ANA CAROLINA COAN

CLINICAL AND BRAIN STRUCTURAL AND FUNCTIONAL DIFFERENCES BETWEEN MESIAL TEMPORAL LOBE EPILEPSIES WITH AND WITHOUT HIPPOCAMPAL SCLEROSIS

DIFERENÇAS CLÍNICAS E DE ALTERAÇÕES CEREBRAIS ESTRUTURAIS E FUNCIONAIS ENTRE EPILEPSIAS DE LOBO TEMPORAL MESIAL COM E SEM SINAIS DE ESCLEROSE HIPOCAMPAL

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Orientador: Prof. Dr. Fernando Cendes

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Tese de Doutorado apresentada ao Programa de Pós Graduação em Fisiopatologia Médica da Faculdade de Ciências Médicas da Universidade Estadual de Campinas para obtenção do título de Doutora em Fisiopatologia Médica, área de concentração Neurociências.

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Leonardo da Vinci

SUMÁRIO

ELTM: epilepsia de lobo temporal mesial

ILAE: *International League Against Epilepsy*

EH: esclerose hipocampal

DCF: displasia cortical focal

RM: ressonância magnética

CPS: crise parcial simples

CPC: crise parcial complexa

CTCG: crise tônico-clônico generalizada

EEG: eletroencefalografia

CF: convulsão febril

DAE: droga anti-epiléptica

ELTM-EH: ELTM com sinais de esclerose hipocampal em exames de RM

ELTM: ELTM com RM normal

T: tesla

VBM: *voxel based morphometry*

SC: substância cinzenta

RMf: ressonância magnética funcional

DMN: default mode network

BOLD: *blood oxygen level dependent*

DEI: descargas epilépticas interictais

PS: período silente

RR: remitente-recorrente

TR: tempo de repetição

TE: tempo de eco

FOV: field of view

FLAIR: *fluid acquisition inversion recovery*

DP: desvio-padrão

MNI: *Montreal Neurologic Institute*

DARTEL: *diffeomorphic anatomical registration using exponentiated lie algebra*

FWE: *family wise error*

SPM8: *Statistical Parametric Mapping 8*

EPI: *eco planar imaging*

AAS: *average artifact subtraction*

MLG: modelo linear geral

BOLDpos: BOLD positivo

BOLDneg: BOLD negativo

LISTA DE ANEXOS

Resumo

Introdução: A epilepsia de lobo temporal mesial (ELTM) não é uma doença única, mas um conjunto de diferentes síndromes com etiologias diversas, que têm uma apresentação clínica e eletroencefalográfica comum. A compreensão dos diferentes tipos de ELTM é fundamental para o desenvolvimento de terapêuticas adequadas e individualizadas.

Objetivo: Avaliar e comparar a ocorrência de alterações estruturais e funcionais na ELTM com (ELTM-EH) e sem (ELTM-NL) sinais de esclerose hipocampal (EH) nas imagens de ressonância magnética (RM) e relacionar essas alterações com a resposta ao tratamento.

Métodos: Pacientes com diagnóstico de ELTM, e sem lesões estruturais exceto por sinais de EH, foram avaliados através de dados clínicos e por exames de RM de 3Tesla estruturais e funcionais. Os pacientes foram classificados em ELTM com (ELTM-EH) ou sem (ELTM-NL) sinais de EH através da quantificação de volume e sinal do hipocampo. Quantificação do volume da amígdala também foi realizada. Análise de volume da substância cinzenta (SC) cerebral foi realizada através da técnica de Morfometria Baseada em Voxel (VBM). Análise de alterações funcionais relacionadas às descargas epilépticas interictais (DEIs) foi realizada com o uso concomitante de EEG e RM funcional (EEG-RMf).

Resultados: A quantificação de volume e sinal hipocampal nos exames de RM de 203 pacientes com ELTM aumentou em 28% a sensibilidade de detecção de sinas de EH em comparação com a análise visual. Subgrupos de pacientes com ELTM-EH e ELTM-NL e hipertrofia de amígdala foram observados. Após exclusão de pacientes com a lateralidade do foco epiléptico indefinida, 172 pacientes (122 ELTM-EH e 50 ELTM-NL) foram avaliados clinicamente e pela técnica de VBM. O grupo ELTM-NL apresentou idade de início de crises mais elevada e menor duração da epilepsia, além de antecedente familiar de

epilepsia mais frequente do que ELTM-EH. ELTM-EH e ELTM-NL apresentaram atrofia de SC difusa, incluindo tálamos e córtex sensório motor bilaterais. Diferentemente do grupo ELTM-EH, os pacientes com ELTM-NL não apresentaram atrofia em regiões temporais e apresentaram atrofia pronunciada em córtex órbito-frontal ipsilateral ao foco epiléptico. A subdivisão dos grupos de acordo com a resposta à droga antiepiléptica (DAE) revelou atrofia de SC difusa em ELTM-EH benignos e refratários, a pesar do segundo grupo apresentar atrofia mais pronunciada principalmente em áreas sem conexões diretas com o hipocampo. Diferentemente, atrofia de SC foi observada apenas nos pacientes com ELTM-NL e crises refratárias. As redes neuronais funcionais relacionadas com as DEIs deferiram entre os grupos ELTM-EH e ELTM-NL e foram distintas das redes estruturais detectadas pelo VBM. Nos exames funcionais, em ambos os grupos, supressão da atividade em áreas da Default Mode Network foi observada concomitantemente às DEIs e esse padrão foi relacionado a melhor prognóstico cirúrgico em pacientes com crises refratárias.

Conclusão: Alterações estruturais e funcionais são distintas em ELTM-EH e ELTM-NL. Diferentes redes neuronais estão relacionadas ao prognóstico clínico e cirúrgico na ELTM. Conhecimento detalhado das redes neuronais envolvidas nos diversos tipos de ELTM e da interação dinâmica entre elas deve contribuir para o aprimoramento do tratamento desses pacientes.

Palavras-chave: Imagem por Ressonância Magnética; Eletroencefalografia; Redes Neurais; Prognóstico.

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Abstract

Introduction: Mesial temporal lobe epilepsy (MTLE) is not a single disease but a group of different diseases with distinct etiologies that share common clinical and EEG characteristics. Understanding the different types of MTLE is fundamental to the development of more appropriate and individualized therapies for ictal phenomena and comorbidities of each patient.

Objective: To evaluate and compare the occurrence of structural and functional abnormalities of MTLE with (MTLE-HS) and without (MTLE-NL) signs of hippocampal sclerosis (HS) in magnetic resonance imaging (MRI) and to correlate these abnormalities with the response to treatment.

Methods: Patients diagnosed with MTLE defined by clinical and electroencephalographic, and without structural lesions except for signs of HS were evaluated with clinical data and structural and functional 3T MRIs. Patients were classified as MTLE with (MTLE-HS) or without (TLE-NL) signs of HS by quantifying and hippocampal volume and signal. Amygdala volume quantification was also performed. Analysis of volume of brain gray matter (GM) of both groups was performed using the technique of voxel-based morphometry (VBM). Analysis of functional changes related to interictal epileptic discharges (IED) in both groups was performed with concomitant use of EEG and functional MRI (EEG-fMRI).

Results: The quantification of volume and hippocampal signal in MRI scans of 203 patients with MTLE increased in 28% the sensitivity of detecting signs of HS compared with the visual analysis. Subgroups of patients with MTLE-HS and MTLE-NL and amygdala hypertrophy were observed. After exclusion of patients with undefined or bilateral epileptic focus, a group of 172 patients (122 ELTM-HS and 50 ELTM-NL) were evaluated with VBM technique. Patients with MTLE-NL had higher age of epilepsy onset

and shorter duration of epilepsy as well as more frequent family history of epilepsy than patients with MTLE-HS. MTLE-HS and MTLE-NL showed diffuse GM atrophy, including bilateral sensorimotor cortex and thalamus. Different from MTLE-HS group, patients with MTLE-NL showed no atrophy in mesial and neocortical temporal regions and had pronounced atrophy in the orbito-frontal cortex ipsilateral to the epileptic focus. The subdivision of the groups according to the response to antiepileptic drug (AED) revealed diffuse GM atrophy in both benign and refractory and MTLE-HS, despite the second group exhibit more pronounced atrophy specially in areas with no direct connections with the hippocampus. Differently, GM atrophy was observed only in patients with MTLE-NL and refractory seizures. The functional neuronal networks related to IED were different in MTLE-HS and MTLE-NL groups and were distinct from the structural networks detected by VBM technique. Functional analysis revealed in both groups suppression of activity in brain areas compatible with the Default Mode Network (DMN) concomitantly with IED and this pattern was related to better surgical outcome in patients with AED resistant seizures.

Conclusion: Structural and functional networks abnormalities are distinct in MTLE-HS and MTLE-NL. Different neural networks are related to surgical and clinical prognosis in MTLE. Detailed knowledge of the neural networks involved in various types of MTLE and the dynamic interaction between them might contribute to improving the treatment of seizures and comorbidities in these patients.

Keywords: agnetic Resonance Imaging; Electroencephalography; Neural Networks; Prognosis.

1. Introdução

1.1. Epilepsia de Lobo Temporal Mesial (ELTM)

A epilepsia de lobo temporal mesial (ELTM) é definida pela Liga Internacional Contra Epilepsia (International League Against Epilepsy – ILAE) como epilepsia focal com características semiológicas e eletroencefalográficas que sugerem início de crises localizado nas regiões temporais mesias (1). A ELTM é a mais prevalente epilepsia focal no adulto (2). ELTM, no entanto, não é uma doença única, mas um conjunto de diferentes patologias com etiologias diversas, que dividem uma apresentação clínica e eletroencefalográfica comum.

O substrato patológico mais comum da ELTM é a esclerose hipocampal (EH), que corresponde a cerca de 70% das ELTM do adulto (3). Outras causas de ELTM incluem lesões estruturais diversas localizadas nos lobos temporais, como tumores, displasias corticais focais (DCF) ou mal formações vasculares. No entanto, cerca de 20% dos pacientes com diagnóstico de ELTM apresentam exames de ressonância magnética (RM) normais (ELTM criptogênica) (4). Os subtipos de ELTM podem apresentar respostas diversas aos tratamentos medicamentoso e cirúrgico, além de evolução e incidência de comorbidades distintas.

A compreensão adequada dos diferentes tipos de ELTM, suas evoluções e resposta ao tratamento são fundamentais para identificação dos mecanismos envolvidos na patogênese de cada uma dessas epilepsias, o que deve propiciar o desenvolvimento de terapêuticas mais adequadas e individualizadas para os fenômenos ictais e comorbidades de cada paciente.

1.2. Características clínicas e eletroencefalográficas da ELTM

Como uma síndrome clínica e eletroencefalográfica, a ELTM apresenta características específicas de semiologia de crises e anormalidades neurofisiológicas, independentemente do seu substrato patológico. As crises parciais simples (CPS) na ELTM se caracterizam por sintomas típicos como sensação epigástrica ascendente (auras viscerosensoriais), fenômenos psíquicos e experiências emocionais como medo, sensação de "*deja vu*" ou "*jamais vu*" (auras experienciais), e, menos comumente, sintomas autonômicos e auras cefálicas, alucinações olfatórias e gustatórias (5,6). As crises podem evoluir para crises parciais complexas (CPC), com perda da consciência associada comumente a automatismos oroalimentares e manuais. Crises tônico-clônico generalizadas (CTCG) secundárias são infrequentes (6).

Na ELTM, o eletroencefalograma (EEG) de escalpo interictal demonstra descargas epileptiformes tipo onda aguda, ou onda aguda-onda lenta, com distribuição localizada nas regiões temporais anteriores, que podem ocorrer de forma bilateral em quase metade dos casos. A atividade ictal em EEG de escalpo é composta por ondas tetas rítmicas, com amplitudes crescentes e frequências decrescentes, mais comumente observada ipsilateral à zona de início ictal, mas que pode se apresentar no lobo temporal contralateral, como confirmado por registros de EEG invasivos (7).

1.3. ELTM associada à EH

O substrato patológico mais comum da ELTM é a EH, que se caracteriza histopatologicamente por perda neuronal característica, sobretudo nas sub-regiões CA1

(corno de Ammon 1), CA3 e hilo, gliose e reorganização sináptica das células sobreviventes (8,9).

A epileptogênese da EH resulta da perda de neurônios específicos do hipocampo e da reorganização sináptica dos elementos celulares sobreviventes que levam à hipersincronização e hiperexcitabilidade (9). Os eventos que iniciam o processo da EH ainda não são bem elucidados. Desde os anos 50, relaciona-se a EH a patologia crônica associada a injúria cerebral precoce (10). Estudos demonstram associação da ELTM com EH a fatores de risco precoces (ou eventos precipitantes iniciais), como insultos cerebrais perinatais, traumas e infecções do sistema nervoso central, sobretudo em pacientes que evoluem com crises refratárias, destacando-se o antecedente convulsões febris (CF) prolongadas (11). Porém, essas associações são decorrentes apenas de dados retrospectivos e os mecanismos que levam à perda neuronal característica da EH nesses casos não são bem compreendidos (11). Da mesma forma, os mecanismos envolvidos no desenvolvimento do foco epileptogênico na ELTM associada à EH derivam em sua maioria de estudos experimentais e, entre algumas teorias, podem estar implicados neurotoxicidade pelo glutamato, disfunção mitocondrial, fatores imunes e predisposição genética (11). Possivelmente, mais de um fator deve estar envolvido na gênese da EH em cada indivíduo, como, por exemplo, um fator precipitante inicial associado a outro fator individual que pode aumentar a vulnerabilidade do hipocampo à perda neuronal característica (12).

Da mesma forma, a história natural da ELTM associada à EH não é completamente conhecida (13). De um modo geral, a gestação, parto e desenvolvimento dos indivíduos com ELTM são normais. As crises epilépticas, em geral, têm início no final da primeira década de vida, após um período latente, de duração variável, que se segue aos possíveis

fatores precipitantes iniciais. As crises inicialmente respondem adequadamente à terapia com drogas antiepilépticas (DAEs) e podem entrar em remissão, mas geralmente retornam na adolescência ou idade adulta (6,7). No entanto, as crises epilépticas podem ter início mais precoce ou apenas na idade adulta. Da mesma forma, apesar da prevalência de pacientes com crises refratárias, um *contínuo* entre pacientes com crises facilmente controláveis com DAEs (14), pacientes com padrão de crises alternante entre períodos de remissão e de refratariedade (15) e aqueles com crises de difícil controle é observado.

O estudo de imagens de RM permite a detecção *in vivo* da EH (ELTM-EH). Na RM, a EH é caracterizada por redução do volume e perda da estrutura interna do hipocampo, melhor visualizadas nas imagens ponderadas T1, além de aumento de sinal nas imagens ponderadas T2 (16). Estima-se que cerca de 70% dos pacientes com ELTM apresentem EH (17). No entanto, a incidência exata de pacientes com EH é desconhecida, uma vez que a confirmação desta patologia só acontece nos casos de indivíduos com crises refratárias que são submetidos a resecção cirúrgica das estruturas temporais mesiais e sabe-se que a EH é também encontrada em indivíduos com controle adequado ou remissão de crises (18, 19). Outra dificuldade de se estimar a prevalência da EH é que o diagnóstico precoce, ainda na primeira década de vida, da ELTM-EH é dificultado devido à detecção pouco frequente de sinais desta patologia em imagens de RM de crianças, levando à hipótese de que a EH seja uma lesão que se desenvolve ao longo do tempo (13).

1.4. ELTM e RM normal

A ELTM com RM normal (ELTM-NL) é um grupo de pacientes não tão minuciosamente estudados como a ELTM-EH. De acordo com a classificação vigente da ILAE (1), a ELTM-NL é considerada epilepsia criptogênica (ou de origem desconhecida, de acordo com a nova proposta de classificação das epilepsias (20)). O número exato de pacientes com ELTM-NL não é conhecido, mas estima-se que eles correspondam a cerca de 30% dos casos de ELTM sem lesões estruturais que não a EH (4).

Dados sobre a história natural da ELTM-NL são mais escassos na literatura. Na ELTM-NL, as crises epilépticas, em geral, têm início no final da segunda década de vida. Nestes indivíduos, antecedente de convulsão febril prolongada é pouco frequente (16, 21-23).

Dentre os indivíduos com ELTM-NL, é possível que um grupo apresente EH sutil, sem redução de volume ou aumento de sinal T2 que possam ser detectados pelas técnicas atuais de neuroimagem. No entanto, de acordo com resultados recentes de estudos com protocolos adequados de RM de alto campo e anatomopatológicos de pós-operatórios de pacientes com ELTM-NL com crises refratárias às DAEs, o percentual de anormalidades histopatológicas compatíveis com EH nestes indivíduos é pequeno. Em uma série de pacientes ELTM-NL selecionados para a ressecção do lobo temporal com base em um protocolo RM 1,5Tesla (T), EH foi observada em 30% dos casos (24). Em estudo mais recente, com pacientes selecionados através de um protocolo "moderno" para avaliação pré-cirúrgica, incluindo RM de 3T, EH só foi identificada em 18% dos casos de ELTM-NL (25). Ainda, é possível que parte dos pacientes com ELTM-NL apresentem outros tipos de

patologias sutis não detectadas nos exames de imagens, como DCF (4, 24, 25) ou ainda que representem um subtipo diferente de epilepsia focal sem alteração estrutural específica (4).

Apesar dos avanços de neuroimagem, genética e patologia nas últimas décadas, cerca de um terço das epilepsias são classificadas como causa desconhecida (20). Dentro do grupo das epilepsias de causa desconhecida, a ELTM-NL apresenta a oportunidade única de comparação com a ELTM-EH, um grupo de pacientes com fenótipo semiológico e eletroencefalográfico semelhante, porém dano estrutural conhecido. Nesse sentido, o estudo da ELTM-NL é importante como uma comparação com ELTM-EH a fim de se compreender o papel da etiologia no prognóstico e evolução dessas epilepsias.

1.5. Avaliação dos sinais de EH em exames de RM

A quantificação do volume da estrutura hipocampal através do uso de softwares apropriados adiciona informações ao estudo desta patologia, como a detecção de anormalidades sutis ou EH bilateral (17). Da mesma forma, a avaliação do sinal hipocampal, por técnicas como a relaxometria de T2, é capaz de identificar e quantificar a presença de anormalidades em pacientes com volume hipocampal reduzido (26, 27) ou mesmo naqueles com volume hipocampal normal (28). Além de aumentar a sensibilidade de detecção de sinais de EH, a quantificação de volume e sinal hipocampal na ELTM tem boa correlação com os achados histopatológicos (29). A redução do volume hipocampal nas imagens ponderadas em T1 se correlaciona com a perda neuronal da EH (29). Diferentemente, apesar da relação entre hipersinal em imagens T2 e histopatologia compatível com EH e estudos que relacionam essa alteração do sinal ao aumento de água

livre no tecido, o significado histológico do aumento de sinal hipocampal detectado pela relaxometria não é completamente compreendido (26, 30).

Atualmente, estas técnicas são consideradas confiáveis e reproduzíveis para a detecção da patologia hipocampal (31). A maioria dos estudos utilizando técnicas de quantificação das anormalidades hipocampais foram realizados em imagens de RM de 1,5T (26, 32). Mais atualmente, a disponibilidade de aparelhos de RM de 3T tornou a determinação visual dos sinais de EH mais fácil e mais acurada (33) e o valor de técnicas de quantificação de sinal e volume da estrutura hipocampal nessas imagens de alto campo ainda não foi bem avaliado.

1.6. ELTM e resposta a droga antiepiléptica

Epilepsias relacionadas a diferentes alterações histopatológicas provavelmente apresentam mecanismos de epileptogênese diversos, com respostas ao tratamento e evoluções distintas. No entanto, mesmo em grupos de epilepsias com achados histopatológicos semelhantes, como a ELTM-EH, encontramos pacientes com respostas distintas ao tratamento com DAEs. Na ELTM-EH, são observados desde pacientes com crises resistentes a politerapias com doses de DAEs otimizadas, àqueles com crises bem controladas ou em remissão com ou sem o uso de medicação (11). Para grupos de etiologia desconhecida como a ELTM-NL, essa variabilidade também ocorre, a pesar de indivíduos com bom controle de crises serem observados com maior frequência (19, 34).

Entre todos os tipos de epilepsias, 60-70% dos pacientes apresentam remissão de crises com o uso adequado de DAEs (35). Em epilepsias focais, a presença e o tipo de lesão

estrutural relacionada às crises (epilepsias sintomáticas) são as principais implicações para a resposta individual às DAEs (19). Epilepsias focais criptogênicas (ou com RM normal) têm uma maior taxa de remissão de crises com o uso de DAEs e EH é a patologia relacionada à ELTM com as taxas mais elevadas de crises refratárias (19). Além disso, os pacientes com sinais de EH e livres de crise necessitam, com maior frequência, uso de politerapia para controle adequado (36).

A real frequência de pacientes com ELTM e crises refratárias ao tratamento medicamentoso é desconhecida, sobretudo pela dificuldade de estudos adequados que incluam grandes populações de pacientes com ELTM seguidos em todos os diferentes níveis de complexidade dos serviços de saúde. Em estudos populacionais, síndromes epilépticas específicas como ELTM são mal classificadas. Em contraste, os estudos que investigam mais detalhadamente e categorizam a ELTM são realizados em centros terciários, nos quais a maioria dos indivíduos são resistentes às DAEs.

Nas últimas décadas, diferentes estudos têm demonstrado a presença de sinais EH em exames de RM de pacientes com bom controle de crises em tratamento com DAEs ou mesmo com remissão de crises (12, 37, 38). Uma vez que confirmação histopatológica de EH não está disponível para estes pacientes com evoluções "benignas", sua real prevalência em indivíduos com ELTM com bom controle de crises é também desconhecida e apenas avaliações de exames de RM pode nos dar uma estimativa dessa associação.

Revisão detalhada sobre a reposta ao tratamento clínico e cirúrgico na ELTM-EH e ELTM-NL é descrita no *Capítulo 1*.

1.7. ELTM e redes neuronais

A patologia da ELTM se estende além da formação hipocampal (39). Como outras epilepsias, a ELTM pode ser considerada uma doença de redes neuronais funcionalmente e anatomicamente conectadas, em que a atividade em qualquer parte afeta todas as demais (39). Além disso, diferenças entre redes neuronais relacionadas às epilepsias podem ser capazes de explicar não só a variabilidade individual de fenômenos ictais, mas também o comportamento interictal, bem como a resposta individual ao tratamento (40). Na ELTM, o funcionamento patológico de redes neuronais pode estar relacionado com anormalidades clínicas frequentemente observadas nesses indivíduos, como deficiência de memória e comorbidades psiquiátricas (41, 42).

A rede neural ictal associada com a ELTM tem sido abordada em estudos com humanos e experimentais (43-45), e inclui as regiões bitemporais medial e lateral, bem como áreas extra-temporais, como tálamos mediais e lobos frontais inferiores (39). No entanto, variabilidades individuais podem ocorrer e não está claro qual o papel de diferentes lesões estruturais na variabilidade dessas redes neuronais (40). Os estudos de neuroimagem desempenham papel fundamental na compreensão das redes neuronais relacionadas às ELTMs e de suas complexas interações.

1.7.1 ELTM e redes neuronais estruturais

Nas últimas décadas, os avanços nas técnicas de neuroimagem detectaram anormalidades estruturais e funcionais na ELTM-EH e ELTM-NL. Um dos métodos utilizados para avaliar estas alterações estruturais é a técnica da morfometria baseada em voxel (voxel based morphometry - VBM), a qual se baseia na comparação das

concentrações locais dos tecidos cerebrais entre diferentes grupos a partir de exames de RM (46). Na técnica de VBM, as diferenças de concentração ou volume dos tecidos são detectadas através da comparação das intensidades locais de sinal de mapas segmentados (47), com a possibilidade de avaliação da substância cinzenta (SC) ou branca.

Estudos de quantificação de SC na ELTM-EH demonstram atrofia além das estruturas mesiais ou neocorticais temporais, incluindo predominantemente outras áreas límbicas como tálamos mediais bilaterais, mas também outras estruturas como os lobos frontais (45, 48, 49). Embora estes danos de SC sejam mais evidente na ELTM-EH refratária e sua ocorrência seja, por vezes, relacionada com a ocorrência de crises epilépticas (50), também há evidências de atrofia difusa em pacientes com ELTM-EH com bom controle de crises (15, 51). Alterações sutis de substância branca também foram identificadas na ELTM-EH, no entanto, até agora estes estudos têm focado sobretudo na ELTM-EH fármaco resistente (52). Estas anormalidades estruturais detectadas por técnicas de pós-processamento de dados de RM permanecem com significado incerto. É possível que essas anormalidades se relacionem com o mecanismo primário de epileptogênese ou sejam decorrentes de crises epilépticas recorrentes (45). Por outro lado, é também incerto se essas anormalidades podem contribuir para diferentes respostas às DAEs ou ao tratamento cirúrgico em diferentes pacientes com ELTM-EH, bem como para ocorrência de comorbidades nesses indivíduos.

Diferentemente da ELT-EH, poucos estudos avaliaram a ocorrência de atrofia de substância cinzenta na ELTM-NL e seus resultados são diversos. Um estudo avaliou um número pequeno de pacientes com ELTM-NL refratária e não encontrou nenhuma alteração de volume de SC (22), enquanto em um estudo diferente, também avaliando ELTM-NL

refratária, atrofia de substância cinzenta foi observada em córtex frontal e órbito frontal, cerebelo, regiões temporais neocorticais e córtex parahipocampal (23). Em outros estudos, anormalidades estruturais na ELTM-NL com bom controle de crises foram detectadas em regiões semelhantes às observadas na ELTM-EH, envolvendo tálamos e parahipocampo (51), além de redução da espessura cortical em córtex sensitivo-motor bilateral (53).

1.7.2. ELTM e redes neuronais funcionais

Estudos com RM funcional (RMf) demonstram anormalidades em redes funcionais de repouso em de pacientes com ELTM refratária (54), como menor conectividade entre as estruturas mesiais ipsilaterais ao foco epiléptico (55). Da mesma forma, disfunção de conectividade funcional nas regiões da *default mode network* (DMN) é observada na ELTM-EH refratária à DAE (54, 55). A DMN (ou "rede de funcionamento padrão") é uma rede neuronal composta por regiões do cérebro que estão ativas durante o repouso vigil (introspecção ou pensamento auto-referencial), sendo seus principais constituintes o córtex prefrontal medial, cíngulo posterior, lobo parietal inferior, córtex temporal lateral e hipocampo, bilateralmente (56). O significado e importância da DMN não são totalmente compreendidos, mas sabe-se que a atividade nessa rede é suprimida durante a atenção dirigida e tarefas (57, 58).

1.7.3. ELTM e redes neuronais funcionais: EEG-RMf

O EEG e a RM são os aparatos de maior importância na avaliação complementar de pacientes com epilepsias. Nas últimas décadas, o uso combinado do EEG com a RMf (EEG-RMf) tem adicionado conhecimento ao estudo das epilepsias, além de auxiliar a detecção da zona de início ictal nos pacientes com crises refratárias em investigação para tratamento cirúrgico (59-61).

A técnica de EEG-RMf propicia a avaliação não invasiva simultânea da atividade neuronal e da hemodinâmica cerebral e permite o estudo do acoplamento neurovascular através da variação do sinal BOLD (nível dependente de oxigênio no sangue - Blood Oxygen Level Dependent) (59, 62). Estas técnicas combinadas podem revelar alterações hemodinâmicas relacionadas com atividade neuronal patológica ictal ou interictal, auxiliando a determinação da região cerebral responsável pelo início e propagação das crises epilépticas (60, 61, 63, 64).

As primeiras aquisições de EEG dentro do campo da RM ocorreram no início da década de 90 (65), com o objetivo já direcionado para o estudo da epilepsia. No entanto, os estudos iniciais, sobretudo na primeira década do uso da técnica, foram direcionados para dificuldades metodológicas relativas ao aparato do EEG (66), algoritmos de redução de artefatos do EEG (67, 68) e análise estatística (69). A partir de então, estudos com questionamentos clínicos em epilepsias generalizadas (70) e epilepsias focais (71) usando a técnica de EEG-RMf passaram a surgir. Na última década, o uso da técnica de EEG-RMf tem se mostrado promissor não apenas para o estudo da zona de início ictal em pacientes com epilepsias refratárias, mas tem também auxiliado a compreensão de redes neuronais em epilepsias generalizadas (70, 72) e focais (73-75), além dos mecanismos de propagação de crises (76, 77).

Redes neuronais relacionadas às descargas epileptiformes interictais (DEIs) foram investigadas com a técnica de EEG-RMf (73-75). Estudos incluindo pacientes com ELT de diversas etiologias demonstram áreas comuns de alterações hemodinâmicas associadas com DEIs evolvendo as estruturas mesiais temporais ipsilaterais, além de putamen, giro temporal superior e ínsula bilaterais (74, 75). Ainda, supressão da atividade em áreas

compatíveis com a *default mode network* (DMN) relacionada às DEIs tem sido descrita em estudos de EEG-RMf, tanto em pacientes com descargas generalizadas (70) quanto na ELT (73, 75). O real significado deste achado não é totalmente conhecido, mas propõe-se que possa estar relacionado a anormalidades sutis de consciência durante as DEIs (70).

A importância da técnica de EEG-RMf na definição da zona de início ictal tem sido investigada em estudos retrospectivos de controle de crises no pós-operatório de pacientes com epilepsias focais. No entanto, até o momento um número limitado de estudos incluindo pequenos grupos de pacientes com síndromes epilépticas ou etiologias diversas foram apresentados (60, 61). Estes demonstram que indivíduos com resultados de EEG-RMf pré-operatórios discordantes da zona de início ictal removida no procedimento cirúrgico apresentam pior prognóstico de controle de crises. Não há, até o momento, estudos que avaliem o papel de exames de EEG-RMf na definição do prognóstico cirúrgico especificamente em pacientes com ELTMs refratárias.

2. Objetivos

2.1. Objetivo Geral e Hipótese

Avaliar e comparar a ocorrência de alterações clínicas, estruturais e funcionais na ELTM com (ELTM-EH) e sem (ELTM-NL) sinais de EH na RM e relacionar essas alterações com a resposta ao tratamento.

Nossa hipótese é que, apesar das semelhanças semiológicas e eletroencefalográficas dos indivíduos com ELTM-EH e ELTM-NL, estas devem se tratar de doenças distintas. A caracterização das diferenças clínicas e de neuroimagem nesses grupos pode auxiliar a melhor compreensão de seus mecanismos de epileptogênese, além das razões da diversidade de evolução e respostas aos tratamentos observadas nesses pacientes.

2.2. Objetivos Específicos de Cada Artigo

Artigo 1: Understanding the spectrum of mesial temporal lobe epilepsy (MTLE) with and without hippocampal sclerosis: contributions for the development of individualized therapies.

Revisão sobre resposta ao tratamento clínico e cirúrgico na ELTM com e sem sinais de EH.

Artigo 2: Multimodal neuroimaging: Potential Biomarkers for response to AEDs?

Revisão sobre o papel da neuroimagem na detecção de biomarcadores que auxiliem o desenvolvimento de novas terapêuticas para as epilepsias.

Artigo 3: 3T MRI quantification of hippocampal volume and signal in mesial temporal lobe epilepsy.

Comparar a análise visual com a quantificação de volume e sinal hipocampal em exames de RM de 3T na detecção de sinais de EH.

Artigo 4: Hippocampal sclerosis and antiepileptic drug response are associated to the pattern of gray matter atrophy in mesial temporal lobe epilepsy.

Avaliar e comparar a ocorrência de atrofia de SC em pacientes com ELTM com e sem sinais de EH em exames de RM e sua relação com a respota ao tratamento.

Artigo 5: Patterns of antiepileptic drug response in patients with mesial temporal lobe epilepsy with and without signs of hippocampal sclerosis.

Avaliar a resposta a longo prazo à DAE em pacientes com ELTM com e sem sinais de EH em exames de RM.

Artigo 6: Amygdala enlargement occurs in patients with temporal lobe epilepsy and hippocampal sclerosis with early epilepsy onset.

Descrever as características clínicas de um grupo de ELTM-EH com aumento de volume da amígdala.

Artigo 7: Amygdala enlargement in patients with temporal lobe epilepsy without hippocampal sclerosis.

Descrever as características clínicas de um grupo de ELTM-NL com aumento de volume da amígdala.

Artigo 8: EEG epileptiform discharges with similar morphology and location have different hemodynamic responses in mesial temporal lobe epilepsy with and without hippocampal sclerosis.

Investigar os padrões de alterações hemodinâmicas relacionadas às DEIs em exames de EEG-RMf e comparar esses padrões funcionais com alterações estruturais sutis em pacientes com ELTM com e sem sinais de EH em exames de RM.

Artigo 9: EEG-fMRI in the pre-surgical evaluation of temporal lobe epilepsy patients.

Avaliar o papel da técnica de EEG-RMf na definição do prognóstico cirúrgico de pacientes com ELTM refratária.

Artigo 10: Epilepsy as progressive disorders: what is the evidence that can guide our clinical decisions and how can neuroimaging help?

Revisão da ocorrência de progressão de dano nas epilepsias, com ênfase para a ELTM.

3. Métodos

3.1. Aspectos éticos

Todos os pacientes incluídos no estudo foram devidamente informados a respeito da natureza do trabalho e de seus riscos. Todos assinaram termos de consentimento informado, aprovados pelo Comitê de Ética da UNICAMP, antes da realização de cada exame de RM ou de EEG-RMf (Anexos 2 e 3).

Parte dos pacientes incluídos no *Capítulo 9* foram selecionados em dois outros centros de epilepsia: Instituto de Neurologia da University College London (Londres, Reino Unido) e na Unidade de Avaliação Pré-operatória de Epilepsia da University of Geneva (Genebra, Suíça). Esses pacientes assinaram termos de consentimento informado de seus institutos antes da realização dos exames de EEG-RMf.

3.2. Identificação dos pacientes

Foram selecionados para o presente estudo pacientes com pelo menos 18 anos de idade, com diagnóstico clínico e eletroencefalográfico de ELTM, de acordo com os critérios da ILAE (1), em tratamento clínico no Serviço de Epilepsia do Hospital de Clínicas da Universidade de Campinas (UNICAMP). Foram excluídos pacientes com ELTM sintomáticas secundárias a doenças cerebrovasculares, infecciosas, tumorais, traumáticas ou mal formações do desenvolvimento cortical, pacientes com contraindicação para realização de exames de RM e aqueles que se recusaram a assinar o termo de consentimento informado.

A semiologia de ELTM foi caracterizada através de questionário estruturado aplicado ao paciente e a pelo menos um acompanhante. Pacientes com auras sugestivas de acometimento temporal neocortical não forma incluídos. Foram selecionados apenas pacientes com DEIs observadas em EEG de escalpo compostas por ondas agudas, ou complexos onda aguda-onda lenta, localizadas exclusivamente nas regiões temporais anteriores. Pacientes com DEIs localizadas ou com repercussão para regiões temporais posteriores ou extra-temporais, bem como pacientes com atividade epileptiforme interictal tipo poliespícula ou com bissincronias secundárias não foram selecionados.

Preencheram os critérios de seleção 320 pacientes. No entanto, após a análise inicial foram excluídos: i) pacientes com alterações estruturais nos exames de RM não relacionados à etiologia das crises epilépticas, mas que poderiam comprometer avaliações quantificativas dos exames (meningeoma, hidrocefalia, cisto aracnóideo, sequela de trauma crânio-encefálico e acidente vascular cerebral) (N=22); ii) pacientes com EEGs interictais sem alterações epileptiformes detectáveis (a pesar de alterações epileptiformes não serem mandatórias para a classificação de ELTM, consideramos que sua ausência diminui a especificidade para seu diagnóstico) (N=57); iii) dúvida em relação ao diagnóstico de ELTM por semiologia ou alterações eletroencefalográficas controversas (N=24). Após análise visual cuidadosa dos exames de RM, outros 14 pacientes foram excluídos da amostra por apresentarem sinais indiretos sutis que podem ser compatíveis com DCF (descrição no *Capítulo 3*).

Dessa forma, 203 pacientes com ELTM foram submetidos à quantificação de volume e sinal hipocampal em exames de RM (*Capítulo 3*).

A lateralidade do foco epiléptico foi definida através das alterações ictais ou interictais dos EEGs de escalpo. A lateralidade do foco epiléptico no EEG ictal foi considerada como o registro de todas as crises com início localizado em um dos lobos temporais anteriores (N=64/203; 32%). A lateralidade do foco epiléptico por EEGs interictais foi considerada como a ocorrência de pelo menos 80% de atividade epileptiforme interictal localizada em um dos lobos temporais. Não houve discordância entre a lateralidade definida por EEGs ictais ou inter-ictais de acordo com a definição proposta em nenhum dos pacientes. Para avaliação dos dados demográficos e da alteração de volume de SC nos pacientes com ELTM-EH e ELTM-NL, os indivíduos com foco epiléptico bilateral, indefinido ou discordante dos sinais de EH à RM foram excluídos (N=30).

Dessa forma, 172 pacientes com ELTM e foco epiléptico unilateral foram avaliados nos *Capítulos 4 e 5* (nesses capítulos, foi ainda excluído o único paciente que apresentou discordância entre a avaliação visual e de quantificação hipocampal descrita no *Capítulo 3*).

No *Capítulo 6*, foram avaliados os 102 pacientes com ELTM-EH, definidos apenas pela avaliação visual das imagens de RM, que apresentavam lateralidade definida do foco epiléptico. No *Capítulo 7*, foram avaliados os 56 pacientes com ELTM-NL definida pela análise visual e pela quantificação de sinal e volume hipocampal, independentemente da lateralidade definida do foco epiléptico.

Ainda, dentre os 172 pacientes com diagnóstico de ELTM e foco epiléptico unilateral bem definido, um subgrupo foi avaliado com a técnica de EEG-RMf (N=29) e os resultados estão descritos nos *Capítulos 8 e 9*. Para a definição desse subgrupo, foram selecionados apenas pacientes com crises epilépticas refratárias ao tratamento com DAEs e

que apresentavam DEIs nos três últimos EEGs de rotina, realizados de acordo com o protocolo de avaliação pré-operatória.

O *Capítulo 9* foi realizado como estudo multicêntrico em conjunto com o Instituto de Neurologia da University College London (Londres, Reino Unido) e a Unidade de Avaliação Pré-operatória de Epilepsia da University of Geneva (Genebra, Suíça). Foram incluídos neste estudo nove dos pacientes pertencentes aos demais *Capítulos* (pacientes selecionados para exame de EEG-RMf e que foram submetidos posteriormente a tratamento cirúrgico para controle de crises epilépticas refratárias), além de 21 pacientes com diagnóstico de ELT de diferentes etiologias (EH, DCF, tumor ou de origem desconhecida) selecionados nos dois outros centros participantes. Como critérios para inclusão neste *Capítulo*, foram selecionados, nos três centros todos, os pacientes com diagnóstico clínico e eletroencefalográfico de ELT, de acordo com os critérios da ILAE, os quais apresentavam crises refratárias ao tratamento medicamentoso e que foram submetidos a tratamento cirúrgico posteriormente à aquisição de exame de EEG-RMf. Ainda como critério de inclusão, de acordo com a metodologia de análise dos exames de EEG-RMf (item 3.5.4), os pacientes deveriam apresentar EEG adquirido fora do ambiente do aparelho de RM com DEIs semelhantes às observadas no EEG adquirido dentro da RM.

3.3. Dados clínicos e definições

Todos os pacientes incluídos foram avaliados em consulta inicial de triagem e então seguidos em consultas com intervalos entre quatro e seis meses, com questionário padronizado. Dados de história pregressa foram avaliados em consulta inicial por

questionário estruturado aplicado ao paciente e seu acompanhante, incluindo os seguintes dados: antecedentes gestacionais e possíveis eventos precipitantes iniciais (CF, trauma crânio encefálico, meningoencefalites, hipóxia/anóxia peri-parto), idade de início das crises, antecedente familiar de epilepsia, DAEs utilizadas, história de estado de mal epiléptico, história de traumas ou outras complicações decorrentes das crises epilépticas, comorbidades, semiologia detalhada das crises epilépticas atuais e pregressas. Fatores modificáveis, como frequência de crises, medicações antiepilépticas em uso, efeitos colaterais, foram avaliados por questionário estruturado, repetidamente, em cada consulta. Dados de evolução do controle das crises ao longo da vida foram coletados retrospectivamente através de questionário estruturado aplicado ao paciente e seu acompanhante, além de registro de prontuário.

Resposta à DAE foi avaliada de forma transversal no *Capítulo 4* e os pacientes foram classificados como bom controle de crises (aqueles que nos dois anos anteriores à aquisição da RM apresentaram até três CPC ao ano, independentemente do número de CPS, e nenhuma CTCG). Os pacientes que não preencheram esse critério foram classificados como refratários. No *Capítulo 5*, a resposta à DAE foi considerada desde o início das crises e foram classificados como bom controle de crises aqueles pacientes que desde o início da terapêutica com DAEs em doses otimizadas mantiveram bom controle das crises, isto é, até três CPC ao ano, independente do número de CPS, e nenhuma CTCG. Neste capítulo, os pacientes que não preencheram esses critérios foram classificados como refratários.

Período silente (PS) foi definido como crises epilépticas com início e remissão na primeira década de vida e recorrência na segunda década, após pelo menos cinco anos de remissão. Padrão de crises remitente-recorrente (RR) foi definido como pelo menos um

período de remissão completa de crises por período igual ou maior a dois anos em uso ou não de DAE.

Foram considerados como antecedente familiar positivo aqueles pacientes que apresentavam pelo menos um parente de primeiro ou segundo grau com história de epilepsia. Duração da epilepsia foi definida como a idade na aquisição da RM menos a idade de início das crises. Tempo de epilepsia ativa foi definido como a idade na aquisição da RM menos a idade de início das crises, menos períodos de remissão das crises iguais ou superiores a dois anos.

3.4. Grupo controle

Para definição dos limites da normalidade, foi utilizado grupo controle composto por indivíduos que não apresentavam qualquer antecedente patológico. Após análise visual das imagens de RM dos controles para exclusão de possíveis artefatos das imagens, o grupo controle foi composto por 82 indivíduos. No entanto, teste de homogeneidade das imagens de RM realizado através do programa SPM8/VBM8 demonstrou três indivíduos com qualidade das imagens fora da média dos demais controles (avaliação da RM detalhada no item 3.5.3). O grupo controle final foi, então, composto por 79 indivíduos.

3.5. Aquisição e análise de exames de RM

Exames de RM de pacientes e controles foram realizados em aparelho de 3T Philips Intera Achieva (Philips, Best, Holanda), com aquisições nos planos coronal, sagital e axial,

com cortes coronais obtidos em plano perpendicular ao longo eixo da formação hipocampal, a fim de melhor estudo desta estrutura.

Protocolo de aquisição de RM:

- Imagens coronais: (a) Imagens ponderadas em T2 multi-eco (3 mm espessura, tempo de repetição (TR)=3300ms, tempo de eco (TE)=30/60/90/120/150ms, matriz=200X180, *field of view* (FOV)=180X180); (b) Imagens ponderadas em T1 "inversion recovery" (3 mm espessura, TR=3550ms, TE=15ms, inversion time=400, matriz=240X229, FOV=180x180), (c) Imagens Fluid Acquisition Inversion Recovery (FLAIR) (Supressão de gordura, 4 mm espessura, TR=12000ms, TE=140ms, matriz=180x440, FOV=200x200);
- Imagens axiais: Imagens FLAIR (Supressão de gordura, 4 mm espessura, TR=12000ms, TE=140ms, matriz=224x160, FOV=200x200);
- Imagens ponderadas em T1 volumétricas: voxels isotrópicos de 1 mm, adquiridas no plano sagital (1 mm de espessura, flip angle=8°, TR=7,0ms, TE=3,2ms, matriz=240x240, FOV=240x240);
- Imagens ponderadas em T2 volumétricas: voxels isotrópicos de 1,5 mm, adquiridas no plano sagital (TR=1800ms, TE=340ms, matriz=140X140, FOV=230x230).

3.5.1. Análise do volume hipocampal e de amígdala (*Capítulos 3, 4, 6, 7*)

Volumetria automática de hipocampo e amígdala de pacientes e controles foi realizada através do programa FreeSurfer (versão 5.1.0; http://surfer.nmr.mgh.harvard.edu/), com o uso de imagens ponderadas em T1 volumétricas

(voxels isotrópicos de 1 mm, 1 mm de espessura, flip angle=8°, TR=7,0ms, TE=3,2ms, matriz=240x240, FOV=240x240). Após definição automática dessas estruturas pelo programa, a delimitação das regiões de interesse foi visualmente checada em cada em cada grupo de imagens. Devido à adequada delimitação das estruturas e a fim de se evitar o viés da segmentação manual, nenhuma região de interesse foi corrigida manualmente. Foram extraídos os volumes absolutos para cada hipocampo e amígdala, os quais foram posteriormente corrigidos pelo volume supratentorial individual. Da mesma forma, foram obtidos índices de assimetria hipocampal, definidos como a razão do volume da estrutura do lado menor pelo lado maior de cada indivíduo. Para a determinação dos parâmetros da normalidade, os volumes de hipocampo e amígdala foram também obtidos em um grupo de 79 controles sadios, pareados para sexo e idade com os pacientes. Todos os valores obtidos foram transformados em Zscore, a fim de se calcular a distância dos volumes de cada paciente da média dos controles. Os volumes corrigidos ou índices de assimetria que se apresentaram abaixo de –2 desvios-padrão (DP) da média do grupo controle (Zscores menores ou iguais a -2) foram considerados sinal indicativo de EH.

3.5.2. Análise do sinal hipocampal e de amígdala (*Capítulos 3, 4, 6, 7*)

Para a quantificação do sinal, foi utilizada a análise de relaxometria multi-eco de T2 (3 mm de espessura; TR = 3300; TE = 30/60/90/120/150; matriz = 200X180; FOV = 180X180). O tempo de relaxometria de T2 pode ser quantificado através da medida de decaimento da intensidade de sinal em diferentes tempos de eco em uma série de imagens T2 ponderadas adquiridas no mesmo corte. Cada pixel do mapa de T2 resultante é derivado da intensidade em cada uma dessas múltiplas imagens no mesmo corte (78).

A análise de relaxometria de T2 foi realizada com o programa Aftervoxel (Figura 3 – Anexo 1), uma ferramenta de visualização de imagens médicas escrita por Felipe Bergo (http://www.liv.ic.unicamp.br/ ~ Bergo / aftervoxel). Para esta análise, uma região de interesse foi definida manualmente em diferentes cortes da sequência de RM de cada indivíduo (três cortes para o hipocampo, sendo um na região da cabeça, um no corpo e um na cauda do hipocampo; dois cortes para a amígdala), por um investigador cego para o resultado da análise visual, divisão de controles e pacientes e dados clínicos dos pacientes. Para a determinação dos parâmetros da normalidade, os sinais de hipocampo e amígdala foram também obtidos para todos os indivíduos controles que apresentavam sequências T2 duplo-eco (N=76). A média do sinal T2 de todos os cortes foi utilizada como a medida final. Os valores de sinal de hipocampo e amígdala superiores a 2DP da média do grupo de controle (o valor absoluto e/ou índice de assimetria, definido pela razão entre o lado de maior pelo lado de menor sinal de cada estrutura de cada indivíduo) foram considerados como hipersinal.

3.5.3. Análise de volume da SC: VBM (*Capítulos 4 e 8*)

Para a detecção de alteração de volume da SC, análise automática da estrutura do cérebro como um todo foi realizada pela da técnica de VBM. A análise foi realizada em imagens sagitais 3D ponderadas em T1 (1 mm de espessura, flip angle=8°, TR=7,0ms, TE=3,2ms, matriz=240x240, FOV=240x240), através do programa SPM8/VBM8 (Wellcome Dept Cogn. Neurol, London, http://www.fil.ion.ucl.ac.uk), com a plataforma MATLAB 8.0 (MathWorks, Natrick, MA).

Antes das etapas de processamento das imagens pelo programa SPM8/VBM8, as imagens de pacientes com o foco epiléptico à direita foram invertidas na orientação esquerda-direita de modo que o foco epiléptico de todos os pacientes ficasse alinhado à esquerda. Um grupo de controles composto por 82 indivíduos saudáveis (pareados para sexo e idade com os pacientes) foi usado para comparação e uma porcentagem de controles comparáveis com cada grupo de pacientes nas diferentes análises foi também invertida na orientação direita-esquerda.

