UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS: FARMACOLOGIA E TERAPÊUTICA

Gabriela Gregory Regner

AVALIAÇÃO PRÉ-CLÍNICA DA ESTIMULAÇÃO TRANSCRANIANA POR CORRENTE CONTÍNUA: UMA NOVA ABORDAGEM TERAPÊUTICA PARA O TRATAMENTO DA EPILEPSIA

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Elaborada por

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Como requisito parcial para a obtenção do título de

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“Em terra de egos, quem vê o outro é rei”

Resumo

Epilepsia é o termo utilizado para descrever uma síndrome cerebral crônica de diversas etiologias caracterizada por recorrentes crises convulsivas devido a descargas neuronais excessivas, sendo um dos principais transtornos neurológicos em adultos e crianças. Apesar da grande variedade de fármacos antiepiléticos, muitos pacientes relatam efeitos adversos que impedem o seu uso continuado. Além disso, há um contingente de pacientes refratário aos tratamentos farmacológicos disponíveis. Portanto, o desenvolvimento de novas abordagens terapêuticas que possam fazer uso de mecanismos múltiplos de ação e efeitos adversos minimizados – como a estimulação transcraniana por corrente contínua (ETCC) – é considerado de grande interesse na terapêutica. Poucos estudos pré-clínicos utilizando a técnica de ETCC foram traduzidos em pesquisa clínica (número de sessões e efeitos na frequência de crises). Além disto, a maioria dos achados não foi testada (efeitos de ETCC no estado de mal epilético, crises de ausência, efeitos neuroprotetores no hipocampo e seu uso combinado com um fármaco específico). Já os estudos clínicos com ETCC foram testados em várias síndromes epilépticas, mas grande parte destes estudos clínicos não foram previamente avaliada em estudos pré-clínicos (encefalite de Rasmussen, epilepsia resistente a medicamentos e epilepsia por esclerose hipocampal). É importante salientar que, a maioria dos estudos envolvendo epilepsia e ETCC mostra resultados positivos. Nesta tese foi avaliado o efeito da ETCC em ratos submetidos ao modelo de *kindling* por pentilenotetrazol (PTZ), sobre parâmetros comportamentais convulsivos e bioquímicos, bem como comparou o efeito da estimulação anodal e catodal associadas ou não ao fármaco anticonvulsivante diazepam, buscando avaliar um possível sinergismo entre ETCC e o tratamento farmacológico. Os parâmetros neuroquímicos avaliados foram níveis de IL1-β, TNFα, NGF e BDNF) em hipocampo e córtex, após o tratamento de

kindling e após a ETCC. Nem a a estimulação transcraniana por corrente contínua anodal (ETCC-a) nem a a estimulação transcraniana por corrente contínua catodal (ETCC-c) reduziram a ocorrência de convulsões clônicas do membro anterior. Porém quando associado ao diazepam (DZP), a ETCC-c (ETCC-c/DZP0.15) aumentou a latência para a primeira convulsão no quarto e sexto dias. Os níveis de IL-1β do hipocampo foram reduzidos por ETCC-c e ETCC-c/DZP0.15. Por outro lado, esses tratamentos induziram um aumento nos níveis corticais de IL-1β. Os níveis de TNF-α no hipocampo não foram alterados por ETCC-c ou ETCC-a, mas ETCC-c e ETCC-c/DZP0.15 aumentaram seus níveis no córtex cerebral. Os níveis de NGF cortical foram aumentados pela ETCC-c e pela ETCC-c/DZP0.15. ETCC-a/DZP0.15 reduziu os níveis de BDNF no hipocampo e ETCC-c/DZP0.15 aumentou esses níveis no córtex cerebral. Em conclusão, a ETCC-c isolada ou em combinação com uma dose baixa de DZP mostrou efeitos neuroprotetores, melhorando os níveis de neurotrofinas centrais e diminuindo os níveis de IL-1β hipocampal após o estímulo repetido induzido por PTZ, sem efeito estatisticamente significativo no comportamento convulsivo. Esta técnica pode ter uso potencial na epilepsia muito provavelmente como adjuvante na farmacoterapia convencional.

Palavras-chave: *kindling*, estimulação transcraniana por corrente contínua (ETCC), inflamação

Abstract

Epilepsy is the term used to describe a chronic cerebral syndrome of various etiologies characterized by recurrent seizures due to excessive neuronal discharges being one of the major neurological disorders in adults and children. Despite the wide variety of antiepileptic drugs, many patients report adverse effects that prevent their continued use. Besides, there is a contingent of patients refractory to available pharmacological treatments. Therefore, the development of new therapeutic approaches that may make use of multiple mechanisms of action and minimized adverse effects - such as transcranial direct current (tDCS) stimulation - is considered of great therapeutic interest. Few preclinical studies using the tDCS technique have been translated into clinical research (number of sessions, effects on the frequency of seizures). In addition, most of the findings were not tested (tDCS effects on the status of epilepsy, absence crises, neuroprotective effects on the hippocampus and their combined use with a specific drug). Clinical studies with tDCS have been tested in several epilepsy syndromes, but most of these clinical studies have not been previously evaluated in preclinical studies (Rasmussen encephalitis, drug resistant epilepsy and epilepsy due to hippocampal sclerosis). It is important to note that most studies involving epilepsy and tDCS show positive results. In this thesis, the effect of tDCS on rats submitted to the kindling model by pentylenetetrazole (PTZ) on behavioral and biochemical parameters was evaluated, as well as the effect of anodal and catodal stimulation associated or not with the anticonvulsant drug diazepam, in order to evaluate a possible synergism between the tDCS and the pharmacological treatment. The neurochemical parameters evaluated were levels of IL1-β, TNFα, NGF and BDNF) in hippocampus and cortex, after the kindling treatment and tDCS. Neither anodal-tDCS (a-tDCS) nor cathodal-tDCS (c-tDCS) reduced the occurrence of clonic seizures of the anterior limb.

Associated with diazepam (DZP), c-tDCS (c-tDCS/DZP0.15) increased the latency for the first seizure on the 4th and 6th days. The levels of IL-1β from the hippocampus were reduced by c-tDCS and c-tDCS/DZP0.15. On the other hand, these treatments induced an increase in the cortical levels of IL-1β. TNF-α levels in the hippocampus were not altered by c-tDCS or a-tDCS, but c-tDCS and c-tDCS/DZP0.15 increased their levels in the cerebral cortex. Cortical NGF levels were increased by c-tDCS and c-tDCS/DZP0.15. a-tDCS/DZP0.15 reduced levels of BDNF in the hippocampus and c-tDCS/DZP0.15 increased those levels in the cerebral cortex. In conclusion, c-tDCS alone or in combination with a low dose of DZP showed neuroprotective effects, improving central neurotrophin levels and decreasing levels of hippocampal IL-1β after repeated PTZ-induced stimulation, with no statistically significant effect on convulsive behavior. This technique may have potential use in epilepsy most likely as adjuvant in conventional pharmacotherapy.

Keywords: kindling, transcranial direct-current stimulation (tDCS), inflammation

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Lista de Abreviaturas

a-tDCS	Anodal transcranial direct-current stimulation
BHE	Barreira hematoencefálica
BDNF	Fator Neurotrófico Derivado do Cérebro (Brain derived neurotrophic factor, em inglês)
c-tDCS	Cathodal transcranial direct-current stimulation
Cl-	Íon cloreto
COX-2	Ciclo-oxigenase-2
DZP	Diazepam
EEG	Eletroencefalograma
ELTM	Epilepsia do lobo temporal mesial
ERNS	Espécies reativas de nitrogênio
EROS	Espécies reativas de oxigênio
EMT	Estimulação magnética transcraniana
ETCC	Estimulação transcraniana por corrente contínua
ETCC-a	Estimulação transcraniana por corrente contínua anodal
ETCC-c	Estimulação transcraniana por corrente contínua catodal
GABA	Ácido-γ-aminobutírico
$GABA_A$	Receptor $GABA_A$
GluN2B	Subunidade do receptor NMDA (NMDAR2)
IL-1 β	Interleucina -1 β
IL-6	Interleucina -6

ILAE	International League Against Epilepsy
iNOS	Óxido nítrico sintase induzível
K^+	Potássio
LTD	Depressão de longa duração
LTP	Potenciação de longa duração
Na^+	Sódio
Na^+/K^+ ATPASE	Bomba de sódio/potássio
NGF	Fator de crescimento neural (Nerve growth factor, em inglês)
NADPH oxidase	Nicotinamida adenina dinucleotídeo fosfato-oxidase
NMDA	N-metil-D-Aspartato
OMS	Organização Mundial da Saúde
PTZ	Pentilenotetrazol
RM	Ressonância magnética
RNAm	Ácido ribonucleico mensageiro
Sal	Solução salina
SNC	Sistema Nervoso Central
TCG	Crises tônico-clônicas generalizadas
tDCS	Transcranial direct-current stimulation
TGF-β	Fator de crescimento transformador beta (Transforming Growth Factor beta, em inglês)
TNF-α	Fator de necrose tumoral-alfa (Tumor Necrosis Factor-alpha, em inglês)
TNFR1	Receptor TNF tipo 1
TNFR2	Receptor TNF tipo 2

WKY Ratos Wistar Kyoto

SUMÁRIO

1 INTRODUÇÃO

Epilepsia é um termo utilizado para descrever uma síndrome cerebral crônica de várias etiologias caracterizada por recorrentes crises convulsivas devido a descargas neuronais excessivas (Chindo et al., 2014), sendo um dos principais transtornos neurológicos em adultos e crianças. Segundo dados da OMS (2018), 50 milhões de pessoas são acometidas pela síndrome ao redor do mundo. Atualmente, a epilepsia tem sido reconhecida como um transtorno da excitabilidade cerebral caracterizada por crises recorrentes não provocadas (Chao et al., 2013) que resultam da atividade anormal, excessiva e sincronizada dos diferentes agrupamentos de células nervosas do cérebro (Amiri et al., 2012). Epilepsia está associada a uma variedade de sintomas clínicos como alterações da consciência, de movimentos e de sensações. É importante salientar que após longo tempo de evolução da doença, verifica-se uma deterioração de conduta sob a forma de impulsividade, irritabilidade, ataques de fúria, rebaixamento da cognição, concentração e juízo crítico, hipobulia, diminuição do altruísmo, criatividade e compaixão, enquanto aumentam o egocentrismo, individualismo, instintos e impulsos primitivos (Sugimoto et al., 2012).

Epilepsia afeta aproximadamente 0,5-1% da população mundial (Dhir et al., 2005). Contudo, apesar da introdução de uma variedade de novos fármacos antiepiléticos, muitos pacientes relatam efeitos adversos que impedem o uso continuado dos mesmos, e dados epidemiológicos indicam que 20-40% dos pacientes com diagnósticos recentes de epilepsia se tornarão refratários ao tratamento (French, 2007; Loscher e Schmidt, 2004). Portanto, o desenvolvimento de novas abordagens terapêuticas, que possam fazer uso de mecanismos múltiplos de ação, com menos efeitos adversos é considerado de grande interesse na terapêutica (White, 1997; Schmidt e Rogawski, 2002; Wolfgang, 2005).

Mais especificamente, a epilepsia do lobo temporal mesial (ELTM) apresenta esclerose hipocampal como uma alteração histológica comum nestes pacientes (Andrade-Valença et al., 2008). Um estudo utilizando morfometria por ressonância magnética (RM) demonstrou que o dano neuronal em pacientes com ELTM se estende além do hipocampo e acomete regiões que se conectam funcionalmente e anatomicamente ao hipocampo. Tal achado sugere que exista lesão abrangendo uma rede neuronal, o que pode ser responsável em conjunto pelas manifestações clínicas

observadas nesses pacientes (Holmes et al., 2013). As causas da refratariedade ao fármaco e ao tratamento cirúrgico ainda são desconhecidas, porém supõe-se que um dos motivos seja a presença de lesão neuronal acometendo outras áreas cerebrais além do hipocampo (Andrade-Valença et al., 2008). Considerando que uma grande parcela de indivíduos é refratária aos tratamentos existentes a busca de novas alternativas terapêuticas, profícua.

Muitas das sequelas da epilepsia crônica resultam do estresse oxidativo e excitotoxicidade com dano mitocondrial e morte celular (Cárdenas- Rodriguez et al., 2013; Li et al., 2013; Chindo et al., 2014). Espécies reativas de oxigênio (EROS) e espécies reativas de nitrogênio (ERNS) têm despertado grande interesse da medicina clínica e experimental. O estresse oxidativo está fortemente implicado em convulsões induzidas por excitotoxicidade, devido à geração de EROS na mitocôndria. Comparado a outros tecidos, o cérebro é altamente suscetível a dano por estresse oxidativo devido ao alto nível de metabolismo oxidativo e baixos níveis tanto de enzimas varredoras de radicais livres como de moléculas antioxidantes (Zhen et al., 2014). Neste contexto, estudos experimentais têm relacionado fortemente o estresse oxidativo e dano celular a convulsões induzidas por fármacos como pentilenotetrazol (PTZ) e pilocarpina (Costa et al., 2012), tornando-se estes, importantes modelos experimentais para a avaliação de potenciais terapias anticonvulsivantes (Zhao et al., 2014).

Estudos clínicos e experimentais demonstram o envolvimento do eixo neuroimune na fisiopatologia da epilepsia. A indução experimental de epilepsia em áreas corticais de roedores na região CA3 do hipocampo induz aumento da expressão de RNAm de diversas citocinas, tais como TNFα, IL-1β e IL-6. Da mesma forma, em pacientes humanos, uma única convulsão aumenta os níveis de IL-1β em células do sistema nervoso central (SNC). A remoção cirúrgica do foco epiléptico por lobectomia temporal anterior não só impede quaisquer outras crises epilépticas nestes pacientes, mas também reduz acentuadamente os níveis séricos de TNFα e de IL-1β. Considerados em conjunto, estes dados sugerem que durante crises epilépticas a atividade neuronal ativa as células da glia promovendo a liberação de citocinas pró-inflamatórias, e envolve vários parâmetros adicionais de inflamação neurogênica (Xanthos e Sandkuhler, 2014).

Além de tratamento farmacológico, o tratamento não farmacológico da epilepsia inclui cirurgia, estimulação do nervo vago, dieta cetogênica e outras terapias

alternativas e/ou complementares. Terapias alternativas incluem técnicas como yoga, acupuntura, quiropraxia, massagem terapêutica, biofeedback de EEG, aromaterapia, homeopatia, ervas medicinais (medicina tradicional chinesa), etc. Tais intervenções são muitas vezes complementares ao tratamento farmacológico (Saxena e Nadkarni, 2011).

Métodos relacionados à estimulação do SNC também têm sido estudados, tais como o uso da estimulação cortical invasiva e da estimulação cerebral não invasiva; esta última dividida em duas modalidades: estimulação magnética transcraniana (EMT) e estimulação transcraniana por corrente contínua (ETCC). Modalidades menos invasivas de estimulação cerebral, como a ETCC, têm mostrado bons resultados em pacientes com depressão, acidente vascular cerebral e alterações da excitabilidade cortical como distonia focal, cefaleia, dor crônica, depressão e epilepsia (Liebetanz et al., 2006a).

ETCC induz mudanças na excitabilidade cortical, apresenta baixo risco e pouco desconforto, e com a utilização em sessões repetidas o efeito pode ser duradouro (Nitsche et al., 2008). ETCC envolve aplicação de corrente galvânica constante de baixa intensidade (1-2 mA) no escalpo por meio de eletrodos (20-35 cm^2) para modular a excitabilidade de áreas corticais. Estimulação anodal tipicamente despolariza (aumenta excitação) e estimulação catodal hiperpolariza (diminui excitação) os neurônios (Nitsche et al., 2008; Rosen et al., 2009).

ETCC tem sido aplicada no tratamento da epilepsia, espasticidade, transtornos do movimento, doença vascular periférica e de certos transtornos psiquiátricos (Raghavan et al., 2008). O efeito agudo da ETCC (efeito imediato) ocorre devido a uma diminuição (anódica) ou aumento (catódica) do limiar de repouso neuronal (Ruscheweyh et al., 2011). No entanto, os efeitos em longo prazo envolvem a participação do fator neuronal derivado do cérebro (BDNF) e de receptores glutamatérgicos NMDA em mecanismos de plasticidade sináptica (Fertonani et al., 2010).

Kamida e colaboradores (2013) demonstraram que estimulação catodal tem efeito anticonvulsivante por pelo menos um dia em modelo de convulsão induzida por estimulação elétrica na amígdala de ratos, e com efeitos positivos na performance cognitiva. Atualmente não existem trabalhos demonstrando o efeito da ETCC em modelos animais de epilepsia induzida quimicamente. Portanto, a ETCC de fraca intensidade é uma modalidade de estimulação cerebral não-invasiva, indolor, que é bem tolerada e não apresenta nenhuma sensação auditiva ou sensitiva desagradável como

outras técnicas de estimulação cerebral (Nitsche et al., 2003). A segurança dessa técnica também tem sido constantemente observada. Não há possibilidade de causar lesão cerebral pela formação de produtos tóxicos, pois não há interação dos eletrodos com o córtex cerebral (Nitsche et al., 2003). Estudo com ressonância magnética antes e após 30 e 60 minutos da estimulação cerebral aplicada em córtex motor ou pré-frontal não indicou alteração patológica, concluindo que a ETCC não induz edema cerebral, alterações da barreira hematoencefálica ou do tecido cerebral (Nitsche et al., 2004). Por fim, estudo de Accornero e colaboradores (2007) mostrou que durante e após 20 minutos do término da estimulação não houve variações em batimento cardíaco, pressão arterial ou temperatura. Trata-se de método seguro para ser empregado em seres humanos e apresenta vantagem de poder ser combinada com outras intervenções. Com isso, a ETCC poderá ser um método adicional no tratamento da epilepsia, porém estudos adicionais focando mudanças neuroquímicas e moleculares devem ser realizados para confirmar essa hipótese.

2 REVISÃO DA LITERATURA

2.1 Epilepsia

Epilepsia é considerada um transtorno neurológico multifatorial e altamente diversificado sintomatologicamente, caracterizada por crises recorrentes não provocadas. Estudos fisiológicos mostram que as convulsões refletem hiperatividade transitória, anormal e síncrona de uma população de neurônios no cérebro. Tal disfunção cerebral pode ser acompanhada por alterações motores, sensoriais e autonômicas dependendo da região cerebral envolvida na origem e/ou na propagação das crises (Lasoń et al., 2013).

Em 2014, a Liga Internacional Contra Epilepsia (ILAE – *International League Against Epilepsy*) divulgou uma definição conceitual de convulsões e epilepsia, seguida de uma definição operacional (prática). As principais mudanças foram: (1) epilepsia pode existir após duas convulsões não provocadas com mais de 24 horas de intervalo (definição antiga) ou uma crise não provocada quando se sabe que o risco para outro é alto (> 60%); (2) convulsões reflexas e convulsões que fazem parte de uma síndrome de epilepsia constituem epilepsia; (3) epilepsia pode ser considerada resolvida quando uma síndrome dependente da idade é superada ou quando uma pessoa está livre de crises por pelo menos dez anos, sendo os últimos cinco anos livre fármacos anti- epilépticos (Fisher et al., 2014).

A presença de epilepsia é definida pela recorrência de crises epilépticas espontâneas (pelo menos duas) - não provocadas por febre, insultos agudos SNC ou desequilíbrios tóxico-metabólicos graves (Gallucci Neto e Marchetti, 2005; Marchetti, 2001). Epilepsias podem ser classificadas de acordo com dois grandes eixos: topográfico e etiológico. No eixo topográfico, epilepsias são separadas em generalizadas e focais. As generalizadas caracterizam-se por crises epilépticas cujo início envolve ambos os hemisférios simultaneamente. De forma geral, são geneticamente determinadas e acompanhadas de alteração da consciência; quando presentes, as manifestações motoras são sempre bilaterais. Como seus principais exemplos temos as crises de ausência, crises mioclônicas e crises tônico-clônicas generalizadas (TCG) (Figura 1) (Berg et al., 2010; Guilhoto, 2011).

Já nas epilepsias focais, as crises epilépticas iniciam de forma localizada numa área específica do cérebro, e suas manifestações clínicas são dependentes do local de início, bem como da velocidade de propagação da descarga epileptogênica. As crises dividem-se em focais simples (sem comprometimento da consciência) e focais complexas (com comprometimento ao menos parcial da consciência durante o episódio). Por fim, uma crise focal, seja simples ou complexa, quando propagada para todo o córtex cerebral, pode terminar numa crise TCG, recebendo a denominação de crise focal secundariamente generalizada (Elger e Schmidt, 2008).

Figura 1. Classificação de crises expandida

Fonte: Berg et al., (2010); Guilhoto, (2011)

No eixo etiológico, as epilepsias são divididas em genética, estrutural/ metabólica e de causa desconhecida. O conceito de epilepsia genética é de que a síndrome é resultado direto de um defeito (s) genético (s) conhecido ou presumido (s) em que as convulsões são a principal expressão desta alteracão, por exemplo, esclerose tuberosa e malformações do desenvolvimento cortical. No entanto, não é excluída a possibilidade de que fatores ambientais (externos ao indivíduo) possam contribuir para a expressão da doença (Berg et al., 2010). Entende-se por causa estrutural/ metabólica o fato de que, conceitualmente, há uma outra condição estrutural ou metabólica distinta ou outra doença que demonstraram estar associadas ao risco aumentado de desenvolver epilepsia (lesões estruturais, como acidente vascular cerebral, trauma e infecção). no entanto, existe um transtorno distinto entre o defeito genético e a epilepsia. Epilepsias de causa desconhecida devem ser vistas de forma neutra e indicar que a natureza da

causa subjacente ainda é desconhecida; podem ser oriundas de um defeito genético fundamental em sua essência ou ainda a consequência de uma outra alteraçao ainda não reconhecida (Berg et al., 2010).

Os mecanismos celulares de geração de convulsões envolvem excitação "runaway" rítmica ou tônica ou a interação sincronizada e rítmica entre neurônios excitatórios e inibitórios e condutâncias de membrana (Lasoń et al., 2013). Tem sido aceito que as crises epiléticas podem ser geradas em resposta a desbalanço entre influências excitatórias e inibitórias, resultando em despolarização tônica ou descargas neuronais repetitivas e rítmicas (Sterman e Thompson, 2014). O primeiro mecanismo compreende hiperatividade da transmissão glutamatérgica e transtornos funcionais de canais de sódio e cálcio ligantes ou voltagem-dependentes. A deficiência nos processos de inibição está relacionada principalmente à inibição insuficiente mediada pelo receptor $GABA_A$ e por correntes de potássio extracelulares (Lasoń et al., 2013).

2.2 Fisiopatologia da epilepsia

Caracterizada por descargas neuronais anormais, a epilepsia é considerada uma desordem paroxística. Apesar da sua etiologia ser diversa, a alteração fundamental é secundária às descargas sincrônicas de uma rede de neurônios, devido às alterações nas membranas neuronais ou desequilíbrio entre influências excitatórias e inibitórias (Browne e Holmes, 2008).

Com a finalidade de manter o potencial de repouso, a enzima transmembrana Na^+/K^+ ATPase libera Na^+ para o meio extracelular e K^+ para o meio intracelular, na proporção de 3 $Na^+/2K^+$, permitindo que o interior da membrana fique polarizada, ou seja, com carga elétrica negativa em relação ao seu exterior. A despolarização (potencial de ação) ocorre com o estímulo nervoso, quando acontece a abertura de canais de Na^+, ocasionando a sua entrada para o ambiente intracelular. Em seguida, com a saída de K^+ pela abertura de seus canais e por meio do transporte ativo de Na^+ para o meio extracelular através da Na^+/K^+ ATPase, ocorre a repolarização da membrana. É denominada de impulso nervoso essa sequência de despolarizações/repolarizações que se propagam ao longo do neurônio (Glynn, 1993; Skou & Esmann, 1992; Jorgensen, 1990; Skou, 1990).

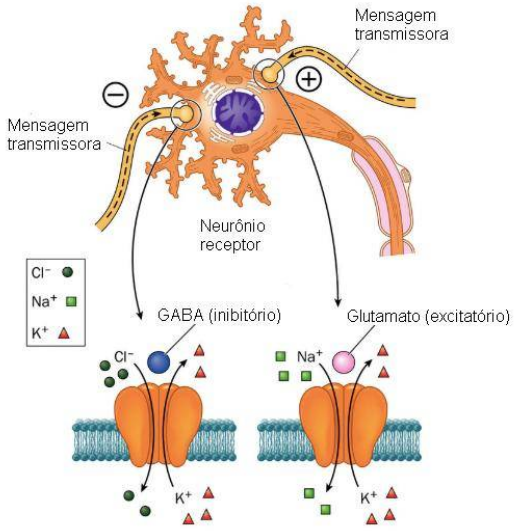
Intimamente controlada pela abertura ou bloqueio de canais iônicos operados por voltagem, que são regulados pelo influxo de cátions para o interior do neurônio, essa excitabilidade intrínseca do sistema nervoso tem papel importante na deflagração das crises epilépticas. Algumas formas de epilepsias são relacionadas a mutações em subunidades dos canais de Na^+ dependentes de voltagem no SNC, importantes para a rápida despolarização da membrana neuronal, que ocorre amplamente e de forma desordenada nos processos epilépticos (Porto et al., 2007).

Evitando a repetição do potencial de ação, os canais de K^+ dependentes de voltagem participam da repolarização e hiperpolarização da membrana que segue as alterações paroxísticas da despolarização. A geração de hiperexcitabilidade pode estar relacionada a mutações nos genes responsáveis pela formação dos canais de potássio, devido à diminuição na repolarização (Porto et al., 2007).

2.2.1 Glutamato e GABA

O glutamato é o principal neurotransmissor excitatório no SNC, enquanto que o ácido γ-aminobutírico (GABA) é o principal neurotransmissor inibitório (Figura 2). O balanço do tônus glutamatérgico e gabaérgico é crucial para a função neurológica normal (Guerriero et al., 2015).

Figura 2. Efeitos do GABA e glutamato na transmissão do impulso nervoso dos neurônios transmissores aos neurônios receptores



Fonte: Adaptado de https://es.dreamstime.com/foto-de-archivo-neurotransmisores-implicados-en-epilepsia-image12522920

Por um lado, a liberação aguda de glutamato após trauma causa excitotoxicidade induzindo lesão cerebral, decorrente de lesão neuronal, morte celular e disfunção de neurônios sobreviventes. Por outro lado, a interrupção retardada de circuitos excitatórios de glutamato leva a deficiências na função cognitiva e motora e na plasticidade experiência-dependente (Guerriero et al., 2015).

GABA modula vias excitatórias no SNC e, após lesão, a perda de células produtoras de GABA altera o balanço entre excitação e inibição levando a lesão celular e apoptose. Os resultados da excitotoxicidade glutamatérgica compartilham elementos comuns observados em traumatismos crânio-encefálicos leves (Giza e Hovda, 2014) a severos, estado de mal epilético, isquemia, e doenças neurodegenerativas (Arundine e Tymianski, 2004).

A ruptura do equilíbrio entre excitação e inibição é o mecanismo mais aceito para o estabelecimento de hiperexcitabilidade. No SNC adulto, este mecanismo implica em aumento da neurotransmissão glutamatérgica e/ou em supressão da neurotransmissão GABAérgica (DiNuzzo et al., 2014). Além disto, lesões neuronais traumáticas podem levar a danos celulares por meio de disfunção mitocondrial, perda axonal, estresse oxidativo e anormalidades vasculares cerebrais (Guerriero et al., 2015). Portanto, é importante salientar que as crises epilépticas não são somente o resultado de hiperexcitação, mas também o resultado de um desequilíbrio entre influências inibitórias e excitatórias (Scharfman, 2007). Algumas vezes, a perda da inibição, ao contrário de aumento específico na excitação, pode estar no cerne da condição epiléptica (Yu et al., 2006; Ogiwara et al., 2007).

O receptor do tipo NMDA de glutamato (N-metil-D-aspartato) pós-sináptico possui atuação importante nas alterações despolarizantes capazes de produzir descargas epilépticas (França, 1998). A perda de função de receptores $GABA_A$ também tem sido relacionada a síndromes epilépticas tanto em humanos como em roedores (DiNuzzo et al., 2014), assim como a ineficiência do neurotransmissor GABA tem sido relacionada a maior excitabilidade neuronal (Błaszczyk, 2016). Tais anomalias na neurotransmissão, seja o aumento da transmissão excitatória, a redução da transmissão inibitória ou ambas

situações (Figura 2), alteram a excitabilidade neuronal e suas conexões sinápticas resultando em crises convulsivas (Meldrum, 1984).

2.3 Neuroinflamação

Epileptogênese está associada, em conjunto com danos neuronais sutis, gliose e microgliose, a um estado inflamatório exacerbado e persistente no microambiente do tecido neural (Alyu and Dikmen, 2016). Os processos inflamatórios podem ter origem no SNC ou serem decorrentes da circulação sistêmica por meio de ruptura na barreira hematoencefálica (BHE) (Choi and Koh, 2008). Neuroinflamação é caracterizada por ativação da microglia, astrócitos e células endoteliais da barreira hematoencefálica, assim como por infiltração de proteínas plasmáticas e células imunológicas. Os membros mais conhecidos dentre as moléculas inflamatórias podem ser classificados em enzimas pró-inflamatórias, incluindo COX-2, óxido nítrico sintase induzível (iNOS), e NADPH oxidase (NOX); citocinas como IL-1β, IL-6, e TNF-α; e fatores de crescimento como o NGF (fator de crescimento neuronal) e de plasticidade como o BDNF (fator neurotrófico de derivado do cérebro) (Dey et al., 2016). As citocinas, proteínas que modulam processos inflamatórios, são principalmente produzidas por células gliais e neurônios durante o processo neuroinflamatório (Alyu and Dikmen, 2016). Citocinas pró-inflamatórias, IL-Iβ, IL-2 e IL-6, tipicamente encontradas em pequenas quantidades no encéfalo, tem seus níveis aumentados após crises convulsivas (Scorza et al., 2018).

IL-Iβ, uma citocina pró-inflamatória, expressa em micróglia ativada e astrócitos, induz o aumento da liberação de glutamato em astrócitos e a diminuição da recaptação de glutamato com consequente hiperexcitabilidade neuronal (Alyu and Dikmen, 2016). Tem sido sugerido que a IL-1β induz convulsões por meio da supra-regulação de receptores NMDA em células pós-sinápticas via ativação da subunidade GluN2B do receptor NMDA (Viviani et al., 2003). Também tem sido relatado que a citocina IL-1β está significativamente aumentada no líquor em criancas epilépticas sugerindo o papel importante desta citocina no início e na progressão da epilepsia (Shi et al., 2017; Rana e Musto, 2018).

Alterações moleculares e neuroquímicas são comumente observadas *post-mortem* em cérebro de pacientes com epilepsia e em estudos pré-clínicos utilizando

modelos animais de epilepsia (Vezzani et al., 2011). Além disso, estudos recentes demonstram que crises convulsivas induzem aumento da permeabilidade da BHE, intensificando e perpetuando a neuroinflamação decorrente do extravasamento de leucócitos e moléculas inflamatórias de vasos sanguíneos no parênquima cerebral (Gorter et al., 2015). Em geral, convulsões prolongadas iniciais podem provocar respostas imunológicas e inflamatórias agudas no encéfalo, enquanto que convulsões espontâneas e recorrentes subsequentes sustentam a neuroinflamação crônica (Dey et al., 2016).

2.4 Tratamentos farmacológicos

Também conhecidos como anticonvulsivantes, os fármacos antiepilépticos são usados no tratamento da epilepsia e de alterações convulsivas não epileptiformes (Rang e Dale, 2011). Quando administrados por um determinado período, os anticonvulsivantes diminuem a incidência ou a severidade das crises epilépticas. Várias propostas terapêuticas para o tratamento das epilepsias foram utilizadas antes do desenvolvimento dos anticonvulsivantes, dentre elas oculosterapia, ligadura das artérias vertebrais, compressão testicular, trepanação, aplicação de ventosas, histerectomia e o uso de preparações a base de ervas e extratos animais (Porto et al., 2007).

