} were recorded in four subjects (patients 1, 2, 6 and 7). Three of them (patients 1, 2 and 6) showed clear CCEPs (Fig. 5). Stimulation of the posterior language electrodes elicited CCEP\textsubscript{PL-AL} in patients 1 and 6. In patient 2, in whom no posterior language electrodes were identified by standard cortical stimulation, CCEP\textsubscript{PL-AL} responses were obtained by stimulating two pairs of electrodes at the SMG (pairs 1 and 2 in Fig. 5). One of the stimulating pairs (pair 2) showed the largest CCEP\textsubscript{AL-PL} upon stimulation of the anterior language area. CCEP\textsubscript{PL-AL} were also recorded at surrounding electrodes in the face motor area in patient 1 and in the silent prefrontal area in patients 2 and 6. In patients 2 and 6, both N1 and N2 were seen with a peak latency of 23–39 and 90–161 ms, respectively. In patient 1, however, N1 and N2 peaks were poorly defined partially due to the stimulus artefact. In patient 1, CCEP\textsubscript{PL-AL} consisted of a single negative potential with a peak latency of 67–80 ms.

**Cortico-cortical connection between the perisylvian and extrasylvian language areas**

Cortico-cortical connections between the perisylvian language and basal temporal areas were studied using CCEPs (Fig. 6). The same electrode pairs used for recording CCEP\textsubscript{AL-PL} and CCEP\textsubscript{PL-AL} were stimulated at the anterior and posterior language areas, respectively.

CCEP\textsubscript{AL-BT} were recorded at the anterior to middle portions of the inferior temporal and fusiform gyri in all three subjects studied (Fig. 6). CCEP\textsubscript{AL-BT} consisted of clearly defined peaks of N1 at 19–47 ms and N2 at 120–187 ms. As for the N2 potential in patient 1, a negative peak was observed in the inferior temporal gyrus simultaneously with a positive peak of identical latency in the fusiform and parahippocampal gyri, suggesting a dipolar activity in the inferior temporal sulcus. In two subjects (patients 4 and 6), the face motor area was also stimulated but no CCEP\textsubscript{FaceMI-BT} were obtained.

Stimulation of the posterior language area, which elicited CCEP\textsubscript{PL-AL} in patients 1 and 2, did not evoke clear CCEP\textsubscript{PL-BT} responses at the basal temporal area. On the other hand, in patient 1, stimulation of more caudally situated electrode pairs (pairs 1 and 2 in Fig. 6) at the temporoparietal junction elicited small N1 and N2 potentials in
Fig. 3  Circle maps for CCEPs_{AL→PL} in patients 2–5. Each circle map shows the amplitude percentage distribution in relation to the maximal response displayed below each map. The middle and right columns show the distribution of N1 and N2 activities, respectively, for each subject. The result of the cortical mapping and the distribution of recording electrodes (coloured black) are shown in the left column. Language electrodes denote those which, when stimulated, elicited language impairment, and auditory electrodes denote those which elicited auditory response in the absence of language impairment. Major sulci are highlighted by white lines (CS = central sulcus). The area of the language electrodes is highlighted with a dotted circle in the middle and right columns. Other conventions are the same as for Fig. 2. CCEPs_{AL→PL} were recorded in the posterior part of the superior temporal gyrus, adjacent portion of the middle temporal gyrus and the supramarginal gyrus in and immediately surrounding the language electrode. The area of N2 activity was significantly larger than that of N1 ($P < 0.01$).
Fig. 4 CCEPs_{AL→PL} and CCEPs_{FaceMI→SI} in patients 6 and 7. Note that the distribution of CCEPs_{FaceMI→SI} in the ventral postcentral and adjacent anterior inferior parietal areas differs significantly from the respective distribution of CCEPs_{AL→PL} in both subjects. In patient 6, stimulation at the anterior language area elicited CCEPs_{AL→FrOp} (a) in addition to CCEPs_{AL→PL} (b). The N1 peak latency of CCEPs_{AL→FrOp} (19 ms) was shorter than that of CCEPs_{AL→PL} (25 ms). Similar CCEPs_{AL→FrOp} were recorded in patient 4 (see the activity 'a' for patient 4 in Fig. 3). x = CCEP not available due to high impedance (left column). Other conventions are the same as for Fig. 3.
the inferior temporal gyrus with a latency of 31–32 and 117–128 ms, respectively.