Os passos de processamento das imagens de RM pelo programa SPM8/VBM8 incluem: 1) normalização espacial das imagens para o mesmo espaço estereotáxico (template MNI-152); 2) modulação das imagens, a fim de corrigir as possíveis variações de volume da normalização e permitir a avaliação de anormalidades de volume (79, 80); 3) segmentação em diferentes tecidos, incluindo SC, substância branca e líquido cefalorraquidiano. O algoritmo DARTEL (Diffeomorphic Anatomical Registration using Exponentiated Lie Algebra) foi ainda utilizado nas etapas de pré-processamento, a fim de aumentar a precisão do alinhamento entre os indivíduos (81). As imagens de SC resultantes foram suavizadas com um kernel gaussiano isotrópico de 8 mm.

Depois do processamento das imagens pelo programa SPM8/VBM8, um teste de qualidade foi realizado a fim de se observar a homogeneidade e coregisto entre os indivíduos. No caso de detecção de imagens com qualidade inferior à média das demais (*outliers*), essas devem ser excluídas e as etapas de processamento refeitas sem esses indivíduos. Imagens de três controles foram excluídas após essa análise e o grupo final foi composto por 79 indivíduos.

As imagens de SC pós-processadas dos diferentes grupos (ELTM-EH, ELTM-NL, controles, além de outros subgrupos) foram comparadas através de uma análise estatística

(Teste-T de duas amostras) baseada na comparação de voxel a voxel. Limite estatístico inicial de p<0,001, sem correção adicional para múltiplas comparações e clusters mínimo de 30 voxels contíguos foi utilizado. Como um segundo passo, os resultados foram corrigidos com um limiar estatístico mais rigoroso, com p<0,05, corrigido para múltiplas análises (Family Wise Error – FWE), a fim de se reduzir a possibilidade da ocorrência de resultados falsos positivos.

3.5.4. Análise de redes neuronais funcionais: EEG-RMf

Capítulo 8: Aquisição e análise de EEG-RMf

Uma parte dos pacientes foi submetida a co-registro de EEG-RMf. A aquisição do EEG foi realizada através de 64 eletrodos compatíveis com o campo magnético, e o sinal transmitido através de amplificador BrainAmp (Brain Products, München, Alemanha) para um terminal de registro.

Foram adquiridas sequências funcionais de RM (imagens eco-planares - Echo Planar Imaging – EPI; TE=30ms, TR=2s, FOV de 240x240x117mm³, 39 cortes e voxel de 3x3x3mm³), além de uma sequência anatômica (imagem 3D, ponderada em T1, voxels isotrópicos de 1 mm, adquiridas no plano sagital, 1 mm de espessura, flip angle=8°, TR=7,0ms, TE=3,2ms, matriz=240x240, FOV=240x240). Para cada indivíduo, duas sequências de EPIs de 24 minutos foram realizadas. As imagens EPIs foram posteriormente realinhadas, corrigidas pelo tempo, normalizadas (Template MNI) e suavizadas (kernel gaussiano isotrópico de 6mm) através do programa SPM8.

Os traçados de EEG foram corrigidos para artefatos de gradiente e de batimento cardíaco através do programa Brain Vision Analyzer2 (Brain Products, München, Alemanha), utilizando o método AAS (*Average Artifact Subtraction*) (67). Após remoção

dos artefatos, os traçados de EEG foram analisados visualmente e as DEIs marcadas, com o registro dos instantes de cada marcação (marcações realizadas no pico da onda aguda, com duração de zero segundos). Esses instantes foram então utilizados como paradigmas na análise estatística realizada com o programa SPM8.

Como primeiro passo, foram realizadas análises individuais das respostas BOLD. A série temporal das DEIs foram convoluídas com a função resposta hemodinâmica canônica. A fim de aumentar a sensibilidade de detecção de respostas hemodinâmicas relacionadas com as DEIs (82), para cada indivíduo foram criadas nove matrizes estatísticas, com variação do pico da função resposta hemodinâmica entre -10 a +10 segundos a partir do instante da marcação das DEIs (funções de respostas hemodinâmicas com picos em -5, -2, zero, 3, 5, 7, 9, 11, 14 segundo das DEIs). As derivadas temporal e de dispersão das funções de respostas hemodinâmicas foram utilizadas como regressores no modelo estatístico (modelo geral linear – MGL). Seis parâmetros de realinhamento (três parâmetros de rotação e três de translação) foram incluídos no modelo estatístico, a fim de se considerar os erros relacionados aos artefatos de movimento. Mapas de contraste BOLD positivo e negativo foram obtidos para cada intervalo de função de resposta hemodinâmica.

Como um segundo passo, para cada grupo de ELTM (ELTM-EH e ELTM-NL) foram realizadas análises estatísticas de segundo nível utilizando-se os mapas de contraste normalizados criados para a DEI ipsilateral para a zona de início das crises na análise individual. O co-registro espacial desses mapas foi verificado e um teste de covariância foi realizado. Os mapas de contrastes das DEIs temporais à direita foram invertidos no sentido direita-esquerda. Assim, todos os resultados são descritos como ipsilateral (lado esquerdo) ou contralateral (lado direito), referente às DEIs marcadas nos EEGs.

A análise estatística foi realizada através de testes-T de duas amostras ($p < 0,005$, sem correção adicional; limite mínimo de 5 voxels agrupados) para respostas BOLD positivo e negativo em cada grupo de ELTM.

Capítulo 9: Aquisição e análise de EEG-RMf

Esse capítulo foi realizado como estudo multicêntrico e a metodologia de análise dos exames de EEG-RMf foi adaptada a fim de se manter homogênea entre os três centros envolvidos (University College London, Londres, Reino Unido; Universidade de Genebra, Genebra, Suíça; Universidade de Campinas, Campinas, Brasil).

Exames de RM foram adquiridos em aparelhos de 3T (Campinas: 3T Philips Intera Achieva; Londres: 3T Signa Excite HDX, GE MedicalSystems; Genebra: 3T Siemens Magnetom Trio). O protocolo de RMf consistiu na aquisição de EPIs com duração entre 20 e 48 minutos (Londres: TR = 3000 ms, voxel: 3,75x 3,75x3mm^3, 43 fatias; Genebra: TR = 1500 ms, voxel: 3,75x3,75x5,5mm^3, 25 fatias; Campinas: TR = 2000ms, voxel 3x3x3mm ^{3}, 39 fatias). EEGs com 32-256 eletrodos compatíveis com o campo magnético foram adquiridos concomitantemente aos exames de RM, com o sinal processado pelo amplificador BrainAmp (Brain Products, Alemanha) e transmitido por meio de cabos de fibra óptica para um terminal de gravação.

O processamento das imagens de RMf e dos traçados de EEG foram realizados conforme descrito acima. Os instantes de atividade epileptiforme interictal foram utilizados como paradigmas nos exames de RMf, assim como a correlação de um mapa topográfico médio da mesma DEI com cada instante do EEG adquirido dentro do aparelho de RM (83). Esse segundo paradigma (correlação de mapas topográficos com o traçado de EEG) baseia-se na possibilidade de se detectar variações do sinal BOLD relacionados à presença de

atividade epileptiforme de difícil detecção visual. Para a construção desse mapa topográfico, foram utilizados EEGs adquiridos fora do ambiente de RM de acordo com o sistema 10-10 ou 10-20. Esses EEGs foram revisados por neurofisiologistas e as DEIs temporais à direita ou esquerda foram marcadas. Mapas topográficos médios de cada tipo de DEI foram calculados através do programa Cartool (84). Os EEGs adquirido dentro do aparelho de RM foram interpolados a fim de se ter o número de eletrodos coincidente com os EEGs adquiridos fora do ambiente de RM e filtros passa-banda entre 1-30Hz foram aplicados. Foi, então, calculada a correlação entre os mapas topográficos e cada ponto amostral (*time-frame*) do EEG adquirido dentro do aparelho de RM. Por fim, o quadrado dessa correlação foi convoluído com a resposta hemodinâmica canônica e utilizado como paradigma nos exames de RMf.

À análise estatística dos exames de RMf foi realizada através de MGL, ao qual foram ainda adicionados os seguintes regressores: 24 parâmetros de realinhamento (seis parâmetros de realinhamento e a expansão Voltera destes) (85); volumes nulos para movimentos maiores que 0,2mm (86); modelação de variações do sinal de RM associados com a pulsação cardíaca (87); instantes e durações de artefatos de movimento e piscamento detectados no EEG adquirido dentro do ambiente de RM convoluídos com a função de resposta hemodinâmica (a fim de se reduzir a contaminação dos resultados com resposta BOLD fisiológica) (88).

Para pacientes que apresentaram DEIs durante a aquisição do exame de EEG-RMf, tanto as DEIs detectadas no EEG adquirido dentro da RM quanto a correlação do mapa topográfico (83) foram incluídos no MGL como efeitos de interesse (Teste-F, $p<0,05$, corrigido para FWE; nos casos de mapas nulos, os dados foram explorados com limiar estatístico inferior, com $p<0,001$, sem correções adicionais). Para os pacientes sem DEIs

detectadas no EEG adquirido dentro da RM, apenas a correlação do mapa topográfico foi considerada como um efeito de interesse.

3.6. Análise estatística

A análise estatística foi realizada utilizando o programa SYSTAT9®. Testes estatísticos paramétricos e não paramétricos foram utilizados de acordo com a distribuição dos dados.

3.7. Apresentação e análise dos dados

No *Capítulo 1*, é apresentada revisão sobre as diferenças de resposta ao tratamento clínico e cirúrgico de pacientes com ELTM com ou sem sinais de EH em exames de RM. No *Capítulo 2*, é apresentada revisão sobre o papel da neuroimagem na detecção de biomarcadores que auxiliem o desenvolvimento de novas terapêuticas para as epilepsias.

A composição dos grupos ELTM-EH e ELTM-NL é apresentada no *Capítulo 3*, o qual ainda demonstra comparação entre a análise visual e a quantificação de volume e sinal da estrutura hipocampal para a definição de EH em exame de RM de 3T.

Os dados demográficos dos pacientes selecionados são descritos no *Capítulo 4*. Esse capítulo apresenta ainda a análise de quantificação de SC pela técnica de VBM nos pacientes com ELTM-EH e ELTM-NL.

O *Capítulo 5* complementa a avaliação dos dados clínicos dos pacientes selecionados e descreve, através da coleta de dados retrospectivos, o padrão a longo prazo da resposta à DAE nos grupos ELTM-EH e ELTM-NL.

Os *Capítulos 6 e 7* descrevem o achado de aumento de amígdala em subgrupos de pacientes com ELTM-EH e ELTM-NL e avalia os dados clínicos desses pacientes.

O *Capítulo 8* descreve os achados da análise de grupo de EEG-RMf dos pacientes com ELTM-EH e ELTM-NL, enquanto os resultados da técnica de EEG-RMf na avaliação pré-operatória dos pacientes com ELT são descritos no *Capítulo 9*.

No *Capítulo 10* é apresentada revisão sobre a progressão de dano nas ELTMs.

4. Resultados

CAPÍTULO 1

Understanding the spectrum of mesial temporal lobe epilepsy (MTLE) with and without hippocampal sclerosis: contributions for the development of individualized therapies

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Expert Review of Neurotherapeutics (invited review)

Understanding the spectrum of mesial temporal lobe epilepsy (MTLE): contributions for the development of individualized therapies

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Summary: Mesial temporal lobe epilepsy (MTLE) is a subtype of epilepsy in that individuals present with seizure semiology and electroencephalographic characteristics that point to an ictal onset in mesial temporal structures. The diagnosis of MTLE involves different etiologies, the most common being hippocampal sclerosis (HS) but up to 20% of MTLE patients have no detectable structural lesions visible on modern MRIs. A variability of antiepileptic drug response and surgical prognosis is observed in MTLE. The understanding of the differences among patients with MTLE can facilitate the development of individualized and more efficient therapeutics. In this paper we will address the recent contributions of neuroimaging, neurophysiology, genetics and histopathology to the comprehension of the spectrum of MTLE with and without signs of HS and how the advances in these areas have helped to improve pharmacological and surgical treatments.

Keywords: mesial temporal lobe epilepsy; hippocampal sclerosis; MRI-negative temporal lobe epilepsy.

1- Introduction

According to the classification of the International League Against Epilepsy[1], mesial temporal lobe epilepsy (MTLE) is a group of individuals with similar seizure semiology and electroencephalographic characteristics that point to an ictal onset zone in the temporal structures. The diagnosis of MTLE involves different etiologies, the most common the MTLE associated with hippocampal sclerosis (HS; MTLE-HS) which is responsible for 60-70% of the cases. Other different structural lesions, as tumors, focal cortical dysplasias, vascular or ischemic lesions, account for 10-15% of MTLE cases. However, there are still 15-20% of individuals with MTLE with no detectable structural lesions even in modern MRI protocols (MTLE-NL)[2].

Among diverse epilepsy etiologies, it is expected different prognosis and evolution. However, even in MTLE associated with specific lesions, distinct patterns of anti-epileptic drug (AED) response and surgical resection outcomes are observed. In MTLE-HS a vast range of AED response is observed, with patients with seizures resistant to high doses of AED to those with well controlled or even few seizures during life. Also, in drug resistant MTLE, the same type of surgical resection including the anterior temporal structures is associated with different seizures control outcomes. These divergent patterns of AED and surgical responses lead to the assumption that MTLE-HS is not a homogeneous entity but rather a group of distinct pathologies[3]. For groups of MTLE and unknown etiology as MTLE-NL, the variability of outcomes is even wider, with patients with few seizures to those refractory to AEDs and also poorer surgical outcomes after temporal lobe resections[4,5]. The understanding of the differences among patients with MTLE-HS and MTLE-NL can facilitate the development of individualized and more efficient therapeutics,

not only concerning their seizures but also the comorbidities and possible progressive damage of some types of MTLE.

In this paper we will address the contribution of neuroimaging, neurophysiology, genetics and histopathology to the comprehension of the spectrum of MTLE-HS and MTLE-NL and how the advances in these areas have helped to improve and to individualize the pharmacological and surgical treatment of these different individuals.

In the section "Advances in the knowledge of AED response in MTLE", we will discuss the current knowledge about the clinical, neuroimaging and genetic characteristics of MTLE individuals that respond or not to AEDs in both MTLE-HS and MTLE-NL groups. In the section "Advances in the knowledge of surgical outcomes in MTLE" we will discuss the current knowledge about the understanding of patients with MTLE and good or poor surgical outcome following anterior temporal lobectomy in both MTLE-HS and MTLE-NL.

2- Advances in the knowledge of AED response in MTLE:

Among all types of epilepsies, 60-70% of patients will achieve seizure remission under AED treatment[6]. In focal epilepsies, the presence and type of a structural lesion related to the seizure onset zone (symptomatic epilepsies) are the major implications for the individual AED response[7]. Cryptogenic (or MRI negative) focal epilepsies have a higher rate of seizure remission under AED treatment and HS is the pathology related to MTLE with the higher rates of seizures refractory to AEDs[7]. However, the scenario of seizure control under AED treatment is more complex in both MTLE-HS and MRI negative MTLE (MTLE-NL).

The real frequency of drug-resistant seizures in patients with MTLE is unknown. In population-based studies, specific epileptic syndromes as MTLE are poorly classified. In contrast, the studies that investigate in depth and categorize MTLE are conducted in tertiary epilepsy centers where the majority of individuals are AED resistant. Recent, in a non surgical series of MTLE with or without MRI signs of HS conducted in a tertiary epilepsy center, about two-thirds of the patients had mild course with good seizure control at long follow-up[8].

Some clinical characteristics are consistent related to worse AED responses in MTLE irrespective of its etiology or MRI findings. For example, early age at seizure onset and epileptiform discharges detected in the interictal scalp EEG are frequently associated with lower rates of AED response[4,9]. The comprehension of the differences of MTLE-HS and MTLE-NL that respond or not to AED will significantly improve our knowledge and the development of new targets for epilepsy therapeutics.

2.1- AED response and MTLE-HS spectrum

Neuroimaging, through its proven correlation with histopathology from surgical specimens, plays an important role in the understanding of the differences of AED responsive and AED resistant MTLE. Drug resistant MTLE-HS has histological confirmation of hippocampal pathology once these patients are often submitted to surgical resection of mesial temporal lobe structures aiming seizure control. Differently, patients with MTLE-HS and good AED seizure control do not have histopathology confirmation of the HS since they are not surgery candidates; however, they can be studied with MRI. Sings of HS can be reliably detected in MR images as hippocampal volume reduction, loss of its

internal structure and signal hyperintensity in T2-weighted sequences and MRI quantification methods can improve the sensitivity to detect this pathology[10,11].

MTLE-HS is classically associated with a high percentage of patients with drug resistant seizures[7] (Semah 1998). However, in the last decades different studies have demonstrated the presence of MRI signs of HS in patients with good seizure control under AED treatment or even with seizure remission[12-14]. Once the majority of epilepsy studies are conducted in tertiary centers and histology is not available for these "benign" patients, the real prevalence of HS in MTLE individuals with good seizure control is unknown. In a group of familial MTLE, MRI signs of HS were observed in 46% of patients with seizure remission, 51% of those with good seizure control under medication and in all patients with refractory seizures[12]. More recently in a group of "benign" MTLE patients with no family history of epilepsy, MRI evidence of HS was detected in 39% of the individuals[14].

Different clinical characteristics and past history in patients with MTLE-HS can be associated with distinct patterns or degrees of HS observed in MR images as well as with different seizure control prognosis. For example, the extent of hippocampal damage in MTLE-HS has been correlated with the number of secondary generalized tonic-clonic seizures (SGTCS) in the individual lifetime and past history of febrile seizures (FS) are more often related to unilateral than bilateral HS[15]. One study has also demonstrated that the presence of reduced hippocampal volume is associated with seizure recurrence in focal epilepsies after long term seizure remission[16].

In the last decades, advances in neuroimaging techniques have detected subtle structural and functional abnormalities in MTLE-HS which are also possibly associated to

worse AED response. Studies of quantification of gray matter in MTLE-HS have demonstrated subtle gray matter atrophy beyond the mesial or neocortical temporal structures, including predominantly other limbic areas as bilateral thalamus, but also extra-limbic structures as the frontal lobes[17-19]. Although this subtle gray matter damage is more evident in drug-resistant MTLE-HS and it has been sometimes correlated with the occurrence of seizures[20], there is also evidence of diffuse atrophy in MTLE-HS patients with well controlled seizures[21,22]. A more recent study has also detected diffuse cortical thinning in MTLE-HS patients with seizure remission, more significantly in the bilateral sensoriomotor cortex[23].

Subtle white matter abnormalities, specifically detected with the use of diffusion tensor imaging (DTI) analysis, have also been identified in MTLE-HS; however, so far these studies have focused only in AED resistant MTLE-HS[24]. Similarly, functional MRI analysis has observed dysfunction of functional connectivity in drug-resistant MTLE-HS[25,26].

These structural and functional abnormalities detected by refined process of MRI data remains with uncertain significance. Some studies claim that these abnormalities might be related to the primary mechanism of epileptogenesis or to the burden of repeated seizures rather than being part of the epileptogenic zone[17]. However, there is also evidence that these abnormalities might contribute to AED or surgical responses as well as to the comorbidities of MTLE[21,27,28]. Further studies comparing abnormal functional and structural networks in MTLE-HS with good and poor AED seizure control are necessary to understand how these detected abnormalities can contribute to the development of new treatments for drug-resistant MTLE-HS.

2.2- AED response and MTLE-NL spectrum

MRI-negative MTLE (MTLE-NL) is a group not as thoroughly studied as MTLE-HS. According to the ILAE classification[1], these patients are considered cryptogenic (or as unknown origin according to the proposal of the new ILAE classification[29]). Although neuroimaging, genetics and pathology have advanced in the last decades, there are still 30% of patients with epilepsies of unknown origin[29]. MTLE-NL can be a prototype to deeply study patients with normal MRI and undefined etiology with the unique opportunity of comparison with a similar EEG/semiological phenotype but with diverse etiology. The study of MTLE-NL is also important as a comparison with MTLE-HS to understand to role of the etiology on prognosis and evolution.

As described before, cryptogenic epilepsies are easily controlled with medications than symptomatic epilepsies[7], so it is expected a significantly larger proportion of MTLE-NL patients with adequate seizure control than in MTLE-HS group. A recent study conducted in a tertiary epilepsy center described a cumulative probability of seizure remission in MTLE-NL of 47.7% at 2 years, 54.4% at 5 years and 55.4% at 10 years follow-ups[4].

Although similar semiology and EEG characteristics, MTLE-NL and MTLE-HS patients have some consistent distinct features, specifically older age of seizures onset and lower incidence of a past history of febrile seizures in MTLE-NL[5,15,30,31]. Even in studies with MTLE-HS, these same characteristics are related to better AED response[9] and one study have found that older age of epilepsy onset is an independent prognostic factor irrespective of the presence or not of MRI HS signs[4]. The weight of HS, febrile seizures

and age of epilepsy onset on prognosis of seizure control in MTLE must be deeply evaluated.

Few studies have looked for subtle gray matter atrophy in MTLE-NL and the results are diverse. One study evaluated a small number of drug-resistant MTLE-NL and found no gray matter abnormality[30] while in a different study, also evaluating refractory MTLE-NL, gray matter atrophy was observed in frontal and orbito frontal cortex, cerebellum, neocortical temporal regions, and parahippocampal cortex[31]. Network structural abnormalities in MTLE-NL with well-controlled seizures have been so far evaluated by a limited number of studies, but a pattern of cortical thinning similar to patients with well-controlled MTLE-HS was observed, involving mainly the bilateral sensorimotor cortex[23].

2.3. Genetics and "benign" MTLE-HS and MTLE-NL:

A type of MTLE with excellent seizure control with or without AED ("benign MTLE", defined as at least 24 months of seizure freedom) is observed in patients with or without signs of HS. For these patients, genetic factors are considered the major etiological determinant according to their extensive family history of epilepsy[32]. The actual incidence of HS among these patients can only be estimated by MRI evaluations, so these benign patients will be discussed together irrespective of MRI findings of HS or normal MRI.

These benign MTLE patients usually have the age of epilepsy onset between adolescence and mid-adult life and family history of seizures or FS is observed in about 30% of the cases[32]. Seizures are easily treated with up to 90% seizure-free under a single AED at low dosage[8,32]. Interictal EEG is normal in around 61% of patients. Long-term follow-ups suggest that later seizure intractability in benign familial MTLE is unlikely[33].

Despite the high incidence of family history of epilepsy in these patients, the weight of genetics in the etiology of "benign" MTLE is still controversy, since some studies also demonstrated sporadic benign MTLE with similar characteristics of those with family history of MTLE[34]. Similarly, the role of genetics in the determination of HS is not well understood. MRI evidence of HS has been observed in asymptomatic relatives of MTLE-HS subjects suggesting that HS can be inherited[35]. Recent evidences suggest a model of complex inheritance for familial MTLE[36].

The influence of genetics is also present in drug resistant MTLE-HS. A recent study demonstrated that widespread structural gray and white matter atrophy and IQ performance are worse in negative family history MTLE-HS patients. The authors suggest that HS in patients with positive family history of epilepsy might be determined by a stronger genetic predisposition differently from those with negative family history for whom the influence of environmental factors, as initial precipitating injuries (IPI) might be higher[28].

3- Advances in the knowledge of surgical outcome in MTLE:

For patients with focal epilepsies and AED resistant seizures, surgical treatment with the removal of the seizure onset zone is the best treatment option[37]. MTLE patients account for the vast majority of epilepsy patients submitted to surgical procedures, with MTLE-HS among the higher rates of successful seizure control[38]. Differently, in patients with negative pathology after temporal lobe resection, as is still the most common scenario in MTLE-NL, the rates of seizure remission after the surgical procedures are much lower[39]. Currently, inadequate or incomplete excision of the epileptogenic tissue is considered the major aspect related to surgical failure after temporal lobe resections. The study of surgical

prognosis not only helps to better define the correct surgical approach to different individuals or to define who are less likely to become seizure -free with surgical interventions but also can increase the understand of the different pathologies behind the clinical, EEG and MRI picture of MTLE-HS or MTLE-NL.

3.1- Surgical outcome and MTLE-HS spectrum

Anterior temporal lobectomy is the standard procedure for patients with MTLE who fail to achieve good seizure control with two first-line AEDs[37,40]. Classically, 60-70% of drug-resistant MTLE-HS submitted to anterior temporal lobe resection become seizure-free in two years follow-up[37,40]. However, recently, longer follow-ups have demonstrated that after ten years, only 50% of patients remain free of seizures[38]. Also, a low percentage of MTLE-HS individuals submitted to surgery become seizure free off medication[37]. The "cure" of MTLE-HS with the removal of the temporal mesial structures happens in a low percentage of individuals. The most plausible reason for this failure is the incomplete removal of the epileptogenic zone. In that sense, in the last decade efforts have been made to better understand the extent of the epileptogenic zone in MTLE-HS and its difference in possible sub-groups of MTLE-HS.

An open divergence in the literature is whether selective amygdalohippocampectomy or the complete removal of the anterior temporal lobe including the neocortical structures have different seizure control outcomes[41]. The major benefit of selective amygdalohippocampectomy is the possible better neuropsychological outcomes due to the preservation of temporal structures associated to memory performance. On the opposite, anterior lobe resections have the advantage of including the removal of

possible epileptic tissue located in the neocortical temporal cortex of a subgroup of MTLE-HS patients. The data available in the literature so far do not show consistent differences of outcomes between the two approaches, however the lack of adequate class I studies hampers a final verdict in favor or either procedure[41].

Shorter or long term outcomes after temporal lobe resections in MTLE-HS have been correlated to different patient's characteristics. One study found that the history of SGTCS is a predictor of poor surgical outcomes in the 2-years outcome while ictal dystonia is predictive of poor seizure outcomes after 2 and 3 years follow-up. According to the authors, these data suggest that early surgical failure in MTLE-HS might be related to the propagation of the epileptic focus outside the mesial temporal region and to a more extensive epileptogenic area[42]. In this same study, longer epilepsy duration was associated with poor surgical outcome in the 3 and 5 years follow-up, suggesting that the chronicity of seizures may lead to additional brain damage and possible secondary epileptogenesis[42]. Also, a study including MTLE of different etiologies but mainly composed by MTLE-HS (60%) confirmed that clinical markers of diffuse or poorly localized epileptogenicity, such as frequent preoperative seizures, generalized motor seizures, are related to worse surgical outcomes[43].

MRI signs of lesions outside the hippocampus (dual pathology) or of bilateral HS are classically associated with poor surgical outcomes[44,45]. Concerning the extent of surgical resection, visual and quantification evaluations of MRIs have helped to identify sub-groups of MTLE-HS patients with damage in other mesial temporal structures, as amygdala[10], parahippocampus[46] and entorhinal cortex[47] or in the neocortical temporal cortex and white matter[48,49]. Quantification of brain tissue in the post-operative MRI have

demonstrated a better seizure outcome according to the extension of hippocampal removal and in one study even better outcomes were obtained extending the mesial temporal resection to the entorhinal cortex[50].

The concept of a more extensive epileptic network in MTLE-HS patients with poor surgical outcomes have also been emphasized by different structural and functional neuroimaging studies. In a study of fluorodeoxyglucose-positron emission tomography (FDG-PET) predominantly composed by MTLE-HS patients, extratemporal cortical hypometabolism outside the seizure focus was associated with a poorer postoperative seizure outcome[51]. In a different FDG-PET study, multiple logistic regression analysis also identified the extent of remote hypometabolism as predictor of seizure outcome[52]. Also the extent of the resection of the FDG-PET hypometabolism significantly correlated with surgical outcome, independent of the presence of HS[53]. The histological meaning of this extra-hippocampal PET hypometabolism is not fully understood since the histopathology of the removed neocortical temporal tissue do not always demonstrated significant abnormalities. One study, however, demonstrated focal cortical dysplasia (FCD) pathology associated with more prominent lateral temporal metabolic dysfunction in PET exams of MTLE-HS patients[54].

The extent of abnormalities detected with other functional neuroimaging techniques are not easily related to surgical outcomes in MTLE-HS. One study evaluating the ictal SPECT patterns in temporal lobe resection of drug resistant MTLE of different etiologies, including HS, failed to demonstrate that extended patterns of ictal perfusion could predict poor surgical outcome. The authors discuss that the extended ictal SPECT hyperperfusion probably represents seizure propagation and not epileptogenic tissue[55]. Diffuse subtle gray

matter atrophy detected with refined pos-process MRI tools has also been associated with poor surgical outcome[27,28], suggesting the participation of some of these extra medial temporal structures in the epileptogenic process of a subgroup of MTLE-HS who do not achieve seizure control after temporal lobe resection. On the opposite, diffuse white matter abnormalities detected with DTI may not necessarily be implicated in the surgical outcome of MTLE-HS as suggested by one study[56]. However, in this specific study the number of non seizure-free patients was very limited (three) so, although, white matter abnormalities might not be related to the epileptic networks possibly involved in the persistency of seizures after surgical resection, more evidence is necessary to confirm these findings.

The presence and extent of scalp and invasive EEG abnormalities have also been implicated in surgical outcomes in MTLE-HS. Patients with interictal epileptiform discharges exclusively localized in one temporal lobe have significant better outcomes and so does the patients with regionalized ictal EEG without contralateral propagation[57]. In a recent study with scalp EEG, it was observed that a higher frequency of interictal temporal spikes in the preoperative EEG is correlated with worse surgical outcome. The authors suggest that the increased frequency of scalp EEG spikes may indicate a more wide epileptogenic region[58]. Another study demonstrated that interictal spikes detected on the 6-month postoperative EEG were a predictor of seizure recurrence in patients who had been seizure free after the procedure up to that moment[43].

Scalp EEG has the advantage of being widely available for all patients with epilepsy. However, the use of intracranial EEG (icEEG) recordings can substantially increase the accuracy to detect the epileptic focus and it can give substantial information

about the real epileptogenicity of brain tissue outside the mesial temporal region in MTLE-HS.

The role of mesial structures other than hippocampus in the origin of seizures in MTLE-HS has been well documented with icEEG, including a substantial intra-patient variability of the localization of seizure onset, what corroborates the hypothesis of dynamic networks responsible for seizure generation in MTLE-HS[59]. Other important information about the distinctive epileptogenic networks of MTLE-HS has come from icEEG studies in the last decades. It has been suggest, through different patterns of ictal onsets that distinct subtypes of MTLE-HS may occur: the temporopolar subtype, the mesiolateral subtype, the lateral subtype and also a widely extended temporal-plus subtype, with multilobar ictal onset zones[60]. In that sense, the success of the surgical procedure would be directly related to the identification of these specific subtypes and to including in the resection all the brain tissue localized in this extended epileptogenic region outside the mesial temporal structures.

The involvement of different brain areas during either seizure onset or affected during seizure spread has also been implicated in postoperatory outcomes in MTLE-HS. Temporopolar involvement before or concurrently with the hippocampus at the onset of seizure has been associated with better surgical outcome after anterior temporal lobe resections[61]. On the opposite, the secondary involvement of the insula during seizure spread was associated with poorer seizure outcomes[62]. And icEEG evidence that the duration of the epilepsy might influence the development of the different epileptogenic networks has also emerged in the last decades. One icEEG study suggested that in MTLE there is a progressive recruitment of epileptogenic structures with time and that the surgical prognosis is related to the extent of the epileptogenic network[63].

Despite the important contributions of icEEG recordings for the knowledge of the extent of the epileptogenic zone in MTLE, a significant proportion of MTLE-HS patients remain with unsatisfactory surgical outcomes[37]. The main disadvantage of icEEG so far is the impossibility of covering large extensions of brain tissue and the sum of increase morbidity of the use of a large number of electrodes[64]. For this reason, non invasive techniques able to accurately detect epileptogenic tissue are still necessary to solve this problem. **S**imilar surgical outcome has been described for both sporadic and familial MTLE-HS[33,65].

Histology specimens of HS also can be divided in distinct subtypes according to the amount and localization of neuronal loss and different histopathology features are implicated in different seizure outcomes[66]. Patients with Type 1 (severe cell loss in all hippocampus subfields, excluding (1a) or not (1b) sector CA2) and Type 2 (severe cell loss restricted to sector CA1) are associated with good surgical outcome, while the atypical variant Type 3 (severe cell loss restricted to the hilar region) is associated with poor surgical outcome[67]. In a recent series, Type 3 HS was observed in only 4% of the specimens and with seizure freedom rates of only 28% after one year follow-up[67].

Proven histological neocortical abnormalities associated with HS have been described in some series. In one study, temporal lobe sclerosis (characterized by reduction of neurons from cortical layers II/III and laminar gliosis) was detected in 11% of cases of HS and these patients had a higher incidence of FS and IPI, but no different in surgical outcomes were observed[68]. According to the authors, this pattern of neuronal loss and gliosis observed in the temporal lobes had been previously interpreted as FCD, however these abnormalities do not easily fit in the new FCD classification[69].

Difference of early and late seizure recurrence: Possibility of "de novo" epileptogenesis after surgical treatment might be related to the etiology of the epilepsy (or the etiology of the HS). Longer epilepsy duration associated with poorer outcomes suggests that secondary epileptogenesis distant to the lesion may develop with years of uncontrolled seizures; however, there is no conclusive evidence for that in humans. Studies that describe the site of seizure origin in early and late seizure recurrence are necessary for a better comprehension of the possibility of secondary epileptogenesis[42].

3.2- Surgical outcome and MTLE-NL spectrum

MTLE with normal MRI is a heterogeneous group with different pathologic substrates and postoperative seizure outcomes[70]. Successful surgical outcomes in MTLE-NL, as in other MRI-negative epilepsies, are significantly lower than in MTLE-HS[39].

In one surgical series of MTLE-NL patients, subtraction of SPECT coregistered to MRI (SISCOM) abnormality localized to the resection site and subtle nonspecific MRI findings in the mesial temporal lobe concordant to the resection[39]. The authors emphasize the consistency of the results with the small number of patients with chronic intracranial monitoring (25%) in this series[39].

Special attention has been given in the last decades to a sub-group of MTLE-NL patients with temporal hypometabolism in FDG-PET images (MRI negative-PET positive MTLE)[71]. These patients have an intermediate surgical outcome that is significantly better than patients with MTLE-NL and no detectable hypometabolism on PET and that in some studies are very close to the prognosis of MTLE-HS[72]. Despite the clinical appearance of mesial MTLE, MTLE-NL with PET positive hypometabolism is consider to involve

primarily lateral temporal neocortical rather than mesial temporal structures[71,72]. Similarly to MTLE-HS, the extent of the resection including the area of PET hypometabolism in MTLE-NL has also been implicated in surgical outcome[73].

In MTLE-NL, scalp EEG also has important prognostic implications in surgical outcomes. Absence of contralateral or extratemporal interictal epileptiform discharges is associated with better surgical outcomes[39,74]. Also, type I ictal scalp EEG patterns (regular temporal rhythm of 5-9 Hz) [75] has been associated with better surgical outcomes also in MTLE-NL[39,74]

When HS is found in the surgical specimen patients usually have good surgical outcome. If FCD is found it still depends on the completeness of the resection. If the pathology is normal poor surgical outcome occurs in the majority of patients. In this sense, high resolution MRI has helped to improve surgical prognosis with the detection of more subtle pathologies as HS or FCDs.

In recent series using high field MRI and adequate protocols, histopathology of HS is found in a low percentage of surgical specimens of MTLE-NL. In one series of MTLE-NL patients selected to temporal lobe resection based on a 1.5T MRI protocol, HS histopathology was observed in 30% of the cases[74]. In a more recent series drug-resistant MTLE-NL selected to surgery on a basis of a "modern" protocol for pre-surgical evaluation which included 3T MRI, HS was only identified in 18% of the cases[39]. One study compared histopathology of MTLE-NL patients with onset of hippocampal seizure confirmed by long-term intracranial monitoring with a MTLE-HS group. Substantial difference was observed in the histopathology of the hippocampus, with MTLE-NL patients with

significant smaller loss of pyramidal cells in the CA1 subfield and maximal neuron loss in the CA4 region (end folium sclerosis) in a subgroup of MTLE-NL[5].

Other histopathologic abnormalities detected in surgical specimens of MTLE-NL are gliosis (which accounted for 80% of one recent surgical series[39]) and FCD (only one out of 40 cases in this same series[39]. In another series, histopathologic examination failed to reveal any focal pathology in 68% of the MR-negative cases[72]. The detection of subtle FCD can be a challenge and new classification schemes have been trying to solve the high variability and low inter observer agreement in the histological determination of FCD[69]. In this context, it is possible that a larger number of histopathologic samples from surgical resection of patients with drug resistant MTLE classified as normal may in fact be mild forms of FCD.

4. Expert commentary: what are the differences from good and bad responders?

Neuroimaging, EEG, genetics and histopathology data show that both MTLE-HS and MTLE-NL are not single entities but a group of distinct pathologies for which there is a wide range of AED responses and surgical outcomes.

Classically, population-based and tertiary centers conducted studies show that MTLE-HS presents with higher rates of patients with AED resistant seizures while MTLE-NL has higher rates of patients with adequate seizure control under AED treatment. On the opposite, drug-resistant MTLE-HS has higher rates of seizure freedom after anterior temporal lobe resection than MTLE-NL. However, more recent data from neuroimaging studies shows that HS is not exclusive observed in drug-resistant MTLE but it also can be

seen in patients with good seizure control and mild epilepsy course, as well as in asymptomatic relatives.

According to recent studies, some clinical characteristics are linked to better or worse AED response. In both MTLE-HS and MTLE-NL, an early age of epilepsy onset and a past history of FS are associated with AED drug-resistant seizures. In MTLE, a genetic background might be associated with higher rates of AED response and less extensive neocortical atrophy. However, patients with familial forms of MTLE-HS and drug-resistance seizures have similar rates of seizure freedom after surgical removal of the epileptic focus as patients sporadic MTLE-HS. So far, these distinct clinical features are important for an early selection of MTLE patients who might benefit from surgical interventions due to lack of AED seizure control.

Concerning surgical treatment of drug resistant MTLE, in MTLE-HS failures of seizure freedom after removal of the epileptic focus are associated with more extensive epileptic brain tissue. Data from neuroimaging and EEG studies also shows that these extended epileptic networks might be associated with the chronicity of the epilepsy what reinforces the fact that AED refractoriness must be early defined for each patient and surgery, if indicated, should not be postponed. However, stronger evidence is needed to better define the role of these extended epileptic networks in seizure generation and for which patients the removal of extra brain regions are necessary for adequate seizure control.

In MTLE-NL, an extensive epileptic brain tissue might also be the cause of failure of seizure freedom after the surgical treatment; however, due to the frequent reports of

normal histology in the surgical specimens, one must keep in mind the possibility of wrong seizure focus localization or also a subgroup of non surgical remediable pathologies among MTLE-NL. Histopathology advances have indicated that part of these normal histology specimens from MTLE-NL surgeries are, in fact, subtle FCD and more studies are necessary to unify histopathology diagnosis in order to better understand the causes of surgical failure in patients with MTLE and normal MRIs.

In summary, the increased comprehension of the differences of individuals with MTLE-HS and MTLE-NL will help not only the early detection of epilepsy surgery candidates but also to improve the knowledge of the mechanisms of AED resistance. Also, adequate trials will help to define how extended surgical resections may help seizure control in drug-resistance MTLE. Further detailed information from both clinical and surgical outcomes in distinct subgroups of MTLE will help the development of more appropriated and individualized targets for the treatment of seizures and co-morbidities in this prevalent type of epilepsy.

5. Five-year view: how will we improve MTLE treatment?

Longer follow-ups of epidemiological studies with more adequate characterization of the epileptic syndromes will help to define the prevalence of good and bad AED responders among MTLE-HS and MTLE-NL. Also, although the efforts of epilepsy studies are mainly concentrated on drug-resistant epilepsies, in the last two decades important cohorts of “benign” MTLE have better characterized these individuals. The longer follow-up of these cohorts will also improve our knowledge about the specific characteristics of MTLE patients with good seizure control.

Improvement of neuroimaging techniques, with higher field MRIs and other sophisticated equipments as PET and SPECT will also have a considerable impact in the understanding of MTLE. Probably, the two major contributions of neuroimaging in the near future will be the early definition of the probability of AED response in specific patients and the more accurate delimitation of the extent of the seizure onset zone in patients with drug-resistant MTLE selected to surgical treatment. Controlled surgical trials evaluating the removal of extended epileptic tissue or also the role of subtle structural abnormalities detected by sophisticated neuroimaging analysis in the surgical outcome will propitiate a more adequate selection of patients to be submitted to surgical procedures.

Important contribution for the understanding of different types of MTLE and the development of more appropriated therapies will come from genetic studies. The identification of different genes associated with MTLE but, probably more important, the degree of influence of the genetic background in the AED response will certainly improve AED treatment through both more adequate choice of a specific mechanism of action as well as to the development of new AEDs with diverse brain targets.

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CAPÍTULO 2

Multimodal neuroimaging: Potential Biomarkers for response to AEDs?

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Epilepsia (aceito para publicação)

Multimodal neuroimaging: Potential Biomarkers for response to AEDs?

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Summary:

Neuroimaging techniques in epilepsy are widely used to the definition of epileptogenic zone and surgical decision. However, its application extends to the knowledge of epileptic mechanisms and includes the identification of prognostic features that can help our decisions for the appropriated treatment of different patients. Structural neuroimaging is able to differentiate some good or worse anti-epileptic drug responders and those patients who will benefit from surgical treatment or not. In the last decades, quantitative analysis have also improved this knowledge. New advanced neuroimaging techniques as functional MRI and the development biotracers that could be associated with inflammation and specific genetic patterns will add a great improvement in the field of epilepsy in the near future.

At least 30% of patients with epilepsy will fail antiepileptic drug (AED) treatment (Kwan & Brodie, 2000). For those, the best approach is surgical treatment with resection of the epileptogenic zone. However, around 53% of patients submitted to surgery will have seizures relapse after a period of 10 years (de Tisi et al., 2011). The early identification of patients who will not respond to AEDs or that will fail surgical treatment can save time and reduce morbidity in individuals with epilepsy. Neuroimaging techniques allow non-invasive detection of subtle structural and functional brain abnormalities that may be linked to better or worse response to AED or surgical treatments in these patients.

The field of neuroimaging has improved in the last decades and the use of these techniques to define biomarkers of neurologic disease, including epilepsy, has grown widely. The most commonly used neuroimaging method is structural MRI, but functional images as functional MRI (fMRI) and PET have improved the searching for biomarkers. MRI quantification analysis has allowed the identification of subtle structural abnormalities related, for example, to clinical features as family history (Yasuda et al., 2010) and seizure recurrence after AED withdrawal (Cardoso et al., 2006). Recently, refined brain imaging targeting abnormalities related to inflammation, pharmacology and genetics are under development.

It is classically known that only about 60% of individuals with epilepsy will respond to the first two AEDs and less than 4% with further AED trials (Kwan & Brodie, 2000). Recent data have added the knowledge that while 59% of patients with epilepsies will remain constantly seizure free with AEDs and 25% will never achieve seizure control with medication, 16% of patients with epilepsy will develop a relapse-remitting pattern of response to AED (Brodie et al., 2012). For this last pattern of AED response, surgical

decision may be delayed, enhancing the morbidity of these individuals. Efforts must be made to the identification of biomarkers that could allow the early detection of patients who will fail treatment. Accordingly, a study in our center demonstrated that patients with pharmaco-resistant and relapse-remitting mesial temporal lobe epilepsy (MTLE) have a similar pattern of gray matter atrophy detected by voxel-based morphometry (VBM), and it was more widespread than in AED responders (Bilevicius et al., 2010). The authors concluded that AED response in MTLE is multifactorial appears to be related to the underlying pattern of brain atrophy that extends beyond the hippocampus and age at seizure onset. It may also imply that the damage and morbidity in pharmacoresistant and relapse-remitting MTLE is similar and that to individuals in the former group surgical treatment should be readily considered. The question that remains is how one could precociously identify AED responders. Another study from our center demonstrated that proton MR spectroscopy (MRS) maybe a reliable biomarker to TLE patients who will respond to the first AED (Campos et al., 2010). Reduced NAA/creatine ration was found in hippocampi of patients who fail the first AED trial but not in those who achieve seizure freedom indicating that patients with TLE who respond well to the first AED have significantly less evidence of neuronal and axonal damage/dysfunction.

More appropriate biomarkers should be directed for specific types of epilepsies. The majority of studies evaluate TLE patients and less is known about the possible markers of treatment response of individuals with other localization-related epilepsies. Even in studies with TLE associated with hippocampal sclerosis (HS), one must have in mind that this is a syndrome rather than a specific disease. Refined analysis emphasizing the possible different etiologies is the gold standard for the definition of accurate biomarkers. For example, we

were able to identify predictive factors of poor outcome in a cohort of individuals with familial mesial TLE (FMTLE) after a mean follow-up of 7.6 years. In this study, the presence of HA and interictal epileptiform discharges (IEDs) were related to worse outcome (Morita et al., *in press*).

Besides structural abnormalities, functional MRI (fMRI) has also helped to improve the knowledge of epilepsy damage and prognosis. Moreover, by examining brain systems and their functional dynamics, fMRI may be able to optimize the discovery of new drugs for neurological conditions, including epilepsy, in the near future (Borsook, Becerra & Hargreaves, 2006). The study of different brain networks abnormalities in specific epilepsy groups and the association with AED response is a rich field to be exploited by future researches. Also, the role of fMRI in the prediction of surgical outcome in epilepsy have been investigated in some studies. The use of IEDs triggered fMRI (EEG-fMRI), for example, can identify not only hemodynamic abnormalities in the seizure onset zone of patients with epilepsy but it also can detect abnormal networks that may have implications in surgical outcome. EEG-fMRI has the advantage of relying on single-subject analysis what can be readily used in the clinical setting for decisions for a specific patient. For example, we observed with EEG-fMRI studies that the hemodynamic abnormalities related to temporal IEDs in patients with non-lesional TLE is often localized in extra-temporal regions and it may be diffuse (Coan et al., 2012). Other studies have demonstrated that the concordance of IEDs triggered hemodynamic abnormalities on EEG-fMRI studies with the localization of surgical resection is associated to a better surgical outcome (Thornton R et al., 2010; Thornton R et al., 2011).

Pharmacogenetic may be one additional variable in AED response. For example, there is a strong association in Han Chinese between the leukocyte antigen HLA-B1502 and Stevens-Johnson syndrome induced by carbamazepine (Chung et al., 2004). In our service, we found association between pharmaco-resistance to AEDs in MTLE patients and drug-transporter (ABCC2) and drug-metabolism genes (CYP1A2 and CYP2E1). In our sample, ABCC2 was up-regulated in tissue samples obtained from patients with pharmacoresistant MTLE (Silva et al., 2010). Neuroimaging may help to clarify the relation of genotype and clinical characteristics and treatment response. Fedi et al, for example, in a with [^{11}C]flumazenil PET, demonstrated that patients with the GABRG2(R82Q) mutation express reduced $GABA_A$ receptors and it was detected by the [^{11}C]flumazenil binding mostly in the cingulate and insular cortices (Fedi *et al.* 2006).

In the past decade, attention has been paid to the role of inflammation in the epileptogenesis and seizure recurrence or epilepsy progression. Drugs with anti-inflammatory mechanisms are promising targets to treat epilepsy. Preliminary data of a Phase II trial indicate a possible beneficial effect in seizure reduction of patients with epilepsy with VX-765, a novel Interleukin-1β -Converting Enzyme/Caspase 1 inhibitor, which reduces the production and release of IL-1 β (French et al., 2011). Neuroimaging may be used to diagnosis and follow inflammatory abnormalities. This evidence is already available in animal models. Filibian *et al*. demonstrated that proton MRS measurements can be used to explore glia activation as a biomarker during epileptogenesis and in the chronic epileptic phase in rat hippocampus (Filibian et al., 2012). In another study with rat lithium–pilocarpine model, Duffy *et al*. verified that vascular cell adhesion molecule 1 antibody labelled iron oxide can be a potential targeted for MRI contrast agent to image the

inflammatory (Duffy et al., 2012). The authors observed marked focal hypointensities caused by contrast agent binding *in vivo* MRIs in the group of animals with induced status epilepticus, particularly in the periventricular organs, the hippocampus and the cerebral cortex. In humans, the use of neuroimaging technique to demonstrate neuroinflammation is still scarce, with few case reports. Butler et al. used [C11]PK11195 PET, a marker of activated microglia, to visualize neuroinflammation in a patient with focal cortical dysplasia (Butler et al., 2011). They observed an area of increased radiotracer uptake in the right frontal lobe and it was concordant to the identified seizure focus.

