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Imaging small animal models of epileptogenesis

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Abstract

In-vivo neuroimaging of small animal models of epilepsy offers the ability to serially examine the progressive structural and functional changes that occur during the development of chronic epilepsy (i.e. epileptogenesis). Such approaches allow the correlation of imaging findings to the etiology, development, progression, treatment and prognosis of epilepsy in animal models, associations that are poorly understood clinically. MRI and PET imaging of the kindling and kainic acid models of TLE have begun to piece together the relationship of structural and functional changes in the brain during epileptogenesis and their relationship to seizure and histological outcomes.

Magnetic resonance imaging (MRI) and positron emission tomography (PET) are powerful diagnostic and research tools for *in-vivo* detection of structural and functional brain changes in patients with epilepsy. Well-documented abnormalities are present on these imaging modalities in patients with certain chronic epilepsy syndromes, particularly temporal lobe epilepsy (TLE). The relationship of these abnormalities to the development (i.e. epileptogenesis), progression and prognosis of epilepsy, and their underlying pathophysiological basis are largely unknown. Such questions are difficult to address in clinical studies due to the practical limitations associated with investigating the processes of epileptogenesis in humans, whereby early and pre-epileptic states are largely un-assessable. To date only a few clinical studies have attempted to address the relationship of the neuroimaging abnormalities with epileptogenesis and disease progression.[1,2]

Animal models of epileptogenesis provide a way to circumvent the practical issues associated with human studies by allowing the reproducible induction of epilepsy with short latent periods, reliable quantification of seizure burden and the ability to correlate findings to histological measures. The investigator is in control of the epileptogenic insult making it possible to assess the pre-, developing and chronic epileptic states.

While small animal models of epileptogenesis have been used for a number of years to study the neurobiological basis of epileptogenesis, small animal imaging is a new approach to address this problem. Imaging provides the ability to investigate changes serially *in-vivo*, providing better controls, more statistical power as well as the ability to compare *in-vivo* and *in-vitro* data. For this reason small animal imaging has become an increasingly utilized technique in translational research.

To date a number of studies of *in-vivo* neuroimaging have been published, using both PET and MRI, in animal models of epilepsy.[3-8] The majority have investigated post status epilepticus induced epilepsy, but more recently models incorporating less damaging insults, including kindling and febrile convulsions, have also been studied.[4,5]

To further investigate the relationship of the development of imaging changes and the process of epileptogenesis, we utilised serial MRI and ^{18}F-flurodeoxyglucose PET (FDG-PET) imaging of two rat models of TLE, the amygdala kindling model and the post kainic acid induced status epilepticus (SE) model. These two models were chosen because they are associated with contrasting histological findings; the later being associated with significant neuronal loss and reorganisation in the hippocampus resembling that seen in TLE patients with mesial temporal sclerosis, while the former shows minimal changes[9] as seen in approximately 30% of TLE patients.[10]

METHODS

Four sequential T_2 weighted MR and FDG-PET image acquisitions were acquired over six

weeks in male rats implanted with bipolar MRI compatible electrodes[11] that had either been kindled[12] (n=7), or received kainic acid induced SE[13] (n=4). The acquisitions were: (i) one week after electrode implantation - prior to SE/first electrical stimulation, (ii) one week post SE/24 electrical stimulations, (iii) three weeks after SE/ completion of 48 electrical stimulations, and (iv) five weeks post SE/ two weeks after the final stimulation. Kainic acid animals received an extra imaging session 24hrs following SE. Control animals (n=11) receiving sham stimulations or saline injections were also serially imaged at the corresponding time points to those of their relevant epileptic counterparts.

AMYGDALA KINDLING INDUCES HIPPOCAMPAL SIGNAL INCREASE IN T_2 MRI

As reported in a previous publication, kindled rats were found to show focal regions of increased T_2 signal in the rostral hippocampus ipsilateral to the site of stimulation on the final MR acquisition two weeks post kindling in 5 of 6 rats imaged (Figure 1).[5] The earliest increased T_2 signal was seen after 24 stimulations (1/7 rats), changes were also first seen in fully kindled animals at the third imaging acquisition (3/7 rats). In these cases the T_2 signal changes intensified further on the subsequent MR imaging acquisitions (compare Figures 1A & 1B). Signal increase was observed without concurrent volume change in the hippocampus. No imaging changes were detected in control animals. These findings suggest that T_2 signal changes in the hippocampus can occur independent to cell loss and their variable onset. Furthermore, their persistence following the cessation of the stimulations suggests that the imaging changes and the seizures are not directly related.

AMYGDALA KINDLING INDUCES DECREASED GLUCOSE UPTAKE IN THE HIPPOCAMPUS

On FDG-PET fully kindled rats were found to have decreased glucose uptake in the ipsilateral hippocampus at the end of the kindling period that persisted at the two weeks post-kindling scan (Figure 2). The mean ipsilateral to contralateral hippocampal decrease was 6% (range –11% to 23%). Control animals showed no relative change in hippocampal FDG-PET intensity. These findings indicate that amygdala kindling induces focal hypometabolism in the ipsilateral hippocampus. The persistence of this finding following the cessation of the stimulations suggests that it is not a transient post-ictal effect but likely a permanent epileptogenic change in the metabolic state of the kindled hippocampus.