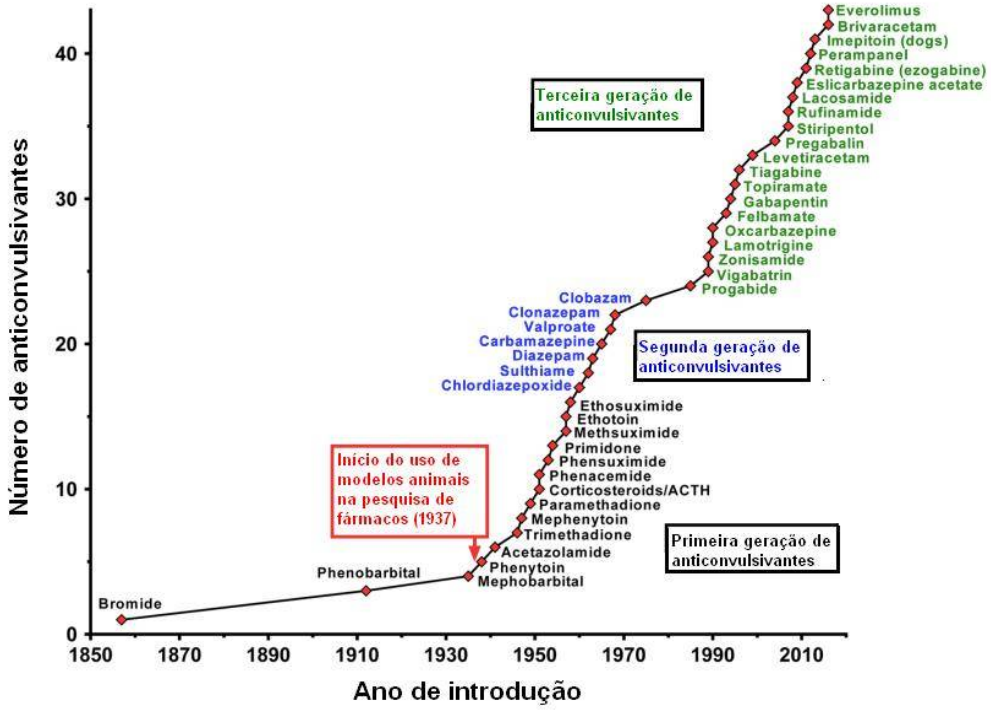
Fármacos antiepilépticos agem no SNC de duas maneiras: reduzindo as descargas elétricas patológicas ou inibindo a propagação de atividade elétrica aberrante. Isto pode ocorrer por meio de efeitos em canais iônicos específicos, neurotransmissores inibitórios ou excitatórios. Embora haja múltiplos efeitos neurofisiológicos dos anticonvulsivantes teorizados e hipotetizados, é importante reconhecer que os verdadeiros mecanismos de ação desses agentes são pouco compreendidos e podem ser multifatoriais (Dichter, 1994).

Em cerca de dois terços dos pacientes, as crises convulsivas podem ser suprimidas com o uso dos anticonvulsivantes. Os pacientes devem tomar o fármaco diariamente, mesmo que na maioria dos dias fiquem sem crises (French, 2017). Apesar da otimização da terapia farmacológica, cerca de 10% dos pacientes permanecem experimentando crises com intervalos de um mês ou menos, comprometendo a qualidade vida e a atividade laboral (Rang e Dale, 2011).

O sal de brometo de potássio foi introduzido por Locock (1857) e utilizado como primeiro fármaco antiepiléptico eficaz, baseando-se o na ideia errônea de que os pacientes com epilepsia tinham hipersexualidade (Figura 3). Locock usou o sal e observou resposta positiva em 14 de 15 mulheres com epilepsia catamenial (Porto et al., 2007).

Durante as últimas três décadas, a introdução de mais de 15 fármacos antiepilépticos de terceira geração forneceu aos médicos e pacientes mais opções para o tratamento de muitos tipos de convulsões (Loscher et al., 2013). Dentre o rol de antiepilépticos disponível no mercado (Tabela 1), temos os fármacos de primeira geração (fenitoína, carbamazepina, fenobarbital, diazepam e valproato de sódio), que mesmo apresentando um número significativo de efeitos adversos, permanecem sendo amplamente utilizados (Porto et al., 2007; Rang e Dale, 2011). Entretanto, com o surgimento dos anticonvulsivantes de segunda (lamotrigina, vigabatrina, tiagabina, topiramato, gabapentina e leviracetam) e de terceira gerações, o tratamento da epilepsia tem apresentado muitos avanços. Todavia, um número considerável de pacientes continua sem reduções significativas das crises. Ademais, um contigente de pacientes sofre mais danos em decorrência do tratamento farmacológico do que em relação à condição epiléptica, o que justifica a busca por novas alternativas terapêuticas (Porto et al., 2007).

Figura 3. Número de anticonvulsivantes e ano de introdução



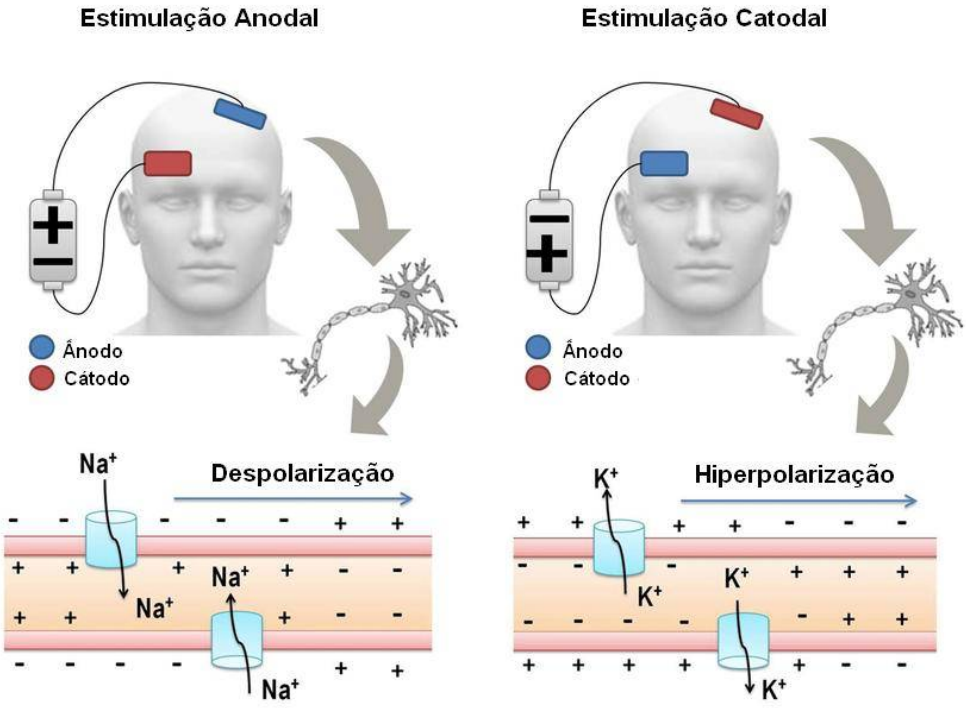
Fonte: Loscher, 2017

2.5 Estimulação Transcraniana por Corrente Contínua (ETCC)

Estimulação transcraniana por corrente contínua (ETCC) é uma técnica de estimulação cerebral não invasiva baseada na alteração do potencial de repouso da membrana neuronal para induzir alterações da excitabilidade cortical. ETCC induz um campo elétrico constante transcraniano, induzindo mudanças na excitabilidade cortical instantâneas (‘online’) e de longa duração (‘offline’) em nível de excitabilidade cortical por meio de um processo de polarização da membrana celular (Paulus, 2003). Originalmente aplicado através de dois eletrodos embebidos com solução salina colocados sobre o couro cabeludo e com intensidades na faixa de 0,5-2 mA, ETCC demonstrou modulação significativa da fisiologia de vários sistemas cerebrais. Os efeitos da ETCC vão desde a modulação em níveis de excitabilidade cortical até redes cognitivas de alta-ordem (Santarnecchi et al., 2015), com resultados promissores para o tratamento de condições neurológicas e psiquiátricas (Liew et al., 2014). São utilizados dois eletrodos, ânodo e cátodo, que, dispostos em diferentes montagens, criam um fluxo

de corrente elétrica contínua de baixa intensidade que atinge uma região específica do córtex cerebral, modulando-a de acordo com a polaridade: a estimulação anodal ou anódica induz despolarização da membrana neuronal enquanto a estimulação catodal ou catódica induz hiperpolarização (Brunoni et al., 2012). Durante a ETCC anodal, a corrente fornecida no "ânodo" atrai íons negativos nos tecidos abaixo do eletrodo: isto reduz o limiar de repouso da membrana, facilitando assim o disparo neuronal (aumenta a excitabilidade cortical). Pelo contrário, os eletrodos com carga negativa (ETCC catodal) afetam regiões próximas ao atrair cargas positivas (Figura 4), aumentando o limiar e fazendo a área estimulada menos propensa a ser ativada em resposta a estímulos endógenos ou exógenos (redução da excitabilidade cortical) (Rossi et al., 2016).

Figura 4. Efeitos da ETCC catódica e anódica



Fonte: Adaptado de http://www.gtmedi.com/proDetail?id=19

Em uma recente revisão (Boon et al., 2018), foram reunidas evidências de qualidade muito baixa a moderada que não foram capazes de demonstrar efeitos antiepilépticos consistentes a partir da realização de uma única sessão de ETCC. Uma única sessão de ETCC, no entanto, não foi associada a nenhum evento adverso grave.

Foram reunidas também evidências de qualidade muito baixa a moderada, demonstrando uma redução na frequência de crises após três ou cinco sessões de ETCC aplicadas em dias consecutivos, sem relatos de eventos adversos graves. Atualmente, as evidências são limitadas a pacientes com síndromes epilépticas específicas, como pacientes com epilepsia do lobo temporal mesial (ELTM) com esclerose hipocampal ou pacientes com síndrome de Lennox-Gastaut. Devido à falta de estudos incluindo pacientes com síndromes epilépticas mais diversas, questões secundárias não foram avaliadas para ETCC.

2.6 Modelo de *Kindling*

Kindling é um modelo crônico de epilepsia em que a administração repetitiva e intermitente de estímulos químicos ou elétricos subconvulsivantes pode levar à amplificação progressiva das convulsões, culminando em atividade convulsiva generalizada (McNamara, 1984). A primeira descrição de *kindling* foi dada por Graham V. Goddard em 1969 (Goddard et al., 1969). Goddard descreveu que o estímulo subconvulsivo inicial (estímulo que não produz nenhum sinal comportamental ou eletrográfico de convulsões), quando aplicado repetidas vezes em ratos, induziria a convulsões completas após algumas estimulações (Goddard et al., 1969). O princípio clássico, "convulsões geram convulsões" (Ben-Ari, 2006), descreve com precisão o princípio subjacente e o resultado do modelo de *kindling*. *Kindling* pode ser induzido por métodos químicos e elétricos (Karler et al., 1989). O *kindling* químico envolve a administração sistemática de certas substâncias químicas convulsivas (Dhir et al., 2012). A indução do *kindling* químico é mais fácil e consome menos tempo se comparado ao *kindling* elétrico. Dentre os protocolos de *kindling* destaca-se o induzido por PTZ, um antagonista do receptor $GABA_A$, que induz convulsões severas após sua administração (Orloff et al., 1949). Sendo este o modelo animal mais amplamente aceito na busca de novos fármacos antiepilépticos. Corda e colegas (1990) associaram o desenvolvimento de *kindling* induzido por PTZ a diminuição da atividade de canais de cloreto acoplados a GABA como demonstrado pela ligação específica de *t*-[^{35}S] butilbiciclofosforotionato e absorção de $^{36}Cl^-$ a preparações de membrana do córtex cerebral de ratos.

Uma vez que as convulsões tenham sido generalizadas, as alterações produzidas pelo modelo de *kindling* geralmente persistem por meses ou anos. É um dos

modelos mais utilizados de epileptogênese e epilepsia do lobo temporal mesial (ELTM). As taxas de animais que atingem o estado epilético no modelo, podem variar entre 20 e 80% (Bascuñana et al., 2016).

Tabela 1. Características dos fármacos antiepilépticos aprovados e utilizados na clínica

Fármaco	Laboratório	Ano de aprovação	Principais mecanismos de ação presumidos	Indicações aprovadas	Utilidade principal	Principais limitações
Anticonvulsivantes de primeira geração						
Brometo de potássio	Dow	1857‡	Potenciação do GABA?	TCGs, convulsões mioclônicas	Convulsões focais e generalizadas	Uso adjuvante, sedativo,
Fenobarbital	Bayer	1912‡	Potenciação do GABA	Convulsões parciais e generalizadas, sedação, transtornos de ansiedade, alterações de sono	Convulsões focais e generalizadas	Indutor enzimático; não é útil para crise de ausência, hipersensibilidade da pele
Fenitoína	Parke-Davis/ Pfizer	1938	Bloqueador de canal de sódio	Convulsões parciais e generalizadas	De primeira linha com uso venoso	Indutor enzimático; hipersensibilidade da pele, farmacocinética não-linear, não é útil para crise de ausência e convulsões mioclônicas
Trimetadiona	Abbott	1946	Bloqueador de canal de cálcio tipo T	Crises de ausência	Crises de ausência	Teratogênico
Primidona	Imperial Chemical Industries	1954	Potenciação do GABA	Convulsões parciais e generalizadas,	Convulsões focais e generalizadas	Indutor enzimático; hipersensibilidade da pele, não é útil para crise de ausência, sedativo
Etossuximida	Parke-Davis/ Pfizer	1958	Bloqueador de canal de cálcio tipo T	Crises de ausência	De primeira linha sem	Sonolência, perda de apetite, náusea,

Continuação

					hipersensibilidade da pele	vômito, depressão, episódios psicóticos, insônia, anemia aplástica rara
Anticonvulsivantes de segunda geração						
Diazepam	Roche	1963	Potenciação do GABA	Transtornos convulsivos, estado de mal epiléptico, ansiedade, abstinência alcoólica	Convulsões focais e generalizadas, uso i.v, sem hepatotoxicidade clínica ou hipersensibilidade cutânea	Uso como adjuvante em emergência; sedativo; induz tolerância farmacológica
Carbamazepina	Novartis	1964	Bloqueio do canal de sódio	Convulsões parciais e generalizadas, dor trigeminal, transtorno bipolar	De primeira linha	Indutor enzimático; hipersensibilidade da pele; não é útil para crises de ausência ou convulsões mioclônicas
Valproato	Sanofi/Abbott	1967	Múltiplo (potenciação do GABA, inibição do glutamato (NMDA), bloqueio dos canais de sódio e cálcio tipo T)	Convulsões parciais e generalizadas, crises de ausência, profilaxia de enxaqueca, transtorno bipolar	De primeira linha, convulsões focais e generalizadas uso i.v, sem hipersensibilidade cutânea	Inibidor enzimático; teratogenicidade substancial; ganho de peso
Clonazepam	Roche	1968	Potenciação do GABA	Síndrome de Lennox–Gastaut, convulsões	Convulsões focais e generalizadas, sem	Uso como adjuvante; sedativo; induz tolerância

Continuação

				mioclônicas, transtornos de pânico	hepatotoxicidade	farmacológica
Clobazam	Hoechst Roussel/ Lundbeck/Sanofi	1975	Potenciação do GABA	Síndrome de Lennox–Gastaut, transtornos de ansiedade	Convulsões focais e generalizadas, sem hepatotoxicidade	Uso como adjuvante; sedativo; induz tolerância
Anticonvulsivantes de terceira geração						
Progabida	Synthelabo	1985	Potenciação do GABA	Síndrome de Lennox–Gastaut, convulsões mioclônicas, hipertonia muscular	Uso raro para convulsões focais	Hepatotoxicidade, não é mais usado amplamente
Vigabatrina	Sanofi/Lundbeck	1989	Potenciação do GABA	Espasmos infantis, crises parciais complexas (atualmente apenas para uso adjunto)	Sem hepatotoxicidade	Não é útil para crises de ausência ou convulsões mioclônicas; perda de visão; ganho de peso
Lamotrigina	GlaxoSmithKline	1990	Bloqueador de canal de sódio	Convulsões parciais e generalizadas, Síndrome de Lennox–Gastaut, transtorno bipolar	De primeira linha, convulsões focais e generalizadas,	Indutor enzimático; hipersensibilidade cutânea
Oxcarbazepina	Novartis	1990	Bloqueador de canal de sódio	Crises parciais	De primeira linha	Indutor enzimático; hipersensibilidade da pele; não é útil para crises de ausência ou convulsões

Continuação

						mioclônicas
Felbamato	Carter-Wallace/ MedPointe Pharmaceuticals	1993	Múltiplo (potenciação do GABA, inibição do glutamato (NMDA), bloqueio dos canais de sódio e cálcio)	Convulsões parciais e generalizadas, Síndrome de Lennox–Gastaut	Convulsões focais e generalizadas	Uso como adjuvante; anemia aplástica; hepatotoxicidade; hipersensibilidade da pele
Gabapentina	Parke-Davis/ Pfizer	1993	Bloqueador de canal de cálcio (subunidade α2δ)	Convulsões parciais e generalizadas, neuralgia pós-herpética e diabética, síndrome das pernas inquietas	Uso como adjuvante; sem hepatotoxicidade	Ganho de peso; não é útil para crises de ausência ou convulsões mioclônicas
Topiramato	Janssen/Johnson & Johnson	1995	Múltiplo (potenciação do GABA, inibição do glutamato (AMPA), bloqueio dos canais de sódio e cálcio)	Convulsões parciais e generalizadas, Síndrome de Lennox–Gastaut, profilaxia de enxaqueca	De primeira linha, convulsões focais e generalizadas, sem hepatotoxicidade	Sonolência, tontura, comprometimento cognitivo, problemas de fala, pedras nos rins, perda de peso
Tiagabina	Novo Nordisk	1996	Potenciação do GABA	Crises parciais	Uso como adjuvante; sem hepatotoxicidade	Não é útil para crises de ausência ou convulsões mioclônicas
Levetiracetam	UCB Pharma	2000	Modulação de SV2A	Convulsões parciais e generalizadas, crises parciais, convulsões tônico-clônicas generalizadas; epilepsia mioclônica	De primeira linha, uso i.v, sem hepatotoxicidade	Não é útil para crises de ausência ou convulsões mioclônicas

Continuação

				juvenil		
Zonisamida	Elan/Eisai	2000	Bloqueador de canal de sódio	Crises parciais	Convulsões focais e generalizadas, sem hepatotoxicidade	Uso como adjuvante; sedativo
Estiripentol	Biocodex	2002	Potenciação do GABA, bloqueador de canal de sódio	Síndrome de Dravet	Sem hepatotoxicidade	Uso como adjuvante
Pregabalina	Pfizer	2004	Bloqueador de canal de cálcio (subunidade α2δ)	Crises parciais, dor neuropática, transtorno de ansiedade generalizada, fibromialgia	Sem hepatotoxicidade	Atualmente apenas para uso adjuvante; não é útil para crises de ausência ou convulsões mioclônicas; ganho de peso
Rufinamida	Eisai	2004	Bloqueio do canal de sódio	Síndrome de Lennox–Gastaut	Uso como adjuvante, sem hepatotoxicidade	Uso como adjuvante
Lacosamida	UCB Pharma	2008	Inativação lenta aprimorada de canais de sódio dependentes de voltagem	Crises parciais	Uso como adjuvante, sem hepatotoxicidade	Uso como adjuvante
Eslicarbazepina (acetato)	Bial/Eisai	2009	Bloqueador de canal de sódio	Crises parciais	Uso como adjuvante para crises parciais	Indutor enzimático; uso como adjuvante
Retigabina (ezogabina)	GlaxoSmithKline	2011	Ativador de canal de potássio	Crises parciais	Uso como adjuvante em crises parciais, quando outros anticonvusivantes	Uso como adjuvante; coloração azul de lábios e unhas; disfunção retiniana;

Continuação

					falharam	não é útil para crises de ausência ou convulsões mioclônicas
Perampanel	Eisai	2012	Antagonista do receptor de Glutamato (AMPA)	Crises parciais	Uso como adjuvante em crises parciais	Uso como adjuvante, não é útil para crises de ausência ou convulsões mioclônicas

Fonte: Loscher et al., (2013)

Considerando o exposto acima e em virtude da refratariedade aos tratamentos farmacológicos apresentada por um percentual dos pacientes com epilepsia, há um crescente interesse em terapias alternativas como a ETCC (Assenza et al., 2017). ETCC catodal em modelos animais de epilepsia mostrou um aumento do limiar para a atividade convulsiva localizada (Liebetanz et al., 2006b) e diminuição do brotamento em hipocampo imaturo (Kamida et al., 2011). Estudo prévio mostrou que a ETCC catodal reduziu as convulsões em até 21% (Kamida et al., 2011), outros mostraram uma redução no número e na duração média do pico e descargas de ondas lentas após a ETCC (Zobeiri e van Luijtelaar, 2013). Além disso, estudos em animais não mostraram qualquer lesão provocada pela estimulação (Liebetanz et al., 2006b; Kamida et al., 2011). Estudos clínicos com ETCC são promissores, com 4 de 6 (67%) estudos mostrando uma redução efetiva nas crises epilépticas e 5/6 (83%), uma redução da atividade epileptiforme (San-Juan et al., 2015). Importante salientar que tem sido demonstrado que a ETCC é segura em humanos (pacientes adultos e pediátricos) (Fregni et al., 2006; San-Juan et al., 2011; Varga et al., 2011; Yook et al., 2011). No entanto, alguns resultados não são conclusivos ou negativos por limitações metodológicas e diferenças nos parâmetros de avaliação. Por exemplo, o número de pacientes dos estudos tem sido pequeno e heterogêneo. Além disto, a ETCC foi aplicada com diferentes parâmetros (Fregni et al., 2006; San-Juan et al., 2011; Varga et al., 2011; Yook et al., 2011; Auvichayapat et al., 2013). Desta forma, mais estudos são necessários para definir os melhores protocolos de estimulação e entender os efeitos em longo prazo da ETCC (San-Juan et al., 2015).

3 OBJETIVOS

3.1 Objetivo Principal

Considerando a relevância do tema, esta tese tem por objetivo avaliar, por meio de ensaios pré-clínicos, a estimulação transcraniana por corrente contínua (ETCC), como uma nova estratégia terapêutica neuromodulatória para o tratamento da epilepsia, bem como determinar o efeito desta estratégia sobre a neuroinflamação induzida em modelo experimental de epilepsia. Para tanto deve avaliar o efeito da ETCC em ratos submetidos ao modelo de *kindling* por pentilenotetrazol, sobre parâmetros de comportamentais convulsivos e bioquímicos, bem como comparar o efeito da estimulação anodal e catodal associadas ou não ao fármaco anticonvulsivante diazepam, buscando avaliar um possível sinergismo entre ETCC e o tratamento farmacológico.

3.2 Objetivos Específicos

- Realizar revisão sistemática sobre a utilização da ETCC em epilepsia, no intuito de resumir as evidências relacionadas aos efeitos da ETCC nesta patologia, tanto em estudos clínicos quanto pré-clínicos, através de um *framework* para fornecer informações sobre a taxa de tradução da pesquisa pré-clínica em estudos clínicos. Além disso, tentar determinar áreas importantes para testes clínicos, verificar se os resultados são complementares e identificar possíveis limitações dos estudos (Artigo I).

- Avaliar os efeitos da ETCC-a ou ETCC-c em ratos submetidos ao modelo de *kindling* induzido por pentilenotetrazol (PTZ), sobre os seguines parâmetros:

    - comparar o efeito da estimulação transcraniana por corrente contínua (ETCC) anodal e catodal com fármaco anticonvulsivante - diazepam - no modelo de *kindling* por PTZ sob parâmetros de comportamento convulsivo;
    - avaliar o uso paralelo da estimulação transcraniana por corrente contínua (ETCC) anodal e catodal e tratamentos com fármaco anticonvulsivante no modelo de *kindling* por PTZ sob parâmetros de comportamento convulsivo;
    - avaliar o efeito da estimulação transcraniana por corrente contínua (ETCC) anodal e catodal sob parâmetros bioquímicos após o modelo de *kindling* e após a ETCC-a ou ETCC-c, através da determinação da concentração de IL-1β, TNF-α, BDNF e NGF em hipocampo e córtex cerebral (Artigo II).

4 ARTIGOS CIENTÍFICOS

ARTIGO I: Preclinical to clinical translation of studies of transcranial direct-current stimulation in the treatment of epilepsy: A systematic review

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Preclinical to Clinical Translation of Studies of Transcranial Direct-Current Stimulation in the Treatment of Epilepsy: A Systematic Review

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Epilepsy is a chronic brain syndrome characterized by recurrent seizures resulting from excessive neuronal discharges. Despite the development of various new antiepileptic drugs, many patients are refractory to treatment and report side effects. Non-invasive methods of brain stimulation, such as transcranial direct current stimulation (tDCS), have been tested as alternative approaches to directly modulate the excitability of epileptogenic neural circuits. Although some pilot and initial clinical studies have shown positive results, there is still uncertainty regarding the next steps of investigation in this field. Therefore, we reviewed preclinical and clinical studies using the following framework: (1) preclinical studies that have been successfully translated to clinical studies, (2) preclinical studies that have failed to be translated to clinical studies, and (3) clinical findings that were not previously tested in preclinical studies. We searched PubMed, Web of Science, Embase, and SciELO (2002–2017) using the keywords "tDCS," "epilepsy," "clinical trials," and "animal models." Our initial search resulted in 64 articles. After applying inclusion and exclusion criteria, we screened 17 full-text articles to extract findings about the efficacy of tDCS, with respect to the therapeutic framework used and the resulting reduction in seizures and epileptiform patterns. We found that few preclinical findings have been translated into clinical research (number of sessions and effects on seizure frequency) and that most findings have not been tested clinically (effects of tDCS on status epilepticus and absence epilepsy, neuroprotective effects in the hippocampus, and combined use with specific medications). Finally, considering that clinical studies on tDCS have been conducted for several epileptic syndromes, most were not previously tested in preclinical studies (Rasmussen's encephalitis, drug resistant epilepsy, and hippocampal sclerosis-induced epilepsy). Overall, most studies report

positive findings. However, it is important to underscore that a successful preclinical study may not indicate success in a clinical study, considering the differences highlighted herein. Although most studies report significant findings, there are still important insights from preclinical work that must be tested clinically. Understanding these factors may improve the evidence for the potential use of this technique as a clinical tool in the treatment of epilepsy.

Keywords: animal models, clinical trials, epilepsy, non-invasive brain stimulation, transcranial direct current stimulation

INTRODUCTION

Techniques involving stimulation of the central nervous system have been extensively studied in recent years. These techniques have been shown to improve symptoms in a range of neurological disorders. Both invasive and non-invasive brain stimulation techniques have been described. Non-invasive techniques can be divided into transcranial magnetic stimulation (TMS), transcranial alternating current stimulation, and transcranial direct current stimulation (tDCS) (Woods et al., 2016).

tDCS relies on the modification of the neuronal resting membrane potential to induce changes in cortical excitability. This technique consists on applying a weak, direct, constant, and low intensity electric current over the scalp using two electrodes: an anode and a cathode (Gomez Palacio Schjetnan et al., 2013). The electrodes are arranged in different assemblies, creating a flow of low-level continuous electrical current targeting a specific region of the cerebral cortex. Anodal stimulation induces depolarization of the neuronal membrane, and therefore facilitates neuronal firing. In contrast, cathodal stimulation has the opposite effect, hyperpolarizing the neuronal membrane (**Figure 1**; Jackson et al., 2016). tDCS is applied at intensities ranging from 0.5–2 mA across saline-soaked electrodes placed on the human or animal scalp (**Figure 2**) [from author].

tDCS has been shown to improve symptoms in patients with depression, stroke, focal dystonia, migraine, chronic pain, and epilepsy (Liebetanz et al., 2006a). Epilepsy represents a chronic brain syndrome of diverse etiology, characterized by recurrent seizures resulting from excessive neuronal discharge (Chindo et al., 2014). This syndrome affects ~0.5–1% of the world's population (Dhir et al., 2005), and is associated with a variety of clinical symptoms such as impaired consciousness, movement and sensation (Chindo et al., 2014). Epileptical discharges are generated in response to a loss of balance between excitatory and inhibitory connections, resulting in tonic depolarization of brain circuits (McCormick and Contreras, 2001). The pathophysiology of epilepsy includes hyperactivity of excitatory glutamatergic transmission, and a deficit of inhibitory signaling, mainly resulting from insufficient neurotransmission mediated by γ-aminobutyric acid A receptors ($GABA_{AR}$) (Lason et al., 2013).

Despite the diversity of new antiepileptic drugs, a large proportion of individuals suffering from epilepsy is refractory to pharmacology treatment (Loscher, 2002), and/or reports side effects which hinder the use of drugs. Epidemiological data indicate that 20–40% of patients with newly diagnosed epilepsy become refractory to treatment (Loscher and Schmidt, 2004; French, 2007). For this reason, there is a continuous search for new therapeutic strategies, from which tDCS has emerged as a possible alternative. In this study, we aimed to summarize the evidence concerning the effects of tDCS in epilepsy in both clinical and preclinical studies. We used a framework to provide insight into the translation rate of preclinical into clinical studies. In addition, we attempted to determine important areas for clinical testing, to verify if these results are complementary, and to identify possible limitations of these studies.

METHODS

Search Strategy

This systematic review was based on a literature search using PubMed, Web of Science, Embase and SciELO. The keyword "tDCS" was used in combination with other keywords such as "epilepsy," "clinical trials," and "animal models." The term "AND" was used in each combination (**Figure 3**). In addition, the reference sections of the studies that met our inclusion criteria were manually screened for relevant publications.

Inclusion and Exclusion Criteria

Studies had to meet the following criteria: (1) publication in English between 2002 and 2017, (2) report original research, and (3) report case reports. Exclusion criterion were: (1) lack of original data (e.g. review articles, editorial material, articles reporting duplicate data); (2) articles addressing only effects of other brain stimulation techniques, such as alternating electrical current stimulation, or TMS.

Data Extraction and Outcomes

Two investigators (GGR and CO) extracted data from the full articles independently. Any disagreements were resolved by a third investigator (PP). However, we summarized the results in a narrative format. The primary outcome of our study was seizure suppression (SS). Relevant articles reporting the outcome of interest were identified, and standardized tables were utilized to extract the following variables: experiment, total sessions, interval between sessions, number of individuals (N), montage, contact area of electrodes, sham group and results/insights. The primary author collected the name of the authors, titles and study design. When further information about the study was needed, the authors were contacted by email.

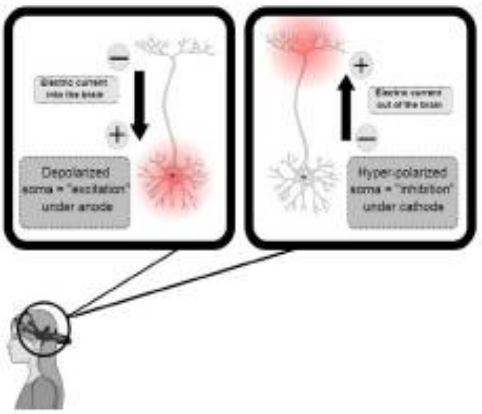

FIGURE 1 | Transcranial direct-current stimulation design, demonstrating anodal, and cathodal stimuli with consequent depolarization (increase in excitability) and hyperpolarization (decrease in excitability), respectively. Adapted from Jackson et al. (2016).

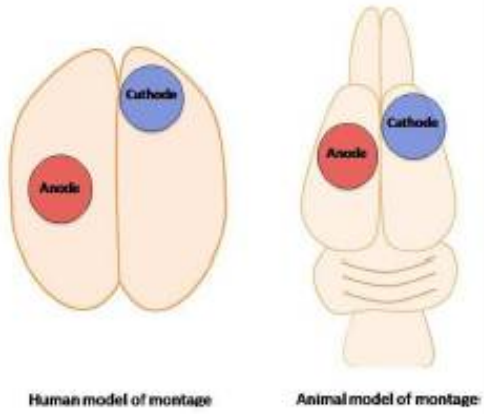

FIGURE 2 | Transcranial direct-current stimulation: human montage vs. transcranial direct-current stimulation animal model (from author).

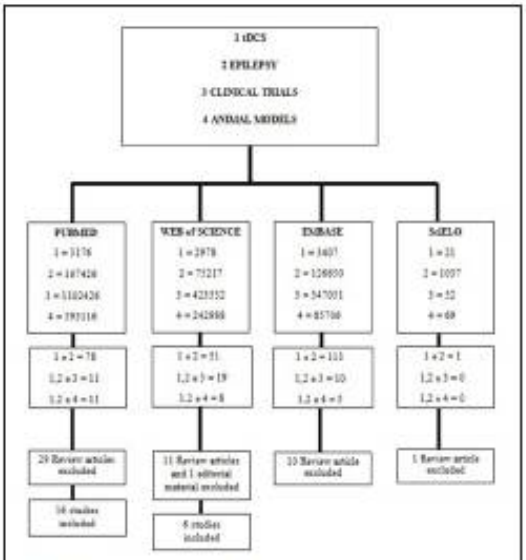

FIGURE 3 | Flowchart of the query on transcranial direct-current stimulation in major databases.

