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EFEITOS DA ADMINISTRAÇÃO DE MEMANTINA SOBRE CRISES EPILÉPTICAS INDUZIDAS POR PENTILENOTETRAZOL NO MODELO DE MALFORMAÇÃO DO DESENVOLVIMENTO CORTICAL

PORTO ALEGRE

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Trabalho de Conclusão de Curso apresentado ao Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul como requisito parcial para obtenção do título de bacharela em Biomedicina.

Orientadora: Profa. Dra. Maria Elisa Calcagnotto

Co-orientadora: Kamila Cagliari Zenki

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RESUMO

Malformações do desenvolvimento cortical são uma das principais causas de epilepsia refratária em crianças em todo o mundo. A busca por novas terapias, além do procedimento cirúrgico, pode ser útil para esses pacientes. Nosso objetivo foi avaliar o reposicionamento controverso da memantina, um antagonista dos receptores NMDA, aprovado pelo FDA para tratar a Doença de Alzheimer moderada a grave (DA), como potencial fármaco antiepiléptico observando seus efeitos sobre crises epilépticas induzidas por pentilenotetrazol (PTZ) em um modelo animal de malformação cortical. Para isso, foi induzido microgiria no córtex somatosensorial primário (bilateral) em filhotes de ratos Wistar (P0) por meio da criolesão (FL: do inglês *freeze-lesion*). Os animais Sham (controles) foram submetidos ao mesmo procedimento, porém sem a criolesão. Em P30 todos os animais foram anestesiados para a cirurgia de implantação de eletrodos. Sete dias após, registros de vídeo-EEG basal foram realizados em cada animal (FL ou Sham) por 10 min, seguido da administração intraperitoneal de memantina (30mg/kg, i.p) (FL-M ou Sham-M) ou salina 0,9% (FL-S ou Sham-S). Após mais 30 minutos de vídeo-EEG, os animais receberam pentilenotetrazol (PTZ,70mg/kg, i.p) para a indução das crises epilépticas. A latência, duração e o número de crises induzidas por PTZ foram avaliadas por vídeo-EEG durante o período de 1 hora após a injeção. Nossos resultados mostraram que a memantina aumentou o número de crises epilépticas de todos os graus de severidade em ambos os grupos, tanto FL como Sham. Porém, foi incapaz de modificar a latência para a primeira crise e a duração das crises em ambos os grupos. Com base nos nossos achados podemos concluir que a memantina aumentou a frequência de crises epilépticas no modelo animal. Portanto, é necessária uma cuidadosa avaliação antes de considerar o uso da memantina como possível fármaco antiepiléptico.

Palavras-chave: Memantina. Crises epilépticas. Malformação do desenvolvimento cortical. PTZ

ABSTRACT

Developmental cortical malformations (DCM) are one of the main causes of refractory epilepsy in children worldwide. Search for new therapies, besides surgical procedure can be helpful for these patients. Here we aimed to investigate the effects of a non-competitive antagonist of NMDA receptors, memantine, a FDA-approved drug to treat moderate to severe Alzheimer Disease (AD), on epileptic seizures in an animal model of cortical malformation. Experimental microgyria in primary somatosensory cortex (bilateral) were induced in P0 Wistar male rat pups by freeze lesioning (FL). The Sham animals (controls) underwent to the same procedure without the freeze lesion. At P30, all animals were anesthetized for electrodes implantation. Seven days later baseline video-EEG was recorded for 10min from each animal (FL or sham). Afterwards each animal received either memantine (20mg/kg, i.p.) (FL- M or sham-M) or 0.9% saline (i.p.) (FL-S or sham-S) and the video-EEG was recorded for 30min more. The seizure was induced by pentylenetetrazole (PTZ – 70mg/kg, i.p) to evaluate the latency, duration and number of seizures during one additional hour of video-EEG recording. Our data showed that memantine increased the number of seizures of all stages in controls and FL animals and was unable to modify the latency for the first seizure and seizure duration in both groups. Using an experimental rat model of cortical malformation exposed to PTZ to induce seizures, we provide evidences that memantine worsened the seizure frequency of all stages of severity. Therefore, careful evaluation should be considered before suggesting the use of memantine as a potential antiepileptic drug.

Keywords: Memantine. Seizures. Developmental cortical malformations. PTZ

SUMÁRIO

1. INTRODUÇÃO

1.1 MALFORMAÇÃO DO DESENVOLVIMENTO CORTICAL

O desenvolvimento do córtex cerebral humano é um processo complexo e bem organizado. A interrupção de qualquer passo na formação cortical pode acarretar em vários tipos de malformações do desenvolvimento cortical (MDC) (GUERRINI E DOBYNS, 2014). Esse termo engloba muitos distúrbios que diferem em sua etiologia: causas genéticas e/ou fatores ambientais que interferem em eventos biológicos (mitose, apoptose, migração neuronal, função citoesqueleto) e consequentemente alteram a formação adequada do córtex cerebral (SISODIYA, 2004). Essas malformações podem causar perturbações de circuitos neuronais e predispor a uma variedade de consequências clínicas, sendo a mais comum as epilepsias (LEVENTER *ET AL.*, 2008).

A classificação dessas malformações se baseia em alterações que ocorrem em uma das três etapas do desenvolvimento primário: proliferação celular, migração neuronal ou organização cortical (BARKOVICH *ET AL.*, 2012). As malformações originadas de anormalidades de proliferação celular podem causar microcefalia, macrocefalia ou displasia cortical (área cortical com citoarquitetura anormal). As desordens do início da migração neuronal podem resultar em heterotopia periventricular (nódulos anormais de neurônios localizados ao longo da parede ventricular), já as desordens da migração neuronal tardia e/ou motilidade causam uma anomalia das seis camadas corticais como lisencefalia (“cérebro liso”) e banda heterotópica subcortical (neurônios heterotópicos localizados a meio caminho entre a superfície do cérebro e os ventrículos laterais). Por fim, as desordens de organização neuronal geram polimicrogiria (múltiplos pequenos giros separados por pequenos sulcos) ou esquizencefalia (“cérebro com grandes fissuras”) (PANG *ET AL.*, 2008; KANEKAR E GENT, 2011). Embora as fases do desenvolvimento cortical estejam separadas, é evidente que perturbações anteriores afetarão todas as etapas seguintes da formação do cérebro, podendo existir uma sobreposição significativa entre as fases e muitas anormalidades podem causar disfunções em mais de um nível (PANG *ET AL.*, 2008; KUZNIECKY, 2015).

1.2 EPILEPSIAS E MALFORMAÇÃO DO DESENVOLVIMENTO CORTICAL

A epilepsia é uma desordem neurológica caracterizada por crises epilépticas espontâneas e recorrentes. As crises epilépticas resultam de descargas paroxísticas, hipersincrônicas e anormais de populações de neurônios do cérebro (FISHER *ET AL.*, 2017). As malformações do desenvolvimento cortical são cada vez mais reconhecidas como causas de epilepsia refratária e distúrbios do neurodesenvolvimento (SISODIYA, 2004). Porém, o mecanismo básico por trás da origem da epilepsia nas MDC é mal compreendido apesar de numerosos estudos (KUZNIECKY, 2015).

O tratamento com drogas antiepilépticas geralmente é ineficiente. Quando refratária a drogas antiepilépticas (DAE), a epilepsia é passível de abordagem cirúrgica, porém, quando a MDC é difusa, envolvendo a maior parte do cérebro, o tratamento é mais limitado (GUERRINI *ET AL.*, 2008). Portanto epilepsias refratárias associadas às malformações corticais permanecem sendo um problema clínico, social e econômico muito importante.

1.3 MODELO DE MALFORMAÇÃO CORTICAL

Os modelos de MDC podem ser experimentalmente induzidos por manipulações químicas, físicas ou manipulações genéticas que levam a alterações do cérebro em desenvolvimento que se assemelham em muitos aspectos à patologia e fisiopatologia das malformações do córtex humano (LUHMANN, 2016). Essas manipulações interferem no processo normal de desenvolvimento, alterando a geração de neurônios e as redes corticais (ROSEN *ET AL.*, 1992).

Um desses modelos consiste em induzir uma lesão cortical por congelamento (criolesão) em animais recém-nascidos através do posicionamento de um *probe* metálico congelado no crânio desses animais no dia 0 ou 1 pós-natal. Esse modelo reproduz a patologia de malformações como polimicrogiria, displasia cortical e esquizencefalia (LUHMANN, 2016). A lesão resultante é um microgiro formado por quatro camadas corticais, como descritos na patologia humana (DVORÁK E FEIT, 1977; MCBRIDE E KEMPER, 1982) (Fig. 1). Esse insulto gera uma completa

necrose do tecido subjacente a *probe.* Para reparar o dano, ocorre um crescimento de fibras gliais radiais e migração neuronal para a zona danificada (ROSEN *ET AL.*, 1992; PATRICK *ET AL.*, 2006). Adjacente ao microgiro forma-se a região paramicrogiral, apresentando uma desorganização das camadas corticais, hiperexcitabilidade cortical e conexões aferentes e eferentes anormais (JACOBS *ET AL.*, 1999; ROSEN *ET AL.*, 2000; KAMADA *ET AL.*, 2013). Apesar de a criolesão alterar o desenvolvimento, esse modelo não apresenta crises epilépticas espontâneas e recorrentes sem um insulto, ou são crises muito raras (LUHMANN, 2016). Por isso uma das estratégias é avaliar a susceptibilidade das crises epilépticas induzidas por um agente convulsivante como o pentilenotetrazol (PTZ), antagonista dos receptores $GABA_A$. O PTZ é amplamente utilizado em modelos animais para *screening* de DAE e avaliação de susceptibilidade às crises epilépticas em modelos animais (CREMER *ET AL.*, 2009).