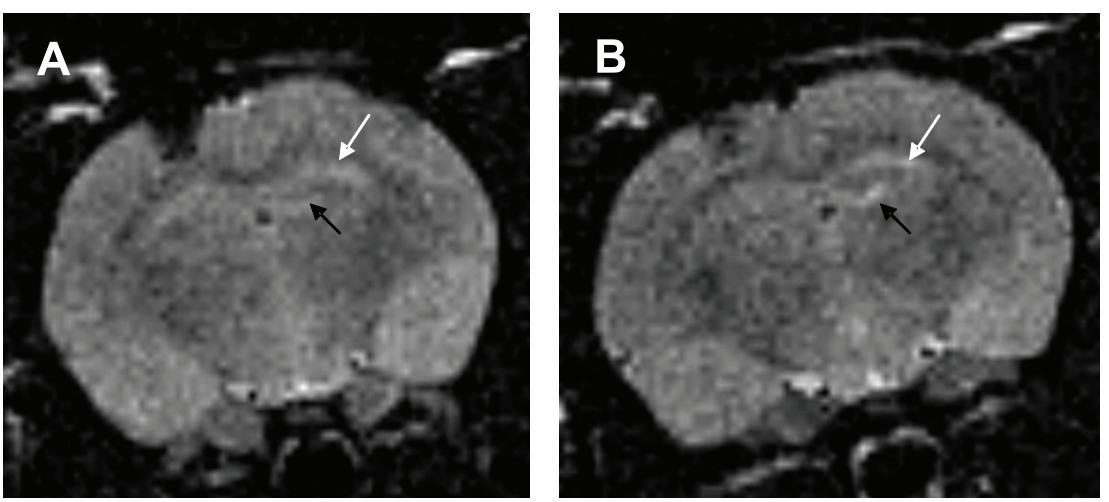

Figure 1: Serial T_2-weighted axial MRI images of a kindled rat brain demonstrating the development of increased T_2 signal intensities in the rostral CA1 (white arrows) and dentate (black arrows) hippocampal regions the ipsilateral to the side of the electrical amygdala stimulations. (A) T_2-weighted MRI following four weeks of stimulations (imaging session three) showing visually apparent increased T_2 signal in the ipsilateral hippocampus. (B) T_2-weighted MRI in the same rat two weeks following the cessation of stimulations showing that the T_2 signal changes in these regions had become more intense. (Modified from Reference 5 with permission).

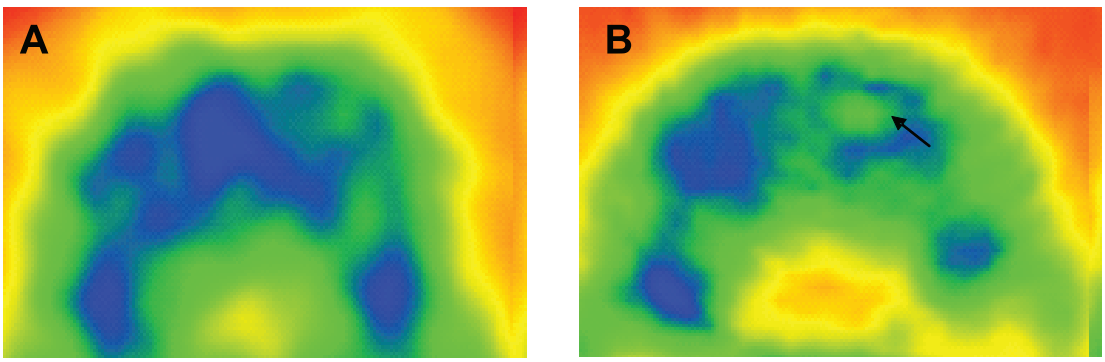

Figure 2: Axial FDG-PET of a kindled rat demonstrating region of decreased glucose uptake in the ipsilateral hippocampus. (A) FDG PET one week following electrode implantation prior to any kindling stimulations. (B) FDG PET two weeks following the cessation of kindling. Note the decreased glucose uptake in the ipsilateral hippocampus (arrow).

