Clinical Imaging of GABAergic Neuronal Activity by Neuropharmacological fMRI.

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Introduction
Recent fMRI studies, using cerebral blood oxygenation level dependent (BOLD) contrast, have reported cerebral signal changes following vascular dilation with medications such as acetazolamide (1). Since fMRI would also be expected to provide insight into the chronological response of the brain to medications (2), we have applied fMRI to the clinical study of the cerebral effects of benzodiazepines (BZs). The mechanism of action of BZs is through the type A benzodiazepine receptor (BZR) which facilitates neurotransmission by gamma-aminobutyric acid (GABA). Epileptics suffer from abnormal cortical excitation, produced by glutamate, that is suppressed by GABAergic inhibition. Both the BZ agonist diazepam (DZ), and its antagonist flumazenil (FMZ), have widespread daily clinical usage, particularly in the management and treatment of epilepsy, and therefore, would be safe agents with which to develop novel neuro-pharmacological fMRI techniques. We performed the following clinical fMRI study, using both a BZR agonist (DZ) and a BZR antagonist (FMZ), in order to determine if their differential modulation of the GABAergic neurotransmitter system and hence, neuronal activity, could provide a useful clinical adjunct. In this respect, the images we obtained were expected to reflect the elevated GABAergic neuronal activity.

Methods
Subjects: Nine patients from our neurosurgical unit, who suffered from intractable epilepsy, and who were scheduled to have surgery, gave their written informed consent to be subsequently enrolled in this clinical research study. Five of these patients had unilateral temporal lobe epilepsy, while the other four suffered from frontal focal seizures. All patients underwent neurosurgery, during which cortical electroencephalography was used in order to localize the seizure focus. The sites of the epileptic foci were easily visible in the MR images as resected areas.

Instrumentation: MRI was performed with a 1.5 T clinical scanner (Philips GYROSCAN) using a FLASH sequence with the following parameters: TR/TE, 100/45 msc; flip angle, 15 degrees; matrix size, 128*128; 2 averages; for 64 sequential image acquisitions. Four control images were taken prior to the administration of the medication. All measurements were completed in ca. 32 min.

Medication protocol: Two drugs were administrated, the BZR agonist diazepam (DZ) and its competitive antagonist, flumazenil (FMZ). First, 2 mg of DZ were administrated intravenously at the beginning of the 5th, 15th, 25th and 35th acquisitions. Then, FMZ was administrated at the beginning of the 45th (0.2 mg) and 55th (0.3 mg) acquisitions in order to reverse the effect of DZ.

Data Analysis: All images were digitally registered using a standard protocol. The effect of DZ and FMZ was evaluated by chronological analysis employing the Information Criterion (3) to detect significant signal changes in each 5x5 pixel region of interest, and to determine at what time that change was recognized after administration of the medication. Summary images were prepared by plotting the cerebral location of the changes so that these could be compared with the images of the surgically resected sites responsible for the epileptic disorders.

Results
All the measurements were performed without any complications. In general, the fMR image intensity was found to be significantly decreased after the administration of DZ and increased following the FMZ administration. In the patients with temporal lobe epilepsy, all the cases demonstrated areas with significant intensity changes ipsilateral to the surgical site. In the cases with focal seizures, the areas which demonstrated significant fMR image intensity changes surrounded the surgical defects, although some areas with significant intensity changes were also seen in the uninvolved cortical gray matter.

Discussion
A novel concept suggested by this work, is that fMRI is capable of demonstrating neuronal activity. This new method of neuro-functional MRI may allow aspects of the function of the neurotransmitter system in humans to be ascertained in vivo. In this respect, a good candidate system for investigation is that of a BZR agonist and its antagonist. BZ affects the GABAergic receptor through allosteric modulation to facilitate GABA’s action on the neuronal synaptic chloride channel. There is evidence that the concentration of the excitatory neurotransmitter, glutamate, gradually increases prior to seizure activity at the epileptic focus, and that this is accompanied by decreasing GABA inhibition in the case of temporal epilepsy. The perifocal areas around the epileptic focus are thought to be accompanied by focally elevated GABAergic activity to prevent ictal attacks in the interictal state as was measured in our study. Our resulting fMR images would, presumably, reflect the elevated GABAergic activity, since both the BZR, and the GABA A receptor, are uniformly distributed in the brain. Our current study was performed safely, for the drug dosages utilized were the minimum single doses in current clinical usage and both of the agents employed have an anti-epileptic effect.

Conclusion
This paper presents a new application of fMRI, neuro-pharmacological fMRI, which appears to be capable of probing the function of the GABAergic neurotransmitter system, in patients with intractable epilepsy. We anticipate that this MRI technique will have broader applications in elucidating the functions of other neurotransmitter systems, as well.

References