Risk of Bias Assessment

Risk of bias was assessed by two reviewers (GGR and CO), for each included preclinical study, using SYRCLE's Risk of Bias tool for animal studies (Hooijmans et al., 2014). We extracted study characteristics related to construct and external validity (Henderson et al., 2013). For construct validity, we included sex, species and strain, type of epilepsy model, total treatment sessions and the interval between them, and the use of any other intervention. For clinical studies, we ranked each of the following as having a high, low, or unclear risk of bias when included allocation concealment, blinding of participants, personnel and outcome assessors, incomplete outcome data, and selective outcome reporting (Higgins and Green, 2011). Since the primary outcome included only the tDCS effectiveness, not harmful effects, results may be biased.

Framework of Translation From Preclinical Studies to Clinical Studies

In order to analyze the results for this review, we used a framework to understand whether preclinical findings were translated into clinical studies, and whether or not this translational research confirmed preclinical findings. Therefore, we created three main categories: (1) preclinical studies that have been successfully translated in clinical studies, (2) preclinical studies that have failed to translate in clinical studies, (3) clinical findings that were not tested previously in preclinical studies.

RESULTS

The final search identified 64 studies. After applying the inclusion and exclusion criteria, we included 17 articles with different types of designs (5 preclinical/12 clinical) for full-text analysis. We screened the articles according to the main outcome, the suppression of seizures, and summarized the results separately for basic (**Table 1**) and clinical (**Table 2**) research. Interestingly, the results obtained from the search in PubMed and Web of Science were the same. We summarized our findings using the framework discussed above.

From **Tables 1,2**, we observed that most clinical studies showed significant results with respect to epileptiform activity, though only 11 studies (91.67%) had positive results in clinical

TABLE 1 | Summary of tDCS and animal models of epilepsy.

Author (year)	Title	Type of article	Experiment	Total sessions	Interval between sessions	*N*	Montage	Contact area of electrodes	Sham group	Results/insights
Liebetanz et al., 2006b	Anticonvulsant effects of tDCS in the rat cortical ramp model of focal epilepsy	Original article	(1) c-tDCS (100 μA) for 30 and 60 min, anodal tDCS (100 μA) for 60 min, and 60 min of c-tDCS (n = 7). (2) c-tDCS (200 μA) for 15 and 30 min, anodal tDCS (200 μA) for 30 min, and c-tDCS for 30 min (n = 8)	(1) 4 sessions–210 min (2) 4 sessions–105 min	1 week	65 male Wistar rats	2 mm left and 2 mm anterior to the bregma	3.5 mm^3	No	The anticonvulsive effect induced by c-tDCS depends on stimulation duration and current strength and may be associated with the induction of alterations of cortical excitability that outlast the actual stimulation.
Kamida et al., 2011	tDCS decreases convulsions and spatial memory deficits following SE in immature rats	Original article	Daily c-tDCS, for 30 min with an intensity of 200 μA, for 2 weeks (n = 6)	14 sessions–420 min	24 h	18 male Wistar rats	1.5 mm to the right and 2 mm anterior to the bregma	3.5 mm^3	No	Reduction of SE-induced hippocampal cell loss, supragranular and CA3 mossy fiber sprouting, and convulsions (reduction of 21%) in immature rats. tDCS treatment also reduced cognitive impairment following SE.
Kamida et al., 2013	c-tDCS affects SZs and cognition in fully amygdala-kindled rats	Original article	Daily 30 min c-tDCS with an intensity of 200 μA for 1 week (n = 6)	7 sessions–210 min	24 h	18 male Wistar rats	1.5 mm to the right and 2 mm anterior to the bregma	3.5 mm^3	Yes	c-tDCS treatment improved the SZ stage and decreased ADT together with elevated ADT 1 day after the last tDCS session. The treatment also yielded significant improvement in the performance of WMT. c-tDCS has anticonvulsive after-effects that last at least 1 day in the amygdala-kindled rats and positively affects cognitive performance.
Zoheir and van Luijtelaar, 2013	Non-invasive tDCS in a genetic absence model	Original article	(1) 4 series of 15 min cathodal and anodal stimulation of 100 μA with an interval of 105 min in counter balanced order (n = 10). (2) 4 sessions of 15 min of cathodal stimulation of 100 μA (n = 8). (3) Similar protocol to (2), except that intensity was 150 μA (n = 8)	(1), (2) and (3) = 4 sessions–80 min	105 min in all protocols	26 male WAG/Rij rats	Stimulation electrodes were placed on both hemispheres considering that the foci are bilateral. The two stimulation electrodes were fixed onto the cranium above the right and left somatosensory cortices (Vt. −4.6 and Vt. +4.6, respectively) and the reference electrode onto the cranium above the frontal cortex with no specific coordinate.	3.5 mm^3	No	c-tDCS reduced the number of SWDs during stimulation and affected the mean duration after stimulation both in an intensity-dependent manner. Behavior was changed after the highest stimulation intensity.
Dhamne et al., 2015	Acute SZ suppression by tDCS in rats	Original article	(1) Rats received tDCS sham tDCS, c-tDCS 1,000 μA, or c-tDCS 100 μA for 20 min (n = 23, 22, 20, respectively). (2) 2 animal groups received a subtherapeutic lorazepam dose and then verum tp-tDCS 1000 μA (n = 11) or sham tDCS (n = 10).	(1) and (2) = 1 session–20 min		114 male Long Evans rats	Disk (active), and sponge (reference) electrodes were secured to the rat's scalp and torso, respectively.		Yes	Cathodal 1 mA tDCS reduced EEG spike bursts, and suppressed clinical SZs in combination with lorazepam and was more effective in SZ suppression and improved the clinical SZ outcomes compared to either tDCS or lorazepam alone.

ADT, After-discharge duration; AD, After-discharge threshold; c-tDCS, Cathodal transcranial direct current stimulation; EEG, Electroencephalogram; SE, Status epilepticus; SWDs, Spike and slow-wave discharges; SZ, Seizure; tDCS, Transcranial direct-current stimulation; WMT, Water maze test.

TABLE 2 | Summary of tDCS and epilepsy clinical trials.

Author (year)	Title	Type of article	Experiment	Total sessions	Interval between sessions	N	Montage	Contact area of electrodes	Sham group	Results/insights
Fregni et al., 2006	A controlled clinical trial of cathodal DC polarization in patients with refractory epilepsy	Randomized, sham-controlled clinical trial	Single session of 1 mA c-tDCS for 20 min	1 session – 20 min		19 subjects (11 male and 8 female)	Cathodal electrode has been placed over the epileptogenic focus and the anode electrode over a silent area (without epileptogenic activity)	35 cm^2	Yes (10 active and 9 sham)	c-tDCS reduced EDs (64.3%) and SZ frequency (44%) when compared with sham group (5.6%) and (11.1%) respectively.
Yook et al., 2011	Suppression of SZs by c-tDCS in an epileptic patient-a case report	Case report	5 days a week, during 2 weeks. Repeating procedure after 2 month, 20 min (2 mA for 20 min)	10 sessions – 200 min	24 h	1 subject (female)	Cathode electrode applied on midpoint between F4 and T4 area and anode electrode on left supraorbital area.	25 cm^2		During the first 2 months after treatment, the patient had only six SZs, with an evident clinical improvement, after the second intervention the patient had just one SZ attack over 2 months.
San-Juan et al., 2011	tDCS in adolescent and adult Rasmussen's encephalitis	Case report	60 min in 4 sessions (on days 0, 7, 30, and 60) 1 mA for patient (1) and 2 mA for patient (2)	4 sessions – 240 min	7, 23 and 30 days respectively	2 subjects (male)	(1) IC3 [-/+cathode]/contralateral supraorbital area [-/+anode] (2) IF2 [-/+cathode]/FT8 [+/anode]]	Subdermal needle 12 mm in length and 0.4 mm in diameter		One patient was SZ free and another patient showed 50% SZ frequency reduction within 6 month of follow-up.
Fiella et al., 2012	Feasibility of focal transcranial DC polarization with simultaneous EEG recording: preliminary assessment in healthy subjects and human epilepsy	Cross-over controlled trial with 15 healthy subjects and preliminary effects of its use, testing repeated tDCS sessions, in two patients with drug-refractory Continuous Spike-Wave Discharges During Slow Sleep (CSWS)	Once weekly, to 3 afternoon sessions of 30 min each. Current was ramped in steps of 0.1 mA, with a duration of 10 s each, until the target current of 1 mA.	3 sessions – 90 min	7 days	2 subjects (male)	Biased in 10-10 International system positions in a cap (mostly C5-C6)	35 cm^2		A large reduction after c-tDCS was found in EDs in C5 (mean 32.1%) during and after tDCS (10 min).
Auvichayapat et al., 2013	tDCS for treatment of refractory childhood focal epilepsy	Controlled study	Single session of 1 mA c-tDCS for 20 min	1 session – 20 min		36 subjects (26 male and 10 female)	Cathodal electrode was placed over the epileptogenic focus, contained on the electrode with the international 10-20 EEG electrode placement system location where spikes of sharp waves were greatest in amplitude, and the anodal electrode was placed over the contralateral shoulder area.	35 cm^2	Yes @7 active and 9 sham)	c-tDCS can suppress EDs frequency in 57.6% for 48 h, but the effect of a single session on EEG abnormalities was not sustained for 4 weeks. A statistical reduction in the frequency of SZs was found (48%) in the post-hoc analysis.
Assenza et al., 2014	Efficacy of c-tDCS in drug-resistant epilepsy: a proof of principle	Single blind and sham-controlled study	Two sessions, (1 sham and 1 real on the 8th and 22th day) 1 mA intensity applied for 9 min	1 real session – 9 min		2 subjects (male)	Cathodal electrode has been placed over the epileptogenic focus and the anode electrode over the contralateral homologous region	12.25 cm^2		Patients showed a consistent reduction of the SZ frequency: about 70% for Patient 1 and about 50% for Patient 2.

(Continued)

TABLE 2 | Continued

Author (year)	Title	Type of article	Experiment	Total sessions	Interval between sessions	*N*	Montage	Contact area of electrodes	Sham group	Results/insights
Tekturk et al., 2016b	The effect of transcranial direct current stimulation on SZ frequency of patients with medial temporal lobe epilepsy with hippocampal sclerosis	A randomized cross-over study	2 mA for 30 min on 3 consecutive days	3 real sessions –90 min	24 h	12 subjects (6 male/6 female)	Active electrode placed over the pathologically affected HS side (temporal region, either T3 or T4 electrode placed), which was determined by both concordant cranial MRI and ictal or interictal EEG findings, depending on the availability of the seizure records, and reference electrode over the contralateral supraorbital region	35 cm^2		Ten patients showed a more than 50% decrease in their SZ frequency after c-tDCS. Six patients were SZ-free in the post c-tDCS period of 1 month.
Auvichayapat et al., 2016	Transcranial Direct Current Stimulation for Treatment of Childhood Pharmacoresistant Lennox-Gastaut Syndrome: a Pilot Study	A randomized, double-blind controlled trial	Five consecutive days of 2 mA c-tDCS for 20 min	5 sessions – 100 min	24 h	22 subjects (14 male and 8 female)	The stimulation site over the left M1, located based on the international electroencephalography (EEG) 10/20 electrode placement system. The reference electrode was placed over the right shoulder area.	35 cm^2	Yes (15 active and 7 sham)	Participants assigned to the active tDCS condition reported significantly more pre- to post-treatment reductions in SZ frequency and epileptic discharges that were sustained for 3 weeks after treatment.
Tekturk et al., 2016a	Transcranial direct current stimulation improves SZ control in patients with Rasmussen's encephalitis	Descriptive study of a small case series	First cathodal, then anodal (2 mA for 30 min on 3 consecutive days for non-sham stimulations), and finally sham stimulation with 2 month intervals	3 sessions - 90 min	24 h	5 subjects (2 male/3 female)	Active electrodes placed over the mostly affected area and reference electrodes over the contralateral mastoid region	35 cm^2		After cathodal stimulation, all but one patient had a greater than 50% decrease in SZs frequency. Two patients who received modulated c-tDCS had better results. The longest positive effect lasted for 1 month.
Zoghi et al., 2016	The effects of cathodal transcranial direct current stimulation in a patient with drug-resistant temporal lobe epilepsy (case study)	Case report	2 sessions of 1 mA c-tDCS (9–20–9 protocol) during a total of 15 min, with 20 min rest after the first 9 min	2 sessions – 16 min	20 min	1 subject (female)	The active electrode (cathode, 3 × 4 cm) was placed over the right temporal lobe, and the return electrode (anode, 5 × 7 cm) was placed over the left supraorbital area	Cathode, 12 cm^2 and anode, 35 cm^2		SZs reduced from 8-10 per day to 0–3 SZs per day. SZ frequency remained as low as 0–3 per day for 4 months, and then started to increase again.
Assenza et al., 2017	Cathodal transcranial direct current stimulation reduces seizure frequency in adults with drug-resistant temporal lobe epilepsy: a sham controlled study	A double-blind, randomized, sham-controlled, crossover, monocentric study	1 real session of 1 mA c-tDCS during 20 min	1 session – 20 min	30 days	10 subjects (male)	The cathode was placed over the epileptic focus, localized by means of EEG interictal and ictal activity, and the anode over the contralateral homologous region	35 cm^2		c-tDCS reduced the percent weekly seizure frequency more than sham stimulation, without any change in interictal epileptiform activity

(Continued)

TABLE 2 | Continued

Author (year)	Title	Type of article	Experiment	Total sessions	Interval between sessions	N	Montage	Contact area of electrodes	Sham group	Results/insights
San-Juan et al., 2017	tDCS in Mesial Temporal Lobe Epilepsy and Hippocampal Sclerosis	A randomized, double-blinded, placebo-controlled, 3-arm parallel group (placebo, 30 min/2 mA daily sessions for 3 days, and 30 min/2 mA daily sessions for 5 days) clinical trial	2 mA for 30 min on 3 or 5 consecutive days of treatment	3 sessions –90 min or 5 sessions –150 min	24 h	26 subjects (16 male and 12 female)	The cathode was positioned over the most active IED area (defined as the zone [electrode] with the highest discharge amplitude and/or frequency located with the 10/20 system) as observed on the scalp EEG immediately before applying the tDCS. The anode electrode was placed over a silent supraorbital area (i.e., without epileptogenic activity contralateral to the stimulated MTLE-HS side	35 cm^2	Yes (20 active and 6 sham)	c-tDCS of 3 and 5 sessions decreased the frequency of SZs and IEDs (baseline vs. immediately post-tDCS).

c-tDCS: Cathodal transcranial direct-current stimulation; IED: Epileptiform discharges; EEG: Electroencephalogram; MTLE-HS: Mesial temporal lobe epilepsy with hippocampal sclerosis; SZs: Seizures; tDCS: Transcranial direct-current stimulation.

outcomes such as seizure frequency reduction. For safety, none of them showed an increase in seizure frequency or moderate to severe adverse effects. On the other hand, two preclinical studies confirmed the initial hypothesis that cathodal-tDCS induces anticonvulsant effect.

Preclinical Studies That Have Been Successfully Translated to Clinical Studies

Reduction in Seizure Frequency by Cathodal tDCS

The first study analyzed results of tDCS in rats subjected to a cortical ramp model of focal epilepsy. In this model, the anticonvulsive effect induced by cathodal-tDCS (c-tDCS) varied according to the duration of stimulation and strength of the current. In addition, the effect may be associated to modulation of cortical excitability that outlasts the actual stimulation (Liebetanz et al., 2006b). Kamida et al. (2013) reported similar results using amygdala-kindled rats; c-tDCS treatment significantly improved the seizure stage, decreased after-discharge duration, and elevated after-discharge threshold 1 day after the last tDCS session. Similar findings have also been reported in clinical studies by Fregni et al. (2006), Yook et al. (2011), San-Juan et al. (2011), Auvichayapat et al. (2013), Assenza et al. (2014), Tekturk et al. (2016a,b), Auvichayapat et al. (2016), Zoghi et al. (2016), Assenza et al. (2017), and San-Juan et al. (2017), conducted in a total of 138 patients.

Preclinical Studies That Have Failed to Be Translated to Clinical Studies

Status Epilepticus

In 2011, Kamida et al. (2011) used a model of pilocarpine-induced status epilepticus (SE) in immature rats, and demonstrated a 21% reduction in convulsions on postnatal day 55. There are no studies evaluating the effects of tDCS in patients experiencing SE. However, there is a case report (that does not meet the inclusion criteria for this review) that has tested this approach (Grippe et al., 2015). Therefore, this design might be a good opportunity for a future trial.

Neuroprotective Effects in the Hippocampus

c-tDCS has been reported to exert neuroprotective effects in the immature rat hippocampus, reducing SE-induced hippocampal cell loss, as well as supragranular and CA3 sprouting (Kamida et al., 2011). This hippocampal impairment is caused by seizure-induced neuronal damage and synaptic reorganization, which starts soon after SE (Covolan and Mello, 2000). This type of a study is difficult to perform in a clinical setting; however, it would clearly be worthwhile to test, for example after brain injury that may lead to further impairment in cortical areas and result in epileptogenic foci (D'Ambrosio and Perucca, 2004).

Effects in Absence Epilepsy

Zobeiri and van Luijtelaar (2013) using a genetic absence model of epilepsy, showed that c-tDCS reduces slow-wave discharges (SWDs) in rats during stimulation and affects the mean duration of SWDs after stimulation, both in an intensity-dependent manner. Behavioral changes were also observed in response to the highest stimulation intensity. Spectral analysis of EEG during

stimulation revealed an increase in sub-delta and delta frequency ranges, suggesting that cortical cells were hyperpolarized. These preclinical findings have not been replicated clinically, as no clinical study has evaluated tDCS in patients with absence epilepsy. This constitutes another opportunity for future studies.

Combination of tDCS With Specific Drugs to Test Synergistic Effects

In an acute seizure induced by pentylenetetrazole, c-tDCS reduced EEG spike bursts, and suppressed clinical seizures in rats. c-tDCS, in combination with lorazepam, was more effective in SS compared with either tDCS or lorazepam alone, and prevented loss of motor cortex inhibition during paired-pulse transcranial magnetic stimulation (ppTMS) accompanied by pentylenetetrazole injection. This study provides evidence of the neural substrate of the antiepileptic effects of tDCS through ppTMS measures and demonstrates that c-tDCS enhances GABAergic intracortical inhibition mediated by $GABA_A$ signaling. Further, c-tDCS prevented the loss of GABAergic ppTMS inhibition that is expected with PTZ-mediated $GABA_A$ antagonism. This corroborates the hypothesis that tDCS may influence neurotransmitter levels and receptor function in humans (Medeiros et al., 2012). Thus, a combination of c-tDCS and GABAergic pharmacotherapy could be proposed, for example with benzodiazepine treatment (Dhamne et al., 2015).

Clinical Findings Not Replicated in Preclinical Studies

Epilepsy is characterized by multiple heterogeneous syndromes with various etiologies and symptoms, insufficiently addressed in current animal models despite the number of experimental options (Kandratavicius et al., 2014; Depaulis and Hamelin, 2015). The choice of appropriate protocol remains a challenge; most animal models used in epilepsy research are models of epileptic seizures rather than epilepsy *per se*, making differentiation subjective (Löscher, 2011). Thus, there are still limitations and shortcomings regarding models of refractory epilepsy and epilepsy because of hippocampal sclerosis, which often compromise the translational application of preclinical findings. Nonetheless, such clinical findings represent an opportunity to perform preclinical studies in an attempt to establish reproducible animal models and clarify the mechanisms involved in both pathology and treatment with tDCS.

Effects in Rasmussen's Encephalitis

Rasmussen's encephalitis is a rare and progressive inflammatory disease that reaches one cerebral hemisphere, and leads to intractable partial-onset seizures. Currently, the only effective treatment is hemispherectomy, but this procedure may cause irreversible neurological deficits. In a case report, San-Juan et al. (2011) reported that one patient with Rasmussen's encephalitis was seizure free and another showed a 50% reduction in seizure frequency within 6 months of follow-up after tDCS treatment. In a recent study, two patients with Rasmussen's encephalitis received modulated c-tDCS (2 mA for 30 min on 3 consecutive days) and demonstrated reduced seizure frequency following stimulation. One patient showed more than 50% reduction in seizure frequency, and the longest positive effect lasted for 1 month (Tekturk et al., 2016a). Thus, tDCS may be used to treat this pathology in order to avoid or delay surgical intervention (San-Juan et al., 2011).

In another study, patients with Lennox-Gastaut syndrome received pharmacological treatment for 5 consecutive days and 2 mA c-tDCS over the primary motor cortex (M1) for 20 min. This combination was more effective in reducing seizure frequency and epileptic discharges than pharmacological treatment alone. This reduction was sustained for 3 weeks after treatment (Auvichayapat et al., 2016).

Effects in Drug Resistant Epilepsy

Approximately one-third of epilepsy patients develop drug resistance, and only 50% can take benefit from the surgical removal of an epileptic focus (Assenza et al., 2017). Although surgery is an option in cases of drug resistant epilepsy, many patients have no access to medical centers that perform respective epilepsy surgery. Furthermore, some patients may have a seizure focus located in eloquent cortex where resection is likely to cause deficit (Auvichayapat et al., 2013).

Many models of refractory epilepsy have been developed over the past 20 years, which use two approaches: (1) seizures or epilepsy models resistant to antiepileptic drugs (for example, 6-Hz psychomotor seizure model in mice) and (2) chronic epilepsy models, such as kindling. Kindling involves the application of repeated excitatory stimuli to induce partial seizures, followed by subsequent generalized seizures. This leads to increased seizure length and severity with continuous stimulation (Löscher, 2011). It is important highlight that we did not find preclinical studies relating the use of tDCS in refractory epilepsy, resulting in a lack of mechanistic and neurochemical clarifications. An alternative treatment is neuromodulation, which represents an attempt to improve the quality of life of patients with refractory epilepsy.

In 2006, Fregni et al. (2006) conducted a controlled study applying c-tDCS in 19 patients with refractory epilepsy; this unprecedented study investigated the electrographic and clinical response to c-tDCS in this epileptic condition. Patients underwent one session of c-tDCS (20 min, 1 mA) targeting the epileptogenic focus. The active stimulation did not induce seizures and was well-tolerated, and the treatment promoted a large reduction in number of epileptiform discharges (EDs) in the EEG and in the frequency of seizures. These parameters were measured and compared before (baseline), immediately after, and 15 and 30 days after either sham or active stimulation.

Yook et al. (2011) demonstrated in a case report of bilateral perisylvian syndrome that tDCS, when applied over the midpoint between P4 and T4, had a lasting effect over a 2-month period following treatment termination, decreasing the duration of each seizure episode. For 2 months after the second treatment session, only one seizure attack occurred, a considerable improvement over the eight seizure attacks per month prior to tDCS, when the patient was treated only with antiepileptic drugs.

The large reduction in interictal epileptiform EEG discharges in two subjects with drug-refractory continuous spike-wave

discharges during slow sleep suggests that the simultaneous application of tDCS treatment and EEG recording allows the assessment of safety parameters during treatment. This methodology ensures that the stimulation is sufficiently focal and provides a detailed evaluation of epileptic activity changes induced by tDCS, representing an attractive outlook for epilepsy treatment (Faria et al., 2012).

Auvichayapat et al. (2013) showed that a single session of active tDCS treatment was associated with significant reductions in epileptic discharge frequency in children with refractory epilepsy immediately, 24, and 48 h after tDCS treatment. In addition, 4 weeks after treatment, a small decrease in seizure frequency was detected.

In focal resistant epilepsy, two patients received c-tDCS (constant current of 1 mA) during a real session in a single-blind, sham-controlled study, followed by 1 month of observation. During this period, the patients or caregivers provided a detailed seizure calendar (frequency per week at basal, post-sham and post-tDCS time points). These patients experienced reduction in seizure frequencies of ~70 and 50% (Assenza et al., 2014).

Effects in Epilepsy Due to Hippocampal Sclerosis

Animals subjected to kainate or pilocarpine-induced SE develop spontaneous seizures after a pre-epileptic period or seizure free, this can be due hippocampal injury, resulting in an animal model of mesial temporal lobe epilepsy (MTLE) with hippocampal sclerosis (Sloviter, 2008). Although MTLE is well-described in in clinical studies, with respect to electrophysiological and histological parameters, it remains partially reproduced in most rodent models (Depaulis and Hamelin, 2015). Even if there animal models described, remains some doubt about their reliability and extrapolation of their findings to the clinical setting, which represents a limitation in the conduction of preclinical studies using tDCS in models of MTLE due to hippocampal sclerosis.

In a randomized, placebo-controlled, double-blinded clinical trial with 3 sessions, 5 sessions and placebo stimulation, 3 and 5 sessions of c-tDCS stimulation decreased the frequency of seizures and interictal epileptiform discharge (immediately post-tDCS vs. baseline) in adults with MTLE and hippocampal sclerosis, compared to sham tDCS (San-Juan et al., 2017). It is known that MTLE with hippocampal sclerosis is a drug-resistant focal epilepsy syndrome. Another study showed that 83.33% of MTLE patients who received modulated c-tDCS (2 mA for 30 min on 3 consecutive days) showed more than 50% reduction in seizure frequency during a 1-month follow-up. Moreover, 50% these patients were seizure-free in the 1-month period post-tDCS (Tekturk et al., 2016b).

Recently, the case of a patient with drug-resistant temporal lobe epilepsy was reported. tDCS reduced seizure frequency from 6–10 per day to 0–3 per day. Seizure diaries revealed that seizure rates remained low (from 0 to 3 per day) for 4 months, and then began to increase (Zoghi et al., 2016). A recent database of published tDCS clinical trials, authored by Lefaucheur, presents a detailed list of studies assessing the clinical effect of tDCS, including in epileptic patients (Lefaucheur, 2016).

DISCUSSION

In this study, we performed a systematic review of clinical and preclinical studies using tDCS as a therapeutic approach in the treatment of epilepsy. Most studies presented here involved the use of c-tDCS, and several have investigated the effects of c-tDCS on spontaneous neural activity and evoked motor responses of the central and peripheral nervous system. These studies provide evidence that the effects of tDCS involve a non-synaptic mechanism of action, based on changes in neural membrane function (Ardolino et al., 2005).

tDCS has been applied in the treatment of epilepsy, spasticity, movement disorders, peripheral vascular disease, and certain psychiatric disorders (Raghavan et al., 2008). The acute effects of weak tDCS on ongoing epileptiform activity are well-established in animal models. However, the underlying mechanism by which prolonged tDCS modulates seizure initiation propensity and epileptogenesis remains unknown (Jackson et al., 2016). In addition, animal studies suggest that prolonged cathodal tDCS (c-tDCS) has anticonvulsant effects. On the other hand, anodal tDCS (a-tDCS) has contrary effects, decreasing the threshold for producing the seizure activity (evident in EEG), while behavioral changes are not observed (Hayashi et al., 1988; Liebetanz et al., 2006b). Nonetheless, Tekturk et al. attempted to prevent the generation and propagation of seizures by applying a-tDCS. This attempt was based on the hypothesis that even if c-tDCS decreases cortical excitability, a-tDCS increases the effects of inhibitory connections (Tekturk et al., 2016a). Therefore, they used c-tDCS targeting the epileptic foci and a-tDCS targeting the surrounding normal cortical tissue, an approach that was not effective in reducing the frequency of seizures.

Epilepsy is a pathology with the intrinsic characteristic of hypersynchronous brain activity. Therefore, epilepsy represents a model for abnormal hyperexcitatory plastic changes within cortical circuitry (San-Juan et al., 2017). The heterogeneity reported when using tDCS to treat refractory epilepsy may partly be attributed to the different etiology of that pathology. Accordingly, different approaches may be assessed in distinct types of epilepsy, due to the paucity of studies available. For example, in drug-resistant post-traumatic epilepsy patients, only one double-blinded randomized control trial has been published (Fregni et al., 2006). The causes of refractoriness of epilepsy to drugs and surgical treatment remain unknown. However, one possible explanation is the presence of neuronal damage affecting other brain areas besides the hippocampus (Petrovski et al., 2010; Zhang et al., 2017). A morphometric study using magnetic resonance imaging showed that neuronal damage in patients with temporal lobe epilepsy extends beyond the hippocampus, and affects regions that connect to the hippocampus functionally and anatomically (van Elst et al., 2000). This finding suggests the presence of a neural network injury which underlies the clinical manifestations in these patients (Andrade-Valença et al., 2008). More specifically, MTLE is commonly associated with hippocampal sclerosis (Andrade-Valença et al., 2008).

A previous review by our research group (Medeiros et al., 2012) discusses the presumed mechanisms of action of tDCS,

attempting to elucidate the underlying neurobiology and cell-signaling pathways involved. There, we suggest that tDCS induces plasticity, improves neuronal viability and morphology, modulates synaptic transmission, and biosynthesis of molecules.

tDCS has consistently been reported to be safe, and a recent review confirmed the absence of evidence for serious adverse effects (Bikson et al., 2016). tDCS is a technique that can be applied with low risk and little discomfort, and when used in repeated sessions, can have long-lasting effects (Nitsche et al., 2008). The effects of tDCS in the short term occur due to a decrease (anodal) or increase (cathodal) in neuronal firing threshold (Ruscheweyh et al., 2011). However, long-term effects involve the participation of brain-derived neuronal factor (BDNF) and glutamatergic N-methyl-d-aspartate (NMDA) receptors in synaptic plasticity mechanisms (Fertonani et al., 2010). Brain damage induced by the formation of toxic products does not occur using this technique, because there is no direct contact of electrodes with the cerebral cortex (Nitsche et al., 2003). Magnetic resonance imaging before and after 30 and 60 min of stimulation applied to the prefrontal and motor cortex did not exhibit pathological signal alterations. As such, it was concluded that tDCS does not induce cerebral edema, or render abnormal the blood brain barrier or brain tissue (Rosen et al., 2009). Finally, Accornero et al. (2007) showed no abnormal variations in heart rate, blood pressure, or temperature during and 20 min after the end of the stimulation. Therefore, tDCS is a safe method for use in humans, and has the advantage of being easily combined with other interventions, as pharmacological treatment.

San-Juan et al. (2015) reviewed the efficacy and safety of tDCS in epilepsy. The authors analyzed 9 articles using different methodologies (3 pre-clinical/6 clinical). Moreover, *in vivo* and *in vitro* animal studies demonstrated that direct current stimulation could induce suppression of epileptiform activity without neurological injury. Four out of six (67%) clinical studies revealed an effective decrease in epileptic seizures, and five out of six (83%) showed a reduction of interictal epileptiform activity (San-Juan et al., 2015; Scorza and Brunoni, 2015). In fact, in this review we did not find evidence that tDCS in epilepsy may lead to an increase in seizures or any other significant adverse effects (Pereira et al., 2016). Additional studies involving a large cohort of patients are required to investigate the effects of tDCS in drug-resistant epilepsy.

