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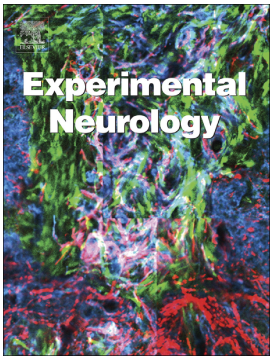

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Functional Imaging of the Piriform Cortex in Focal Epilepsy

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Summary:
Experiments in animal models have identified specific brain regions such as the deep anterior piriform cortex as important for controlling the initiation or propagation of both generalized and focal seizure activity. However, there is little experimental evidence to translate these observations to the control of focal seizures in humans. Here, we summarize findings from different hemodynamic and neurotransmitter functional imaging studies in groups of patients with focal epilepsies arising from different cortical locations in support of a common area of brain dysfunction in focal epilepsies.

Keywords: focal epilepsy, Flumazenil-PET, BOLD, EEG/fMRI, subcortical

Abbreviations: EEG, electroencephalography; MRI, magnetic resonance imaging; fMRI, functional MRI; EEG/fMRI, EEG combined with simultaneous fMRI; PET, positron emission tomography; FMZ, flumazenil; FMZVD, FMZ volume of distribution; IED, interictal epileptic discharge; GABA, gamma aminobutyric acid;

Introduction

In animal models and human patients , electrical discharges oscillate between cerebral cortex, thalamus and basal ganglia during generalized epileptic seizures (Depaulis et al., 1994; Deransart et al., 1998; Piredda and Gale, 1985; Steriade, 2005). During focal cortical seizure activity, specific cortical-subcortical circuits contribute to sustaining and propagating the seizure discharge. Experiments in animal models have identified the substantia nigra and the deep anterior piriform cortex as important brain regions for controlling the initiation or propagation of both generalized and focal seizure activity (Biraben et al., 2004; Bouilleret et al., 2005; Depaulis et al., 1994; Deransart et al., 1998; Piredda and Gale, 1985). In rats and monkeys, a discrete site within the deep piriform (olfactory) cortex, termed *area tempestas* or "ventrostriatal anterior piriform cortex", is critical for modulating focal seizures (Ekstrand et al., 2001; Piredda and Gale, 1985). However, there is little experimental evidence to translate these observations to the human situation (Blumenfeld et al., 2004). Observations during deep brain stimulation in a variety of subcortical structures suggest that the circuitry has the potential to be harnessed for therapeutic benefit in patients with epilepsy (Morrell, 2006).

Positron emission tomography

Akin to Karen Gale's experiments in non-human primates, GABA-mediated mechanisms ,common to focal epilepsies, were studied *in vivo* by performing a group analysis of carbon-11 labeled flumazenil (FMZ) positron emission tomography (PET) studies in 18 patients with different extra-temporal epilepsy syndromes. (Laufs et al, 2011) Compared to 24 controls, FMZ volume of distribution (V_T) was significantly increased in the putamen bilaterally. Restricting the analysis only to those regions identified in the first analysis, the difference in FMZ-V_T was correlated with seizure-frequency as a measure of epilepsy severity: the higher the FMZ-V_T the lower was the seizure frequency over the preceding month in an area in the vicinity of the deep anterior piriform cortex, adjacent to putamen and claustrum.

These results are concordant with chemical stimulation of this region in the deep piriform cortex through unilateral microinjection of a GABA receptor antagonist or glutamate receptor agonists, which triggered limbic motor seizures in the rat (Piredda and Gale, 1985; Doherty et al., 2000) and limbic seizures and status epilepticus in non-human primates (Gale, 1995; Gunderson et al., 1999). Local application of the GABA agonist muscimol prevented seizures, which would have occurred following microinjection of all convulsant agents examined (Piredda and Gale, 1985). Immunostaining revealed particular features of this site that could alter excitability, including a near-absence of GABAergic "cartridge" endings on axon initial

segments, and very low gamma-aminobutyric acid transporter-1 (GAT1)-like immunoreactivity. Normally, the function of this area may be to shape neuronal activity through inhibitory processes so that this region is no longer susceptible to pathological behaviour (Ekstrand et al., 2001). Increased FMZ-V_T in this area and the observed association of increased binding with a reduced seizure frequency might reflect compensatory mechanisms of seizure suppression: postsynaptic increases in the number of $GABA_A$ receptors have been described in the kindling model underlying inhibitory potentiation (Nusser et al., 1998). Such an increase in post-synaptic binding sites will lead to a measurable increase in FMZ-V_T. Similarly, pre- and postsynaptic changes of GABA transmission involving changes of $GABA_A$ ß receptor subunit composition have been found using the pilocarpine model (Brooks-Kayal et al., 1998).