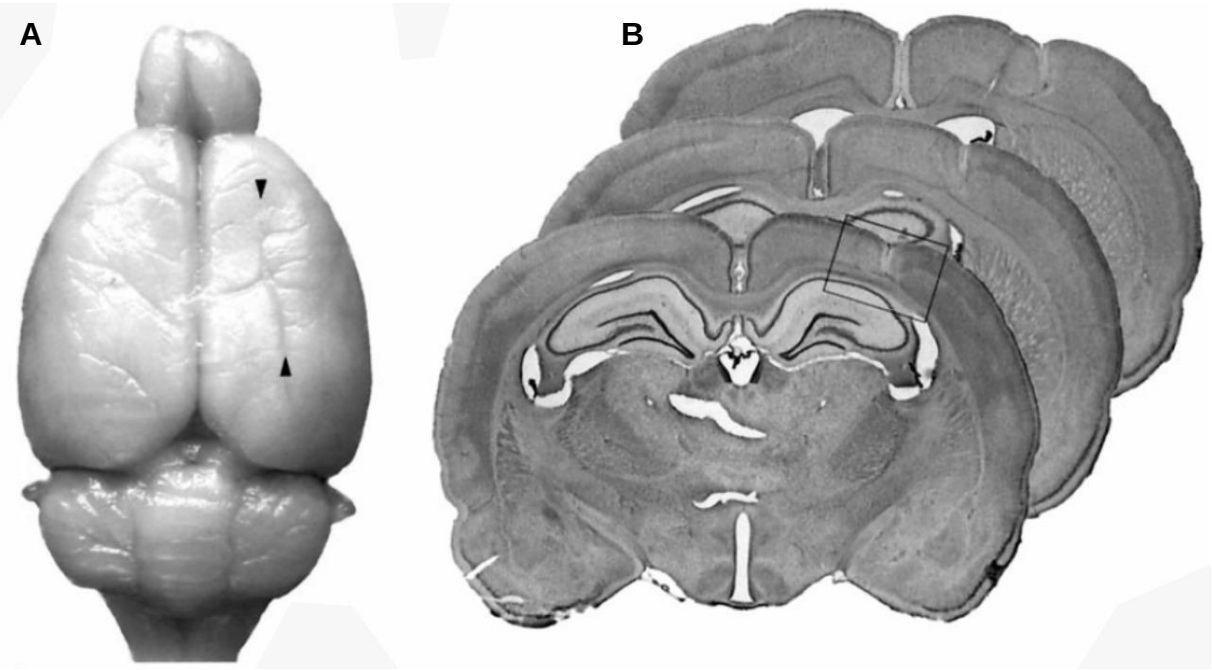

Figura 1: Imagens representativas de animais sujeitos a lesão por congelamento. A, cérebro de rato adulto que recebeu uma lesão congelada no dia do nascimento, resultando em um microgiro longitudinal. B, cortes coronais mostrando a lesão por congelamento, associado a perda de camadas corticais e a formação de um microssulco. Adaptado de (Redecker *et al.*, 2000).

1.4 REPOSICIONAMENTO FARMACOLÓGICO: MEMANTINA

A memantina é um fármaco amplamente empregado no tratamento da doença de Alzheimer (HUANG E MUCKE, 2012). O mecanismo de ação envolve um antagonismo não competitivo de baixa afinidade no receptor NMDA, se ligando perto ou no sítio de Mg^{2+}, levando a um bloqueio parcial do influxo excessivo de cálcio decorrente do aumento de glutamato na fenda sináptica (JOHNSON E KOTERMANSKI, 2006; LIPTON, 2004). Além de bloquear todos os receptores NMDA que possuam as subunidades NR1 e NR2, a memantina também atua bloqueando os receptores nicotínicos α7, α4ß2, α9α10 e receptores 5-HT_3 (REISER *ET AL.*, 1988; BRESINK *ET AL.*, 1996; ARACAVA *ET AL.*, 2005) (Fig. 2). A molécula possui várias características importantes: estrutura de três anéis; uma amina terminal (–NH_2) que em condições fisiológicas fica protonada (–NH_3^+) e representa a região da memantina onde ela se liga ao receptor e as cadeias laterais do grupo metil (-CH_3), que prolongam o tempo de abertura no canal, diminuem a taxa de fechamento do canal e aumentam a afinidade ao receptor (LIPTON, 2006).

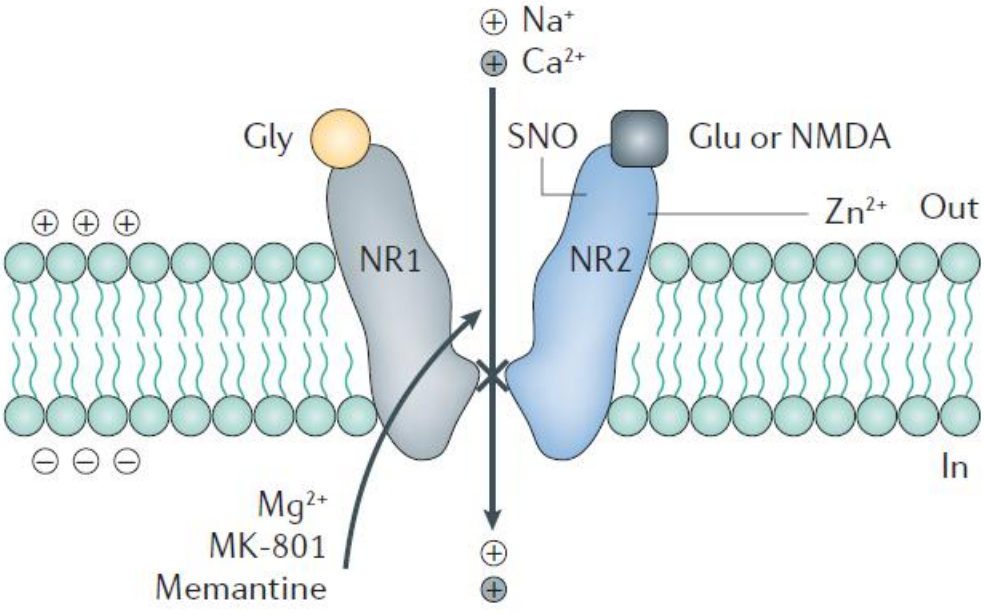

Figura 2: Figura representativa do receptor NMDA mostrando importantes sítios de ligação. Adaptado de (Lipton, 2006).

Apesar de ser utilizada para o tratamento da doença de Alzheimer, esse fármaco tem potencial para ser empregado em outras doenças associadas com a excessiva ativação NMDA, como glaucoma, esclerose múltipla, dor neuropática e

epilepsias (JOHNSON E KOTERMANSKI, 2006). Entretanto o reposicionamento da memantina como potencial anticonvulsivante ainda é controverso, visto que alguns estudos mostram que memantina não foi capaz de reduzir ou prevenir crises epilépticas em modelos animais (OLNEY *ET AL.*, 1986; KOPLOVITZ, 1997; CREELEY *ET AL.*, 2008) e aumentou a frequência de crises epilépticas em pacientes com epilepsia refratária (KYRIAKOPOULOS *ET AL.*, 2018). Existe ainda relato de ocorrência crise epiléptica tônico-clônica generalizada (PELTZ *ET AL.*, 2005) e inesperada perda da consciência (SAVIĆ E MIMICA, 2013) em pacientes com Doença de Alzheimer após o uso de memantina.

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1.5 JUSTIFICATIVA

As malformações corticais são uma das principais causas de epilepsia refratárias ao tratamento farmacológico em crianças. Apesar da existência de vários fármacos antiepilépticos, estima-se que 25% a 40% das epilepsias refratárias sejam atribuídas as MDC e que pelo menos 70% dos pacientes com MDC apresentam epilepsia (LEVENTER *ET AL.*, 1999; LEVENTER *ET AL.*, 2008). Sendo assim, é de grande importância o estudo de novos fármacos que possam ser utilizados na clínica em pacientes com crises refratárias aos tratamentos convencionais. O reposicionamento da memantina em prevenir ou diminuir as crises epilépticas parece controverso como exposta acima. Portanto, são necessários estudos para avaliar o possível efeito da memantina em melhorar ou piorar as crises epilépticas em modelo animal.

1.6 OBJETIVOS

Investigar o efeito da memantina sobre as manifestações clínicas e eletroencefalográficas das crises epilépticas induzidas por pentilenotetrazol em um modelo de malformação do desenvolvimento cortical.

Objetivos específicos:

- Investigar o efeito da memantina na latência e na duração das crises epilépticas induzidas por pentilenotetrazol em animais com criolesão.