**Sites of CCEP generation relative to brain pathology**

Since the epileptogenic focus was located outside the lateral temporo-parietal area in all the subjects studied, the majority of CCEP$_{\text{AL}}$-PL were recorded from the normal brain tissue. In some subjects, however, CCEPs were also recorded from the cortical area including brain pathology. CCEP$_{\text{AL}}$-PL were obtained within a MRI abnormality in the temporal lobe in patients 3 and 5, consistent with Sturge–Weber syndrome and cortical dysplasia, respectively. In patient 5, two separate posterior language areas were defined by standard cortical stimulation. It is possible that these two distinct regions had been separated by abnormal dysplastic cortex. In this case, CCEP$_{\text{AL}}$-PL were also distributed in these two separate regions. In patients 1 and 4, CCEPs in the basal temporal area overlapped mesially with the epileptogenic zone, while the maximum of CCEPs was situated laterally away from the epileptogenic zone.

In patient 3, the EEG seizure onset zone was situated in the basal temporal area, while interictal epileptiform discharges were observed diffusely in the left temporo-occipital region. This Danish patient was bilingual, and language was studied by testing with English phrases. Consequently, he underwent a large resection in the left temporo-occipital area. In Fig. 3, the posterior margin of the resection is shown in relation to language electrodes and those with CCEPs. The resection margin spared the language area identified by standard cortical stimulation but included electrodes that gave rise to N1 responses in the CCEP$_{\text{AL}}$-PL study. Postoperatively, this patient developed language disturbance (difficulty in word finding) only in Danish. At the 1 year follow-up, it was clear that he continued to have word retrieval difficulties in his native Danish. In five other subjects with temporal foci, anterior temporal lobectomies were performed, sparing both...
language electrodes and other electrodes showing CCEPsAL→PL responses. Two of them (patients 1 and 5) showed only mild transient disturbance in a confrontation naming task after surgery.

Discussion
To the best of our knowledge, the present study demonstrated for the first time inter-areal connections in the human language system in vivo using CCEPs. Several attempts have been made to track neuronal connectivity non-invasively by stimulating the cortex with the technique of transcranial magnetic stimulation (TMS) and by recording neural activities from the remote cortical areas with PET (Fox et al., 1997; Paus et al., 1997, 2001). The poor time resolution of this methodology has greatly limited its success. Using subdural electrodes chronically implanted for presurgical evaluation of patients with intractable partial epilepsy, we were able to track neuronal pathways with excellent temporal and spatial resolution by directly stimulating a focal area of the cortex and recording CCEPs from the adjacent as well as remote cortical areas.

Implication of CCEPs
It has been shown that single pulse electrical stimulation of the motor cortex with a pair of subdural electrodes elicits motor evoked potentials in the electromyogram (EMG) in humans (Ikeda et al., 2000; Hanajima et al., 2002). It is postulated that anodal and cathodal stimulation generates direct and indirect orthodromic discharges, respectively, in the corticospinal pathway. With an anodal stimulus, a surface positive current induces an electrical current flow into the vertically oriented dendrites of pyramidal neurons or their cell bodies, which then...
excite or depolarize the initial segment or the first node of the axon. Conversely, a cathodal stimulus excites pyramidal neurons indirectly by activating chains of interneurons in the outer cortical layers, as is postulated to occur with TMS (Amassian et al., 1990). Therefore, by analogy to the motor cortex stimulation, direct cortical electrical stimulation seems to generate both direct and indirect orthodromic discharges at the site of stimulation. In principle, the following two mediators can convey the orthodromic discharges to elicit CCEPs in the adjacent or remote cortex: (i) a direct cortico-cortical pathway; and (ii) an indirect cortico-subcortico-cortical pathway.

In the first case, cortical surface stimulation generates orthodromic discharges in cortico-cortical projection neurons situated mainly in layer III (Jones and Wise, 1977), thus activating the cortico-cortical association fibres to convey the impulses to the cortical area where they project. Two important questions that arise from the present finding are which white matter tract is involved in the transmission of these responses and the reason for the relatively late latency of N1. Let us discuss them focusing on CCEPAL.