In pre-surgical patients monitored continuously with video-EEG telemetry in the week prior to their FMZ-PET scan, the reduction of FMZ-V_T in the mesial temporal structures was greatest the closer the PET scan was performed following a seizure. (Bouvard et al., 2005) The authors did not specifically report on sub-cortical changes, but abnormalities can be seen not only in area of the piriform cortex, but also in the brainstem, possibly substantia nigra. As in the Laufs study, the greater the increase in FMZ binding in the piriform cortex, the fewer the seizures were observed.

Functional Magnetic Resonance Imaging

Akin to the group analysis of FMZ-PET studies, Laufs and colleagues (Laufs et al, 2011) performed a group analysis of functional MRI (fMRI) studies acquired with simultaneous electroencephalography (EEG) recordings in patients with clear-cut focal epilepsies arising from a wide variety of cortical locations. In essence, fMRI was correlated with a measure of epilepsy activity, i.e. the interictal epileptic discharge (IED), with the aim to detect hemodynamic changes common to all patients, but independent of the site of focal IEDs.

The fMRI data from 19 patients with focal epilepsies was analyzed in an event-related fashion (Salek-Haddadi et al., 2006). Patients were selected from a larger pool of 63 patients on the basis of a spiking rate in the mid-range level of activity, between one and 20 IED per minute. This was done to ensure a balanced design by only including patients with a similar number of IED (Friston et al., 2005; Laufs et al., 2007).

To test for any common patterns across the group of patients, a random effects model was used to identify any typical responses consistent across patients (Friston et al., 1999b) and to test the hypothesis of activation in the region of the presumed *area tempestas*. Accordingly,

bilateral 0.7 cm x 0.7 cm x 0.7 cm search volumes (2744 mm^3) were each centred between the tip of the temporal pole and the orbitofrontal gyrus based on the aneurysm case report of Mizobuchi et al. (Mizobuchi et al., 1999). FMRI signal changes within in these regions were considered significant at $P<0.05$ corrected for multiple comparisons. In addition, positive responses were explored across the whole brain at a significance threshold of $P<0.001$ (uncorrected at the voxel level).

There was a significant correlation between IED and haemodynamic response ($p<0.05$ corrected for multiple comparisons) common to all 19 patients in an area in the vicinity of the deep anterior piriform cortex, adjacent to putamen and claustrum on the same side as the presumed cortical epileptic focus. These findings (Laufs et al. , 2011, fig 1A) were replicated in three large studies from Canada (Fahoum et al. 2012, fig 1B), Brazil (Coan et al 2014, fig 1C) and Australia (Flanagan et al. 2014, fig 1D). All these studies found a common brain region on fMRI that was recruited during epileptiform discharges despite having variable seizure foci including both temporal lobe epilepsy and extratemporal focal epilepsy patients. The brain area common to focal epilepsy patients in all of these studies was the ipsilateral piriform cortex.

Figure 1 - here

An analysis using task-free MRI was performed in 14 patients with extra-temporal focal epilepsy to calculate voxel-wise regional connectivity with regional homogeneity (ReHo) and weighted degree centrality (DCw). (Pedersen et al., 2016) Despite heterogenous sites of seizure origin, these patients had common areas of abnormality:

- increased regional network connectivity was observed in the ipsilateral piriform cortex, insula, and thalamus, in addition to the dorsal anterior cingulate cortex and lateral frontal cortices.
- decreased regional connectivity was observed in the ventromedial prefrontal cortex, as well as lateral temporal cortices.

Proof of concept: post-operative seizure outcome

A case report of a patient with seizure relapse three years following an initially successful right temporal lobectomy for ipsilateral medial temporal sclerosis, was the first to demonstrate a potential role for the piriform cortex/area tempestas for generating or modulating seizure activity in humans, if not completely resected. Interictal EEG–fMRI revealed significant

BOLD signal changes over the inferior, basal and lateral temporal and temporooccipital cortices posterior to the resection margin, plus a significant BOLD signal change over the ipsilateral basal frontal region, closely corresponding to the piriform cortex. (K. Garganis et al; 2013)

The current assumption is that successful epilepsy surgery depends on the complete removal of the tissue that is involved in seizure generation, and thus, inclusion of the piriform cortex within the temporal lobe resection would provide proof-of-concept for the clinical relevance of this area.