- Investigar o efeito da memantina sobre pentilenotetrazol severidade das crises epilépticas induzidas por pentilenotetrazol em animais com criolesão.

2. ARTIGO CIENTÍFICO

Elaborado conforme as normas da revista Epilepsia.

Tipo de artigo: Brief Communication.

Effect of memantine on Pentylenetetrazole-induced seizures in animal model of cortical malformation

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Abstract

Despite the availability of several antiepileptic drugs (AED), refractory epilepsy in children associated with Developmental Cortical Malformation (DCM) remains a significant problem. Here we evaluate the controversial repositioning of a non-competitive NMDA receptors antagonist, memantine, a FDA-approved drug to treat Alzheimer Disease, as a potential AED investigating the effect on pentylenetetrazole (PTZ)-induced seizures in an animal model of DCM. Experimental microgyria in primary somatosensory cortex (bilateral) were induced in P0 male Wistar rats by freeze lesioning (FL). Sham animals underwent the same procedure without the freeze-lesion. At P30, all animals were anesthetized for electrodes implantation. Seven days later baseline video-EEG was recorded for each animal receiving either memantine (20mg/kg, i.p.) (FL-M or sham-M) or 0.9% saline (i.p.) (FL-S or sham-S) followed by (PTZ; 70mg/kg, i.p) to evaluate the latency, duration and number of seizures. Memantine increased the number of seizures of all stages but was unable to modify the latency for the first seizure or seizure duration in all animals. Using an experimental rat model of DCM with PTZ-induced seizures, we provide evidences that memantine worsened the seizure frequency of all stages of severity. Therefore, careful evaluation should be considered before suggesting the use of memantine as a potential AED.

Keywords: memantine, seizures, developmental cortical malformations, PTZ

1. Introduction

Developmental cortical malformations (DCM) are abnormalities of the cerebral cortex originated from a disruption of the normal steps in the cortical plate formation[1]. A common form of cortical malformation is the focal cortical dysplasia (FCD), classified as “malformation due to abnormal postmigrational development” and consists of discrete localized regions of disorganized cell structure and altered cortical lamination commonly associated with microgyria[1]. DCM are a heterogeneous group of disorders associated with 25% to 40% of refractory epilepsy in children[2].

Several animal models of cortical malformation are available to study epilepsy[3]. One of the primary experimental animal model of focal malformation is the neonatal freeze lesion (FL) that results in distinct structural abnormalities and exhibits similar pathology to microgyria in humans[4]. However, despite the structural modifications in the cortex, spontaneous seizures in this model are rare. The use of pentylenetetrazole (PTZ), a $GABA_A$ receptor antagonist, is widely apply to study antiepileptic drug screening seizure susceptibility and can be used to test seizures susceptibility in animal models[3].

Despite the availability of several antiepileptic drugs (AED), refractory epilepsy in children associated with DCM remains a significant medical and economic problem. One strategy is to investigate the possible application of one approved drug to treat a different disease as therapy in epilepsy. Memantine, an adamantine, is a FDA-approved drug to treat moderate to severe Alzheimer Disease[5]. It is an NMDA receptor antagonist that binds to or close to the Mg^{2+} site[6], preventing the pathological activation of NMDA receptors and yet, maintaining their physiological function due to the noncompetitive antagonism[7]. For that reason, it has been recently investigated as a possible treatment for other neurological disorders including

epilepsy[8,9]. However, memantine repositioning to prevent or decrease epileptic seizure are still under debate[10,11]. Therefore, here we aimed to evaluate the possible effects of memantine on preventing or worsening the PTZ-induced seizures in a freeze lesion model in rats.

2. Materials and Methods

2.1. Animals

Pregnant Wistar rats (gestational age:19 d, n=8) were obtained from the Animal Facility of the Biochemistry Department at UFRGS. Animals were housed in 40.5 x 33.5 x 18.0 cm Plexiglass cages containing sawdust, in an acclimatized room (22–26°C), with a 12-h light/dark cycle. The animals had access to water and food *ad libitum*. Day of parturition was considered 0 day of age (P0). On P0, litters were culled to 8-10 pups, and male pups were used for this study. All efforts were made to reduce the number of animals and to minimize their suffering. Animal procedures were performed in strict accordance with the Brazilian Society for Neurosciences (SBNeC) and the Brazilian Law on the Use of Animals (Federal Law 11.794/2008) recommendations and were approved by the Institutional Ethical Committee (CEUA UFRGS protocol no. 31727).

2.2. Freeze lesion

Experimental microgyria on the bilateral primary somatosensory cortex were induced in P0 (within the first 24 h after birth) Wistar male rat pups by freeze lesioning as described previously[4]. Briefly, the animals were anesthetized by hypothermia, the skull was exposed through a scalp incision and a probe with a

rectangular tip, 5.2 mm, cooled to -50 to -60°C (FL) or at room temperature (Sham), was placed on the skull over somatosensory cortex for 10s. The scalp was then glued with surgical glue, and the pup warmed and returned to the dam. Each litter was randomically divided in half to perform either FL or Sham procedure.

2.3. Electrodes implantation

At P30 rats were anesthetized with ketamine:xilazine (100mg/kg:10mg/kg) for electrodes implantation. Four recording stainless steel subdural electrodes were implanted bilateral in somatosensory cortex and temporal cortex (±3.0mm LL; -2,8 and -4,16mm AP from bregma)[12]. The reference electrode was placed in the frontal bone. Electrodes were secured in place with dental cement and fixation screws. After surgery, each animal was place in an individual Plexiglass cage for recovery.

2.4. Drugs

Memantine (memantine chloridrate) was acquired in film-coated tablets with 10mg each and the solution was prepared one day before its use. To prepare the solution, a tablet (10mg) was gently crushed with a pestle and dissolved in 2.5mL of 0.9% saline. A 0.22μm filter was used to remove the excipients and the solution was stored in a refrigerator. Memantine was used in a dose of 20mg/kg i.p[13]. Pentylenetetrazole (PTZ- Sigma-Aldrich) at a dose of 70mg/kg i.p. was freshly dissolved in saline and stored at 5^{o}C. The drugs were injected in a volume of 10 mL/kg.

2.5. Video-EEG recordings

Seven days after electrode implantation, each animal (FL or Sham) was transferred to the observation box and the electrodes were connected to an amplifier

(MAP-32, Plexon, Inc.). Baseline video-EEG recordings were performed for 10min-period. Afterwards each animal received either memantine (20mg/kg, i.p.) (FL-M or Sham-M) or 0.9% saline (i.p.) (FL-S or Sham-S) and the video-EEG was recorded for additional 30min. The seizure was induced by PTZ (70mg/kg, i.p) and recorded during further 1 hour. EEG signals were filtered at 0.1-500Hz followed by digitalization at 1kHz for posterior analysis.

2.6. Video-EEG analysis

The EEG analysis was carried out using the pClamp 10.6 software (Molecular Devices, CA, USA. Seizures were evaluated simultaneously and were classified according Racine scale[14] as following: “mouth and facial movements” (stage I); “head nodding” (stage II); “forelimb clonus” (stage III); seizures characterized by rearing, (stage IV) and seizures characterized by rearing and falling (stage V). Parameters as latency for the first PTZ-induced seizure, seizure duration and frequency were evaluated during 1h-period. Afterwards the animals were sacrificed and the brain removed for posterior histological analysis.

2.7. Histology

After de video-EEG recordings the animals were anesthetized and decapitated. The brain was removed, left in 4% PFA for 12h and then stored in 30% sucrose solution at 4^{o}C. Each brain was cut (40µm) in a cryostat (Leica, Germany), stained with hematoxylin-eosin (HE) and mounted into slides to identify the cortical microgyria induced by freeze-lesion (Fig.1A,B).

2.8. Data analysis

Parameters were analyzed using two-way ANOVA (F1: Lesion, F2: treatment) and the data reported as Mean ± SD. The significance level (α) of 0.05 was used to analyze latency, duration and number of seizures stages II-III; and 0.01 to analyze number of seizures stages IV-V that did not fit in a normal distribution.

3. Results

3.1. Effect of Memantine on PTZ- induced seizures

PTZ induced seizures in all animals. We observed that the latency for the first PTZ-induced seizure and the duration of seizures were similar for all group tested (Table 1, Figure 1C,D). There were no effect of memantine ([F(1,33)=0.80; P=0.38]; [F(1,33)=0.83; P=0.37]) or freeze lesion ([F(1,33)=0.05; P=0.82]; [F(1,33)=1.4; P= 0.24]) on both seizure parameters. No interaction was observed ([F(1,33)=0.91; P= 0.35]; [F(1,33)=0.078; P=0.78]). Therefore, memantine was not able to alter the latency to begin the seizures or the duration of PTZ-induced seizures. On the other hand, both groups of animals that received memantine (Sham-M and FL-M) had increased number of seizures stages II-III when compared to the animals that received saline (Sham-S and FL-S) [F(1,29)=24,4; P<0.0001]. No difference was found between Sham and FL groups [F(1,29)=0.28; P=0.61] and no interaction effect was detected [F(1,29)=3,6; P=0.068] (Table 1, Figure 3E,3G). Memantine also increased the number of seizures stages IV-V in Sham-M and FL-M groups when compared to the saline groups (Sham-S and FL-S) [F(1,33)=15.6; P<0.0001]. There was no difference between Sham and FL groups [F(1,33)=0.79; P=0.38] and no interaction effect were detected [F(1,33)=0.04; P=0.84] (Table 1, Figure 3F,3H).