In the first case, cortical surface stimulation generates orthodromic responses and the reason for the relatively late latency of N1. Let us discuss them focusing on CCEPAL. As for the former question, the arcuate fasciculus is a possible mediator of CCEPAL responses. This is mostly speculation because direct recordings from white matter tracts were not performed. In our view, that is a reasonable assumption since this is the best known anatomical substrate connecting Broca’s and Wernicke’s areas. Regarding the latter question, the N1 peak of CCEPAL ranged from 22 to 36 ms. In macaque monkeys, the earliest monosynaptic response via the frontoparietal circuit has a latency of 2–5 ms in the intracortical microstimulation study (Goldschalk et al., 1984). Taking account of the considerable size difference of the brain between the two species, the conduction time for the earliest orthodromic response via the arcuate fasciculus in humans would range approximately 4–12 ms. In the present study, this earliest response through the largest fibres might have been missed due to the stimulus artefact contaminating the initial 5–10 ms segment of CCEPs. If that is the case, the N1 could be the later responses mediated by smaller fibres in the arcuate fasciculus. According to Bishop and Smith (1964) who studied the surgical specimen in the human frontal lobe, the diameter of most axons was between 0.8 and 2 μm, with a very small portion of axons lying outside of this range. Applying the conduction velocity of central axons derived from Waxman and Swadlow (1977) to the distance of 10–12 cm between the anterior and posterior language areas measured on the patients’ MRI, the expected conduction time through these small fibres is 10–30 ms. By taking into account the local synaptic delay of 1–2 ms per synapse at the site of stimulation and at the cortex where CCEPs were evoked, this assumption could be regarded as one of the generator mechanisms of N1 of CCEPs. In fact, this estimated value is in good agreement with the peak latency (average of 22 ms) of the evoked potential recorded at the contralateral homologous area in response to TMS of the hand motor area via transcallosal connection (approximately 10 cm) (Komssi et al., 2002).

Alternatively, the relatively blunt peak of the N1 waveform may suggest a different generator mechanism. Review of previous studies on ‘direct cortical response’ may provide a clue for the late N1 response. In 1936, Adrian first described a ‘direct cortical response’ and found that a local surface negative potential, termed the primary negative potential, is evoked by electrical stimulation of the cortical surface in various species. This surface negative response has also been recorded in humans in the intraoperative setting, and distributed within 10 mm from the site of stimulation with its peak at around 10 ms (Purpura et al., 1957; Goldring et al., 1994). Simultaneous surface and intracellular recording in animals revealed that this negative potential reflects predominantly postsynaptic events, representing excitatory postsynaptic potentials (EPSPs) (Li and Chou, 1962; Sugaya et al., 1964). In fact, most spikes were preceded by a synaptic potential and the majority of neurons fired within the time course of the primary negative potential in the intracellular recording. This response is local in origin since it is also observed in completely isolated cortex of animals (Jerv et al., 1960). Taking account of the relatively long duration (~20 ms) of this local negative response, it is possible that a single pulse stimulation of the subdural electrode actually produced activation of the pyramidal neurons not only just beneath the electrode but also in the local surrounding cortex with a variable jitter of <20 ms. If this is the case, it follows that this jitter was reflected in generation of CCEPs at the remote target cortex via large fibres, resulting in a rather blunt late peak of N1. Furthermore, in addition to the jitter taking place at the stimulus site, the arriving impulse itself might have also caused the local jitter of synaptic activity at the target cortex through a similar mechanism.

