Investigating Drug-induced Seizures in Rats using BOLD-fMRI and Cluster Analysis

L. I. Stanberry[1], B. P. Keogh[1], R. R. Nandy[1], D. Cordes[1], K. Maravilla[1]

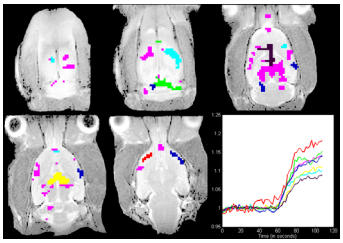
[1]University of Washington, Seattle, WA, United States

Introduction The fundamental question underlying the study of epilepsy is determination of the anatomic location of seizure origination and how neural activation associated with epileptiform discharge propagates through the brain. Functional MRI studies of drug-induced epileptic seizures have unknown, complicated and temporally unstable hemodynamic response patterns. Generalized seizure associated activation, under some circumstances, spreads from an initial focus to encompass some or all of the brain. For accurate investigation of seizure dynamics, conventional model-driven methods are not appropriate. In this paper, we used a Hierarchical Clustering combined with Dendrogram Sharpening. In the context of seizure studies, we seek to cluster voxels according to their temporal responses. We hypothesize that seizure activation has significant spatial and temporal structure that can be grouped into a few types of responses. Subsequently, temporal and spatial characteristics of the obtained clusters can be analyzed with regard to descriptive parameters (magnitude, onset, tissue sensitivity, drug dosage etc.)

Theory Hierarchical clustering combined with dendrogram sharpening [1,2] is a model free approach that does not require prior assumption about the number and location of the clusters. Dendrogram sharpening removes observations from low-density regions producing a clear representation of the modal peaks. The similarity between two voxels is expressed in terms of the correlation coefficient of the corresponding time courses which is then converted into a distance according to **d(i,j)=1 - cc(i,j),** where **cc(i,j)** is the correlation coefficient between voxels i and j. Voxels are grouped into a binary tree using the single linkage method where the distance between two clusters is equal to the minimal distance of all pairs of voxels in the two clusters [3]. In order to make the structure of the data more apparent the tree is pruned by discarding all small-sized children-nodes with a large-sized parent node. Clusters in the modified tree are identified using the method of inconsistent edges, where the value of median edge length of the left (right) subtree plus twice the interhindge spread is the proposed threshold, beyond which edge is considered inconsistent with respect to its left (right) child. Once the cores are identified, voxels discarded during sharpening are assigned to the cluster group, to which they are joined by the link of minimal length.

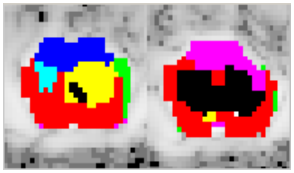
Methods The animals used in this study were adult male, 350-450 gm Sprague-Dawley rats. Rodents were fully anesthetized with halothane. Femoral artery, vein and intraperitoneal catheterization were performed for further drug administration. Blood pressure and heart rate were monitored during the course of the experiment. After a tracheotomy was performed, the animal was paralyzed and mechanically ventilated. Data recording were delayed at least 1 hour after discontinuation of halothane anesthesia to allow adequate wash-out. The convulsant Pentylenetetrazol (PTZ) (Sigma, St. Louis, MO) was given intravenously at 50mg/kg to anesthetized animals. At the end of the experiment the animal was euthanized via established procedures (Nembutal injection). Functional imaging was performed on a 1.5T GE (Milwaukee, WI) MRI scanner equipped with echo-speed gradients and a special rat brain imaging surface coil with session parameters TR/TE 2s/50ms, 128x96 (interpolated to 128x128 upon reconstruction), slice thickness/gap 1mm/0.1mm, 102 time points, 5 axial slices. Extracted images were checked for scanner drift and motion.

Cluster analysis Only voxels with cross-correlation coefficient of at least 0.3 were considered. The amount of voxels was reduced to about 1000. Upon the grouping of the remaining voxels into a binary tree, the dendrogram sharpening was performed twice with parameters: *(fluff-value, core-value)* set to (2,20) and (10,20), respectively, where *fluff-value* is the maximum size of a child cluster that is discarded if it has a parent node of a size greater than a *core-value*. Cluster cores were identified using the method of inconsistent edges. The final classification was run on voxels, set-aside during sharpening, in order to assign them to the found clusters.



Results Intravenous PTZ administration is known to produce synchronous brain activations [4, 5]. Our algorithm produced several clusters (Fig.1). Mean time courses for these five clusters showed rapid (40-60) seconds onset of generalized seizures that began synchronously over the brain. The observed magnitude of the BOLD contrast was on the order of 10%. The difference in the signal magnitude could be attributed in part to heterogeneity of vascular inflow and susceptibility differences in the respected brain regions.

Fig.1 Five color-coded clusters resulting from the cluster analysis combined with the sharpening algorithm are shown overlaid on high-resolution anatomical images. On the last plot, mean time courses, matching in color to the corresponding cluster, show rapid (40-60) secs onset of generalized seizures, which began synchronously over the brain.



In order to examine the progression of seizures, we used an alternative intraperitoneal (IP) administration known to produce gradual activation of the brain. The time to onset of generalized seizures from convulsants given parenterally varies with route, dosage, and drug. Given the onset of seizures after IP administration is slower, we expected to see temporally distinguishable activation areas.

Fig.2 Seven clusters derived from data (IP-PTZ administration). Though spatial location was not included in the analysis, voxels clustered in anatomically cohesive groups (cortex, thalamus, hippocampus, amygdala), suggesting that each tissue has a unique activation behavior. The onset of seizures varied from 2-9min, similar to other published results.

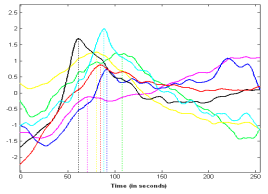


Fig.3 Smooth piecewise-polynomial approximation of the average time series, matching in color to the corresponding cluster, suggests the gradual progress of seizures from one structure to another, with overlap of activation during the process. Though the temporal patterns of the characteristic time courses look somewhat similar, there is significant variability in the magnitude of the signals (time courses shown in black and light blue exhibit identical activation pattern, but the latter achieves a maximum 20s later than the first one). The delay between local maxima for other time courses is on the order of 10s.

Conclusion Our preliminary studies show the ability of hierarchical cluster analysis combined with dendrogram sharpening to differentiate between pharmacologically different seizure events and support the relevance of the neurobiological assumption that the response of a particular region to a convulsant is a function of its anatomy and drug administration. It remains open to investigate whether the observed sequential onset of seizure activity represents propagation or is solely due to the difference in tissue sensitivity and variation in receptors distribution. We also found negatively signed responses in some trials that may be a consequence of brain oxygen demand exceeding vascular reserve

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