In order to develop optimal stimulation protocols and long-term follow-up, animal studies and larger prospective clinical trials with homogenous epileptic conditions are needed. Every study uses different patient categories, stimulation protocols, electrode sizes, stimulation sites, and stimulation current strength. Therefore, conclusions drawn from the comparison of these studies should be used to provide standardized measures, in order to improve reproducibility of outcomes (Gschwind and van Mierlo, 2016). Epileptogenesis involves an increase in excitatory synaptic strength, and seizure foci are characterized by a pathological reduction of inhibitory (GABA-releasing) terminals and an increase in excitatory (glutamatergic) terminals (**Figure 4**; Fregni and Pascual-Leone, 2007). Hence, the principle mechanism of action of tDCS might be the induction of long-term-depression-like (LTD) effects, i.e., reducing cortical excitability and the probability of paroxysmal activity in epileptogenic cortical regions (Nitsche and Paulus, 2009). While the immediate anticonvulsant effects of c-tDCS involve the hyperpolarization of neuronal soma and desynchronization of

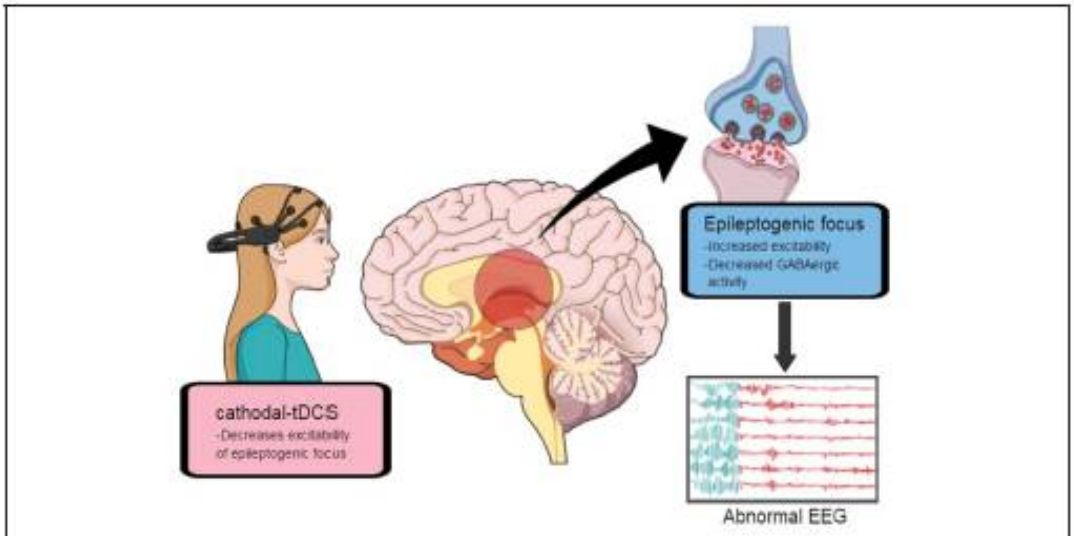

FIGURE 4 | Excitability in epileptogenic focus might be decreased by cathodal transcranial direct-current stimulation. Adapted from Fregni and Pascual-Leone (2007).

neuronal activity, its long-term effects seem to occur through the modulation of synaptic transmission, causing LTD in the thalamus-cingulate pathway. This process appears to be N-methyl-D aspartate (NMDA) receptor- and duration-dependent (Chang et al., 2015), thus c-tDCS seems to promote intracortical inhibition. On the other hand, a-tDCS facilitates synaptic plasticity mediated by a long-term potentiation (LTP)-like mechanism, as well as previous studies presented that brief seizures could induce LTP and mossy fiber sprouting in the hippocampus; therefore, the mechanism of LTP formation might be similar to the mechanism of epileptogenesis (Chang et al., 2015; Rroji et al., 2015). Finally, the mechanisms underlying the effects of tDCS seem to be involved not only in local polarity-related modifications of cortical excitability, but also in more complex interhemispheric connections (Tatti et al., 2016).

CONCLUSIONS

Considering the data obtained in this review, we conclude that tDCS should be considered a viable therapeutic option in refractory epilepsy, particularly in patients who are unable to undergo surgery. In general, animal studies used c-tDCS with currents ranging from 100 to 200 μA; even with defined montages, the stimulation appears to be bicephalic, due to the animal's skull size. At present, clinical studies involving c-tDCS use ranging from 1 to 2 mA, and the cathode is placed over the epileptic foci in majority. Cathodal tDCS appears to decrease excitability through hyperpolarization associated to LTD-like mechanisms. Moreover, because tDCS is simple to use, low cost, and easily accessible, it is a good option in countries with limited resources (Scorza and Brunoni, 2015; Zoghi et al., 2016). Therefore, despite the intriguing possibility of modulating neural networks, tDCS still requires a more in-depth analysis of the most beneficial protocols and elucidation of the underlying mechanism of action. Thus, this non-invasive technique still requires further sham-controlled, double-blind larger multi-center studies, which may be justified for cost-effectiveness and surgery complication avoidance. Novel methods of real-time assessment with EEG, and use of other neural markers, may also help with understanding the clinical effects of tDCS in epilepsy (Faria et al., 2012; Leite et al., 2017). Although there are several trials published in this field, further evidence is needed to understand the potential role of using tDCS to treat epilepsy.

AUTHOR CONTRIBUTIONS

All authors participated in the design of the study and drafted the manuscript. IT and PP participated in study coordination and helped draft the manuscript. FF and DL helped finalize the manuscript. GR, CdO, and RV have designed and prepared the manuscript figures. All authors read and approved the final manuscript.

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REFERENCES

Accornero, N., Li Voti, P., La Riccia, M., and Gregori, B. (2007). Visual evoked potentials modulation during direct current cortical polarization. *Exp. Brain Res.* 178, 261–266. doi: 10.1007/s00221-006-0733-y

Andrade-Valença, L. P., Valença, M. M., Velasco, T. R., Carlotti, C. G., Assirati, J. A., Galvis-Alonso, O. Y., et al. (2008). Mesial temporal lobe epilepsy: clinical and neuropathologic findings of familial and sporadic forms. *Epilepsia* 49, 1046–1054. doi: 10.1111/j.1528-1167.2008.01551.x

Ardolino, G., Bossi, B., Barbieri, S., and Priori, A. (2005). Non-synaptic mechanisms underlie the after-effects of cathodal transcutaneous direct current stimulation of the human brain. *J. Physiol.* 568, 653–663. doi: 10.1113/jphysiol.2005.088310

Assenza, G., Campana, C., Assenza, F., Pellegrino, G., Di Pino, G., Fabrizio, E., et al. (2017). Cathodal transcranial direct current stimulation reduces seizure frequency in adults with drug-resistant temporal lobe epilepsy: a sham controlled study. *Brain Stimul.* 10, 333–335. doi: 10.1016/j.brs.2016.12.005

Assenza, G., Campana, C., Formica, D., Schena, E., Taffoni, F., Di Pino, G., et al. (2014). Efficacy of cathodal transcranial direct current stimulation in drug-resistant epilepsy: a proof of principle. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2014, 530–533. doi: 10.1109/EMBC.2014.6943645

Auvichayapat, N., Rotenberg, A., Gersner, R., Ngodklang, S., Tiamkao, S., Tassaneeyakul, W., et al. (2013). Transcranial direct current stimulation for treatment of refractory childhood focal epilepsy. *Brain Stimul.* 6, 696–700. doi: 10.1016/j.brs.2013.01.009

Auvichayapat, N., Sinsupan, K., Tunkamnerdthai, O., and Auvichayapat, P. (2016). Transcranial direct current stimulation for treatment of childhood pharmacoresistant lennox-gastaut syndrome: a pilot study. *Front. Neurol.* 7:66. doi: 10.3389/fneur.2016.00066

Bikson, M., Grossman, P., Thomas, C., Zannou, A. L., Jiang, J., Adnan, T., et al. (2016). Safety of transcranial direct current stimulation: evidence based update 2016. *Brain Stimul.* 9, 641–661. doi: 10.1016/j.brs.2016.06.004

Chang, W. P., Lu, H. C., and Shyu, B. C. (2015). Treatment with direct-current stimulation against cingulate seizure-like activity induced by 4-aminopyridine and bicuculline in an *in vitro* mouse model. *Exp. Neurol.* 265, 180–192. doi: 10.1016/j.expneurol.2015.02.002

Chindo, B. A., Ya,'U. J., Danjuma, N. M., Okhale, S. E., Gamaniel, K. S., and Becker, A. (2014). Behavioral and anticonvulsant effects of the standardized extract of Picus platyphylla stem bark. *J. Ethnopharmacol.* 154, 351–360. doi: 10.1016/j.jep.2014.03.061

Covolan, L., and Mello, L. E. (2000). Temporal profile of neuronal injury following pilocarpine or kainic acid-induced status epilepticus. *Epilepsy Res.* 39, 133–152. doi: 10.1016/S0920-1211(99)00119-9

D'Ambrosio, R., and Perucca, E. (2004). Epilepsy after head injury. *Curr. Opin. Neurol.* 17, 731–735. doi: 10.1097/00019052-200412000-00014

Depaulis, A., and Hamelin, S. (2015). Animal models for mesiotemporal lobe epilepsy: the end of a misunderstanding? *Rev. Neurol.* 171, 217–226. doi: 10.1016/j.neurol.2015.01.558

Dhamne, S. C., Ekstein, D., Zhuo, Z., Gersner, R., Zurakowski, D., Loddenkemper, T., et al. (2015). Acute seizure suppression by transcranial direct current stimulation in rats. *Ann. Clin. Transl. Neurol.* 2, 843–856. doi: 10.1002/acn3.226

Dhir, A., Naidu, O. S., and Kulkarni, S. K. (2005). Effect of naproxen, a non-selective cyclo-oxygenase inhibitor, on pentylenetetrazol-induced kindling in mice. *Clin. Exp. Pharmacol. Physiol.* 32, 579–584. doi: 10.1111/j.1440-1681.2005.04233.x

Faria, P., Fregni, F., Sebastião, F., Dias, A. I., and Leal, A. (2012). Feasibility of focal transcranial DC polarization with simultaneous EEG recording: preliminary assessment in healthy subjects and human epilepsy. *Epilepsy Behav.* 25, 417–425. doi: 10.1016/j.yebeh.2012.06.027

Fertonani, A., Rosini, S., Cotelli, M., Rossini, P. M., and Miniussi, C. (2010). Naming facilitation induced by transcranial direct current stimulation. *Behav. Brain Res.* 208, 311–318. doi: 10.1016/j.bbr.2009.10.030

Fregni, F., and Pascual-Leone, A. (2007). Technology insight: noninvasive brain stimulation in neurology-perspectives on the therapeutic potential of rTMS and tDCS. *Nat. Clin. Pract. Neurol.* 3, 383–393. doi: 10.1038/ncpneuro0530

Fregni, F., Thome-Souza, S., Nitsche, M. A., Freedman, S. D., Valente, K. D., and Pascual-Leone, A. (2006). A controlled clinical trial of cathodal DC polarization in patients with refractory epilepsy. *Epilepsia* 47, 335–342. doi: 10.1111/j.1528-1167.2006.00426.x

French, J. A. (2007). Refractory epilepsy: clinical overview. *Epilepsia* 48, 3–7. doi: 10.1111/j.1528-1167.2007.00992.x

Gomez-Palacio Schjetnan, A., Faraji, J., Metz, G. A., Tatsuno, M., and Luczak, A. (2013). Transcranial direct current stimulation in stroke rehabilitation: a review of recent advancements. *Stroke Res. Treat.* 2013:170256. doi: 10.1155/2013/170256

Grippe, T. C., Brasil-Neto, J. P., Boechat-Barros, R., Cunha, N. S., and Oliveira, P. L. (2015). Interruption of Epilepsia Partialis Continua by Transcranial Direct Current Stimulation. *Brain Stimul.* 8, 1227–1228. doi: 10.1016/j.brs.2015.08.004

Gschwind, M., and van Mierlo, P. (2016). Difficulty of comparing the multiple heterogeneous approaches: comment to transcranial direct current stimulation in epilepsy. *Brain Stimul.* 9, 459–461. doi: 10.1016/j.brs.2016.03.003

Hayashi, Y., Hattori, Y., Asaki, H., Mortwaki, A., and Hori, Y. (1988). Effects of prolonged weak anodal direct current on electrocorticogram in awake rabbit. *Acta Med. Okayama* 42, 293–296.

Henderson, V. C., Kimmelman, J., Fergusson, D., Grimshaw, J. M., and Hackam, D. G. (2013). Threats to validity in the design and conduct of preclinical efficacy studies: a systematic review of guidelines for *in vivo* animal experiments. *PLoS Med.* 10:e1001489. doi: 10.1371/journal.pmed.1001489

Higgins, J., and Green, S. (eds.). (2011). *Cochrane Handbook for Systematic Reviews of Interventions. Version 5.1.0.* The Cochrane Collaboration.

Hooijmans, C. R., Rovers, M. M., de Vries, R. B., Leenaars, M., Ritskes-Hoitinga, M., and Langendam, M. W. (2014). SYRCLE's risk of bias tool for animal studies. *BMC Med. Res. Methodol.* 14:43. doi: 10.1186/1471-2288-14-43

Jackson, M. P., Rahman, A., Lafon, B., Kronberg, G., Ling, D., Parra, L. C., et al. (2016). Animal models of transcranial direct current stimulation: methods and mechanisms. *Clin. Neurophysiol.* 127, 3425–3454. doi: 10.1016/j.clinph.2016.08.016

Kamida, T., Kong, S., Eshima, N., Abe, T., Fujiki, M., and Kobayashi, H. (2011). Transcranial direct current stimulation decreases convulsions and spatial memory deficits following pilocarpine-induced status epilepticus in immature rats. *Behav. Brain Res.* 217, 99–103. doi: 10.1016/j.bbr.2010.08.050

Kamida, T., Kong, S., Fahima, N., and Fujiki, M. (2013). Cathodal transcranial direct current stimulation affects seizures and cognition in fully amygdala-kindled rats. *Neurol. Res.* 35, 602–607. doi: 10.1179/1743132813Y.0000000170

Kandratavicius, L., Balista, P. A., Lopes-Aguiar, C., Ruggiero, R. N., Umeoka, E. H., Garcia-Cairasco, N., et al. (2014). Animal models of epilepsy: use and limitations. *Neuropsychiatr. Dis. Treat.* 10, 1693–1705. doi: 10.2147/NDT.S50371

Lason, W., Chlebicka, M., and Rejdak, K. (2013). Research advances in basic mechanisms of seizures and antiepileptic drug action. *Pharmacol. Rep.* 65, 787–801. doi: 10.1016/S1734-1140(13)71060-0

Lefaucheur, J. P. (2016). A comprehensive database of published tDCS clinical trials (2005–2016). *Neurophysiol. Clin.* 46, 319–398. doi: 10.1016/j.neucli.2016.10.002

Lefte, J., Morales-Quezada, L., Carvalho, S., Thibaut, A., Doruk, D., Chen, C. F., et al. (2017). Surface EEG-transcranial direct current stimulation (tDCS) closed-loop system. *Int. J. Neural Syst.* 27:1750026. doi: 10.1142/S0129065717500265

Liebetanz, D., Fregni, F., Monte-Silva, K. K., Oliveira, M. B., Amâncio-dos-Santos, A., Nitsche, M. A., et al. (2006a). After-effects of transcranial direct current stimulation (tDCS) on cortical spreading depression. *Neurosci. Lett.* 398, 85–90. doi: 10.1016/j.neulet.2005.12.058

Liebetanz, D., Klinkerm, F., Hering, D., Koch, R., Nitsche, M. A., Potschka, H., et al. (2006b). Anticonvulsant effects of transcranial direct-current stimulation (tDCS) in the rat cortical ramp model of focal epilepsy. *Epilepsia* 47, 1216–1224. doi: 10.1111/j.1528-1167.2006.00539.x

Loscher, W. (2002). Current status and future directions in the pharmacotherapy of epilepsy. *Trends Pharmacol. Sci.* 23, 113–118. doi: 10.1016/S0165-6147(00)01974-X

Löscher, W. (2011). Critical review of current animal models of seizures and epilepsy used in the discovery and development of new antiepileptic drugs. *Seizure* 20, 359–368. doi: 10.1016/j.seizure.2011.01.003

Loscher, W., and Schmidt, D. (2004). New horizons in the development of antiepileptic drugs: the search for new targets. *Epilepsy Res.* 60, 77–159. doi: 10.1016/j.eplepsyres.2004.06.004

McCormick, D. A., and Contreras, D. (2001). On the cellular and network bases of epileptic seizures. *Annu. Rev. Physiol.* 63, 815–846. doi: 10.1146/annurev.physiol.63.1.815

Medeiros, L. F., de Souza, I. C., Vidor, L. P., de Souza, A., Deitos, A., Volz, M. S., et al. (2012). Neurobiological effects of transcranial direct current stimulation: a review. *Front. Psychiatr.* 3:110. doi: 10.3389/fpsyt.2012.00110

Nitsche, M. A., Cohen, L. G., Wassermann, E. M., Priori, A., Lang, N., Antal, A., et al. (2008). Transcranial direct current stimulation: state of the art. *Brain Stimul.* 1, 206–223. doi: 10.1016/j.brs.2008.06.004

Nitsche, M. A., Liebetanz, D., Lang, N., Antal, A., Tergau, F., and Paulus, W. (2003). Safety criteria for transcranial direct current stimulation (tDCS) in humans. *Clin. Neurophysiol.* 114, 2220–2222. doi: 10.1016/S1388-2457(03)00235-9

Nitsche, M. A., and Paulus, W. (2009). Noninvasive brain stimulation protocols in the treatment of epilepsy: current state and perspectives. *Neurotherapeutics* 6, 244–250. doi: 10.1016/j.nurt.2009.01.003

Pereira, L. S., Müller, V. T., da Mota Gomes, M., Rotenberg, A., and Fregni, F. (2016). Safety of repetitive transcranial magnetic stimulation in patients with epilepsy: a systematic review. *Epilepsy Behav.* 57, 167–176. doi: 10.1016/j.yebeh.2016.01.015

Petrovski, S., Szoeke, C. E. I., Jones, N. C., Salzberg, M. R., Sheffield, L. J., Huggins, R. M., et al. (2010). Neuropsychiatric symptomatology predicts seizure recurrence in newly treated patients. *Neurology* 75, 1015–1021. doi: 10.1212/WNL.0b013e3181f25b16

Raghavan, S., Eldabe, S., and Strachan, R. (2008). Novel trends in pain management – Neuromodulation. *Curr. Anaesth. Crit. Care* 19, 344–348. doi: 10.1016/j.cacc.2008.07.005

Rosen, A. C., Ramkumar, M., Nguyen, T., and Hoeft, F. (2009). Noninvasive transcranial brain stimulation and pain. *Curr. Pain Headache Rep.* 13, 12–17. doi: 10.1007/s11916-009-0004-2

Rroji, O., van Kuyck, K., Nuttin, B., and Wenderoth, N. (2015). Anodal tDCS over the primary motor cortex facilitates long-term memory formation reflecting use-dependent plasticity. *PLoS ONE* 10:e0127270. doi: 10.1371/journal.pone.0127270

Ruscheweyh, R., Wilder-Smith, O., Drdla, R., Liu, X. G., and Sandkühler, J. (2011). Long-term potentiation in spinal nociceptive pathways as a novel target for pain therapy. *Mol. Pain* 7:20. doi: 10.1186/1744-8069-7-20

San-Juan, D., Calcáneo, Jde, D., González-Aragón, M. F., Maldonado, L. B., Avellán, A. M., Argumosa, E. V., et al. (2011). Transcranial direct current stimulation in adolescent and adult Rasmussen's encephalitis. *Epilepsy Behav.* 20, 126–131. doi: 10.1016/j.yebeh.2010.10.031

San-Juan, D., Espinoza López, D. A., Vázquez Gregorio, R., Trenado, C., Fernández-González Aragón, M., Morales-Quezada, L., et al. (2017). Transcranial direct current stimulation in mesial temporal lobe epilepsy and Hippocampal Sclerosis. *Brain Stimul.* 10, 28–35. doi: 10.1016/j.brs.2016.08.013

San-Juan, D., Morales-Quezada, L., Orozco Garduño, A. J., Alonso-Vanegas, M., González-Aragón, M. F., Espinoza López, D. A., et al. (2015). Transcranial direct current stimulation in epilepsy. *Brain Stimul.* 8, 455–464. doi: 10.1016/j.brs.2015.01.001

Scorza, F. A., and Brunoni, A. R. (2015). Transcranial direct current stimulation against sudden unexpected death in epilepsy: press that button again, please. *Brain Stimul.* 8, 839–840. doi: 10.1016/j.brs.2015.04.006

Sloviter, R. S. (2008). Hippocampal epileptogenesis in animal models of mesial temporal lobe epilepsy with hippocampal sclerosis: the importance of the "latent period" and other concepts. *Epilepsia* 49, 85–92. doi: 10.1111/j.1528-1167.2008.01931.x

Tatti, E., Rossi, S., Innocenti, I., Rossi, A., and Santarnecchi, E. (2016). Non-invasive brain stimulation of the aging brain: state of the art and future perspectives. *Ageing Res. Rev.* 29, 66–89. doi: 10.1016/j.arr.2016.05.006

Tekturk, P., Erdogan, E. T., Kurt, A., Kocagoncu, E., Kucuk, Z., Kinay, D., et al. (2016a). Transcranial direct current stimulation improves seizure control in patients with Rasmussen encephalitis. *Epileptic Disord.* 18, 58–66. doi: 10.1684/epd.2016.0796

Tekturk, P., Erdogan, E. T., Kurt, A., Vanli-Yavuz, E. N., Ektiroglu, E., Kocagoncu, E., et al. (2016b). The effect of transcranial direct current stimulation on seizure frequency of patients with mesial temporal lobe epilepsy with hippocampal sclerosis. *Clin. Neurol. Neurosurg.* 149, 27–32. doi: 10.1016/j.clineuro.2016.07.014

van Elst, L. T., Woermann, F. G., Lemieux, L., Thompson, P. J., and Trimble, M. R. (2000). Affective aggression in patients with temporal lobe epilepsy: a quantitative MRI study of the amygdala. *Brain* 123, 234–243. doi: 10.1093/brain/123.2.234

Woods, A. J., Antal, A., Bikson, M., Boggio, P. S., Brunoni, A. R., Celnik, P., et al. (2016). A technical guide to tDCS, and related non-invasive brain stimulation tools. *Clin. Neurophysiol.* 127, 1031–1048. doi: 10.1016/j.clinph.2015.11.012

Yook, S. W., Park, S. H., Seo, J. H., Kim, S. J., and Ko, M. H. (2011). Suppression of seizure by cathodal transcranial direct current stimulation in an epileptic patient – a case report. *Ann. Rehabil. Med.* 35, 579–582. doi: 10.5535/arm.2011.35.4.579

Zhang, Z., Liao, W., Xu, Q., Wei, W., Zhou, H. J., Sun, K., et al. (2017). Hippocampus-associated causal network of structural covariance measuring structural damage progression in temporal lobe epilepsy. *Hum. Brain Mapp.* 38, 753–766. doi: 10.1002/hbm.23415

Zoberi, M., and van Luijtelaar, G. (2013). Noninvasive transcranial direct current stimulation in a genetic absence model. *Epilepsy Behav.* 26, 42–50. doi: 10.1016/j.yebeh.2012.10.018

Zoghi, M., O'Brien, T. J., Kwan, P., Cook, M. J., Galea, M., and Jaberzadeh, S. (2016). The effects of cathodal transcranial direct current stimulation in a patient with drug-resistant temporal lobe epilepsy (case study). *Brain Stimul.* 9, 790–792. doi: 10.1016/j.brs.2016.05.011

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ARTIGO II: Transcranial direct current stimulation (tDCS) affects neuroinflammation parameters and behavioral seizure activity in pentylenetetrazole-induced kindling in rats

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Transcranial direct current stimulation (tDCS) affects neuroinflammation parameters and behavioral seizure activity in pentylenetetrazole-induced kindling in rats

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Abstract
Despite the introduction of new antiepileptic drugs, about 30% of patients with epilepsy are refractory to drug therapy. Thus, the search for non-pharmacological interventions such as transcranial direct current stimulation (tDCS) may be an alternative treatment either alone or in combination with low doses of anticonvulsants. This study evaluated the effect of anodal (a-tDCS) and cathodal tDCS (c-tDCS) on seizures induced by pentylenetetrazole (PTZ) in rats submitted to the kindling model using diazepam (DZP) as anticonvulsant gold standard. Neither a-tDCS nor c-tDCS reduced the occurrence of clonic forelimb seizures. Associated with diazepam, c-tDCS (c-tDCS-DZP0.15) increased the latency to first clonic forelimb seizure on the 4^{th} and 6^{th} days. Hippocampal IL-1β levels were reduced by c-tDCS and c-tDCS-DZP0.15. On the other hand, these treatments induced an increase in cortical IL-1β levels. Hippocampal TNF-α levels were not altered by c-tDCS or a-tDCS, but c-tDCS and c-tDCS-DZP0.15 increased those levels in cerebral cortex. Cortical NGF levels were increased by c-tDCS and c-tDCS-DZP0.15. a-tDCS-DZP0.15 reduced hippocampal BDNF levels and c-tDCS-DZP0.15 increased those levels in cerebral cortex. In conclusion, c-tDCS alone or in combination with a low dose of DZP showed neuroprotective effects, improving central neurotrophin levels and decreasing hippocampal IL-1β levels after PTZ-induced kindling without statistically significant effect on seizure behavior.

Keywords: Transcranial direct current stimulation; Kindling; Pentylenetetrazole; Neuroinflammation; Cytokines

1. Introduction

With detrimental effects on health and life quality, epilepsy is a chronic neurological disorder characterized by recurrent seizures [1,2]. These effects include complex psychiatric, behavioral, cognitive, and social problems in more than 50% of patients with drug-resistant epilepsy [3]. Additionally, to poor drug effectiveness, patients may suffer from adverse effects such as depression, irritability, and loss of cognitive functions [4]. In the past two decades more than 15 antiepileptic drugs (AEDs) were developed to treat epilepsy, most of which work based on a single action mechanism [5,6]. Despite these developments, about 30% of adults and adolescents with common forms of epilepsy still suffer from seizures. This underscores the failure of drug-based treatments, either in monotherapy regimens or in combinations of AED [7,4].

Considering this scenario, alternative treatment strategies such as transcranial direct-current stimulation (tDCS) provide a non-invasive tool to control drug-resistant seizures [8]. tDCS has been shown to improve symptoms of patients with Alzheimer's disease [9], Parkinson'sdisease [10], post-stroke motor deficit [11], and untreatable seizures [12]. The detailed mechanism of action of tDCS remains unknown, but it appears to involve a combination of hyperpolarization and depolarization of axons besides changes in synaptic functions [13]. Several clinical studies have demonstrated that tDCS may produce both short- and long-term suppressive effects on seizures, suggesting a complex mechanism underlying its effects [8,14]. A previous study using deep brain stimulation (DBS) showed that along-term-depression-like (LTD) stimulation protocol (0.1-Hz stimulation) delayed basolateral amygdala kindling [15]. Also, low-frequency stimulation induces LTD, decreasing the frequency and amplitude of the seizure-like activity in hippocampal slices [16]. This allows suggesting that the induction of LTD might be helpful in suppressing seizure, which in turn may be the main action

mechanism of tDCS on seizures. Consequently, cortical excitability decreases, along side the probability of paroxysmal activity in epileptogenic cortical regions [17]. Moreover, the immediate anticonvulsant effects of cathodal-tDCS (c-tDCS) consist of the hyperpolarization of neuronal soma and the desynchronization of neuronal activity [8]. The long-term effect of c-tDCS has been suggested to occur through the modulation of synaptic transmission, inducing NMDA receptor-dependent LTD in the thalamocingulate pathway [8]. Although preclinical studies suggest that c-tDCS has anticonvulsant effect, the effects of anodal-tDCS (a-tDCS) seem to be the opposite. Research shows that a-tDCS decreases the threshold for seizure activity – as evident in electroencephalogram evaluations – without behavioral changes [18,19]. In the attempt to prevent the generation and propagation of seizures with a-tDCS, a survey was conducted based on the hypothesis that, even if c-tDCS decreases cortical excitability, a-tDCS increases the activity of inhibitory connections. However, this a-tDCS approach was not effective in reducing seizure frequency [20].

The importance of inflammatory processes and their relationship with the etiology of epilepsy has been investigated in recent years [21]. Characterized primarily by astrogliosis, microglial activation, and the production of cytokines and chemokines, neuroinflammation has been observed both in human and experimental models of epilepsy [22]. Experimental data have demonstrated the intrinsic relationship between epilepsy and inflammation, showing that inflammatory molecules are involved in significant loss of neuronal cells after cerebral insults from seizures [23,24]. Evidence such as the fact that anti-inflammatory therapies appear to have anticonvulsant effect on drug-resistant epilepsy supports the role of inflammation in epilepsy [25,26]. Also, seizure activity may activate immune cells of the brain or microglia, consequently producing inflammatory cytokines [24]. Additionally, spontaneous seizures may

perpetuate chronic inflammation [27]. Increased levels of cytokines were observed in rodent brain submitted to seizures induced chemically and electrically [28,29,30]. Accordingly, besides the fact that inflammation is a consequence of seizure activity, it appears to play a key role in epileptogenesis.

In this scenario, the aim of this study was to determine the effects of a-tDCS or c-tDCS in rats submitted to the pentylenetetrazole (PTZ)-induced kindling model. Also, behavior and neurochemical parameters were evaluated, and the effect of combined treatments of a-tDCS or c-tDCS with diazepam (DZP), a therapeutically relevant anticonvulsant, was assessed.

2. Material and Methods

2.1. Animals

The experiment was carried out using 152 male Wistar rats (2 months of age, 300-400 g). Animals (three per cage) were kept in polypropylene home cages (49 × 34× 16 cm) in a temperature-controlled environment (22 ± 2 °C) with access to water and food *ad libitum.* The floor of cages was lined with sawdust, and animals were maintained under a standard 12 h dark/light cycle (lights on at 7:00 a.m. and off at 7:00 p.m.). Rats were assigned randomly to eight groups. All experiments and procedures were approved by the Institutional Committee for Animal Care and Use (GPPG/HCPA protocol No. 160265) and conducted in compliance with Brazilian laws [31-33] and the Laboratory Guide for the Care and Use of Animals [34], which regulates the scientific use of animals. Vigorous attempts were made to minimize the number of animals required to produce reliable scientific data and mitigate external sources of pain and discomfort.

2.2. Pharmacological agents

PTZ was purchased from Sigma-Aldrich Co. (St. Louis, MO, U.S.A.). DZP (Compaz® 5 mg/mL, Cristália Ltda., São Paulo, Brazil) was provided by Hospital de Clínicas de Porto Alegre, Porto Alegre, Rio Grande do Sul, Brazil and was used as the reference anticonvulsant drug for comparison (positive control). PTZ and DZP were dissolved in normal saline (SAL) and administered (10 mL/kg body weight) intraperitoneally (i.p.) [35].

2.3. Kindling procedure

The PTZ-induced kindling model was carried out as described by De Oliveira et al. [36] with minor modifications. The severity of seizures was evaluated using the Racine scale. The PTZ dose used was 50 mg/kg i.p. based on a pilot test. This convulsant agent was administered every three days, totaling six treatments (16 days). Rats were distributed into eight groups: (i) sham/SAL/PTZ (submitted to PTZ and to SHAM tDCS); the groups submitted to tDCS were divided in three groups: (ii) tDCS/SAL/SAL – received only SAL; (iii) tDCS/SAL/PTZ (received SAL and PTZ); (iv) and tDCS/DZP0.15/PTZ (received DZP 0.15 mg/kg and PTZ);the groups that were not submitted to tDCSwere divided in four groups: (v) SAL/SAL (received only SAL); (vi) SAL/PTZ (received SAL and PTZ); (vii) DZP3/PTZ (received DZP 3 mg/kg and PTZ); and (viii) DZP0.15/PTZ (received DZP 0.15 mg/kg and PTZ). A schematic diagram demonstrating the experimental design used is shown in Figure 1.

Immediately after PTZ injection, rats were placed individually in acrylic observation chambers for 30 min, and behavioral seizure was observed. The parameters evaluated were latency to first clonic forelimb seizure and occurrence of clonic forelimb seizures longer than 3 s (Racine Scale Stage 3). Animals were observed on each day PTZ was administered. DZP, a gamma-aminobutyric acid A receptors (GABAAR) agonist, was used in this study as a positive control (3 mg/kg). Also, a low DZP dose (0.15 mg/kg)

was used to assess its association with tDCS in the PTZ-induced kindling model. DZP doses were determined according to pilot tests (dose-response). All behavioral experiments were carried out between 7 a.m. and 11 a.m.

2.4. tDCS treatment

Animals received tDCS on interval days between the PTZ administrations throughout the experimental protocol (a total of 10 sessions). Rats of the active treatment groups were submitted toa 20-min daily session of a-tDCS or c-tDCS every morning for 10 days, as described by Adachi et al. [37] and Laste et al. [38]. A constant direct current of 0.5 mA was delivered from a battery-powered stimulator using electrocardiogram electrodes with conductive adhesive hydrogel. The rats' heads were shaved to ensure proper adherence andelectrodes were trimmed to 1.5 cm^2 for better fit. The electrodes were fixed to the head with adhesive tape (Micropore™) and covered with a protective mesh to prevent removal. For cathodal assembly, the anode was positioned at the midpoint between the lateral angles of both eyes (supraorbital area), and the cathode was placed on the head using landmarks of the neck and shoulder lines as a guide (the anterior and posterior regions in the midline between the two hemispheres of the parietal cortex, as described by Dhamne et al. [39] with minor modifications). This technique mirrors human tDCS protocols used in epilepsy treatment [12,40,41,42,14,43,44,20,45] and has been used by several research groups. It changes cortical excitability, suppressing seizures [39,46,47,18,48]. For the anodal assembly, the anodal electrode was positioned between the ears, from the neck of the rat (parietal cortex) [49, with modifications], while the cathodal electrode was positioned at the midpoint of the lateral angle of the eyes (supraorbital area). The electrodes were placed on the skin [37]. For sham stimulation, the electrodes were placed and fixed on

the same sites as used for real stimulation; however, they were not connected to the battery.