Neuroimaging biomarkers may also be used to evaluate progressive structural and functional abnormalities in chronic epilepsies. There is extensive evidence that TLE-HS is a progressive disorder and neuroimaging studies, especially MRI, have contributed to this knowledge (Coan et al., 2009; Conz et al., 2011; Morita et al., *in press*). For other types of epilepsies, the evidence of disease progression is not so clear. Neuroimaging studies with homogenous types of epilepsies can provide evidence of specific pattern and intensity of progression what can propitiate more adequate clinical decisions as well as the development of mechanisms to stop this progression. We have observed progressive gray matter atrophy correlated with seizure frequency and epilepsy duration in a VBM study of patients with MTLE and that the progressive abnormalities were more pronounced in patients with seizure focus on the left side (Coan et al., 2009). Progression of hippocampal atrophy defined by volume measures was also detected in sporadic and familial MTLE, although in the former group this progression maybe slower, what emphasize that the basic mechanism of epileptogenesis may play a role in the progressive burden of chronic epilepsy (Conz et al., 2011; Morita et al., *in press*).

Conclusion

Prediction of response to AEDs and surgical outcome are essential to improve treatment in patients with epilepsy. Neuroimaging techniques, especially quantitative MRI, have already helped to increase our knowledge about the differences of structural abnormalities in diverse groups of patients and its relation to AED response. New neuroimaging modalities, as fMRI, will bring further information and help the development of new AEDs directed to specific networks targets. Imaging associated to inflammation and specific genetic patterns are under development and will help us learn more about the different epileptogenic mechanisms.

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CAPÍTULO 3

3T MRI quantification of hippocampal volume and signal in mesial temporal lobe epilepsy

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Quantification of hippocampal volume and signal in mesial temporal lobe epilepsy improve detection of hippocampal sclerosis

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Abbreviation Key

HS: hippocampal sclerosis

MTLE: mesial temporal lobe epilepsy

ILAE: International League Against Epilepsy

T: Tesla

SD: standard deviation

Abstract:

Background: In mesial temporal lobe epilepsy (MTLE), MRI quantification of hippocampal volume and T2 signal can improve the sensitivity for detecting hippocampal sclerosis (HS). However, it is not clear what are the current contributions of these analyses for diagnosis of HS in 3T MRIs.

Purpose: To compare visual analysis, volumetry and signal quantification of hippocampus for detecting HS in 3T MRIs.

Methods: 203 MTLE patients defined by clinical and electroencephalogram criteria had 3T MRIs visually analyzed by imaging epilepsy experts. As a second step, we performed automatic quantification of hippocampal volumes with FreeSurfer and T2 relaxometry with an in-house software. MRIs of 79 healthy controls were used for comparison.

Results: Visual analysis classified 125 patients (62%) as having signs of HS and 78 (38%) as normal MRIs. Automatic volumetry detected atrophy in 119 (95%) patients with visually detected HS and in 10 (13%) with visually normal MRI. Relaxometry analysis detected hyperintense T2-signal in 103 (82%) patients with visually detected HS and in 15 (19%) with visually normal MRI. Considered together, volumetry plus relaxometry detected signs of HS in all but one (99%) patients with visually detected HS and in 22 (28%) with visually normal MRI.

Conclusion: In 3T MRIs visually inspected by experts, quantification of hippocampal volume and signal can increase the detection of HS in 28% of patients with MTLE.

Introduction:

HS is the main pathologic substrate in patients with MTLE, which is the most common form of epilepsy in adults[1]. HS can be reliably detected in MRIs[2] and quantitative analysis of hippocampal formation improve the sensitivity for detecting this pathology[3,4]. With the advances in the MRI field in the last decades, it is not clear what are the current contributions of these post-processing MRI tools for the detection of hippocampal pathology, especially in tertiary epilepsy centers.

The histopathology of HS is characterized by loss of specific neurons and synaptic reorganization of surviving cells[6] and in MR images this is observed as volume reduction and signal hyperintensity in T2 images[7,8]. Quantification of hippocampal volume has advantages over the visual analysis for the detection of subtle and bilateral hippocampal abnormalities[2,5]. Equally, the quantification of hippocampal T2 signal, especially with relaxometry, can improve the MRI diagnosis of HS[9,10]. Both volume and hippocampal signal quantification measurements have good correlation with histopathologic findings of HS[4].

Currently, these techniques are considered reliable and reproducible for the detection of hippocampal pathology[11]. However, hippocampal volumetry by manual segmentation is time consuming and depends on the expertise of the examiner. These two facts have limited its use in clinical practice. More recent automatic analyses have shown to be promising, however, there are few studies comparing its efficacy with the visual analyses of high quality MRI by experts in the field[12,13].

Although it is clear that in studies with 1.5T MRI volumetry and relaxometry have significantly higher sensitivity than the qualitative analysis of MRI[5,9], today the majority of epilepsy centers work with 3T MRIs and specific epilepsy protocols which makes the determination of MRI signs of HS by visual analysis easier and more acurate[14]. It has been reported that there is no difference of the hippocampal volume measures of 1.5 and 3T[15,16]; however, quantitative measures in 3T MRIs can demonstrate ultra structural details of HS pathology not detectable with lower fields scanners[17]. Once the sensitivity to visually detect signs of HS at 3T is higher[14], the question that remains is whether the use of hippocampal measurements in these higher field MRIs still add information to the clinical practice.

In this study, we aimed to evaluate the contribution of automatic hippocampal volume and signal quantification in 3T MRIs to detect signs of HS after visual evaluation of the images by epilepsy imaging experts.

Methods

Patients

This is a prospective study of patients followed at the Epilepsy Clinic of University of Campinas with clinical and electroencephalographic diagnosis of MTLE according to ILAE criteria[18] who were submitted to 3T MRIs between August 2009 and April 2012. Informed consent form approved by the Ethics Committee of UNICAMP was signed by all patients prior to acquisition of MRI. Patients with symptomatic MTLE due to lesions other that HS (tumor, vascular malformations, gliosis, focal cortical dysplasia) were excluded.

MRI epilepsy protocol and visual analysis

All patients were submitted to a MRI epilepsy protocol in a 3 Tesla Philips Intera Achieva scanner (Philips, Best, Netherlands) which included:

- Coronal images, perpendicular to the long axis of the hippocampus, defined at the sagital image: (a) T2WI multi-eco image (3mm thick, TR=3300, TE= 30/60/90/120/150, matrix=200X176, FOV=1802X180); (b) T1WI "inversion recovery" (3 mm thick, TR=3550, TE=15, inversion time=400, TSE factor=7, matrix=240X229, FOV=180x180), (c) FLAIR images (Fat-suppressed, 4 mm thick, TR=12000, TE=140, matrix=224x160 ;
- Axial images parallel to the long axis of the hippocampus: FLAIR images (Fat-suppressed, 4 mm thick, TR=12000, TE=140, matrix=224x160, FOV=220X186);
- T1WI volume: with isotropic voxels of 1 mm, acquired in the sagital plane (1 mm thick, flip angle=8°, TR=7.1, TE=3.2, matrix=240x240, FOV=240x240);
- T2WI volume: with isotropic voxels of 1.5 mm, acquired in the sagital plane (TR=1800, TE=342, matrix=140X139, FOV=210x210).

As a first step, MRIs were visually analyzed by two epilepsy experts (ACC and FC) and the images were classified as normal or with signs of HS. Classical signs of HS were considered as follows: reduction of volume and abnormal shape observed on T1 images and increased signal observed in T2 and FLAIR images. Images were carefully examined by the investigators in the light of the clinical and EEG data of each patient as it is the routine of MRI evaluation for the investigation of focal epilepsies in most epilepsy centers. Special attention was given to rule out subtle signs of focal cortical dysplasia, as sulcal morphology abnormalities, focal increase of cortical thickness, FLAIR focal signal hyperintesities or small transmantle signs.

Hippocampal Volumetry

As the second step, quantification of hippocampal volume and signal was performed. A group of 79 healthy controls was used for comparison. Automatic volumetric analysis was performed with FreeSurfer software (version 5.1.0; http://surfer.nmr.mgh.harvard.edu/) using 3-D T1W images (1 mm slices, TR=22ms, TE=9ms, flip angle=35°, matrix=256x220). Hippocampal volumes were corrected for individual's brain (supratentorial) volumes and corrected hippocampal volumes smaller than 2SD (absolute value and/or asymmetry index, defined by the ratio of smaller over the larger hippocampus of each individual) from the mean of controls were considered as atrophy.

Hippocampal signal quantification

For signal quantification, we used relaxometry analysis of T2 multi-eco images (3mm slices; TR= 3300; TE=30/60/90/120/150; matrix=200X176; FOV=1802X180) with Aftervoxel, a medical image visualization tool written by Felipe Bergo (http://www.liv.ic.unicamp.br/~bergo/aftervoxel). For this analysis, a ROI was manually defined in three different MRI slices including the hippocampus of each individual (one in the head, one in the body and one in the tail of the hippocampus) by an investigator blind to the result of the MRI visual evaluation (BK). The mean T2 signal from the three slices of each hippocampus was used as the final measurement. Hippocampal signal values higher than 2SD from the mean of the control group (absolute value and/or asymmetry index, defined by the ratio of higher and lower hippocampal signal of each individual) were considered as hyperintense signal.

Results

Two hundred and seventeen patients fulfilled the inclusions criteria. However, after detailed MRI visual analysis, fourteen patients were considered to have subtle signs of focal cortical dysplasia and were excluded. The final group was then composed by 203 patients (129 female, 74 male, medium age of 46 years, range 17-74). According to ictal and interictal scalp EEG, 173 patients had unilateral temporal epileptic focus (111 left MTLE and 62 right MTLE) and 30 had bitemporal or undefined epileptic focus.

MRI visual analysis

MRI visual analysis detected 125 (62%) patients with signs of HS (62 left, 54 right, 6 bilateral with left-sided predominance, 3 bilateral with right-sided predominance). For the remaining 78 (38%) patients, MRIs were considered normal by visual analyses.

MRI quantification analysis

Automatic volumetry analysis detected hippocampal atrophy in 119 (95%) patients with visual signs of HS and in 10 (13%) patients with visually normal MRI. Relaxometry detected hyperintense T2 signal in 103 (82%) patients with visual signs of HS and in 15 (19%) patients with visually normal MRI (Figure 1). Considered together, volumetry plus relaxometry detected signs of HS in all but one (124; 99%) patients with visual signs of HS and in 22 (28%) patients with visually normal MRI.

Volumetry detected bilateral hippocampal atrophy in nine patients (eight with visual signs of HS and one with visually normal MRI). Relaxometry detected bilateral hippocampal hyperintense signal in 22 patients (20 with visual signs of HS and two with

visually normal MRI). In patients who had bilateral hippocampal abnormalities detected by the quantifications methods, there was a marked asymmetry.

Concordance of MRI visual and quantification analysis

The side of the HS detected by MRI visual and volumetry analysis was concordant in all but one case (118/119, 99%). The patient with discordant volumetry and MRI visual analysis had a subtle hippocampal atrophy and clear hyperintense T2 signal on the left hippocampus by visual analysis which was concordant with the T2 relaxometry.

The side of the abnormal hippocampal signal detected by relaxometry was concordant with the visual analysis in 96% (99/103). In the remaining four patients, two had bilateral asymmetrical hippocampal abnormalities on visual analyses and the relaxometry lateralized to the side with less hippocampal atrophy by both visual and automatic volumetry; and the other two patients had unilateral hippocampal atrophy (concordant by visual and automatic volumetry) and the relaxometry lateralized to the side contralateral to the atrophy and hyperintense T2 signal defined by visual analysis.

Concordance of MRI quantification analysis and the EEG epileptic focus

From the 129 MTLE patients with hippocampal atrophy detected by volumetry, 120 had unilateral epileptic focus (defined by ictal and interictal EEG) and in 95% (114/120) the side of epileptic focus was correctly lateralized by volumetry. From the 118 MTLE patients with hippocampal signal hyperintensity detected by relaxometry, 106 had unilateral epileptic focus and in 94% (100/106) the side of epileptic focus was correctly lateralized by volumetry.

Discussion

The detection of MRI signs of HS can help to define seizure etiology and to indicate surgical treatment for patients with drug resistant MTLE. We demonstrated here that even in 3T MRIs analyzed in tertiary centers by epilepsy experts, hippocampal volume and signal quantification can significantly improve the detection of signs of HS in patients with otherwise normal MRIs using an epilepsy protocol.

MRI has significantly improved the detection of pathologies related to epilepsy[19]. It is safe, non-invasive and widely available in epilepsy centers. However, a variable, but significant number of patients with focal epilepsies have normal MRIs and unknown seizure etiology[20-24].

From the beginning of MRI use in epilepsy, special attention has been given to HS since it is the main pathologic feature associated with the most common epilepsy in adults[1]. Hippocampal volume and signal have been used for research purposes but also in epilepsy clinics to help the evaluation of drug resistant focal epilepsies. Quantification of hippocampal volume and signal in MRIs can not only detect signs of HS but can consistently help to lateralize the seizure focus in MTLE patients who are surgical candidates[5]. For these individuals, the prognosis of surgical resection of the temporal lobe ipsilateral to HS detected by MRIs is excellent[21]. Today, most of the tertiary epilepsy centers have 3T MRIs available and visual signs of HS have been more easily detected[14]. In this context, the contribution of MRI quantification methods to detect HS in 3T MRIs had not been previously evaluated. Here we demonstrated that with 3T MRIs, adequate epilepsy

protocols and experts visual evaluation, quantification analysis can still improve in 28% the detection of subtle signs of HS.

The detection of more subtle MRI abnormalities in patients with focal epilepsies depends both on the quality of MRI acquisition protocol and the experience of the examiner in reading MRIs from patients with epilepsies. A previous study [20] showed that "non-experts" reported 61% of standard MRI as normal or as showing no focal abnormality, while epilepsy "expert" examiners' reassessment of the same standard MRIs classified 28% of these scans as technically inadequate and considered only 22% of these standard MRI scans as normal. More importantly, using a dedicated epilepsy MRI protocol, the same group of "experts" described focal MRI abnormalities in 91% of the same group of patients (they did not include hippocampal volumetry or T2 relaxometry) [20]. Also, in this context, MRIs are always evaluated in the light of clinical, neuropsychological and EEG data. In this paper, we reproduced this optimal visual evaluation of MRIs of patients with MTLE: two epileptologists with expertise in MRI evaluation of patients with focal epilepsies reviewed the images of all patients (acquired with an extensive epilepsy protocol) in the context of clinical and EEG data. Even in this most favorable circumstance, quantification of hippocampal volume and signal significantly increased the detection of signs of HS.

The increase of patients with detectable MRI abnormalities compatible with the site of the seizure onset origin is significantly important for drug resistant focal epilepsies. With quantitative analysis of 1.5T MRIs, a group of MTLE patients remains with no detectable structural abnormalities even when HS is confirmed after surgical removal of the mesial temporal structures[22]. For patients with drug resistant MTLE and normal MRI, invasive procedures, which have high costs and morbidity, are often necessary to evaluate the

potential target for surgical intervention[23] and yet for these individuals the rates of seizure freedom are lower than for those with MRIs signs of HS[24,25]. Histopathology of HS is only found in a limited number of MTLE patients with normal MRI submitted to surgery, but those with positive hippocampal pathology are the ones with better surgical outcome[24,26]. Efforts are necessary to improve non invasive techniques that could more efficiently select the MTLE individuals with these subtle HS in order to better select patients and improve surgical outcomes.

Up to today, the majority of studies about hippocampal volumetry applies manual hippocampal delimitation and it is still a debate whether manual[27] or automatic analysis has higher sensitivity and specificity[12,13]. Despite this controversy, in the clinical context the quantification of hippocampal abnormalities must be as fast and practical as possible. In this paper, we used automated volumetry and a simple manual signal quantification in which it is only necessary to define a small region of interest in three slices of the T2 scan of each patient, without the need to define precisely the borders of hippocampus. We think that this optimized hippocampal quantification protocol can be easily applied to all patients with drug resistant focal epilepsies as a parallel and additional analysis for the routine diagnosis of MRIs in specialized epilepsy centers.

The major problem of our study is the absence of histopathological correlation with the MRI findings. However, it has been previously demonstrated a good correlation of volume and signal abnormalities detected by quantification analysis and histopathology of HS[28]. Also, in our group of patients the laterality of abnormal hippocampal volume and signal was highly concordant to the laterality of the epileptic focus defined by EEG recordings.

Conclusions

In MRIs acquired in a 3T scanner and visually inspected by experts, quantification of hippocampal volume and T2 signal can increase the detection of signs of HS in about 28% of patients with MTLE. Today, these MRI quantification methods are easily available and not very time consuming and they can be used as routine diagnostic tools for patients with drug resistant focal epilepsies and visually normal MRIs.

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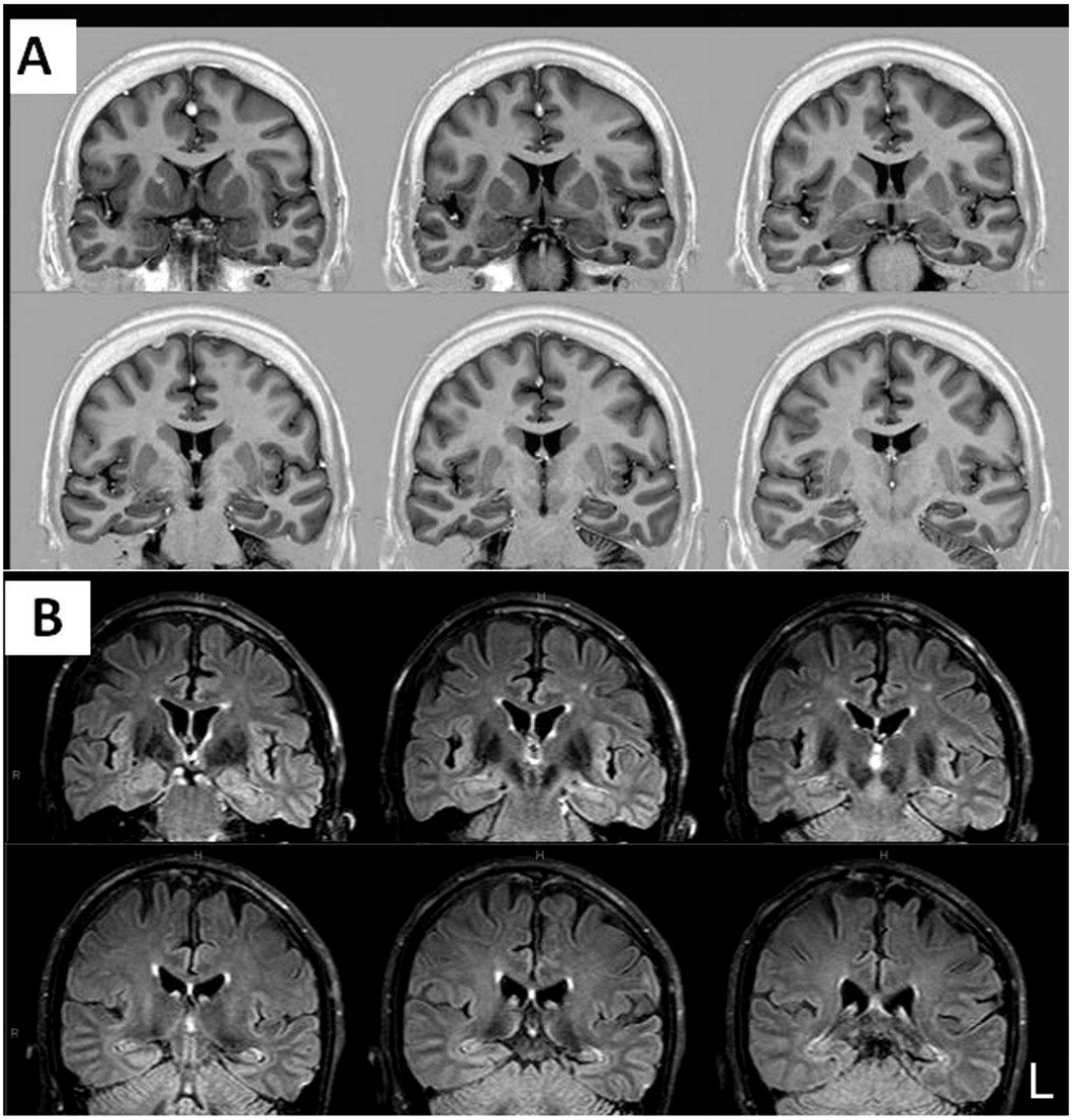
Figure 1: MRI signs of HS detected by quantification analysis. Examples of two patients with normal MRI visual analysis (MTLE-NL) and HS signs detected by quantification techniques. **A:** Patient with MTLE and left seizure focus; MRI volumetry detected significant left reduced hippocampal volume. **B:** Patient with MTLE and left seizure focus; MRI T2 relaxometry detected significant left increased hippocampal signal. HS: hippocampal sclerosis; MTLE-NL: mesial temporal lobe epilepsy without visual MRI signs of hippocampal sclerosis.



CAPÍTULO 4

Hippocampal sclerosis and antiepileptic drug response are associated to the pattern of gray matter atrophy in mesial temporal lobe epilepsy

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Submetido para *Epilepsia*

Hippocampal sclerosis and antiepileptic drug response are associated to the pattern of gray matter atrophy in mesial temporal lobe epilepsy

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Abstract:

Purpose: Patients with mesial temporal lobe epilepsy (MTLE) with hippocampal sclerosis (HS) have diffuse subtle gray matter (GM) atrophy detected by MRI quantification analysis. However it is not clear if the same pattern of extra-hippocampal atrophy occurs in MTLE of different etiologies and whether anti-epileptic drug (AED) response influence the GM atrophy in different subtypes of MTLE. Therefore, we aimed to evaluate the occurrence of GM atrophy and the influence of AED response in patients with MTLE and normal MRI (MTLE-NL) and MTLE-HS. **Methods:** We evaluated a group of 172 patients with unilateral MTLE and signs of hippocampal sclerosis (HS) or normal MRI (NL) as defined by hippocampal volumetry and signal quantification (122 MTLE-HS and 50 MTLE-NL). For the detection of GM atrophy, voxel-based morphometry (VBM) was performed with VBM8/SPM8 in 3T MRIs. Clinical characteristics were compared between the groups. Patients with up to 3 complex partial seizures in the previous year were considered as good seizure control. Those who did not fulfill this criterion were considered refractory. **Results:** Patients with MTLE-HS had more pronounced GM atrophy, including more importantly the ipsilateral mesial temporal structures, temporal lobe, bilateral thalami and pre/post-central gyri. Patients with MTLE-NL had more subtle GM atrophy, including ipsilateral orbitofrontal cortex, bilateral thalami and pre/post-central gyri. Both MTLE-HS and MTLE-NL had increased GM volume in the contralateral pons. Refractory MTLE-HS patients had more pronounced GM atrophy in extra-temporal regions than MTLE-HS with good seizure control. Patients with MTLE-NL and good seizure control had no detectable GM atrophy. **Conclusion:** A network of diffuse GM atrophy occurs in both MTLE-HS and MTLE-NL, and the atrophy in some extra-temporal regions is common for both groups, although in MTLE-NL there is no detectable atrophy in the mesial temporal structures. In MTLE-HS, GM atrophy is more pronounced and occurs in patients with refractory and good seizure control while in MTLE-NL it is only observed in refractory patients.

Introduction:

The structural damage of patients with mesial temporal lobe epilepsy (MTLE) with hippocampal sclerosis (HS) extends beyond the mesial temporal structures as it has been detected by different MRI quantification analysis (Bonilha, 2004). This knowledge corroborates with the hypothesis that MTLE is in fact a network disease of functionally and anatomically connected structures (Spencer, 2002). However, what are the specific contributors for the extra-hippocampal gray (GM) and white matter (WM) atrophy in MTLE is not well established.

MTLE is the most frequent form of partial epilepsy in adults and HS is its most common pathological substrate, especially in patients with drug resistant seizures (Tatum, 2011). However, MTLE is not a homogeneous group and among patients with this type of epilepsy the phenotypic presentation, natural history and response to treatment can vary significantly. Although 60-70% of cases of MTLE show MRI signs of HS, a significant number of patients have normal MRI exams ("MRI-negative" MTLE) (Hauser, 1992). Surgical specimens from drug-resistant MRI-negative MTLE submitted to anterior temporal lobectomy rarely show hippocampal cell loss compatible with HS (Cohen-Gadol, 2005; Bell, 2009) and MTLE with a normal MRI using an epilepsy protocol is considered a distinct pathophysiological entity from MTLE-HS (Bell, 2009).

There are few quantitative MRI studies of patients with MRI-negative MTLE accessing the extra-hippocampal atrophy and their results are distinct, from absence of detected abnormalities (Mueller, 2006), to extra-hippocampal atrophy with a distinct pattern from MTLE-HS (Rieder, 2008) or more subtle but similar pattern of temporal and extra-temporal atrophy than MTLE-HS (Labate, 2008). Small number of enrolled patients,

variability in the clinical characteristics of the individuals and the definition of MRI-negative are some of the possible factors responsible for the variability of the results.

In this study we aimed to evaluate and compare the occurrence of GM atrophy in patients with MTLE and normal MRI (MTLE-NL) and MTLE-HS. Additionally, we tried to assess the relationship of the presence of HS and the response to the antiepileptic drug (AED) and the extra-hippocampal GM atrophy by comparing subgroups of MTLE-NL and MTLE-HS. For this purpose, we used a whole brain morphometric analysis (voxel-based morphometry (VBM)) from 3 Tesla MRIs. We hypothesized that in patients with clinical and electroencephalographic characteristics of MTLE and appropriately defined absence of MRI signs of HS, the network of structural damage observed might differ from those with MRI signs of HS. Also, in these groups, the patterns of structural damage might be related to different patterns of AED response. The knowledge of the different structural networks related to specific types of MTLE might help the understanding of each epileptogenic mechanism as well as the particular characteristics of MTLE patients with good or poor AED response.

Methods

Patient's selection and clinical data

We evaluated a group of 172 patients with diagnosis of unilateral MTLE and MRI signs of hippocampal sclerosis (HS) or normal MRI (NL) as defined by hippocampal volumetry and signal quantification (122 MTLE-HS and 50 MTLE-NL).

All patients had the epileptic focus lateralized by ictal and/or interictal scalp EEG. All patients with MRI signs of HS had the epileptic focus ipsilateral to the HS. Also, in order to decrease the possibility of neocortical epilepsy and to ensure the homogeneity of

the individuals, only those with interictal epileptiform discharges characterized by isolated anterior-mid temporal spikes followed or not by slow waves were selected. Those with spikes localized in the posterior temporal lobe, polyspikes or secondary bisynchronous discharges were not included.

All patients signed informed consent form before enroll in the study and were followed in visits with intervals of four to six months with a standardized questionnaire. Data as age of epilepsy onset, pattern of anti-epileptic drug (AED) response, family history of epilepsy, history of febrile convulsion, initial precipitating injury (IPI) and status epilepticus were retrospectively collected. Seizure frequency was accessed at each visit.

Family history was defined as at least one first degree family member with any type of epilepsy. Duration of epilepsy was defined as the age at MRI acquisition minus the age of epilepsy onset while the time of active epilepsy was defined as the age at MRI acquisition minus the age of epilepsy onset minus the sum of periods of seizure remission higher than two years. Patients with up to three complex partial seizures (CPS) and no generalized tonic-clonic seizures (GTCS) in the previous year were considered with good seizure control. Those who did not fulfill this criterion were considered refractory. Patients who were seizure free for at least two years were considered in remission.

MRI acquisition and GM atrophy analysis

For the detection of GM atrophy, automated analysis of brain structure as a whole was performed with VBM (voxel based morphometry). VBM consists of an automatic image analysis that allows comparison of the local intensity brain tissues in groups of individuals, without the need for prior definition of a region of interest (Ashburner 2000). VBM8/SPM8 toolbox was used (Wellcome Department of Cognitive Neurology, http://www.fil.ion.ucl.ac.uk) with 3D sagittal T1-weighted images (voxel size=1x1x1mm³,

TR=7ms, TE=3.2ms, flip angle=8°, matrix=240x240) acquired in a 3T MRI scanner (Philips Medical Systems, Best, The Netherlands). Images from patients with the epileptic focus in the right side were flipped in left-right orientation so the epileptic focus of all patients was aligned in the left side. A control group of 82 healthy subjects (age and sex matched with patients) was used for comparison (60% female; median age 40 years, range 21-70) and a proportion of controls comparable with each patient group was also flipped in the right-left orientation.

After VBM preprocess, a test of quality was performed to observe homogeneity and co-registration between the data and 10 outliers were detected: three images from controls, four from MTLE-HS and three from MTLE-NL groups. These individuals were therefore excluded from the final analysis and the preprocess steps were redone. The images preprocess steps included spatial normalization to the same stereotaxic space (MNI-152 template), modulation (in order to correct the possible volume changes of normalization and to allow evaluation of abnormal volumes) (Good, 2001; Keller, 2004), segmentation into different tissues, including GM, white matter and cerebrospinal fluid. DARTEL algorithm was used in the pre-process steps in order to increase the accuracy of the alignment between subjects (Ashburner, 2007). The resultant GM images were smoothed with an 8-mm full width at half maximum (FWHM) isotropic Gaussian kernel.

The GM post-processed images of both groups were compared using a voxel-wise statistical analysis looking for the areas of volume reduction or increase in patients. The statistical analysis was performed with grand mean scaling, proportional threshold masking, and implicit masking. Two sample T-tests with an initial statistical threshold of $p<0.001$,

uncorrected and minimum clusters of 30 voxels was used. As a second step, the results were corrected with a more stringent statistical threshold of $p<0.05$ FWE corrected.

GM atrophy: response to AED

As a second step, patients were divided in four subgroups according to the response to the AED: i) MTLE-NL with good seizure control; ii) refractory MTLE-NL; iii) MTLE-HS with good seizure control; iv) refractory MTLE-HS. It has been observed in previous studies that patients with MTLE-NL and MTLE-HS have significant clinical differences (VanPaesschen, 1997; Cohen-Gadol, 2005; Mueller, 2008; Riederer, 2008). In other to avoid the differences of clinical data that might contribute to the pattern of GM atrophy in MTLE, as side of epileptic focus (Keller, 2002; Riederer, 2008), duration of epilepsy (Keller, 2002; Bonilha, 2006) or family history (Yasuda, 2010a), these four groups were paired according to the clinical characteristics. For that, the sub-group with the smaller number of patients was considered the model, and an equal number of patients from all the other three sub-groups were chosen in consecutive order until similar characteristics were achieved. Two-sample T-tests for each patient subgroup versus controls were done with an initial statistical threshold of $p<0.001$, uncorrected and minimum clusters of 30 voxels. As a second step, the results were corrected with a threshold of $p<0.05$ FWE corrected.

Results

Clinical characteristics of MTLE patients:

One hundred and sixty five patients were included in VBM analysis: 118 MTLE-HS and 47 MTLE-NL. There was no significant difference of sex or age distribution between the groups. Demographic and clinical data is summarized on Table 1.

Age of epilepsy onset was higher in patients with MTLE-NL than in those with MTLE-HS. Duration of epilepsy and time of active epilepsy were longer in patients with MTLE-HS than in those with MTLE-NL. Family history of epilepsy was significantly more frequent in patients with MTLE-NL than MTLE-HS.

Although not significantly, the proportion of patients with good seizure control was higher in MTLE-NL than in MTLE-HS as was the proportion of patients in seizure remission. The frequency of seizures (CPS with or without SGTCS) did not differ between MTLE-HS than MTLE-NL, however, the former group had a higher incidence of patients with SGTCS (Table 1).

There was no difference in the distribution of different types of auras in MTLE-HS and MTLE-NL groups. Viscerosensorial auras were observed in 53/118 (45%) of MTLE-HS and 20/47 (43%) of MTLE-NL; experiential auras were observed in 32/118 (27%) of MTLE-HS and 15/47 (32%) of MTLE-NL; cephalic or autonomic auras were observed in 15/118 (13%) of MTLE-HS and 8/47 (17%) MTLE-NL.No aura was reported by 18/118 (15%) of MTLE-HS and 4/47 (8%) of MTLE-NL.

Gray matter atrophy: MTLE-HS versus MTLE-NL

Patients with MTLE-HS had more pronounced GM atrophy, including more importantly the ipsilateral mesial temporal structures and anterior and inferior portion of the temporal lobe, bilateral thalami, pre/post-central gyri and cuneus and ipsilateral precuneus, (Table2) (Two-sample T-test, $p<0.05$, FWE corrected, minimum cluster of 30 voxels). In patients with MTLE-NL, VBM analysis did not survive the FWE correction. However, GM atrophy was observed with a statistical threshold of $p<0.001$ uncorrected (minimum cluster of 30 voxels). In this group, GM atrophy was observed in ipsilateral orbitofrontal cortex,

bilateral thalami, pre/post-central gyri and cuneus, ipsilateral inferior and medial frontal gyri and contralateral middle occipital gyrus (Table 2).

Figure 1 shows GM atrophy detected by VBM analysis in MTLE-HS and MTLE-NL. For more appropriated comparison, the results for both groups are shown with the same statistical threshold ($p<0.001$ uncorrected, minimum cluster of 30 voxels). All the clusters of GM atrophy observed in the MTLE-NL group were also observed in MTLE-HS. When the VBM threshold was set to $p=0.05$ FWE corrected in the MTLE-HS group, still the same clusters with atrophy detected in the MTLE-NL group remained, except for the orbitofrontal cortex (Figure 2).

Gray matter increase: MTLE-HS versus MTLE-NL

In MTLE-HS, GM increase was observed in contralateral uncus, fusiform gyrus, lingual gyrus and cingulate gyrus, ipsilateral middle temporal gyrus and in MTLE-NL in contralateral middle temporal gyrus, anterior cingulate. As shown in Figure 4, the majority of increased GM clusters detected by VBM analysis were localized in the edges of GM limits. Additionally, both groups had GM volume increase in the contralateral dorsolateral portion of pons (Two-sample T-tests, $p<0.001$, uncorrected, minimum of 30 voxels). No clusters of GM volume increase persisted after the correction of the T-tests for multiple comparisons (FWE) (Figure 3; Table 3).

Gray matter atrophy: response to AED

Patients were evaluated according to the four sub-groups: i) MTLE-NL with good seizure control; ii) refractory MTLE-NL; iii) MTLE-HS with good seizure control; iv) refractory MTLE-HS. MTLE-NL with good seizure control was the group with the smaller

number of patients (N=16) and the other groups were composed based on their clinical characteristics. The characteristics of each of the four groups are described in Table 4. In these VBM analysis, only the results of the subgroups refractory MTLE-HS and MTLE-HS with good seizure control survived the statistical threshold of $p<0.05$, FWE corrected. Therefore, for adequate comparison, all the results described are referent to $p<0.001$, uncorrected.

In MTLE-HS, in both groups of patients with good seizure control or refractory, GM atrophy was observed in the ipsilateral mesial temporal structures, putamen, caudate, bilateral thalamus and contralateral precentral cortex. However, differently from MTLE-HS with good seizure control, patients with refractory MTLE-HS also presented more diffuse GM atrophy including other neocortical structures, mainly in the ipsilateral frontal lobe (Figure 4; Table 5).

In MTLE-NL, patients classified as refractory presented GM atrophy in the ipsilateral orbitofrontal cortex, bilateral thalamus and precentral cortex, while those classified as good seizure control had no detectable GM atrophy (Figure 4; Table 5).

Gray matter increase: response to AED

No GM increase was observed in any of the four MTLE subgroups.

Discussion

We demonstrated here that a network of diffuse GM atrophy occurs in both MTLE-HS and MTLE-NL, and that the atrophy in some regions is common for both groups

although in MTLE-NL there is no detectable atrophy in the mesial temporal structures. In MTLE-HS, GM atrophy is more pronounced and occurs in both patients with drug-resistant and good seizure control, while in MTLE-NL it is only observed in those with AED-resistant seizures.

Although diffuse GM atrophy has been consistently described in MTLE patients (Keller, 2002; Keller, 2004; Bonilha, 2004; Bonilha, 2006; Riederer, 2008), the determinants of this structural damage remains unclear. In the present study, from a large group of individuals with MTLE with detailed clinical information, we were able not only to demonstrate the differences of GM atrophy in patients with and without HS, but also to compose very homogeneous subgroups in order to isolate specific characteristics as AED response. Moreover, the definition of MRI signs of HS in our group was done by combined visual analysis of MRIs by epilepsy experts plus hippocampal volumetry and signal quantification. With this approach, we were able to significantly decrease the odds to keep patients with subtle signs of HS not detectable by visual analysis in the MTLE-NL group, what was corroborated by the absence of mesial structures atrophy in the VBM analysis.

Surgical series of refractory MTLE-NL defined in modern MRI protocols demonstrate that histopathology of HS is found in a small percentage of the patients (Cohen-Gadol, 2005; Bell, 2009). MTLE-NL is, in fact, considered a heterogeneous group of individuals with different pathologic substrates (Engel, 1996). As other MRI-negative focal epilepsies, in MTLE-NL, drug-resistant cases remain a challenge once recognizing the potential target for surgical intervention demands extensive and usually invasive procedures. Also, the rates of seizure freedom are significantly lower in MRI-negative epilepsies than in those with MRI identifiable lesions (Immonen, 2010). Studies that can

better characterize the individuals with MTLE-NL might help the comprehension of the differences between MTLE-HS and MTLE-NL as well as the physiopathology of the MRI-negative patients.

Methodological considerations

VBM is an automatic method for quantification of brain structures that allows the evaluation of the whole brain without the need of *a priori* definition of a region of interest (Yasuda, 2010b). Despite some methodological differences, it has been used in the study of epilepsy with a high agreement of the results. However, the majority of previous VBM studies of MTLE were based on 1.5T MRIs and in an old version of VBM software (SPM2) (Keller, 2002; Keller, 2004; Bonilha, 2004; Bonilha, 2006; Mueller, 2006; Riederer, 2008; Labate, 2008). The use of a more recent version of the software (SPM8) together with DARTEL algorithm and 3T MRIs allows the detection of more subtle abnormalities with lower likelihood of false positive findings.

In the present study, we chose to evaluate together patients with EEG epileptic focus in the right or left side. We are aware that there are differences in the anatomy and function of the hemispheres as well as in the patterns of structural damage of right or left MTLE. However, the aim of the present study was to compare MTLE-HS and MTLE-NL irrespective to the side of the epileptic focus. Also, to limit the anatomical variation, an adequate proportion of controls (composed by an extensive number of individuals) was also flipped in the same orientation as the patients.

Other consideration is the statistical threshold defined for the VBM analysis. MTLE-NL patients did not survive additional correction for multiple comparisons (FWE), and the results of the four subgroups were also better characterized without this additional

correction. There is no consensus of the better statistical threshold for VBM analysis. The additional correction can decrease the false-positive results. However, comparing the results of our groups, it is possible to assume that the majority of the clusters of GM atrophy observed are not random, once they are replicable in the different categories.

Clinical characteristics

The group of MTLE patients included in this study has important peculiarities. Although the patients have been selected in a tertiary center of epilepsy, we found a high incidence of patients with good seizure control under AED treatment and who had not been considered for surgical treatment. In fact, 21% of MTLE-NL and 10% of MTLE-HS patients had been completely seizure-free under AED for at least two years by the time of MRI acquisition. So, this cohort is different from what is observed in the majority of studies conducted in tertiary epilepsy centers, which are only able to evaluate patients under investigation for surgical treatment due to AED-resistant seizures. Here we had the advantage of assess a large cohort of patients with distinct seizure control and compare the structural damage of good and poor AED responders.

We observed in our group that the age of epilepsy onset was higher in patients with MTLE-NL while, consequently, duration of epilepsy and time of active epilepsy were longer in patients with MTLE-HS. Similar results have been described previously (Cohen-Gadol, 2005; Muller, 2006; Riederer, 2008), although a study that also selected MTLE-HS and MTLE-NL based on hippocampal quantification analysis failed to demonstrate this difference (Carne, 2004). Interestingly, there was no difference in the distribution of types of auras in MTLE-HS and MTLE-NL, with a prevalence of viscerosensorial auras in both groups, what confirms the semiological homogeneity of our patients.

Areas of GM atrophy in MTLE-HS and MTLE-NL

Although VBM analysis demonstrated diffuse GM atrophy that was strikingly more evident in MTLE-HS, we were able to demonstrate that extra-temporal GM atrophy also occurs in MTLE-NL. GM atrophy in extra-hippocampal regions has been consistently reported in previous investigations of MTLE-HS patients (Keller, 2002; Keller, 2004; Bonilha, 2004; Bonilha, 2006); however, for MTLE-NL this is a matter of debate with significantly different results in previous studies (Mueller, 2006; Riederer, 2008; Labate, 2008).

Mueller et al. reported no GM atrophy in a group of 17 drug-resistant MTLE-NL (Mueller, 2006), while Riederer et al. reported decreased GM volume in frontal and orbitofrontal cortex, thalamus and bilateral postcentral gyri but also in hippocampal and parahippocampal formation in 17 drug-resistant MTLE-NL (Riederer, 2008). Other VBM study selected only "mild" MTLE-NL (patients with seizure remission for at least two years) and reported GM atrophy that, although obtained with a less stringent statistical threshold, had the same pattern as in mild MTLE-HS group, including bilateral thalamus and ipsilateral hippocampus (Labate, 2008). In our group of MTLE-NL, although similarities of GM atrophy were detected in comparison with MTLE-HS, these were all in extra-temporal regions and no mesial temporal atrophy was detected. We justify this finding, and its divergence with the previous reports, by a more rigorous selection of MTLE-NL individuals in our study, which was done not only by visual analysis of MRI images but also by quantification of hippocampal volume and T2 relaxometry. Therefore, we did not expect to observe hippocampal abnormalities in these individuals. Also, the previous VBM studies of MTLE-NL patients were based on 1.5T MRIs and used an old

version of VBM software (SPM2) (Mueller, 2006; Riederer, 2008; Labate, 2008). As described previously, in our study, with the use of 3T MRI and more refined version of VBM software, we expected higher sensitivity and specificity of our findings than previously described and, therefore, we consider the absence of volume loss in the mesial structures of MTLE-NL as an accurate finding.

VBM analysis demonstrated regions of GM atrophy that were common in MTLE-HS and MTLE-NL and, interestingly, these were localized outside the temporal lobes. In both groups, GM atrophy was observed in bilateral thalamus, pre/postcentral gyri, bilateral cuneus, middle frontal gyrus and orbitofrontal region. Also, although we used a less stringent statistical threshold in order to compare MTLE-HS and MTLE-NL, when the threshold was additionally corrected for multiple comparisons (FWE) in MTLE-HS, all the similar clusters (except for the ipsilateral frontal lobe) shared by MTLE-HS and MTLE-NL were still significant (Figure 2). That is, the areas of extra-temporal atrophy detected in MTLE-HS with a more stringent statistical threshold are compatible with those detected in MTLE-NL in a less robust statistical analysis.

Bilateral thalamic atrophy has been repeatedly described in MTLE-HS in VBM studies (Keller & Roberts, 2008; Li, 2012), as well as in manual volumetry reports (McDonald, 2008b). Studies that investigated the thalamic nucleus in MTLE have demonstrated predominance of the atrophy in the anterior group or dorsomedial nucleus (Bonilha, 2005; Barron, 2012), confirming the increased damage in the nucleus with connections to limbic pathways. The main hypothesis is that the thalamic structural damage is secondary to the excitoxicity of the input from the hippocampus (Barron, 2012). However, in our results, although MTLE-NL patients had no detectable atrophy in the

mesial temporal structures, a pattern of thalamic atrophy similar to the MTLE-HS group was observed. Despite the absence of structural damage, we cannot state that there is no epileptiform input from hippocampus to thalamus in these patients. In fact, evidence of seizure onset in the hippocampus of MTLE-NL patients from icEEG studies is reported (Cohen-Gadol, 2005). We hypothesized that the hippocampus and other mesial structures are part of the epileptic network of MTLE-NL although no atrophy or cellular loss are detected in these regions. This is in concordance with the knowledge that epileptic networks of patients with MTLE are a complex interaction of areas with structural damage and/or functional abnormalities that pathologically interact to determine ictal and interictal behavior in each individual (Spencer, 2002).

Structural damage in pre/postcentral cortex has also been described in other MRI quantification studies of MTLE-HS (Bonilha, 2006; McDonald, 2008; Labate, 2010; Labate, 2011) and MTLE-NL (Mueller, 2008; Labate, 2011). Neuronal loss secondary to the excitoxicity of seizure spread has been advocated as a possible cause of the atrophy detected in these regions (McDonald, 2008a; Mueller, 2008) and duration of epilepsy has also been associated with this damage (Bonilha, 2006). Hyperperfusion in the ipsilateral precentral and contralateral postcentral gyrus has also been demonstrated by ictal SPECT studies of MTLE, what corroborates the hypothesis of seizure propagation to these areas (Van Paesschen, 2003). In our study, pre/postcentral cortex atrophy was detected in refractory MTLE-HS and refractory MTLE-NL, but also in MTLE-HS with good seizure control. This last group was composed by patients with a low frequency of seizures and 38% was actually free of seizures for at least two years. This finding do not contradict the hypothesis of seizure burden as a possible cause of atrophy in the sensoriomotor cortex,

once our data is transversal and the exact quantification of number of seizures since the onset of epilepsy is practically impossible. Moreover, while in the AED refractory group the atrophy in the pre/postcentral cortex was bilateral, in MTLE-HS with good seizure control it was only observed contralateral to the epileptic focus and in a smaller cluster of voxels.

Although thalamic or cortical atrophy has not been detected in patients with extra-temporal lobe epilepsies (Natsume, 2003), an open question facing our results is whether the atrophy detected in MTLE-NL is specific of a mesial temporal lobe epileptogenic zone and seizure pathways or is a common finding in other focal epilepsies. Despite the difficulty of studying structural damage in groups of extra-temporal lobe epilepsies due the heterogeneity of localization of their epileptic focus (Bonilha, 2006b), studies of large cohorts of extra-temporal patients are necessary to answer this question.

In our MTLE-NL group, differently from MTLE-HS, no GM atrophy was identified in mesial temporal structures, as well as in other regions anatomically or functionally connected to the hippocampus as insula and lentiform nucleus. Similar with a previous report, significant GM atrophy was detected in the orbitofrontal region (Riederer, 2008). Absence of atrophy in the mesial structures despite the clinical and electroencephalographic MTLE characteristics also demonstrates that MTLE-NL is a complex group of patients and justifies the lower rates of surgical success after anterior temporal lobe resections in these patients.

Areas of GM increase in MTLE-HS and MTLE-NL

Areas of GM volume increase are less often reported in MTLE and it is mostly appreciated in patients with epilepsy and suspect focal cortical dyplasias (FCD) (Bonilha,

2006b). Similar to our results, GM increase in VBM studies of MTLE are more often described in the temporal lobes (Keller, 2007). Although it can represent subtle FCDs that are frequently observed in the neocortical temporal tissues of surgical specimens of MTLE patients (Diehl, 2003), to the moment there is no study correlating these VBM findings and histopathology. In our opinion, most of the increased GM described in VBM studies of patients with MTLE and also observed in our patients can be representative of the asymmetry of the whole brain volume between patients and controls once it is mainly observed in the edges of the temporal lobes

However, in our study we also observed GM increase in the dorsolateral portion of the pons contralateral to the epileptic focus in both MTLE-HS and MTLE-NL. The participation of the pons in the network of MTLE has not been consistently evaluated. Bilateral hypermetabolism of this structure has been demonstrated in the post-ictal phase in SPECT studies (Blumenfeld, 2004) and more recently decreased functional connectivity between the left amygdala and the bilateral paramedian pontine area in MTLE patients was observed (Pittau, 2012). The dorsal pons is connected to the hippocampus through the locus coeruleus nucleus, providing the source of noradrenaline to the hippocampal neurons and probably participating to the memory formation (Samuels, 2008). Due to the small size of the pons and the limitation of the present method to identify the specific regions of the brainstem, a consistent hypothesis of the involvement of the pons in the structural network of MTLE cannot be formulated. Further studies with more appropriate techniques are encouraged.

