

Using fMRI to Elucidate the Pathways Responsible for Seizure Genesis in a Conscious Animal Model.

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Introduction:

Electrophysiologic and lesion structures have identified brain structures important in epileptogenesis. Members of the papez circuit, primarily the anterior thalamus, mammillary bodies, and cingulate cortex are all important structures. The model of general seizure, PTZ, was used to investigate the whole brain during the period of epileptogenesis and these structures do indeed activate first and foremost in the period leading up to a generalized seizure episode. Further studies with an anti-convulsant, ethosuximide, support the importance of the anterior thalamus in this pathway. Paramount to this investigation was the development of a robust and repeatable protocol for inducing seizures while conducting high resolution, multi slice, sub-second fMRI.

Methods:

Male SD rats (N=6) were anesthetized with medetomidine (1mg/Kg, Pfizer) and ketamine (10mg/Kg) IM. An intracerebroventricular cannula was placed in the lateral ventricle and subcutaneous electrodes were placed above the hemispheres. Four animals were placed into their home cage, while 2 animals had a femoral artery cannulated and were placed into a mock MRI environment. After anesthesia was reversed with atipamizole (Pfizer), animals were exposed to PTZ from 1-4mg in 5-40 μ l and time to seizure onset was noted.

For imaging studies, rats (N=6) were prepared as above and placed in an animal restrainer with built in RF electronics (Insight Neuroimaging Sys.). This system was placed in a 4.7T/40cm imager (Bruker) with a 12 cm, 22 G/cm gradient. Anatomical images were acquired; TR= 2s, TE= 48 ms, 8 echos, 10 NEX, 256x256 matrix, 2.8 cm FOV, 6 slices, 1.5 mm slice thickness. BOLD fMRI images were acquired with a spin-echo EPI; TR= 0.6s, TE= 55 ms, 64x64 matrix, 200 repetitions, and same geometry as the above. One hundred acquisitions (1min) were acquired for baseline, at the 100th acquisition, 20 μ l of vehicle (artificial CSF) was injected ICV and another 100 repetitions were acquired. Three additional trials were conducted at 20 minute intervals using ICV PTZ (1.5mg). Six additional animals were imaged as above, but were pre-treated one hour prior to imaging with Ethosuximide (250mg/kg IP).

Dose response, and physiologic data was tabulated. Seventeen bilateral ROIs were defined to cover the brain and raw data for each ROI was averaged together into a composite dataset. Inclusion of every voxel from each brain eliminated sampling bias in the final data set. An ROI covering the injection site was used to synchronize the injection time for each trial as the injection caused rapid hyperintensity of BOLD signal. This analysis was conducted for each cohort; vehicle, PTZ and PTZ + ESM. Retrospective analysis was conducted for vehicle distribution through the ventricles and for activational lateralization.

Results:

Dose response studies determined that 1.5 mg could reproducibly initiate repeated seizures in 30s. Physiology did not alter significantly in the first minute of seizure, except for an increase in the respiration rate in animals treated with PTZ alone. Vehicle injection, which circulated through the ventricular system in 3 seconds, produced no significant activation. PTZ produced robust activations in several structures independent of cannula placement. First and foremost was a rapid and coupled increase in the mammillary bodies, and anterior thalamus within 5 seconds (Fig 1a). The cingulate and hippocampus also activated but the frontal cortex did not. Other areas showed a mild activation including the piriform, temporal, and parietal cortices and the amygdala (Fig 2a). Treatment with ESM produced decreased activation in all regions (Fig 1b, 2b). The anterior thalamus however, still showed the largest increase in BOLD, but the mammillary bodies decoupled from this structure (Fig 1b).

Conclusions:

These data indicate the feasibility of imaging the rapid events of epileptogenesis in conscious animals using ICV PTZ. Seizure produces strong negative BOLD effects, the source of which are uncertain. The papez circuit, and more specifically, the anterior thalamus plays an important role in this seizure genesis as previous studies have also suggested.

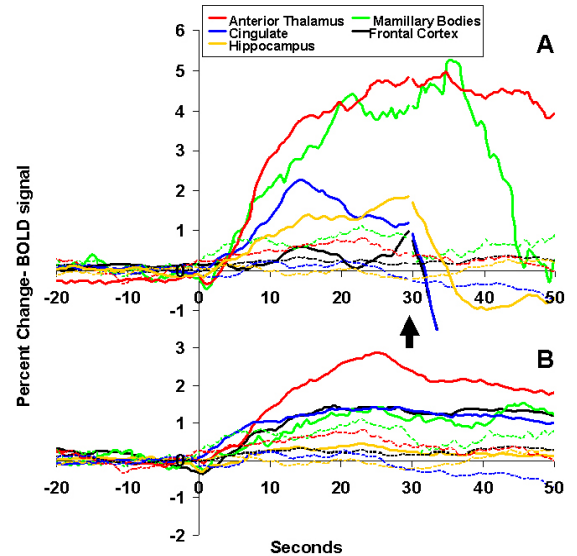


Figure 2: Activation in response to ICV PTZ before (A) and after (B) ESM treatment in significant regions. Arrow indicates seizure onset. Time lines for vehicle are dashed.

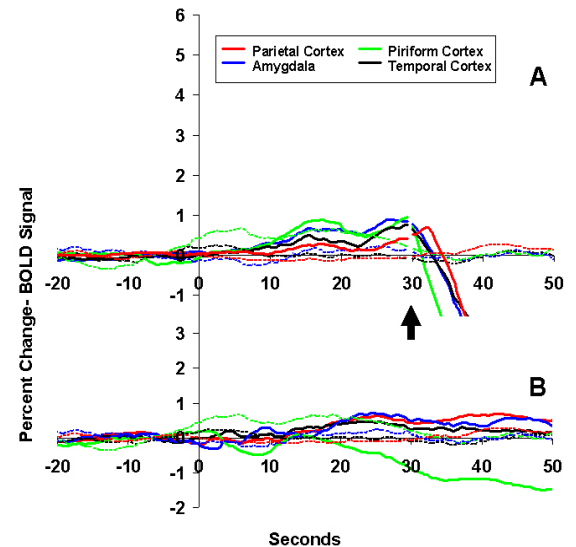


Figure 2: Activation in response to ICV PTZ before (A) and after (B) ESM treatment in areas previously cited as important in seizure.