2.5. Tissue Collection

Rats were killed by decapitation at the end of kindling protocol, immediately after the observation. After decapitation, the hippocampus and cerebral cortex were removed, stored in independent slots (without buffer solution), and frozen at -80ºC for subsequent analysis.

2.6. Biochemical Assays

The hippocampus and cerebral cortex of each rat was collected and frozen at −80°C upon analysis. The cerebral structures were homogenized in a mixture of Protease Inhibitor Cocktail (Sigma® #P8340) and phosphate buffered saline (PBS) (1:100) at pH 7.2 using a handheld homogenizer. The homogenate was centrifuged for 5 min at 10,000 rpm. The resultant supernatant was used for the BDNF, IL-1β, NGF, and TNF-α assays. The levels of BDNF, IL-1β, NGF, and TNF-α were determined by sandwich enzyme-linked immunosorbent assay (sandwich-ELISA) using monoclonal antibodies specific for each measurement (R&D Systems, Minneapolis, United States, #DY248, #DY501, #DY556 and #DY510 respectively). Procedures were performed in accordance with the manufacturer's protocol. Optical density was measured using an ELISA reader at450 nm. Data were expressed in pg/mgof protein. Total protein was measured using Bradford's method and bovine serum albumin asstandard [50].

2.7. Statistical analysis

The results of PTZ-induced kindling, occurrence of clonic forelimb seizures, and latency time to its onset were analyzed using Fisher's exact probability test and the Generalized Estimating Equation (GEE) followed by the Bonferroni test, respectively.

Biochemical data were expressed as means ± standard error of the mean (SEM) and analyzed by one-way ANOVA. Means were compared using the Student Newman-Keuls (SNK) test, and the P-value< 0.05 was considered as statistically significant. All experiments were independently repeated at least three times, with triplicate samples for each treatment. Data were analyzed using the GraphPad Prism v.5 program (Intuitive Software for Science, San Diego, CA, U.S.A.) and Statistical Package for Social Sciences (SPSS, Chicago, USA) version 20.0.

3. Results

3.1. Effects of tDCS on parameters of seizures

The results shown in Table 1 demonstrate that DZP 3 mg/kg blocked the occurrence of clonic forelimb seizures for more than 3 s (Fisher's exact test, P< 0.001). c-tDCS did not change the convulsive behavior of the animals (occurrence of seizure or latency to the first bilateral forelimb clonus lasting more than 3 s) (Table 1 and 2; Fisher's exact test, P >0.05 and GEE: Wald χ^2= 111.84; 28; P>0.05, respectively). In contrast, a-tDCS increased the number of seizures and reduced the latency to first seizure, as observed on the 4th and 5th days into exposure to PTZ, similarly to what was observed for the negative control (SAL/PTZ) (Table 1 and 2; Fisher's exact test, P > 0.05 and GEE, P > 0.05, respectively).

The association of c-tDCS and a low dose of DZP (c-tDCS/DZP0.15/PTZ) induces an increase the latency to first seizure, as shown on the 4th and 6th days (Table 2; GEE, P < 0.01). It is possible to observe that the behavior of the c-tDCS/DZP0.15/PTZ group differed from that of the negative control in the number of seizures on the 3rd, 4th, 5th, and 6th days, though with no statistically difference when compared to the SAL/PTZ group (Table 1; Fisher's exact test, P > 0.05)

Associated with a-tDCS, a low dose of DZP (a-tDCS/DZP0.15/PTZ) induced an increase in the occurrence of seizures on the 2nd, 4th, and 5th days; however, there was no statistically significant difference when compared to the SAL/PTZ group (Table 1; Fisher's exact test, P >0.05). Following the same trend, this association reduced the latency to the first bilateral forelimb clonus lasting more than 3 s, also evidenced on the 2nd, 4th, and 5thdays, though with nostatistically significant difference when compared to the negative control (Table 2; GEE, P >0.05).

c-tDCS alone orassociated with DZP 0.15mg reduced PTZ-induced mortality of kindled rats; however, there was no statistically significant difference (Figure 2; Kaplan-Meier/Mantel-Cox, P >0.05). DZP 3 mg/kg prevented mortality when compared to the SAL/PTZ group (Kaplan-Meier/Mantel-Cox, P <0.01). However, the association of a-tDCS with DZP 0.15mg induced the same mortality levels, as observed for the negative control group (SAL/PTZ) (Figure 2; Kaplan-Meier/Mantel-Cox, P = 0.96).

3.2. Effects of tDCS on parameters of neuroinflammation

Hippocampal IL-1β levels were increased by PTZ, as shown in Figure 3A (SAL/PTZ) compared to the SAL/SAL group (one-way ANOVA/SNK, P< 0.05). In contrast, we observed a decrease in the basal levels of IL-1β in the c-tDCS/SAL/SAL group, compared tothe total control group (SAL/SAL) (Figure 3A; one-way ANOVA/SNK, P < 0.05). Oppositely, the a-tDCS/SAL/SAL group had increased levels of IL-1β, similarly to the group treated with PTZ (Figure 3A; one-way ANOVA/SNK, P <0.05).

Figure 3B shows that the groups that received c-tDCS alone (c-tDCS/SAL/PTZ) or in association with DZP 0.15 mg/kg (c-tDCS/DZP0.15/PTZ) had reduced IL-1β levels compared to the SAL/PTZ group (one-way ANOVA/SNK, P <0.05). There was no significant difference between hippocampal IL-1β levels in the groups that received

anodal treatments (a-tDCS/SAL/PTZ and a-tDCS/DZP0.15/PTZ; one-way ANOVA/SNK, P > 0.05). Also, the group treated with DZP 3 mg/kg or DZP 0.15 mg/kg showed no significant difference compared to the SAL/PTZ group (one-way ANOVA/SNK, P>0.05).

PTZ did not alter cortical IL-1β levels (Figure 3C, one-way ANOVA/SNK, P >0.05). Also, neither c-tDCS nor a-tDCS altered the levels of this interleukin (Figure 3C, one-way ANOVA/SNK, P> 0.05).

c-tDCS alone or associated with DZP 0.15 mg/kg raised IL-1β levels compared to SAL/PTZ (Figure 3D; one-way ANOVA/SNK, P < 0.05). a-tDCS did not induce any change in cortical IL-1β levels (one-way ANOVA/SNK, P > 0.05).

Hippocampal TNF-α levels did not differ across treated groups when compared to the negative control (SAL/PTZ) (Figures 4A and 4B; one-way ANOVA/SNK, P > 0.05). In the cerebral cortex, PTZ did not affect TNF-α levels; identical results were observed for the groups treated with c-tDCS or a-tDCS compared to the SAL/SAL group (Figure 4C; one-way ANOVA/SNK, P > 0.05).

c-tDCS alone or in association with DZP 0.15 mg/kg induced a significant increase incortical TNF-α levels, as shown in Figure 4D (one-way ANOVA/SNK, P < 0.05). On the other hand, a-tDCS did not induce alterations in the levels of this cytokine (one-way ANOVA/SNK, P > 0.05).

Figure 5 shows that PTZ did not alter NGF levels in the hippocampus and cerebral cortex (Figure 5A and 5C; one-way ANOVA/SNK, P > 0.05). Also, neither c-tDCS nor a-tDCS changed the levels of this neurotrophin compared to the SAL/SAL group in both brain structures (Figure 5A and 5C; one-way ANOVA/SNK, P > 0.05). The groups treated with c-tDCS alone or associated with DZP 0.15 mg/kg showed a significant

difference in NGF levels compared to the SAL/PTZ group in cerebral cortex (Figure 5D; one-way ANOVA/SNK, $P < 0.05$).

Figure 6A shows that PTZ and c-tDCS did not alter the levels of BDNF in hippocampus when compared to the SAL/SAL group (one-way ANOVA/SNK, $P > 0.05$). However, a-tDCS was able to reduce the levels of this neurotrophin (one-way ANOVA/SNK, $P < 0.05$). Following the same trend, a-tDCS associated with DZP also reduced hippocampal BDNF levels (Figure 6B; one-way ANOVA/SNK, $P < 0.05$). Figure 6C shows that PTZ did not alter cortical BDNF levels (one-way ANOVA/SNK, $P > 0.05$). The same effect was observed after c-tDCS and a-tDCS (one-way ANOVA/SNK, $P > 0.05$). The group treated with c-tDCS associated to DZP 0.15 mg/kg showed a significant difference in the cortical BDNF levels compared to the SAL/PTZ group (Figure 6D; one-way ANOVA/SNK, $P < 0.05$).

4. Discussion

In this study we show that there is a tendency to increase latency to first seizure on the 4^{th} and 6^{th} days after c-tDCS/DZP0.15/PTZ treatment. This contrasts with the negative control both in the number of seizures and latency to first seizure. Also, a-tDCS increased the number of seizures, reducing the latency to first seizure as seen on the 4^{th} and 5^{th} days and increasing the mortality of kindled rats. On the other hand, c-tDCS alone or associated with DZP reduced mortality, though with no statistically significant difference.

It is important to highlight that this study is the first to analyze the protective effect of tDCS in the PTZ-induced kindling model and to compare the effect of a-tDCS and c-tDCS. Our data confirm the findings of astudy [39] that showed that the combined use of c-tDCS and lorazepam is more effective in suppressing seizures than in

improving clinical outcome, when compared to c-tDCS or the drug administered individually. It is important to emphasize that the association with a therapeutic alternative such as tDCS may allow reducing drug doses, thereby decreasing undesirable effects without loss of anticonvulsive effect.

It is important to note that the kindling model is induced by pentylenetetrazole, which is a GABA receptor antagonist that binds to the picrotoxin recognition site on the GABA-A benzodiazepine-chloride-ionophore complex, decreasing GABA inhibition [51]. Benzodiazepines such as DZP are positive allosteric modulators of synaptic GABAA receptors that are responsible for classical phasic inhibition [52]. This occurs because channel opening frequency increases, rising conductance of the chloride ion and inhibiting action potential [53]. Although benzodiazepines are considered potent anticonvulsants, their clinical use is limited due to the associated adverse effects, including psychomotor impairment, sedation, ataxia, and risk of dependence [23]. Even though c-tDCS alone did not produce a consistent improvement in seizure behavior, it presented better results when compared to a-tDCS, a finding that was discussed in other studies [54,41,55]. Nevertheless, it should be underscored that a-tDCS induced higher mortality in the animals submitted to kindling.

Moreover, c-tDCS reduced the hippocampal IL-1β levels in the c-tDCS/SAL/SAL group compared to the control group (SAL/SAL). This result confirms the findings of a study recently published by our research group that showed that tDCS reduced TNF-α and IL-1β protein levels in the Wistar Kyoto rats (WKY) [56]. It suggests the modulation of inflammatory response by tDCS in the control animals. We also observed that c-tDCS and its association with diazepam (c-tDCS/DZP0.15/PTZ) reduced the levels of IL-1β raised by PTZ in hippocampus. However, c-tDCS increased cortical IL-1β levels. a-tDCS increased IL-1β in the SAL/SAL (a-tDCS/SAL/SAL)

group, similarly to what was observed for the negative control (SAL/PTZ) in hippocampus. It is also important to highlight that this result differs from the effect caused by c-tDCS in the c-tDCS/SAL/SAL group, since IL-1β levels were even lower than in the total control (SAL/SAL).

In addition, we observed an increase in IL-1β in the DZP3 group. This finding was unexpected and suggests the modulation of inflammatory response by anticonvulsive activity, confirming the results of a previous study [57]. Kolosowska et al. [58] found that IL-1β, but not TNF-α, appears to play an important role in the kindling process as a good marker for fully developed seizures. The authors further demonstrated an increase in IL-1β concentration in the final stage of kindling, when tonic-clonic seizures are established. Thus, IL-1β is a critical factor involved in the generation and in the spread of seizures [59,60]. In addition, pro-inflammatory mediators such as cytokines like IL-1β and TNF-α and growth factors such as BDNF undergo positive regulation during neuroinflammation. This process is involved in the activation of microglia, astrocytes, and endothelial cells of the blood-brain barrier [61]. Contrary to what we observed in the hippocampus of animals submitted to kindling, in the cerebral cortex we observed an increase in IL-1β levels induced by c-tDCS. This finding confirms the results published by Sayya et al. [62] in a study that suggested that IL-1β could be linked to neuroprotection to the damage induced by epilepsy due to altered epileptogenesis by delaying the acquisition of generalized seizures. It has also been shown that infusion of chronic central IL-1β appears to delay the acquisition of kindling by blocking the generalization of seizures during the early stages of epileptogenesis [58]. It is important to note that modified cytokine levels are linked to increased sensitivity to pain and kindling stimuli [63]. A consistent body of experimental evidence suggests that neuroinflammation processes contribute to the

etiopathogenesis of convulsions [64]. Therefore, such changes are observed in the brain of epileptic patients and in brain tissue samples from animals submitted to experimental models of epilepsy [21].

We observed a reduction in cortical TNF-α levels of animals submitted to kindling and to a-tDCS, besides an increase in the parameter in those receiving c-tDCS. This suggests that TNF-α is important in the activation, differentiation, proliferation, and infiltration of immune cells in the neuroinflammation process [65]. It is interesting to clarify dual role of TNF-α in the pathophysiology of seizures and epilepsy. TNF receptor type 1 (TNFR1 or p55) activation induces a pro-convulsive effect; on the other hand, receptor 2 (TNFR2 or p75) activation induces an anti-convulsive effect [66,67]. Thus, inhibitory or stimulatory effects will be observed in the emergence of seizures, depending on the concentration and type of receptor involved in this process [68]. On the other hand, no significant changes were detected in TNF-α levels during PTZ-induced kindling; however, increased levels of IL-1β were observed in rats that had fully developed seizures [58]. This may be due to the fact that this cytokine is more active at the early and late inflammatory stages [69]. The different TNF-α levels reported in the literature may be attributed to the administration of different doses of the seizure agent as well as to the inter-strain sensitivity in PTZ-induced seizures.

Finally, c-tDCS alone or in association with DZP induced an increase in the levels of NGF and BDNF in cerebral cortex. As already mentioned, excess IL-1β production may induceneurodegeneration in *in vivo* models [70]. However, the *in vitro* effect appears to be the opposite, when IL-1β hasneuroprotective effect in degenerative process induced by excitatory amino acids, which seems to be linked to NGF activity [71]. c-tDCS during kindling significantly increased levels of NGF and BDNF. Moreover, we observed a reduction in the hippocampal levels of BDNF in the a-

tDCS/SAL/SAL group, compared to the negative control. Likewise, we observed that a-tDCS associated with DZP also reduced hippocampal BDNF levels. It is well known that NGF and BNDF are neurotrophins produced in the brain by neuronal and non-neuronal cells [72]. They regulate neuronal survival, differentiation, and reorganization of connectivity in developing and adult brains as well as in neuronal excitability through the synthesis of neurotransmitters, synapse formation, and axon elongation [72,73]. Humpel et al. [51] showed that BDNF levels might be linked to protection mechanisms against damage after kindling seizures and in sprouting responses. It is important to note that BDNF levels were increased in the hippocampus of animals 3 h and 24 h as well as up to 3 days after the last PTZ injection. It is known that various brain lesions increase the activity of neurotrophic factors in the affected areas and around them, which can regulate axonal and dendritic remodeling, which is required in order to maintain damaged neurons and even to prevent the cells from dying [74]. This may explain the improvement in the convulsive behavior in the groups that received c-tDCS, especially in those associated with a low dose of DZP, since their levels of NGF and BDNF increased. Furthermore, it has been proposed that these neurotrophins can mediate anticonvulsive actions associated with other drugs reduce susceptibility to PTZ [72].

The results observed in the present study confirm the findings published in previous research, which showed the ability of tDCS to modulate seizures in a PTZ-induced kindling model based on its beneficial effects on inflammatory parameters [23,64]. Previous studies provide evidence that tDCS reduces inflammatory markers in neuropathic pain and chronic stress-induced pain models [75,37] confirming our results. However, the modulation of neuroinflammatory pathways by tDCS has not been completely clarified. A previous study suggested that tDCS might activate glial cells [76], indicating the pro-inflammatory effect of stimulation. In agreement with another

study [77], our results demonstrate that these effects on inflammation might depend on stimulation parameters. Central and peripheral neuromodulatory techniques like electro acupuncture [78], vagus nerve stimulation [79], epidural motor cortex stimulation [80], and deep brain stimulation [81] have been associated with anti-inflammatory effects in pathological scenarios, supporting the role of tDCS in neuroinflammatory conditions.

Thus, our findings demonstrate that c-tDCS did not significantly decrease PTZ-induced seizures in the kindling model, but c-tDCS alone or associated with a low dose of DZP had neuroprotective effects, improving neurotrophin levels and decreasing IL-1β levels in the hippocampus after PTZ-induced kindling. Instead, a-tDCS has been shown to worsen seizure parameters, in addition to overturning NGF and BDNF levels.

Conclusion

In conclusion, c-tDCS alone or in combination with a low dose of DZP had neuroprotective effects, improving central neurotrophin levels and decreasing hippocampal IL-1β levels after PTZ-induced kindling, without a statistically significant effect in the seizure behavior. In this way, c-tDCS should be further investigated in new studies focusing on the elucidation of its mechanism of action and its interaction with other anticonvulsant drugs. This technique may have potential use in epilepsy or as an adjuvant in conventional pharmacotherapy.

Competing interests

The authors declare that they have no competing interests regarding the publication of this paper.

Authors' contributions

IT and PP contributed to the experimental designs. GGR, PF, CO, LSS, VLS, RS and AS collected and analyzed the experimental data. ILST and PP participated in the study coordination and helped to draft the manuscript. FF helped to final draft the manuscript. All authors read and approved the final manuscript.

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References

1. World Health Organization. Epilepsy, http://www.who.int/mediacentre/factsheets/fs999/en/;2017 [accessed 10 January 2018].

2. Harden J, Black R, Chin RF. Families' experiences of living with pediatric epilepsy: A qualitative systematic review. Epilepsy Behav 2016; 60:225–37.

3. Lin JJ, Mula M, Hermann BP. Uncovering the neurobehavioural comorbidities of epilepsy over the lifespan. Lancet 2012; 380:1180–92.

4. Brodie MJ, Besag F, Ettinger AB, Mula M, Gobbi G, Comai S, et al. Epilepsy, Antiepileptic Drugs, and Aggression: An Evidence-Based Review. Pharmacol Rev 2016; 68:563–602.

5. Löscher W, Klitgaard H, Twyman RE, Schmidt D. New avenues for anti-epileptic drug discovery and development. Nat Rev Drug Discov 2013; 12:757–76.

6. Hanaya R, Arita K. The New Antiepileptic Drugs: Their Neuropharmacology and Clinical Indications. Neurol Med Chir (Tokyo) 2016; 56: 205–20.

7. Brodie MJ, Barry SJE, Bamagous GA, Norrie JD, Kwan P. Patterns of treatment response in newly diagnosed epilepsy. Neurology 2012; 78:1548–54.

8. Chang WP, Lu HC, Shyu BC. Treatment with direct-current stimulation against cingulate seizure-like activity induced by 4-aminopyridine and bicuculline in an in vitro mouse model. Exp Neurol 2015; 265:180–92.

9. Hansen N. Action mechanisms of transcranial direct current stimulation in Alzheimer's disease and memory loss. Front Psychiatry 2012; 3:48.

10. Benninger DH, Lomarev M, Lopez G, Wassermann EM, Li X, Considine E, et al. Transcranial direct current stimulation for the treatment of Parkinson's disease. J Neurol Neurosurg Psychiatry 2010; 81:1105–11.

11. Ayache SS, Farhat WH, Zouari HG, Hosseini H, Mylius V, Lefaucheur JP. Stroke rehabilitation using noninvasive cortical stimulation: motor deficit. Expert Rev Neurother 2012; 12:949–72.

12. Yook SW, Park SH, Seo JH, Kim SJ, Ko MH. Suppression of seizure by cathodaltranscranial direct current stimulation in an epileptic patient: a case report. Ann Rehabil Med 2011; 35:579–82.

13. San-Juan D, Morales-Quezada L, Orozco Garduño AJ, et al. Transcranial Direct Current Stimulation in Epilepsy. Brain Stimul 2015; 8:455–64.

14. Auvichayapat N, Rotenberg A, Gersner R, Ngodklang S, Tiamkao S, Tassaneeyakul W, et al. Transcranial direct current stimulation for treatment of refractory childhood focal epilepsy. Brain Stimul 2013; 6:696–700.

15. Velisek L, Veliskova J, Stanton PK. Low-frequency stimulation of the kindling focus delays basolateral amygdala kindling in immature rats. Neurosci Lett 2002; 326:61–3.

16. Albensi BC, Ata G, Schmidt E, Waterman JD, Janigro D. Activation of long-term synaptic plasticity causes suppression of epileptiform activity in rat hippocampal slices. Brain Res 2004; 998:56–64.

17. Nitsche MA, Paulus W. Noninvasive brain stimulation protocols in the treatment of epilepsy: current state and perspectives. Neurotherapeutics 2009; 6:244–50.

18. Liebetanz D, Klinkerm F, Hering D, Koch R, Nitsche MA, Potschka H, et al. Anticonvulsant effects of transcranial direct-current stimulation (tDCS) in the rat cortical ramp model of focal epilepsy. Epilepsia 2006; 47:1216–24.

19. Zobeiri M, van Luijtelaar G. Noninvasive transcranial direct current stimulation in a genetic absence model. Epilepsy Behav 2013; 26:42–50.

20. Tekturk P, Erdogan ET, Kurt A, et al. Transcranial direct current stimulation improves seizure control in patients with Rasmussen encephalitis. Epileptic Disord 2016a; 18:58–66.

21. Vezzani A, et al. The role of inflammation in epilepsy. Nat Ver Neurol 2011; 7:31–40.

22. McElroy PB, Liang LP, Day BJ, Patel M. Scavenging reactive oxygen species inhibits status epilepticus-induced neuroinflammation. Exp Neurol 2017; 298:13–22.

23. Frantz AL, Regner GG, Pflüger P, Coelho VR, da Silva LL, Viau CM, et al. Manual acupuncture improves parameters associated with oxidative stress and inflammation in PTZ-induced kindling in mice. Neurosci Lett 2017; pii:S0304-3940(17)30786-3.

24. Pflüger P, Viau CM, Coelho VR, Berwig NA, Staub RB, Pereira P, et al. Gamma-decanolactone inhibits iNOS and TNF-alpha production by lipopolysaccharide-activated microglia in N9 cells. Eur J Pharmacol 2016; 780:38–45.

25. Walker L, Sills GJ. Inflammation and Epilepsy: The Foundations for a New Therapeutic Approach in Epilepsy? Epilepsy Curr. 2012; 12:8–12.

26. Yu N, Liu H, Dia Q. Modulation of Immunity and the Inflammatory Response: A New Target for Treating Drug-resistant Epilepsy. Curr Neuropharmacol 2013; 11:114–27.

27. Vezzani A, Balosso S, Ravizza T. Inflammation and epilepsy. Handb Clin Neurol 2012; 107:163–75.

28. Lehtimaki KA, Peltola J, Koskikallio E, Keranen T, Honkaniemi J. Expression of cytokines and cytokine receptors in the rat brain after kainic acid-induced seizures. Brain Res Mol Brain Res 2003; 110:253–60.

29. Andrzejczak D. Epilepsy and pro-inflammatory cytokines. Immunomodulating properties of antiepileptic drugs. Neurol Neurochir Pol 2011; 45:275–85.

30. Li G, Bauer S, Nowak M, Norwood B, Tackenberg B, Rosenow F, et al. Cytokines and epilepsy. Seizure 2011; 20:249–56.

31. Brasil. Presidência da República. Lei nº 11.794 de 8 de outubro de 2008. Regulamenta o inciso VII do parágrafo 1º do artigo 225 da Constituição Federal, estabelecendoProcedimentos para o UsoCientífico de Animais, http://bit.ly/1WV52wP; 2008 [accessed 10 January 2018].

32. MCTI. Ministério da CiênciaTecnologia e Inovação. DiretrizBrasileira para o Cuidado e a Utilização de Animais para Fins Científicos e Didáticos e DBCA, Portaria nº 465 de 23 de maio de 2013, http://www.cobea.org.br/arquivo/download?ID_ARQUIVO¼20; 2013a [accessed 10 January 2018].

33. MCTI. Ministério da CiênciaTecnologia e Inovação. Diretrizes da Prática de Eutanásia do Concea, Portaria n. 596 de 25 de junho de 2013, http://www.cobea.org.br/download/download?ID_DOWNLOAD¼14; 2013b [accessed 10 January 2018].

34. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. 8th edition. Washington (DC): National Academies Press (US); 2011.

35. Guimarães SB, Kimura OS, Vasconcelos PR. Dimethylsulfoxide attenuates ischemia-reperfusion injury in rat testis. Acta Cir Bras 2010; 25:357-61.

36. De Oliveira PA, Lino FL, Cappelari SE, Da Silva Brum LF, Picada JN, Pereira P. Effects of gamma-decanolactone on seizures induced by PTZ-kindling in mice. Exp Brain Res 2008; 187:161–66.

37. Adachi LS, Caumo W, Laste G, Fernandes LF, Ripoll JR, De Souza A, et al. Reversal of chronic stress-induced pain by transcranial direct current stimulation (tDCS) in an animal model. Brain Res 2012; 1489:17–26.

38. Laste G, Caumo W, Adachi LN, Rozisky JR, de Macedo IC, Filho PR, et al. After-effects of consecutive sessions of transcranial direct current stimulation (tDCS) in a rat model of chronic inflammation. Exp Brain Res 2012; 221:75-83.

39. Dhamne SC, Ekstein D, Zhuo Z, Gersner R, Zurakowski D, Loddenkemper T, et al. Acute seizure suppression by transcranial direct current stimulation in rats. Ann Clin Transl Neurol 2015; 2:843–56.

40. San-Juan D, Calcáneo J de D, González-Aragón MF, Maldonado LB, Avellán AM, Argumosa EV, et al. Transcranial direct current stimulation in adolescent and adult Rasmussen's encephalitis. Epilepsy Behav 2011; 20:126–31.

41. San-Juan D, Espinoza López DA, VázquezGregorio R, et al. Transcranial Direct Current Stimulation in Mesial Temporal Lobe Epilepsy and Hippocampal Sclerosis. Brain Stimul 2017; 10:28–35.

42. Faria P, Fregni F, Sebastião F, Dias AI, Leal A. Feasibility of focal transcranial DC polarization with simultaneous EEG recording: preliminary assessment in healthy subjects and human epilepsy. Epilepsy Behav 2012; 25:417–25.

43. Auvichayapat N, Sinsupan K, Tunkamnerdthai O, Auvichayapat P. Transcranial Direct Current Stimulation for Treatment of Childhood PharmacoresistantLennox-Gastaut Syndrome: A Pilot Study. Front Neurol 2016; 7:66.

44. Assenza G, Campana C, Formica D, et al. Efficacy of cathodaltranscranial direct current stimulation in drug-resistant epilepsy: a proof of principle. Conf Proc IEEE Eng Med Biol Soc 2014; 2014:530–33.

45. Tekturk P, Erdogan ET, Kurt A, et al. The effect of transcranial direct current stimulation on seizure frequency of patients with mesial temporal lobe epilepsy with hippocampal sclerosis. Clin Neurol Neurosurg 2016b; 149:27–32.

46. Kamida T, Kong S, Eshima N, Abe T, Fujiki M, Kobayashi H. Transcranial direct current stimulation decreases convulsions and spatial memory deficits following pilocarpine-induced status epilepticus in immature rats. Behav Brain Res 2011; 217:99–103.

47. Kamida T, Kong S, Eshima N, Fujiki M. Cathodaltranscranial direct current stimulation affects seizures and cognition in fully amygdala-kindled rats. Neurol Res 2013; 35:602–07.

48. Zobeiri M, van Luijtelaar G. Noninvasive transcranial direct current stimulation in a genetic absence model. Epilepsy Behav 2013; 26:42–50.

49. Takano Y, Yokawac T, Masudac A, Niimic J, Tanakad S, Hironakaa N. A rat model for measuring the effectiveness of transcranial direct current stimulation using fMRI. Neurosci Lett 2011; 491:40–3.

50. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein dye binding. Anal Biochem 1976; 72:248–54.

51. Humpel C, Wetmore C, Olson L. Regulation of brain-derived neurotrophic factor messenger RNA and protein at the cellular level in pentylenetetrazol-induced epileptic seizures. Neuroscience 1993; 53:909-18.

52. Bruun DA, Cao Z, Inceoglu B, Vito ST, Austin AT, Hulsizer S, et al. Combined treatment with diazepam and allopregnanolone reverses tetramethylenedisulfotetramine (TETS)-induced calcium dysregulation in cultured neurons and protects TETS-intoxicated mice against lethal seizures. Neuropharmacology 2015; 95:332–42.

53. Ataee R, Falahati A, Ebrahimzadeh MA, Shokrzadeh M. Anticonvulsant activities of Sambucusnigra. Eur Rev Med Pharmacol Sci 2016; 20:3123–26.

54. Assenza G, Campana C, Assenza F, et al. Cathodaltranscranial direct current stimulation reduces seizure frequency in adults with drug-resistant temporal lobe epilepsy: A sham controlled study. Brain Stimul 2017; 10:333–35.

55. Zoghi M, O'Brien TJ, Kwan P, Cook MJ, Galea M, Jaberzadeh S. The effects of cathodaltranscranial direct current stimulation in a patient with drug-resistant temporal lobe epilepsy (case study). Brain Stimul 2016; 9:790–92.

56. Leffa DT, Bellaver B, Salvi AA, de Oliveira C, Caumo W, Grevet EH, et al. Transcranial direct current stimulation improves long-term memory deficits in an animal model of attention-deficit/hyperactivity disorder and modulates oxidative and inflammatory parameters. Brain Stimul 2018; pii: S1935-861X(18)30102-5.

57. Figueroba SR, Desjardins MP, Nani BD, Ferreira LE, Rossi AC, Santos FA, et al. Effect of diazepam on temporomandibular joints in rats with increased occlusal vertical dimension. Br J Oral Maxillofac Surg 2014; 52:438–44.

58. Kołosowska K, Maciejak P, Szyndler J, Turzyńska D, Sobolewska A, Płaźnik A. The role of interleukin-1β in the pentylenetetrazole-induced kindling of seizures, in the rat hippocampus. Eur J Pharmacol 2014; 731:31–7.

59. De Simoni MG, Perego C, Ravizza T, Moneta D, Conti M, Marchesi F, et al. Inflammatory cytokines and related genes are induced in the rat hippocampus by limbic status epilepticus. Eur J Neurosci 2000; 12:2623–33.

60. Vezzani A, Moneta D, Richichi C, Aliprandi M, Burrows SJ, Ravizza T, et al. Functional role of inflammatory cytokines and antiinflammatory molecules in seizures and epileptogenesis. Epilepsia 2002; 43:30–5.

61. Dey A, Kang X, Qiu J, Du Y, Jiang J. Anti-Inflammatory Small Molecules To Treat Seizures and Epilepsy: From Bench to Bedside. Trends Pharmacol Sci 2016; 37:463–84.

62. Sayyah M, Beheshti S, Shokrgozar MA, Eslami-far A, Deljoo Z, Khabiri AR, et al. Antiepileptogenic and anticonvulsant activity of interleukin 1beta in amygdala-kindled rats. Exp Neurol 2005; 191:145–53.

63. Clinton JM, Davis CJ, Zielinski MR, Jewett KA, Krueger JM. Biochemical Regulation of Sleep and Sleep Biomarkers. J Clin Sleep Med 2011; 7:S38–42.

64. Hoda U, Agarwal NB, Vohora D, Parvez S, Raisuddin S. Resveratrol suppressed seizures by attenuating IL-1β, IL1-Ra, IL-6, and TNF-α in the hippocampus and cortex of kindled mice. Nutr Neurosci 2017; 20:497–504.

65. Sonar S, Lal G. Role of tumor necrosis factor superfamily in neuroinflammation and autoimmunity. Front Immunol 2015; 6:364.