Differences of GM atrophy in good seizure control and drug-resistant MTLE

In this study, we aimed to evaluate the role of HS and the response to AED in the diffuse GM atrophy observed in MTLE. For this reason, in order to eliminate the characteristics that could contribute to the differences classically observed between MTLE-HS and MTLE-NL (Mueller, 2006) we composed four subgroups of patients (good seizure control and refractory MTLE-HS and MTLE-NL). To keep patients with clinical characteristics as close as possible, each individual was randomly selected according to the characteristics of the smallest group (MTLE-NL with good seizure control). Although this selection may seem arbitrary, we also compared the patterns of GM atrophy in these subgroups including all patients irrespective of their clinical data and the results were similar to the ones described here (data not shown).

With this secondary analysis, we demonstrated that although patients with MTLE-HS and good seizure control also have diffuse GM atrophy, this is more restricted to areas with connections to the mesial temporal structures. Other distant regions as frontal, and parietal cortex presented less diffuse damage in MTLE-HS with good seizure control. This result is in concordance with a previous study from our group that demonstrated more extensive GM atrophy in MTLE-HS patients with drug-resistant and relapse-remitting pattern of seizures than in those with good AED response (Bilevicius, 2010). Our results show that in MTLE-HS some regions of GM atrophy cannot be justified only by the continuous occurrence of seizures, once significant atrophy was detected in bilateral thalamus and lentiform nucleus in individuals with good seizure control in a similar pattern of what was observed in refractory MTLE-HS.

A previous study reported similar pattern of GM atrophy in both “mild” and drug-resistant MTLE-HS, which was more restricted than what was observed in our MTLE-HS

patients and included bilateral thalamus and sensoriomotor cortex (Labate, 2010). In our MTLE-HS patients with good seizure control, atrophy of the pre/post-central cortex was only observed contralateral to the epileptic focus. Also, in this group, no atrophy was detected in ipsilateral orbitofrontal region, middle frontal gyrus and ipsilateral pre/post-central as was demonstrated in refractory MTLE-HS. Differently from previous reports, in our study, with the composition of subgroups, we were able to pair in refractory and good seizure control groups all the characteristics previously reported in the literature that might influence the pattern of GM atrophy in MTLE, as age of seizure onset, duration of epilepsy time of active epilepsy, family history of epilepsy, history of febrile convulsion and IPI, occurrence of SGTCS (Keller, 2002; Riederer, 2008; Bonilha, 2006;Yasuda, 2010). We could, therefore, be closer to observe the influence of AED response as well as the occurrence of HS in the GM atrophy of MTLE patients. In contrast, GM atrophy was not observed in MTLE-NL patients with good seizure control and it was restricted to the refractory MTLE-NL group. With these results, we hypothesized that the occurrence of HS is linked to the atrophy of structures connected to it, as the thalamus, while other neocortical extra-temporal atrophy can be related to the occurrence of refractory seizures.

One consideration that must be made to the results of GM atrophy in the different subgroups is concerning the occurrence of SGTCS. SGTCS may contribute to GM atrophy and possibly to the progression of the atrophy in MTLE. In our groups, the proportion of patients who presented GTCS in the previous year before the acquisition of MRI was low what can indicate that other mechanisms influences the GM atrophy in these individuals. However, since this is a transverse study, we were not able to analyze the contribution of the number of GTCS throughout life for GM atrophy in MTLE.

The question that remains is whether the diffuse GM atrophy contributes to drug-resistance in MTLE or these patients have more pronounced GM atrophy because of repeated seizures. Patients with new onset MTLE should be more deeply explored for the understanding of the role of seizures and chronicity in structural abnormalities of MTLE.

In conclusion, a network of diffuse and similar pattern GM atrophy occurs in both MTLE-HS and MTLE-NL, despite the absence of detectable atrophy in the mesial temporal structures in MTLE-NL. The presence of HS is associated with a more pronounced GM volume loss that is observed irrespective to the good or poor AED response while the subtle atrophy detected in MTLE-NL is exclusively detected in patients with drug-resistant seizures. These results add information to the possible mechanisms associated to diffuse GM loss observed in MTLE and reinforces the burden related to the occurrence of seizures in these patients. Further studies able to identify the timing of onset of these structural abnormalities as well as the clinical relevance of their progression are necessary and might contribute to the knowledge of the epileptogenic mechanisms in MTLE.

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Table 1: Demographic and clinical data of MTLE-HS and MTLE-NL patients

	MTLE-HS (n=118)	**MTLE-NL (n=47)**	P value
Sex	74 (63%) female 44 (37%) male	27 (57%) female 20 (43%) male	X^2, p=0.531
Age (range)	46 years (17-73)	43 years (19-74)	T-test, p=0.228
Age of seizure onset (range)	12 years (0-50)	16 years (2-45)	**T-test, p=0.004**
Family history of epilepsy	40 (34%)	27 (57%)	X^2, **p=0.006**
FS/IPI	13(11%)/26(22%)	3(6%)/12(25%)	X^2, p=0.356/ p=0.650
SE	3 (2%)	1 (2%)	Fischer´s exact test, p=0.858
Duration of epilepsy (range)	32 years (2-62)	23 years (3-50)	**T-test, p=0.001**
Time of active epilepsy (range)	28 years (2-62)	22 years (3-47)	**T-test, p=0.008**
AED response	31 (26%) benign 87 (74%) drug-resistant	16 (34%) benign 31 (66%) drug-resistant	X^2, p=0.381
Seizure remission	12 (10%)	10 (21%)	X^2, p=0.063
Laterality of epileptic focus	64 left (54%) 54 right (46%)	37 left (79%) 10 right (21%)	X^2, **p=0.001**
Number of patients with SGTCS in the previous year	14 (12%)	12 (25%)	X^2, **p=0.03**

MTLE-HS: mesial temporal lobe epilepsy with MRI signs of hippocampal sclerosis; MTLE-NL: mesial temporal lobe epilepsy with normal MRI; FS: febrile seizure; IPI: initial precipitating injury; SE: status epilepticus; AED: anti-epileptic drug; SGTCS: secondary generalized tonic-clonic seizures.

	Nº Voxel of the cluster	Area	Side	T score	MNI Coordinates
MTLE-HS	8205	Hippocampus	Left	12.68	-27 -21 -11
(p<0.05, FWE)		Parahippocampal Gyrus	Left	7.66	-8 -36 3
		Thalamus	Left	7.43	-15 -27 1
	1938	Postcentral Gyrus (BA 3)	Left	7.20	-24 -28 66
	1236	Middle Temporal Gyrus (BA21)	Left	6.74	-38 5 -32
		Superior Temporal Gyrus (BA 38)	Left	6.25	-35 9 -24
	1877	Middle Occipital Gyrus (BA 19)	Left	6.64	-24 -93 15
		Cuneus (BA 19)	Left	6.31	-27 -88 27
		Precuneus (BA 19)	Left	5.94	-27 -72 40
	526	Precentral Gyrus (BA 4)	Right	5.96	36 -19 52
	86	Cuneus (BA 7)	Left	5.59	-8 -73 30
		Cuneus (BA 17)	Right	5.42	2 -85 7
	32	Caudate	Left	5.29	-5 15 6
MTLE-NL	727	Superior Frontal Gyrus (BA 11)	Left	4.71	-14 65 -15
(p<0.001)	755	Precentral Gyrus (BA 6)	Right	4.13	44 -12 30
	394	Postcentral Gyrus (BA 3)	Left	3.95	-27 -30 51
	86	Cuneus (BA 17)	Right	3.63	3 -93 1
	477	Thalamus (Ventral Posterior Medial	Right	3.62	15 -22 3
		Thalamus (Medial Dorsal Nucleus)	Left	3.59	-5 -13 9
		Thalamus (Pulvinar)	Right	3.51	3 -27 7
	66	Medial Frontal Gyrus (BA 10)	Left	3.46	-5 62 7
	35	Middle Occipital Gyrus	Right	3.41	29 -87 12

Table 2: Areas of gray matter atrophy in patients with MTLE-HS and MTLE-NL detected by VBM analysis (Two sample T-test; MTLE-HS: $p<0.05$, FWE, minimum of 30 voxels; MTLE-NL: $p<0.001$, uncorrected, minimum of 30 voxels). MTLE-HS: mesial temporal lobe epilepsy with MRI signs of hippocampal sclerosis; MTLE-NL: mesial temporal lobe epilepsy with normal MRI; VBM: voxel based morphometry; FWE: family-wise error.

	Nº Voxel of the cluster	Area	Side	T score	MNI Coordinates
MTLE-HS	164	Pons	Right	4.55	9 -37 -43
(p<0.001)	704	Cingulate Gyrus	Right	5.51	16 9 -31
	117	Uncus	Left	3.93	-13 6 -31
	87	Cerebellum, Posterior Lobe	Right	3.50	54 -69 -22
MTLE-NL	228	Pons	Right	4.27	6 -37 -42
(p<0.001)	48	Inferior Temporal Gyrus	Right	3.60	55 -22 -18
	53	Anterior Cingulate	Right	3.90	1 1 -3

Table 3: Areas of gray matter volume increase in patients with MTLE-HS and MTLE-NL detected by VBM analysis (Two sample T-test; $p<0.001$, uncorrected, minimum of 30 voxels). MTLE-HS: mesial temporal lobe epilepsy with MRI signs of hippocampal sclerosis; MTLE-NL: mesial temporal lobe epilepsy with normal MRI; VBM: voxel based morphometry.

Table 4: Clinical data of subgroups of MTLE-HS and MTLE-NL patients according to the AED response

	MTLE-NL good sz control (n=16)	MTLE-NL Ref (n=16)	MTLE-HS good sz control (n=16)	MTLE-HS Ref (n=16)
Sex	7 female 9 male	7 female 9 male	7 female 9 male	7 female 9 male
Age (range)	41 years (19-74)	42 years (20-55)	46 years (23-61)	42 years (26-62)
Age of seizure onset (range)	14 years (3-25)	18 years (3-31)	18 years (4-36)	17 years (2-30)
Family history of epilepsy	11	10	7	7
FS/IPI	2/7	1/4	1/6	1/6
SE	zero	zero	zero	zero
Duration of epilepsy (range)	24 years (3-48)	23 years (7-50)	25 years (2-54)	26 years (9-45)
Time of active epilepsy (range)	19 years (3-38)	20 years (4-47)	21 years (2-41)	23 years (9-39)
Laterality of epileptic focus	4 Right 12 Left	4 Right 12 Left	4 Right 12 Left	4 Right 12 Left
Patients with GTCS in the previous year	1	2	zero	1
Seizure remission (>2 years)	10	zero	6	zero

MTLE-HS: mesial temporal lobe epilepsy with MRI signs of hippocampal sclerosis; MTLE-NL: mesial temporal lobe epilepsy with normal MRI; good sz control: good seizure control; Ref: refractory; FS: febrile seizure; IPI: initial precipitating injury; AED: anti-epileptic drug; SGTCS: secondary generalized tonic-clonic seizures.

	Nº Voxel of the cluster	Area	Side	T score	MNI Coordinates
Refractory MTLE-HS (p<0.001)	6130	Hippocampus	Left	9.33	-30 -15 -17
		Thalamus (Medial Dorsal	Left	5.14	-5 -16 6
	1875	Superior Temporal Gyrus (BA 38)	Left	5.23	-36 12 -23
		Middle Temporal Gyrus (BA 21)	Left	5.04	-36 5 -33
	202	Superior Parietal Lobule (BA 5)	Right	4.88	20 -43 60
	2383	Medial Frontal Gyrus (BA 9)	Left	4.42	0 50 18
		Superior Frontal Gyrus (BA 8)	Left	4.20	-24 27 49
	284	Caudate	Left	4.27	-6 17 7
	419	Inferior Frontal Gyrus (BA 10)	Left	4.10	-47 45 0
	730	Inferior Parietal Lobule (BA 40)	Right	4.09	51 -27 46
		Precentral Gyrus (BA 4)	Right	4.08	39 -19 54
	139	Middle Frontal Gyrus (BA 10)	Left	3.80	-33 48 6
	188	Postcentral Gyrus (BA 5)	Left	3.79	-21 -42 63
	81	Precentral Gyrus (BA 4)	Left	3.36	-48 -13 42
	42	Precuneus (BA 7)	Right	3.59	12 -5 49
Good control MTLE-HS (p<0.001)	7519	Hippocampus	Left	8.55	-26 -22 -11
		Thalamus (Medial Dorsal	Left	5.45	-3 -15 9
	1941	Caudate	Left	5.24	-8 17 7
	1568	Cerebellum, Posterior Lobe	Left	4.94	-21 -75 -44
	694	Precentral Gyrus (BA 4)	Right	4.35	32 -25 64
	83	Parahippocampal Gyrus	Right	4.07	23 -25 -6
	36	Subcallosal Gyrus (BA 25)	Right	3.33	12 25 -9
Refractory MTLE-NL (p<0.001)	296	Precentral Gyrus (BA 4)	Right	4.25	33 -27 64
	210	Precentral Gyrus (BA 6)	Left	3.81	-36 -18 67
	189	Thalamus	Right	3.70	2 -25 10
	306	Superior Frontal Gyrus	Left	3.70	-21 62 -2
	75	Postcentral Gyrus (BA 3)	Right	3.64	41 -19 48
	68	Precuneus (BA 19)	Left	3.63	-33 -84 42
	49	Middle Temporal Gyrus (BA 21)	Left	3.63	-41 5 -30
	30	Caudate	Left	3.50	-23 -27 64

Table 5: Areas of gray matter atrophy in subgroups of MTLE-HS and MTLE-NL patients with good seizure control and refractory seizures detected by VBM analysis (Two sample T-test; MTLE-HS: $p<0.05$, FWE, minimum of 30 voxels; MTLE-NL: $p<0.001$, uncorrected, minimum of 30 voxels). MTLE-HS: mesial temporal lobe epilepsy with MRI signs of hippocampal sclerosis; MTLE-NL: mesial temporal lobe epilepsy with normal MRI; VBM: voxel based morphometry.

Figure 1:

Title: Gray matter atrophy in MTLE-HS and MTLE-NL

VBM demonstrated significant areas of diffuse gray matter volume loss in MTLE-HS and MTLE-NL. A1 and A2 ("glass view") show the areas of gray matter atrophy in MTLE-HS (Two-sample T-test, $p<0.001$, uncorrected, minimum threshold cluster of 30 voxels); B1 and B2 ("glass view") show the areas of gray matter atrophy in MTLE-NL (Two-sample T-test, $p<0.001$, uncorrected, minimum threshold cluster of 30 voxels). MTLE-HS: mesial temporal lobe epilepsy with MRI signs of hippocampal sclerosis; MTLE-NL: mesial temporal lobe epilepsy with normal MRI; VBM: voxel based morphometry; T: t-value; L: left; R: right.

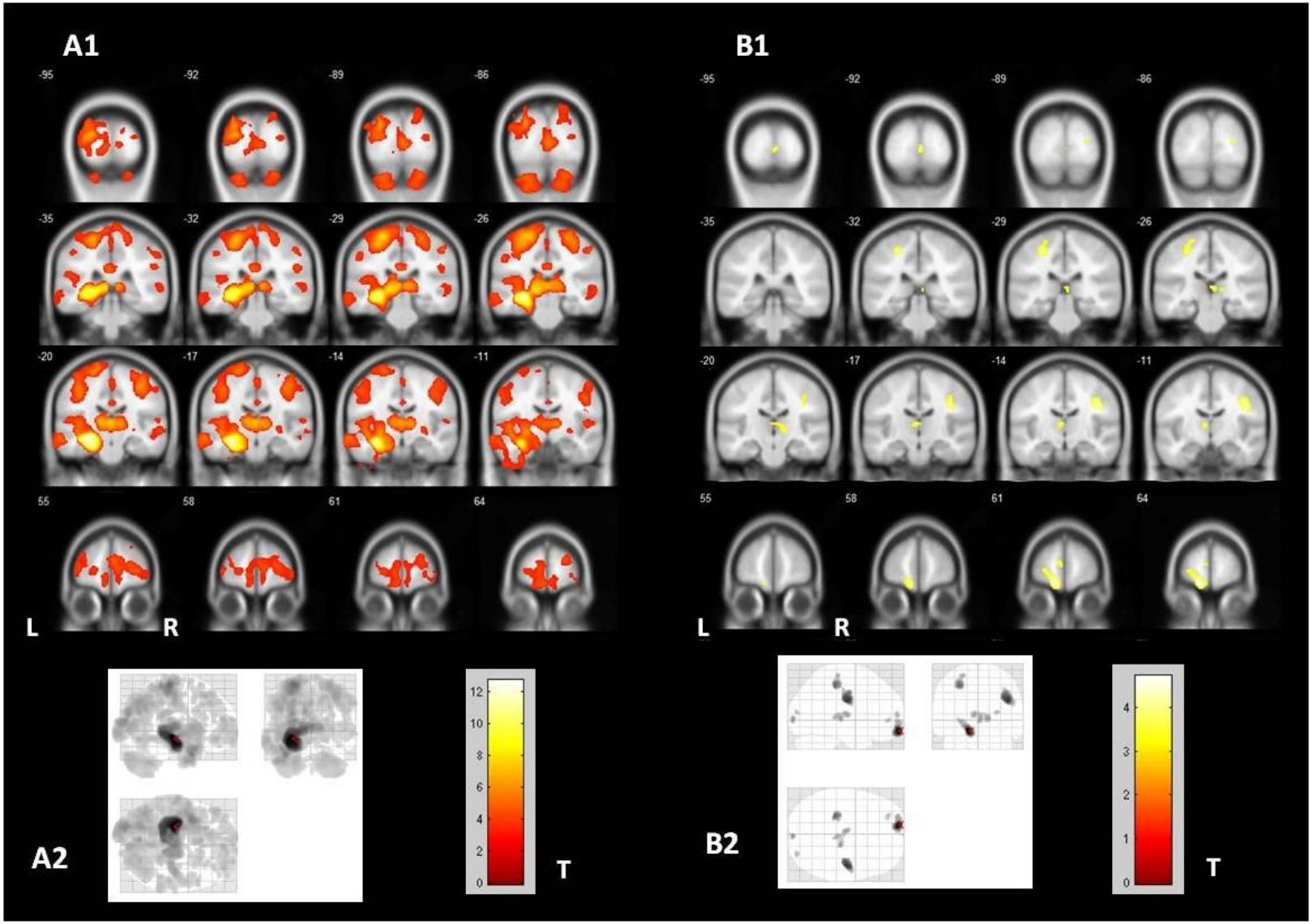
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Figure 2:

Title: Common areas of gray matter atrophy in MTLE-HS and MTLE-NL

The slices demonstrate the common brain areas with gray matter atrophy in MTLE-HS and MTLE-NL. MTLE-HS results are shown with a more stringent statistical significance in order to facilitate the comparison. A1 ("glass view") and A2 show the areas of gray matter atrophy in MTLE-HS (Two-sample T-test, $p<0.05$, FWE corrected, minimum threshold cluster of 30 voxels); B1 ("glass view") and B2 show the areas of gray matter atrophy in MTLE-NL (Two-sample T-test, $p<0.001$, uncorrected, minimum threshold cluster of 30 voxels). MTLE-HS: mesial temporal lobe epilepsy with MRI signs of hippocampal sclerosis; MTLE-NL: mesial temporal lobe epilepsy with normal MRI; VBM: voxel based morphometry; T: t-value; L: left; R: right.

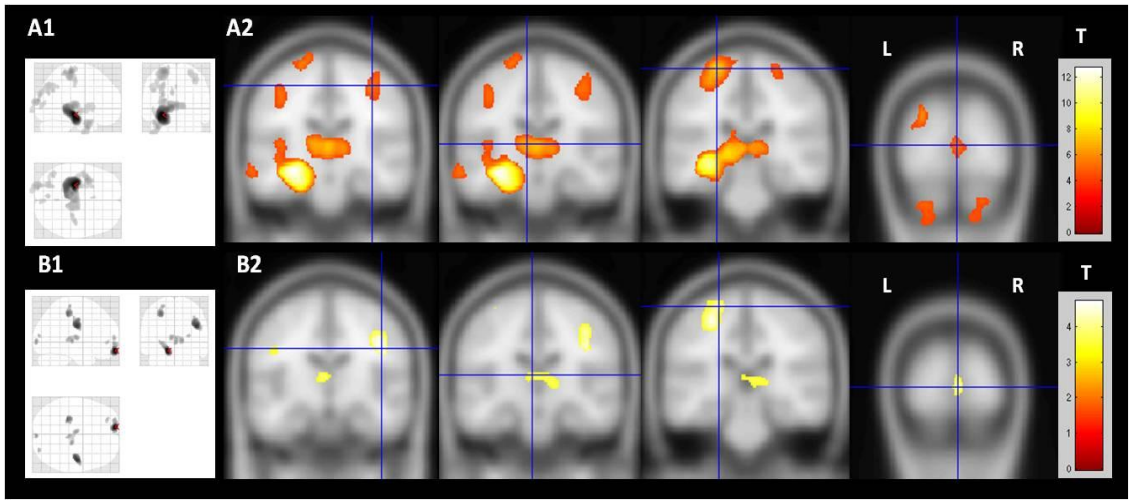


Figure 3:

Title: Gray matter volume increase in MTLE-HS and MTLE-NL

VBM demonstrated areas of gray matter increase in MTLE-HS and MTLE-NL. A shows the areas of gray matter volume increase in MTLE-HS (Two-sample T-test, $p<0.001$, uncorrected, minimum threshold cluster of 30 voxels); B shows the areas of gray matter volume increase in MTLE-NL (Two-sample T-test, $p<0.001$, uncorrected, minimum threshold cluster of 30 voxels). MTLE-HS: mesial temporal lobe epilepsy with MRI signs of hippocampal sclerosis; MTLE-NL: mesial temporal lobe epilepsy with normal MRI; VBM: voxel based morphometry; T: t-value; L: left; R: right.

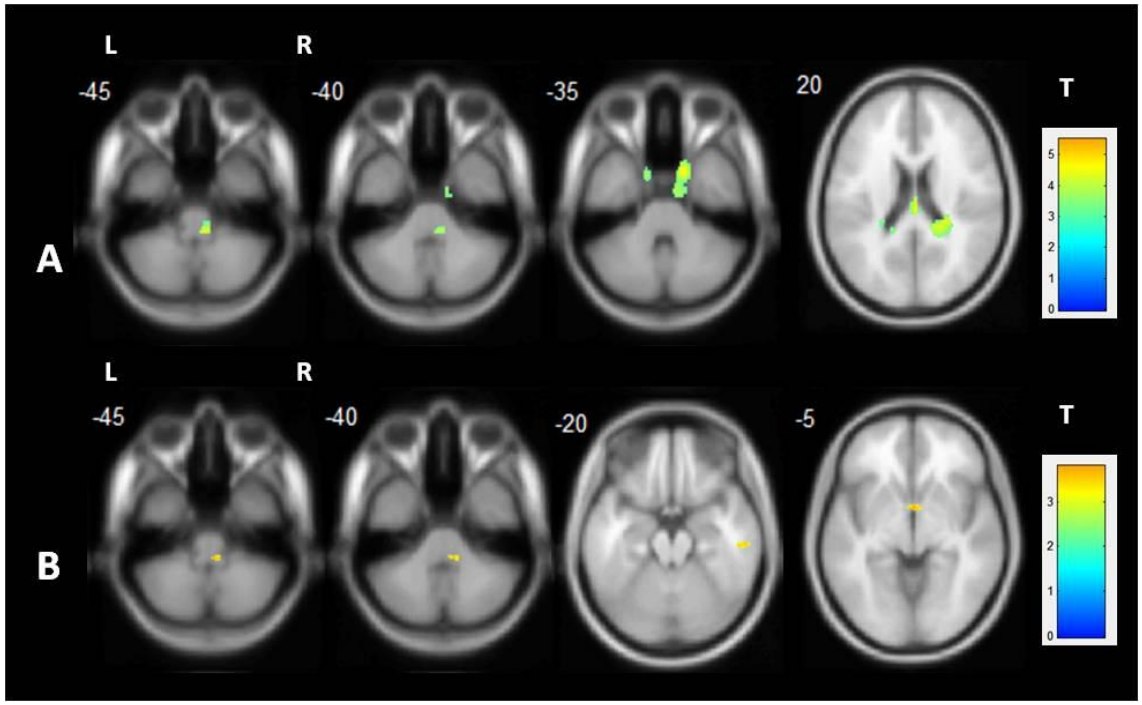
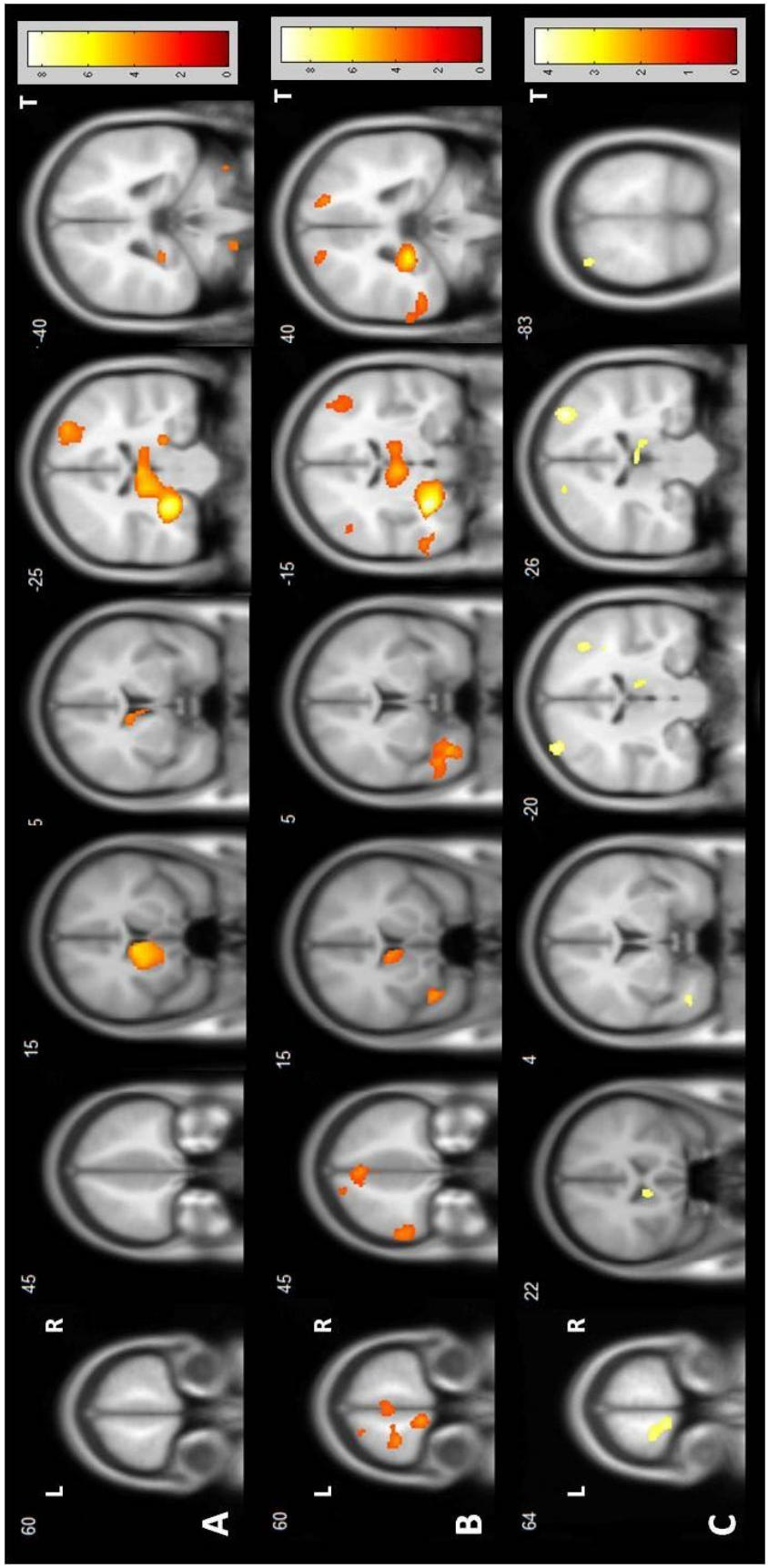


Figure 4:

Title: Patterns of gray matter atrophy according to AED response in MTLE-HS and MTLE-NL

VBM demonstrated significant areas of diffuse gray matter volume loss in MTLE-HS patients with good seizure control and refractory seizures and only in MTLE-NL with refractory seizures. A: areas of gray matter atrophy in MTLE-HS with good seizure control (Two-sample T-test, $p<0.001$, uncorrected, minimum threshold cluster of 30 voxels); B: areas of gray matter atrophy in refractory MTLE-HS (Two-sample T-test, $p<0.001$, uncorrected, minimum threshold cluster of 30 voxels); C: areas of gray matter atrophy in refractory MTLE-NL (Two-sample T-test, $p<0.001$, uncorrected, minimum threshold cluster of 30 voxels). MTLE-HS: mesial temporal lobe epilepsy with MRI signs of hippocampal sclerosis; MTLE-NL: mesial temporal lobe epilepsy with normal MRI; VBM: voxel based morphometry; T: t-value; L: left; R: right.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CAPÍTULO 5

Patterns of antiepileptic drug response in patients with mesial temporal lobe epilepsy with and without signs of hippocampal sclerosis

Coan AC; Cendes F.[1]

Submetido para *Journal of Neurology, Neurosurgery and Psychiatry*

Patterns of antiepileptic drug response in patients with mesial temporal lobe epilepsy with and without signs of hippocampal sclerosis

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ABSTRACT:

Background: Patients with epilepsy may present with an unstable pattern of seizure control with antiepileptic drugs (AEDs) which may be inherent to the pathophysiology of the disease.

Objective: To evaluate the response to AEDs across the lifespan of patients with mesial temporal lobe epilepsy (MTLE) with and without MRI signs of hippocampal sclerosis (HS).

Methods: We retrospectively evaluated the clinical data of 172 patients with MTLE who had signs of HS (MTLE-HS, N=122) or normal MRI (MTLE-NL, N=50) as defined by quantification of hippocampus volume and signal. A silent period was considered as seizures with onset and remission at the first decade of life and seizure recurrence in the second decade after at least five years of seizure freedom. Relapse-remitting (RR) pattern of AED response was defined as periods equal or longer than two years without any type of epileptic seizure intercalated with periods of seizure recurrence. Patients with a stable pattern of AED response were considered good seizure control (goodSC) if after achieving the adequate dose of the first AED they presented only simple partial seizures or up to three complex partial seizures per year. Those who did not fulfill these criteria were classified as Refractory.

Results: In the MTLE-HS group, 84 (69%) were classified as Refractory, one (1%) as goodSC and 37 (30%) as RR while in MTLE-NL group, 31 (62%) were classified as Refractory, one (2%) as goodSC and 18 (36%) as RR. Twelve (10%) patients with MTLE-HS and only one (2%) with MTLE-NL had a silent period. In these individuals, after seizure recurrence, 11 (92%) MTLE-HS evolved as Refractory while the patient with MTLE-NL evolved as goodSC.

Conclusion: While similar number of patients (up to one-third) with MTLE-HS or MTLE-NL may present with a relapse-remitting pattern of AED response, in the latter group the majority remains with a satisfactory control of seizures even during the relapse periods. A silent period is significantly more frequent in patients with MTLE-HS than MTLE-NL and these patients usually have drug-resistant epilepsy after this period.

Introduction:

Patients with epilepsy may present an unstable pattern of seizure control with antiepileptic drugs (AEDs) which may be inherent to the pathophysiology of the disease and it has important implications for appropriated treatment decisions.

Population-based studies demonstrate that nearly two-thirds of patients with epilepsy will achieve seizure remission under AED treatment[1]. Also, it is recognized that although the majority of patients will have a stable pattern of response to the medications through long-term follow-ups, an intermittent pattern with refractory seizures interrupted by periods of remission can occur[2]. It has been estimated that around 16% of patients with epilepsies will have a relapse-remitting course, intercalating periods of seizure remission and refractory seizures[3]. It is also current knowledge that the underlying cause of the epilepsy is the major contributor to define AED response[4]. The role of possible different etiologies to the occurrence of relapse-remitting patterns of AED response has not been properly delineated.

Mesial temporal lobe epilepsy (MTLE) is the most common epileptic syndrome in adults and is most often associated with hippocampal sclerosis (HS)[5]. Due to its elevated prevalence and the high rates of AED-resistant individuals, the natural history of MTLE-HS has been persistently investigated[6]; however, it has not been completely overcome, possibly due to the existence of different underlying causes and additional modifiers, both genetic and environmental [7]. Although relapse-remitting patterns of AED response has been described in MTLE, its frequency in different studies is variable[8,9] and the role of the presence of HS or other underlying pathology in this pattern of AED response has not been properly examined.

Understanding how epilepsies with different etiologies respond to AEDs can provide more adequate and individualized therapies. Moreover, early definition of the long-term outcome of patients with MTLE can contribute to appropriate selection of those who will benefit from surgical interventions due to AED-resistant seizures.

The objective of the present study was to evaluate and compare the response to AEDs response across the lifespan of patients with MTLE with and without MRI signs of HS.

Methods:

Patients´ selection and classification

We retrospectively evaluated the clinical data of 172 consecutive patients with clinical and electroencephalographic diagnosis of MTLE followed in a tertiary epilepsy center (Epilepsy Clinic of Campinas State University).

All patients had either MTLE associated with HS (MTLE-HS) or cryptogenic MTLE (MTLE with normal MRI, MTLE-NL). All patients signed Informed Consent approved by the Ethics Committee of UNICAMP prior to the acquisition of MRI. Patients with symptomatic MTLE due to lesions other than HS (tumor, vascular malformations, gliosis, focal cortical dysplasia) were excluded from the present study.

For the definition of signs of HS, MRIs were acquired in a 3 Tesla Philips Intera Achieva scanner (Philips, Best, Netherlands) with sequences 3D-T1 weighted image (isotropic voxels of 1 mm, acquired in the sagittal plane; 1 mm thick, flip angle=8°, TR=7.0ms, TE=3.2ms, matrix=240x240, FOV=240x240) and T2 weighted image multi-eco image (3mm thick, TR=3300ms, TE= 30/60/90/120/150ms, matrix=200X180, FOV=180X180). HS signs were defined by MRI visual analysis plus MRI quantification of hippocampus volume and T2 signal. According to this analysis, patients were classified as MRI signs of HS (MTLE-HS, N=122) or normal MRI (TLE-NL, N=50).

Clinical data and definitions

The long term AED response of each patient was assessed from the seizure onset period and classified as follows. Patients with a stable pattern of AED response since the onset of the epilepsy were considered long term good seizure control (LT-Good) if after achieving the adequate dose of the first AED they presented only simple partial seizures (SPS) or up to three complex partial seizures (CPS) per year and no generalized tonic-clonic seizures (GTCS) but no periods of complete seizure remission for at least two years. Patients with a stable pattern of AED response but with any period of seizures higher than that were considered long term refractory (LT-Refractory). Patients were considered as relapse-remitting (RR) pattern of AED response if they presented at least one period equal or longer than two years without any type of epileptic seizure intercalated with periods of seizure recurrence.

A second classification of AED response was made according to good or poor seizure control independent of a stable or variable pattern of seizure control and the RR patients were distributed in the good seizure control or refractory group according to the criteria as follows. RR patients who had during all periods of seizure recurrence only SPS or up to three CPS per year and no GTCS were considered as good seizure control and grouped together with the LT-Good patients (Good). RR patients who had any period of seizure recurrence with more than this number of seizures were considered as refractory and grouped with LT-Refractory patients (Refractory).

A silent period was considered as recurrent spontaneous seizures (and therefore the diagnosis of epilepsy) with onset and remission at the first decade of life and seizure recurrence in the second decade after at least five years of seizure freedom. This period of seizure remission was not considered in the classification of RR pattern.

Results:

There was no difference of sex distribution (MTLE-HS: 77 women (63%), 45 men (37%); MTLE-NL: 28 women (56%), 22 men (44%); X^2, p=0.385) or age at the moment of the MRI acquisition (MTLE-HS: medium age 46 years, range from 17 to 73; MTLE-NL: medium age 43 years, range from 19 to 74; T-test, p=0.251) between MTLE-HS and MTLE-NL patients.

In the MTLE-HS group, 84 (69%) were classified as LT-Refractory, one (1%) as LT-Good and 37 (30%) as RR while in MTLE-NL group, 31 (62%) were classified as LT-Refractory, one (2%) as LT-good and 18 (36%) as RR. There was no difference in the distribution of the different AED responses in the MTLE-Hs and MTLE-NL groups (Fisher Exact Test, p=0.211).

In the second classification, 20 patients with RR pattern of AED response and MTLE-HS were classified as Refractory (with a total of 104 (85%) of MTLE-HS patients classified as Refractory) and 17 as Good (with a total of 18 (15%) of MTLE-HS patients classified as Good). In the MTLE-NL group, seven patients with RR pattern of AED response were classified as Refractory (with a total of 38 (76%) of MTLE-NL patients classified as Refractory) and 11 as Good (with a total of 12 (24%) of MTLE-NL patients classified as Good). Chi-square test demonstrated significant higher rates of Refractory patients in the MTLE-HS group (X^2, p<0.001).

Fifty (41%) patients with MTLE-HS and 12 (24%) with MTLE-NL had the seizure onset in the first decade of life. In MTLE-HS group, 86% (43/50) of the patients with seizure onset in the first decade of life and 85% (61/72) of those with later seizure onset

were classified as Refractory (X^2, p=0.841). In MTLE-NL group, 75% (9/12) of the patients with seizure onset in the first decade of life and 76% (29/38) of those with later seizure onset were classified as Refractory (Fisher Exact Test, p=0.601).

Twelve (10%) patients with MTLE-HS and only one (2%) with MTLE-NL had a silent period (respectively, 24% (12/50) and 8% (1/12) of the patients with seizure onset in the first decade of life). Among patients with the seizure onset in the first decade of life, in those with MTLE-HS and history of a silent period, 92% (11/12) evolved as Refractory, while 84% (32/38) of those with MTLE-HS without history of a silent period were classified as Refractory (Fisher Exact Test, p=0.458). The only patient with MTLE-NL and a history of silent period evolved as Good, while 82% (9/11) of the remaining patients with MTLE-NL and seizure onset in the first decade of life were classified as Refractory.

Discussion:

We observed that a pattern of relapse-remitting seizures occurs in nearly one-third of patients with MTLE, independent of the presence of MRI signs of HS or normal MRI. MTLE-HS and MTLE-NL differ, however, in the proportion of patients with good seizure control, with higher rates of AED resistance in MTLE-HS. Also, a progress to refractory seizures after a long period of seizure-freedom in patients with seizure onset in the first decade of life is characteristic of MTLE-HS.

MTLE is the most prevalent epilepsy among adults, with high rates of refractory seizures[4,5]. This is not a single entity, but a group of different conditions that share common clinical and neurophysiological characteristics[10]. In MTLE, it is known that different etiologies imply diverse long term outcomes, with MTLE-HS being the subtype with the

higher rates of drug-resistant seizures[4]. The knowledge of the natural history of the different types of MTLEs, including long-term response to AEDs has important implications in the individual decisions for adequate choices of treatments. Moreover, it is also important for the understanding of the characteristics that lead different patients to good or poor AED responses.

The natural history of drug resistant MTLE-HS has been evaluated in retrospective and longitudinal studies[6]. Classically, the seizures start in the end of the first decade of life after a latent period following what has been considered an initial precipitating injury, most commonly prolonged febrile seizures[6]. Drug-resistant seizures can manifest from the beginning of the disease; however, good seizure control or remission is often achieved with adequate AEDs in the first years after the onset, with later development of Refractoryractoriness[7,11]. For other subtypes of MTLE, as MTLE-NL, the natural history is not well established. It is known that the age of seizures onset in MTLE-NL is higher than for MTLE-HS, with the average start in the end of the second decade of life, and that rates of AED response are higher in these patients[12,13]. However, a significant percentage of patients with MTLE-NL have seizures starting in the first decade of life and the long term AED response in this group of MTLE has not been completely investigated.

Three decades ago, Goodridge and Shorvon[2] noted an intermittent pattern of AED response in which refractory seizures are interrupted by periods of remissions of at least two years in 12% of their patients. Also, French *et al.*[6] reported in a surgical series of adult patients with MTLE that a quarter had experienced previous periods of remission. In our study, we observed higher rates of relapse-remitting pattern in both MTLE-HS and MTLE-NL (approximately one-thirty in both groups). This variability of the frequency of relapse-

remitting patterns in different studies might be related to different aspects. First, the definition of a remission period differs in the studies, with some considering necessary at least one year while others at least two years of seizure freedom for the diagnosis. The other difference is how the data is assessed and the consideration of remission periods with or without the use of AEDs. A third factor that certainly influences the rates of relapse-remitting MTLE patients observed in the different studies is the recruitment of these individuals in tertiary centers specialized in epilepsy surgery, that certainly decreases the possibility of finding patients that can remain for long periods without seizures. This last factor is possibly what influenced the higher rates of relapse-remitting pattern observed in our patients. Although they were selected in a tertiary epilepsy center, our service is composed by two different epilepsy clinics, one for surgical candidates and other for patients with epilepsy having secondary complexity. Therefore, the incidence of individuals with good seizure control in our groups is possibly intermediate from what could be expected in a population-based study or a study conducted exclusively with patients of tertiary complexity.

While both MTLE-HS and MTLE-NL groups presented a similar frequency of relapse-remitting pattern of AED response, in the latter group the majority of the individuals remained with a satisfactory control of seizures even during the relapse periods. Accordingly, as expected, patients with MTLE-NL had a higher frequency of good seizure control than those with MTLE-HS. Although HS is classically associated with AED-resistant seizures, in the last decades it has been demonstrated that it can also be observed in individuals with good seizure control or seizure remission with or without AEDs[14,15]. However, the prevalence of HS in patients without refractory seizures remains unclear[15]. In

our study, 15% of the patients with MTLE-HS were classified as having good seizure control. As described, our patients were selected in an epilepsy service with patients with both secondary and tertiary complexity. In this context, one might expect higher rates of MTLE-HS individuals with good seizure control[16]. However, we opted in this study for a restricted classification of patients and we considered the seizure frequency from the seizure onset. Only patients who never experienced more than three CPS per year and had no history of CTGS were classified as good seizure control. This classification differs from the majority of the studies which consider a transversal period of one or two years of good seizure control for this definition.

In MTLE, seizure onset in the first decade of life is associated with higher incidence of refractoriness[9,17,18], although this is a matter of debate[10]. In the present study, although we did not observe differences in the frequency of refractory patients in those with seizure onset in the first decade of life or after that, the age of epilepsy onset was significantly lower in those classified as refractory. It is interesting to note that earlier age of epilepsy onset was observed in both groups of MTLE patients, independent of the presence of HS signs.

In this study, we also demonstrated that a silent period (i.e., a period of seizure freedom between the first seizures in life and the recurrence of seizures usually in the second decade of life) is more frequent in patients with MTLE-HS than MTLE-NL and it is usually followed by AED-resistant seizures after the silent period. Moreover, in MTLE-HS, the occurrence of a prolonged interval of seizure freedom that starts still in the first decade of life was associated with additional risk of AED resistance in the adult life. In fact, in our group of MTLE-HS, 92% of the individuals with seizure onset in the first decade of life and

a history of a silent period developed refractory seizures in the adult life. This rate of AED resistant seizures was higher than the already elevated rate (84%) of MTLE-HS with seizure onset in the first decade of life but without an early period of prolonged seizure freedom. This finding has important implications for early decisions and investigation of epilepsy surgery of those patients with diagnosis of MTLE in the first decade of life. However, the complicating factor is that studies demonstrate that MRI signs of HS are rare in children, with the hypothesis that this pathology may develop throughout the years[7]. Thus, the early diagnosis of MTLE-HS and the prompt referral of those with refractory seizure to surgical treatment remains a challenge.

In conclusion, although MTLE with or without signs of HS share some characteristics of AED response, as similar rates of relapse-remitting pattern, other aspects differentiate these groups, such as higher rates of AED resistance and the frequent occurrence of a silent period followed by refractory seizures in MTLE-HS. The detailed comprehension of the natural history of different subtypes of MTLE will be helpful for better clinical or surgical treatment on an individual basis.

Acknowledgments:

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CAPÍTULO 6

Amygdala enlargement occurs in patients with temporal lobe epilepsy and hippocampal sclerosis with early epilepsy onset

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Submetido para *Epilepsy and Behavior*

Brief Communication

Amygdala enlargement occurs in patients with mesial temporal lobe epilepsy and hippocampal sclerosis with early epilepsy onset

Running title: Amygdala enlargement in MTLE with HS

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Key words: mesial temporal lobe epilepsy, amygdala, volumetry

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Summary: Mesial temporal lobe epilepsy (MTLE) associated with hippocampal sclerosis (HS) is considered an electro-clinical syndrome and there is a debate whether it is a unique disease or an entity with distinct subtypes. Together with other mesial temporal structures, the amygdala is important in the epileptogenic network of patients with MTLE with HS. During automatic volumetric analysis of mesial structures in a group of 102 MTLE patients with MRI signs of HS, we observed significant amygdala enlargement in 14 (14%) individuals. The increased amygdala volume was contralateral to the epileptic focus and MRI signs of HS in 93% of the patients. MTLE with HS patients and enlarged amygdala had significantly lower age of epilepsy onset than those without increase of amygdala volumes. MTLE with HS and enlarged amygdala might be a part of the spectrum of this condition.

Key words: mesial temporal lobe epilepsy, amygdala, volumetry

Introduction:

Mesial temporal lobe epilepsy (MTLE) associated with hippocampal sclerosis (HS) is a constellation of clinical and electroencephalographic characteristics (Wieser , 2004). The occurrence of different antiepileptic drug response, surgical outcomes and evolution leads to the assumption that MTLE associated with HS is not a single and homogeneous disease but rather a group of distinct pathologies. Likewise, the etiology of MTLE associated with HS is not completely understood, with a high incidence of febrile seizures or other initial precipitating injuries but also genetic factors being considered as possible causes of HS (Wieser , 2004).

Irrespective of its etiology, in MTLE associated with HS not only the hippocampus is abnormal but there is a network of different structures involved in the epileptogenicity and seizure occurrence, in particular other mesial temporal structures such as the amygdala. There is a large incidence of amygdala atrophy observed in neuroimaging studies of patients with MTLE and HS, which is most often ipsilateral to the HS and the site of seizure origin (Cendes et al., 1993). Moreover, depth electrodes studies have shown that seizures may originate in both hippocampus and amygdala, either independently or simultaneously, or even with fast propagation from one to the other structure (Quesney, 1986). In addition, surgery for treatment of drug resistant MTLE with HS have better outcome when the amygdala is included in the resection (Schramm, 2008).

Significant enlargement of amygdala volumes has been reported in MTLE in patients with psychiatric disorders as depression and psychosis (Tebartz et al., 1999; Van Elst et al., 2002). In other studies about MTLE without HS (MRI-negative MTLE),

enlarged amygdalae have been investigated as the possible lesion associated with seizures origin (Bower et al., 2003; Mitsueda-Ono et al., 2011).

In the present study, we investigated the frequency of enlarged amygdala in patients with MTLE and HS and compared the clinical, electroencephalographic and MRI features of individuals with and without increased amygdala volumes.

Methods:

Patients' selection

One hundred and two patients with clinical and electroencephalographic diagnosis of mesial temporal lobe epilepsy (MTLE) and MRI signs of hippocampal sclerosis (HS) followed in a tertiary epilepsy center (Epilepsy Clinic of University of Campinas (UNICAMP)) were consecutively selected for volumetric study of mesial temporal structures. All patients signed informed consent approved by the Ethics Committee of UNICAMP before MRI and clinical data acquisition.