The second possible pathway, i.e. the indirect cortico-subcortico-cortical pathway, may also account for the relatively late N1 response. As proposed by Penfield and Roberts (1959), lesion studies have revealed participation of the thalamus and basal ganglia in the language system (Damasio et al., 1982; Graff-Radford et al., 1985; Segal et al., 2003). An electrical stimulation study also supports involvement of the thalamus in the language system (Ojemann et al., 1975). Although the exact cortico-subcortico-cortical pathway for the language system has not yet been elucidated, it is possible that the earliest volleys upon cortical stimulation travel this large fibre pathway to generate the relatively late N1 response in the remote target cortex with oligosynaptic relay. This pathway may account for the relatively similar N1 latencies observed between CCEPAL and CCEPAL, considering the similar cortico-subcortico-cortical distance in each circuit. One candidate of the cortico-subcortico-cortical pathway is the reciprocal or non-reciprocal cortico-thalamo-cortical connection, which has been documented in non-human primates (Murphy and Sillito, 1996; Darian-Smith et al., 1999; McFarland and Haber, 2002). The interlobar connection could be built through the relay of two reciprocal cortico-thalamo-cortical circuits each within a single lobe.
Alternatively, the interlobar connection might be conveyed via the interlobar non-reciprocal cortico-thalamo-cortical circuit selectively connecting the anterior and posterior language areas, although this sort of selective interlobar connection was reported to be scarce in non-human primates (Giguere and Goldman-Rakic, 1988). In this regard, the diffusion tensor tractography, attempts at which have just begun (Henry et al., 2004), may be of some use in delineating cortico-subcortical connections in the language system.

Regarding a generator mechanism of the N2 potential, it is interesting to note that N2 has a larger distribution than the N1 potential. This observation may suggest a different mechanism of generation for these two components. One possibility is that, after the direct excitation of the cortex by the aforementioned mechanisms generating N1, the N2 potential is then generated in and immediately surrounding the cortex via either a local cortico-cortical or a cortico-subcortical reverberating circuit. Alternatively, the late N2 potential might represent the direct arrival of slow multisinaptic volleys through the cortico-subcortical-cortical pathway.

It is also possible that antidromic activation of the presynaptic axonal terminals of the association fibres could play a role in generating CCEPs. However, this possibility is less likely than the orthodromic activation since extracellular current flow excites large axons such as initial segments of pyramidal neurons more easily than smaller fibres such as the presynaptic terminals (Koester and Siegelbaum, 2000). Moreover, the arrangement of presynaptic terminals is less organized for effective cortical surface stimulation compared with the highly structured vertically arranged pyramidal neurons or tangentially arranged interneurons (Nieuwenhuys et al., 1988). Nevertheless, extensive arborization of the presynaptic terminals may increase the chances to be excited by cortical surface stimulation. If that is the case, CCEPs are actually due to a mixture of both orthodromic and antidromic excitation of the neurons in the remote target cortex.

In the present study, no seizures or other adverse effects were observed during repetitive single pulse stimulation. Valentin et al. (2002) employed a similar single pulse stimulation technique in the epileptic foci in an attempt to investigate cortical hyperexcitability, without any adverse effects. The method of CCEP seems to be a safe technique for exploring neuronal connectivity in vivo in patients who require the investigation of higher cortical functions such as language before surgical treatment.

**Cortico-cortical connection within the perisylvian language area**

A cornerstone in the understanding of language processing has been the belief that there is a direct connection via the arcuate fasciculus between Wernicke’s and Broca’s areas (Geschwind, 1970). Repetitive electrical stimulation of the arcuate fasciculus can elicit language impairment, namely anomia (Duffau et al., 2002). Gross dissection of the white matter, however, failed to identify the exact termination of fibres of the arcuate fasciculus (Dejerine, 1895).

In the present study, stimulation of the anterior language area elicited responses at the STG and SMG (CCEPs\textsubscript{AL→PL}) in all subjects except patient 8 who had a severe closed lip schizencephaly in the corresponding area. On the other hand, stimulation of the posterior language area at the STG or SMG elicited responses at the anterior language area and its surroundings (CCEPs\textsubscript{PL→AL}) in three out of four subjects. Although study of CCEPs\textsubscript{PL→AL} was not performed in all the subjects due to their condition, at least electrophysiologically, the connection between the two areas appears to be bidirectional. However, the waveforms of CCEPs\textsubscript{PL→AL} were less defined with smaller distribution, compared with CCEPs\textsubscript{AL→PL}. The preponderance of evoked responses in the posterior language area, which seems opposite to the classical Wernicke–Geschwind hypothesis, may be due to the different degree of convergence between the two directions; namely more convergent projection from the posterior to anterior area but relatively more divergent projection from the anterior to posterior area (Fig. 7). Because of the scattered distribution of neurons in the posterior area projecting to the anterior area, only a part of the posterior language area can be activated at one time by the stimulation technique employed in the present study, generating less defined responses in the anterior language area. In addition, a different degree of evoked responses might be related to the size difference of pyramidal neurons that are the main target of the cortical surface stimulation. The pyramidal neurons are smaller in the posterior language area (Braak, 1978; Hayes and Lewis, 1993), which could result in weaker neuronal activation at the site of stimulation when the cortex is stimulated with the same current intensity as that for the anterior language area. In summary, the most plausible explanation for the present findings seems to be that these responses are the results of an orthodromic excitation of bidirectional direct and/or indirect cortico-cortical pathways connecting the two language areas. Thus, against the concept