KAINIC ACID INDUCED STATUS EPILEPTICUS RESULTS IN PROGRESSIVE VOLUME LOSS IN THE LIMBIC REGIONS

Twenty-four hours following SE rats demonstrated an increased T_2 signal in the limbic regions of the brain, particularly the amgydala and hippocampus (Figure 3A). This signal increase resolved within one week. Following this time point a significant decrease in the volume of the limbic structures (average 13% reduction) was also observed in some rats. The volume of these structures continued to decrease over time (Figure 3B, C, D). These changes were not seen in control animals. These findings demonstrate that the epileptogenic insult initiates a progressive neurodegenerative process in limbic structures. However, animals demonstrating volume loss did not necessarily

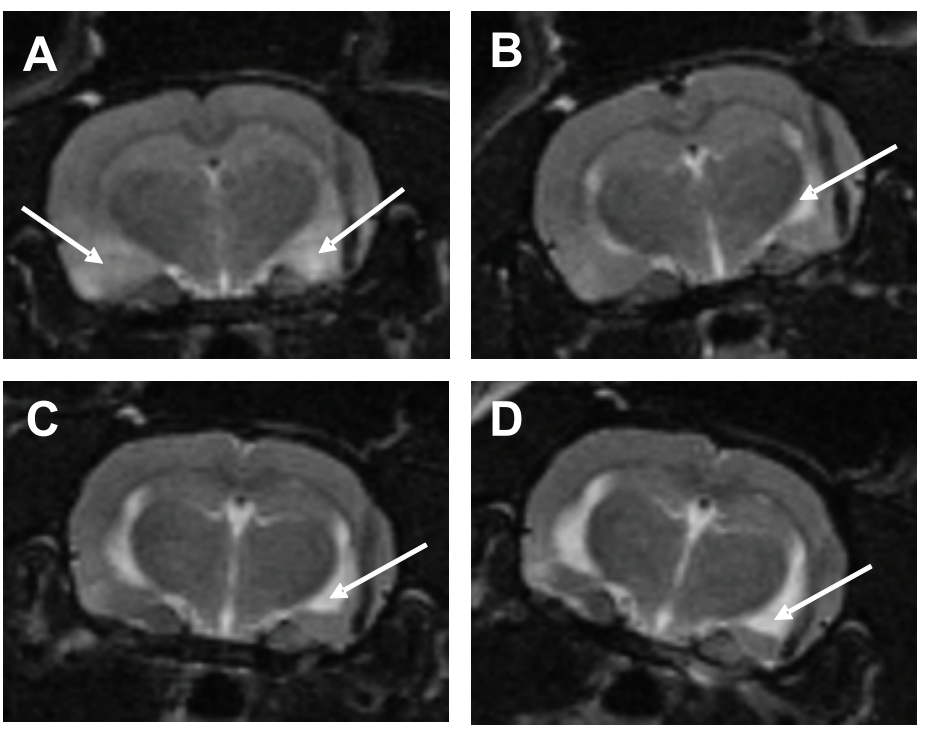

Figure 3: Axial T_2-weighted MRI of a rat following status epilepticus demonstrating volume reduction in the limbic regions of the brain. (A) T_2-weighted MRI 24hrs following status epilepticus. Note the increased signal in the amygdala (arrows). (B, C, D) T_2 weighed MRI 2, 4 and 6 weeks following status epilepticus. Note the increase in ventricular volume (arrows) and decrease in hippocampal and amgydala volumes over time.

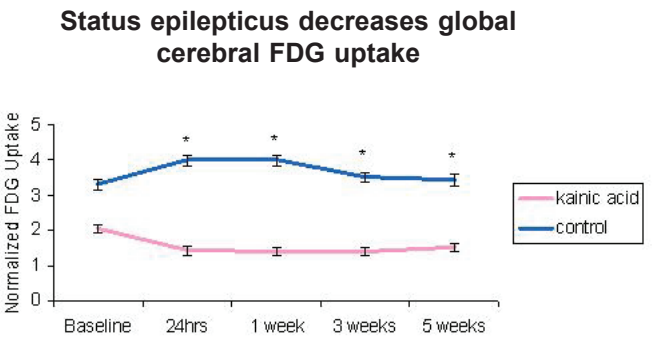

Figure 4: Serial FDG-PET reveals a persistent decrease in normalized glucose uptake globally in the brain from 24 hours following kainic acid induced SE. (*p>0.05 matched pairs t-test). Brain FDG uptake was normalized to bone FDG uptake.

demonstrate seizures, and vice versa, suggesting no direct correlation to the onset of seizures.

KAINIC ACID INDUCED STATUS EPILEPTICUS RESULTS IN GLOBAL CEREBRAL GLUCOSE HYPOMETABOLISM

FDG-PET demonstrated a distinct decrease in total cerebral glucose metabolism 24 hours post SE (average 31% reduction). This hypometabolism remained persistently decreased for the following six weeks (2 weeks 32%, 4 weeks 32%, 6 weeks 26%) (Figure 4). No changes were seen in control animals. The onset of the hypometabolism did not correlate with the presence of spontaneous recurrent seizures and was not affected by the onset of spontaneous recurrent seizures, and occurred prior to the onset of volume loss in limbic structures.

CONCLUSION

The present study has demonstrated that changes in T_2 signal and glucose uptake occur during the process of epileptogenesis in both the kindling and kainic acid models of TLE. These changes do not directly correlate to the onset of seizures or structural volume loss suggesting that they may occur independently or may contribute indirectly to the process of epileptogenesis.

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