4. Discussion

In this study we investigated the effects of memantine on PTZ-induced seizures in an animal model of cortical malformation to evaluate the possible controversial application to treat epileptic seizures. PTZ 70mg/Kg induced seizure in all animals. In spite of the microgyria, we did not find any significance difference of seizure characterization between sham and freeze lesion groups as previous reposted in the litarature[15]. Even though animals with freeze lesion have a local hyperexcitability *in vitro*[4], the increased seizure susceptibility may require additional factors other than regional excitability changes. In addition, compensatory mechanisms could be involved, as described in other types of cortical malformation, such as increased GABAergic synaptic transmission at the dysplastic area in animal models and in humans[16]. The benefits of memantine on preventing or decreasing seizure severity or susceptibility remain quite unclear. Some data reinforce its neuroprotective effect showing improvement of neonatal seizures[8], or preventing PTZ-induced seizures and brain damage in animal models[9]. Moreover, when used as coadjuvant with NBQX (2.5–10mg/kg) an AMPA antagonist, synergistically potentiated the anticonvulsive effects of NBQX alone, indicating that this combination therapy might represent a promising therapeutic approach[17].

However, here we demonstrated that the use of memantine at 20mg/kg increased instead of decreased the number of seizures of all stages in the freeze lesion and in Sham animals without altering the latency to the first seizure or the average of seizure duration. Interestingly enough several publications reported similar results. The *in vitro* electrophysiology recordings of organotypic hippocampal slices kept under memantine for 3 days shown an increase in number and duration of bicuculline-induced seizure-like activity when compared to controls[7]. Memantine

(18mg/kg i.p.) also failed in reduce or prevent the seizures or seizure-related brain damage induced by acetylcholinesterase inhibitors in rats[18]. It is important to mention that cholinesterase inhibitors, such as donepezil, when combined with memantine (at 10 to 30mg/kg), potentiated a neurotoxic effect causing cell death throughout many brain regions, characteristic of an excitotoxic seizure-related brain damage syndrome[19]. Although by blocking NMDA receptors memantine could suppress propagation of seizure activity, it may induce seizure activity through other neurotransmitter pathways, especially those responsive to cholinergic excitation once memantine can also interact with cholinergic receptors[19].

Therapeutically relevant doses (5–10mg/kg) of memantine shown to be ineffective in animal models of focal seizures and higher doses (20mg/kg) actually enhance seizures in amygdala-kindled rats[10]. Interestingly that this same dose of 20mg/Kg has been reported as neuroprotective[13] in other study.

Although memantine has not been systematically evaluated in patients with epileptic seizures, case reports published years ago had shown tonic-clonic seizure[11] after the use of memantine to treat Alzheimer Disease, and worsen seizures frequency in 4 patients with atypical Rett Syndrome and refractory epilepsy with gain-of-function variants in GRIN2B[20]. Therefore, the neuroprotective effect of memantine remains controversial.

In conclusion, our results indicated a negative effect of memantine in controlling seizure activity and together with other reports could suggested that the use of memantine alone as AED is not quite efficient to prevent seizures and careful evaluation should be considered before use as potential antiepileptic drug.

Disclosure of Conflicts of Interest:

None of the authors has any conflict of interest to disclose.

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Figure Legends

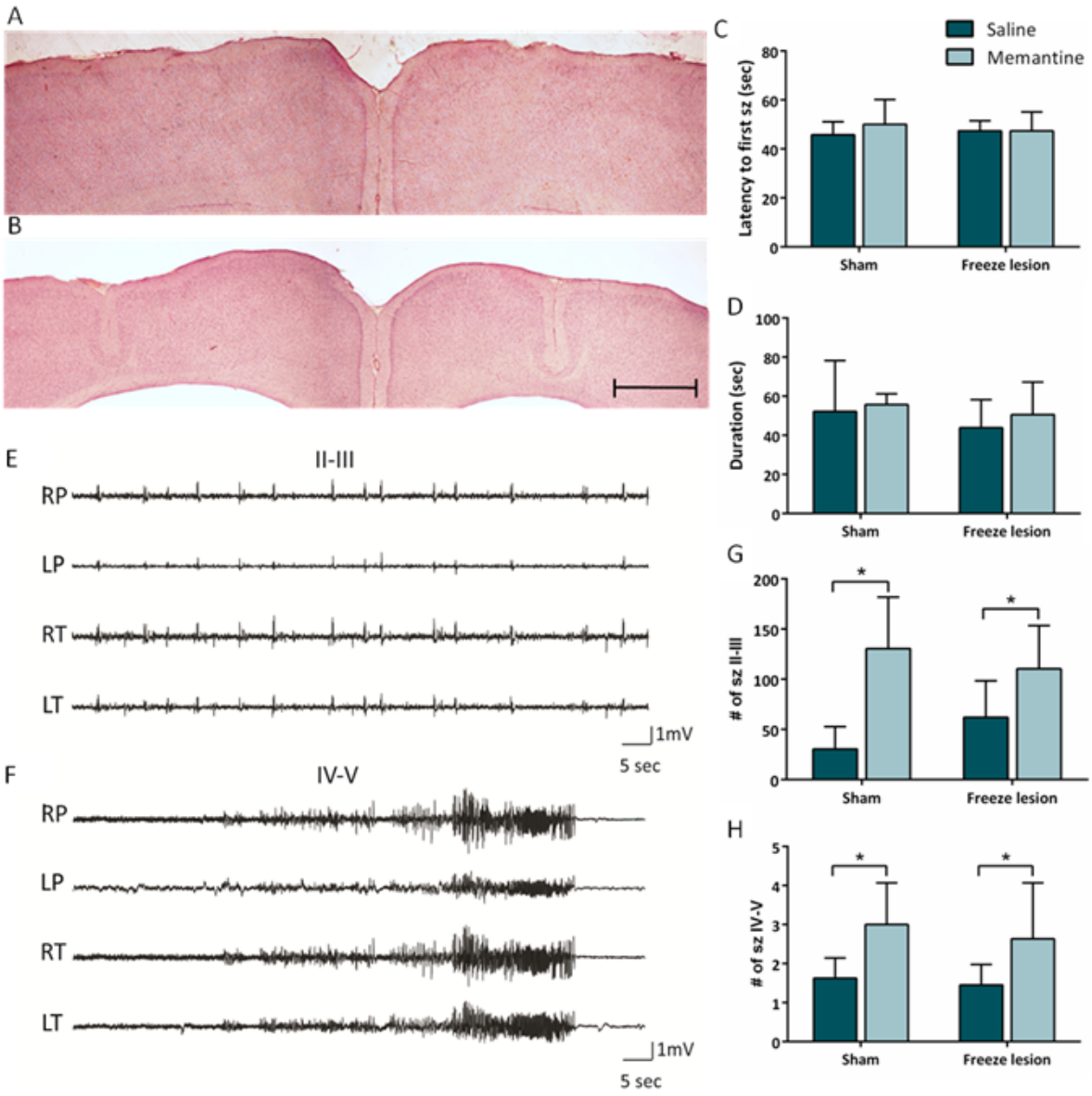
Figure 1. Effects of memantine on PTZ-induced seizures in rats

Photomicrographs of cerebral cortex from coronal brain slices. Representative images (HE) of Sham (A) and Bilateral Cortical Freeze Lesion (B). Magnification: 2x. Scale bar = 1000 μm. Plots of latency for the first seizure (C), and duration of seizures (D) for all animal groups. Representative EEG traces of a seizure stages II-III (E) seizure stages IV-V (F). Plots of number of seizures stages II-III (G) and stages IV-V (H) for all animal groups. Data are presented as mean ± SD. *$p < 0.0001$, Two-way ANOVA (*n* are reported on the Table 1). RP: right parietal, LP: left parietal; RT: right temporal; LT: left temporal.

Table 1: Characteristics of PTZ-induced seizures

	Sham-S	Sham-M	FL-S	FL-M
Latency for 1st Sz (sec)	45.7 ± 5.3	50.1 ± 10.1	47.5 ± 4.2	47.3 ± 7.7
	n=8	n=8	n=10	n=11
IV-V Sz Duration (sec)	52.1 ± 26.0	55.7 ± 5.4	43.9 ± 14.2	50.6 ± 16.5
	n=8	n=8	n=10	n=11
Number of II-III Sz	30.5 ± 22.5	130.5 ± 51.2	66.0 ± 37.0	110.4 ± 42.9
	n=6	n=8	n=8	n=11
Number of IV-V Sz	1.6 ± 0.5	3.0 ± 1.0	1.4 ± 0.5	2.6 ± 1.4
	n=8	n=8	n=10	n=11

Sz: Seizure


A
B
C
Saline
Memantine
Latency to first sz (sec)
Sham
Freeze lesion
D
Duration (sec)
E
II-III
RP
LP
RT
LT
1mV
5 sec
F
IV-V
G
# of sz II-III
H
# of sz IV-V

3. CONCLUSÃO E PERSPECTIVAS

Concluindo, a memantina aumentou o número de crises epilépticas de todos os graus de severidade em todos os grupos sem modificar a latência para a primeira crise ou a duração das crises epilépticas induzidas por PTZ.