66. Weinberg MS, et al. Opposing actions of hippocampus TNF-alpha receptors on limbic seizure susceptibility. Exp Neurol 2013; 247:429–37.

67. Balosso S, et al. The dual role of TNF-alpha and its receptors in seizures. Exp Neurol 2013; 247:267–71.

68. Balosso S, Ravizza T, Perego C, Peschon J, Campbell IL, De Simoni MG, et al. Tumor necrosisfactor-alpha inhibits seizures in mice via p75 receptors. Ann Neurol 2005; 57:804–12.

69. Lai YC, Shaftel SS, Miller JH, et al. Intra-articular induction of interleukin-1b expression in the adult mouse, with resultant temporomandibular joint pathologic changes, dysfunction, and pain. Arthritis Rheum 2006; 54:1184–97.

70. Rothwell NJ, Strijbos PJ. Cytokines in neurodegeneration and repair. Int J Dev Neurosci 1995; 13:179–85.

71. Strijbos PJ, Rothwell NJ. Interleukin-1bet attenuates excitatory amino acid-induced neurodegeneration in vitro: involvement of nerve growth factor. J Neurosci 1995; 15:3468–74.

72. Tirassa P, Costa N. CCK-8 induces NGF and BDNF synthesis and modulates TrkA and TrkB expression in the rat hippocampus and septum: Effects on kindling development. Neurochem Int 2007; 50:130–38.

73. Poo MM. Neurotrophins as synaptic modulators. Nat Rev Neurosci 2001; 2: 24–32.

74. Satfran BN, Crutcher KA.NGF-induced remodeling of mature uninjured axon collaterals. Brain Res 1990; 525:1l–20.

75. Cioato SG, Medeiros LF, Marques Filho PR, Vercelino R, de Souza A, Scarabelot VL, et al. Long-Lasting Effect of Transcranial Direct Current Stimulation in the Reversal of Hyperalgesia and Cytokine Alterations Induced by the Neuropathic Pain Model. Brain Stimul 2016; 9:209–17.

76. King JA, Barkley RA, Delville Y, Ferris CF. Early androgen treatment decreasescognitive function and catecholamine innervation in an animal model ofADHD. Behav Brain Res 2000; 107:35-43.

77. Yoon KJ, Lee YT, Chae SW, Park CR, Kim DY. Effects of anodal Transcranial direct current stimulation (tDCS) on behavioral and spatial memory duringthe early stage of traumatic brain injury in the rats. J Neurol Sci 2016; 362:314-20.

78. Yu X, Li Y, Wen H, Zhang Y, Tian X. Intensity-dependent effects of repetitiveanodal transcranial direct current stimulation on learning and memory in arat model of Alzheimer's disease. Neurobiol Learn Mem 2015; 123:168-78.

79. Pedron S, Monnin J, Haffen E, Sechter D, Van Waes V. Repeated Transcranial direct current stimulation prevents abnormal behaviors associated withabstinence from chronic nicotine consumption. Neuropsychopharmacology 2014; 39:981-8.

80. Kandel ER. The molecular biology of memory: cAMP, PKA, CRE, CREB-1, CREB-2, and CPEB. Mol Brain 2012; 5:14.

81. Rohan JG, Carhuatanta KA, McInturf SM, Miklasevich MK, Jankord R. Modulatinghippocampal plasticity with in vivo brain stimulation. J Neurosci 2015; 35:12824-32.

Figure legends

Figure 1. Diagram illustrating the chronological events of the experiments. (A) groups not treated with tDCS. (B) tDCS groups.

Figure 2. The effect of tDCS on mortality in rats submitted to PTZ-kindling. Cumulative survival (Kaplan-Meier) during PTZ-kindling. N = 9 - 20 animals per group (**$P \leq 0.01$). P-values were calculated using the log-rank (Mantel-Cox) test compared to the SAL/PTZ group.

Figure 3. The effect of tDCS on IL-1β levels in hippocampus and cerebral cortex. Data are presented as the mean ± SEM, n = 4-8 animals per group.

(A) Effect of c-tDCS and a-tDCS on IL-1β levels in hippocampus. One-way ANOVA/SNK. * significant difference compared to the SAL/SAL group, $P < 0.05$.

(B) Effect of c-tDCS and a-tDCS on IL-1β levels in hippocampus. One-way ANOVA/SNK. * significant difference compared to the SAL/PTZ group, $P < 0.05$.

(C) Effect of c-tDCS and a-tDCS on IL-1β levels in cerebral cortex. One-way ANOVA/SNK. There was no significant difference, $P > 0.05$. (D) Effect of c-tDCS and a-tDCS on IL-1β levels in cerebral cortex. One-way ANOVA/SNK. * significant difference compared to the SAL/PTZ group, $P < 0.05$.

Figure 4. The effect of tDCS on TNF-α levels in hippocampus and cerebral cortex. Data are presented as the mean ± SEM, n= 5-11 animals per group.

(A) Effect of c-tDCS and a-tDCS on TNF-α levels in hippocampus. One-way ANOVA/SNK. There was no significant difference, $P > 0.05$.

(B) Effect of c-tDCS and a-tDCS on TNF-α levels in hippocampus. One-way ANOVA/SNK. There was no significant difference, $P > 0.05$.

(C) Effect of c-tDCS and a-tDCS on TNF-α levels in cerebral cortex. One-way ANOVA/SNK. There was no significant difference, $P > 0.05$.

(D) Effect of c-tDCS and a-tDCS on TNF-α levels in cerebral cortex. One-way ANOVA/SNK. * significant difference compared to the SAL/PTZ group, $P < 0.05$.

Figure 5. The effect of tDCS on NGF levels in hippocampus and cerebral cortex. Data are presented as the mean ± SEM, n = 5 - 10 animals per group.

(A) Effect of c-tDCS and a-tDCS on NGF levels in hippocampus. One-way ANOVA/SNK. There was no significant difference, $P > 0.05$.

(B) Effect of c-tDCS and a-tDCS on NGF levels in hippocampus. One-way ANOVA/SNK. There was no significant difference, $P > 0.05$.

(C) Effect of c-tDCS and a-tDCS on NGF levels in cerebral cortex. One-way ANOVA/SNK. There was no significant difference, $P > 0.05$.

(D) Effect of c-tDCS and a-tDCS on NGF levels in cerebral cortex. One-way ANOVA/SNK. * significant difference compared to the SAL/PTZ group, $P < 0.05$.

Figure 6. The effect of tDCS on BDNF levels in hippocampus and cerebral cortex. Data are presented as the mean ± SEM. n = 4 - 11 animals per group.

(A) Effect of c-tDCS and a-tDCS on BDNF levels in hippocampus. One-way ANOVA/SNK. * significant difference compared to the SAL/SAL group, $P < 0.05$.

(B) Effect of c-tDCS and a-tDCS on BDNF levels in hippocampus. One-way ANOVA/SNK. * significant difference compared to the SAL/PTZ group, $P < 0.05$.

(C) Effect of c-tDCS and a-tDCS on BDNF levels in cerebral cortex. One-way ANOVA/SNK. There was no significant difference, $P > 0.05$.

(D) Effect of c-tDCS and a-tDCS on BDNF levels in cerebral cortex. One-way ANOVA/SNK. * significant difference compared to the SAL/PTZ group, $P < 0.05$.

Figure 1

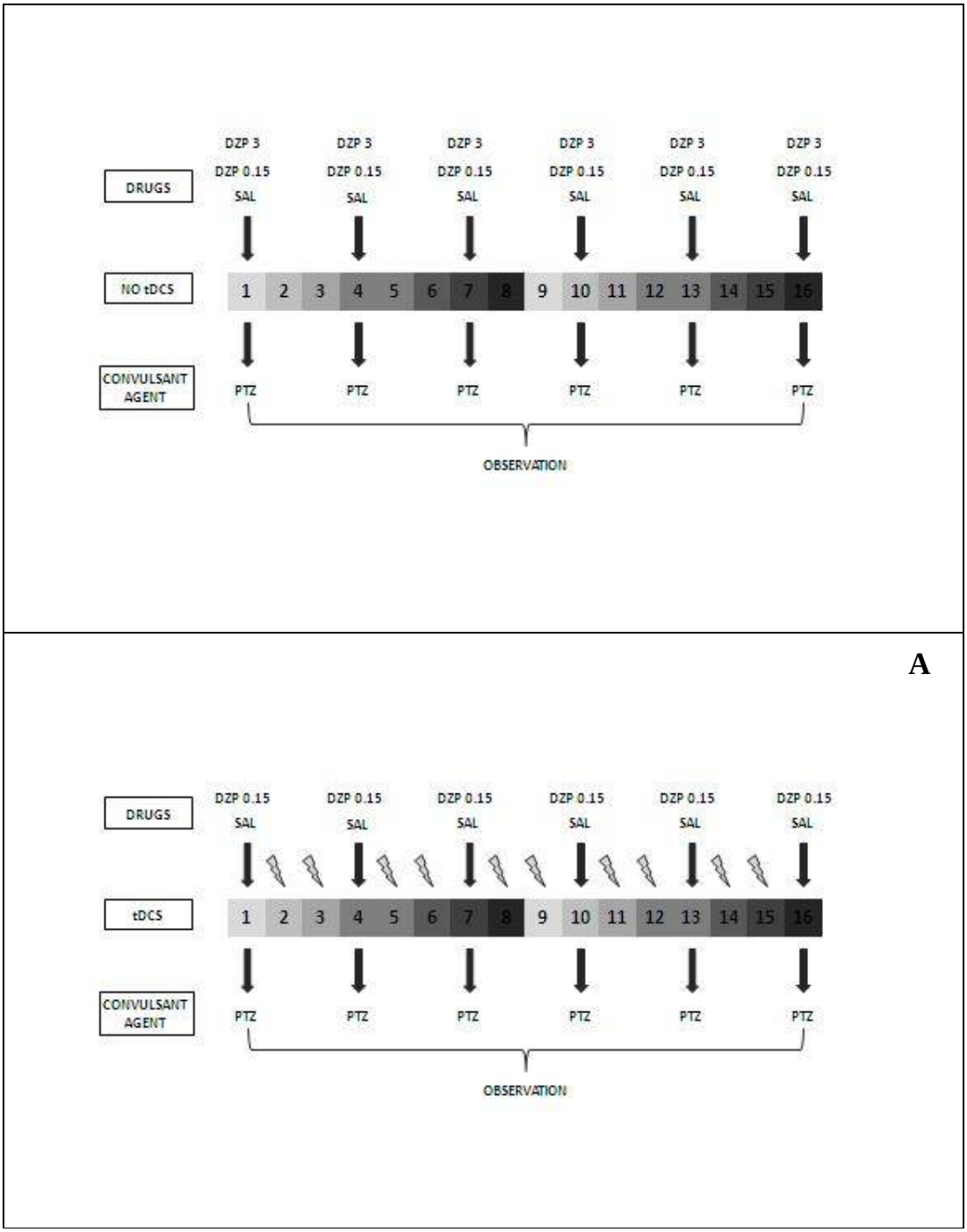

DZP 3
DZP 0.15
SAL
DRUGS
NO tDCS
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
CONVULSANT AGENT
PTZ
OBSERVATION
A
DZP 0.15
SAL
DRUGS
tDCS
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
CONVULSANT AGENT
PTZ
OBSERVATION

Figure 2

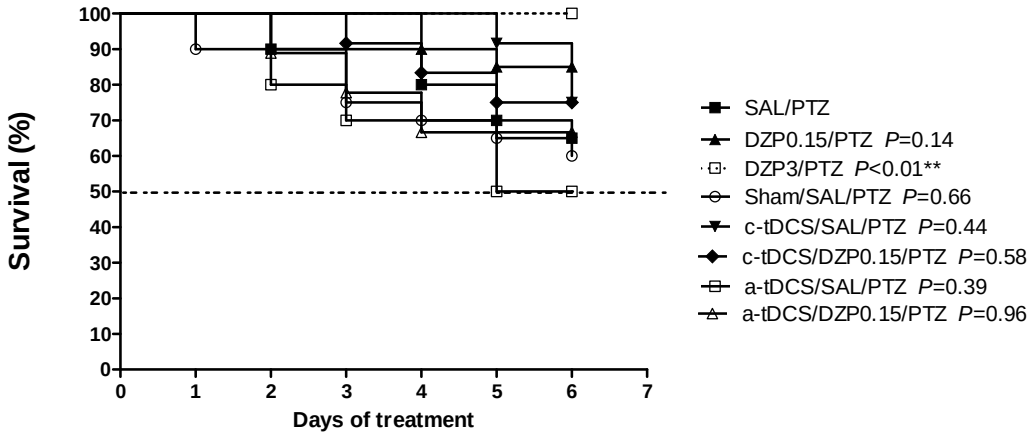
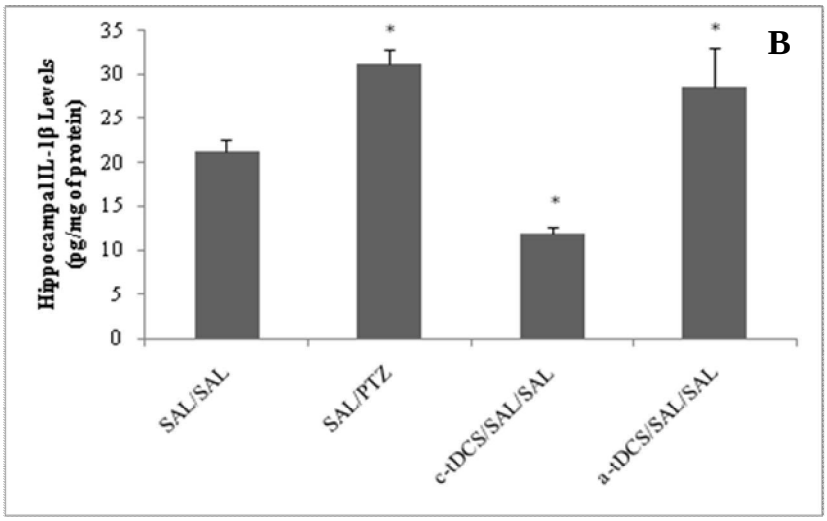
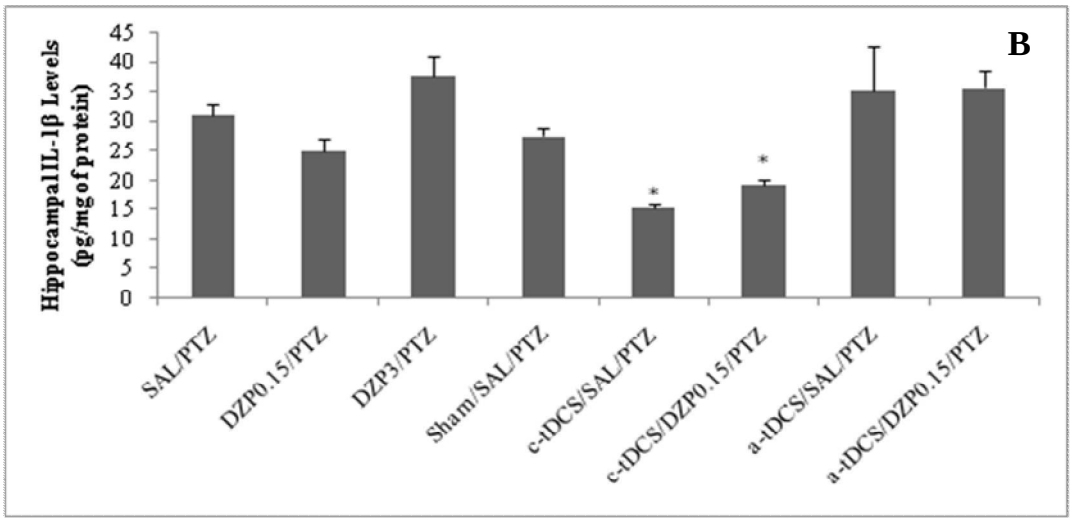

Survival (%)
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
Days of treatment
SAL/PTZ
DZP0.15/PTZ P=0.14
DZP3/PTZ P<0.01**
Sham/SAL/PTZ P=0.66
c-tDCS/SAL/PTZ P=0.44
c-tDCS/DZP0.15/PTZ P=0.58
a-tDCS/SAL/PTZ P=0.39
a-tDCS/DZP0.15/PTZ P=0.96

Figure 3

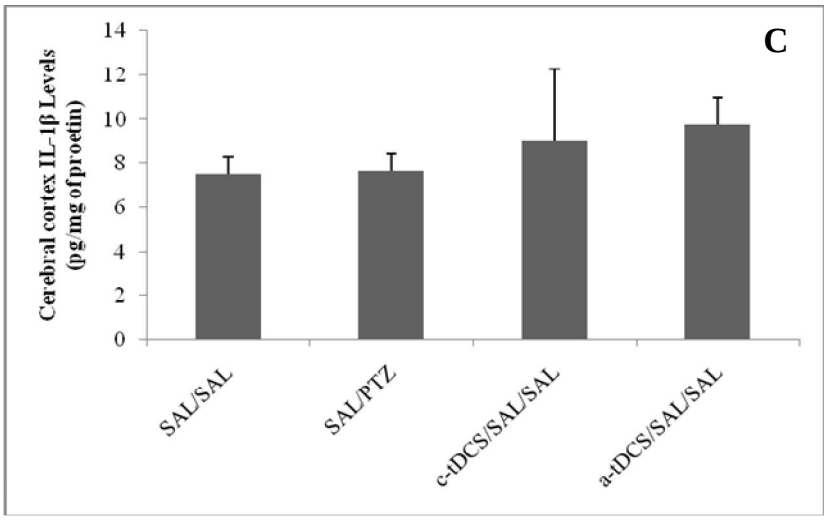
Panel A



Panel B



Panel C



Panel D

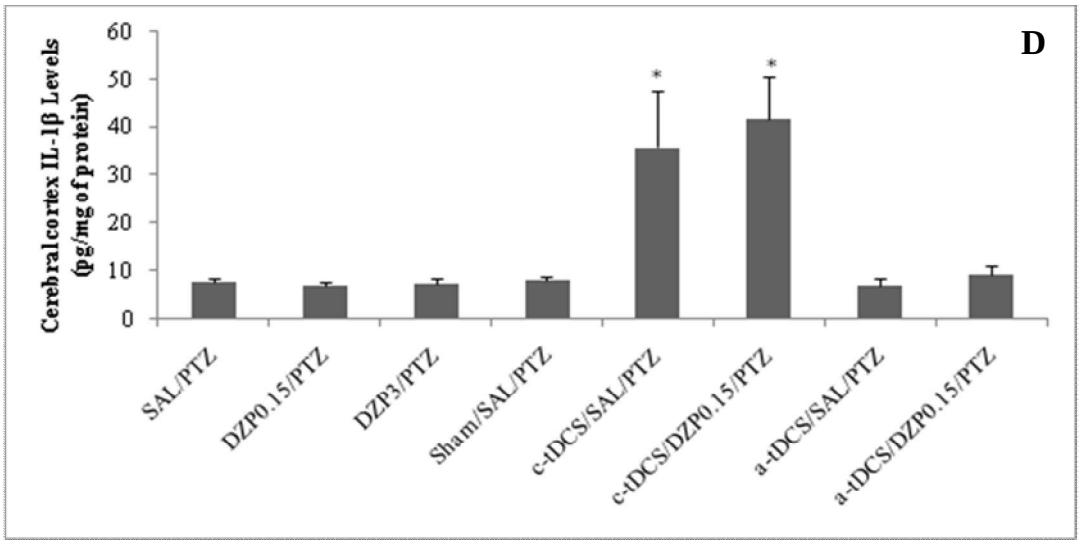
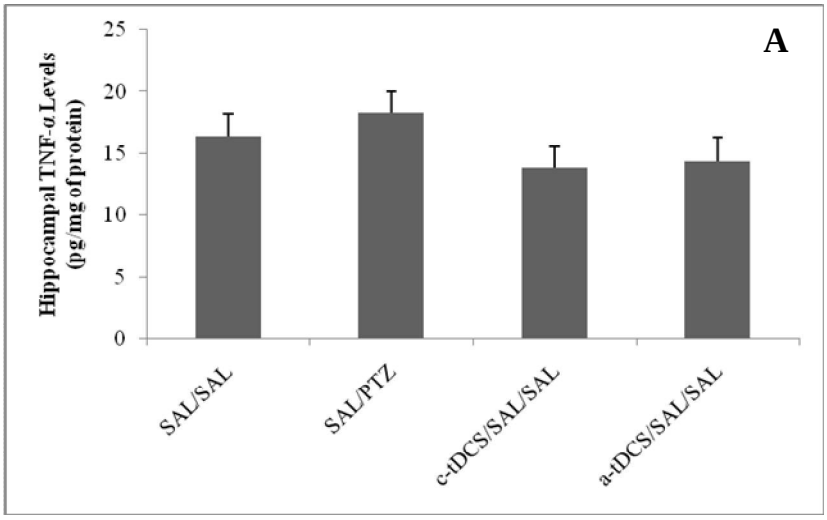


Figure 4

Panel A



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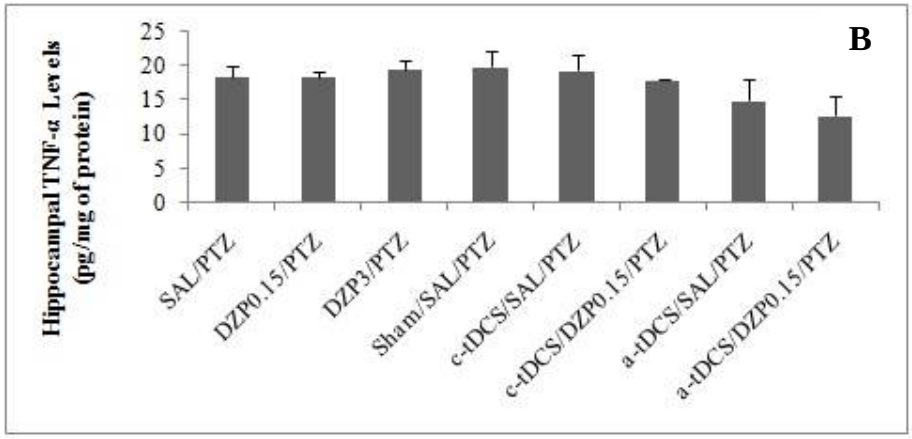

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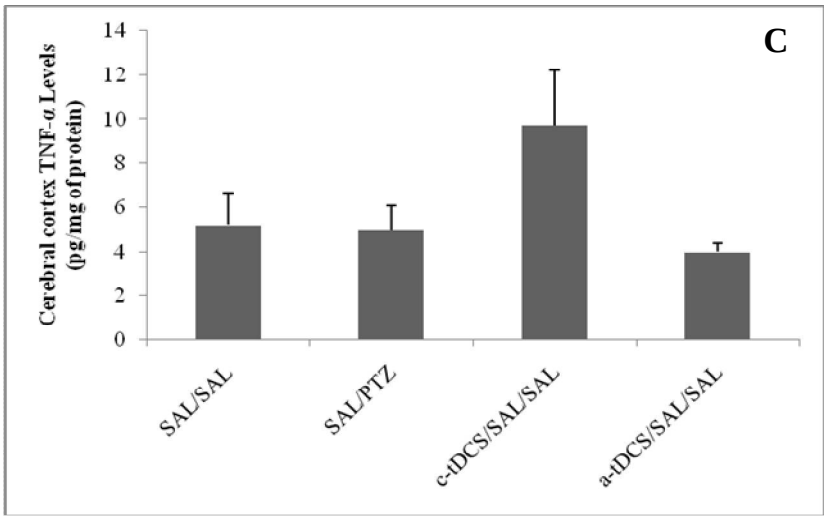


Panel D

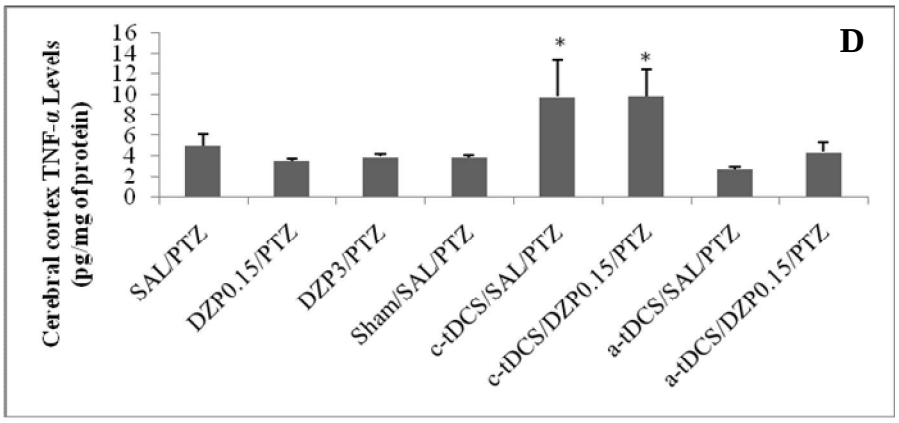
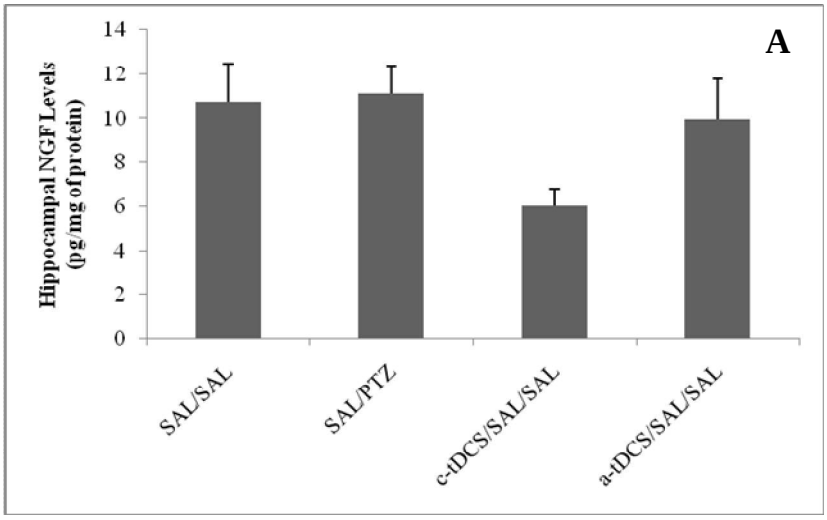
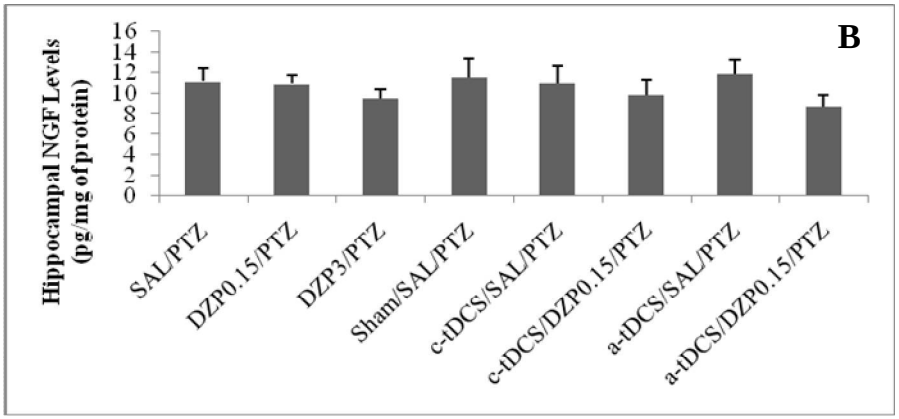


Figure 5

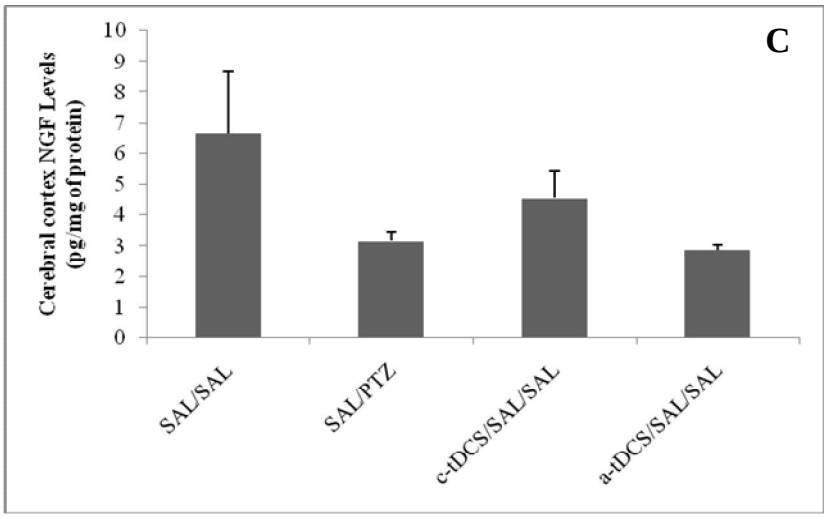
Panel A



Panel B



Panel C



Panel D

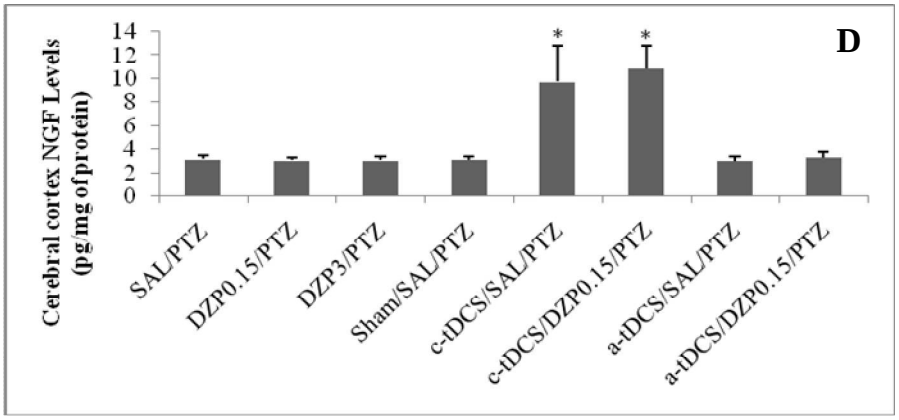
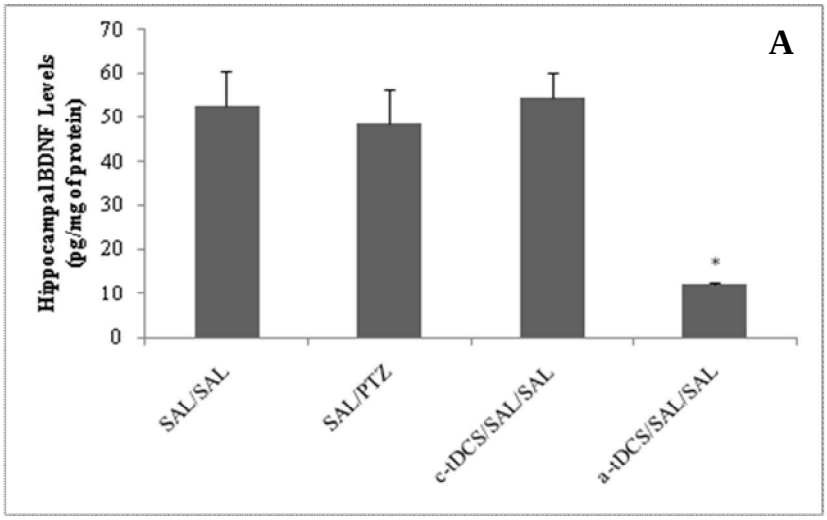
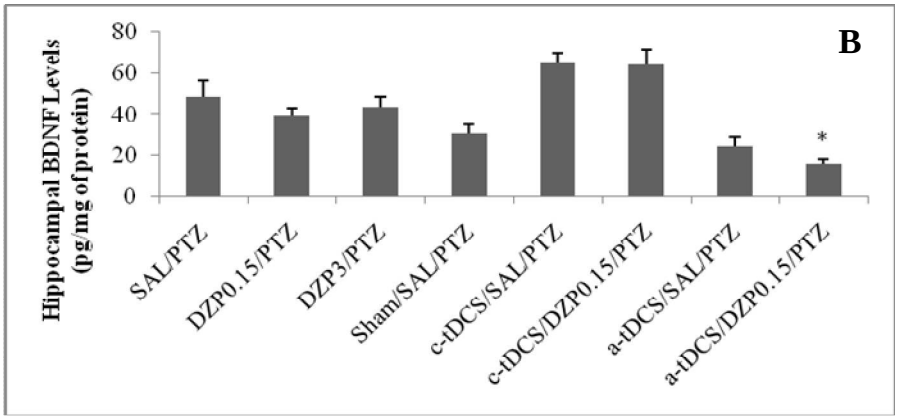


Figure 6

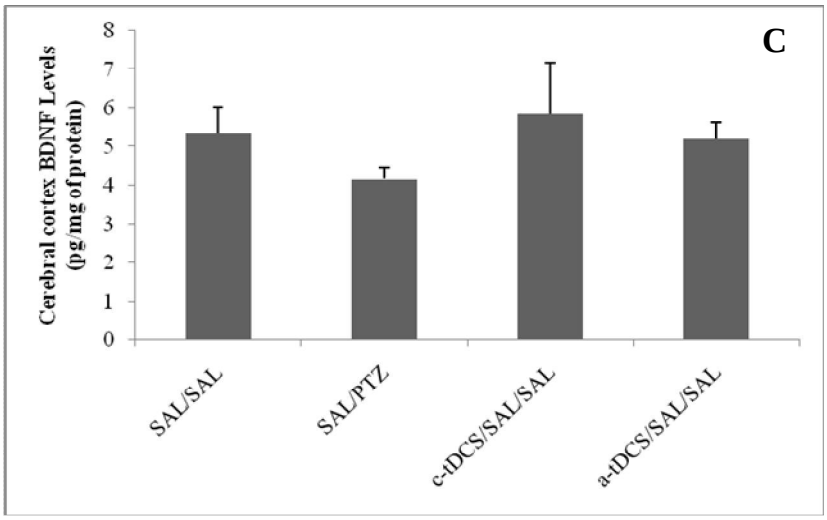
Panel A



Panel B



Panel C



Panel D

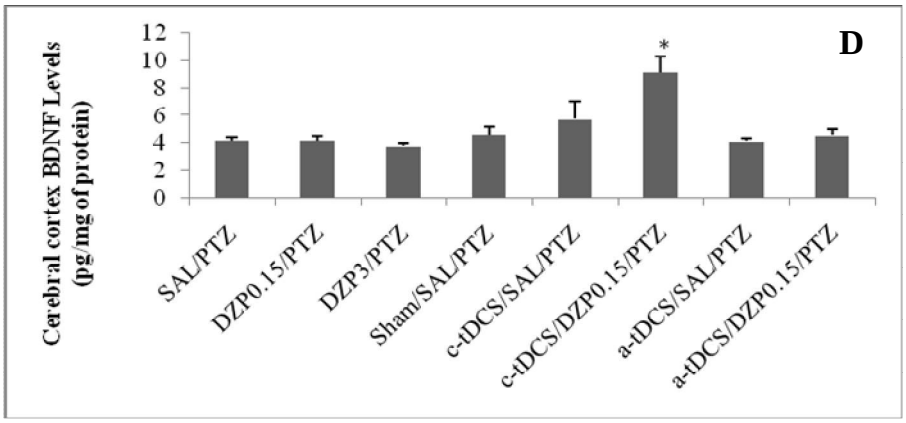


Table 1
Effects of c-tDCS and a-tDCS on the number of animals presenting seizures (bilateral forelimb clonus lasting more than 3 s) during repeated PTZ administration

	Number of seizures (%)				
Groups	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**
SAL/PTZ	40	61.11	66.67	75	50
DZP0.15/PTZ	25	30	45	27.78*	29.41
DZP3/PTZ	0**	0***	0***	0***	0**
Sham/SAL/PTZ	50	38.89	40	50	30.77
c-tDCS/SAL/PTZ	58.33	58.33	66.67	58.33	63.64
c-tDCS/DZP0.15/PTZ	58.33	33.33	36.36	50	22.22
a-tDCS/SAL/PTZ	70	50	71.43	85.71	33.33
a-tDCS/DZP0.15/PTZ	66.67	37.5	85.71	83.33	14.29

N = 9 - 20 animals per group (*P < 0.05, **P < 0.01, and ***P < 0.001; Fisher's Exact Test, compared to the SAL/PTZ group).