MRI acquisition and amygdala and hippocampal volumetry

Images were acquired on a 3T-Achieva MRI (Philips Medical Systems, Best, The Netherlands). Automatic amygdala and hippocampal volumetry was performed with FreeSurfer software (version 5.1.0; http://surfer.nmr.mgh.harvard.edu/) in 3D T1-weighted images (voxel size 1x1x1mm³, TR=7ms, TE=3.2ms, flip angle=8°, matrix=240x240). MRIs of 79 healthy subjects (age and sex matched with MTLE-HS patients) were used for comparison. Amygdala and hippocampal volumes were corrected for the individual brain volumes. Amygdala and hippocampal absolute volumes higher than 2 standard deviations

(SD) from the mean of the control group were considered enlarged (MTLE-EA) and the same was considered for the hippocampal formation. Patients with normal amygdala volumes or amygdala atrophy (volumes lower than 2SD from the mean of the controls) were considered together as a group of MTLE-HS patients without amygdala enlargement (MTLE-no).

For patients with amygdala enlargement, MRI T2 signal quantification (T2 relaxometry, Aftervoxel software; T2-weighted multi-eco image, 3mm thick, TR=3300, TE= 30/60/90/120/150, matrix=200X176, FOV=1802X180) was also evaluated. For this analysis, the same group of 79 healthy subjects was used. Amygdala signal intensities higher than 2 SD from the mean of the control group were considered abnormal.

Clinical data:

Clinical characteristics were compared between MTLE-HS with and without amygdala enlargement. Epileptic focus was defined by ictal and inter-ictal scalp EEG and it was ipsilateral to the MRI signs of HS in all cases. Definition of the laterality of the epileptic focus by ictal EEG was considered the record of all seizures with clear onset exclusively localized in one of the anterior temporal lobes. The laterality of the epileptic focus by inter-ictal EEG was considered as the occurrence of at least 80% of the inter-ictal epileptiform activity located in one of the temporal lobes.

Patients with up to three complex partial seizures (CPS) and no secondary generalized tonic-clonic seizure (SGTCS) in the twelve months prior to the MRI acquisition were considered as having good seizure control. Those who do not fulfill this criterion were considered refractory.

Depression and other psychiatric conditions were assessed from the epilepsy onset with retrospective data collected from the medical charts plus the present symptoms.

Results:

Fourteen patients (14%) with MTLE-HS had significant increase of amygdala volume (13 [94%] contralateral and one ipsilateral to the epileptic focus and MRI signs of HS; no bilateral abnormality was observed). T2 relaxometry demonstrated normal amygdala signal in all individuals with MTLE- EA. The detailed clinical characteristics of the patients with MTLE-EA are described in Table 1.

Six (43%) patients with MTLE- EA also had the hippocampus contralateral to the epileptic focus with significant increased volume while it only happened to 8% of patients with MTLE- no (eight patients).

The only significant difference observed between MTLE-EA and MTLE-no was the age of epilepsy onset, which was significantly lower in individuals with MTLE-EA (medium=6 years, range one to 28 years) than in those with MTLE-no (medium=11 years, range one to 38 years) (Two-sample T-test, p=0.044).

There was no difference between patients with MTLE-EA or MTLE-no with respect to sex (MTLE-EA: 10 (71%) women; MTLE-no: 54 (61%) women; Fischer's Exact Test, p=0.561), age (MTLE-EA: medium age 44 years, range 30-62; MTLE-no: medium age 46 years, range 17-73; T-test, p=0.424), duration of epilepsy (MTLE-EA: medium 37 years, range 13-48; MTLE-no: medium 34 years, range 2-62; T-test, p=0.780), history of initial precipitate injury (MTLE-EA: 2 (14%); MTLE-no: 23 (26%); Fischer's Exact Test, p=0.508), family history of epilepsy (MTLE-EA: 2 (14%); MTLE-no: 34 (39%); Fischer's

Exact Test, p=0.129), frequency of CPS and CPC-SGTCS in the previous year (MTLE-EA: medium 36 seizures per year, range 0-360; MTLE-no: medium 18 seizures per year, range 0-1080; Mann-Whitney Test, p=0.144) and diagnosis of depression or other psychiatric comorbidities (MTLE-EA: 2 (14%); MTLE-no: 15 (17%); Fischer's Exact Test, p=0.576).

Thirteen patients (93%) with MTLE-EA and 67 patients (76%) with MTLE-no were classified as having refractory seizures (Fischer's Exact Test, p=0.189). . There was no difference in the distribution of different types of auras, with a predominance of viscerocensorial auras in both groups.

Four (29%) patients with MTLE-EA were submitted to anterior temporal lobe resection due to drug resistant seizures. Histopathology confirmed the HS diagnosis in all. Due to surgical technique, no amygdala tissue was available for histopathology. In the last clinical visit, three were Engel IA (patients with eight, 18 and 24 months follow-up) and the third was Engel IIB (two years follow-up).

Discussion:

We described a group of patients with MTLE-HS and significant enlarged amygdala volume, which occurs most frequently contralateral to the epileptic focus and MRI signs of HS. These patients consisted of a significant proportion of our MTLE with HS cohort (14%) and had significantly lower age of epilepsy onset.

MTLE with HS is considered a constellation of clinical, EEG and MRI signs. Classically, patients are expected to have antiepileptic drug (AED) resistant seizures but there are consistent descriptions of HS signs in individuals with good seizure control or remission (Kobayashi et al., 2001). Besides the diversity of clinical evolution and AED

response, also the prognosis after the surgical treatment in MTLE with HS is variable, with only 60-70% of the patients with complete seizure control after a follow up of one year (Wiebe et al., 2001). Moreover, the events that culminate with the development of the HS and its epileptogenicity are not fully understood and many different mechanisms, from genetics to the occurrence of precipitating injuries, have been implicated (Wieser , 2004). These facts bring the concept that MTLE-HS is not a single entity but a group of different pathologies with distinct prognosis and evolution.

The amygdala is known to be part of the epileptogenic network of patients with MTLE with HS and reduced volume of this structure ipsilateral to the HS is consistently reported (Cendes et al., 1993). On the opposite, few studies have described amygdala enlargement in MTLE with HS. Enlargement of amygdala is associated with psychiatric conditions as bipolar disorders and equally in MTLE with HS enlarged amygdala has also been associated with depression and psychosis (Tebartz et al., 1999; Van Elst et al., 2002).

In our group of MTLE with HS and enlarged amygdala, no significant increase of psychiatric disorders was observed. Thereby, a consistent hypothesis for the pathophysiology of enlarged amygdala in our subgroup of MTLE with HS remains unresolved. In these patients, a possible etiology for the HS was not clear in the majority of cases. Only one patient had history of febrile seizure and other three had history of possible precipitating injury early in early life. Although three patients had family history of epilepsy, none had a clear diagnosis of familial MTLE (Kobayashi et al., 2001). The main difference from patients with or without amygdala enlargement in our study was earlier age of epilepsy onset in the former group. This fact together with the high percentage of patients (43%) in this group who had concomitant increase of hippocampal volume

contralateral to the HS makes us hypothesized that enlarged amygdala could be a marker of a developmental abnormality but up to this point there is no other evidence to support this hypothesis and further investigations are necessary .

In conclusion, a sub-group of MTLE-HS patients have enlarged amygdala most often contralateral to the epileptic focus. Whether MTLE-HS with enlarged amygdala is a distinct pattern of MTLE with HS or a part of the spectrum of this condition remains to be determined.

Acknowledgments:

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Table 1: Clinical characteristics and amygdala and hippocampal volumes of MTLE-HS patients with enlarged amygdala. MTLE-HS: medial temporal lobe epilepsy and hippocampal sclerosis; F: female; M: male; FS: febrile seizures; IPI: initial precipitating injury; FH: family history of epilepsy; AED: antiepileptic drug; poorSC: poor seizure control; goodSC: good seizure control; LOC: loss of conscious; SGTC: secondary generalized tonic-clonic seizure; LT: left temporal; RT: right temporal; Bil: bilateral; Uni: unilateral; SF: seizure focus; AI: asymmetry index.

Patient No.	Sex	Age at MRI scan (years)	Age of epilepsy onset (years)	FS	IPI	FH	AED response	Seizure semiology	Side of MRI signs of HS	Seizure focus (ictal/interictal EEG)	Epilepsy surgery (Engel outcome)	Amygdala Volume (Zscore): ipsilateral to SF	Amygdala Volume (Zscore): contralateral to SF	Hippocampal Volume (Zscore) ipsilateral to SF/AI Zscore	Hippocampal Volume (Zscore): contralateral to SF
1	F	39	1.5	N	Y	N	poorSC	Rising epigastric sensation / LOC, hypomotor	Left	LT (ictal)	N	1.87cm^3	**2.61cm^3**	3.39cm^3	4.26cm^3
2	M	48	2	N	N	N	poorSC	Epigastric sensation / LOC, manual automatisms	Right	RT (interictal)	Y (IA)	**2.62cm^3 (2.03)**	2.13cm^3	3.30cm^3	**5.24cm^3**
3	M	48	11	N	N	Y	poorSC	Déjà vu / LOC, oral and manual automatisms	Right	RT (ictal)	N	2.19cm^3 (0.30)	**2.56cm^3**	3.45cm^3	**4.89cm^3**
4	F	37	5	N	N	N	poorSC	Nonspecific bad feeling / LOC, hypomotor	Left	LT (ictal)	Y (IIB)	2.13cm^3 (0.98)	**2.72cm^3**	3.09cm^3	4.50cm^3
5	F	62	17	N	N	N	poorSC	Tachycardia, epigastric sensation / LOC, oral automatisms	Left	LT (interictal)	N	1.83cm^3	**2.89cm^3**	3.02cm^3	**5.17cm^3**
6	F	36	4	N	N	N	poorSC	Chest discomfort, déjà vu / LOC, manual automatisms	Right	RT (interictal)	N	2.06cm^3	**2.49cm^3**	3.48cm^3	**5.70cm^3**
7	F	41	28	N	N	Y	poorSC	Epigastric sensation / LOC, ictal speech, hypomotor	Right	RT (ictal)	Y (IA)	2.36cm^3 (0.99)	**2.43cm^3**	3.74cm^3	4.81cm^3
8	F	34	9	Y	Y	N	poorSC	Rising epigastric sensation / LOC, hypomotor, SGTC	Right	RT (ictal)	N	2.55cm^3 (1.75)	**2.91cm^3**	4.69cm^3	**5.37cm^3**
9	M	36	6	N	N	N	goodSC	Déjà vu, headache / LOC, ictal speech, oral automatisms	Right	RT (interictal)	N	1.89cm^3	**3.79cm^3**	3.55cm^3	3.79cm^3
10	F	50	8	N	N	N	poorSC	Chest discomfort, dizziness / LOC, hypomotor	Right	RT (interictal)	N	1.77cm^3	**2.70cm^3**	3.35cm^3	**5.57cm^3**
11	F	47	2	N	N	N	poorSC	Bad sensation / LOC, aphasia, manual automatisms	Left	LT (interictal)	N	2.06cm^3 (0.68)	**2.68cm^3**	2.94cm^3	4.66cm^3
12	F	54	6	N	Y	N	poorSC	Epigastric sensation / LOC, manual automatisms, right arm dystonia	Left	LT (interictal)	N	2.04cm^3 (0.62)	**2.63cm^3**	3.42cm^3	4.90cm^3
13	M	47	6	N	N	N	poorSC	Thirsty, rising abdominal heat / LOC oral automatisms	Right	RT (ictal)	Y (IA)	2.13cm^3 (0.03)	**2.53cm^3**	3.47cm^3	4.50cm^3
14	F	30	17	N	N	N	poorSC	Nausea / LOC, oral and manual automatisms	Left	LT (interictal)	N	2.37cm^3 (1.93)	**2.65cm^3**	3.56cm^3	4.50cm^3

CAPÍTULO 7

Amygdala enlargement in patients with mesial temporal lobe epilepsy without hippocampal sclerosis

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Amygdala enlargement in patients with mesial temporal lobe epilepsy without hippocampal sclerosis

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Running title: Amygdala enlargement in MTLE

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Abstract:

Purpose: Mesial temporal lobe epilepsy (MTLE) with normal MRI represents a challenging subgroup of patients when they are considered for possible surgical treatment due to refractory seizures. The objective of this study was to evaluate the frequency of abnormal amygdala volume and its clinical features in "MRI-negative" MTLE patients.

Methods: We evaluated 56 patients with the diagnosis of MTLE without MRI abnormalities on visual analysis (MTLE-NL) and 82 healthy subjects as control group. MRI was acquired in 3T MRI scanner (Philips Medical Systems, Best, The Netherlands). Morphometric group analysis (Voxel-based morphometry - VBM) was performed with VBM8/SPM8 (two-sample t-Test, $P<0.05$ FDR-corrected) looking for possible decrease or increase amygdala volume. As a second step, we performed automatic amygdala volumetry using Freesurfer software and T2 relaxometry of amygdala to confirm VBM findings.

Key findings: VBM group-analysis demonstrated bilateral increased amygdala volume in the MTLE-NL. Individual volumetric analysis confirmed amygdala enlargement in eight (14%) and no significant amygdala atrophy was detected. T2 relaxometry demonstrated no amygdala hyperintense signal in any individual with significant amygdala enlargement. There were no clinical differences between patients with and without amygdala abnormalities.

Significance: This study demonstrates the occurrence of enlarged amygdala volumes in 14% of patients with MTLE-NL. This finding supports the hypothesis that there might be a subgroup of patients with refractory MTLE-NL in which the enlarged amygdala could be related to the epileptogenic process in these individuals. Further studies are necessary but this finding could be of great importance not only in the understanding of TLE without HS but also in the surgical treatment of this condition.

Key Words: amygdala, temporal lobe epilepsy, MRI-negative, volumetry, VBM

1. Introduction

Mesial temporal Lobe Epilepsy (MTLE) is frequently associated with hippocampal sclerosis (HS), however there is a small group of patients with MTLE that do not have MRI signs of HS nor other lesions on MRI visual analysis, the so called "MRI-negative" MTLE[1].

MTLE with normal MRI (MTLE-NL) is a very challenging condition, especially when patients are under evaluation for epilepsy surgery[2]. Although no obvious epileptogenic lesion is detected on MRI, many of these patients undergo temporal lobectomy after appropriate presurgical evaluation[3-5]. Patients with MTLE-NL often show a different course of the disorder and worse surgical outcome than patients with HS. MTLE-NL can be considered a different syndrome from MTLE with HS[6]. For MTLE-NL patients, it is still unknown whether there is lack of structural abnormality or if there is an underlying cause that we still do not understand.

The amygdala is known for its central role in emotional behavior and it plays an important function in epilepsy and epileptogenesis[7]. The involvement of the amygdala in MTLE has been largely investigated; however, its complete participation in MTLE is still unknown[7]. Findings such as stimulation of the amygdala leading to experiential symptoms[8,9] as well as epileptiform discharges arising from the amygdala in intracranial EEG recordings[10] are corroborating evidence of the importance of this structure in MTLE.

Studies with amygdala volumes in MRI-negative patients have already been done and incidental cases of unexpected amygdala enlargement have been reported[7]. Although some authors even suggest the existence of a subgroup of MTLE patients with amygdala

involvement, the frequency of this finding and the clinical differences of patients with MTLE-NL with or without abnormal amygdala volume have not been evaluated.

Prior to the use of specific epilepsy neuroimaging protocols many patients that today are considered "MRI-positive" were initially considered "MRI-negative" patients. Thus, we aimed to investigate whether a subgroup of MTLE-NL presents with abnormal amygdala volumes and if these patients have specific clinical characteristics.

2. Methods

2.1. Patients´ selection

We included 56 patients with mean age of 41 years, (standard-deviation (SD), ± 12.2 years, ranging from 19 to 74 years, 39 female) who had clinical and electroencephalographic diagnosis of MTLE with normal MRI on visual analysis. They were *followed at the Epilepsy Clinic, University of Campinas.* Prior to acquisition of MRI data *all patients signed an* informed consent form approved by the Ethics Committee of UNICAMP. For the neuroimaging analysis we acquired 3D images, sagittal T1-weighted, with voxel size of 1x1x1mm³ (TR=7ms, TE=3.2ms, flip angle=8°, matrix=240x240) in 3T MRI scanner (Philips Medical Systems, Best, The Netherlands) of all patients and of a control group of 82 healthy subjects. All images underwent visual inspection and only patients with normal MRI by visual analysis were selected. To increase the specificity of the visual MRI analysis and to exclude the individuals with subtle HS signs, we also performed automatic hippocampal volume measurements in all patients and in 82 healthy subjects using FreeSurfer software (version 5.1.0; http://surfer.nmr.mgh.harvard.edu/). Hippocampal volumes were corrected for brain volumes of each individual. Patients with

hippocampal volumes lower than 2 SD (absolute value and/or asymmetry index, defined by the ratio of smaller over the larger hippocampus of each individual) from the mean of the control group were excluded from this analysis. We collected clinical information regarding sex, age, side of epileptic focus, age of seizure onset, time of epilepsy, history of initial precipitate injury or *status epilepticus*, family history of epilepsy, occurrence of generalized tonic clonic seizures (GTCS), frequency of complex partial seizures (CPS) and GTCS in the previous year. The epileptic focus was lateralized by ictal and/or inter-ictal scalp EEG.

2.2. Voxel-based morphometry (VBM)

For VBM we used the acquired 3D images for patients and for a control group. Pre-processing and statistical analysis were performed with VBM8/SPM8 toolbox. The resultant gray matter (GM) images were smoothed to remove large signals discrepancies between neighboring voxels (8mm FWHM). A test of quality was performed to observe homogeneity and co-registration between the data and outliers were excluded from this study (3 controls and 4 with TLE-NL). A two-sample *t-test* ($p<0.05$, FDR-corrected; minimum threshold cluster of 30 voxels) was performed between MTLE-NL and controls.

2.3. Amygdala Volumetry

To confirm VBM findings we performed amygdala volume (AV) measurements in all patients and in 82 healthy subjects using Freesurfer software. Amygdala volumes were corrected for brain volumes of each individual. Patients with amygdala volumes lower or higher than 2 SD (absolute value and/or asymmetry index, defined by the ratio of smaller over the larger amygdala of each individual) from the mean of the control group were

considered abnormal. We also analyzed a subgroup of patients with amygdala volumes between 1.5 - 2 SD from the mean of the control group.

2.4. T2-Relaxometry

T2 relaxometry of amygdala was performed in patients with significant increase or decrease of amygdala volume to investigate signal abnormalities (higher than 2 SD of the mean of the control group, composed by 79 healthy subjects). For this analysis we used T2 multi-eco images (3mm slices; TR= 3300; TE=30/60/90/120/150; matrix=200X176; FOV=1802X180) and Aftervoxel software (http://www.liv.ic.unicamp.br/~bergo/aftervoxel).

2.5. Secondary VBM analysis

In order to cross validate the VBM and volumetry we performed a secondary VBM analysis based on groups defined by amygdala volumetry. Because the binary definition of abnormally enlarged (>2SD from controls) and normal (within 2 SD from controls) amygdala volumes may include some individuals with less pronounced enlargement, we included a third subgroup as follows:

1. MTLE-NL with amygdala enlargement higher than 2 SD from the mean of the control group (enlarged amygdala volume).
2. MTLE-NL with amygdala volumes between 1,5 - 2 SD from the mean (borderline amygdala enlargement).
3. MTLE-NL with amygdala volumes lower than 1,5 SD from the mean (normal amygdala volume).

Pre-processing and statistical analysis were performed with VBM8/SPM8 toolbox. The resultant GM images were smoothed to remove large signals discrepancies between neighboring voxels. A two-sample *t-test* ($p<0.05$, FDR-corrected; minimum threshold cluster of 30 voxels) was performed between the described group and controls.

3. Results:

3.1. VBM group analysis:

The VBM T-score maps demonstrated increased amygdala GM volume in the MTLE-NL group compared to controls. Details of GM volume increase are show in Figure 1.

VBM analysis of areas of GM decrease confirmed that there were no signs of GM atrophy in the amygdala region.

3.2. Amygdala Volumetry

Individual volumetric analysis confirmed increased amygdala volumes in eight (14%) MTLE-NL patients. Five patients had unilateral and three had bilateral but asymmetrical amygdala increase. From the five patients with unilateral amygdala enlargement, two had the increased amygdala ipsilateral to the epileptic focus, two contralateral and one had bilateral temporal focus. From the three patients with bilateral amygdala increase, two had the predominant side of increased volume ipsilateral and one contralateral to the epileptic focus. Overall, from all patients with amygdala enlargement and defined epileptic focus, four (57%) had the increased or predominant increased volume ipsilateral to the epileptic focus.

Five patients had borderline amygdala enlargement.

None had significant decrease of amygdala volume.

3.3. T2-Relaxometry

T2 relaxometry demonstrated no amygdala hyperintense signal in any individual with significant amygdala enlargement.

3.4. Clinical data

There were no clinical differences between the two groups (with and without amygdala enlargement) (Table 1). Same statistical tests were done comparing patients with amygdala volume below 1.5 SD versus patients with amygdala volume higher than 1.5 SD. There were no clinical differences between the two groups.

3.5. Secondary VBM group analysis:

The VBM T-score maps demonstrated bilateral increased amygdala GM volume in the enlarged amygdala group compared to controls. (Figure 2-A)

The increased amygdala GM volume was still present in the VBM T-score maps when comparing controls to the borderline amygdala volumes (between 1.5 - 2 SD from the controls' mean group). (Figure 2-B)

The VBM analysis comparing controls to the patents with normal amygdala volumes did not show areas of increased grey matter. (Figure 2-C)

4. Discussion

In this study we demonstrated an increase of GM amygdala volume using VBM in a group of patients with MTLE-NL. Although the involvement of the amygdala in MRI-negative MTLE has already been suggested, this pattern was not observed in previous VBM studies, which could be explained by the heterogeneity among MRI-negative patients and small number of patients in previous studies.

The hypothesis that a subgroup of MTLE with amygdala involvement may exist was already raised before[7,10]. Results observed after individual automatic volumetry analysis and after secondary VBM analysis strengthen this hypothesis. However, the meaning of the increased volume of the amygdala is still unknown. We could hypothesize that it may represent dysgenesis or other subtle structural abnormality, however only further studies with pathological correlation would help us understand this MRI finding.

Clinically, the importance of this result is that if in the future we confirm that there is indeed a subgroup of patients with abnormalities restricted to the amygdala we could further discuss possible surgical approaches to these cases, either suggesting a selective amygdalectomy or an anterior temporal pole resection that includes the amygdala.

Indeed, a series of 100 MTLE patients submitted to temporal lobectomy with amygdalectomy and minimal hippocampal resection showed similar outcomes as compared to a series of 100 MTLE patients submitted to temporal lobectomy with major hippocampectomy in the same institution[11,12]. This and other series, in addition to SEEG data [13,14] supports the notion that some patients with MTLE may have a major amygdalar seizure focus and may not require removal of hippocampus. This would be particularly

relevant for the patients with normal MRI since these patients are at high risk for memory decline after removal of a normal appearing hippocampus on MRI. In a recent series of patients who were submitted to a tailored resection sparing the hippocampus they showed that 96.8% of patients did not have worsening of post operative memory performance.[15]

The main limitation of the present study is that we did not have ictal intracranial EEG recordings of these patients, nor surgical treatment with sufficient follow up. However, this preliminary finding may give support for further investigations for defining MRI surrogate markers of amygdala pathology and thus, helping to identify patients who would benefit from a selective amygdala removal sparring the hippocampus and parahippocampus.

The results reported herein emphasize the possible role of the amygdala in MTLE-NL, suggesting that there might be, at least in some cases, a structural abnormality in the amygdala that may be involved in the MRI-negative MTLE. The enlargement of the amygdala could be the source of the pathology of these individuals. Further studies are necessary but this finding could be of great importance not only in the understanding of MTLE-NL but also in the surgical treatment of this condition.

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Figure1:

Title: Gray matter volume increase in patients with MTLE-NL

VBM analysis looking for matter volume increase in patients with MTLE-NL demonstrated significant amygdala enlargement on the left side (row A). Othe small clusters of volume increase were only observed near the right cingulate gyrus and brain stem (row B). (VBM, Two-sample T-test, $p<0.05$, FDR corrected, minimum cluster size of 30 voxels). MTLE-NL: mesial temporal lobe epilepsy with normal MRI; VBM: voxel based morphometry; FDR: false discovery rate; T: T score.

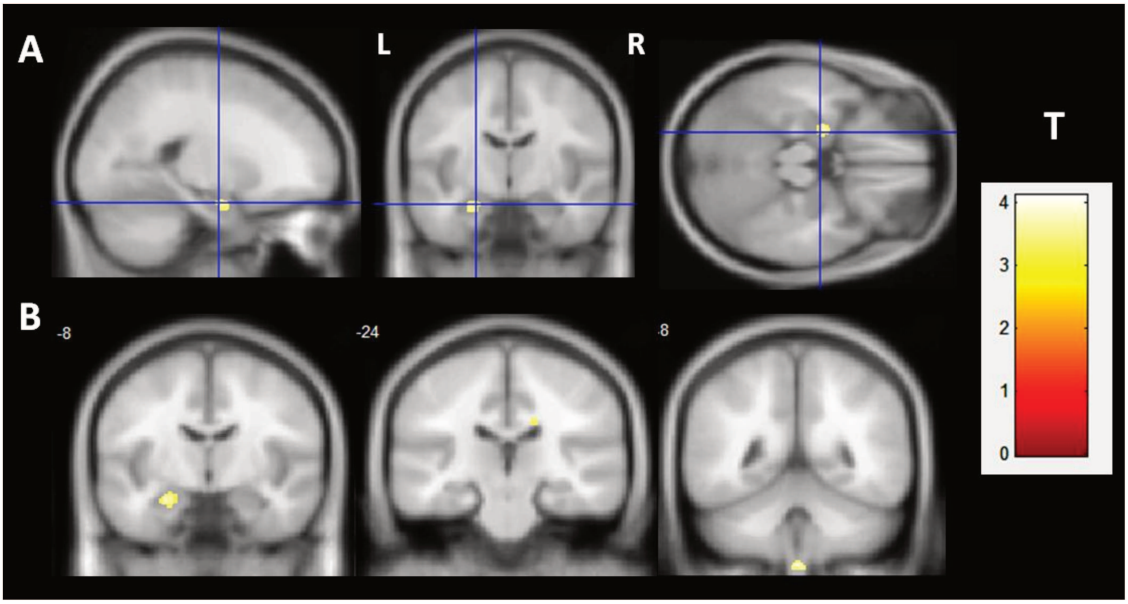

Figure2:

Title: Secondary VBM group analysis

Secondary VBM analysis confirmed the finding of subgroups of MTLE-NL patients with or without amygdala enlargement detected by automatic volumetry. A: VBM demonstrated bilateral increased amygdala gray matter volume in the enlarged amygdala group (N=8); B: VBM also detected increased amygdala gray matter volume in the subgroup of patients with borderline amygdala volumes (amygdala volumes Z-score between 1,5 - 2 SD in the volumetric analysis); C: VBM analysis did not detected increase gray matter volumes in the subgroup of patients with normal amygdala defined by the volumetric analysis. (VBM, Two-sample T-test, $p<0.05$, FDR corrected, minimum cluster size of 30 voxels). MTLE-NL: mesial temporal lobe epilepsy with normal MRI; VBM: voxel based morphometry; FDR: false discovery rate; T: T score.

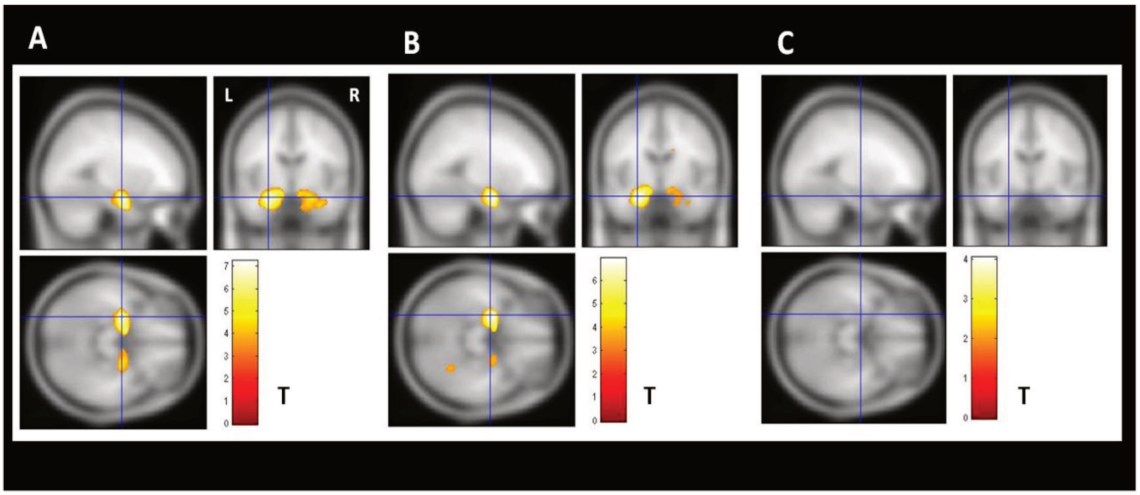

Table 1: Demographic and clinical data MTLE-NL patients with or without increased amygdala.

	Normal amygdala (n=48)	Increased amydgala (n=8)	P value
Sex	M= 26	M= 1	P=0.081 (Pearson Chi-square)
Mean Age (range)	41.7 (19 to 74 years)	47.6 (29 to 68 years)	P=0.274 (t-test)
Mean age of seizure onset (range)	19.8 (2 to 48 years)	17.9 (8 to 47 years)	P=0.702 (t-test)
Family history of epilepsy	29	3	P=0.507 (Pearson Chi-square)
IPI (FS)	12 (3)	2 (0)	P=0.78 (Pearson Chi-square)
SE	1	0	P=0.708 (Pearson Chi-square)
Mean duration of epilepsy	21.9 (1 to 50 years)	25.75 (15 to 48 years)	P=0.144 (t-test)
AED response	Refractory 36	Refractory 5	P=0.981 (Pearson Chi-square)
Seizure remission	12	1	P=0.585 (Pearson Chi-square)
Laterality of epileptic focus	28 (bilateral)	6 (bilateral)	P=0.156 (Pearson Chi-square)
Number of patients with GTCS in the previous year	11	1	P=0.657 (Pearson Chi-square)

MTLE-NL: mesial temporal lobe epilepsy with normal MRI; M: male; IPI: initial precipitating injury; FS: febrile seizure; SE: status epilepticus; AED: anti-epileptic drug; SGTCS: secondary generalized tonic-clonic seizures.

CAPÍTULO 8

EEG Epileptiform Discharges with Similar Morphology and Location Have Different Hemodynamic Responses in Mesial Temporal Lobe Epilepsy with and without Hippocampal Sclerosis.

Ana C. Coan; Brunno M. Campos; Guilherme C. Beltramini; Clarissa L. Yasuda; Roberto J. M. Covolan; Fernando Cendes.

Submetido para *Neuroimage*

EEG Epileptiform Discharges with Similar Morphology and Location Have Different Hemodynamic Responses in Mesial Temporal Lobe Epilepsy with and without Hippocampal Sclerosis.

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Abstract

Introduction: Mesial temporal lobe epilepsy (MTLE) has different underlying pathologies with common clinical and electroencephalographic (EEG) expression. We aimed to investigate patterns of EEG-correlated functional MRI (EEG-fMRI) and subtle structural abnormalities in MTLE patients with hippocampal sclerosis (MTLE-HS) or normal MRI (MTLE-NL).

Methods: We evaluated EEG-fMRI acquisition of the 25 patients with diagnosis of MTLE who had interictal epileptiform discharges (IEDs) in the intra-MRI EEG: 13 MTLE-HS and 12 MTLE-NL. All had similar morphology and location of the IEDs. FMRI was performed using echo-planar images in a 3T MRI coupled with EEG acquired with 64 MRI-compatible electrodes. In the first level analyses, the time of the IEDs ipsilateral to the epileptic focus was used as the paradigm and four variations of the hemodynamic response function (HRF) were used according to the IEDs onset times: HRFs with peaks at zero, 3, 5 and 7 seconds of the IEDs markers. Second level analyses were performed combining the contrasts of MTLE-HS or MTLE-NL patients for each different HRF. Areas of gray matter atrophy were evaluated with Voxel Based Morphometry (VBM) in both groups.

Results: Both MTLE-HS and MTLE-NL had posBOLD detected in the ipsilateral anterior temporal lobe, insula, postcentral gyrus and contralateral precentral gyrus. However, only MTLE-HS had significant posBOLD on the ipsilateral hippocampus, contralateral insula, anterior cingulate and putamen whereas MTLE-NL had more areas of posBOLD on the ipsilateral frontal lobe. Both groups had significant negBOLD responses in areas of the default mode network (DMN), such as posterior cingulate and bilateral precuneus. There was no overlap of both pos and negBOLD and areas of atrophy detected by VBM.

Conclusion: Similar IEDs have different patterns of hemodynamic responses in sub-groups of MTLE. In both MTLE-HS and MTLE-NL, there is a possible suppression of the DMN related to the occurrence of IEDs, as demonstrated by the negBOLD in these areas. The brain areas involved

in the interictal related hemodynamic network are not the regions with the most significant gray matter atrophy in MTLE with or without MRI signs of HS.

Key words: temporal lobe epilepsy; hippocampal sclerosis; functional neuroimaging; EEG, default mode network.

Abbreviations:

MTLE-HS: mesial temporal lobe epilepsy with MRI signs of hippocampal sclerosis; MTLE-NL: mesial temporal lobe epilepsy with normal MRI; IED: interictal epileptiform discharges; posBOLD: positive BOLD; negBOLD: negative BOLD.

1. Introduction

Epilepsies are conditions with functionally and anatomically connected networks (Spencer, 2002) and both ictal and interictal phenomena may be explained by the dysfunction of these networks (Laufs, 2012). The most studied epilepsy network is the one associated with mesial temporal lobe epilepsy (MTLE) (Blumenfeld et al, 2004; Bonilha et al, 2004; King and Spencer, 1995) which is the most common focal epilepsy in adults especially in individuals with drug-resistant seizures (Semah et al., 1998).

Although patients may share a common clinical and electroencephalographic (EEG) expression, MTLE has a constellation of different etiologies which may also imply diverse neuronal networks. Hippocampal sclerosis (HS) is the most common pathological substrate in MTLE refractory to anti-epileptic drugs (AEDs) (Gastaut et al., 1975; Semah et al., 1998); however, a significant number of patients with MTLE have normal MRI (Bell et al.,

2009; Cascino, 2004; Kuzniecky et al., 1987). Better understanding of these neural networks may have important implications in the comprehension of the biology of MTLE and its associated comorbidities (Heuser et al, 2009; Mueller et al., 2006).

The use of combined continuous EEG recording and functional MRI (EEG-fMRI) has been used to investigate neural networks in patients with epilepsies. The combination of these techniques permits non-invasive simultaneous measurement of neural activity and hemodynamics and allows the study of neurovascular coupling through the variation of BOLD (Blood Oxygen Level Dependent) signal (Ogawa et al., 1992). EEG-fMRI can reveal hemodynamic changes related to ictal or interictal epileptiform discharges (IEDs), giving insights for the determination of the seizure onset zone (Moeller et al., 2009; Thornton et al., 2010; Thornton et al., 2011; Zijlmans et al., 2007) but it also provides concomitant pattern of hemodynamic activity of all other brain areas distant from the presumed irritative zone. Previous reports of EEG-fMRI and TLE patients have demonstrated a consistent pattern of BOLD responses related to IEDs in areas as bilateral mesial and neocortical temporal structures but also including extra-temporal regions as insula and anterior cingulate (Laufs et al., 2007; Kobayashi et al., 2009; Fahoum et al., 2012). All these studies have combined individuals with TLE of different underlying pathologies as well as mesial and neocortical temporal presumed seizure origin. Moreover, BOLD responses related to IEDs have been detected in areas in which subtle structural damage has also been described, as anterior cingulate and insula (Bonilha et al, 2004), however it has not been observed in other brain regions with consistent description of atrophy, as thalamus (Fahoum et al, 2012).

In the present study, we used EEG-fMRI in an attempt to infer the brain structures involved in the network related to IEDs of two groups of refractory MTLE: patients with

MRI signs of HS and patients with normal MRI. Our hypothesis was that despite the similar semiology and interictal EEG findings, the underlying cause of the epilepsy might also contribute to the pattern of metabolic changes observed during the IEDs. Additionally, we tried to evaluate whether the functional network defined by EEG-fMRI is related to the network of structural abnormalities defined by MRI voxel-based morphometry (VBM) (Ashburner and Friston, 2000; Bonilha et al., 2004).

2. Methods

2.1. Patients

We included 29 patients (11 men, 18 women; mean age 41 years, range 19-58 years) with clinical-EEG diagnosis of MTLE, followed at the Epilepsy Clinic, University of Campinas. Patients were divided in two groups according to visual MRI analysis: TLE with MRI-signs of HS (MTLE-HS, 14 patients) and MTLE with normal MRI (MTLE-NL, 15 patients). Patients with MTLE secondary to other brain lesions or dual pathology were not selected. In addition, only patients with well-defined ictal and interictal EEG and seizure semiology typical of MTLE were initially screened. Final selection included only patients with refractory epilepsy and routine EEGs with frequent IEDs consisting of spikes restricted to the anterior and medium portion of the temporal lobes. Patients with polyspikes, bisynchronous discharges or rhythmic epileptiform discharges were not selected.

Informed consent form approved by the Ethics Committee of UNICAMP was signed by all patients prior to acquisition of EEG-fMRI data.

The epileptic focus was defined by prolonged ictal and interictal EEG. All patients had unilateral MTLE and all those with MRI signs of HS had ipsilateral epileptic focus.

2.2. EEG-fMRI Acquisition and Pre-Processing

fMRI exams were performed on a 3T-Achieva MRI (Philips Medical Systems, Best, The Netherlands) with EPI (echo-planar image) sequences of 24-48 minutes (mean 38 minutes) (voxel size=3x3x3mm³, 39 slices, no gap, FOV=240x240x117mm³, TE=30ms, TR=2000ms, flip angle=90º). The heads of patients were immobilized with an air cushion and they were oriented to lie still with eyes closed.

The concomitant EEG (sampling rate 5 kHz) was recorded with 64 MR-compatible (Ag/AgCl) electrodes (BrainProducts, Munich, Germany). The signal was amplified with BrainAmp Amplifier (BrainProducts) and transmitted through optic fibers to a recording terminal outside the MRI room. The fMRI data was processed and analyzed using SPM8 (Welcome Trust Center for Neuroimaging, London, UK). The EPIs were realigned, slice timing corrected, normalized according to MNI template, and smoothed to remove large signals discrepancies between neighboring voxels with a Gaussian kernel of 6mm full width at half maximum (FWHM).

The EEG post-processing was performed offline with Brain Vision Analyzer 2.0 (BrainProducts), and gradient and ballistocardiogram artifacts were removed by the AAS (Average Artifact Subtraction) correction (Allen et al., 1998 and 2000).

2.3. Intra-MRI EEG

The filtered EEGs acquired inside the scanner were reviewed by a neurophysiologist (ACC) to mark the IEDs. Spikes were marked as single points and used as an event in an fMRI paradigm to look for BOLD changes in the MR signal. Three patients (all from MTLE-NL group) did not have IEDs during the scan and were excluded from the analysis. No seizures occurred during the fMRI acquisition. The number of spikes detected during

the fMRI varied from 11 to 281 (mean of 77.75 spikes) for MTLE-NL and from 5 to 923 (mean of 141.30) for MTLE-HS. T-test did not show difference of the number of spikes between groups (p= 0.40). In MTLE-HS group, seven patients had bitemporal spikes, five had only left temporal and two only right temporal spikes. In the MTLE-NL group, seven had bitemporal spikes, three had only left temporal and two only right temporal spikes. All had similar morphology and location of the IEDs, which consisted of single spikes restricted to the anterior and medium portion of temporal lobes. The spikes were restricted to electrodes F8, T7, T8, P7, P8, TP9, TP10, FT7, FT8, TP7, TP8, FT9, FT10, with minor difference in the fields between each patient. None had spikes outside the temporal lobes or different from those observed in the routine EEG.

One patient (MTLE-HS) was also excluded because of an artifact in the fMRI scan. Therefore, 25 patients were included in the analysis (13 MTLE-HS and 12 MTLE-NL). Detailed clinical data are described on Table 1.

2.4. EEG-fMRI Statistical Analysis

The temporal series of IEDs were convolved with the canonical SPM8 hemodynamic response function (HRF) (peak at 5s relative to onset, delay of undershoot 16s, ratio of response to undershoot 6, and length of kernel 32s). In order to increase the sensitivity to detect hemodynamic responses related to the IEDs (Bagshaw et al., 2004), for each subject, nine design matrices were created varying the beginning of the HRF from -10 to +10 seconds from the instant of the IEDs (HRFs with peaks at -5, -2, zero, +3, +5, +7, +9, +11, +14 seconds from the IEDs).

The HRF derivatives (temporal and dispersion) were used as regressors. Six realignment regressors (three rotation and three translation parameters) were included in the

design matrix in order to consider errors related to movement artifacts. All temporal spikes observed in the intra-MRI EEG were added to the design matrix; however, only the maps of the temporal spikes ipsilateral to the seizure onset zone defined by prolonged video-EEG and ictal recordings were considered in the group analysis. Positive (posBOLD) and negative BOLD (negBOLD) contrast maps were built for each HRF interval.

Subsequently, for each MTLE group we performed a second level ('random effect') statistical analysis using the normalized contrast maps created for the temporal spike ipsilateral to the seizure onset zone in the single subject analysis (MTLE-HS: five right temporal and eight left temporal; MTLE-NL: five right temporal and seven left temporal) . The spatial co-registration of these maps was checked and a covariance test was performed. The maps built for the right temporal spikes were flipped (right-left orientation). Thus all the results are described as *ipsilateral* (left side) or *contralateral* (right side) referring to the IED marked on the intra-MRI EEG and consequently the seizure onset zone.

One sample T-tests ($P<0.005$, unmasked; uncorrected; minimum threshold cluster of 5 voxels) of posBOLD and negBOLD were performed for each group of individuals. The BOLD maps from all the different HRFs were visually checked and for the final analysis we chose those with any detected BOLD response in all the four subgroups (posBOLD for MTLE-HS and MTLE-NL and negBOLD for MTLE-HS and MTLE-NL). Thus, the results are described for the maps with HRF peaks at zero, +3, +5 and +7 seconds from the IEDs.

2.5. Structural Analysis (Voxel-Based Morphometry – VBM)

For VBM we acquired 3D images, sagittal T1-weighted, (voxel size=1x1x1mm^3, TR=7ms, TE=3.2ms, flip angle=8°, matrix=240x240) for patients and for a control group of 79 healthy subjects (age and sex matched). Three patients with no structural 3T-MRI

previous to epilepsy surgery were excluded from the MTLE-HS group (Table 1). The MRIs of patients with right epileptic focus for both MTLE-HS and MTLE-NL were flipped in left-right orientation.

Pre-processing and statistical analysis were performed with VBM8/SPM8 toolbox. Pre-process included normalization and modulation (MNI template) using DARTEL and segmentation of the images in gray (GM), white matter and cerebrospinal fluid. The resultant GM images were smoothed (10mm FWHM). A test of quality was performed to observe homogeneity and co-registration between the data and no outliers were detected. A two-sample T-Test ($P<0.001$, uncorrected; minimum threshold cluster of 5 voxels) was performed between each MTLE group and controls. Age and sex were used as covariates in the statistical model.

Bilinear interpolation was used in SPM to combine positive and negBOLD maps from all the different HRFs with structural maps for each MTLE group. This step had to be taken to adapt the difference in voxels size from EPI and T1 images. The number of voxels from structural analysis superimposed to voxels from functional analysis was calculated in the resampled maps.

3. Results

3.1. MTLE-HS

3.1.1. EEG-fMRI-posBOLD

In MTLE-HS group, posBOLD was most prominently observed in the ipsilateral anterior and inferior temporal lobe, parahippocampal gyrus and bilateral insula and anterior cingulate. Positive hemodynamic responses were also detected in the ipsilateral putamen, postcentral gyrus and contralateral precentral gyrus (Figure 1-A/B/C/D). Detailed

information of the different clusters of posBOLD detected in MTLE-HS group as well as the distribution in the different peaks of the HRFs is described in Table 2.

3.1.2. EEG-fMRI-negBOLD

The negBOLD detected in MTLE-HS patients included areas overlapping with default mode network (DMN), as bilateral precuneus, posterior cingulate, contralateral supramarginal gyrus, cuneus and middle temporal gyrus (Figure 2-A/B/C/D). NegBOLD was more prominently detected in the hemisphere contralateral to the epileptic focus. Detailed information of the different clusters of posBOLD detected in MTLE-HS group as well as the distribution in the different peaks of the HRFs is described in Table 2.

3.1.3. Structural analysis

VBM structural group analysis in MTLE-HS showed significant GM volume reduction in bilateral hippocampus, temporal lobes, thalamus, occipital regions and ipsilateral caudate (Figure 3-A/B). Detailed information of the different clusters of GM atrophy in MTLE-HS is described in Table 4. Pos and negBOLD responses did not show significant overlap with areas of GM atrophy on VBM analyses. Combined VBM and negBOLD maps showed only 0.1% of superimposed voxels (Figure 3-A) while the combined VBM and negBOLD maps showed just 2% of intersection voxels (Figure 3-B).

3.2. MTLE-NL

3.2.1. EEG-fMRI-posBOLD

In MTLE-NL group, posBOLD was most prominently observed in the ipsilateral anterior temporal lobe and insula. Positive hemodynamic responses were also detected in the ipsilateral frontal regions, postcentral gyrus; contralateral uncus/hippocampus and precentral gyrus and bilateral cerebellum (Figure 1-E/F/G/H). Detailed information of the

different clusters of posBOLD detected in MTLE-NL group as well as the distribution in the different peaks of the HRFs is described in Table 3.

3.2.2. EEG-fMRI-negBOLD

The negBOLD detected in MTLE-HS patients included areas overlapping with default mode network (DMN), as ipsilateral middle frontal gyrus, posterior cingulate; contralateral precuneus and supramarginal gyrus (Figure 2-E/F/G/H). NegBOLD was more prominently detected in the hemisphere contralateral to the epileptic focus. Detailed information of the different clusters of negBOLD detected in MTLE-NL group as well as the distribution in the different peaks of the HRFs is described in Table 3.

3.2.3. Structural analysis

MTLE-NL patients showed subtle GM atrophy, more evident in the ipsilateral neocortical temporal region as shown in Figure 3-C/D and Table 4. No medial temporal abnormalities were observed. As seen in MTLE-HS group, in MTLE-NL VBM analysis did not show significant GM reduction overlapping with the areas positive or negBOLD responses. Combined VBM and posBOLD maps as well as combining VBM map and negBOLD maps showed 0% of superimposed voxels (Figure 3-C and Figure 3-D).

4. Discussion

In this study, we demonstrated that similar IEDs have different patterns of hemodynamic responses in two sub-groups of MTLE: MTLE associated with HS and MTLE with normal MRI. Moreover, we compared these functional maps with subtle GM atrophy and observed that there is no overlap of the functional and structural abnormalities in these groups. Although previous authors had already addressed functional analysis in TLE (Laufs et al., 2007; Kobayashi et al., 2009; Fahoum et al., 2012), our work adds new

information because it includes more homogeneous subtypes of MTLE patients and a simultaneous comparison of structural abnormalities detected by VBM analysis.

The significance of BOLD responses in terms of neuronal activity in different pathologies are under investigation; however, EEG-fMRI can provide a broad overview of metabolic changes in the brain related to the epileptiform discharges (Bagshaw et al., 2004; Hamandi et al., 2008). EEG-fMRI has been demonstrated to be a non-invasive tool for the evaluation of patients with refractory epilepsy because it can determine brain areas involved with the irritative or the epileptogenic zone (Moeller et al., 2009; Zijlmans et al., 2007; Thornton et al., 2010; Thornton et al., 2011). Moreover, it allows the non-invasive study of neurovascular coupling and the advances in the use of this technique might help to improve not only the definition and extent of the seizure onset zone of refractory patients but also the knowledge of epileptogenic networks related to interictal phenomenon and comorbidities.