Nossos resultados enfatizam os efeitos negativos da memantina no controle de crises epilépticas e sugerem cautela antes de considerar o uso da memantina como possível medicamento antiepiléptico.

Temos como perspectiva aumentar o número amostral do estudo e fazer uma análise quantitativa dos registros eletroencefalográficos Isto nos fornecerá informações importantes da sincronia e da conectividade cortical durante os períodos ictais e ictais nos animais com MDC e possíveis efeitos que a memantina pode ter causado para aumentar a hiperexcitabilidade.

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The following types of material may be considered for publication:

(1) Peer-reviewed papers (to be submitted by uploading online via Scholar One Manuscript Central http://mc.manuscriptcentral.com/epilepsia).

a. Critical Reviews and Commentaries. The Editors-in-Chief encourage submission of reviews and commentaries on topical and controversial issues. Authors planning/proposing such papers should contact the Editors-in-Chief at epilepsia@epilepsia.com before submitting their manuscripts. Authors can also approach one of *Epilepsia's* Associate Editors about possible reviews. While there are no strict length limits on this type of paper, manuscripts generally should be around 5000 words and include a maximum of 100 references. Ample figures and tables are encouraged. Longer manuscripts will be considered at the discretion of the Editors-in-Chief, but justification should be provided by the authors.

b. Full-length Original Research Articles. These articles should be limited in length to 4000 words, 50 references and no more than 6 figures and tables (combined). Additional figures and tables will be permitted at the discretion of the Editors or can be submitted as online only Supporting Information (which will be linked to the online version of the published article). Authors should aim for presenting material clearly and completely, in the most concise and direct form possible; the Introduction should be brief (typically less than 600 words), and the Discussion should be restricted to issues directly relevant to the Results (typically less than 1200 words).

c. Brief Communications. These articles including short studies, small series, case reports, etc. should describe previously unpublished material, including original research and/or clinical observations. The papers are limited generally to 1800 words (excluding the summary), 18 references, and no more than 2 figures and tables (combined). Please note that the Editors may use their discretion to request that brief communications be shortened to a length that they feel is appropriate, and may provide for a larger number of figures and tables if justified.

Instructions for Authors

Brief Communications may be published online only (not in the print version of the journal) depending on their impact. They will appear in a specific issue in the electronic (online) version, and will be identified and described (Short Summary) in the Table of Contents of the printed version of that issue. The online versions will be dealt with by PubMed/Medline and other indexing/citation systems, exactly the same way as print articles; they will be referenced by their DOI number and date of online publication.

d. Controversy in Epilepsy: For emerging areas related to epilepsy care and research for which there is more opinion than high quality data, *Epilepsia* uses the Controversy series as a venue. Authors can propose a pro- and con-position each limited to 2000 words. Contact the editors at epilepsia@epilepsia.com before submitting in this series.

(2) Editorially-reviewed material (to be submitted by email to the Editors-in-Chief at epilepsia@epilepsia.com, except letters and commentaries which should be submitted online at http://mc.manuscriptcentral.com/epilepsia)

Other contributions that do not report original research will be published at the discretion of the Editors-in- Chief, with only editorial review. Such material includes: workshop reports and conference summaries, obituaries, letters/commentary to the Editors (500 word limit, and only exceptionally figures or tables), special (brief) reports from ILAE Commissions or other working groups, and announcements. Such material will usually be published in **Gray Matters**.

(3) Supplements (to be submitted as directed by the Editors-in-Chief)

Supplements, including meeting abstracts, will be published only after advance arrangements are made with the Editors-in-Chief. Guidelines for preparing supplements are given below. Proposal for, and questions about supplements should be directed to one of the Editors-in-Chief (epilepsia@epilepsia.com). Such proposals must be explicitly approved by the Editors-in-Chief, who will also confirm the page rate charge for the proposed supplement.

(4) Special reports: In some cases, special reports from ILAE Commissions or other broadly constituted working groups will be published after peer review. The corresponding author of such papers should confer with the Editors-in-Chief to determine if the full manuscript will be peer-reviewed, or whether only a short version will be considered for publication in *Epilepsia's* Gray Matters (see below).

Manuscript Preparation

General Style Guidelines

Manuscripts are to be submitted (and will be published) in English. Writers not fluent in English should seek assistance to ensure proper grammar and syntax, and to help generate a manuscript organization that facilitates reader understanding. Authors for whom English is a second language may choose to have their manuscript professionally edited before submission, to improve the English. A list of independent suppliers of editing services can be found at http://wileyeditingservices.com/en/. All services are paid for and arranged by the author, and use of one of these services does not guarantee acceptance or preference for publication. The Editors will not re-write papers submitted in unacceptable English, and will return such manuscripts for revision before sending them out for review.

Use international non-proprietary (generic) names when referring to drugs; avoid proprietary (brand) names. All acronyms should be spelled out at first mention. Spell out numbers below 10 and all numbers that are used to begin a sentence; use Arabic numerals for numbers above 10 and for units of measure. Manuscript text should be double spaced with at least 1 inch margin on all sides using size 12 font. Word limits for each type of submission will generally be enforced unless there are good reasons not to do so. If manuscripts exceed these guidelines, authors should submit a cover letter explaining why the additional length is necessary.

Authors are encouraged to use the most recent terminology of seizures and epilepsy (Fisher et al., 2014) and epilepsy classification of the ILAE (Berg et al., 2010). Studies involving treatments should adhere to ILAE's classification of medically refractory epilepsy (Kwan et al., 2011).

Manuscript Format

a. Critical Reviews and Invited Commentaries

- ❑ **Title Page** (see Full-Length Original Research below)
- ❑ **Summary and Key Words**

  Reviews and commentaries should generally begin with a summary (less than 300 words) of the content. The unstructured summary should provide the reader an outline of the main points of the paper. The Summary should be followed by a list of 3-6 Key Words; please provide Key Words that will assist in the indexing of your article (i.e., make it easy for individuals who are searching PubMed to find your paper). Do not use words already incorporated into your title (those words are picked up automatically by the indexing service).

- ❑ **Body of review**

  There is no designated structure for the body of Reviews or Commentaries. Authors are encouraged, however, to use sub-headings to separate major sections and to facilitate clarity and to use figures and tables to illustrate the key issues of the document.

  Tables, figures, figure legends, references, acknowledgements, statement of compliance with the Journal's guidelines for ethical standards in publishing, disclosure of conflicts of interest, and Supplementary material as for *Full-Length Original Research* (see below)

b. Full-Length Original Research, Special Reports, and Brief Communications

❑ **Title Page**

Include the following information: Full title of the manuscript which generally should be as concise and precise as possible; authors' names (first and last names, middle initial when commonly used by that author); institutional affiliation for each author (use superscripted numbers after each author's name, and a corresponding superscripted number before each institutional affiliation); contact information for the corresponding author (name, address, telephone number, fax number, e-mail address); Key Words for use by abstracting services (same as following summary); number of text pages; number of words; number of references; number of figures; number of tables.

❑ **Summary and Key Words**

Provide a summary of no more than 300 words (200 words for Brief Communications). The summary for Full Length Original Research reports should consist off our sections, labeled: Objective; Methods; Results; Significance. This structured summary should concisely and specifically describe why and how the study was performed, the essential results, and what the authors conclude from the results. To promote brevity, authors may use phrases rather than complete sentences. The summary for Special Reports, Invited Commentaries, and Brief Communications is not structured, but should cover the same topics as the structured summary. The summary (structured or unstructured) should be followed by 3-6 Key Words (see above). A second short summary (less than 100 words) is required for Brief Communications that can be used in the print issue Table of Contents. Submit the second short summary as a Supporting Document.

❑ **Key Point Box**

Include 3 to 5 key bullet points that summarize your article after the main body of text. Please ensure each bullet point is no longer than 140 characters. (Brief Communications do not have a key point box). An example of a key point box can be found on the *Epilepsia* Scholar One Manuscripts website (http://mc.manuscriptcentral.com/Epilepsia); please click 'Instructions and Forms' at the top right-hand corner of the homepage.

❑ **Introduction**

State the objectives of the study clearly and concisely, and provide a context for the study by referring judiciously to previous work in the area. Do not attempt to present a comprehensive view of the field. Provide a statement about the significance of this research for understanding and/or treating epilepsy.

❑ **Methods**

Describe the research methods in sufficient detail that the work can be duplicated; alternatively, give references (if they are readily accessible) to previous comprehensive descriptions. Identify the statistical procedures that were used and the rationale for choosing a particular method, especially if it is not standard.

Reports of experimental studies on humans must explicitly certify that the research received prior approval by the appropriate institutional review body and that informed consent was obtained from each volunteer or patient. Studies involving animals must include an explicit statement that animal care and use conformed to institutional policies and guidelines. When animals are subjected to invasive procedures, details must be provided regarding the steps taken to eliminate/minimize pain and suffering, including the specific anesthetics, analgesics, or other drugs used for that purpose (amounts, mode of delivery, frequency of administration).