Table 2
Effects of c-tDCS and a-tDCS on latency to the 1st bilateral forelimb clonus lasting more than 3 s in rats submitted to PTZ-kindling

	Latency to first seizure (min.)				
Groups	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**
SAL/PTZ	19.94 ± 2.94	14.76 ± 2.97	12.56 ± 2.59	11.00 ± 2.70	13.99 ± 3.30
DZP0.15/PTZ	24.31 ± 2.28	22.54 ± 2.56*	19.16 ± 2.65	23.12 ± 2.35**	22.29 ± 2.76
DZP3/PTZ	30.00 ± 0.00***	30.00 ± 0.00***	30.00 ± 0.00***	30.00 ± 0.00***	30.00 ± 0.00***
Sham/SAL/PTZ	17.77 ± 3.17	16.79 ± 3.05	19.95 ± 3.13	16.45 ± 3.36	24.32 ± 3.20*
c-tDCS/SAL/PTZ	16.36 ± 3.42	15.99 ± 3.54	14.51 ± 3.25	18.03 ± 3.29	18.83 ± 3.27
c-tDCS/DZP0.15/PTZ	15.54 ± 3.56	21.81 ± 3.35	22.36 ± 2.95**	17.42 ± 3.64	25.86 ± 2.42**
a-tDCS/SAL/PTZ	11.05 ± 3.93	17.58 ± 4.30	12.10 ± 4.12	10.41 ± 3.26	19.97 ± 4.90
a-tDCS/DZP0.15/PTZ	13.49 ± 4.03	20.82 ± 3.98	10.19 ± 3.20	9.09 ± 3.88	22.91 ± 3.33

N = 9 - 20 per group (*P < 0.05 **P < 0.01, and ***P < 0.001, compared to the SAL/PTZ group). The comparisons between the eight groups, according to the number of treatments, were analyzed by using the GEE. Bonferroni multiple comparisons were used to complete the evaluation statistical calculations.

5 DISCUSSÃO

Nesta tese, realizamos uma revisão sistemática de estudos clínicos e pré-clínicos usando a estimulação transcraniana por corrente contínua (ETCC) como uma abordagem terapêutica para a epilepsia. A maioria dos estudos encontrados demonstrou maior eficácia com o uso da estimulação transcraniana por corrente contínua catodal (ETCC-c). Os efeitos antiepilépticos da ETCC-c podem ser explicados pela diminuição da excitabilidade cortical, que induz a hiperpolarização das membranas neuronais e subsequente alteração da eficácia sináptica (Nitsche et al., 2003b). Embora a eficácia antiepiléptica seja promissora com a ETCC-c reduzindo a excitabilidade, tem sido demonstrado que a estimulação transcraniana por corrente contínua anodal (ETCC-a) aumenta a amplitude e reduz o tempo do disparo neuronal glutamatérgico (efeito oposto observado com ETCC-c), consequentemente aumentando a excitabilidade (Pelletier e Cicchetti 2015). ETCC não induz o disparo neuronal por estímulos supralimiares, diferentemente da estimulação magnética transcraniana. Apesar de haver um caso clínico relatando que uma criança teve uma convulsão após um protocolo diário repetido de ETCC (Ekici, 2015), a indução de convulsões por ETCC não é comum.

Embora o completo mecanismo de ação da ETCC permaneça desconhecido, este parece envolver uma combinação de hiperpolarização e despolarização de axônios e alterações das funções sinápticas (Pelletier e Cicchetti 2015). Vários estudos investigaram os efeitos da ETCC-c na atividade neural espontânea e nas respostas motoras evocadas do sistema nervoso central e periférico. Estes relataram que os efeitos resultantes da ETCC envolvem um mecanismo de ação não-sináptico baseado em alterações das funções da membrana neural (Ardolino et al., 2005). Portanto, os autores sugeriram outros mecanismos, como mudanças nas concentrações de íons, alterações das proteínas transmembrana e alterações eletrolíticas relacionadas ao hidrogênio induzidas pela exposição a uma corrente elétrica constante (Ardolino et al., 2005; Nitsche et al., 2005; Fricke et al., 2011).

A aplicação de um campo elétrico constante no escalpo gera dois polos elétricos com cargas opostas, constituídas por dois eletrodos. Durante a ETCC-a, a corrente fornecida no ânodo atrai íons negativos na área abaixo do eletrodo, reduzindo, assim, o limiar e facilitando o disparo neuronal (aumento da excitabilidade cortical). Por outro lado, os eletrodos com carga negativa (ETCC-c) atraem cargas positivas, aumentando o

limiar tornando a área estimulada menos suscetível a estímulos endógenos ou exógenos (reduzindo a excitabilidade cortical) (Rossi et al., 2016; Nitsche et al., 2008; Rosen et al., 2009). Os efeitos da ETCC são dependentes da montagem dos eletrodos, parâmetros de estimulação e local de estimulação. A excitabilidade é alterada e a duração do efeito é dependente dos parâmetros das correntes diretas utilizadas, como intensidade e tempo de aplicação (Nitsche e Paulus, 2000; George e Aston-Jones, 2010).

Em revisão anterior do nosso grupo de pesquisa (Medeiros et al., 2012) foram discutidos os mecanismos presumíveis da ação da ETCC, tentando elucidar as vias subjacentes neurobiológicas e de sinalização celular. Esta revisão evidencia que a ETCC induz plasticidade, melhora a viabilidade e a morfologia neuronal, modula a transmissão sináptica e a biossíntese de moléculas. Além disso, vários eventos de modulação associados à atividade glutamatérgica, GABAérgica, dopaminérgica, serotoninérgica e colinérgica são induzidos pela ETCC.

Recente revisão confirmou a ausência de evidência de efeitos adversos graves (Bikson et al., 2016) confirmando a segurança da ETCC. Esta técnica pode ser aplicada com baixo risco e pouco desconforto e, quando usada em sessões repetidas, pode ter efeitos duradouros (Nitsche et al., 2008). O efeito da ETCC no curto prazo (efeito imediato) ocorre devido a diminuição (anódico) ou aumento (catódico) do limiar de disparo neuronal (Ruscheweyh et al., 2011). No entanto, os efeitos em longo prazo envolvem a participação do BDNF e receptores NMDA nos mecanismos de plasticidade sináptica (Fertonani et al., 2010). Como nesta técnica não há contato direto de eletrodos com o córtex cerebral, não há formação de produtos tóxicos indutores de dano cerebral (Nitsche et al., 2003a). Ressonância magnética antes e após 30 e 60 min de estimulação, aplicada ao córtex pré-frontal e ao córtex motor, não apresentou sinal de alterações patológicas. Desta forma, é possível concluir que a ETCC não provoca anomalias na BHE ou no tecido cerebral, nem edema cerebral (Rosen et al., 2009). Finalmente, Accornero e colaboradores (2007) não verificaram variações anormais na frequência cardíaca, pressão arterial ou temperatura durante e 20 minutos após o término da estimulação. Portanto, ETCC é um método seguro para uso em humanos, e tem a vantagem de poder ser combinado com outras intervenções, como tratamento farmacológico.

ETCC tem sido utilizada no tratamento de epilepsia, espasticidade, alterações de movimento, doença vascular periférica e transtornos psiquiátricos (Raghavan et al.,

2008). Embora os efeitos agudos da ETCC na atividade epileptiforme em curso estejam bem estabelecidos em modelos animais, permanece desconhecido o mecanismo pelo qual a ETCC prolongada modula o início da convulsão e a epileptogênese (Jackson et al., 2016). Além disso, enquanto estudos em animais sugerem que a ETCC-c prolongada pode ter efeito anticonvulsivante; a ETCC-a tem efeitos contrários, mas não consistentes, diminuindo o limiar da atividade convulsiva - evidente no EEG - mesmo não observando mudanças comportamentais (Liebetanz et al., 2006b; Hayashi et al., 1988). Tekturk e colegas buscaram evitar a geração e a propagação das convulsões utilizando ETCC-a. Hipotetizaram que, mesmo que o ETCC-c diminua a excitabilidade cortical, o ETCC-a aumenta os efeitos das conexões inibitórias (Tekturk et al., 2016). Sendo assim, eles usaram ETCC-c direcionando os focos epilépticos e ETCC-a, ao tecido cortical normal circundante, o que não foi eficaz na redução da frequência de convulsões.

Epilepsia é uma doença com característica intrínseca de atividade hipersincrônica. Portanto, a epilepsia representa o modelo para alterações plásticas hiperexcitatórias anormais dentro do circuito cortical (San-Juan et al., 2017). A heterogeneidade entre estudos usando ETCC para o tratamento da epilepsia refratária pode ser parcialmente atribuída à etiologia diversa da patologia. Portanto, diferentes abordagens podem ser avaliadas para diferentes tipos de epilepsia, porque apenas alguns estudos estão disponíveis. Por exemplo, em pacientes com epilepsia pós-traumática resistente a medicamentos, apenas um ensaio clínico randomizado duplo-cego foi publicado (Fregni et al., 2006). As causas da refratariedade da epilepsia aos medicamentos e ao tratamento cirúrgico ainda são desconhecidas. No entanto, um possível motivo é a presença de danos neuronais que afetam outras áreas do encéfalo, além do hipocampo (Petrovski et al., 2010; Zhang et al., 2017). Um estudo morfométrico usando imagens de ressonância magnética mostrou que o dano neuronal em pacientes com epilepsia do lobo temporal se estende para além do hipocampo e afeta regiões que conectam o hipocampo funcional e anatomicamente (van Elst et al., 2000). Esse achado sugere a presença de uma lesão na rede neural, subjacente às manifestações clínicas nesses pacientes (Andrade-Valença et al., 2008). Mais especificamente, a epilepsia do lobo temporal mesial é comumente associada à esclerose hipocampal nos achados histológicos desses pacientes (Andrade-Valença et al., 2008).

San-Juan e colaboradores (2015) publicaram uma revisão sobre a eficácia e segurança da ETCC na epilepsia. Os autores analisaram nove artigos utilizando diferentes metodologias (três pré-clínicas/seis clínicas). Além disso, estudos *in vivo* e *in vitro* demonstraram que a ETCC poderia induzir a supressão da atividade epileptiforme sem lesão neurológica. Quatro de seis (67%) estudos clínicos revelaram uma diminuição efetiva das crises epilépticas e cinco de seis (83%), uma redução da atividade epileptiforme interictal (San-Juan et al., 2015; Scorza e Brunoni, 2015). De fato, não encontramos evidências de que a utilização de ETCC na epilepsia possa provocar um aumento de convulsões, nem gerar qualquer outro efeito adverso significativo (Pereira et al., 2016). Estudos adicionais envolvendo um maior número de pacientes são necessários para investigar os efeitos da ETCC na epilepsia resistente a medicamentos.

A fim de desenvolver protocolos de estimulação ideais e acompanhamento em longo prazo, estudos em animais e ensaios clínicos prospectivos maiores com condições epilépticas homogêneas são necessários. Em pesquisa anterior, foi demonstrado que cada estudo usa diferentes categorias de pacientes, protocolos de estimulação, tamanhos de eletrodos, locais de estimulação e força de corrente de estimulação. Portanto, as conclusões a partir da comparação desses estudos devem ser usadas para fornecer medidas padronizadas, a fim de permitir a reprodutibilidade dos desfechos (Gschwind e van Mierlo, 2016). A epileptogênese envolve um aumento na força sináptica excitatória, e os focos de convulsão são caracterizados por uma redução da atividade em terminais inibitórios (GABAérgicos) e um aumento em terminais excitatórios (glutamatérgicos) (Fregni e Pascual-Leone, 2007). Assim, o principal mecanismo de ação da ETCC pode ser uma indução de efeitos do tipo depressão de longa duração (LTD), ou seja, a redução da excitabilidade cortical e a probabilidade de atividade paroxística em regiões corticais epileptogênicas (Nitsche e Paulus, 2009). Enquanto os efeitos anticonvulsivantes imediatos da ETCC-c envolvem a hiperpolarização do soma neuronal e a dessincronização da atividade neuronal, seus efeitos em longo prazo parecem ocorrer por meio da modulação da transmissão sináptica, podendo causar LTD via tálamo-cingulado, que parece ser dependente da duração e dos receptores NMDA (Chang et al., 2015), assim a ETCC-c parece promover a inibição intracortical. Por outro lado, a ETCC-a facilita a plasticidade sináptica via potenciação de longa duração (LTP), bem como estudos anteriores mostraram que convulsões breves poderiam

induzir LTP e surgimento de fibras musgosas no hipocampo, portanto o mecanismo de formação de LTP poderia ser semelhante ao mecanismo de epileptogênese (Chang et al., 2015; Rroji et al., 2015). Finalmente, os mecanismos subjacentes aos efeitos da ETCC parecem estar envolvidos não apenas em modificações locais relacionadas à polaridade da excitabilidade cortical, mas também em conexões inter-hemisféricas mais complexas (Tatti et al., 2016).

Os resultados obtidos nesta tese demonstram que a associação de ETCC-c e diazepam (DZP) em dose baixa (ETCC-c/DZP0.15) demonstrou uma tendência a aumentar a latência para a primeira convulsão nos dias quatro e seis do protocolo de *kindling*. Isso contrasta com o controle negativo tanto no número de convulsões quanto na latência para a primeira convulsão. Além disso, verificamos que a ETCC-a aumentou o número de convulsões, reduzindo a latência para a primeira convulsão, observada nos dias quatro e cinco, e aumentando a mortalidade dos ratos submetidos ao modelo de *kindling* por pentilenotetrazol (PTZ). Por outro lado, a ETCC-c isolada ou associada ao DZP reduziu a mortalidade, embora sem diferença estatisticamente significativa.

É importante ressaltar que este estudo é o primeiro a analisar o efeito protetor da ETCC no modelo de *kindling* induzido por PTZ e comparar o efeito de ETCC-a e ETCC-c. Nossos dados confirmam os achados de um estudo (Dhamne et al., 2015) que mostrou que o uso combinado de ETCC-c e lorazepam é mais eficaz na supressão de convulsões quando comparado à ETCC-c isolada ou ao fármaco administrado individualmente. É importante enfatizar que a associação com uma alternativa terapêutica, como a ETCC, pode permitir a redução das doses de fármacos, diminuindo os efeitos indesejáveis sem perda do efeito anticonvulsivo.

É necessário salientar que o modelo de *kindling* é induzido pelo pentilenotetrazol, que é um antagonista do receptor GABA que se liga ao sítio de reconhecimento da picrotoxina bloqueando o canal de cloreto (Cl^-) do complexo do receptor $GABA_A$, diminuindo a inibição de GABA (Humpel et al., 1993). Os benzodiazepínicos, como o DZP, são moduladores alostéricos positivos dos receptores $GABA_A$ sinápticos, responsáveis pela inibição fásica clássica (Bruun et al., 2015). Isso ocorre porque a frequência de abertura do canal aumenta, aumentando a condutância do íon Cl^-, inibindo o potencial de ação (Ataee et al., 2016). Embora os benzodiazepínicos sejam considerados anticonvulsivantes potentes, seu uso clínico é limitado devido aos efeitos adversos associados, incluindo comprometimento psicomotor, sedação, ataxia,

tolerância e dependência (Frantz et al., 2017). ETCC-c isolada não produziu melhora consistente no comportamento convulsivo, mas apresentou melhores resultados comparado à ETCC-a, corroborando outros estudos (San-Juan et al., 2017; Assenza et al., 2017; Zoghi et al., 2016), no entanto deve-se ressaltar que a ETCC-a induziu maior mortalidade nos animais submetidos ao *kindling*.

Além disso, a ETCC-c reduziu os níveis de IL-1β no hipocampo no grupo ETCC-c/SAL/SAL em comparação com o grupo controle (SAL/SAL). Este achado corrobora um estudo recentemente publicado pelo nosso grupo de pesquisa que mostrou que a ETCC reduz os níveis de TNF-α e IL-1β nos ratos Wistar Kyoto (WKY) (Leffa et al., 2018). Este achado sugere a modulação da resposta inflamatória pela ETCC nos animais controles, ou seja, na ausência de exposição ao agente convulsivante. Observamos também que a ETCC-c e sua associação com diazepam (ETCC-c/DZP0.15/PTZ) reduziram os níveis de IL-1β gerados pelo PTZ no hipocampo. No entanto, a ETCC-c aumentou os níveis corticais de IL-1β. ETCC-a aumentou IL-1β no grupo SAL/SAL (ETCC-a/SAL/SAL), similarmente ao que foi observado para o controle negativo (SAL/PTZ) no hipocampo. Também é importante ressaltar que esse resultado difere do efeito causado pela ETCC-c no grupo ETCC-c/SAL/SAL, uma vez que os níveis de IL-1β foram ainda menores do que no controle total (SAL/SAL).

Além disto, observamos um aumento na IL-1β no grupo DZP3. Esse resultado foi inesperado e sugere a modulação da resposta inflamatória pela atividade anticonvulsiva, confirmando os resultados de um estudo anterior (Figueroba et al., 2014). Kolosowska e colaboradores (2014) demonstraram que a IL-1β, mas não o TNF-α, parece desempenhar um papel importante no processo de inflamação como um bom marcador para convulsões totalmente desenvolvidas. Os autores observaram ainda um aumento na concentração de IL-1β no estágio final do *kindling*, quando convulsões tônico-clônicas são estabelecidas. Assim, IL-1β é um fator crítico envolvido na geração e na propagação das convulsões (De Simoni et al., 2000; Vezzani et al., 2002). Além disso, mediadores pró-inflamatórios como as citocinas IL-1β e TNF-α e fatores de crescimento como o BDNF sofrem regulação positiva durante a neuroinflamação. Este processo está envolvido na ativação da microglia, astrócitos e células endoteliais da BHE (Dey et al., 2016). Ao contrário do que observamos no hipocampo de animais submetidos ao *kindling*, no córtex cerebral, houve um aumento nos níveis de IL-1β induzidos pela ETCC-c. Esse achado corrobora estudo de Sayya e colegas (2005) que

sugeriram que a IL-1β poderia estar associada à neuroproteção aos danos induzidos pela epilepsia, devido à epileptogênese alterada pelo retardo na aquisição das convulsões generalizadas. Também foi demonstrado que a infusão de IL-1β central crônica parece atrasar a aquisição do *kindling*, bloqueando a generalização das convulsões durante os estágios iniciais da epileptogênese (Kołosowska et al., 2014). Vale ressaltar que os níveis alterados de citocinas estão ligados ao aumento da sensibilidade à dor e aos estímulos induzidos pelo *kindling* (Clinton et al., 2011). Evidências sugerem que processos de neuroinflamação contribuem para a etiopatogênese das convulsões (Hoda et al., 2017), tais alterações são observadas no cérebro de pacientes epilépticos e em amostras de tecido cerebral de animais submetidos a modelos experimentais de epilepsia (Vezzani et al., 2011).

Observamos uma redução nos níveis de TNF-α cortical dos animais submetidos ao *kindling* e à ETCC-a, além de um aumento no parâmetro naqueles recebendo ETCC-c. Isto sugere que o TNF-α é importante na ativação, diferenciação, proliferação e infiltração de células imunes no processo de neuroinflamação (Sonar e Lal, 2015). É interessante salientar o duplo papel do TNF-α na fisiopatologia das convulsões e da epilepsia. A ativação do receptor TNF tipo 1 (TNFR1 ou p55) induz um efeito pró-convulsivo; por outro lado, a ativação do receptor 2 (TNFR2 ou p75) induz um efeito anticonvulsivo (Weinberg et al., 2013; Balosso et al., 2013). Assim, efeitos inibitórios ou estimulatórios serão observados no surgimento de convulsões, dependendo da concentração e do tipo de receptor envolvido nesse processo (Balosso et al., 2005). Em contrapartida, não foram detectadas alterações significativas nos níveis de TNF-α durante o *kindling* induzido por PTZ; no entanto, níveis aumentados de IL-1β foram observados em ratos que tiveram convulsões plenamente desenvolvidas (Kołosowska et al., 2014). Isto pode ser devido a esta citocina ser mais ativa em estágios inflamatórios precoces e tardios (Lai et al., 2006). Os diferentes níveis de TNF-α relatados na literatura podem ser atribuídos à administração de diferentes doses do agente convulsivo, bem como à sensibilidade intercepa em convulsões induzidas por PTZ.

Finalmente, a ETCC-c isolada ou em associação com o DZP induziu aumento nos níveis de NGF e BDNF em córtex cerebral. Como já mencionado, o excesso de produção de IL-1β pode induzir a neurodegeneração em modelos *in vivo* (Rothwell e Strijbos, 1995). No entanto, o efeito *in vitro* parece ser o oposto, quando a IL-1β tem efeito neuroprotetor no processo degenerativo induzido por aminoácidos excitatórios, o

que parece estar ligado à atividade do NGF (Strijbos e Rothwell, 1995). A ETCC-c durante o *kindling* aumentou significativamente os níveis de NGF e BDNF. Além disso, observamos uma redução nos níveis hipocampais do BDNF no grupo ETCC-a/SAL/SAL, comparado ao controle negativo. Da mesma forma, observamos que a ETCC-a associada ao DZP também reduziu os níveis de BDNF no hipocampo. É sabido que o NGF e o BNDF são neurotrofinas produzidas no cérebro por células neuronais e não neuronais (Tirassa e Costa, 2007). Eles regulam a sobrevivência, a diferenciação e a reorganização neuronal em cérebros em desenvolvimento e adultos, bem como na excitabilidade neuronal por meio da síntese de neurotransmissores, formação de sinapses e alongamento de axônios (Tirassa e Costa, 2007; Poo, 2001). Humpel e colaboradores (1993) mostraram que os níveis de BDNF podem estar ligados a mecanismos de proteção contra danos após as convulsões no modelo de *kindling* e em respostas de brotamento. É necessário ressaltar que os níveis de BDNF foram aumentados no hipocampo dos animais 3 h e 24 h, bem como até três dias após a última injeção de PTZ. Sabe-se que várias lesões cerebrais aumentam a atividade de fatores neurotróficos nas áreas afetadas e ao seu redor, o que pode regular o remodelamento axonal e dendrítico, que é imprescindível para manter os neurônios danificados e até mesmo impedir que as células morram (Satfran et al., 1990). Isso pode explicar a melhora no comportamento convulsivo nos grupos que receberam ETCC-c, especialmente naqueles associados com uma dose baixa de DZP, uma vez que seus níveis de NGF e BDNF aumentaram. Além disso, tem sido proposto que essas neurotrofinas podem mediar as ações anticonvulsivas associadas a outros fármacos, reduzindo a suscetibilidade ao PTZ (Tirassa e Costa, 2007).

Os resultados observados na presente tese confirmam os achados publicados em pesquisas anteriores, que mostraram a capacidade da ETCC em modular convulsões em um modelo de *kindling* induzido por PTZ, baseado em seus efeitos benéficos sobre parâmetros inflamatórios (Frantz et al., 2017; Hoda et al., 2017). Estudos anteriores fornecem evidências de que a ETCC reduz os marcadores inflamatórios na dor neuropática e nos modelos de dor induzida por estresse crônico (Cioato et al., 2016; Adachi et al., 2012), confirmando nossos resultados. No entanto, a modulação das vias neuroinflamatórias pela ETCC ainda não foi completamente esclarecida. Um estudo anterior sugeriu que a ETCC poderia ativar células gliais (King et al., 2000), indicando o efeito pró-inflamatório da estimulação. Corroborando outro estudo (Yoon et al.,

2016), nossos resultados demonstram que esses efeitos na inflamação dependem de parâmetros de estimulação. Técnicas neuromodulatórias centrais e periféricas, como eletroacupuntura (Yu et al., 2015), estimulação do nervo vago (Pedron et al., 2014), estimulação do córtex motor epidural (Kandel, 2012) e estimulação cerebral profunda (Rohan et al., 2015), têm sido associadas a efeitos anti-inflamatórios em cenários patológicos, apoiando o papel de ETCC em condições neuroinflamatórias.

Assim, nossos resultados demonstram que a ETCC-c não diminuiu significativamente as convulsões induzidas por PTZ no modelo de *kindling*, mas a ETCC-c isolada ou associada a uma dose baixa de DZP exibiu efeitos neuroprotetores, melhorando os níveis de neurotrofinas e diminuindo os níveis de IL-1β no hipocampo após o *kindling* induzido por PTZ. Por outro lado, a ETCC-a demonstrou agravar os parâmetros de convulsão, além de reduzir os níveis de NGF e BDNF.

6 CONCLUSÃO

A ETCC deve ser considerada uma opção terapêutica viável no tratamento da epilepsia refratária, particularmente em pacientes que não podem se submeter à cirurgia. Em geral, em estudos com animais foram utilizados ETCC-c com corrente variando de 100 a 200μA, e mesmo com montagens definidas, a estimulação parece ser bicefálica, devido ao tamanho dos crânios dos animais. Já em estudos clínicos foi utilizado o ETCC-c com corrente variando de 1 a 2mA e a montagem inclui o cátodo sobre os focos epilépticos, em sua maioria. Ainda são necessários mais estudos multicêntricos, duplo-cego e controlados por sham (placebo), avaliando essa técnica não invasiva, o que pode ser justificado por custo-efetividade e prevenção de complicações cirúrgicas. Novos métodos de avaliação em tempo real com EEG e também o uso de outros marcadores neurais também podem ajudar na compreensão dos efeitos clínicos da ETCC na epilepsia.

Os resultados deste trabalho demonstraram que a ETCC-c isolada ou em combinação com uma dose baixa de DZP demonstrou efeitos neuroprotetores, melhorando os níveis centrais de neurotrofinas além de reduzir os níveis hipocampais de IL-1β após o *kindling* induzido por PTZ, sem efeito estatisticamente significativo no comportamento convulsivo. Apesar da intrigante possibilidade de modular as redes neurais (por exemplo, a ETCC-c parece diminuir a excitabilidade por meio da hiperpolarização associada a mecanismos do tipo LTD), a ETCC ainda requer uma análise aprofundada dos protocolos mais adequados e a elucidação de seu mecanismo de ação. Dessa forma, sugere-se que a ETCC-c deve ser investigada em novos estudos, com enfoque na elucidação de seu mecanismo de ação e sua interação com outros anticonvulsivantes. Embora existam vários ensaios publicados neste campo, ainda há necessidade de mais evidências, a fim de compreender o real papel da ETCC, que pode ter uso potencial na epilepsia ou como adjuvante na farmacoterapia convencional.

7 REFERÊNCIAS BIBLIOGRÁFICAS

Accornero N, Li Voti P, La Riccia M, Gregori B (2007) Visual evoked potentials modulation during direct current cortical polarization. **Experimental Brain Research** 178: 261–266.

Adachi LS, Caumo W, Laste G, Fernandes LF, Ripoll JR, De Souza A, et al. (2012) Reversal of chronic stress-induced pain by transcranial direct current stimulation (tDCS) in an animal model. **Brain Research** 1489:17–26.

Alyu F, Dikmen M (2016) Inflammatory aspects of epileptogenesis: contribution of molecular inflammatory mechanisms. **Acta Neuropsychiatrica** 29(1):1–16.

Amiri M, Bahrami F, Janahmadi M (2012). Functional contributions of astrocytes in synchronization of a neuronal network model. **Journal of Theoretical Biology** 292:60–70.

Andrade-Valença LP, Valença MM, Velasco TR, Carlotti CG Jr, Assirati JA, Galvis-Alonso OY, Neder L, Cendes F, Leite JP (2008). Mesial temporal lobe epilepsy: clinical and neuropathologic findings of familial and sporadic forms. **Epilepsia** 49(6):1046–1054.

Ardolino G, Bossi B, Barbieri S, Priori A (2005). Non-synaptic mechanisms underlie the after-effects of cathodal transcutaneous direct current stimulation of the human brain. **The Journal of Physiology** 568, 653–663.

Arundine M, Tymianski M (2004) Molecular mechanisms of glutamate-dependent neurodegeneration in ischemia and traumatic brain injury. **Cellular and Molecular Life Sciences** 61(6):657–668.

Assenza G, Campana C, Assenza F, et al. (2017) Cathodal transcranial direct current stimulation reduces seizure frequency in adults with drug-resistant temporal lobe epilepsy: A sham controlled study. **Brain Stimulation** 10:333–335.

Ataee R, Falahati A, Ebrahimzadeh MA, Shokrzadeh M (2016) Anticonvulsant activities of Sambucus nigra. **European Review for Medical and Pharmacological Sciences** 20:3123–3126.

Auvichayapat N, Rotenberg A, Gersner R (2013) Transcranial direct current stimulation for treatment of refractory childhood focal epilepsy. **Brain Stimulation** 6(4):696e700.