4.1. Patients´ selection

In the present study, we defined a strict selection of patients with similar clinical and electroencephalographic features, differing only in the presence or not of MRI signs of HS. Moreover, the visual analysis and classification of each individual with or without signs of HS was made with an epilepsy protocol of a high resolution MRI by two different epilepsy experts. In this context, with the use of modern MRIs protocols, the possibility of the occurrence of subtle HS not visually detected in some individuals is remote (Bell et al., 2009). With this patient selection we aimed to evaluate whether EEG discharges with similar morphology and localization, in individuals with comparable seizure semiology and AED response, but possible diverse primary pathology, could elicit different hemodynamic responses.

4.2. MTLE-HS and MTLE-NL IEDs related posBOLD

Despite the similarity of morphology, field distribution and frequency of the IEDs in both MTLE-HS and MTLE-NL, differences were observed in the posBOLD responses of each group of patients. PosBOLD responses related to IEDs have been addressed to reflect areas of discharge generation (Haminadi et al., 2008); however concomitant posBOLD is frequently observed in areas distant from the presumed epileptogenic focus in individuals with refractory epilepsies (Kobayashi et al., 2006). Whether this diffuse posBOLD response related to the IEDs reflects the propagation of the interictal activity remains to be confirmed but the inter-subject variability of BOLD responses in individuals analysis is a challenge for this comprehension.

In our study, the group analysis of IEDs in patients with MTLE-HS demonstrated posBOLD mostly important in the ipsilateral neocortical temporal lobe and parahippocampal gyrus, bilateral insula, anterior cingulate and ipsilateral putamen. Accordingly, previous studies including TLE patients with diverse etiology had demonstrated common areas of posBOLD associated with interictal spikes such as the ipsilateral mesial temporal structures (Laufs et al., 2007; Kobayashi et al., 2009), putamen/globus pallidus, bilateral superior temporal gyrus and inferior insula (Kobayashi et al., 2009). More recently, another study found posBOLD in areas concordant with our MTLE-HS group in TLE with diverse etiologies (Fahoum et al., 2012). Since the majority of individuals included in these previous studies had TLE with MRI findings of HS it is possible that their results reflect, in fact, networks not common to any TLE but more specific of MTLE-HS.

Also, in our study, posBOLD in MTLE-HS was observed in other brain areas distant from the mesial temporal lobe and also with less direct connections with the

hippocampus as pre and postcentral gyrus, medial frontal lobe and superior parietal lobule. The higher number of brain areas with detected posBOLD during IEDs in our study is possibly due to the homogeneity of our group which was composed only by MTLE patients with MRI signs of HS and no other pathologies. Accordingly, previous studies have demonstrated interictal hypoperfusion and ictal hyperperfusion in the primary motor cortex in MTLE-HS (Van Paesschen et al., Brain 2003; Tae et al., 2005) what corroborates the hypothesis of functional impairment of these extra-temporal regions even during interictal periods.

Similar from MTLE-HS, in our group with MTLE-NL the maximum posBOLD was observed in the anterior region of the ipsilateral temporal lobe and insula; also, posBOLD was observed in contralateral precentral gyrus and ipsilateral postcentral gyrus. However, significant difference was observed between the two groups, with no positive hemodynamic responses detected in brain areas as ipsilateral hippocampus/parahippocampus and putamen, contralateral insula and anterior cingulate. In order to evaluate the consistency of the absence of posBOLD in these areas, we also observed the BOLD maps of MTLE-NL group with a decreased statistical threshold ($p=0.01$, $T=2.7$). But even in this less stringent statistics no posBOLD was detected in putamen or anterior cingulate (data not shown).

The anterior cingulate has been repeatedly indicated as part of the of interictal hemodynamic network of TLE patients (Kobayashi et al., 2009; Fahoum et al., 2012) and its importance has been associated to the connections of this structure with the limbic system (Fahoum et al., 2012). It is also interesting to notice that hippocampal posBOLD was only observed contralateral to the epileptic focus in the MTLE-NL group. The absence of hemodynamic response observed in the anterior cingulate and exclusively observed in the contralateral hippocampus in MTLE-NL suggest that, although the semiology and scalp EEG

findings of these individuals do not differ from the MTLE-HS group, these patients may have different spike generators and consequently different patterns of hemodynamic responses propagation. Also, these differences in the posBOLD network may indicate that in the MTLE-NL group there may be a more complex epileptogenic network with seizures originating in both mesial and neocortical temporal structures, as well as seizure onsets in extra-temporal regions mimicking MTLE, as demonstrated by stereotactic intracerebral EEG (Barba et al., 2007). Accordingly, the MTLE-NL group had more areas of posBOLD on the ipsilateral frontal lobe than observed in MTLE-HS.

Although none of our patients had invasive recordings, they all fulfilled our strict selection and definition of MTLE. Also, the consistent posBOLD of the temporal lobe confirms that even if these patients have a more complex epileptogenic zone involving areas distant from the temporal lobes, their interictal activity propagates to the temporal lobe. The question that remains is whether the temporal lobe in these patients is the primary "focus", just a propagation, or—more importantly and in our opinion more probable— may be implicated in their ictal and interictal dysfunction.

In the present study, we opted to combine the BOLD resulting from different HRFs with the aim of increasing the sensitivity to detect hemodynamic responses related to the IEDs as it has been reported by previous studies (Bagshaw et al., 2004). In fact, our results corroborate this proposal with the observation of a variation of posBOLD detected in both MTLE-HS and MTLE-NL with the different HRFs. For example, in MTLE-HS, posBOLD was observed in putamen and contralateral insula, but not in the ipsilateral hippocampus/parahippocampal gyrus in the HRF peak at +5 seconds from the onset of the EEG spikes while a positive hemodynamic response was observed in the ipsilateral hippocampus/parahippocampal gyrus with the HRF peak at zero seconds.

4.3. MTLE-HS and MTLE-NL IEDs related negBOLD

In the MTLE-HS group negBOLD was observed in areas related to the DMN (Raichle et al., 2001; Greicius et al., 2003), such as bilateral precuneus and posterior cingulum and similar results were also found in MTLE-NL.

The meaning of negBOLD and its relation with blood flow and metabolism are not fully understood. Possible mechanisms of negBOLD response are a decrease in blood flow concomitant to a relative decrease of cortical neuronal activity from the baseline (Hamandi et al., 2008); neuronal inhibition (Czisch et al, 2004); or a purely vascular origin ("vascular steal") (Harel et al., 2002). According to previous studies comparing negBOLD responses and perfusion changes specifically related to IEDs, the mechanism of a relative decrease in the basal cortical activity is the most suitable to explain the BOLD observed in areas compatible with DMN (Hamandi et al., 2008).

Suspension of the DMN during generalized spike-and-wave discharges have been previously demonstrated in idiopathic generalized epilepsies (Gotman et al., 2005) and also in MTLE (Laufs et al., 2007). Although a previous study could not observe it in extra-temporal epilepsies (Laufs et al., 2007), a recent study showed deactivation of the DMN in response to IEDs in different groups of focal epilepsies (temporal, frontal and posterior quadrant epilepsies) (Fahoum et al., 2012). Despite these differences, our results and previous fMRI studies consistently demonstrated that the DMN is affected not only by generalized spike-and-wave bursts, which commonly have a clinical manifestation of altered consciousness, but also by isolated spikes in focal epilepsies which are usually not accompanied by any apparent behavioral or cognitive change.

In our MTLE-NL group, the negBOLD maps also included areas of the DMN although this correspondence was not as evident as in MTLE-HS group. With the present

results we cannot formulate an adequate hypothesis for this difference, since the number of individuals and the spikes in both MTLE-HS and MTLE-NL were similar. More studies are necessary to better understand this difference and the real implications of the abnormal DMN in refractory focal epilepsies.

We also observed that in both MTLE-HS and MTLE-NL, but more evident in the former group, there was a predominance of negBOLD in the hemisphere contralateral to the IEDs and the epileptic focus. Abnormal connectivity of the DMN have been described in MTLE and reduced connectivity of the posterior cingulate cortex only with the ipsilateral, and not contralateral, hippocampus has been recently described (McCormick et al., 2013). However asymmetries of the DMN and whether the epileptic focus (specifically the epileptogenic hippocampus) may affect the ipsilateral areas and functioning of the DMN lack deeper investigations.

4.4. Comparison of subtle gray matter atrophy and IEDs related to pos/negBOLD

The VBM analysis of MTLE-HS group demonstrated GM atrophy in medial and neocortical bilateral temporal regions, as well as extra-temporal areas, in concordance with previous published data (Bonilha et al., 2004; Mueller et al., 2006; Riederer et al., 2008; Coan et al., 2009). By contrast, MTLE-NL had only subtle GM atrophy detected by VBM analysis and it was more evident in the ipsilateral neocortical temporal region. A previous VBM study has failed to detect GM atrophy in non-lesional TLE (Mueller et al., 2006), while other has also demonstrated abnormalities in the neocortical temporal areas and in the ipsilateral parahippocampus (Riederer et al., 2008). These results together emphasize the fact that MTLE-NL is a heterogeneous group and that the structural abnormalities in these patients are not as evident as those seen in MTLE-HS.

In our study, the analysis of structural abnormalities with VBM did not show significant GM reduction in the areas with positive or negBOLD changes detected by EEG-fMRI, such as insula and cingulum. This may indicate that the structures involved in interictal network in these two groups of TLE patients do not sustain significant loss of volume. However, previous VBM studies have reported structural abnormalities that also included the areas with posBOLD observed by our EEG-fMRI group analysis (Bonilha et al., 2004; Coan et al., 2009) in both MTLE-HS and MTLE-NL patients (Riederer et al., 2008). For example, we reported posBOLD detected during IEDs in precentral cortex and previous reports have demonstrated GM atrophy as well as cortical thinning in bilateral sensorimotor cortex in patients with MTLE-HS (Bonilha et al., 2006; McDonald Epilepsia 2008). One possibility is that the number of individuals included in each of our groups was too small to detect the subtle structural abnormalities of these brain regions. Yet we were able to show consistent GM reduction in other important brain areas in both groups; therefore, it is still possible that even if there are structural abnormalities in the regions related to IEDs related pos/negBOLD these are not as relevant as seen in other areas, such as thalamus, caudate or occipital regions.

Likewise, although morphometric and volumetric studies of patients with MTLE have demonstrated diverse results with a significant variability of the brain regions with detected atrophy, areas such as bilateral thalamus are consistently reported as atrophic in the studies of refractory MTLE-HS (Keller et al., 2007; Li et al., 2012). In the present study, thalamic atrophy was detected in both MTLE-HS and MTLE-NL but no hemodynamic response was observed in this region. The absence of overlap between IED related hemodynamic response and atrophy demonstrated by our results confirms the complex interactions between functional and structural networks in MTLE. In fact,

although the propagation of ictal activity has been implicated in progressive structural damage, paradoxically it could be expected that in brain regions with atrophy, and consequently a smaller concentration of neurons, a lesser amount of ictal or interictal activity would happen. Also, the neurovascular coupling of brain damaged areas is not completely known and although the atrophy detected in brain regions of MTLE patients distant from the temporal lobe are very subtle and only detected by refined MRI analysis, it is possible that also the perfusion of these areas are compromised.

In conclusion, we were able to show that brain structures involved in the functional network related to IEDs differ in MTLE-HS and MTLE-NL patients and also that the structures involved in these functional networks are not those with most significant structural damage as detected by VBM. The importance of these findings extends beyond the simple definition of the irritative zone and may direct future investigations about the interictal dysfunctions in patients with MTLE. Moreover, by revealing distinct patterns of BOLD response from similar IEDs, EEG-fMRI can add information to the scalp EEGs of patients with MTLE and it can improve the understanding of the functional networks of sub-groups of patients with similar semiology and EEG findings.

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Case	Age	Gender	TLE group	Age 1st seizure	Seizure semiology	Laterality SOZ (interictal/ictal scalp EEG)	Spikes during EEG-fMRI (no. events)	Structural analysis (VBM)
1	24	F	TLE-HS	1 year	Jamais vu, staring, dystonic posture of left arm, right hand automatisms	RT	RT (10)	no
2	40	F	TLE-HS	1 year	Fear, loss of consciousness, staring, oral automatisms	RT	RT(143) / LT(4)	no
3	38	M	TLE-HS	6 months	No aura. Staring, oral and bi-manual automatisms.	RT	RT(32) / LT(203)	yes
4	56	F	TLE-HS	7 years	Abdominal pain, loss of consciousness, staring, oral and manual automatisms	LT	LT(122)	yes
5	36	F	TLE-HS	5 years	Fear, loss of consciousness, staring	LT	RT(13) / LT(3)	yes
6	38	F	TLE-HS	1 year	Epigastric pain, nausea, loss of consciousness, chewing, dystonic posture of right arm	LT	RT(3) / LT(15)	yes
7	50	M	TLE-HS	9 months	Sudden holocranial headache, manual automatisms	LT	LT(17)	yes
8	58	M	TLE-HS	16 years	Jamais vu, speech arrest, lost of consciousness, staring	LT	LT(13)	no
9	46	F	TLE-HS	17 years	Epigastric sensation, nausea, staring, chewing	LT	LT(5)	yes
10	47	M	TLE-HS	14 years	Strange and bad feeling, loss of consciousness, upward eye deviation, oral automatisms	RT	RT(187)	yes
13	38	F	TLE-HS	2 years	No aura, sudden loss of consciousness, say meaningless sentences, manual automatisms	LT	RT(260) / LT(663)	yes
11	28	M	TLE-HS	17 years	No aura, loss of consciousness, bi-manual automatisms	RT	RT(16) / LT(40)	yes
12	19	M	TLE-HS	1 year	Abdominal sensation, starring, walk around	LT	LT(88)	yes
14	20	M	TLE-NL	10 years	Rising epigastric sensation, staring	LT	LT(147)	yes
15	48	F	TLE-NL	14 years	Feeling of discomfort and imminent death, fear, loss of consciousness, hypomotor	LT	RT(46) / LT(36)	yes
16	43	F	TLE-NL	13 years	Fear, loss of consciousness, ictal speech, manual automatisms	RT	RT(26) / LT(13)	yes
17	51	F	TLE-NL	28 years	Bad rising epigastric sensation, staring, chewing, hands automatisms	RT	RT(11) / LT(12)	yes
18	27	M	TLE-NL	03 years	Rising epigastric sensation, buzz in both years, staring/hypomotor	RT	RT(84)	yes
19	47	F	TLE-NL	05 years	No aura, sudden loss of consciousness, manual automatisms	LT	LT(85)	yes
20	56	M	TLE-NL	07 years	Jamais vu, loss of consciousness, hypomotor	LT	LT(13)	yes
21	45	F	TLE-NL	08 years	Epigastric sensation, fear, loss of consciousness, oral and bi-manual automatisms	LT	RT(24) / LT(18)	yes
22	27	F	TLE-NL	27 years	Fear, loss of consciousness, swallow, walk with no purpose	RT	RT(241) / LT(40)	yes
23	37	F	TLE-NL	13 years	Epigastric sensation, loss of consciousness, chewing	LT	RT(24) / LT(28)	yes
24	28	F	TLE-NL	16 years	"Religious" feeling, loss of consciousness, bi-manual automatisms	LT	RT(12) / LT(62)	yes
25	48	M	TLE-NL	19 years	Rising epigastric sensation, staring/hypomotor	RT	RT(11)	yes

Table 1: Clinical data of TLE patients included in functional and structural network analysis. TLE: temporal lobe epilepsy; VBM: voxel based morphometry; F: female; M: male; HS: hippocampal sclerosis; NL: non-lesional; RT: right temporal; LT: left temporal; R: right; L: left; SOZ: seizure onset zone.

Table 2. BOLD - TLE-HS

Area	Lateralization	Nº Voxel	T score	Coordinates	HRF Peak (s)	BOLD Type
Insula	Left/*ipsi*	323	4.21	-39 -4 4	0	Positive
Postcentral Gyrus	Left/*ipsi*	13	3.89	-54 -31 49	0	Positive
Superior Temporal Gyrus	Left/*ipsi*	7	3.61	-27 11 -38	0	Positive
Fusiform Gyrus	Left/*ipsi*	28	3.58	-36 -55 -5	0	Positive
Medial Frontal Gyrus	Left/*ipsi*	5	3.07	-3 -7 49	0	Positive
Parahippocampal Gyrus	Left/*ipsi*	5	3.00	-34 -23 -21	0	Positive
Anteiror Cingulate Gyrus	Right/*contra*	72	3.98	9 -1 43	0	Positive
Precentral Gyrus	Right/*contra*	7	3.55	36 -10 52	0	Positive
Parahippocampal Gyrus	Right/*contra*	8	3.32	36 -13 -11	0	Positive
Precentral Gyrus	Left/*ipsi*	173	4.77	-45 2 7	3	Positive
Anterior Cingulate Gyrus	Left/*ipsi*	191	4.25	-6 8 34	3	Positive
Superior Parietal Lobule	Left/*ipsi*	80	4.20	-30 -49 64	3	Positive
Postcentral Gyrus	Left/*ipsi*	22	4.10	-30 -34 43	3	Positive
Putamen	Left/*ipsi*	14	3.86	-15 5 -11	3	Positive
Medial Frontal Gyrus	Right/*contra*	95	4.15	3 -10 76	3	Positive
Superior Parietal Lobule	Right/*contra*	19	3.49	27 -55 67	3	Positive
Insula	Left/*ipsi*	95	3.68	-42 -1 -8	5	Positive
Postcentral Gyrus	Left/*ipsi*	6	3.22	-30 -46 70	5	Positive
Medial Frontal Gyrus	Right/*contra*	122	4.07	3 -1 49	5	Positive
Anterior Cingulate Gyrus	Right/*contra*	5	3.84	12 23 34	5	Positive
Insula	Right/*contra*	9	3.28	45 2 -5	5	Positive
Parahippocampal Gyrus	Left/*ipsi*	41	4.32	-30 -1 -20	7	Positive
Inferior Frontal Gyrus	Left/*ipsi*	14	3.70	-60 26 7	0	Negative
Precuneus	Right/*contra*	9	4.12	18 -52 34	0	Negative
Supramarginal Gyrus	Right/*contra*	12	4.04	39 -52 31	0	Negative
Precuneus	Left/*ipsi*	13	3.97	-15 -58 34	3	Negative
Posterior Cingulate	Left/*ipsi*	9	3.43	-6 -37 25	3	Negative
Angular Gyrus	Left/*ipsi*	16	3.38	-39 -61 37	3	Negative
Precuneus	Right/*contra*	12	4.06	18 -52 34	3	Negative
Supramarginal Gyrus	Right/*contra*	48	4.03	45 -52 37	3	Negative
Posterior Cingulate	Right/*contra*	46	3.76	12 -37 28	3	Negative
Superior Temporal Gyrus	Right/*contra*	12	3.57	39 -43 13	3	Negative
Inferior Frontal Gyrus	Right/*contra*	6	3.27	33 26 -23	3	Negative
Medial Frontal Gyrus	Left/*ipsi*	11	3.97	-12 59 4	5	Negative
Cingulate Gyrus	Right/*contra*	161	5.16	9 -43 34	5	Negative
Supramarginal Gyrus	Right/*contra*	140	4.69	57 -52 25	5	Negative
Inferior Frontal Gyrus	Right/*contra*	33	4.16	33 26 -23	5	Negative
Superior Frontal Gyrus	Right/*contra*	23	4.14	24 35 64	5	Negative
Cuneus	Right/*contra*	40	3.50	6 -67 31	5	Negative
Middle Temporal Gyrus	Right/*contra*	16	3.38	51 -40 1	5	Negative
Posterior Cingulate	Right/*contra*	7	3.13	18 -61 19	5	Negative
Superior Frontal Gyrus	Left/*ipsi*	39	5.28	-24 47 28	7	Negative
Middle Frontal Gyrus	Left/*ipsi*	17	4.10	-36 56 -5	7	Negative
Inferior Frontal Gyrus	Left/*ipsi*	5	3.71	-51 47 4	7	Negative
Superior Frontal Gyrus	Right/*contra*	198	5.01	12 68 10	7	Negative
Middle Frontal Gyrus	Right/*contra*	103	4.42	33 62 1	7	Negative
Inferior Parietal Lobule	Right/*contra*	41	4.11	66 -37 28	7	Negative
Precuneus	Right/*contra*	61	3.73	6 -58 52	7	Negative
Middle Temporal Gyrus	Right/*contra*	36	3.57	63 -49 -2	7	Negative

Areas with significant BOLD (positive and negative) associated with interictal EEG abnormalities (T-test, p=0.005, uncorrected, limit threshold of 5 voxels). TLE-HS: temporal lobe epilepsy with hippocampal sclerosis; *Ipsi*: ipsilateral to EEG abnormalities recorded inside MRI scanner; *contra*: contralateral to EEG abnormalities recorded inside MRI scanner.

Table 3. Positive BOLD - TLE-NL

Area	Lateralization	Nº Voxels	T Score	Coordinates	HRF Peak (s)	BOLD Type
Superior Temporal Gyrus	Left/*ipsi*	19	4.19	-42 11 -20	0	Positive
Middle Frontal Gyrus	Left/*ipsi*	25	3.88	-39 -1 61	0	Positive
Medial Frontal Gyrus	Left/*ipsi*	17	3.77	-3 -25 61	0	Positive
Uncus/Hippocampus	Right/*contra*	5	4.38	21 -1 -23	0	Positive
Superior Temporal Gyrus	Right/*contra*	9	4.23	45 8 -14	0	Positive
Precentral Gyrus	Right/*contra*	5	3.59	24 -25 61	0	Positive
Paracentral Lobule	Right/*contra*	5	3.59	18 -40 55	0	Positive
Culmen	Left/*ipsi*	5	4.02	-30 -28 -29	3	Positive
Cerebellar Tonsil	Right/*contra*	5	3.27	24 -31 -32	3	Positive
Superior Temporal Gyrus	Left/*ipsi*	92	5.40	-45 8 -11	5	Positive
Postcentral Gyrus	Left/*ipsi*	33	4.07	-15 -49 73	5	Positive
Insula	Left/*ipsi*	92	3.78	-42 -10 -2	5	Positive
Superior Frontal Gyrus	Left/*ipsi*	5	3.70	-15 65 19	7	Positive
Fusiform Gyrus	Right/*contra*	15	4.18	57 -13 -23	7	Positive
Superior Frontal Gyrus	Right/*contra*	14	3.78	12 56 19	7	Positive
Middle Frontal Gyrus	Left/*ipsi*	5	4.69	-24 23 40	0	Negative
Posterior Cingulate	Left/*ipsi*	5	3.29	-3 -58 16	0	Negative
Caudate Body	Right/*contra*	8	5.10	9 17 16	0	Negative
Superior Frontal Gyrus	Right/*contra*	30	3.99	15 20 61	0	Negative
Inferior Temporal Gyrus	Right/*contra*	9	3.83	60 -13 -14	0	Negative
Supramarginal Gyrus	Right/*contra*	77	3.74	51 -52 34	0	Negative
Superior Frontal Gyrus	Left/*ipsi*	24	4.71	-12 68 19	3	Negative
Superior Frontal Gyrus	Left/*ipsi*	13	4.05	-15 65 19	5	Negative
Precuneus	Left/*ipsi*	8	3.35	-21 -64 34	5	Negative
Medial Frontal Gyrus	Right/*contra*	6	3.51	12 53 16	5	Negative
Superior Frontal Gyrus	Left/*ipsi*	5	3.70	-15 65 19	7	Negative
Fusiform Gyrus	Right/*contra*	15	4.18	57 -13 -23	7	Negative
Superior Frontal Gyrus	Right/*contra*	14	3.78	12 56 19	7	Negative

Areas with significant BOLD (positive and negative) associated with interictal EEG abnormalities (T-test, p=0.005, uncorrected, limit threshold of 5 voxels). TLE-NS: temporal lobe epilepsy Non lesional; *Ipsi*: ipsilateral to EEG abnormalities recorded inside MRI scanner; *contra*: contralateral to EEG abnormalities recorded inside MRI scanner.

Table 4. Structural analysis

Area	Side	Nº Voxel	T score	Coord.	TLE group
Parahippocampal Gyrus	Left/*ipsi*	810	5.95	-17, -21, -23	TLE-HS
Cerebellum	Left/*ipsi*	938	5.13	-26, -51, -38	TLE-HS
Middle Occipital Gyrus	Left/*ipsi*	226	4.24	-39, -96, 7	TLE-HS
Caudate	Left/*ipsi*	877	4.55	-33, -25, -2	TLE-HS
Thalamus / Pulvinar	Left/*ipsi*	22	4.55	-7, -31, 1	TLE-HS
Middle Temporal Gyrus	Left/*ipsi*	97	3.97	-39, 2, -30	TLE-HS
Lingual Gyrus	Left/*ipsi*	119	3.93	-9, -104, -9	TLE-HS
Claustrum	Left/*ipsi*	119	3.83	-29, 9, 12	TLE-HS
Precuneus	Left/*ipsi*	142	3.66	-29, -77, 36	TLE-HS
Lingual Gyrus	Left/*ipsi*	5	3.28	-33, -69, -3	TLE-HS
Cuneus	Left/*ipsi*	5	3.27	-12, -99, 29	TLE-HS
Superior Occipital Gyrus	Left/*ipsi*	8	3.26	-32, -92, 29	TLE-HS
Hippocampus	Right/*contra*	1451	4.47	33, -33, -2	TLE-HS
Middle Frontal Gyrus	Right/*contra*	293	4.36	42, 59, -3	TLE-HS
Parahippocampal Gyrus	Right/*contra*	124	4.25	33, -18, -15	TLE-HS
Thalamus/VLN	Right/*contra*	363	4.25	10, -13, 3	TLE-HS
Cerebellum	Right/*contra*	813	4.07	12, -74, -39	TLE-HS
Superior Frontal Gyrus	Right/*contra*	82	3.86	12, 63, -23	TLE-HS
Middle Occipital Gyrus	Right/*contra*	80	3.86	15, -87, 12	TLE-HS
Temporal Pole	Right/*contra*	81	3.77	62, 6, -23	TLE-HS
Middle Temporal Gyrus	Right/*contra*	25	3.58	69, -11, -9	TLE-HS
Superior Temporal Gyrus	Right/*contra*	7	3.30	65, -1, 3	TLE-HS
Lingual Gyrus	Right/*contra*	6	3.30	33, -59, -2	TLE-HS
Inferior Parietal Lobule	Left/*ipsi*	178	4.04	-50, -33, 30	TLE-NL
Middle Temporal Gyrus	Left/*ipsi*	184	3.77	-47, -63, 15	TLE-NL
Inferior Temporal Gyrus	Left/*ipsi*	344	3.75	-29, -7, -42	TLE-NL
Thalamus/MDN	Right/*contra*	97	3.35	5, -21, 7	TLE-NL

Areas with significant gray matter reduction observed with VBM (T-test, p=0.001, uncorrected, limit threshold of 5 voxels). TLE-HS: temporal lobe epilepsy with hippocampal sclerosis; TLE-NL: non-lesional temporal lobe epilepsy; VBM: voxel based morphometry; *Ipsi*: ipsilateral to EEG abnormalities recorded inside MRI scanner; *contra*: contralateral to EEG abnormalities recorded inside MRI scanner; MDN: Medial dorsal Nucleus VLN: Ventral Lateral Nucleus

Figure 1:

Title: EEG-fMRI positive BOLD in MTLE-HS and MTLE-NL

Group analysis of interictal epileptiform discharges related positive hemodynamic responses (posBOLD) in patients with MTLE (T-test, $p<0.005$, uncorrected, minimum threshold cluster of 5 voxels). Boxes A to D show posBOLD of MTLE-HS group with the HRF peaks at zero (A), +3 (B), +5 (C) and +7 (D) seconds from the EEG discharges. Boxes E to H show posBOLD of MTLE-NL group with the HRF peaks at zero (E), +3 (F), +5 (G) and +7 (H) seconds from the EEG discharges. BOLD: Blood Oxygen Level Dependent; MTLE: Mesial Temporal Lobe Epilepsy; posBOLD: Positive BOLD; HS: Hippocampal Sclerosis; NL: normal MRI; HRF: hemodynamic response function; T: T-score.

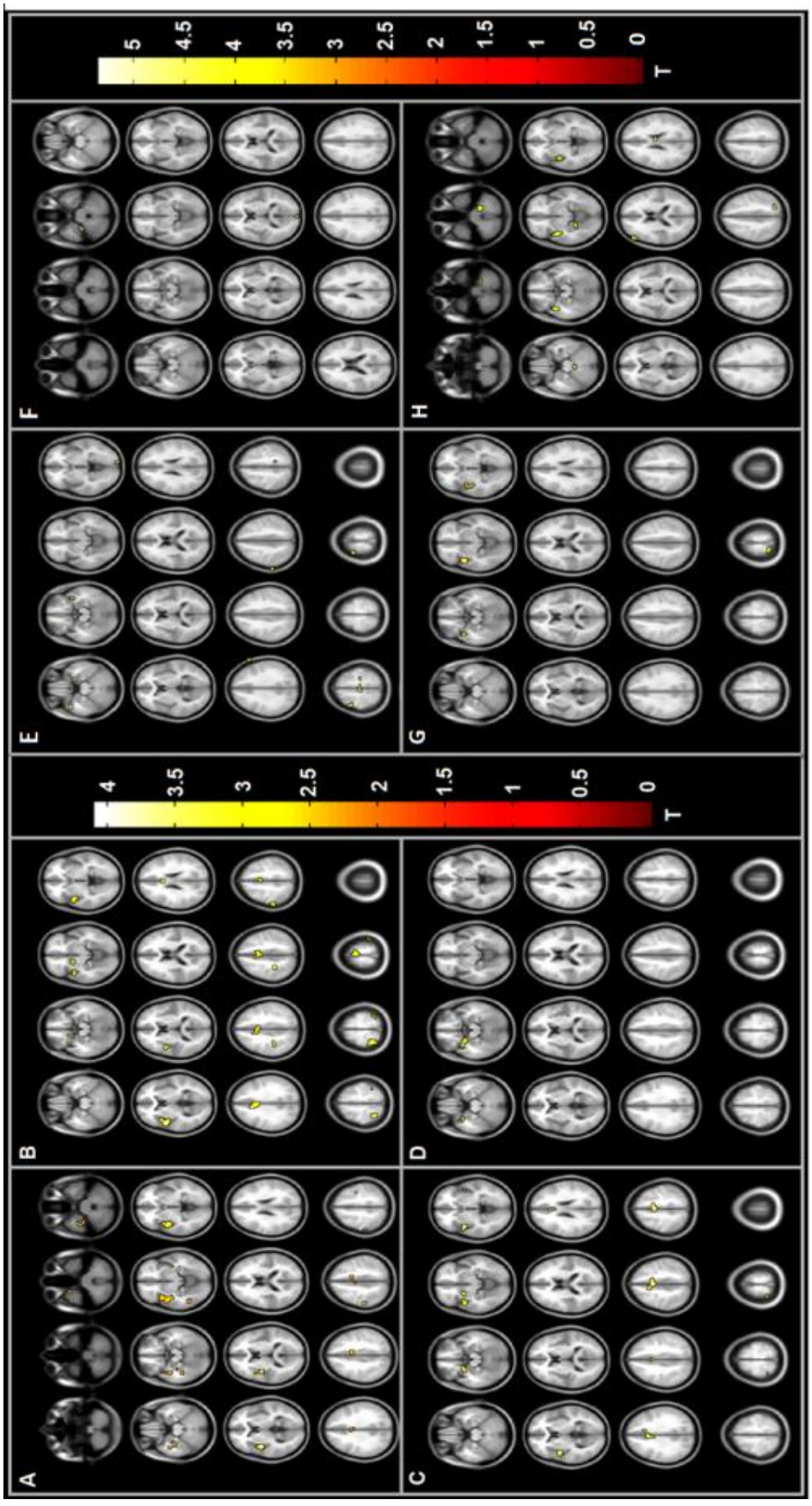
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Figure 2:

Title: EEG-fMRI negative BOLD in MTLE-HS and MTLE-NL

Group analysis of interictal epileptiform discharges related negative hemodynamic responses (negBOLD) in patients with MTLE (T-test, $p<0.005$, uncorrected, minimum threshold of 5 voxels). Boxes A to D show negBOLD of MTLE-HS group with the HRF peaks at zero (A), +3 (B), +5 (C) and +7 (D) seconds from the EEG discharges. Boxes E to H show negBOLD of MTLE-NL group with the HRF peaks at zero (E), +3 (F), +5 (G) and +7 (H) seconds from the EEG discharges. BOLD: Blood Oxygen Level Dependent; MTLE: Mesial Temporal Lobe Epilepsy; negBOLD: Negative BOLD; HS: Hippocampal Sclerosis; NL: normal MRI; HRF: hemodynamic response function.

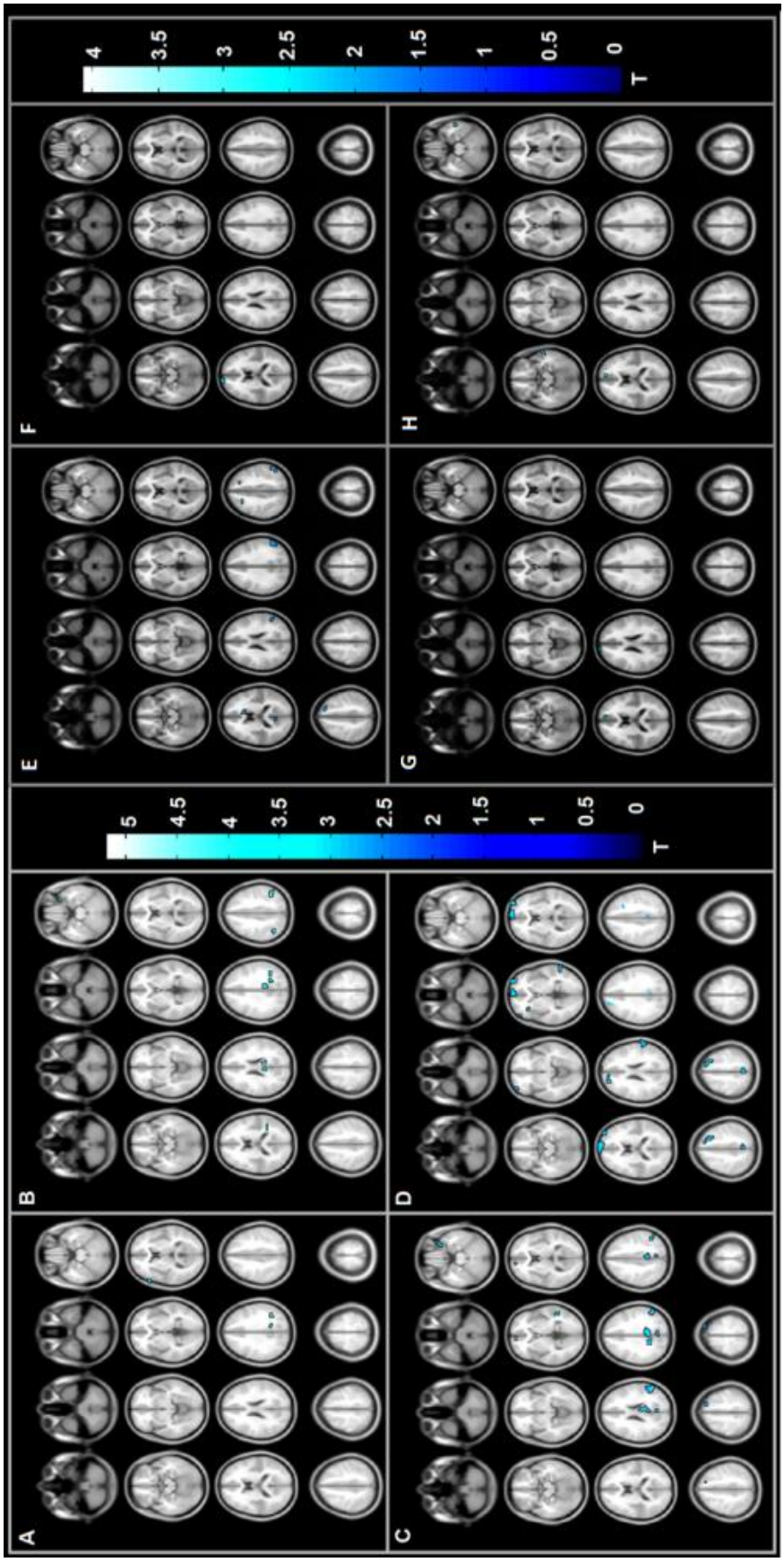
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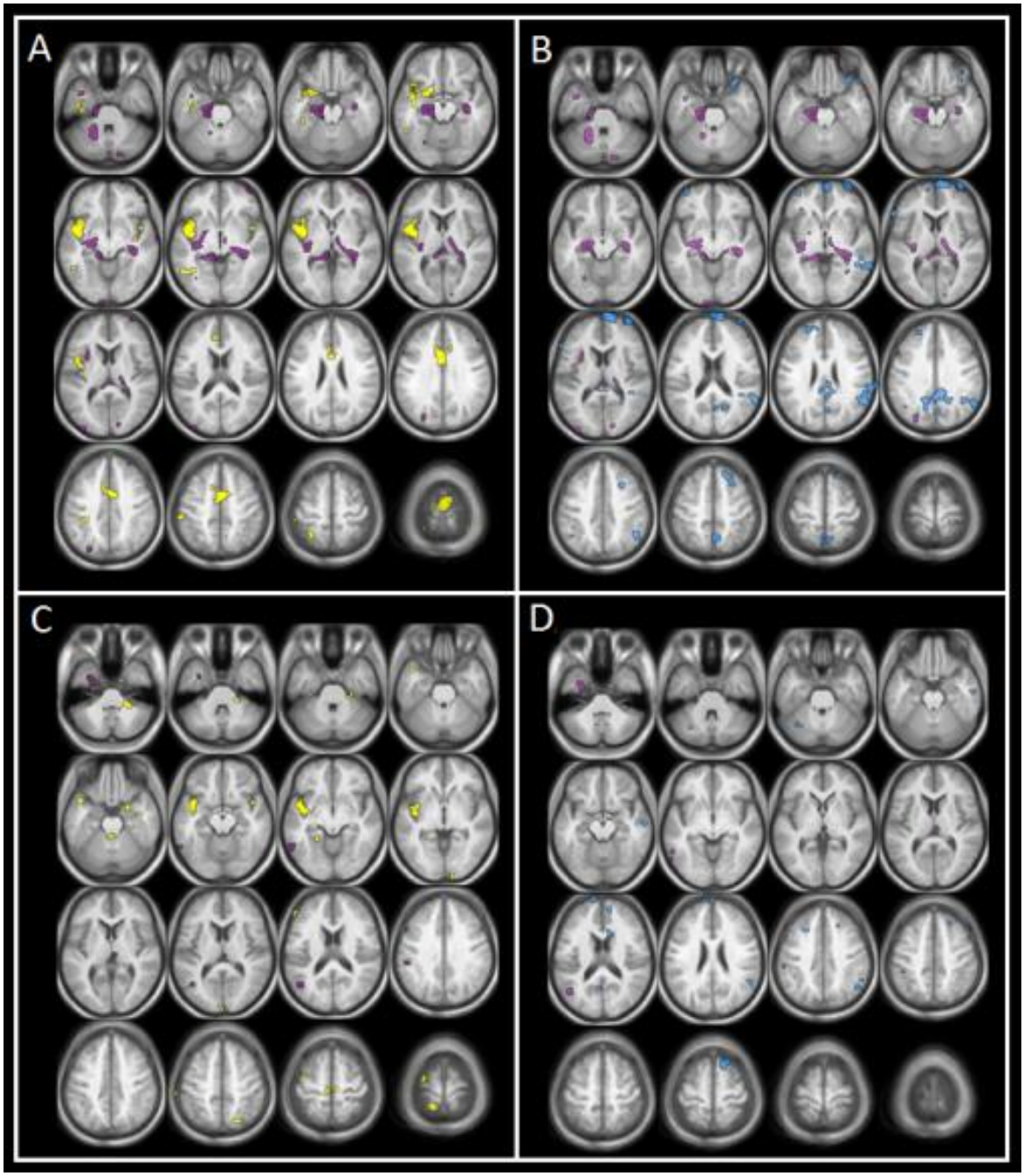
Figure 3:

Title: Combined structural (VBM) and functional (EEG-fMRI) analysis of MTLE-HS and MTLE-NL

Areas of gray matter atrophy detected with VBM *(purple)* did not overlap with interictal epileptiform discharge related posBOLD *(yellow)* and negBOLD *(blue)* (VBM: T-test, $p<0.001$, uncorrected, minimum threshold cluster of 5 voxels; EEG-fMRI: T-test, $p<0.005$, uncorrected, minimum threshold cluster of 5 voxels). A: co-register of MTLE-HS posBOLD maps (HRF peaks at zero, +3, +5 and +7 seconds from the EEG discharge) and gray matter atrophy; B: co-register of MTLE-HS negBOLD maps (HRF peaks at zero, +3, +5 and +7 seconds from the EEG discharge) and gray matter atrophy; C: co-register of MTLE-NL posBOLD maps (HRF peaks at zero, +3, +5 and +7 seconds from the EEG discharge) and gray matter atrophy; D: co-register of MTLE-NL negBOLD maps (HRF peaks at zero, +3, +5 and +7 seconds from the EEG discharge) and gray matter atrophy. VBM: voxel based morphometry; BOLD: Blood Oxygen Level Dependent; MTLE: Mesial Temporal Lobe Epilepsy; posBOLD: Positive BOLD; negBOLD: Negative BOLD; HS: Hippocampal Sclerosis; NL: normal MRI; HRF: hemodynamic response function.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CAPÍTULO 9

EEG-fMRI in the pre-surgical evaluation of temporal lobe epilepsy patients

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Em preparação

EEG-fMRI in the pre-surgical evaluation of temporal lobe epilepsy patients

Running title: EEG-fMRI pre-surgical evaluation in TLE

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Summary

Temporal lobe epilepsy (TLE) is refractory in up to 70% of patients requiring thorough investigation to define the epileptogenic zone for surgical treatment. Simultaneous combined scalp EEG and functional MRI (EEG-fMRI) has been proposed as a non-invasive pre-surgical evaluation tool for patients with focal refractory epilepsy. However, the technique's specific clinical role in different types of focal epilepsy remains to be determined. We used EEG-fMRI to map haemodynamic changes related to interictal epileptiform discharges (IEDs) to evaluate its value as a pre-surgical tool for patients with refractory TLE and to assess its potential post-surgical outcome prediction value. Sixty-seven patients with refractory TLE undergoing presurgical evaluation were invited to have EEG-fMRI. IEDs were identified on the intra-scanner EEG and used to build a model of blood oxygen level-dependant (BOLD) fMRI signal changes in a general linear model (GLM). In addition, EEG topographic correlation maps were calculated between the average IEDs recorded during long-term video-EEG monitoring and the intra-scanner EEG and used as a condition, following a convolution with a haemodynamic kernel. This allowed the analysis of all data irrespective of the presence or absence of IED on the intra-scanner EEG. Thirty of the 67 patients (45%) subsequently underwent unilateral TL resection of different extent according to the pathology. The mean follow-up period after surgery was 22.4 months. Significant IED-related BOLD changes were revealed in all but one case. The interictal BOLD maps were classified as Concordant with conventional electroclinical localization in 4 patients, and of those, 3 (75%) had a good surgical outcome. In 12 patients the EEG-fMRI results were classified as having Some Concordance and nine of these (75%) had a good surgical outcome. By contrast only five of the thirteen patients (38%) classified as Discordant had a good surgical outcome. Patients with BOLD changes in areas of the posterior component of default mode network (DMN) had a better surgical outcome than those without it. These results reveal that EEG-fMRI can provide useful information for the pre-surgical evaluation of patients with TLE and that surgical resection including regions of

IEDs BOLD changes may lead to better postoperative outcome. The presence of BOLD changes in the areas compatible with DMN may imply intact network function and its absence is associated to worse surgical outcome in patients with TLE probably due to more diffuse network abnormalities.

Key words: EEG-fMRI, temporal lobe epilepsy, default mode network

Introduction

Among adult patients with refractory focal epilepsies submitted to surgical treatment, temporal lobe epilepsy (TLE) is the most common pathology (deTisi et al., 2011). For appropriated surgical procedure the accurate definition of the seizure onset zone is necessary and different non-invasive and sometimes invasive procedures are often employed, including special neuroimaging techniques.

In this context, the technique of combined electroencephalography and functional MRI (EEG-fMRI) has proved to be a useful non-invasive approach for the definition of the seizure onset-zone in individuals with focal refractory epilepsies (Salek-Haddad et al, 2006; Thornton et al., 2010). Different studies have tried to define the role of EEG-fMRI in the pre-surgical evaluation and the value of EEG-fMRI in defining the long-term prognosis of TLE and non-TLE patients. The difficulty, so far, is that the number of individuals included in such studies is small, partly due to the high proportion of patients for whom the technique was insensitive due to the lack of interictal epileptiform discharges (IEDs) on intra-scanner EEG.

In TLE, the surgical prognosis is mainly defined by the structural abnormality observed in MRI, with hippocampal sclerosis (HS) having the best long-term results (deTisi et al., 2011). However, even patients with TLE associated with HS, up to one third will present seizure recurrence after a period of five years (Berkovic et al., 1995).

The hypothesis of this study was that EEG-fMRI can be used as a tool for presurgical evaluation and can help defining surgical prognosis in patients with refractory TLE. For this purpose, we evaluated a series of individuals with long-term follow-up. Also,

we used this opportunity to advance the knowledge of EEG-fMRI in this specific group of patients by analyzing a larger number of individuals irrespective the occurrence of visually detectable IEDs on intra-scanner EEG. This was made possible in patients in whom no IED was captured during EEG-fMRI by the use of the correlation of epilepsy-specific voltage maps derived from video-EEG recorded outside the MRI with the intra-scanner EEG to reveal haemodynamic changes associated with EEG activity that matches such maps (Grouiller et al., 2011). A secondary hypothesis was that hemodynamic changes and their relationship with the seizure onset zone on one hand and and networks associated with normal brain activity on the other, may help predict surgical outcome in individuals with TLE.

Method

Patient selection

Patients with TLE and who have been submitted to EEG-fMRI exams between July 2007 and March 2012 at three centres: University College London, London, UK; University of Geneva, Geneva, Switzerland; University of Campinas, Campinas, Brazil were enrolled in the study. The diagnosis of TLE was made based on clinical and scalp EEG recordings data according to ILAE criteria. The availability of a clinical EEG recording showing IEDs was a requirement. A total of 67 individuals fulfilled these criteria, out of which 30 subsequently underwent surgery aimed at stopping refractory seizures and therefore are the subject of the present study (16 women, mean age 32 years, range 13-51). All patients signed specific consent information terms approved by the respective Ethic Committee of each centre.

The EEG-fMRI results were not taken into consideration during the surgical management process. The seizure onset zone was defined according to an expert panel discussion based on clinical, electroencephalographic, neuroimaging or intracranial EEG findings. Surgical outcome was defined according to ILAE outcome classification (Wieser et al., 2001). For the purpose of statistical analysis, ILAE outcomes 1 to 3 were considered good surgical outcome and ILAE 4 and 5 were considered poor surgical outcome.

EEG-fMRI acquisition

MRIs were acquired in 3T MRI scanners (Campinas: 3T Phillips Achieva Medical Systems; London: 3T Signa Excite HDX, GE MedicalSystems; Geneva: 3T Siemens Magnetom Trio). The fMRI protocol consisted of blood oxygen level-dependent (BOLD) sensitive eco-planar image (EPI) time series lasting between 20 and 48 minutes (London: repetition time = 3000 ms, voxel size: 3.75x 3.75x3mm^3, 43 slices Geneva: repetition time = 1500 ms, voxel size: 3.75x 3.75x 5.5mm^3, 25 slices; Campinas: repetition time = 2000ms, 3x3x3mm³ voxel size, 39 slices, no gap, FOV 240x240x117mm³, TE 30ms, TR 2000ms, flip angle 90º). Concomitant EEGs with 32 to 256 channels recorded with MRI compatible electrodes were acquired (Brain Products, Munich, Germany). Patients were asked to lie still with eyes closed with no further instructions; head restraining was used. The EEG signal was amplified and digitized using BrainAmp Amplifier (Brain Products GmbH, Germany) and transmitted via optic fibre cables to a recording terminal outside the scanner room.

EEG-fMRI analysis

<u>fMRI and EEG pre-processing</u>

The fMRI data were processed and analyzed using SPM8 (Wellcome Trust Center for Neuroimaging, London, UK). The fMRI images were realigned, slice timing corrected, and smoothed with a Gaussian kernel of 8 mm full width at half maximum (FWHM).