If extensive descriptions of methods are needed, provide basic information with in the text and submit supplementary information for online Supporting Information.

❑ **Results**

Results should be reported fully and concisely, in a logical order. Do not repeat methodological details from the Methods section. Where possible, use figures and/or tables to present the data in a clear and concise format. Do not repeat data in the text that are given in a table, but refer to the table. Provide textual explanations for all figures, with clear reference to the figure(s) under discussion. Descriptive information provided in figure legends need not be repeated in the text; use the text, however, to describe key features of the figures. When appropriate, give sample numbers, the range and standard deviation (or mean error) of measurements, and significance values for compared populations.

❑ **Discussion**

Provide an interpretation of the results and assess their significance in relation to previous work in the field. Do not repeat the results. Do not engage in general discussion beyond the scope of the experimental results. Conclusions should be supported by the data obtained in the reported study; avoid speculation not warranted by experimental results, and label speculation clearly. Discuss the significance of the data for understanding and/or treating epilepsy.

❑ **Statistical Methods**

The following guidelines assume familiarity with common statistical terminology and methods. We recommend that authors consult a biostatistician during the planning stages of their study, with further consultations during the analytical and interpretational stages.

1. Analysis guidelines:

- Use robust analytic methods when data are skewed.
- Use Kaplan Meier methods, Cox Proportional Hazards, and mixed models analyses for longitudinal data.

Instructions for Authors

- Account properly for statistical outliers.
- Use exact methods as much as possible in analyses of categorical data.
- Use appropriate correction procedures to account for multiple comparisons, and conduct post-hoc comparisons with statistically appropriate methods.

2. Presentation guidelines:

- Report means accompanied by standard deviations; standard errors should not be used.
- Present results with only as much precision as is appropriate.
- Present confidence intervals, whenever possible, including in figures.
- Describe quantity of missingness and methods used for handling such missingness.
- In general, present two-sided p-values. P-values larger than 0.01 should be reported to two decimal places, those between 0.01 and 0.001 to three decimal places, and those smaller than 0.001 should be reported as $p<0.001$.
- In reporting clinical trials, include a flow diagram, a completed trial checklist, and trial registration information. The CONSORT flow diagram and checklist are recommended (http://www.consort-statement.org/).

❑ **Acknowledgments**

Acknowledge sources of support (grants from government agencies, private foundations, etc.); including funds obtained from private industry. Also acknowledge (consistent with requirements of courtesy and disclosure) participation of contributors to the study who are not included in the author list.

❑ **Disclosure of Conflicts of Interest**

In addition, each author should provide full disclosure of any conflicts of interest. One of the following sentences must be included at the end of the paper: either "Author A has received support from, and/or has served as a paid consultant for .... Author B has received support from.... The remaining authors have no conflicts of interest." Or "None of the authors has any conflict of interest to disclose." Note: Disclosure is needed for financial income/payment from commercial sources, the interests of which are relevant to this research activity. Please identify sources from which financial assistance/income was obtained during the period of the research activity and generation of the current report. Grants from government and/or private agencies should be identified in the Acknowledgments section.

❑ **Ethical Publication Statement**

All papers must include the following statement to indicate that the authors have read the Journal's position on issues involved in ethical publication (see below) and affirm that their report is consistent with those guidelines: "We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines."

❑ **References**

Authors are responsible for the accuracy of their references. References should follow a modified Vancouver style format. Citation of references in the text should be in superscript numbers (including those in figure legends and tables). Cite the end references in numerical order. The first three authors should be listed and followed by et al. Use journals' PubMed abbreviations in the reference list at the end of the paper (as opposed to journals' names being written out in full). Reference program patches are available on the *Epilepsia* Scholar One Manuscripts website (http://mc.manuscriptcentral.com/Epilepsia); please click 'Instructions and Forms' at the top right-hand corner of the homepage.

Number of references is limited to the following:
Full Length Original Research Paper – 50
Brief Communications – 18
Reviews – 100
Special Reports – 100

Sample References:

Journal Article

Berg AT, Berkovic SF, Brodie MJ, et al. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005-2009. Epilepsia 2010; 51: 676-685.

Journal article published electronically ahead of print version

Reilly C, Atkinson P, Das KB et al. Academic achievement in school-aged children with active epilepsy: A population-based study. Epilepsia Epub 2014 Oct 20.

Journal article In Press

Battino D, Tomson T, Bonizzoni E, et al. Seizure control and treatment changes in pregnancy: Observations from the EURAP epilepsy pregnancy registry. Epilepsia (in press 2013)

Letter

Marucci G. Commentary on the new ILAE classification system for focal cortical dysplasias. Epilepsia 2012; 1:219-220. Letter

Published Abstract

Noe K, Drazkowski J. Safety of Long-Term Video EEG Monitoring. Epilepsia 2008; 59(suppl 7):1.125. Abstract

Book

Shorvon S. Handbook of the treatment of epilepsy. Oxford: Blackwell Publishing; 2005

Instructions for Authors

Chapter in a Book

Fraser RT, Gumnit RJ, Thorbecke R, et al. Psychosocial rehabilitation: A pre- and postoperative perspective. In Engel J (Ed) Surgical treatment of the epilepsies. 2nd Ed. New York: Raven, 1993:669-667

Online

Russo CA, Elixhauser A. Hospitalizations for Epilepsy and Convulsions, 2005: Statistical Brief #46. Available at:http://www.hcup-us.ahrq.gov/reports/statbriefs/sb46.jsp. Accessed February 12, 2011.

❑ **Figure legends**

Number each legend sequentially to conform to the figure number (e.g., Figure 1, Figure 2...). The legend should provide a brief description of the figure, with explanation of all symbols and abbreviations. Written permission to use non-original material must be obtained (from the original authors (where possible) and publishers) by the authors. Credit for previously published material (author(s), date, journal/book title, and publisher) must be included in the legend. A figure legend should be listed at the end of the manuscript following the list of references.

❑ **Tables**

Tables should be formatted as the authors wish the table to appear in print. Present all tables together at the end of the manuscript, with each table on a separate manuscript page. Each table should be given a number and a descriptive title. Provide notes and explanations of abbreviations below the table, and provide clear headings for each column and row. Do not duplicate data given in the text and/or in figures. Written permission to use non-original material must be obtained (from the original authors (where possible) and publishers) by the authors. Credit for previously published material (author(s), date, journal/book title, and publisher) must be included in the table notes.

❑ **Figures**

All figures should be prepared with care and professionalism. Submissions that do not comply with the following formatting requirements will be returned for correction and re-submission. Figures should be submitted as TIF files in the size expected for final publication– approximately 3 inches (7-8 cm) for half column and 6 to 7 inches (15-17 cm) for double columns. Submit black and white figures with a minimum of 300 dpi (MRI scans) and for line drawings or figures that included imbedded text (bar graphs with numbers) at least 600 dpi. Complex figures (including photographs, micrographs, and MR-related images), either in color, in half-tones, or in black and white, should also be submitted in TIF format with a resolution of at least 600 dpi. We recommend saving the TIF files with LZW compression (an option when you 'save as' in packages like Photoshop), which will make the files smaller and quicker to upload without reducing the resolution/quality. Save each TIF file with a name that includes the first author's last name and the figure number as referenced in the text (e.g., Smith-fig1.tif). Provide clear labels on the ordinate and abscissa. Figures with more than one part should be combined by the authors in the correct orientation and labeled with A, B, C etc. When relevant, include calibration information. Label figures use Calibri font and the authors should make sure that all labels are large enough to be clearly legible when the figure is reduced to fit onto a journal page. The maximum size of any figure is 7x9 in (17×22.5 cm) and 40 mega pixels; the total number of pixels for each figure (i.e., height×width) must be less than 40 megapixels otherwise the image will not convert to PDF for review. There is no charge for color figures. We strongly encourage authors to generate figures in color (to enhance clarity of presentation and aesthetic appeal), using the following color palette:

Color #	RGB Definition	CMYK Definition
#e4b8b4	228/184/180	0/25/15/9
#ce8080	206/128/128	0/50/30/18
#a30234	163/2/52	0/100/60/37
#511d24	81/29/36	42/85/67/60
#f1b682	241/182/130	0/29/50/4
#e37c1d	227/124/29	0/58/100/8
#ffde76	255/223/118	0/11/64/0
#abb47d	171/180/125	13/0/47/27
#67771a	103/119/26	27/0/94/55

Color #	RGB Definition	CMYK Definition
#a1c5cb	161/197/203	25/0/7/16
#5698a3	86/152/163	50/0/14/32
#00545f	0/84/95	100/0/28/64
#002f30	0/47/48	87/34/47/77
#bacfec	186/207/236	25/11/0/0
#0076c0	0/118/192	100/46/0/0
#002157	0/33/87	100/75/0/60
#7a5072	122/80/114	50/73/30/18

Instructions for Authors

Photographs or videos of patients should not reveal patient identity; masking eyes and/or other identifiers is compulsory unless the eyes are essential to the meaning of the photograph or video. In addition, such photographs and videos must be accompanied by a letter saying that signed consent forms authorizing publication have been obtained for all identifiable patients, and that the consents will be maintained by the author for seven years or until the patient reaches 21 years of age, which ever is longer. Do not send *Epilepsia* the consent forms; U.S. Federal privacy rules prohibits ending signed consent forms to *Epilepsia* or Wiley Publishing without written permission from the patient to do so. A sample signed consent form can be found on the *Epilepsia* Scholar One Manuscripts website (http://mc.manuscriptcentral.com/Epilepsia); please click 'Instructions and Forms' at the top right-hand corner of the homepage.