Balosso S, Ravizza T, Perego C, Peschon J, Campbell IL, De Simoni MG, et al. (2005) Tumor necrosis factor-alpha inhibits seizures in mice via p75 receptors. **Annals of Neurology** 57:804–812.

Balosso S, et al. (2013) The dual role of TNF-alpha and its receptors in seizures. **Experimetal Neurology** 247:267–271.

Bascuñana P, Javela J, Delgado M, Fernández de la Rosa R, Shiha AA, García-García L, Pozo MÁ (2016) [18F] FDG PET Neuroimaging Predicts Pentylenetetrazole (PTZ) Kindling Outcome in Rats. **Molecular Imaging and Biology** 18(5):733–740.

Ben-Ari, Y (2006) Seizures beget seizures: The quest for GABA as a key player. **Critical Reviews in Neurobiology** 18:135–144.

Berg AT, Berkovic SF, Brodie MJ, Buchhalter J, Cross JH, van Emde Boas W, Engel J, French J, Glauser TA, Mathern GW, Moshé SL, Nordli D, Plouin P, Scheffer IE (2010) Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005-2009. **Epilepsia** 51(4):676–685.

Bikson M, Grossman P, Thomas C, et al. (2016). Safety of Transcranial Direct Current Stimulation: Evidence Based Update 2016. **Brain Stimulation** 9, 641–661.

Błaszczyk JW (2016) Parkinson's Disease and Neurodegeneration: GABA-Collapse Hypothesis. **Frontiers in Neuroscience** 10: 269.

Boon P, De Cock E, Mertens A, Trinka E (2018) Neurostimulation for drug-resistant epilepsy: a systematic review of clinical evidence for efficacy, safety, contraindications and predictors for response. **Current Opinion in Neurology** 31(2):198–210.

Browne TR, Holmes GL (2008) **Handbook of epilepsy**. 4 ed. Philadelphia: Lippincot, Williams & Wilkins, p. 9.

Brunoni AR, Pinheiro FS, Boggio PS. Estimulação Transcraniana por Corrente Contínua. In: Fregni F, Boggio PS, Brunoni AR (Org). **Neuromodulação Terapêutica**. São Paulo: Sarvier, 2012. p. 65-75.

Bruun DA, Cao Z, Inceoglu B, Vito ST, Austin AT, Hulsizer S, et al. (2015) Combined treatment with diazepam and allopregnanolone reverses tetramethylenedisulfotetramine (TETS)-induced calcium dysregulation in cultured neurons and protects TETS-intoxicated mice against lethal seizures. **Neuropharmacology** 95:332–342.

Cárdenas-Rodríguez N, Coballase-Urrutia E, Rivera-Espinosa L, Romero-Toledo A, Sampieri A 3rd, Ortega-Cuellar D, Montesinos-Correa H, Floriano-Sánchez E, Carmona-Aparicio L (2013). Modulation of antioxidant enzymatic activities by certain antiepileptic drugs (valproic acid, oxcarbazepine, and topiramate): evidence in humans and experimental models. **Oxididative Medicine and Cellular Longevity** 2013.

Chang WP, Lu HC, Shyu BC (2015). Treatment with direct-current stimulation against cingulate seizure-like activity induced by 4-aminopyridine and bicuculline in an in vitro mouse model. **Experimental Neurology** 265, 180–192.

Chao D, Shen X, Xia Y (2013). From Acupuncture to Interaction between δ-Opioid Receptors and Na (+) Channels: A Potential Pathway to Inhibit Epileptic Hyperexcitability. **Evidence-Based Complementary and Alternative Medicine** 2013:216016.

Chindo BA, Ya'U J, Danjuma NM, Okhale SE, Gamaniel KS, Becker A (2014). Behavioral and anticonvulsant effects of the standardized extract of Ficus platyphylla stem bark. **Journal of Ethnopharmacology** 154(2):351–360.

Choi J, Koh S (2008) Role of brain inflammation in epileptogenesis. **Yonsei Medical Journal** 49(1):1–18.

Cioato SG, Medeiros LF, Marques Filho PR, Vercelino R, de Souza A, Scarabelot VL, et al. (2016) Long-Lasting Effect of Transcranial Direct Current Stimulation in the Reversal of Hyperalgesia and Cytokine Alterations Induced by the Neuropathic Pain Model. **Brain Stimulation** 9:209–217.

Clinton JM, Davis CJ, Zielinski MR, Jewett KA, Krueger JM (2011) Biochemical Regulation of Sleep and Sleep Biomarkers. **Journal of Clinical Sleep Medicine** 7:S38–42.

Corda MG, Giorgi O, Longoni B, Orlandi M, Biggio G (1990) Decrease in the function of the gamma-aminobutyric acid-coupled chloride channel produced by the repeated administra- tion of pentylenetetrazol to rats. **Journal of Neurochemistry** 55:1216–1221.

Costa DA, de Oliveira GA, Lima TC, dos Santos PS, de Sousa DP, de Freitas RM (2012). Anticonvulsant and antioxidant effects of cyano-carvone and its action on acetylcholinesteraseactivity in mice hippocampus. **Cellular and Molecular Neurobiology** 32(4):633–640.

De Simoni MG, Perego C, Ravizza T, Moneta D, Conti M, Marchesi F, et al. (2000) Inflammatory cytokines and related genes are induced in the rat hippocampus by limbic status epilepticus. **The European Journal of Neuroscience** 12:2623–2633.

Dey A, Kang X, Qiu J, Du Y, Jiang J (2016) Anti-Inflammatory Small Molecules To Treat Seizures and Epilepsy: From Bench to Bedside. **Trends in Pharmacological Sciences** 37:463–484.

Dhamne SC, Ekstein D, Zhuo Z, Gersner R, Zurakowski D, Loddenkemper T, et al. (2015) Acute seizure suppression by transcranial direct current stimulation in rats. **Annals of Clinical and Translational Neurology** 2:843–856.

Dhir A (2012) Pentylenetetrazol (PTZ) kindling model of epilepsy. **Current Protocols in Neuroscience** 9: 9.37.

Dhir A, Naidu OS, Kulkarni SK (2005). Effect of naproxen, a non-selective cyclo-oxygenase inhibitor, on pentylenetetrazol-induced kindling in mice. **Clinical and Experimental Pharmacology & Physiology** 32:579–584.

Dichter MA (1994) Emerging insights into mechanisms of epilepsy: implications for new antiepileptic drug development. **Epilepsia** 35(4):S51–S57.

DiNuzzo M, Mangia S, Maraviglia B, Giove F (2014) Physiological bases of the K^+ and the glutamate/GABA hypotheses of epilepsy. **Epilepsy Research** 108(6):995–1012.

Elger CE, Schmidt D (2008) Modern management of epilepsy: a practical approach. **Epilepsy & Behavior** 12(4):501–539.

Ekici B (2015). Transcranial direct current stimulation-induced seizure: analysis of a case. **Clinical EEG and Neuroscience** 46, 169.

Fertonani, A, Rosini S, Cotelli M, Rossini P M, Miniussi C (2010) Naming facilitation induced by transcranial direct current stimulation. **Behavioural Brain Research** 208(2):311–318.

Figueroba SR, Desjardins MP, Nani BD, Ferreira LE, Rossi AC, Santos FA, et al. (2014) Effect of diazepam on temporomandibular joints in rats with increased occlusal vertical dimension. **The British Journal of Oral & Maxillofacial Surgery** 52:438–444.

Fisher RS, Acevedo C, Arzimanoglou A, Bogacz A, Cross JH, Elger CE, Engel J Jr, Forsgren L, French JA, Glynn M, Hesdorffer DC, Lee BI, Mathern GW, Moshé SL, Perucca E, Scheffer IE, Tomson T, Watanabe M, Wiebe S (2014) ILAE official report: a practical clinical definition of epilepsy. **Epilepsia** 55(4):475-482.

França GV (1998). Epilepsias. In: **Medicina legal**. 5 ed. Rio de Janeiro: Guanabara-Koogan, 351–353.

Frantz AL, Regner GG, Pflüger P, Coelho VR, da Silva LL, Viau CM, et al. (2017) Manual acupuncture improves parameters associated with oxidative stress and inflammation in PTZ-induced kindling in mice. **Neuroscience Letters** pii:S0304–3940(17)30786-3.

Fregni F, Pascual-Leone A (2007). Technology insight: noninvasive brain stimulation in neurology-perspectives on the therapeutic potential of rTMS and tDCS. **Nature Clinical Practice. Neurology** 3, 383–393.

Fregni F, Thome-Souza S, Nitsche MA, Freedman SD, Valente KD, PascualLeone A (2006) A controlled clinical trial of cathodal DC polarization in patients with refractory epilepsy. **Epilepsia** 47(2):335e42.

French JA (2007). Refractory epilepsy: clinical overview. **Epilepsia** 48:3–7.

French JA (2017) Withdrawal of antiepileptic drugs: an individualised approach. **The Lancet. Neurology** 16(7):493–494.

Fricke K, Seeber AA, Thirugnanasambandam N, Paulus W, Nitsche MA, Rothwellet JC (2011). Time course of the induction of homeostatic plasticity generated by repeated transcranial direct current stimulation of the human motor cortex. **Journal of Neurophysiology** 105, 1141–1149.

Gallucci Neto J, Marchetti RL (2005) Epidemiologic aspects and relevance of mental disorders associated with epilepsy. **Revista Brasileira de Psiquiatria** 27(4):323–328.

George MS, Aston-Jones G (2010). Noninvasive techniques for probing neurocircuitry and treating illness: vagus nerve stimulation (VNS), transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS). **Neuropsychopharmacology** 35, 301–316.

Giza CC, Hovda DA (2014) The new neurometabolic cascade of concussion. **Neurosurgery**75:S24–33.

Glynn IM (1993) All hands to the sodium pump. **The Journal of Physiology** 462:1–30.

Goddard GV, McIntyre DC, Leech CK (1969) A permanent change in brain function resulting from daily electrical stimulation. **Experimental Neurology** 25:295–330.

Gorter JA, van Vliet EA, Aronica E (2015). Status epilepticus, blood-brain barrier disruption, inflammation, and epileptogenesis. **Epilepsy & Behavior** 49:13–16.

Gschwind M, van Mierlo P (2016). Difficulty of comparing the multiple heterogeneous approaches: comment to transcranial direct current stimulation in epilepsy. **Brain Stimulation** 9, 459–461.

Guerriero RM, Giza CC, Rotenberg A (2015) Glutamate and GABA Imbalance Following Traumatic Brain Injury. **Current Neurology and Neuroscience Reports** 15: 27.

Guilhoto LMFF (2011) Revised terminology and concepts for organization of seizures and epilepsies: Report of the ILAE Commission on Classification and Terminology, 2005-2009. New paradigms? **Journal of Epilepsy and Clinical Neurophysiology** 17(3):100-105.

Hayashi Y, Hattori Y, Asaki H, Moriwaki A, Hori Y (1988). Effects of prolonged weak anodal direct current on electrocorticogram in awake rabbit. **Acta Medica Okayama** 42, 293–296.

Hoda U, Agarwal NB, Vohora D, Parvez S, Raisuddin S (2017) Resveratrol suppressed seizures by attenuating IL-1β, IL1-Ra, IL-6, and TNF-α in the hippocampus and cortex of kindled mice. **Nutritional Neuroscience** 20:497–504.

Holmes MJ, Yang X, Landman BA, Ding Z, Kang H, Abou-Khalil B, Sonmezturk HH, Gore JC, Morgan VL (2013). Functional networks in temporal-lobe epilepsy: a voxel-wise study of resting-state functional connectivity and gray-matter concentration. **Brain Connectivity** 3(1):22–30.

Humpel C, Wetmore C, Olson L (1993) Regulation of brain-derived neurotrophic factor messenger RNA and protein at the cellular level in pentylenetetrazol-induced epileptic seizures. **Neuroscience** 53:909-918.

Jackson MP, Rahman A, Lafon B, et al. (2016). Animal models of transcranial direct current stimulation: Methods and mechanisms. **Clinical Neurophysiology** 127, 3425–3454.

Jørgensen PL (1990) Structure and molecular mechanisms of the Na,K-pump. **Monovalent Cations in Biological Systems** 117–154.

Kamida T, Kong S, Eshima N, Abe T, Fujiki M, Kobayashi H (2011) Transcranial direct current stimulation decreases convulsions and spatial memory deficits following pilocarpine-induced status epilepticus in immature rats. **Behavioural Brain Research** 217(1):99e103.

Kamida T, Kong S, Eshima N, Fujiki M (2013) Cathodal transcranial direct current stimulation affects seizures and cognition in fully amygdala-kindled rats. **Neurological Research** 35:602–607.

Kandel ER (2012) The molecular biology of memory: cAMP, PKA, CRE, CREB-1, CREB-2, and CPEB. **Molecular Brain** 5:14.

Karler R, Murphy V, Calder LD, Turkanis SA (1989) Pentylenetetrazol kindling in mice. **Neuropharmacology** 28:775-780.

King JA, Barkley RA, Delville Y, Ferris CF (2000) Early androgen treatment decreases cognitive function and catecholamine innervation in an animal model of ADHD. **Behavioural Brain Research** 107:35–43.

Kołosowska K, Maciejak P, Szyndler J, Turzyńska D, Sobolewska A, Płaźnik A (2014) The role of interleukin-1β in the pentylenetetrazole-induced kindling of seizures, in the rat hippocampus. **European Journal of Pharmacology** 731:31–37.

Lai YC, Shaftel SS, Miller JH, et al. (2006) Intra-articular induction of interleukin-1b expression in the adult mouse, with resultant temporomandibular joint pathologic changes, dysfunction, and pain. **Arthritis and Rheumatism** 54:1184–1197.

Lasoń W, Chlebicka M, Rejdak K (2013) Research advances in basic mechanisms of seizures and antiepileptic drug action. **Pharmacological Reports** 65(4):787–801.

Leffa DT, Bellaver B, Salvi AA, de Oliveira C, Caumo W, Grevet EH, et al. (2018) Transcranial direct current stimulation improves long-term memory deficits in an animal model of attention-deficit/hyperactivity disorder and modulates oxidative and inflammatory parameters. **Brain Stimulation** 11(4):743–751.

Li B, Tang F, Wang L, Liu L, Zhao J, Zhou Y, Wang Y, Song Y, Li Y, Cui R (2013). Anticonvulsant effects of Fuzi total alkaloid on pentylenetetrazole-induced seizure in mice. **Journal of Pharmacological Sciences** 123(2):195–198.

Liebetanz D, Fregni F, Monte-Silva KK, Oliveira MB, Amâncio-dos-Santos A, Nitsche MA, Guedes RC (2006a). After-effects of transcranial direct current stimulation (tDCS) on cortical spreading depression. **Neuroscience Letters** 398(1-2):85–90.

Liebetanz D, Klinker F, Hering D, et al (2006b) Anticonvulsant effects of transcranial direct-current stimulation (tDCS) in the rat cortical ramp model of focal epilepsy. **Epilepsia** 47(7):1216e24

Liew SL, Santarnecchi E, Buch ER, Cohen LG (2014). Non-invasive brain stimulation in neurorehabilitation: local and distant effects for motor recovery. **Frontiers in Human Neuroscience** 8:1–15.

Loscher W, Schmidt D (2004). New horizons in the development of antiepileptic drugs: the search for new targets. **Epilepsy Research** 60:77–159.

Loscher W, Klitgaard H, Twyman RE, Schmidt D (2013) New avenues for anti-epileptic drug discovery and development. **Nature Reviews. Drug Discovery** 12(10):757–776.

Loscher W (2017) Animal Models of Seizures and Epilepsy: Past, Present, and Future Role for the Discovery of Antiseizure Drugs. **Neurochem Research** 42(7):1873–1888.

Marchetti RL. Terapêutica nos transtornos mentais associados à epilepsia. In: Cordás TA, Moreno RA, editors. **Condutas em psiquiatria**. 4a ed. São Paulo: Editora Lemos; 2001. p. 349–384.

McNamara JO (1984) Kindling: An animal model of complex partial epilepsy. **Annals of Neurology** 16:S72S76.

Medeiros LF, de Souza IC, Vidor LP, et al. (2012). Neurobiological effects of transcranial direct current stimulation: a review. **Frontiers in Psychiatry** 3, 110.

Meldrum B (1984) Amino acids neurotransmitters in new approaches to anticovulsivant drug action. **Epilepsia** 22:140–149.

Nitsche MA, Cohen LG, Wassermann EM, Priori A, Lang N, Antal A, Paulus W, Hummel F, Boggio PS, Fregni F, Pascual-Leone A (2008). Transcranial direct current stimulation: state of the art 2008. **Brain Stimulation** 1(3):206–223.

Nitsche MA, Liebetanz D, Lang N, Antal A, Tergau F, Paulus W (2003a). Safety criteria for transcranial direct current stimulation (tDCS) in humans. **Clinical Neurophysiology** 114:2220–2222.

Nitsche MA, Fricke K, Henschke A, et al. (2003b). Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans. **The Journal of Physiology** 553, 293–301.

Nitsche MA, Niehaus L, Hoffmann KT, Hengst S, Liebetanz D, Paulus W, Meyer BU (2004). MRI study of human brain exposed to weak direct current stimulation of the frontal cortex. **Clinical Neurophysiology** 115(10):2419–2423.

Nitsche MA, Seeber A, Frommann K, et al. (2005). Modulating parameters of excitability during and after transcranial direct current stimulation of the human motor cortex. **The Journal of Physiology** 568, 291–303.

Nitsche MA, Paulus W (2000). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. **The Journal of Physiology** 527, 633–639.

Nitsche MA, Paulus W (2009). Noninvasive brain stimulation protocols in the treatment of epilepsy: current state and perspectives. **Neurotherapeutics** 6, 244–250.

Ogiwara I, Miyamoto H, Morita N, Atapour N, Mazaki E, Inoue I, Takeuchi T, Itohara S, Yanagawa Y, Obata K, Furuichi T, Hensch TK, Yamakawa K (2007) Nav1.1 localizes to axons of arvalbumin-positive inhibitory interneurons: a circuit basis for epileptic seizures in mice carrying an scn1a gene mutation. **The Journal of Neuroscience** 27:5903–5914.

OMS. Epilepsia. Disponível em http://www.who.int/mediacentre/factsheets/fs999/es/. Acesso em 26 de junho de 2018.

Orloff MJ, Williams HL, Pfeiffer CC (1949) Timed intravenous infusion of metrazol and strychnine for testing anticonvulsant drugs. **Proceedings of the Society for Experimental Biology and Medicine** 70:254–257.

Paulus W (2003) Transcranial direct current stimulation (tDCS). **Supplements to Clinical Neurophysiology** 56:249–254.

Pedron S, Monnin J, Haffen E, Sechter D, Van Waes V (2014) Repeated Transcranial direct current stimulation prevents abnormal behaviors associated with abstinence from chronic nicotine consumption. **Neuropsychopharmacology** 39:981–988.

Pelletier SJ, Cicchetti F (2015). Cellular and Molecular Mechanisms of Action of Transcranial Direct Current Stimulation: Evidence from In Vitro and In Vivo Models. **International Journal of Neuropsychopharmacoly** 18, pyu047.

Pereira LS, Müller VT, da Mota Gomes M, Rotenberg A, Fregni F (2016). Safety of repetitive transcranial magnetic stimulation in patients with epilepsy: A systematic review. **Epilepsy & Behavior** 57, 167–176.

Petrovski S, Szoeke CEI, Jones NC, et al. (2010). Neuropsychiatric symptomatology predicts seizure recurrence in newly treated patients. **Neurology** 75, 1015–1021.

Poo MM (2001) Neurotrophins as synaptic modulators. **Nature Reviews. Neuroscience** 2: 24–32.

Porto LA, Siqueira JS, Seixas LN, et al. (2007) O papel dos canais iônicos nas epilepsias e considerações sobre as drogas antiepilépticas – uma breve revisão. **Journal of Epilepsy and Clinical Neurophysiology** 13(4):169–175.

Raghavan S, Eldabe S, Strachan R (2008). Novel trends in pain management – Neuromodulation. **Current Anaesthesia & Critical Care** 19:344–348.

Rana A, Musto AE (2018) The role of inflammation in the development of epilepsy. **Journal of Neuroinflammation** 15(1):144.

Rang HP, Dale MM (2011) **Farmacologia.** 7 ed. Rio de Janeiro: Elsevier, p. 540-543.

Rohan JG, Carhuatanta KA, McInturf SM, Miklasevich MK, Jankord R (2015) Modulating hippocampal plasticity with in vivo brain stimulation. **The Journal of Neuroscience** 35:12824–12832.

Rosen AC, Ramkumar M, Nguyen T, Hoeft F (2009). Noninvasive transcranial brain stimulation and pain. **Current Pain and Headache Reports** 13:12–17.

Rossi S, Santarnecchi E, Valenza G, Ulivelli M (2016) The heart side of brain neuromodulation. **Philosophical Transactions. Series A, Mathematical, Physical and Engineering Sciences** 374(2067). pii: 20150187.

Rothwell NJ, Strijbos PJ (1995) Cytokines in neurodegeneration and repair. **International Journal of Developmental Neuroscience** 13:179–185.

Rroji O, van Kuyck K, Nuttin B, Wenderoth N (2015). Anodal tDCS over the Primary Motor Cortex Facilitates Long-Term Memory Formation Reflecting Use-Dependent Plasticity. **PLoS One** 21, 10(5):e0127270.

Ruscheweyh R, Wilder-Smith O, Drdla R, Liu XG, Sandkühler J (2011). Long-term potentiation in spinal nociceptive pathways as a novel target for pain therapy. **Molecular Pain** 7:20.

San-Juan D, Calcáneo Jde D, González-Aragón MF, et al (2011). Transcranial direct current stimulation in adolescent and adult Rasmussen's encephalitis. **Epilepsy & Behavior** 20(1):126e31.

San-Juan D, Morales-Quezada L, Orozco Garduño AJ, et al. (2015). Transcranial Direct Current Stimulation in Epilepsy. **Brain Stimulation** 8:455–464.

San-Juan D, Espinoza López DA, Vázquez Gregorio R, et al. (2017). Transcranial Direct Current Stimulation in Mesial Temporal Lobe Epilepsy and Hippocampal Sclerosis. **Brain Stimulation** 10, 28–35.

Santarnecchi E, Brem AK, Levenbaum E, Thompson T, Cohen Kadosh R, Pascual-Leone A (2015). Enhancing cognition using transcranial electrical stimulation. **Current Opinion in Behavioral Sciences** 4:171–178.

Satfran BN, Crutcher KA (1990) NGF-induced remodeling of mature uninjured axon collaterals. **Brain Research** 525:1l–20.

Saxena VS, Nadkarni VV (2011). Nonpharmacological treatment of epilepsy. **Annals of Indian Academy of Neurology** 14(3):148–152.

Sayyah M, Beheshti S, Shokrgozar MA, Eslami-far A, Deljoo Z, Khabiri AR, et al. (2005) Antiepileptogenic and anticonvulsant activity of interleukin 1beta in amygdala-kindled rats. **Experimental Neurology** 191:145–153.

Scharfman HE (2007) The neurobiology of epilepsy. **Current Neurology and Neuroscience Reports** 7:348–354.

Schmidt D, Rogawski MA (2002). New strategies for the identification of drugs to prevent the development or progression of epilepsy. **Epilepsy Research** 50:71–78.

Scorza FA, Brunoni AR (2015). Transcranial Direct Current Stimulation Against Sudden Unexpected Death in Epilepsy: Press That Button Again, Please. **Brain Stimulation** 8, 839–840.

Scorza C, Marques M, Gomes da Silva S, Naffah-Mazzacoratti M, Scorza F, Cavalheiro E (2018) Status epilepticus does not induce acute brain inflammatory response in the Amazon rodent Proechimys, an animal model resistant to epileptogenesis. **Neuroscience Letters** 668:169–173.

Shi L, Chen R, Zhang H, Jiang C, Gong J (2017) Cerebrospinal fluid neuron specific enolase, interleukin-1β and erythropoietin concentrations in children after seizures. **Child's Nervous System** 33(5):805–811.

Skou JC (1990) The energy coupled exchange of Na+ for K+ across the cell membrane. The Na+, K(+)-pump. **FEBS Letters** 268:314–324.

Skou JC, Esmann M (1992) The Na,K-ATPase. **Journal of Bioenergetics and Biomembranes** 24:249–261.

Sonar S, Lal G (2015) Role of tumor necrosis factor superfamily in neuroinflammation and autoimmunity. **Frontiers in Immunology** 6:364.

Sterman MB, Thompson LM. Neurofeedback for Seizure Disorders. In: Cantor DS e Evans JR (Ed). **Clinical Neurotherapy: Application of Techniques for Treatment**. Londres: Elsevier, 2014. p. 301-318.

Strijbos PJ, Rothwell NJ (1995) Interleukin-1bet attenuates excitatory amino acid-induced neurodegeneration in vitro: involvement of nerve growth factor. **The Journal of Neuroscience** 15:3468–3474.

Sugimoto A, Futamura A, Kawamura M (2012). Epilepsy and dementia. **Brain and Nerve** 64(12):1399–1404.

Tatti E, Rossi S, Innocenti I, Rossi A, Santarnecchi E (2016). Non-invasive brain stimulation of the aging brain: State of the art and future perspectives. **Ageing Research Reviews** 29, 66–89.

Tekturk P, Erdogan ET, Kurt A, et al. (2016). Transcranial direct current stimulation improves seizure control in patients with Rasmussen encephalitis. **Epileptic Disorders** 18, 58–66.

Tirassa P, Costa N (2007) CCK-8 induces NGF and BDNF synthesis and modulates TrkA and TrkB expression in the rat hippocampus and septum: Effects on kindling development. **Neurochemistry International** 50:130–138.

van Elst LT, Woermann FG, Lemieux L, Thompson PJ, Trimble MR (2000). Affective aggression in patients with temporal lobe epilepsy: a quantitative MRI study of the amygdale. **Brain** 123, 234–243.

Varga ET, Terney D, Atkins MD, et al (2011) Transcranial direct current stimulation in refractory continuous spikes and waves during slow sleep: a controlled study. **Epilepsy Research** 97(1e2):142e5.

Vezzani A, Moneta D, Richichi C, Aliprandi M, Burrows SJ, Ravizza T, et al. (2002) Functional role of inflammatory cytokines and antiinflammatory molecules in seizures and epileptogenesis. **Epilepsia** 43:30–35.

Vezzani A, French J, Bartfai T, Baram TZ (2011). The role of inflammation in epilepsy. **Nature Reviews. Neurology** 7(1):31–40.

Viviani B, Bartesaghi S, Gardoni F, Vezzani A, Behrens MM, Bartfai T, Binaglia M, Corsini E, Di Luca M, Galli CL, Marinovich M (2003) Interleukin-1beta enhances NMDA receptor-mediated intracellular calcium increase through activation of the Src family of kinases. **The Journal of Neuroscience** 23:8692–8700.

Weinberg MS, et al. (2013) Opposing actions of hippocampus TNF-alpha receptors on limbic seizure susceptibility. **Experimental Neurology** 247:429–437.

White HS (1997). Clinical significance of animal seizure models and mechanisms of action studies of potential antiepileptic drugs. **Epilepsia** 38:9–17.

Wolfgang L (2005). How to explain multidrug resistance in epilepsy? **Epilepsy Currents** 5:107–112.

Xanthos DN, Sandkühler J (2014). Neurogenic neuroinflammation: inflammatory CNS reactions in response to neuronal activity. **Nature Reviews. Neuroscience** 15(1):43–53.

Yook SW, Park SH, Seo JH, Kim SJ, Ko MH (2011) Suppression of seizure by cathodal transcranial direct current stimulation in an epileptic patient - a case report-. **Annals of Rehabilitation Medicine** 35(4):579e82.

Yoon KJ, Lee YT, Chae SW, Park CR, Kim DY (2016) Effects of anodal Transcranial direct current stimulation (tDCS) on behavioral and spatial memory during the early stage of traumatic brain injury in the rats. **Journal of the Neurological Sciences** 362:314–320.

Yu FH, Mantegazza M, Westenbroek RE, Robbins CA, Kalume F, Burton KA, Spain WJ, McKnight GS, Scheuer T, Catterall WA (2006) Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy. **Nature Neuroscience** 9:1142–1149.

Yu X, Li Y, Wen H, Zhang Y, Tian X (2015) Intensity-dependent effects of repetitive anodal transcranial direct current stimulation on learning and memory in a rat model of Alzheimer's disease. **Neurobiology of Learning and Memory** 123:168–178.

Zhang Z, Liao W, Xu Q, et al. (2017) Hippocampus-associated causal network of structural covariance measuring structural damage progression in temporal lobe epilepsy. **Human Brain Mapping** 38, 753–766.

Zhao RR, Xu XC, Xu F, Zhang WL, Zhang WL, Liu LM, Wang WP (2014) Metformin protects against seizures, learning and memory impairments and oxidative damage induced by pentylenetetrazole-induced kindling in mice. **Biochemical and Biophysical Research Communications** 448(4):414–417.

Zhen J, Qu Z, Fang H, Fu L, Wu Y, Wang H, Zang H, Wang W (2014) Effects of grape seed proanthocyanidin extract on pentylenetetrazole-induced kindling and associated cognitive impairment in rats. **International Journal of Molecular Medicine** 34(2):391–398.

Zobeiri M, van Luijtelaar G (2013) Noninvasive transcranial direct current stimulation in a genetic absence model. **Epilepsy & Behavior** 26(1):42e50.

Zoghi M, O'Brien TJ, Kwan P, Cook MJ, Galea M, Jaberzadeh S (2016). The effects of cathodal transcranial direct current stimulation in a patient with drug-resistant temporal lobe epilepsy (case study). **Brain Stimulation** 9, 790–792.

8 ANEXOS

ANEXO A – Carta de aprovação do projeto pelo CEUA/HCPA.

HCPA - HOSPITAL DE CLÍNICAS DE PORTO ALEGRE
GRUPO DE PESQUISA E PÓS-GRADUAÇÃO

COMISSÃO DE ÉTICA NO USO DE ANIMAIS

A Comissão de Ética no Uso de Animais (CEUA/HCPA) analisou o projeto:

Projeto: 160265
Data da Versão do Projeto: 20/05/2016

Pesquisadores:
IRACI LUCENA DA SILVA TORRES
PRICILA FERNANDES PFLUGER
CASSIANA MACAGNAN VIAU
CARLA DE OLIVEIRA
GABRIELA GREGORY REGNER

Título: Avaliação Pré-Clínica da Estimulação Transcraniana por Corrente Contínua (ETCC), uma Nova Abordagem Terapêutica para o Tratamento da Epilepsia.

Este projeto foi APROVADO em seus aspectos éticos e metodológicos de acordo com as Diretrizes e Normas Nacionais e Internacionais, especialmente a Lei 11.794 de 08/10/2008, que estabelece procedimentos para o uso científico de animais.

- Os membros da CEUA/HCPA não participaram do processo de avaliação de projetos onde constam como pesquisadores.
- Toda e qualquer alteração do Projeto deverá ser comunicada à CEUA/HCPA.
- O pesquisador deverá apresentar relatórios semestrais de acompanhamento e relatório final ao CEUA/HCPA.

Porto Alegre, 12 de agosto de 2016.

Biol. Michael Everton Andrades
Coordenador CEUA/HCPA

ANEXO B – Comprovante de submissão do artigo 2 para publicação na Revista Epilepsy & Behavior.

Your co-authored submission

Epilepsy & Behavior <EviseSupport@elsevier.com>

qui 14/06/2018 15:18

Para:gabigregory@hotmail.com <gabigregory@hotmail.com>;

Dear Mrs. REGNER,

You have been listed as a Co-Author of the following submission:

Journal: Epilepsy & Behavior

Title: Transcranial direct current stimulation (tDCS) affects neuroinflammation parameters and behavioral seizure activity in pentylenetetrazole-induced kindling in rats.

Corresponding Author: Iraci Lucena da Silva Torres

Co-Authors: GABRIELA G REGNER, PRICILA F PFLÜGER, CARLA DE OLIVEIRA, LISIANE S SILVA, Vanessa Scarabelot, Roberta Ströher, Andressa Souza, Felipe Fregni, Patricia Pereira

Iraci Lucena da Silva Torres submitted this manuscript via Elsevier's online submission system, EVISE®. If you are not already registered in EVISE®, please take a moment to set up an author account by navigating to http://www.evise.com/evise/faces/pages/navigation/NavController.jspx?JRNL_ACR=EB

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If you did not co-author this submission, please contact the Corresponding Author directly at 87605@ufrgs.br.

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