Intra-scanner EEG was reviewed after removing scanner and pulse-related artifacts using Brain Vision Analyzer 2 (Brain Products GmbH, Germany) (Allen et al., 1998; Allen et al., 2000) as implemented in Brain Vision Analyzer 2.0. IEDs on intra-scanner EEG were visually marked by experienced neurophysiologists and modeled as single zero-duration events regardless of the duration. The zero-duration stick functions marking the onset of each IED were convolved with the canonical hemodynamic response function (HRF) and used as a condition in a GLM to find the associated BOLD responses. One patient had ictal activity during EEG-fMRI: this activity was also modeled to account for the maximum amount of variance but the results will not be discussed here. In all patients who had IED during EEG-fMRI these were exclusively in the temporal regions. In cases with bilateral temporal independent IEDs these were included in the design matrix as separate regressors. BOLD maps resulting from the temporal IEDs ipsilateral to the surgical resection were considered in this analysis.

The following confounding effects were modeled: Motion-related effects were corrected using 24 regressors (six scan realignment parameters and the Voltera expansion of these) (Friston et al., 1996). fMRI signal effects associated with large motion events (defined as inter-scan movement higher than 0.2mm) were specifically accounted for (Lemieux et al., 2007). For four patients, large motion events were defined as 0.5mm due to model estimability problems at 0.2mm. fMRI signal variations associated with the cardiac pulse were modeled (Liston et al., 2006). To avoid the contamination of physiological BOLD, EEGs were visually inspected and movement or eye blink artifacts were marked.

The time and duration of these markers were convolved with the HRF and added as a regressor in the GLM (Chaudhary et al., 2012).

Long-term video-EEG monitoring and topographic maps

In addition to the BOLD signal changes associated with IED recorded during EEG-fMRI as described in the previous section, we used the Grouiller approach to map BOLD signal variations correlated with the projection of the intra-scanner EEG onto topographic maps derived from IED recorded on the patients' EEG recorded during long-term video-EEG monitoring (Grouiller et al, 2011). The idea of this approach is to attempt to account for BOLD signals associated with weak, visually undetected, epileptic discharges during EEG-fMRI. All patients underwent EEG exams according to 10-10 system, with 18 to 256 electrodes. To calculate the Grouiller model, the clinical EEG of each patient was used to build a topographic map of the most clinically relevant IED. EEGs were reviewed by experienced neurophysiologists and right or left temporal IED were marked. Corresponding IEDs were averaged and corresponding topographic maps calculated using the software *Cartool* (Brunet et al., 2006). The artifact corrected intra-scanner EEG was interpolated to match the number of electrodes used for the long-term video-EEG monitoring and a band pass filter 1-30Hz applied. The correlation between the topographic maps was calculated for each intra-scannerEEG time point, then squared and convolved with the canonical HRF, giving the Grouiller predictor of BOLD changes.

For patients with IED during EEG-fMRI, both the intra-scanner IED and Grouiller regressors were included in the GLM as effects of interest; for those with no IED during EEG-fMRI, only the Grouiller effect was included as an effect of interest.

Statistical analysis

The statistical analyses were performed using the software *SPM8*. For patients with IED during EEG-fMRI scanner an F-contrast was calculated across the IED and Grouiller effects; for those with no IED during EEG-fMRI, an F-contrast over the Grouiller regressor was used. A threshold of $p<0.05$ (FWE corrected) was applied to the SPM{F}-maps; in cases when this resulted in null maps, the data was further explored by applying a lower statistical threshold of $p<0.001$ (uncorrected for multiple comparisons).

Postoperative MRIs

To assess the spatial relationship between the BOLD changes and area of surgical resection in a given patient, the former was co-registered with the patient's postoperative MRI.

EEG-fMRI BOLD concordance

We classified cases according to the relationship between the IED-related BOLD maps and two independent methods of localization: laterality of the ictal onset zone (IOZ) (lobar concordance) and area of resected tissue (sublobar concordance). Degree of concordance with surgical resection: 1) lobar concordance: BOLD located in the temporal lobe (TL) ipsilateral to the surgical resection; 2) Sub-lobar concordance: BOLD in the TL ipsilateral to the surgical resection and this BOLD area resected in the postoperative MRI. For each of these two approaches, BOLD maps were considered: Concordant if the statistical global maxima abnormality was located in the TL ipsilateral to the resected area (*Con*) or in the brain area removed during surgical procedure (*Con-resected*); Some Concordance (*SCon or SCon-resected*) maps were defined as a significant cluster detected in these areas although the global maxima abnormality was located in a different region;

Discordant (*Dis or Dis-resected*) maps were defined as no significant cluster in these defined regions.

Results

On MRI, 13/30 patients had signs of hippocampal sclerosis (HS), 6 had TL tumors, 2 had signs of focal cortical dysplasia (FCD) and 9 had normal MRI (Table 1).

The mean follow-up time after surgery was 22.4 months (range 4-53). In the last follow-up thirteen patients were classified as ILAE outcome classification 1 (ILAE-1); two as ILAE outcome classification 2 (ILAE-2); two as ILAE outcome classification 3 (ILAE-3); eight as ILAE outcome classification 4 (ILAE-4) and five as ILAE outcome classification 5 (ILAE-5). Clinical data is summarized on Table 1.

Fifteen patients had IEDs (mean: 75.2; range: 3-532) in the intra-scanner EEG. Fourteen individuals had bi-temporal discharges.

In one patient there was no significant BOLD change associated with the IED ipsilateral to the seizure onset zone. In the remaining 29 patients, a BOLD abnormality was revealed for each type of temporal IED.

EEG-fMRI *vs* surgical outcome

1) Lobar concordance:

Regarding the presence of BOLD abnormalities in the ipsilateral TL, four patients were classified as *Con*, 12 as *SCon* and 13 as *Dis*. 75% of patients with *Con* results had good surgical outcomes: two had outcome ILAE-1, one had ILAE-3 and one had ILAE-4. 75% of patients with *SCon* results had good surgical outcomes: six had ILAE-1, two had ILAE-2, one had ILAE-3 and three had ILAE-4. 38% of patients with *Dis* results had good surgical outcomes: five had ILAE-1, four had ILAE-4, and four had ILAE-5 (Table 2).

1.1) Lobar concordance and etiology

Two patients with HS were classified as *Con* (one ILAE-3 and one ILAE-4), seven as *SCon* (two outcome ILAE-1, two ILAE-2, one ILAE-3, two ILAE-4) and four as *Dis* (one outcome ILAE-1 and 3 ILAE-4). Patients with cryptogenic TLE had BOLD abnormalities classified as follows: two *Con* (both with ILAE-1), 1 *SCon* (ILAE-4), 5 Dis (one outcome ILAE-1, one ILAE-4, three ILAE-5). Both patients with FCD had *Dis* BOLD results (one ILAE-1 and one ILAE-5). Four patients with tumor were classified as *SCon* and two as *Dis* and all of them had ILAE-1 (Table 2).

1.2) Lobar concordance and presence of default mode network (DMN) component

Sixteen patients (55%) had a significant BOLD change in the posterior component of the DMN (precuneus or posterior cingulate cortex (PC/PCC)). Twelve of those (75%) had good surgical outcome (ten outcome 1, two ILAE-2 and four had ILAE-4), as compared to 5/13 (38%) patients who did not have significant BOLD changes in the posterior component of DMN and had a good surgical outcome (three outcome ILAE-1, two ILAE-3, four ILAE-4 and four ILAE-5). Patients with posterior DMN component had significantly higher chance of good surgical outcome (Chi-square test, p=0.02). DMN was specially frequent in the precuneus ipsilateral to the IED.

There was no difference in the distribution of etiologies in the groups of patients with or without BOLD abnormality in the DMN. In the group of patients with DMN component, 62% had visual IEDs during EEG-fMRI while 38% of patients without DMN component had IEDs. However, if we consider only the results of patients with IEDs during EEG-fMRI, the proportion of good or poor surgical outcome related to the presence or not of the DMN component remains similar (80% of good outcome for patients with

DMN and only 40% of good outcome for those without DMN). The number of IEDs during EEG-fMRI in thses two groups was not significantly different (t-test: p=0.15)

There was also no difference of the duration of follow-up between the groups with or without DMN component (t-test: p=0.67). There was no difference of age of epilepsy onset or epilepsy duration between the groups with or without DMN component (t-test: p=0.18 and p=0.65).

2) Sub-lobar concordance

Two of the 3 cases classified as *Con-removed* had a good surgical outcome: one had outcome ILAE-1, one ILAE-3 and one ILAE-4. All 6 patients classified as *SCon-removed* had good surgical outcome (four ILAE-1, one ILAE-2 and one ILAE-3). 45% of the remaining 20 patients in whom no BOLD cluster overlapped with the resection had good surgical outcome (eight outcome ILAE-1, one ILAE-2, seven ILAE-4 and four ILAE-5) (Table 2).

Discussion

In this study we demonstrated that EEG-fMRI can be a reliable tool in the pre-surgical evaluation of patients with TLE. This study benefitted from a major methodological advance which made it possible to evaluate all individuals submitted to the technique even in the absence of IED during the scan (Grouiller et al, 2011). We were able to show in patients with TLE that

- The presence of a significant BOLD abnormality in the ipsilateral TL detected by EEG-fMRI is consistent with a better surgical outcome.

- Moreover, we demonstrated for the first time that the involvement of the DMN is also related to a better surgical outcome.

EEG-fMRI is a non-invasive technique that allows the mapping of BOLD signal changes related to epileptic discharges (Lemieux et al., 2001). Previous reports have shown its importance in the definition of the seizure onset zone in patients with focal refractory epilepsies (Salek-Haddadi et al., 2006; Moeller et al., 2009; Thornton et al., 2011). Moreover, EEG-fMRI has helped the understanding of network abnormalities in different types of epilepsies (Laufs 2007, Fahoum 2012, Laufs 2012).

In our study we evaluated the possible contribution of EEG-fMRI for predicting surgical outcome in patients with TLE. TLE is the most common form of epilepsy in adults and individuals are frequently refractory to AED (Semah et al., 1998), and surgical treatment remains the best approach to seizure control. However, the prognosis of seizure control after surgery is heterogeneous with a high number of patients presenting seizure recurrence after a period of long follow-up (deTisi et al., 2011). The etiology of the epilepsy is the major factor influencing the surgical prognosis but even among patients with TLE with HS only 60-70% will remain seizure-free after the procedure (Berkovic et al., 1995; deTisi et al., 2011). Longer epilepsy duration may influence surgical outcome in TLE with HS but the other factors influencing the surgical outcome in patients with TLE with HS or other etiologies are not fully understood (Janszky et al., 2005).

We demonstrated that the presence of BOLD abnormalities detected by EEG-fMRI in the TL ipsilateral to the defined SOZ can predict a good surgical outcome in patients with TLE. Previous studies had similar results in smaller groups of heterogeneous

refractory patients (Thornton et al., 2010; Zijlmans et al., 2007) or in patients with FCD (Thornton et al., 2011). Although etiology is the main predictor of surgical outcome in TLE, there was no difference in the surgical outcome *vs* BOLD concordance in respect to the etiology.

In different EEG-fMRI studies, up to 50% of patients are excluded from the final analysis for not presenting relevant BOLD abnormalities. In the present study we had the opportunity of comparing the concordance of EEG-fMRI results with an extended follow-up in a group of homogenous epileptic syndrome. The clearly higher proportion of concordant EEG-fMRI studies among individuals with good surgical outcome corroborates the hypothesis that BOLD abnormalities outside the seizure onset area are not exclusively related to methodological issues and may be related to network abnormalities.

We also observed that the presence of interictal BOLD changes in the posterior component of DMN (precuneus and posterior cingulate cortex - PC/PCC) is strongly associated with a good surgical outcome in patients with TLE. It has been proposed that the PC/PCC may play a central role in how intrinsic activity is mediated throughout the DMN (Fransson et al., 2008) and by PET finding of resting-state elevated metabolic activity in the PC/PCC compared to all other regions (Raichle et al.,2001; Gusnard &Raichle, 2001). The suppression of areas compatible with DMN related to IEDs have been consistently emphasized in studies with EEG-fMRI and different epilepsy syndromes (Gotman et al., 2005; Laufs et al., 2006; Fahoum et al., 2012), however the significance and importance of these findings are not fully understood. It has been proposed that it may reflect subtle abnormalities of awareness during IEDs (Gotman et al., 2005). Similarly, DMN is suppressed during directed attention and tasks (Greicius & Menon, 2004; Singh & Fawcett,

2008). Previous studies of patients with refractory TLE using fMRI have also revealed abnormalities of the resting state networks at the group level (Zhang et al., 2010). We hypothesized that IEDs may act to disrupt the processes that sustain "mind wondering" and reflected in a suppression of DMN areas. In that sense, the presence of the DMN component could imply integrity of normal brain networks. Patients with epilepsy who do not have detectable DMN component during IED may have more diffuse abnormal brain networks reflecting more widespread disease consisting in worse surgical outcome. This finding may have important implications for surgical management of patients with drug-resistant TLE, with the possibility of more appropriate choice of the patients who will be submitted to the procedure or not. Moreover, it suggests that these abnormalities are somehow related to the maintenance of the functionality of the epileptic network even after the removal of the defined epileptogenic zone.

In our patients, the presence of a significant BOLD abnormality in any part of the temporal lobe ipsilateral to the surgical resection was associated with good surgical outcome irrespective of whether the region of BOLD change was resected. Previous publications have demonstrated a better surgical outcome in patients with FCD in which the BOLD cluster was resected (Thornton et al., 2011). In our data, only two patients had FCD. The majority of our patients had epilepsy related to HS or tumor and some had cryptogenic TLE. In these patients the complete removal of the BOLD associated with the IEDs is not imperative for a good surgical outcome, probably because in TLE it is just necessary to disrupt the epileptic network for a good seizure control (Arruda et al., 1996). Furthermore, due to various factors linked to the sensitivity of fMRI the lack of significant BOLD does not mean lack of involvement in process, and therefore it is likely that haemodynamic

changes linked to interictal epileptic activity extended beyond the observed clusters. In FCD the complete resection of the BOLD abnormality related to the IED is associated to a better surgical outcome probably reflecting the fact that complete removal of the lesion is the most important definer of good outcome (Krsek et al., 2009; Kim et al., 2009).

In EEG-fMRI studies of epileptic activity, significant BOLD abnormalities are frequently observed outside the defined SOZ and the meaning of these diffuse clusters are not fully understood. One may argue that these spread abnormalities are false positive findings due to artefacts. However, in recent years different approaches have been proposed to eliminate false results, including corrections for movements, cardiac artefacts and physiological noise (Liston et al., 2006; Lemieux et al., 1007; Chaudhary et al., 2012). Moreover, we could demonstrate here, in agreement with previous articles (Thornton et al., 2011) that the occurrence of BOLD abnormalities outside the expected SOZ and in areas compatible with DMN may reflect pathological mechanisms once these findings are clearly associated to surgical outcome in TLE.

In conclusion, EEG-fMRI is a non-invasive tool that may add information in the pre-surgical evaluation of individuals with refractory TLE. Patients with EEG-fMRI results concordant with the defined SOZ and with hemodynamic abnormalities detected in the areas of DMN have a better surgical outcome.

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Table 1: Clinical data of TLE patients submitted to EEG-fMRI exams acquired previous to surgical treatment due to refractory seizures.

	Gender	Age (years)	Age of epilepsy onset (years)	Etiology	Surgery Localization	Follow-up after surgery (months)	ILAE surgical outcome
1	F	29	15	HS	LT resection	44	4
2	M	18	13	Crypt	Taylored LT resection	15	1
3	M	33	5	HS	LT resection	43	3
4	M	20	10	Crypt	LT resection	5	1
5	M	24	19	FCD	RT resection	23	5
6	F	31	6	Crypt	LT resection	36	5
7	M	50	1	HS	LT resection	6	3
8	M	13	10	Tumor	LT resection	15	1
9	F	42	28	HS	RT resection	6	4
10	M	28	10	Crypt	RT resection	26	4
11	F	47	4	HS	RT resection	4	1
12	M	20	10	Tumor	RT resection	28	1
13	M	48	33	Tumor	LT resection	15	1
14	F	50	16	HS	RT resection	17	2
15	F	18	7	Tumor	RT resection	26	1
16	F	36	5	HS	LT resection	28	4
17	M	48	7	HS	LT resection	6	1
18	F	51	4	HS	RT resection	4	2
19	F	26	21	Crypt	LT resection	21	5
20	F	19	17	Tumor	LT cyst resection	14	1
21	F	28	3	Crypt	Taylored LT	36	1
22	F	23	14	Crypt	LT tailored	42	5
23	M	30	12	HS	RT resection	53	4
24	F	24	15	Crypt	LT resection	50	4
25	F	46	17	HS	RT resection	14	4
26	M	21	17	HS	LT resection	10	4
27	F	46	3	HS	LT resection	15	1
28	F	27	27	Tumor	RT resection	16	1
29	M	33	10	FCD	RT resection	6	1
30	F	31	24	Crypt	Taylored LT resection	36	5

F: female; M: male; HS: hippocampal sclerosis; Crypt: cryptogenic; FCD: focal cortical dysplasia; LT: left temporal; RT: right temporal.

Table 2

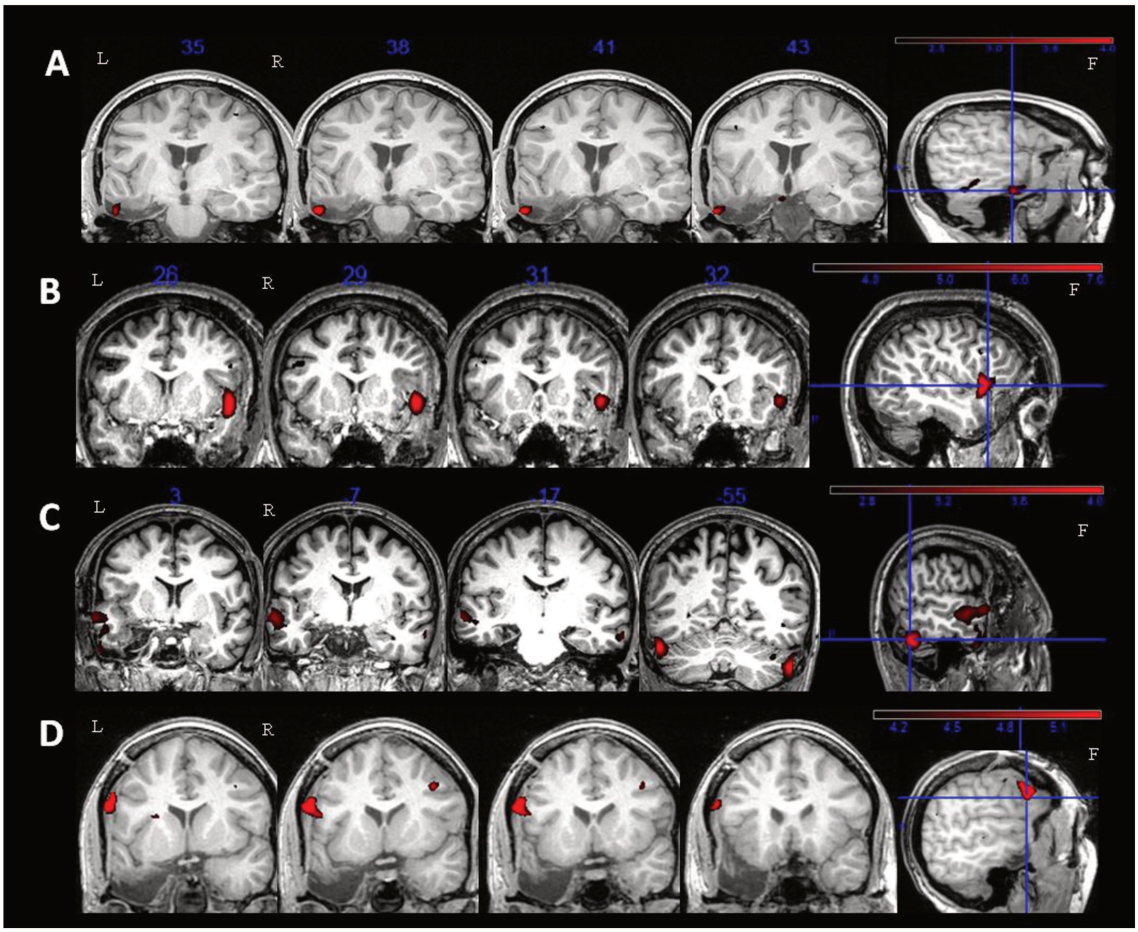
	Intra-scannerEEG marker	BOLD response GMax	Temporal ipsilateral cluster	DMN	Number of intra-scannerIEDs	Lobar concordance	Sublobar concordance
1	LT	LT (FWE)	Y	N	34	Con	Con
2	LT	LT (FWE)	Y	Y	152	Con	Con
3	LT	LT	Y	N	23	Con	Con
4	LT	LT	Y	Y	147	Con	Dis
5	LT	R Cerebelum (FWE)	Y	N	17	SCon	SCon
6	LT	L Anterior Cingulate (FWE)	Y	Y	532	SCon	SCon
7	RT	R Insula	Y	N	1	SCon	Dis
8	RT	R Posterior Cingulate	Y	Y	no IED	SCon	Dis
9	RT	LT	Y	Y	39	SCon	Dis
10	RT	R Frontal (FWE)	Y	Y	89	SCon	Dis
11	LT	R Frontal (FWE)	Y	Y	21	SCon	SCon
12	RT	L Thalamus	Y	Y	no IED	SCon	SCon
13	RT	L Anterior Cingulate	Y	N	no IED	SCon	SCon
14	LT	L Parietal	Y	Y	3	SCon	Dis
15	LT	R Cerebelum (FWE)	Y	N	no IED	SCon	SCon
16	RT	L Cerebelum	Y	Y	15	SCon	Dis
17	LT	R Frontal	N	N	no IED	Dis	Dis
18	LT	L Brain Stem	N	N	no IED	Dis	Dis
19	LT	L Parietal	N	Y	11	Dis	Dis
20	LT	R Cerebelum	N	N	30	Dis	Dis
21	RT	L Frontal	N	N	no IED	Dis	Dis
22	LT	R Parietal	N	Y	no IED	Dis	Dis
23	LT	L Parietal	N	Y	5	Dis	Dis
24	LT	R Frontal	N	N	no IED	Dis	Dis
25	LT	L Frontal	N	Y	no IED	Dis	Dis
26	RT	L Frontal	N	Y	no IED	Dis	Dis
27	RT	R Parietal	N	Y	no IED	Dis	Dis
28	RT	R Occipital	N	N	no IED	Dis	Dis
29	LT	L Frontal	N	N	no IED	Dis	Dis
30	LT	(no BOLD)	N	(no BOLD	no IED	(no BOLD)	(no BOLD)

BOLD: blood oxygen level dependent; DMN: default mode network; IEDs: interictal epileptiform discharges; FWE: LT: left temporal; RT: right temporal; FWE: family-wise error; Y: yes; N: no; Con: concordant; SCon: some concordance; Dis: discordant.

Figure 1: Results of EEG-fMRI in four different patients. The descriptions of the results are on the right side of each image. The crosshair at the sagittal images indicates the GM. (F-test, $p<0.001$, uncorrected).

A: Patient with *Concordant* result. The BOLD cluster in the TL ipsilateral to the spikes was completely removed in the post-op MRI. Surgical outcome ILAE class 3 (GLM model: 23 LT spikes + TM); B: Patient with *Concordant* result. The BOLD cluster in the TL ipsilateral to the spikes was not removed in the post-op MRI. Surgical outcome ILAE class 1 (GLM model: No intra-MRI spikes/ TM); C: Patient with *Some Concordance* result. The BOLD cluster in the TL ipsilateral to the spikes was partially removed in the post-op MRI. Surgical outcome ILAE class 3 (GLM model: 17 LT spikes + TM); D: Patient with *Discordant* result. IcEEG had showed two ictal onset zones: one in the LT and other in the LF lobe (and this was concordant with the BOLD cluster). Surgical outcome ILAE class 5. (GLM model: No intra-MRI spikes/ TM).

L: left side; R: right side; GM: global statistical maximum; TL: temporal lobe; FL: frontal lobe; TM: topographic map; IcEEG: intra-cranial EEG.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CAPÍTULO 10

Epilepsy as progressive disorders: what is the evidence that can guide our clinical decisions and how can neuroimaging help?

Ana C. Coan; Fernando Cendes.

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Review

Epilepsy as progressive disorders: What is the evidence that can guide our clinical decisions and how can neuroimaging help?☆

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ABSTRACT

There is evidence that some types of epilepsy progress over time, and an important part of this knowledge has derived from neuroimaging studies. Different authors have demonstrated structural damage more pronounced in individuals with a longer duration of epilepsy, and others have been able to quantify this progression over time. However, others have failed to demonstrate progression possibly due to the heterogeneity of individuals evaluated. Currently, temporal lobe epilepsy associated with hippocampal sclerosis is regarded as a progressive disorder. Conversely, for other types of epilepsy, the evidence is not so clear. The causes of this damage progression are also unknown although there is consistent evidence that seizure is one of the mechanisms. The conflicting data about epilepsy progression can be a challenge for clinical decisions for an individual patient. Studies with homogenous groups and longer follow-up are necessary for appropriate conclusions about the real burden of damage progression in epilepsies, and neuroimaging will be essential in this context.

This article is part of a Special Issue entitled "The Future of Translational Epilepsy Research".

1. Current evidence of epilepsy progression

"Seizures beget seizures" has been a famous citation by William Gowers [1] for more than 100 years. It brings the implicit concept that epilepsy may be a progressive disorder and, more specifically, related to the occurrence of seizures. However, as clinical neurologists, how does it affect (or how should it affect) our decisions facing a specific patient with epilepsy?

Epilepsy progression can be verified or described as the worsening of seizure control, cognition, behavior, structural abnormalities, and EEG patterns as well as social interactions over time. Overall, it is not possible to say that all types of epilepsy are progressive conditions [2,3]. While some epilepsy syndromes are clearly progressive, others do not appear to progress over time, and yet it is unclear if in some localization-related epilepsies, the progression of damage depends on the underlying etiology, seizure type (i.e., presence or not of secondary generalized seizures), duration and frequency of seizures, other environmental factors (e.g., viral infections and head trauma), or a combination of several of these factors. For example, prolonged focal seizures, prolonged generalized seizures, isolated or clusters of brief seizures, or seizures with a longer seizure-free interval may have distinct effects on brain integrity.

The controversy begins in studies about the natural history of epilepsies. Some of these show worse prognosis of seizure control associated with the number of seizures prior to treatment and a tendency to progressive reduction of seizure-free intervals in populations without treatment [4–7]. However, other authors disagree with these results emphasizing that this tendency of worsening over time may be related to an inherent severity of the disease in these individuals [8,9]. Similarly, community-based studies of patients with several years of delay before starting antiepileptic drug (AED) therapy show similar patterns of response than studies with newly diagnosed epilepsies [10,11].

Studies conducted in tertiary centers with patients with drug-refractory seizures have diverse results. In this context, experimental, neuroimaging, EEG, and clinical studies have consistently pointed towards a tendency of progression among the years in some types of epilepsy, especially drug-refractory temporal lobe epilepsy (TLE) associated with hippocampal sclerosis (HS) (TLE–HS), which can be identified in vivo by MRI (Fig. 1) [12–15]. Studies of TLE have shown clinical [7], cognitive [16,17], electroencephalographic [18,19], and neuroimaging evidence of progressive damage [12–15]. However, lack of evidence exists in other types of focal or generalized epilepsies. Moreover, the knowledge of what contributes to this progression, even in TLE, is not well understood.

Some studies show an important role of seizures in this progressive damage [14,15] while others do not [20]. For example, some experimental data suggest that the recurrence of seizures may be responsible

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[1] Rising star.

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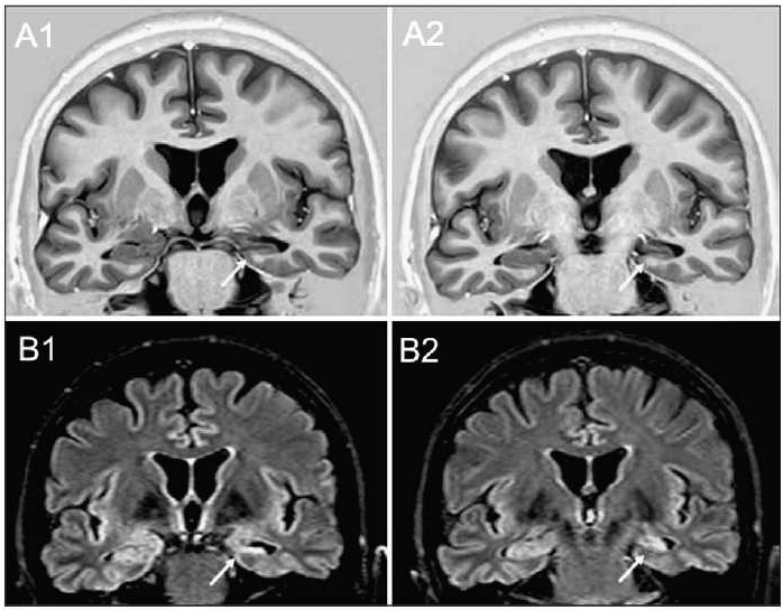

Fig. 1. 3-Tesla MRI coronal T1-weighted inversion recovery (A1 and A2) and T2-FLAIR (fluid-attenuated inversion recovery) images (B1 and B2) from a patient with left mesial TLE showing signs of left hippocampal sclerosis (arrows): hippocampal atrophy with abnormal shape and hyperintense signal on T2-FLAIR images.

for the progressive neuronal damage [21], and some histopathological evidence in patients with epilepsy correlates the occurrence of drug-refractory seizures with lower hippocampal neuronal density [22]. Conversely, a post-mortem study showed that not all patients with drug-refractory seizures have significant hippocampal neuronal loss [23].

Recently, important data about the role of uncontrolled inflammation in the progress of epilepsy damage have emerged [24]. In vitro and in vivo data support the idea that inflammation may play a role in the epileptogenic process. In addition, it is possible that uncontrolled inflammation may be implicated in the chronic epileptic process of TLE–HS [24,25], thus, adding additional neuronal damage over years.

In this review, we will focus on neuroimaging evidence of progressive damage in epilepsy. Although the gold standard for brain damage related to progression of epilepsy is the pathological examination, MRI – especially the newer scans with larger fields of 3 T to 7 T – can provide an in vivo analysis that can approach what is seen in histopathology. We will focus on focal symptomatic/cryptogenic and idiopathic generalized epilepsies, while clearly progressive disorders (e.g., progressive myoclonic epilepsies or West syndrome) will not be discussed. We will specifically try to emphasize the evidence that is already relevant for clinical practice. Moreover, we will try to point out the gaps and the perspectives and look ahead to the knowledge that is likely to emerge in the next years and what relevance it may have in our daily actions as clinical neurologists. Special attention will be given to the important role that neuroimaging techniques may play in filling these gaps.

2. Neuroimaging evidence of epilepsy progression

Magnetic resonance imaging is the most important neuroimaging tool for the evaluation of epilepsies. It can image the structural substrate responsible for the epileptic process [26] and reveal signs of HS, malformations of cortical development, tumors, or other underlying conditions. It is particularly sensitive for detecting signs of HS in TLE patients, which can be seen as a reduction of volume, loss of hippocampal internal structure, and increased hippocampal T2 signal [27,28]. In the last two decades, with the emergence of more sophisticated scans and quantitative analysis, the literature about progressive epilepsy damage and MRI techniques has grown. For other neuroimaging techniques, such as PET or SPECT, the literature regarding the progressive damage of epilepsies is not so vast.

2.1. Cross-sectional neuroimaging evidence of epilepsy progression

2.1.1. MRI manual and automatic volume measures

Manual volumetry has been repeatedly used to evaluate structural progression in epilepsy (Fig. 2). In the early 1990s, an MRI volumetric study composed predominantly of patients with drug-refractory TLE (but which also included extra-temporal epilepsies) did not observe a relation of repeated seizures or longer duration of epilepsy with the degree of atrophy of the amygdala and hippocampus [29]. Conversely, more homogeneous studies including only individuals with TLE demonstrated that hippocampal [30,31] and amygdala [31] volumes were negatively correlated with the duration of epilepsy. Although the majority of published papers include TLE–HS patients, a study with cryptogenic TLE [14] also demonstrated that those with the epileptic focus in the left hippocampus had the ipsilateral hippocampal volume inversely correlated with the estimated total number of partial or generalized seizures.

Manual and automatic volumetric and signal quantification techniques have helped to determine that the structural damage of epilepsies extends beyond the epileptic focus, and authors have also tried to correlate this diffuse damage with the occurrence of seizures or duration of the disease. Semi-automated volumetric study of unilateral TLE patients demonstrated that duration of epilepsy was associated with reduced ipsilateral hippocampal volume and bilateral extra-temporal (frontal and parietal) white matter volume [32]. Similarly, childhood onset (but not adult onset) TLE has been associated with significantly reduced total brain tissue [33]. Another study, with refined cortical analysis, demonstrated that neocortical atrophy

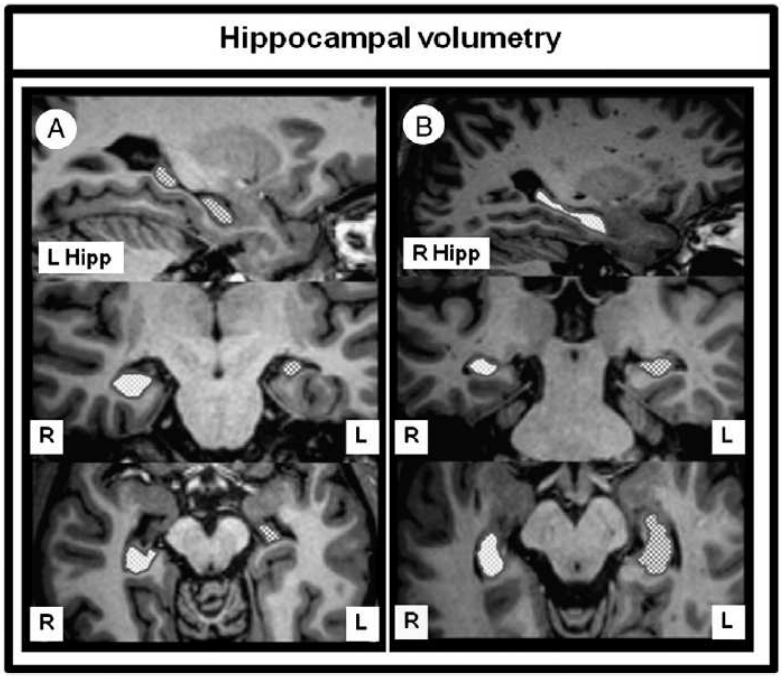

Fig. 2. Manual volumetry of hippocampi of two patients with temporal lobe epilepsy. Image A shows the quantification of volume in a patient with visually detected left hippocampal atrophy. Image B shows reduction of volume in the right hippocampus, which was not detected by visual analysis. The quantification of hippocampal and other brain structure volumes can help to evaluate progression of structural damage in patients with epilepsy.

in mesial and supero-lateral frontal and parietal cortices in TLE is correlated with longer epilepsy duration [34]. However, other studies have failed to demonstrate that structural damage outside the presumed epileptogenic zone can be progressive. For example, Moran et al. [35] found significant atrophy of mesial and lateral temporal lobe substructures, but there was no correlation between the degree of atrophy in the extra-hippocampal temporal lobe structures and the duration of epilepsy or occurrence of generalized seizures.

Other automatic MRI quantitative analyses, such as voxel-based morphometry (VBM), also helped to address the question of brain damage progression. Voxel-based morphometry is a technique that allows the MRI evaluation of gray and white matter concentrations or volumes through an automatic analysis of the whole brain [36]. A VBM analysis of unilateral TLE patients showed that only extra-temporal (bilateral thalamic, prefrontal, and cerebellar) but not mesial temporal gray matter concentration was related to duration or age of onset of epilepsy [37]. Similarly, another VBM study [38] demonstrated that gray matter concentration in the ipsilateral hippocampus, temporal lobes, and extra-temporal limbic structures in patients with MTLE is negatively correlated with the duration of epilepsy.

The data about epilepsy progression for other focal or idiopathic generalized epilepsies (IGE) are scarce. In IGE, while some studies have failed to demonstrate thalamic atrophy in small groups of patients [39–41], a more recent study [42] including a larger number of IGE individuals who presented only generalized tonic-clonic seizures demonstrated bilateral thalamic atrophy. Moreover, the authors observed that the thalamic volumes and the fronto-central and limbic cortices were negatively correlated with duration of epilepsy, and this progression occurred even faster in patients with poorer seizure control. These results emphasize the concept that different factors may influence structural progressive damage in individuals with epilepsies, and the homogeneity of the patients included in the analysis may contribute to a better understanding of these issues.

2.1.2. Other neuroimaging techniques

2.1.2.1. MRI T2 relaxometry. Magnetic resonance imaging T2 relaxometry is a technique that allows the quantification of signal intensity in MRI scans (Fig. 3) and can help to improve the detection of structural brain pathologies such as HS [43]. There are contradictory data about T2 relaxometry and epilepsy progression. Kalviainen et al. [14] demonstrated that patients with cryptogenic TLE and the epileptic focus in the left hippocampus had a T2 relaxation time that positively correlated with the estimated total number of partial or generalized seizures. Conversely, Grünewald et al. [44] could not observe any correlation of hippocampal T2 relaxation times with seizure frequency or duration of epilepsy in individuals with partial epilepsy.

In a group of 43 patients with drug-refractory TLE and MRI signs of HS, we did not find a significant relationship between frequency and the duration of seizures and hippocampal T2 relaxometry (unpublished data).

2.1.2.2. MRI spectroscopy. Magnetic resonance imaging spectroscopy allows the observation of cerebral metabolites, and studies in epilepsy have shown that it can help to demonstrate neuronal damage that correlates with clinical–electroencephalographic lateralization in TLE [45]. Some cross-sectional studies using MRI spectroscopy have demonstrated correlation of seizure frequency [46] and epilepsy duration [30] with a reduction in the *N*-acetylaspartate-to-creatine ratio (NAA/Cr) in patients with medically intractable TLE. Conversely, other studies have failed to demonstrate this correlation [47].

2.1.2.3. PET. Positron emission tomography provides images of different biochemical functions in the human brain, and abnormal glucose metabolism has been associated with the epileptic focus [26]. Gaillard et al. [48] demonstrated that abnormalities of glucose utilization seen in [^{18}F]-fluorodeoxyglucose (^{18}FDG)-PET are less common in children

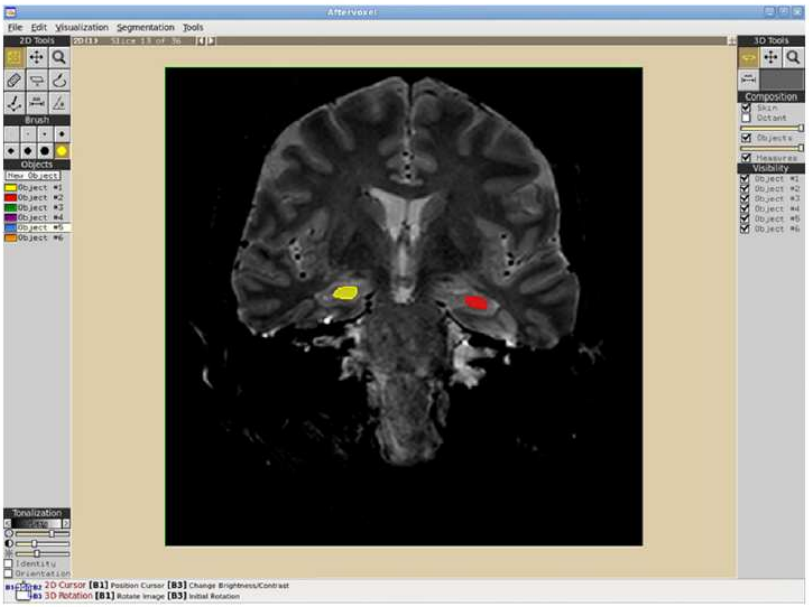

Fig. 3. Screenshot from the program *Aftervoxel* (http://www.liv.ic.unicamp.br/~bergo/aftervoxel) used to measure T2 signal (relaxometry), showing a coronal T2-weighted image with regions of interest (ROIs) painted on each hippocampus. A T2 map is obtained from a T2 multi-echo sequence (in this case, six echoes). The program calculates the signal intensity of the ROIs that are chosen by the operator: in this example one ROI is placed in the left (red) and another in the right (yellow) hippocampus. It is also possible to obtain several ROIs in the same structure (for example, ROIs along the hippocampus) and calculate the average signal from ROIs in each structure. Different from hippocampal volumetry, the ROIs here do not need to cover the entire structure, and its shape is not important because we are obtaining an average of signal intensity. It is important, however, to avoid contamination of surrounding tissues.

with new-onset partial seizures than in adults with chronic partial epilepsy. Similar results have been found by Theodore et al. [49], who demonstrated with PET exams that longer duration of epilepsy in TLE is associated with greater hypometabolism in the hippocampus ipsilateral to the epileptic focus.

2.2. Longitudinal neuroimaging evidence of epilepsy progression

To evaluate the evolution of a disease, longitudinal studies are more appropriate than cross-sectional analysis, and extensive data with neuroimaging follow-up have been published in the last decades. However, most of the studies have a short follow-up period, usually less than 5 years.

2.2.1. MRI manual and automatic volume measures

Longitudinal studies including community-based or newly diagnosed epilepsies (which are invariably composed of highly heterogeneous individuals) show that epilepsy, as a single group, is not consistently a progressive disorder. For example, Van Paesschen et al. [50] evaluated patients with newly diagnosed partial epilepsy with MRI scans at a one-year interval and demonstrated subtle changes in only three individuals. Similarly, a study that followed adult patients with newly diagnosed seizures with two MRIs 3.5 years apart showed that quantitative analysis of the hippocampus, cerebellum, and total brain volume did not differ between patients and controls or patients with or without further seizures [2]. Recently, the same group [51] found a significant difference in neocortical volume loss between controls and patients with chronic epilepsies in MRIs 3.5 years apart but not between controls and newly diagnosed epilepsies. However, this increased risk of cerebral atrophy was not related to a history of seizures.

In more homogenous groups including only TLE patients, usually associated with HS, the results of longitudinal quantitative MRI analyses with manual or automated techniques point towards a slow but consistent structural progression. This progression is seen not only in the mesial temporal region but also in extra-temporal areas from the early phases of the disease. For example, Briellmann et al. [12] showed in a group of newly diagnosed TLE patients an ipsilateral hippocampal volume decrease of 9% over a mean period of 3.5 years. They also verified that the hippocampal volume loss was correlated to the number of generalized seizures between the scans. A comparable result was demonstrated by Fuerst et al. [13]. In this study, in which patients with TLE and unilateral hippocampal sclerosis had repeat volumetric magnetic resonance imaging scans with a mean of 3.4-year interval, seizure-free patients showed no change in hippocampal volume, while those with continuing seizures had a decline in ipsilateral hippocampal volume that correlated with seizure frequency.

Results of longitudinal studies were similar to cross-sectional analysis regarding progressive abnormalities distant from the epileptic focus. In the study from Liu et al. [51], the ongoing cerebral atrophy observed was widespread and remote from the epileptic focus. Also, Bernhardt et al. [34] evaluated neocortical atrophy in TLE in a longitudinal analysis with a mean of 2.5 years of follow-up and detected progression of cortical atrophy in the ipsilateral temporopolar and central areas, and contralateral orbitofrontal, insular, and angular regions. In a VBM analysis of TLE patients performed in our center [15], we observed progressive gray and white matter reduction not only in the ipsilateral mesial temporal region but also in the ipsi- and contra-lateral neocortical areas of temporal, frontal, and occipital regions. This progressive damage was associated with poorer seizure control and a longer duration of epilepsy.

2.2.2. Other neuroimaging techniques

2.2.2.1. MRI T2 relaxometry. A study with patients with chronic focal and generalized epilepsies failed to demonstrate quantitative changes of hippocampal T2 relaxation times after a follow-up period of 115–331 days [44].

2.2.2.2. PET. One study with PET demonstrated dynamic changes of cortical glucose hypometabolism in children with intractable non-lesional partial epilepsy, and these changes were related to the frequency of seizures [52].

2.3. The role of seizures and other clinical evidence of epilepsy progression

In humans, there are numerous descriptions of *status epilepticus* (SE) leading to neuronal changes, especially in the hippocampus [53,54]. There is extensive MRI evidence that febrile and afebrile SE can damage the brain and that the mesial structures are especially vulnerable to the hypoxia that occurs in association with status epilepticus [55]. In contrast, for brief recurrent seizures, the relation with brain damage is not so clear. Despite the evidence of progressive structural damage in some types of epilepsy, the causative relation of this with sporadic or refractory seizures is still controversial [56].

A few case reports have described clear progressive hippocampal damage that could be secondary to brief and sporadic generalized or focal seizures. O'Brien et al. [57] reported a case of long-standing intractable complex partial and secondary generalized seizures whose MRI scans 4 years apart documented a progressive decrease in the left hippocampal volume. Briellman et al. [58] described a patient who developed reduced hippocampal volume and increased hippocampal T2 signal after six generalized tonic-clonic seizures (GTCS). Worrell et al. [59] described MRI signs of hippocampal atrophy that developed in an adult after the occurrence of new-onset partial seizures and only one brief GTCS from acute venous thrombosis.

One major problem of determining whether seizures cause progressive damage is that almost invariably, the researchers can rely only on patients' and observers' reports. In this context, the number or type of seizures presented in a defined interval is not always precise since the majority of patients with epilepsy have unnoticed seizures [60]. So, any study addressing the number of seizures will report only a proportion of possible seizures that occurred in that period. In addition, different types of seizures have probably different weights in the progression of epilepsies. Moreover, different types of seizures may involve different patterns of neural networks in different individuals, and it may also influence the structural abnormalities observed in each patient. According to the evidence that we have so far, we may infer that some types of seizures in some specific epilepsies and in individuals with some distinct characteristics may lead to progressive structural damage and cognitive dysfunction.

Another difficulty is to differentiate if the association of more extensive structural damage and higher seizure frequency is due to the consequence of recurrent seizures per se or to the vulnerability that patients with more aggressive disease have from the beginning of their refractory seizures.

Some cross-sectional and longitudinal MRI studies support the hypothesis that seizures may influence structural damage. Bilevicius et al. [61] demonstrated in a quantitative MRI cross-sectional analysis more pronounced and widespread gray matter abnormalities in AED-resistant and relapse-remitting TLE individuals when compared with AED responders. A longitudinal VBM analysis in patients with TLE [15] also demonstrated progressive mesial temporal and neocortical damage associated with poorer seizure control. Also, a study including patients with IGE with only tonic-clonic seizures [42] observed that the reduction of thalamic volumes and fronto-central and limbic cortices occurred faster in patients with poorer seizure control.

Other studies support the hypothesis that GTCS and not partial seizures may be causative of damage [30,62].

Conversely, some studies contradict the theory that seizures cause further injury in individuals with epilepsy [2,29,51]. However, the majority of these are composed of community-based or very heterogeneous groups of individuals.

Besides the heterogeneity of the individuals included in the community-based studies, according to epidemiological data, up to 70% of patients with epilepsy will have adequate seizure control with AED therapy [3]. Hence, the question arises if the study of more homogenous groups of patients with well-controlled seizures could demonstrate progression even in some patients with more "benign" epilepsy. Andrade-Valenca et al. [63] showed in an MRI volumetric analysis that TLE–HS, half of which with good seizure control, failed to demonstrate any correlation of seizures with the degree of hippocampal atrophy. Recently, a cross-sectional quantitative MRI study [64] showed cortical thinning, mainly in the sensoriomotor cortex in individuals with "benign" TLE patients with or without MRI signs of HS. All patients had been free of major seizures for at least 1 year before the MRI, which supports the idea that the pathology in neocortical regions may be implicated in pathophysiology of TLE. Our group also showed [65], in an extensive follow-up with a medium of 90 months, progressive hippocampal volume reduction in a group of familial mesial TLE (FMTLE) in which 71% of the individuals were classified as benign. In this study – compared with a group of individuals with sporadic MTLE, in which only 12% were considered benign – FMTLE had a slower progression of hippocampal volume reduction. This evidence supports the hypothesis that although seizures may contribute to additional injury in some types of epilepsy, a slower progressive damage may be intrinsic to the pathophysiology of MTLE.