❑ **Supporting Information**

Supporting information, to be published online only, can be submitted for review. Such material may include: additional figures, large tables, videos, etc. that cannot be accommodated within the normal printed space allocation for an article–but provide important complementary information for the reader. As determined by the reviewers and Editors, supporting information will be posted on the Wiley Online Library *Epilepsia* server and directly integrated into the full-text HTML article. Explicit reference to the supporting information in the main body of the text of the article is recommended, and the material must be captioned at the foot of the text, below the reference list. Supporting information will be published as submitted and will not be corrected or checked for scientific content, typographical errors or functionality. Although hosted on Wiley Online Library, the responsibility for scientific accuracy and file functionality remains entirely with the authors. A disclaimer will be displayed to this effect with any supporting information published.

Supporting Information files should be accompanied by detailed information (if relevant) about what they are and how they were created (e.g., a native dataset from a specific piece of apparatus). Acceptable formats for supporting information include:

General – Standard MS Office format (Word, Excel, PowerPoint, Project, Access, etc.); PDF

Graphics – GIF; TIF (or TIFF); EPS; PNG; JPG (or JPEG); BMP; PS (postscript); embedded graphics (e.g. a GIF pasted into a Word file) are also acceptable.

Video–QuickTime; MPEG; AVI. All video clips must be created with commonly-used codecs, and the codec used should be noted in the supplementary material legend. Video files should be tested for playback before submission, preferably on computers not used for its creation, to check for any compatibility issues. Video clips are likely to be large; try to limit their size to less than 10 MB.

c. Gray Matters

❑ **Title**

Letters, workshop reports, etc. should be given a brief title. Letters should start with the opening *To the Editors*:

❑ **Authors and affiliations**

Provide authors' names (first and last names, middle initial when commonly used by that author); institutional affiliation for each author (use superscripted numbers after each author's name, and a corresponding superscripted number for each institutional affiliation); e-mail contact address for the corresponding author.

❑ **Body of submission**

Letters and commentaries should be restricted to 500 words or less, unless other wise allowed by the Editors. Figures and tables will be included only in exceptional cases. Gray Matters will not be used to publish case reports. Tables, figures, figure legends, references, acknowledgements, disclosure of conflicts of interest, ethical publication statement and Supporting Information–as for *Full Length Original Research* (see above).

(3) Details of Preparation

Detailed instructions for all aspects of electronic manuscript submission (including useful information on image files) is available on the *Epilepsia* Scholar One Manuscripts website (http://mc.manuscriptcentral.com/Epilepsia); please click 'Instructions and Forms' at the top right-hand corner of the home page; then click on the link 'Instructions to Authors'.

a. Text

Manuscripts should be prepared using a word processing program. Save text and tables as a Microsoft Word document. Place the lead author's name and the page number in the upper right hand corner of each page. Begin numbering with the Title Page as #1, and number pages consecutively including references, figure legends, and tables. Text (including acknowledgements, disclosure statement, and figure legends) and references should be double-spaced, and be composed in 12 point font (preferably Times New Roman). When generating a revised manuscript, identify the altered portions of the manuscript with highlighted text, underlined, colored or bold font to indicate where changes to the original version of the text have been made.

b. Tables, Figures, and Supporting Information

See above.

Manuscript Submission

(1) Online submission via Manuscript Central

Manuscripts should be submitted via the Journal's website on Scholar One Manuscripts at http://mc.manuscriptcentral.com/epilepsia. Instructions at the site will guide the author through the submission process.

Separate files should be submitted for: Cover letter to editors, manuscript text, tables, each figure, supplemental material, permissions to use previously-published material, patient consent declaration.

(2) Cover letter

All manuscripts should be submitted with a cover letter, addressed to the Editors-in-Chief, which explains why the manuscript should be published in *Epilepsia*. In particular, authors should identify novel findings, innovative approaches, and important insights that would make the manuscript of particular value to the broad readership of *Epilepsia*.

(3) Text, table and figure files

All files should be given a label that includes the first author's last name and the nature of the file (e.g., Smith-manuscripttext.doc; Smith-Fig1.tif).

(4) Other materials/forms

At the time of submission, all other materials (e.g., permission forms, supplemental material, patient consent) must be uploaded onto Manuscript Central, faxed to the editorial office (Fax: +1-702-548-0706) or emailed to epilepsia@epilepsia.com.

(5) Questions/Contacts

Questions and request for assistance should be addressed to the Journal at epilepsia@epilepsia.com. The Managing Editor, Ms. Laurie Beninsig will in most cases be able to provide direction, or will contact the Editors-in-Chief for further assistance.

MANUSCRIPT PUBLICATION

(1) The Editors may approach authors to provide one or two of their figures as possible cover material for the printed journal. These figures will need to be large enough and with the appropriate dpi.

(2) Online tracking of your article

Online production tracking of your article is available through Wiley's Author Services. Author Services enables authors to track their article once it has been accepted through the production process to publication online and in print. Authors can check the status of their articles online and choose to receive automated e-mails at key stages of production. The corresponding author will receive an e-mail with a unique link that enables him/her to register and have the article automatically added to the system. To facilitate this service, please ensure that you provide a complete e-mail address when submitting the manuscript. Visit http://authorservices.wiley.com/bauthor/ for more details on online production tracking and for other publication resources (including FAQs and tips on article preparation, submission and more).

(3) Proofs

Proofs are sent electronically in a PDF format, and must be returned within 48 hours of receipt. Late returns of proofs will cause substantial delay in article publication. It is the corresponding author's responsibility to see that the proof is accurately checked and corrected, and to return the proofs promptly to avoid publication delays. Please check the spelling of coauthors' names and affiliations, text, tables, legends, and references carefully. It is the authors' responsibility to make sure that the information is accurate. Indicate corrections either using the PDF editor function (so as to return proofs electronically to eeeproofs@aol.com), or with clear hard-copy indications which should be faxed to +1 508-586-4024. The proof corrections stage is not the time for fine-tuning language or making any other substantive changes. Confine corrections to errors in printing; authors may be charged for major author-initiated changes.

(4) Early View

The publication-ready PDF of an article will be published initially online. Early View publication will precede print publication by a variable time period. The online publication date will be considered the official publication date. Early View published material will be indexed by PubMed, and can be cited by DOI number. In general, manuscripts will be published on Early View within 28 days of the publisher's receipt of the complete accepted manuscript (including CAF and permission forms).

(5) Print issue publication

Publication of an article in a print issue will typically occur after Early View publication. Print issue articles carry their electronic publication date.

(6) Public access of accepted/published articles

Prior to acceptance, articles may be shared (print or electronic copies) with colleagues; at this time the article may be posted on the author's personal website, on his/her employer's website, and/or on free public servers in the author's subject area - with the acknowledgement that the article has been submitted to *Epilepsia*. After an article has been accepted, authors may share print or electronic copies of the article (accepted and revised to address peer review) with colleagues, and may use the material in personal compilations, other publications of his/her own work, and for educational/research purposes. Articles published in *Epilepsia* are freely accessible to the public via the Wiley Online Library website one year after publication. *Epilepsia* will automatically upload NIH-supported studies to PubMed Central after a 12 month moratorium (provided the appropriate funding acknowledgement has been

provided). Similarly, at this time authors may post an electronic version of the article on their own personal websites, on their employer's website/repository, and on free public servers in the relevant subject area. Electronic versions of the accepted (or published) article must include a link to the published version of the article, together with the following text: "The definitive version is available at http://www3.interscience.wiley.com/journal/117957420/home." Authors can also choose to make their articles open access and available free for all readers through the payment of an author fee. This facility allows authors to fulfill the requirements for studies supported by agencies requiring open access before 12 months. For full details visit http://authorservices.wiley.com/Bauthor/onlineopen.asp

(7) Reprints

An order form for reprints will be included with the electronic transmission of initial proofs. For pricing of quantities in excess of 500 copies, please contact Helane Silverman (email: hsilverman@wiley.com).

SUPPLEMENT PUBLICATION

(1) Policy

A decision to publish a supplement is based on the topic, Guest Editor, proposed table of contents and contributing authors, and availability of necessary funding. Supplement topics must be of importance to *Epilepsia* readers, and supplements will be published only if there is scientific or educational rationale for combining papers on a given theme within one publication. The number and quality of the articles must be sufficient to constitute a body of important information. Each supplement will have a Guest Editor who is an expert on the theme of the supplement. The Guest Editor is responsible for compiling articles and assisting with the editorial process, and is responsible for the overall quality and integrity of the supplement. The publication of a supplement usually incurs charges, payable to Wiley Publishing.

(2) Publishing guidelines

Articles in a supplement are subject to the same copyright regulations and ethical publishing guidelines that apply to articles published in regular issues of *Epilepsia*. All supplement articles are peer-reviewed; the first level of review is carried out by the Guest Editor and his/her designates. The second level of review will include the articles being sent out for peer review.