In such a proof-of-concept study, Galovic et al (2019) correlated the extent of surgical resection with postoperative outcome in 107 drug-refractory TLE patients. Using voxel-based morphometry comparing MRI before and after standard anterior 2/3 temporal lobe resection and correlating the difference with post-operative outcome, seizure-free patients showed more pronounced grey matter reductions in the ipsilateral piriform cortex than patients who suffered from postoperative seizures. Comparing the volumes of the piriform cortex and three other mesiotemporal regions involved in TLE (hippocampus, amygdala, entorhinal cortex), a preoperatively reduced volume of the ipsilateral piriform cortex was significantly ($p=0.02$) associated with postoperative seizures, whereas there was no association for the hippocampus, amygdala, or entorhinal cortex between resection volume and seizure outcome.

Comparing directly the extent of resection between the two outcome groups, a significantly larger proportion of the piriform cortex had been resected in seizure-free patients compared to the surgically refractory group (83% vs. 52%, $p<0.001$). In contrast, the resected proportion of the hippocampus, amygdala, or entorhinal cortex and the overall resection volume did not correlate with postsurgical outcome.

Resected proportion of the piriform cortex was a good predictor of postoperative seizure outcome in individual patients with an area under the receiver operating characteristics curve (AUC) of 0.80 ($p<0.001$, left AUC 0.82, right AUC 0.77). Only 7% (2/30 patients) became seizure free if less than 50% of the piriform cortex were resected, compared to 57% (44/77 patients) if ≥ 50% were removed. Resection of at least half of the piriform cortex increased the odds of complete seizure-freedom by a factor of 19 (95% confidence interval [CI] 4-84).

Figure 2 - here

Discussion

The ipsilateral piriform cortex is likely to constitute an important node in focal epilepsy. This area corresponds in location to the physiologically defined "deep piriform cortex" (or *area tempestas*) from which pro-convulsants initiate temporal lobe seizures (Fornai et al., 2005; Maggio et al., 1993), and blockade of glutamate (Fornai et al., 2005; Millan et al., 1986; Piredda and Gale, 1985; Piredda and Gale, 1986) or application of a GABA agonist in this area (Piredda and Gale, 1986) reduce limbic motor seizures.

Signal changes common to all cases were detected by averaging imaging data across a group of patients with different sites of seizure onset. Inherent to the group analyses performed in the studies reported here, signal changes associated with varying sites of seizure onset are eliminated. There is likely to be considerable individual variability in potential “epileptogenic networks”, but some areas are common to all networks. The piriform cortex was altered in focal epilepsy subjects, whether temporal or extra-temporal lobe epilepsy, and regardless of the functional imaging method used, be it FMZ-PET imaging the major inhibitory neurotransmitter system or EEG-fMRI and task—free resting-state fMRI analysed with various regional connectivity methods: in this region close to the human frontal olfactory cortex, cerebral blood flow correlates with interictal EEG spikes, and benzodiazepine-$GABA_A$ receptor complex expression with seizure frequency. This region closely corresponds to a zone, referred to as *area tempestas*, which is highly epileptogenic in rodents and non-human primates. Taken together, previous functional imaging findings and the most recent post-operative results provide compelling evidence for a critical epileptic area in the human piriform cortex, i.e. the possible location of a human area tempestas.

The piriform cortex is the most susceptible area for epileptogenic stimulation (Gale 1988, Piredda and Gale, 1985; McIntyre DC and Kelly ME, 2000; Roch C et al, 2002; Vaudano et al, 2012) and a node for the spread of epileptic discharges in TLE (Laufs et al, 2011; Vaughan and Jackson, 2014; Flanagan et al, 2014) Hence, we presume that seizures originating from different parts of the temporal lobe can lead to an extension of the epileptic network into the piriform cortex (Roch et al, 2002, 2007; Loescher and Ebert, 1996) Such a spread of the epileptogenic zone, indicated by a presurgical volume-loss in the ipsilateral piriform cortex, was prognostic of poor postsurgical outcome.

Furthermore, if the epileptic network in the piriform cortex is not sufficiently disrupted by removing at least half of this area, a patient is almost 20-times more likely to suffer seizures postoperatively. Resection of at least 50% of the piriform cortex is a prerequisite to achieve postoperative seizure-freedom in most TLE patients. The extent of piriform cortex resection

was prognostic of postsurgical seizure-outcome and it explained one third of outcome variability. In line with previous research[20], other mesiotemporal regions were not prognostic. Removal of the piriform cortex was not associated with negative neuropsychological and psychiatric postsurgical outcome. Further prospective studies, using both structural data and diffusion tensor imaging / tractography are required to correlate the extent of resection at the time of surgery with both neuropsychological as well as seizure outcome. Ideally, together with repeat post-operative imaging studies in short intervals, this will allow to determine, whether the observed association between size of the post-operative piriform remnant and seizure outcome, is due to the surgical resection, or consecutive Wallerian degeneration in those who are post-operatively seizure-free.