The duration of epilepsy, independent of the occurrence or frequency of seizures, has also been addressed as involved in the process of epilepsy progression, affecting regions in the epileptic focus [66,67], as well as distant areas as thalamus [67] and neocortical structures [15] (Fig. 4). Similarly, the age of epilepsy onset has also been described as related to worsening of structural damage in TLE [67,68], and some authors have proposed that childhood onset epilepsy may be associated with an adverse neurodevelopmental impact on brain structure and function [68]. However, other studies failed to support this evidence [66].

Some more specific individual characteristics have been related to progressive MRI structural damage in epilepsies. In one study with TLE patients [14], only individuals with an epileptic focus on the left side had a hippocampal volume and signal that correlated with the number of partial or generalized seizures, while in another study [15], gray and white matter diffuse progressive abnormalities were more pronounced in individuals with a left-sided EEG focus (Fig. 5). Briellmann et al. [69] proposed that seizure frequency may be a factor contributing to reduced brain volume in men but not in women with TLE. In a VBM study of individuals with drug-refractory TLE, Yasuda et al. [70]

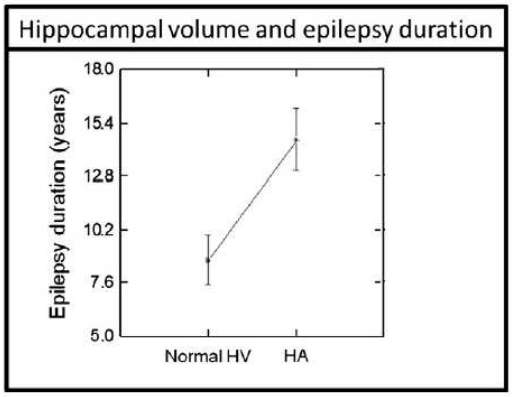

Fig. 4. In a group of 84 patients with benign focal epilepsies, those with hippocampal atrophy detected by manual volumetry had significantly longer epilepsy duration than those with normal hippocampal volume (two-sample *T*-test, $p=0.003$). HV: hippocampal volume and HA: hippocampal atrophy.

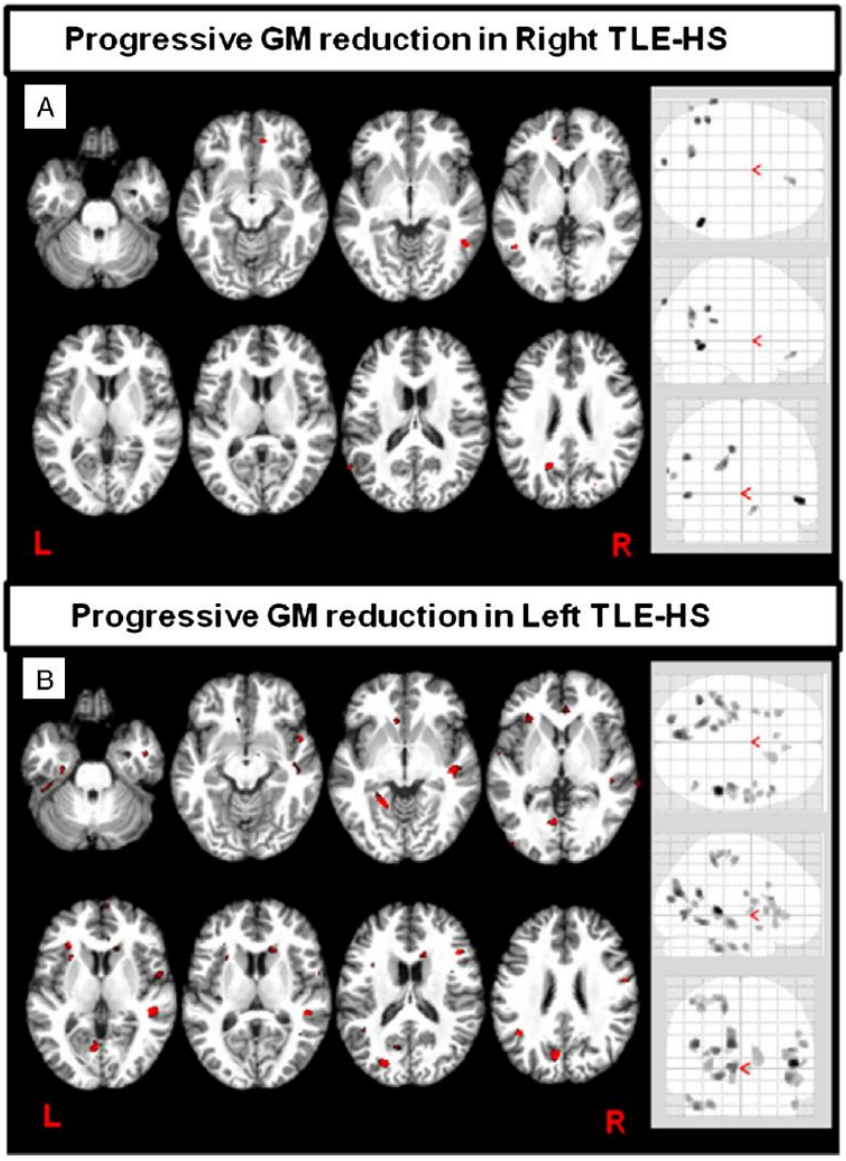

Fig. 5. Longitudinal analysis of GM volumes in MRI scans of patients with TLE–HS (13 with right HS and nine with left HS) and refractory seizures. The images where acquired at a mean interval of 16 months (range 6–29 months), with no significant difference in the follow-up interval between patients with right or left HS (T-test = 0.30). A: areas of progressive GM reduction in patients with right TLE–HS compared with left TLE–HS; B: areas of progressive GM reduction in patients with left TLE–HS compared with right TLE–HS (VBM analysis: vbm8 toolbox, longitudinal analysis; two-sample T-test, $p = 0.001$, FDR corrected). The glass brain images on the right columns of Panels A and B clearly show that patients with left HS have more pronounced GM progressive reduction (B) than those with right HS. GM: gray matter; TLE–HS: temporal lobe epilepsy associated with hippocampal sclerosis; VBM: voxel-based morphometry; and FDR: false discovery rate.

showed that those with a negative family history of epilepsy had a more widespread pattern of gray matter abnormalities as well as lower IQ scores than those with a positive family history, suggesting that in the first group, a stronger environmental influence may be related to the more intense clinical and structural abnormalities.

The influence of AED exposure in epilepsy progression is also not well understood. One study [51] has observed generalized brain atrophy more commonly in patients with increased exposure to AEDs, independently of seizure control. However, no large available datasets have been published, and the difficulties of this type of evaluation are vast. The majority of individuals with refractory epilepsies, which according to the data available are the ones more vulnerable to epilepsy progression, are exposed to AED polytherapy with frequent modification of dosages and types of AEDs.

2.4. The contribution and perspectives of new neuroimaging techniques

In the last decade, an extensive number of refined quantitative MRI post-processing tools have emerged. While for some tools the technical issues still need to be solved, others have already given us new

important clues about the pathophysiology of epilepsies. However, the use of these techniques to answer the questions about epilepsy progression is still scarce.

2.4.1. Diffusion tensor imaging/tractography

Diffusion tensor imaging (DTI) can study the white matter integrity through the evaluation of water diffusion [71]. Diffusion tensor imaging abnormalities have been described in malformations of cortical development and idiopathic generalized epilepsies [72,73], but the majority of studies have focused so far on TLE. White matter abnormalities in cross-sectional DTI studies of TLE patients were observed in bilateral temporal regions [74] as well as in bilateral extra-temporal areas belonging to the limbic system [75].

It is possible that these white matter abnormalities may be secondary to seizure activity [64]. However, the correlation of these abnormalities with seizures and epilepsy duration has not yet been adequately evaluated and long-term follow-up studies are still necessary. Some DTI studies with TLE patients found a correlation between abnormalities in white matter and duration of epilepsy, especially in patients with HS [76,77]. However, this has been contradicted by other studies [74,78].

More recently, in one study with DTI, Kim et al. [79] also demonstrated widespread disturbance of white matter integrity in the frontal lobe and corpus callosum of patients with juvenile myoclonic epilepsy, and the number of GTCS was correlated with these abnormalities.

2.4.2. Functional MRI (fMRI)

Functional magnetic resonance imaging allows the measurement of brain activity by detecting signal changes associated with changes in blood flow and changes in blood oxygenation [80]. It has been used as an important clinical tool for evaluation of memory performance [81,82] and to map eloquent areas for surgical planning [83]. This capacity of a non-invasive evaluation of hemodynamic changes associated with neural activity allows the possibility of evaluating different aspects related to the epilepsy progression. For example, Cheung et al. [84] demonstrated that the longer the duration of epilepsy, the lower the brain activation in a visual scene-encoding task with TLE patients and that this reduction of brain activation negatively affects memory function. However, the available fMRI data concerning progressive damage are still scarce.

Other promising results regarding the epilepsy process and progression may come from fMRI studies of resting state and default mode network (DMN) (which appears to include areas involved in the maintenance of baseline activities related to the modulation during internal and external tasks, self-awareness, and episodic memory) [85,86]. Pieces of evidence suggest that the DMN is abnormal in patients with TLE [87,88] and IGE [89] and that the inter-ictal epileptiform discharge may be implicated in the deactivation of the DMN in IGE [90], TLE [91,92], and other types of focal epilepsies [92]. Functional magnetic resonance imaging studies also demonstrated abnormal functional connectivity in DMN areas in TLE [93,94]. Extensive work is necessary to understand if these abnormalities in the DMN and specific connectivity patterns have any relation to the occurrence of seizures or duration of epilepsy and if it can be part of the progression of some types of epilepsy.

Multimodal neuroimaging techniques, such as the use of concomitant EEG and fMRI (EEG–fMRI), may be able in the future to add more knowledge through a refined non-invasive evaluation. Electroencephalography–functional magnetic resonance imaging has been proven to help in the identification of the epileptogenic focus in focal refractory epilepsies [95–98] as well as to evaluate networks involved in different types of epilepsies [90–92]. This technique may be able to provide signatures that could enable the classification of more homogeneous groups, and it could also help to evaluate differences and long-term modifications of networks implicated in ictal and inter-ictal abnormalities in different groups of patients with epilepsy.

2.4.3. Other promising neuroimaging techniques

Other promising MRI techniques that may contribute to the study of epilepsy progression have also emerged in the last two decades. Higher field (3 T) MRIs have allowed the improvement of some techniques, such as spectroscopy [99] and arterial spin label [100,101]. 7 T MRI will be able to add to our knowledge once it allows an anatomic observation that is closer to the histopathological findings [102,103]. However, no data regarding progressive damage in epilepsy have yet resulted from these advances.

With the emerging knowledge about the role of inflammation in epileptogenesis and possibly in the chronicity and progression of some types of epilepsies such as TLE–HS, researchers have also focused on neuroimaging techniques that may be able to image inflammation, with promising results from PET and MRI studies [104,105].

3. Translational knowledge: epilepsy progression and clinical decisions

Despite the controversial results of different studies – which may be mainly the result of the heterogeneity of the patients included – most current evidence indicates that TLE–HS is a progressive disorder.

In this context, for patients with TLE–HS, refractoriness to AEDs must be defined early in the treatment, and surgery must be considered as soon as possible. For this specific group of individuals, the early control of seizures may decrease the risk of progressive structural, cognitive, and behavior damage related to repeated seizures. Even with some contradictory results [106], different studies have confirmed that for TLE–HS, age at surgery [107,108] and epilepsy duration are important predictors for long-term surgical outcome [109]. The remaining question is whether avoiding the seizures will also be able to block the possible progression related to the intrinsic pathophysiology of this type of epilepsy. In fact, one study suggested that stopping seizures after surgical treatment may reverse some of the brain damage in TLE [110], and other studies further demonstrated that postoperative seizure freedom reverses part of the metabolic dysfunction of patients with TLE [111,112].

4. Perspectives for future studies

The progressive characteristic of the epilepsies is still controversial. Current knowledge indicates that TLE–HS is a progressive disorder. The contradictory data of different studies may be related to the heterogeneity of individuals included. The natural history of the many diverse types of epilepsies may be related to the etiology and initial epileptogenic process but may also be associated with many distinct individual characteristics concerning genetic background and exposure to environmental factors.

Therefore, in order to achieve a satisfactory answer about the progression of epilepsies, studies must evaluate larger series of individuals for longer periods of follow-up. Moreover, it is important to control the several genetic and environmental factors that may influence the epilepsy burden and to give the appropriate importance for each of them. Only with an appropriate multivariate analysis will we be able to have a better view of what is the weight of each intrinsic and extrinsic characteristic for individuals with epilepsy.

With the advances in neuroimaging techniques, in vivo and non-invasive structural and functional studies will help to improve the understanding of epilepsy progression.

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5. Discussão

A ELTM, além de ser a epilepsia mais frequente no adulto, representa uma das epilepsias com o maior número de pacientes com crises refratárias às DAEs, sobretudo quando relacionada à EH. Nas últimas décadas, a pesar dos progressos no conhecimento dessa patologia, pouco se avançou no sentido de significativa melhora no controle de crises e de comorbidades nesses indivíduos. Uma das dificuldades do desenvolvimento de novas terapêuticas para o tratamento das epilepsias é o desconhecimento dos fatores que levam a variabilidade de respostas aos tratamentos clínico e cirúrgico observada em indivíduos com fenótipos semelhantes. Dessa forma, a compreensão detalhada das diferenças individuais nos subtipos de ELTM e quais os elementos associados à resposta adequada ao tratamento são fundamentais para o desenvolvimento de terapêuticas mais apropriadas.

Neste trabalho, nós procuramos avaliar as diferenças clínicas e de neuroimagem estrutural e funcional em dois grupos de ELTM: ELTM com sinais de EH em exames de RM e ELTM criptogênica (com RM normal). Nossa hipótese era que esses subgrupos de ELTM, a pesar da semelhança semiológica e eletroencefalográfica, devem apresentar características clínicas e de neuroimagem distintas. Séries recentes de pacientes com ELTM-NL refratários avaliados com RM de alto campo e protocolo adequado para epilepsias, demonstram que histopatologia do EH é encontrada em um baixo percentual desses pacientes (25) o que reinforça o fato de a ELTM-EH e a ELTM-NL tratarem-se de doenças diferentes.

A fim de aumentarmos a sensibilidade da detecção de sinais de EH em nossos pacientes e melhor caracterizar os indivíduos com ELTM-NL, a definição de sinais de EH em nosso estudo foi baseada não apenas na análise visual de exames de RM, mas também na quantificação de volume e sinal hipocampal. Técnicas de quantificação das anormalidades hipocampais são amplamente utilizadas para pesquisas com pacientes com

ELTM (26, 32). No entanto, com análises quantitativas de exames de RM de 1,5T, um grupo de pacientes com ELTM permanece sem alterações estruturais detectáveis, mesmo quando EH é confirmada após a remoção cirúrgica das estruturas mesiais temporais (89). Não está claro, até o momento, qual o papel dessas técnicas para o auxílio da determinação de alterações hipocampais em pacientes com ELTM e RM visualmente normal após o uso de escâneres de alto campo (3T) na prática clínica (33).

Em nosso estudo, a quantificação de sinal e volume da estrutura hipocampal nos exames de RM de 3T aumentou em 28% a detecção de sinais de EH em pacientes com exames de RM visualmente normais, mesmo tendo sido estas avaliadas por especialistas e com protocolo adequado para o estudo de epilepsias. Além disso, a elevada concordância entre a lateralidade dos sinais de EH detectados por essas técnicas e a lateralidade do foco epiléptico tem elevada importância clínica, pois pode auxiliar na indicação de tratamento cirúrgico para pacientes com ELTM e imagens de RM visualmente normais. Ainda, a quantificação das anormalidades hipocampais foi realizada por volumetria automatizada e quantificação do sinal manual simples. Este protocolo otimizado pode ser facilmente aplicado em centros especializados de epilepsia a pacientes com ELTM e crises refratárias às DAEs como uma análise adicional de rotina para o diagnóstico de sinais de EH.

Em relação às diferenças entre os pacientes com ELTM-EH e ELTM-NL, clinicamente observamos que os pacientes com ELTM-NL apresentam idade de início de crises mais elevada e, consequentemente, menor duração da epilepsia e menor tempo de epilepsia ativa. Nesse grupo, observamos ainda, maior frequência de antecedente familiar de epilepsia. Idade média das crises na segunda década de vida em pacientes com ELTM-NL, diferente do que ocorre na ELTM-EH, em que as crises mais comumente têm início no final da primeira década de vida (6), é frequentemente descrito na literatura (21-23), apesar

de poucos artigos discordantes (4). Por outro lado, antecedente familiar de epilepsia, especificamente em indivíduos com ELTM-NL, é dado pouco avaliado. Um trabalho recente não mostrou diferença entre a frequência de antecedente familiar de epilepsia em pacientes com ELTM-EH ou ELTM-NL e este variou entre 12 a 15% (90).

A elevada incidência de antecedente familiar em nossos pacientes, assim como a baixa incidência de CF e outros eventos precipitantes iniciais, diferem em relação à descrição de outras coortes de pacientes com ELTM-EH (6). Antecedente de CF em pacientes com ELTM-EH é descrito em torno de 30% dos casos (11), porém esse dado é variável na literatura, com estudos demonstrando taxas de até 94% em pacientes com crises refratárias à DAE (6). Estudos com pacientes com ELTM-EH e adequado controle de crises reportam menor incidência de CF, chegando a até 15% dos casos (14). Da mesma forma, a incidência de CF é também baixa em estudos com ELTM familiar (18, 91). Em nosso trabalho, observamos antecedente de CF em apenas 11% dos pacientes com ELTM-EH e 6% dos pacientes com ELTM-NL. Em nossos pacientes, consideramos que fatores relacionados aos antecedentes familiares possam estar envolvidos na gênese da EH nos pacientes com ELTM-EH, mas também na ocorrência de crises epilépticas, a despeito da possível ausência de EH, nos pacientes com ELTM-NL. Fatores genéticos podem estar associados ao desenvolvimento da epilepsia em uma parcela significativa dos nossos pacientes, mas outros possíveis fatores ambientais compartilhados pelos membros da uma mesma família não podem ser descartados.

Em relação à resposta à DAE, observamos algumas diferenças entre os pacientes com ELTM-EH e ELTM-NL. Sabe-se que pacientes com epilepsias criptogênicas, como a ELTM-NL, apresentam maior taxa de pacientes com bom controle ou remissão de crises do que aqueles com epilepsias sintomáticas (19). Sabe-se, ainda, que a EH é a patologia

relacionada à ELTM com as maiores taxas de crises refratárias às DAEs (19, 90). Em nosso estudo, não observamos diferença significativa da frequência de pacientes com bom controle de crises ou refratários entre os grupos ELTM-EH e ELMT-NL quando consideramos esse dado de forma transversal (considerados como bom controle de crises aqueles com baixa frequência de CPC e ausência de CTCG nos últimos dois anos) (*Capítulo 4*). Porém, quando consideramos a resposta à DAE desde o início das crises, e classificamos como bom controle de crises aqueles indivíduos que desde o início da terapia otimizada com DAEs mantiveram uma frequência baixa de crises, verificamos que o grupo ELTM-EH apresenta maior frequência de pacientes refratários (*Capítulo 5*).

Verificamos, ainda, que um padrão intermitente de resposta à DAE, alternando períodos de remissão de crises com outros de crises refratárias (padrão remitente-recorrente) ocorre em cerca de um terço dos pacientes com ELTM, independente da presença ou não de sinais EH nos exames de RM. Padrão intermitente de controle de crises em pacientes com ELTM-EH é descrito em estudos prévios, porém com menor prevalência (6, 92) e sem uma comparação direta entre pacientes com ELTM-NL. A elevada frequência desse padrão remitente-recorrente em nossos pacientes deve estar possivelmente relacionada à maior frequência de pacientes com bom controle de crises em nosso grupo.

Uma diferença interessante observada entre os pacientes com ELTM-EH e ELMT-NL é que a ocorrência de um PS, isto é, um longo intervalo livre de crises que se inicia ainda na primeira década de vida, seguido por recorrência das crises refratárias ao tratamento medicamentoso, parece ser característica da ELTM-EH. Este dado tem implicações importantes para as decisões iniciais e investigação de cirurgia de epilepsia dos pacientes com diagnóstico de ELTM na primeira década de vida.

Neste trabalho, nós avaliamos as diferenças estruturais de pacientes com ELTM-EH e ELTM-NL através da detecção de alterações de volume de SC pela técnica de VBM. Nós observamos que há atrofia difusa de SC tanto na ELTM-EH quanto na ELTM-NL, e que a atrofia em algumas regiões é comum para ambos os grupos. Por outro lado, diferentemente da ELTM-EH, no grupo ELTM-NL, não foi observada atrofia em estruturas mesiais temporais, bem como em outras regiões anatomicamente ou funcionalmente ligadas ao hipocampo como ínsula e núcleo lentiforme. Atrofia de SC foi observada em ambos os grupos nos tálamos bilaterais, giros pré e pós-central, cuneus bilaterais, giro frontal médio e região orbitofrontal. Observamos também aumento de SC que foi semelhante em ambos os grupos, acometendo a porção dorsolateral da ponte contralateral ao foco epiléptico.

Na ELTM-EH, a atrofia de SC foi mais acentuada e ocorreu tanto em pacientes com bom controle de crises quanto nos pacientes refratários, enquanto na ELTM-NL atrofia de SC só foi observada em pacientes com crises refratárias. Ainda, nos pacientes com ELTM-EH e bom controle de crises, a atrofia de SC poupou estruturas corticais extra-temporais como regiões do córtex frontal e occipital bilaterais e se localizou em áreas mais próximas ao hipocampo, como tálamos e núcleo lentiforme. De acordo com estes resultados, existe a possibilidade de que a presença da EH esteja relacionada à atrofia das estruturas a ela conectadas, como o tálamo, quer seja por dano secundário ou por dano primário concomitante ao desenvolvimento da EH e relacionado ao processo de epileptogênese. Por outro lado, atrofia extra-temporal neocortical pode estar relacionada com a ocorrência de crises epilépticas refratárias.

Em nosso estudo, nós observamos, ainda, pacientes com aumento do volume de amígdala, tanto no grupo ELTM-EH quanto ELTM-NL. A amígdala é conhecida por ser parte da rede epileptogênica de pacientes com ELTM-EH e redução do volume desta

estrutura ipsilateral à EH e ao foco epiléptico são consistentemente relatados (32). Nós descrevemos um grupo de pacientes com ELTM-EH e aumento significativo de volume de amígdala (correspondendo a 14% dos pacientes com ELTM-EH), que ocorre mais frequentemente contralateral ao foco epiléptico e aos sinais de EH. Estes pacientes apresentaram idade significativamente menor de início de crises. A pesar de descrições prévias de aumento de amígdala em pacientes com distúrbios psiquiátricos como depressão e psicose (93, 94), no nosso subgrupo isso não foi observado. Assim, uma hipótese consistente para a fisiopatologia do aumento de amígdala no nosso subgrupo de ELTM-EH não pode ser definida.

Da mesma forma, observamos um subgrupo de pacientes com ELTM-NL com aumento significativo de volume da amígdala (14% dos pacientes ELTM-NL). Diferentemente dos pacientes com ELTM-EH, nos pacientes com ELTM-NL esse aumento de volume foi mais frequentemente observado ipsilateral ao foco epiléptico. O envolvimento de amígdala como possível lesão estrutural relacionada ao foco epiléptico de pacientes com ELTM foi descrito previamente (95, 96). Em nosso subgrupo, mostramos, porém, a frequência com que anormalidade da amígdala pode ser observada em uma coorte de pacientes com ELTM e imagens de RM visualmente normais e sem sinais de EH à análise quantificativa. Nenhuma diferença clínica foi observada entre os pacientes com ou sem aumento de amígdala. O significado histopatológico desse achado, bem como sua relação com a ocorrência de crises nesses pacientes demandam estudos adicionais.

Investigamos ainda, neste estudo, as diferenças de alterações em exame de RMf dos pacientes com ELTM-EH e ELTM-NL. Através do uso da técnica de EEG-RMf demonstramos que DEIs semelhantes apresentam diferentes padrões de respostas hemodinâmicas nesses dois grupos. A análise de grupo das respostas hemodinâmicas

relacionadas às DEIs nos pacientes com ELTM-EH demonstrou resposta BOLD positivo (BOLDpos) no lobo temporal e giro parahipocampal ipsilaterais ao foco epiléptico, além de ínsula bilateral, cíngulo anterior e putâmen ipsilaterais. Esses resultados são concordantes com estudos prévios que investigaram pacientes com ELTM de diversas etiologias agrupados, porém em todos estes estudos havia predomínio de pacientes com ELTM-EH (73-75). Diferentemente, no grupo ELTM-NL, a pesar de BOLDpos também ter sido observado na região anterior do lobo temporal e ínsula ipsilaterais ao foco epiléptico, BOLDpos não foi observado em hipocampo/parahippocampo, putâmen e cíngulo anterior ipsilaterais e ínsula contralateral ao foco epiléptico. Essas diferenças de resposta hemodinâmica observadas entre a ELTM-EH e a ELTM-NL sugerem que, embora a semiologia e os achados eletroencefalográficos destes indivíduos sejam semelhantes, estes pacientes podem ter diferentes geradores das DEIs e, consequentemente, diferentes padrões de propagação da resposta hemodinâmica. Além disso, estas diferenças, incluindo a ausência de resposta BOLDpos em áreas temporais mesiais e áreas conectadas ao sistema límbico como o cíngulo anterior, indicam que no grupo ELTM-NL pode haver uma rede epiléptica que, a pesar de incluir a região temporal mesial, deve ter origem e distribuição distintas do observado na ELTM-EH. É possível que nos pacientes com ELTM-NL, a despeito da clínica compatível com ELTM, existam estruturas responsáveis por geração de crises em regiões temporais neocorticais ou mesmo extra-temporais, como o que se observa em estudos com monitorização eletroencefalográfica invasiva (97).

Compararmos, ainda, os mapas funcionais relacionados às DEIs ipsilaterais ao foco epiléptico com a atrofia de SC nos mesmos indivíduos, e observamos que não há sobreposição das alterações funcionais e estruturais nestes grupos. Isto pode indicar que as estruturas envolvidas na rede interictal nestes dois grupos de pacientes com ELTM não

apresentam perda significativa de volume. No entanto, estudos de VBM anteriores, assim como nossos resultados descritos no *Capítulo 4*, relatam anormalidades estruturais que também incluem as áreas com BOLDpos observados pela nossa análise de EEG-RMf, como atrofia em córtex sensório-motor bilateral (23, 45, 50). Uma possibilidade é que o número de indivíduos incluídos em cada um dos nosso grupos na análise de EEG-RMf foi demasiado pequeno para detectar as atrofias sutis destas regiões. No entanto, como foram observadas atrofias consistentes em algumas regiões mesmo nesse grupo pequeno de indivíduos, consideramos possível que, mesmo que existam anomalias estruturais nas regiões de resposta BOLDpos e BOLDneg relacionadas com as DEIs, estas não são tão relevantes como o observado em outras áreas, tais como tálamos, núcleo caudado ou córtex occipital.

Dentro desse contexto, consideramos a hipótese de que existam redes neuronais distintas que participem da patogênese das ELTMs: 1) uma rede neuronal funcional ictal, composta por regiões que obrigatoriamente participam do fenômeno ictal (39); 2) uma rede neuronal funcional interictal, composta por regiões que apresentam atividade anormal durante as DEIs (e que podem estar relacionadas a outras disfunções, que não as crises, como déficits cognitivos e comorbidades psiquiátricas); 3) uma rede neuronal estrutural, composta por regiões com redução ou aumento de volume e que podem ser decorrentes do processo de epileptogênese, de dano secundário decorrente de crises repetidas ou do próprio processo biológico na evolução da patologia. Essas redes devem interagir entre si e se sobrepõem, porém não são idênticas.

Observamos reposta BOLDneg relacionada às DEIs em áreas compatíveis com a DMN, como precuneus bilaterais e cíngulo posterior (56) tanto na ELTM-EH quanto na ELTM-NL. Esses resultados, assim como o demonstrado em estudos de anteriores (73-75),

demonstram que a DMN é afetada não apenas por descargas epileptiformes generalizadas, que frequentemente se apresentam com quadro clínico de alteração breve de consciência (70), mas também por DEIs isoladas em epilepsias focais, as quais normalmente não são acompanhadas por qualquer alteração aparente comportamental ou cognitiva.

Ainda, observamos que a presença de uma resposta BOLDneg no componente posterior (precuneus/cíngulo posterior) da DMN pode prever um bom resultado cirúrgico em pacientes com ELT independente da etiologia. A atividade da DMN normalmente é suprimida durante a atenção dirigida a tarefas (interrupção da introspecção) (57, 58). Nossa hipótese é que processo semelhante esteja relacionado às DEIs. Dessa forma, a presença de BOLDneg no componente posterior da DMN relacionado às DEIs pode indicar funcionamento normal dessa rede neuronal e a supressão de sua atividade durante essas descargas pode implicar integridade das redes cerebrais normais. Portanto, os pacientes com ELT nos quais não se observa resposta BOLDneg relacionada às DEIs podem apresentar redes cerebrais anormais mais difusas, o que pode estar implicado no pior prognóstico cirúrgico.

Da mesma forma, a detecção de resposta BOLDpos relacionada às DEIs no lobo temporal ipsilateral ao foco epiléptico pela técnica de EEG-RMf pode prever um bom resultado cirúrgico em pacientes com ELT, independentemente de sua etiologia. Estudos prévios demonstraram a importância da técnica de EEG-RMf para a definição, de forma não invasiva, da zona de início ictal em pacientes com epilepsias focais e crises refratárias (60, 64, 71). Nossos resultados mostram, que o uso da técnica de EEG-RMf na avaliação pré-operatória pode auxiliar na definição do prognóstico cirúrgico nesses indivíduos, o que

pode aprimorar a escolha adequada de pacientes que devem ser submetidos a tratamento cirúrgico para controle de crises.

Perspectivas

De acordo com os resultados apresentados, novos trabalhos devem ser desenvolvidos a fim de que os resultados possam ser confirmados e melhor compreendidos. Assim, seguimento dos indivíduos com ELMT-EH e ELTM-NL e avaliação longitudinal de exames de RM com quantificação dos danos estruturais desses pacientes pode auxiliar a compreensão das causas relacionadas a esses danos, bem como verificar sua possível progressão, conforme descrito no Capítulo 10. Da mesma forma, avaliação longitudinal das redes neuronais em pacientes com ELTM-EH ou ELTM-NL, bem como a correlação das áreas de resposta BOLD relacionadas às DEIs com diferentes aspectos clínicos, como tempo de doença e comorbidades, podem revelar distintos padrões de funcionamento de redes neuronais patológicas e detectar áreas-alvo para o desenvolvimento de terapêuticas específicas. Por fim, a avaliação prospectiva dos padrões de resposta BOLD, incluindo a detecção de BOLDneg relacionado às DEIs em regiões da DMN, pode comprovar a hipótese da contribuição de anormalidades dessas redes neuronais com o prognóstico cirúrgico nos pacientes com ELTM e crises refratárias.

6. Conclusões

- Em exames de RM adquiridos em escâner de 3T e inspecionados visualmente por especialistas, a quantificação do volume e sinal hipocampal pode aumentar a detecção de sinais de EH em 28% dos pacientes com ELTM.

- Padrão remitente-recorrente de resposta à DAE ocorre com frequência semelhante em pacientes com ELTM-EH e ELTM-NL, porém neste segundo grupo há maior proporção de pacientes que apresentam bom controle de crises, sobretudo quando se observam as respostas à DAE desde o início da epilepsia.

- A ocorrência de PS seguido por recorrência de crises refratárias às DAEs é característica dos pacientes com ELTM-EH.

- Aumento de volume de amígdala contralateral ao foco epiléptico é observado em um subgrupo de pacientes com ELTM-EH e idade de início de crises precoce.

- Um subgrupo de pacientes com ELTM-NL apresentam aumento significativo de volume da amígdala, mais frequentemente observado ipsilateral ao foco epiléptico.

- Há atrofia difusa de SC em pacientes com ELTM-EH e ELTM-NL e essa atrofia, em algumas regiões, é comum para ambos os grupos, apesar da ausência de atrofia detectável nas estruturas mesiais temporais no grupo ELTM-NL.

- A presença de sinais de EH em exames de RM está associada a atrofia mais acentuada de SC, a qual é obsevada tanto em pacientes refratários quanto com bom controle de crises.

- A atrofia de SC nos pacientes com ELTM-NL é observada apenas naqueles com crises refratárias às DAEs.

- DEIs semelhantes apresentam padrões de respostas hemodinâmicas diferentes em pacientes com ELTM-EH ou ELTM-NL.

- As estruturas cerebrais envolvidas na rede funcional relacionada às DEIs diferem entre a ELTM-EH e a ELTM-NL e as estruturas envolvidas nessas redes funcionais não são aquelas com dano estrutural mais significativo detectadas pela técnica de VBM.

- Tanto na ELTM-EH quanto na ELTM-NL, BOLDneg relacionado às DEIs é observado em áreas compatíveis com a DMN.

- A detecção, através de exames de EEG-RMf, de resposta BOLD relacionada às DEIs no lobo temporal ipsilateral à zona de início ictal de pacientes com ELT está relacionada a melhor prognóstico cirúrgico.

- A detecção de resposta BOLDneg relacionada às DEIs em regiões compatíveis com a DMN está relacionada a melhor prognóstico cirúrgico na ELT.

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Anexos

Anexo 1: Termo de consentimento informado

UNICAMP

Universidade Estadual de Campinas

Departamento de Neurologia

FORMULÁRIO DE CONSENTIMENTO PARA PESQUISA MÉDICA, *Página 1 de 3*

Título do projeto: **A Neuroimagem nas Epilepsias**

Investigador principal: Dr. Fernando Cendes

OBJETIVO DA PESQUISA:

Eu _____ entendo que fui convidado (a) a participar em um projeto de pesquisa envolvendo pacientes com epilepsia. O objetivo geral do estudo é o de determinar a utilidade da Imagem e Espectroscopia por Ressonância Magnética para identificar e quantificar alterações estruturais e metabólicas do sistema nervoso central. A identificação e quantificação dessas anormalidades no cérebro, pode eventualmente melhorar o diagnóstico e levar a um melhor tratamento dessa doença. As informações médicas a meu respeito que forem obtidas para esse estudo, poderão ser compartilhadas com outros pesquisadores que trabalham com epilepsia. Podendo assim ser utilizadas eventualmente para outros fins de pesquisa sobre as epilepsias. O sigilo será mantido em todos os estudos colaborativos através da utilização de um número de código para a identificação dos indivíduos participantes.

A ressonância magnética é uma técnica capaz de produzir imagens de alta qualidade e resolução (nitidez) anatômica, assim como informações sobre a bioquímica dos tecidos. A ressonância magnética produz imagens em cortes que são parecidos com as imagens produzidas pela tomografia computadorizada, porém com maior resolução (nitidez) e sem a exposição aos raios X. Essas imagens também irão produzir informações bioquímicas que serão úteis para melhor definição do diagnóstico e tratamento. O objetivo principal desse estudo é determinar a importância dessas informações bioquímicas e estruturais.

PROCEDIMENTO:

Eu entendo que se concordar em participar desse estudo, os pesquisadores participantes farão perguntas a respeito dos meus antecedentes médicos e de minha família. Eu serei submetido a um exame físico neurológico para estabelecer meu estado clínico. Além disso, poderei ser submetido a um eletroencefalograma (EEG) além dos exames de ressonância magnética. Hospitalização não será necessária.

O procedimento de ressonância magnética é semelhante a uma tomografia. Eu fui informado que eu serei colocado em uma maca e serei movido lentamente para dentro do aparelho de ressonância magnética. Um alto falante dentro do campo magnético possibilita a minha constante comunicação com as pessoas responsáveis pelo exame. Durante todo o tempo o pessoal médico e paramédico pode me ver e ouvir, e eu posso ser removido(a) se for preciso. O procedimento pode durar entre 45 a 90 minutos. Durante a primeira parte do exame eu irei ouvir ruídos, tipo marteladas, por alguns minutos enquanto o aparelho faz as imagens do meu cérebro. O restante do exame será relativamente silencioso.

VANTAGENS:

Eu entendo que não obterei nenhuma vantagem direta com a minha participação nesse estudo e que o meu diagnóstico e o meu tratamento provavelmente não serão modificados. Contudo, os resultados desse estudo podem, a longo prazo, oferecer vantagens para os indivíduos com epilepsia, possibilitando um melhor diagnóstico e um tratamento mais adequado. Os resultados do meu exame de ressonância magnética ficarão a disposição dos médicos responsáveis pelo meu tratamento, e poderão ser úteis no futuro.

Título do projeto: A neuroimagem nas epilepsias parciais.

Investigador principal: Dr. Fernando Cendes

RISCO E DESCONFORTO:

O único desconforto relacionado a este exame é o ruído intermitente durante os primeiros 15 minutos. Depois disso o ruído será muito menor. O pessoal técnico providenciará tapa-ouvidos para me deixar mais confortável.

Uma das principais vantagens da ressonância magnética e que esta não utiliza raios X ou outro tipo de radiação ionizante, ao contrário de outros tipos de exame radiológicos. As imagens são obtidas graças a um campo magnético (imã), um transmissor e receptor de ondas de rádio e um computador que é utilizado para obter as informações bioquímicas e imagens da anatomia interna. Não existem efeitos nocivos associados com a ressonância magnética dentro das condições utilizadas atualmente.

REQUERIMENTOS

É **muito importante** informar aos médicos(as) e técnicos(as) caso eu tenha um **marca-passo cardíaco, um clipe de cirurgia para aneurisma cerebral ou qualquer outro objeto metálico em meu corpo,** que tenha sido implantado durante uma cirurgia ou alojado em meu corpo durante um acidente, pois estes podem parar de funcionar ou causar acidentes devido ao forte campo magnético que funciona como um imã muito forte. Eu também devo remover todos os objetos metálicos que estiverem comigo (relógio, canetas, brincos, colares, anéis, etc), pois estes também podem movimentar ou aquecer dentro do campo magnético.

SIGILO:

Eu entendo que todas as informações médicas decorrentes desse projeto de pesquisa farão parte do meu prontuário médico e serão submetidos aos regulamentos do HC- UNICAMP referentes ao sigilo da informação médica.

Se os resultados ou informações fornecidas forem utilizados para fins de publicação científica, nenhum nome será utilizado.

FORNECIMENTO DE INFORMAÇÃO ADICIONAL:

Eu entendo que posso requisitar informações adicionais relativas ao estudo a qualquer momento. O Dr. Fernando Cendes, tel (019) 3521-9217 estará disponível para responder minhas questões e preocupações. Em caso de recurso, dúvidas ou reclamações contactar a secretaria da Comissão de Ética da Faculdade de Ciências Médicas-UNICAMP, tel. (019) 3521-7232.

RECUSA OU DESCONTINUAÇÃO DA PARTICIPAÇÃO:

Eu entendo que a minha participação é voluntária e que eu posso me recusar a participar ou retirar meu consentimento e interromper a minha participação no estudo a qualquer momento sem comprometer os cuidados médicos que recebo atualmente ou receberei no futuro no HC- UNICAMP. Eu reconheço também que o Dr. Fernando Cendes pode interromper a minha participação nesse estudo a qualquer momento que julgar apropriado.

Título do projeto: A neuroimagem nas epilepsias parciais.

Investigador principal: Dr. Fernando Cendes

Eu confirmo que o(a) Dr(a)._____

me explicou o objetivo do estudo, os procedimentos aos quais serei submetido e os riscos, desconforto e possíveis vantagens advindas desse projeto de pesquisa. Eu li e compreendi esse formulário de consentimento e estou de pleno acordo em participar desse estudo.

_____

Nome do participante ou responsável

_____ _____

Assinatura do participante ou responsável data

_____

Nome da testemunha

_____ _____

Assinatura da testemunha data

RESPONSABILIDADE DO PESQUISADOR:

Eu expliquei a _____ o objetivo do estudo, os procedimentos requeridos e os possíveis riscos e vantagens que poderão advir do estudo, usando o melhor do meu conhecimento. Eu me comprometo a fornecer uma cópia desse formulário de consentimento ao participante ou responsável.

_____

Nome do pesquisador ou associado

_____ _____

Assinatura do pesquisador ou associado data

Anexo 2: Termo de consentimento informado para realização de EEG-RMf

UNICAMP

Universidade Estadual de Campinas

Departamento de Neurologia

FORMULÁRIO DE CONSENTIMENTO PARA PESQUISA MÉDICA,

Título do projeto: **Eletroencefalografia e Ressonância Magnética funcional**

Investigador principal: Dra. Ana Carolina Coan

Orientador: Dr. Fernando Cendes

OBJETIVO DA PESQUISA:

Eu _____ entendo que fui convidado (a) a participar em um projeto de pesquisa envolvendo pacientes com epilepsia. O objetivo geral do estudo é o de determinar a utilidade do uso conjunto dos exames de Eletroencefalografia e Ressonância Magnética, para identificar e quantificar alterações relacionadas às descargas neuronais. A identificação e quantificação dessas anormalidades no cérebro, pode eventualmente melhorar o diagnóstico e levar a um melhor tratamento dessa doença. As informações médicas a meu respeito que forem obtidas para esse estudo, poderão ser compartilhadas com outros pesquisadores que trabalham com epilepsia. Podendo assim ser utilizadas eventualmente para outros fins de pesquisa sobre as epilepsias. O sigilo será mantido em todos os estudos colaborativos através da utilização de um número de código para a identificação dos indivíduos participantes.

A ressonância magnética é uma técnica capaz de produzir imagens de alta qualidade e resolução (nitidez) anatômica, assim como informações sobre a bioquímica dos tecidos. A ressonância magnética produz imagens em cortes que são parecidos com as imagens produzidas pela tomografia computadorizada, porém com maior resolução (nitidez) e sem a exposição aos raios X.

A eletroencefalografia é uma técnica capaz de avaliar a atividade neuronal, através do registro da corrente elétrica cerebral por eletrodos colocados no couro cabeludo. Permite observar descargas de ondas anormais que ocorrem em indivícuos com epilepsia.

PROCEDIMENTO:

Eu entendo que se concordar em participar desse estudo, os pesquisadores participantes farão perguntas a respeito dos meus antecedentes médicos e de minha família. Eu serei submetido a um exame físico neurológico para estabelecer meu estado clínico. Hospitalização não será necessária.

Antes de entrar no aparelho de ressonância magnética, entendo que serei submetido à colocação de eletrodos no couro cabeludo, fixados com gel e faixa, da mesma forma como é realizado o exame de eletroencefalografia habitualmente. Esses eletrodos ficarão conectados a uma caixa (amplificador), que será apoiada em uma mesa, próxima a minha cabeça, durante o exame.

O procedimento de ressonância magnética é semelhante a uma tomografia. Eu fui informado que eu serei colocado em uma maca e serei movido lentamente para dentro do aparelho de ressonância magnética. Um alto falante dentro do campo magnético possibilita a minha constante comunicação com as pessoas responsáveis pelo exame. Durante todo o tempo o pessoal médico e paramédico pode me ver e ouvir, e eu posso ser removido(a) se for preciso. O procedimento pode durar entre 45 a 90 minutos. Durante esse tempo, eu irei ouvir ruídos, tipo marteladas, enquanto o aparelho faz as imagens do meu cérebro.

VANTAGENS:

Eu entendo que não obterei nenhuma vantagem direta com a minha participação nesse estudo e que o meu diagnóstico e o meu tratamento provavelmente não serão modificados. Contudo, os resultados desse estudo podem, a longo prazo, oferecer vantagens para os indivíduos com epilepsia, possibilitando um melhor diagnóstico e um tratamento mais adequado. Os resultados do meu exame de ressonância magnética e eltroencefalografia ficarão a disposição dos médicos responsáveis pelo meu tratamento, e poderão ser úteis no futuro.

FORMULÁRIO DE CONSENTIMENTO PARA PESQUISA MÉDICA,

Título do projeto: **Eletroencefalografia e Ressonância Magnética funcional**

Investigador principal: Dra. Ana Carolina Coan

Orientador: Dr. Fernando Cendes

RISCO E DESCONFORTO:

Os desconfortos relacionados a este exame são o incoveniente de sujar o cabelo com o gel dos eletrodos (que é facilmente removido após lavagem), e o ruído intermitente do aparelho de ressonância magnética. O pessoal técnico providenciará tapa-ouvidos para me deixar mais confortável.

Uma das principais vantagens da ressonância magnética e que esta não utiliza raios X ou outro tipo de radiação ionizante, ao contrário de outros tipos de exame radiológicos. As imagens são obtidas graças a um campo magnético (imã), um transmissor e receptor de ondas de rádio e um computador que é utilizado para obter as informações bioquímicas e imagens da anatomia interna. Não existem efeitos nocivos associados com a ressonância magnética ou com o registro do eletroencefalograma dentro das condições utilizadas atualmente.

REQUERIMENTOS

É **muito importante** informar aos médicos(as) e técnicos(as) caso eu tenha um **marca-passo cardíaco, um clipe de cirurgia para aneurisma cerebral ou qualquer outro objeto metálico em meu corpo,** que tenha sido implantado durante uma cirurgia ou alojado em meu corpo durante um acidente, pois estes podem parar de funcionar ou causar acidentes devido ao forte campo magnético que funciona como um imã muito forte. Eu também devo remover todos os objetos metálicos que estiverem comigo (relógio, canetas, brincos, colares, anéis, etc), pois estes também podem movimentar ou aquecer dentro do campo magnético.

SIGILO:

Eu entendo que todas as informações médicas decorrentes desse projeto de pesquisa farão parte do meu prontuário médico e serão submetidos aos regulamentos do HC- UNICAMP referentes ao sigilo da informação médica. Se os resultados ou informações fornecidas forem utilizados para fins de publicação científica, nenhum nome será utilizado.

FORNECIMENTO DE INFORMAÇÃO ADICIONAL:

Eu entendo que posso requisitar informações adicionais relativas ao estudo a qualquer momento. A Dra. Ana Carolina Coan, tel (19) 3521-9217, estará disponível para responder minhas questões e preocupações. Em caso de recurso, dúvidas ou reclamações contactar a secretaria da Comissão de Ética da Faculdade de Ciências Médicas-UNICAMP, tel. (19) 3521-7232.

RECUSA OU DESCONTINUAÇÃO DA PARTICIPAÇÃO:

Eu entendo que a minha participação é voluntária e que eu posso me recusar a participar ou retirar meu consentimento e interromper a minha participação no estudo a qualquer momento sem comprometer os cuidados médicos que recebo atualmente ou receberei no futuro no HC- UNICAMP. Eu reconheço também que a Dra. Ana Carolina Coan pode interromper a minha participação nesse estudo a qualquer momento que julgar apropriado.

FORMULÁRIO DE CONSENTIMENTO PARA PESQUISA MÉDICA,

Título do projeto: **Eletroencefalografia e Ressonância Magnética funcional**

Investigador principal: Dra. Ana Carolina Coan

Orientador: Dr. Fernando Cendes

Eu confirmo que o(a) Dr(a)._____me explicou o objetivo do estudo, os procedimentos aos quais serei submetido e os riscos, desconforto e possíveis vantagens advindas desse projeto de pesquisa. Eu li e compreendi esse formulário de consentimento e estou de pleno acordo em participar desse estudo.

_____

Nome do participante ou responsável

_____ _____

Assinatura do participante ou responsável data

_____

Nome da testemunha

_____ _____

Assinatura da testemunha data

RESPONSABILIDADE DO PESQUISADOR:

Eu expliquei a _____ o objetivo do estudo, os procedimentos requeridos e os possíveis riscos e vantagens que poderão advir do estudo, usando o melhor do meu conhecimento. Eu me comprometo a fornecer uma cópia desse formulário de consentimento ao participante ou responsável.

_____

Nome do pesquisador ou associado

_____ _____

Assinatura do pesquisador ou associado data