![Fig. 7 Schematic illustration of presumed connections between the anterior and posterior language areas. The grey circle denotes the site of stimulation. Arrows indicate the direction of impulse projection evoked by single pulse stimulation. The excited fibres are shown as thick black lines, and those not excited as thin grey lines. Waveforms show representative CCEPs. AL = anterior language area; PL = posterior language area.](image-url)
postulated in the Wernicke–Geschwind model, the arcuate fasciculus may not be a solely unidirectional pathway conveying information from Wernicke’s to Broca’s area, but rather a bidirectional system which reciprocally connects the two language areas. Taking into account the larger distribution of the N2 than the N1 potential, the distribution of CCEPs suggests the existence of a rather broad neuronal network surrounding the previously recognized core region of the posterior language area, defined either anatomically (posterior sector of BA 22) or functionally (cortical stimulation).

Comparison of the circuitry between non-human primates and humans provides an insight into the acquisition of language functions. Based on similarities in cytoarchitecture and anatomical location, non-human primate homologues of Broca’s area, Wernicke’s area and the SMG have been postulated to be the rostral part of the ventral premotor region (areas 6 and 45), the temporoparietal area and area 7b, respectively (Eidelberg and Galaburda, 1984; Aboitiz and Garcia, 1997). The evidence in the non-human primate brain points to a much more extensive parietal than temporal projection to the ventral premotor area. While a heavy reciprocal connection exists between the ventral premotor area and area 7b (Pandya and Kuypers, 1969; Godschalk et al., 1984; Cavada and Goldman-Rakic, 1989), only a scarce fibre tract connects the ventral premotor area and the temporoparietal area (Petrides and Pandya, 1988; Deacon, 1992). One may speculate that the increased connectivity demonstrated in the present study between Broca’s and Wernicke’s areas in humans is the result of evolution and acquisition of language function. On the other hand, in view of the presence of an equivalent premotor–parietal circuit in monkeys, a tight reciprocal connection between Broca’s area and the SMG seems to have been established in the early stage of evolution. This connection may play an important role in phonological processing, as revealed in neuroimaging studies which showed activation of SMG in phonological storage and of Broca’s area in subvocal rehearsal or phonological segmentation (Zatorre et al., 1992, 1996; Paulesu et al., 1993).

In addition, two short circuits with shorter N1 peak latencies (11–24 ms) were observed within the perisylvian language area. One is the connection between the face motor area and the primary sensory cortex, and the other is a connection between the anterior language area and the frontal operculum or the anterior insula. Functionally, the former circuit could play a role in sensorimotor coupling in the motor control of speech, and the latter probably connects the anterior language area to areas participating in articulatory planning (Dronkers, 1996; Wise et al., 1999) for motoric processing of language. The existence of these circuits is supported by similar connections seen in the non-human primate brain (Barbas and Pandya, 1987).

In this study, we report on inter-areal connections in the language dominant hemisphere. Future CCEP studies in the non-dominant hemisphere could also contribute to the understanding of the language system by assessing inter-hemispheric network asymmetries.

**Cortico-cortical connection between the perisylvian and extrasylvian language areas**

By a series of lesion, electrophysiology and neuroimaging studies, the basal temporal area has been shown to have a language function, namely lexical retrieval (Semenza and Zettin, 1989; Lüders et al., 1991; Nobre et al., 1994; Damasio et al., 1996; Price, 2000). The present study shows evidence for strong connectivity between the anterior language area and basal temporal area. These connections were unique to Broca’s area, and no CCEPs were elicited by stimulating the adjacent face motor area. Connection studies in monkeys showed reciprocal connections between the inferior temporal areas (TE and TEO) and the ventral frontal area including the homologue of Broca’s area (area 45) via the uncinate fasciculus (Ungerleider et al., 1989; Webster et al., 1994). Both the ventral frontal convexity and inferotemporal cortex are known to be involved in object recognition tasks in monkeys (Wilson et al., 1993). Similar direct projection in humans, as shown in the present study, may serve as an alternative pathway that transmits linguistically relevant object information between the anterior language area and basal temporal area. Simultaneous activation of the inferior frontal gyrus and the middle and inferior temporal cortex during a task of linking semantics of two objects in a PET study further supports the significance of this circuit in semantic processing (Vandenberghhe et al., 1996).