(3) Online only and print supplements

Abstract supplements, from meetings or congresses sponsored by the ILAE or its chapters, will generally be published online only. Longer articles will be published in print supplements (these articles will also appear online). Print supplements may be generated from proceedings of symposia organized by an independent body of professionals in which the funding organization does not have a controlling voice on scientific content. The Guest Editor and/ororganizers of such symposia should be members of ILAE chapters. Supplements from other sources including invited supplements initiated by the Editors-in-Chief will also be considered.

(4) Supplement content

The content of supplements must not be biased in the interest of any sponsor. *Epilepsia* does not permit presentations that extol a commercial product, and supplements should not be perceived as endorsing a particular product. Publication of supplements does not constitute product or sponsor endorsement by *Epilepsia* or ILAE. In most cases, supplements should not focus on a single product; however, when a new product is introduced, a single product focus will be considered by the Editors-in-Chief. In all cases, the content of a supplement must be determined by a body of professionals working independently of the sponsor. The Guest Editor is charged with assuring that the material presented in the supplement is not biased toward the interests of the product manufacturer.

(5) Supplement sponsorship

Most supplements require external sponsorship. When a supplement proposal is presented to the Editors-in-Chief, they will fix appropriate fees. Supplement costs may be negotiated with the Editors-in-Chief and the publisher's supplement representative. The Editors-in-Chief may choose to publish a supplement of particular academic and clinical value without external sponsorship.

(6) Instructions for submitting supplements

Agreement to publish a supplement must be obtained from the Editors-in-Chief prior to submission. Proposals for supplements should be submitted to the Editors-in-Chief (Epilepsia@epilepsia.com) well in advance of desired publication date, so that the proposal can be evaluated and discussed. Timing is especially critical if the supplement is linked to asymposium or congress, since rapid publication is often important to assure that the information is current. The proposals should identify the Guest Editor and include a list of topics and potential authors. The proposal should include an estimate of supplement length so that the Editors-in-Chief can provide reasonable information about the cost of publication. The cost of any supplement, and related financial issues, should be discussed with Joann Mitchell at Wiley Publishing (jmitchell@wiley.com). Collection of manuscripts, as well as initial editing and reviewing should be

carried out by the Guest Editor on a schedule predetermined in discussion with the Editors-in-Chief. The Guest Editor is responsible for timely submission of articles, and should expect to assist the Editors-in-Chief in collecting final revised manuscripts (including any required permissions).

(7) Format of supplement articles

In general, articles should follow the format described above for Critical Reviews (in regular issues of the Journal). Contact the Editors-in-Chief for additional information and special instructions.

Epilepsia's POSITION ON ISSUES INVOLVED IN ETHICAL PUBLICATION

(1) Authorship/Credit

Epilepsia follows the guidelines of the International Committee of Medical Journal Editors regarding criteria for authorship (http://www.icmje.org/). The author list should include those who have made substantial intellectual/conceptual contributions to the work. Such contributions should include participation in: (a) experimental design, data acquisition, and analysis and interpretation of data; (b) drafting and/or critically revising the article with respect to intellectual content; and (c) final approval of the manuscript version to be published. We strongly discourage the inclusion of "honorary" authors (individuals who are listed as authors but have not contributed to the work/manuscript - e.g., heads of departments) and "ghost" authorship (individuals who have substantively contributed to the work and/or manuscript but are not listed as authors or contributors). In cases where writing support is necessary, the writer(s)should be acknowledged in the Acknowledgments section, and the source of funding for writing support should be provided under Disclosure of Conflicts of Interest. The corresponding/submitting author must, when submitting a manuscript, give assurance that all authors have read and approved the submitted manuscript. The corresponding/submitting author should also give assurance that all authors have seen and approved the final (accepted) manuscript, and that the manuscript includes all conflict of interest declarations. All individuals who have contributed to the work but do not meet criteria for authorship should be cited in the Acknowledgment section.

(2) Funding

Sources of funding (for the research, data analysis, and manuscript generation) should always be disclosed in the Acknowledgements section. Sources may include government funding agencies, institutions and departments, private industry, and charitable organizations and foundations. Funding for all authors should be acknowledged.

If no funding has been provided for the research, please include the following sentence: "This research did not receive any grant from the public, commercial, or not-for-profit sector funding agencies."

(3) Procedures involving Human and Animal Subjects

The authors should include within the manuscript an explicit statement indicating that the submitted study was approved by the relevant research ethics committee or institutional review board (IRB).When the study involves human participants (including material from human subjects), authors should also provide assurance that appropriate consent was obtained. When studies involve animal subjects, authors should provide methodological details about steps taken to minimize pain/discomfort. Such papers must contain a statement that affirms that the experimental protocols were approved by the institutional animal care and use committee (IACUC).

(4) Confidentiality

In all cases, information and images derived from individual patients must be presented with assurance of appropriate consent and with details removed that might reveal identity of the individual.

(5) Disclosure

All authors are required to disclose associations which might affect their ability to present and/ or interpret data objectively, particularly financial ties to funding sources for the work under review (e.g., membership on corporate scientific boards, stock ownership, consultant arrangements, patent ownership or application, etc.). Disclosure of such associations for the Editorial personnel of *Epilepsia* (Editors-in-Chief, Associate Editors, Editorial Board members) will be published each year. Reviewers will also be asked to affirm that they have no conflict of interest when critiquing a manuscript.

(6) Research Misconduct (Data Fabrication/ Falsification)

Epilepsia will attempt to ensure that any allegations of misconduct are properly investigated. In the case of any allegations, authors will be given a right to respond. While the Journal is limited in its ability to investigate misconduct, we will seek COPE's advice and alert appropriate bodies and encourage them to investigate.

Instructions for Authors

Epilepsia's Position on Issues Involved in Ethical Publication

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Epilepsia requires that work submitted for publicationis the authors' own work and has not been misappropriated. When previously published material is used, appropriate credit must be given and written permission obtained (for use of copyrighted material). *Epilepsia* also explicitly discourages duplication of published material and redundant publication. All manuscripts submitted to *Epilepsia* are checked with the iThenticate software to detect instances of overlapping and similar text. In the case of apparent or substantial overlap, authors will be asked to rewrite their article.

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Authors are expected to proof-read their articles carefully before returning page proofs for publication. They should make needed corrections at this time. We recognize that it is only human to err occasionally, and the Journal is committed to correcting mistakes when those errors affect the interpretation of data or information presented in an article. Such corrections will be published in the form of an Erratum, and linked to the original article electronically. Errors that result from author oversight in the proofing process, and that do not affect data interpretation, will not be corrected.

(9) Peer Review

Epilepsia is committed to a peer-review system that is fair to the author and enhances the value of the articles published in the Journal. In order to encourage qualified reviewers to offer their time and efforts to the Journal, reviewer identity is kept confidential. Reviewers are chosen for their expertise in the field; conflicts of interest are avoided whenever the Editors are aware of such issues, and reviewers are asked to affirm that they have no conflicts of interest in reviewing a given *Epilepsia* manuscript. Authors are encouraged to identify specific individuals who, they believe, cannot provide unbiased review. While the Editors-in-Chief reserve the right to make the final decision to accept or reject an article, appeals will be seriously considered. Address appeals to the Editors-in-Chief, who will examine the reviews and the author responses, consult the relevant Associate Editor, and seek additional reviewer input if deemed necessary.

ANEXO – CARTA DE APROVAÇÃO DA COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA)

CEUA
UFRGS

PRÓ-REITORIA DE PESQUISA

COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CARTA DE APROVAÇÃO/ADENDO

Processo Nº: 31727

Título: Modulação colinérgica da transmissão sináptica em regiões displásicas em modelo animal de malformação cortical associada à epilepsia

Pesquisador Responsável: Maria Elisa Calcagnotto

Comissão De Ética No Uso De Animais aprovou o Adendo ao Projeto 31727 - Modulação colinérgica da transmissão sináptica em regiões displásicas em modelo animal de malformação cortical associada à epilepsia, em reunião realizada em 19/03/2018- Sala 323 do Anexo 1 da Reitoria - Campus Centro - Porto Alegre - RS, em seus aspectos éticos e metodológicos, para utilização de 65 ratos Wistar fêmeas, adultas, e 19 ratos Wistar machos, adultos, provenientes do Departamento de Bioquímica da UFRGS; 70 ratos WAR fêmeas e 18 ratos WAR machos, adultos, provenientes do Departamento de Fisiologia da Faculdade de Medicina de Ribeirão Preto USP; autoriza ainda a utilização de 321 neonatos (fêmeas e machos) Wistar e 346 neonatos (fêmeas e machos) WAR, de acordo com os preceitos das Diretrizes e Normas Nacionais e Internacionais, especialmente a Lei 11.794 de 08 de novembro de 2008, o Decreto 6899 de 15 de julho de 2009, e as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), que disciplinam a produção, manutenção e/ou utilização de animais do filo Chordata, subfilo Vertebrata (exceto o homem) em atividade de ensino ou pesquisa. Este documento revoga a Carta de Aprovação emitida anteriormente.

Porto Alegre, 29 de março de 2018

Marcelo Meller Alievi

Coordenador da CEUA/UFRGS