Whilst the reported imaging findings suggest that this area may contribute to seizure modulation and that it may be an attractive target for neurosurgical or targeted pharmacological epilepsy therapy, placement of electrodes for either recording or stimulation is difficult due to the close proximity to large vessels as well as anterior perforating arteries. This area is usually avoided by the neurosurgeon because of the high risk of vascular accidents during surgery. To date, planned and implemented trajectories for stereotactic approaches have been straight line of sight. Work on nonlinear trajectories and appropriate delivery methods are required to target brain areas, like the piriform cortex, that are not otherwise accessible.

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Legends:

Figure 1: Group findings of EEG-fMRI studies from London (A), Montreal (B), Campinas (C) and Melbourne (D) A: Results of group analysis for EEG-fMRI (yellow) and correlation between FMZ binding and seizure frequency (blue) are superimposed on T1 template. B-D: group analysis of EEG-fMRI (B: Fahoum et al, 2012; C= Coan et al, 2014; D: Flanagan et al, 2014)

Figure 2: Effect of resection of piriform cortex and four temporal areas on seizure outcome after anterior temporal lobe resection. The position of the left and right piriform cortex is illustrated using 3D reconstructions in panel (A).

Volumetric results in panel (B) are displayed as median and standard deviation (vertical lines) of the resected proportion of the piriform cortex, hippocampus, amygdala, and entorhinal cortex and the overall resection volume in the overall cohort (n=107). Comparing postoperatively seizure-free (SF, n=46) with non-seizure-free (NSF, n=61) patients, there was a significant (***, $p<0.001$) difference in piriform cortex volumes whereas no differences were observed in all other regions.

References

Biraben A, Semah F, Ribeiro MJ, Douaud G, Remy P, Depaulis A. PET evidence for a role of the basal ganglia in patients with ring chromosome 20 epilepsy. Neurology 2004; 63: 73-7.

Blumenfeld H, McNally KA, Vanderhill SD, Paige AL, Chung R, Davis K, et al. Positive and negative network correlations in temporal lobe epilepsy. Cereb Cortex 2004; 14: 892-902.

Bouilleret V, Semah F, Biraben A, Taussig D, Chassoux F, Syrota A, et al. Involvement of the basal ganglia in refractory epilepsy: an 18F-fluoro-L-DOPA PET study using 2 methods of analysis. J Nucl Med 2005; 46: 540-7.

Bouvard S, Costes N, Bornefoi F, Lavenne F, Mauguière F, Delforge J, Ryvlin P. Seizure-related short-term plasticity of benzodiazepine receptors in partial epilepsy: a [11C]flumazenil-PET study. Brain. 2005 Jun;128(Pt 6):1330-43

Brooks-Kayal AR, Shumate MD, Jin H, Rikhter TY, Coulter DA. Selective changes in single cell GABA(A) receptor subunit expression and function in temporal lobe epilepsy. Nat Med 1998; 4: 1166-72.

Centeno, M. et al. Structural changes in the temporal lobe and piriform cortex in frontal lobe epilepsy. Epilepsy Res 108, 978–981 (2014).

Coan AC, et al. Distinct functional and structural MRI abnormalities in mesial temporal lobe epilepsy with and without hippocampal sclerosis. Epilepsia, 55(8):1187–1196, 2014

Depaulis A, Vergnes M, Marescaux C. Endogenous control of epilepsy: the nigral inhibitory system. Prog Neurobiol 1994; 42: 33-52.

Deransart C, Vercueil L, Marescaux C, Depaulis A. The role of basal ganglia in the control of generalized absence seizures. Epilepsy Res 1998; 32: 213-23.

Doherty J, Gale K, Eagles DA. Evoked epileptiform discharges in the rat anterior piriform cortex: generation and local propagation. Brain Res 2000; 861: 77-87.

Ekstrand JJ, Domroese ME, Johnson DM, Feig SL, Knodel SM, Behan M, et al. A new subdivision of anterior piriform cortex and associated deep nucleus with novel features of interest for olfaction and epilepsy. J Comp Neurol 2001; 434: 289-307.