Regarding connectivity from the posterior language area to the basal temporal area, no clear response was recorded by stimulating the language electrode in the STG or SMG. On the other hand, small amplitude responses were recorded in the inferior temporal gyrus by stimulating caudally situated electrodes in the temporo-occipital junction (patient 1). These results are too preliminary to draw any strong conclusions. However, they may suggest that the functional connectivity of the posterior language area to the basal temporal area is not as tight as that of the anterior language area. Further studies focusing on this connection will be required to elucidate detailed connectivity between those two areas.

The basal temporal area was not stimulated by either the conventional repetitive or the single pulse stimulation due to the limited time allowed for invasive evaluation. CCEP_{BT→AL} and CCEP_{BT→PL} studies, after identifying the basal temporal language area by conventional stimulation, will be another interesting subject of investigation.

**Clinical relevance**

The present study revealed cortico-cortical connections within the perisylvian language area as well as between the perisylvian and extrasylvian language areas, via long and short association fibres and/or through the cortico-subcortico-cortical pathway. The functional connectivity demonstrated here supports the contemporary concepts of language organization, namely that neuronal groups participate as components of a network by means of feed-forward and feed-back projections (Damasio and Damasio, 2000). In contrast to classical aphasiology descriptions, the
conventional electrical stimulation of either Broca’s, Wernicke’s or the basal temporal language area can interfere with both language production and comprehension (Lüders et al., 1991; Schaffler et al., 1993, 1996). It is likely that the language network itself is functionally disturbed by high frequency stimulation, because even a single pulse stimulus in one language area reaches the other language areas via different cortico-cortical connections.

The connections revealed within the perisylvian area most probably reflect normal brain function since the epileptogenic focus or EEG seizure onset zone was outside the perisylvian area. It is interesting, however, to note that CCEPs could also be recorded from pathological brain tissue (calcification in patient 3, cortical dysplasia in patient 5) as well as from the epileptogenic focus (patients 1 and 4). The CCEP study may help in identifying normal cortico-cortical connections in the brain region where normal brain function co-exists with pathology.

The location of N1 identified by the CCEP$_{AL}$—$_{PL}$ study was not always identical to that of the language electrode in the posterior language area. Indeed, patient 3, who was bilingual in Danish and English, developed language deficit only in Danish after the removal of the area with the N1 electrodes, but sparing the language electrodes identified by English testing. Although this is a single bilingual case with a large resection, it may suggest that a restricted region of N1 potential is essential for language processing. As there was only an approximate relationship between the maximum location of N1 and N2 and the language electrode in general, further cases should be accumulated to establish the relationship.

Conventional cortical electrical stimulation has served as a gold standard to map language functions and to predict deficits in functional neurosurgery. In contrast to the rather restricted location of the anterior language area in the posterior third of the inferior frontal gyrus, the core region of the posterior language area in the temporoparietal cortex, defined by cortical stimulation, varies among subjects (Penfield and Roberts, 1959; Ojemann et al., 1989; Schaffler et al., 1996). Interestingly, despite the variable location of the core region or the language electrode, the present preliminary results showed that the area of CCEP$_{AL}$—$_{PL}$, in particular that of N2, reflected the large posterior language network and contained the language electrode. Therefore, the study of CCEP$_{AL}$—$_{PL}$ after identifying more localized Broca’s area at BA 44/45 by standard cortical stimulation, may be useful for guiding the clinician to determine the area of the conventional stimulation study in the temporoparietal cortex. This method does not require any attention or cooperation of the subject, and would be particularly useful in paediatric patients and in the setting of intraoperative language mapping. In this regard, the influence of anaesthetics upon CCEPs is an important subject for future investigation.

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References