Fahoum F, Lopes R, Pittau F, Dubeau F, Gotman J. Widespread epileptic networks in focal epilepsies: EEG-fMRI study. Epilepsia. 2012 Sep;53(9):1618-27Fahoum et al. 2012

Flanagan D, Badawy RAB, Jackson GD. EEG-fMRI in focal epilepsy: local activation and regional networks. Clin Neurophysiol 2014;125, 21–31

Fornai F, Busceti CL, Kondratyev A, Gale K. AMPA receptor desensitization as a determinant of vulnerability to focally evoked status epilepticus. Eur J Neurosci 2005; 21: 455-63.

Friston KJ, Worsley KJ, Frackowiak RSJ, Mazziotta JC, Evans AC. Assessing the significance of focal activations using their spatial extent. Human Brain Mapping 1994; 1: 210-20.

Gale K. Chemoconvulsant seizures: advantages of focally-evoked seizure models. Ital J Neurol Sci 1995; 16: 17-25.

Galovic M, Baudracco I, Wright-Goff E, Pillajo G, Nachev P, Wandschneider B, et al, Association of Piriform Cortex Resection With Surgical Outcomes in Patients With Temporal Lobe Epilepsy. JAMA Neurol. 2019 Jun 1;76(6):690-700

Garganis K et al; EEG–fMRI findings in late seizure recurrence following temporal lobectomy: A possible contribution of area tempestas. Epilepsy & Behavior Case Reports 2013;157–160

Gunderson VM, Dubach M, Szot P, Born DE, Wenzel HJ, Maravilla KR, et al. Development of a model of status epilepticus in pigtailed macaque infant monkeys. Dev Neurosci 1999; 21: 352-64.

Laufs H, Hamandi K, Salek-Haddadi A, Kleinschmidt AK, Duncan JS, Lemieux L. Temporal lobe interictal epileptic discharges affect cerebral activity in "default mode" brain regions. Hum Brain Mapp 2007; 28: 1023-32.

Laufs, H. et al. Converging PET and fMRI evidence for a common area involved in human focal epilepsies. Neurology 2011; 77, 904–910

Maggio R, Lanaud P, Grayson DR, Gale K. Expression of c-fos mRNA following seizures evoked from an epileptogenic site in the deep prepiriform cortex: regional distribution in brain as shown by in situ hybridization. Exp Neurol 1993; 119: 11-9.

McIntyre DC, Kelly ME. The parahippocampal cortices and kindling. *Ann. N. Y. Acad. Sci.* 2000; 911, 343–354

Millan MH, Patel S, Mello LM, Meldrum BS. Focal injection of 2-amino-7-phosphonoheptanoic acid into prepiriform cortex protects against pilocarpine-induced limbic seizures in rats. Neurosci Lett 1986; 70: 69-74.

Mizobuchi M, Ito N, Tanaka C, Sako K, Sumi Y, Sasaki T. Unidirectional olfactory hallucination associated with ipsilateral unruptured intracranial aneurysm. Epilepsia 1999; 40: 516-9.

Morrell M. Brain stimulation for epilepsy: can scheduled or responsive neurostimulation stop seizures? Curr Opin Neurol 2006; 19: 164-8.

Nusser Z, Hajos N, Somogyi P, Mody I. Increased number of synaptic GABA(A) receptors underlies potentiation at hippocampal inhibitory synapses. Nature 1998; 395: 172-7.

Pedersen, M., Curwood, E. K., Vaughan, D. N., Omidvarnia, A. H. & Jackson, G. D. Abnormal Brain Areas Common to the Focal Epilepsies: Multivariate Pattern Analysis of fMRI. *Brain Connect* 2016; **6,** 208–215

Piredda S, Gale K. A crucial epileptogenic site in the deep prepiriform cortex. Nature 1985; 317: 623-5.

Piredda S, Gale K. Anticonvulsant action of 2-amino-7-phosphonoheptanoic acid and muscimol in the deep prepiriform cortex. Eur J Pharmacol 1986; 120: 115-8.

Roch C, Leroy C, Nehlig A, Namer IJ. Predictive value of cortical injury for the development of temporal lobe epilepsy in 21-day-old rats: an MRI approach using the lithium-pilocarpine model. *Epilepsia* 2002; 43, 1129–1136.

Roch C, Leroy C, Nehlig A, Namer IJ. Magnetic resonance imaging in the study of the lithium-pilocarpine model of temporal lobe epilepsy in adult rats. *Epilepsia* 2002; 243, 325–335

Salek-Haddadi A, Diehl B, Hamandi K, Merschhemke M, Liston A, Friston K, et al. Hemodynamic correlates of epileptiform discharges: an EEG/fMRI study of 63 patients with focal epilepsy. Brain Res 2006; 1088: 148-66.

Steriade M. Sleep, epilepsy and thalamic reticular inhibitory neurons. Trends Neurosci 2005; 28: 317-24.