ISSN 1676-2649

# JECN

## Journal of Epilepsy and Clinical Neurophysiology

Volume 21 | Number 3 | Year 2015


- Beta-tubulin expression in the hippocampus of patients with mesial temporal lobe epilepsy
- Epilepsy and eeg abnormalities in children with autism spectrum disorder
- Inflammatory reaction in epilepsy
- Closed-loop optogenetic strategy in experimental epilepsy: how affordable is the implementation of this emergent technique?


J Epilepsy Clin Neurophysiol 2015; 21(3): 089-120

www.jecn.org

JECN Journal of Epilepsy and Clinical Neurophysiology

Órgão Oficial da Liga Brasileira de Epilepsia

Indexada no LILACS – Index Medicus Latino Americano.



**EXPEDIENTE**

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**Editora Executiva –** Ana Carolina de Assis

**Editora Administrativa –** Atha Comunicação Editora
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**Ficha Catalográfica**

Journal of Epilepsy and Clinical Neurophysiology (Revista de Epilepsia e Neurofisiologia Clínica) / Liga Brasileira de Epilepsia. – Vol. 21, n.1, mar 2015.

v.1, 1995 – JLBE: Jornal da Liga Brasileira de Epilepsia
v.2 a 7 (n. 2, jun. 2001) Brazilian Journal of Epilepsy and Clinical Neurophysiology (Jornal Brasileiro de Epilepsia e Neurofisiologia Clínica)
Publicação trimestral.
ISSN 1676-2649

CDD: 616.8
CDU: 616.853(05)
616.8-092(05)
616.8-073(05)

**Índice para Catálogo Sistemático:**

Epilepsia – Periódicos – 616.853(05);
Neurofisiologia – Periódicos – 616.8-092(5);
Eletroencefalografia – Periódicos – 616.8-073(05);
Eletroneuromiologia – Periódicos – 616.8.073(05);
Neurologia – Fisiologia – Periódicos – 616.8-092(05).

Coordenação editorial, criação, diagramação, tradução e produção gráfica Atha Comunicação e Editora - E-mail: 1atha@uol.com.br

JECN Journal of Epilepsy and Clinical Neurophysiology

# Instructions for authors / Instruções aos autores / Instrucciones para los autores

Most recent revision: March 2015

The Journal of Epilepsy and Clinical Neurophysiology (JECN) is the Official Body of the Brazilian Epilepsy League, whose purpose is to publish original scientific-technological articles about epilepsy and clinical neurophysiology, resulting from ethically developed and approved clinical and experimental research. Volumes are published annually, with quarterly editions, in March, June, September and December of each year. The articles submitted must be original and concise, written in English, Portuguese or Spanish. The text should be prepared in accordance with the technical standards, and sent via the publications management system. In order to be approved, the articles will be submitted for evaluation by a panel of reviewers (peer review), who will receive the text anonymously and decide on its publication, suggest changes, request clarification from the authors, and provide recommendations to the Editor-in-Chief. The concepts and statements contained in the work are the sole responsibility of the authors. The Journal Epilepsy and Clinical Neurophysiology follows, in full, the international trend of the Vancouver style, which is available at www.icmje.org.br. We thank the authors, in advance, for their collaboration in following the instructions.

## FORMATTING OF ARTICLES

**LIMITS FOR EACH TYPE OF PUBLICATION (Extension):** The following criteria must be observed for each type of publication. The electronic word count must include: the title page and text.

| Type of Article | Abstract | Number of words | References | Figures | Tables |
|---|---|---|---|---|---|
| Original | Structured with up to 250 words | 6.000 not including the abstract, references, tables and figures | 45 | 10 | 6 |
| Update / Review Case Report | It is not structured with up to 250 words | 6.000 not including the abstract, references, tables and figures | 60 | 3 | 2 |
| Editorial | 0 | 500 | 5 | 0 | 0 |

**MANUSCRIPT PREPARATION:** The Journal of Epilepsy and Clinical Neurophysiology receives the following types of manuscript for publication: Original Articles, Update and Reviews Articles, Case Report, Editorial. The manuscripts should be submitted in accordance with PC standard, in Word files, double spaced, with wide margins, and the author shall include a signed letter of authorization for publication, declaring that it is an original work, and that it has not been, or is not being submitted for publication in any other journal. Ensure that the manuscript is fully in accordance with the instructions.

**CLINICAL TRIALS:** The Journal of Epilepsy and Clinical Neurophysiology supports the policies for the recording of clinical trials of the World Health Organization (WHO) and the International Committee of Medical Journal Editors (ICMJE), recognizing the importance of these initiatives for the recording and international disclosure of information on clinical trials, in open access. Accordingly, only clinical research articles that have received an identification number in one of the Clinical Trial Records validated by the WHO and ICMJE criteria will be accepted for publication. The addresses for these records are available on the ICMJE website (www.icmje.org). The identification number should be declared in the text.

**CONFLICTS OF INTEREST:** According to the requirements of the International Committee of Medical Journal Editors (ICMJE), the Vancouver group, and Resolution no. 1595/2000 of the Federal Council of Medicine Resolution, the authors have a responsibility to recognize and declare any conflicts of interest, financial or otherwise (business, personal, political, etc.) involved in the development of work submitted for publication. The authors must declare, and may acknowledge, in the manuscript, any financial support received for the work, as well as others parties involved in its development.

**CORRECTION OF GRAPHIC PROOFS:** As soon as they are ready, graphic proofs in electronic format shall be sent by email to the author responsible for the article. Authors must return the graphic proofs with the necessary corrections, also by email, within 48 hours of their receipt. The sending and the return of graphic proofs by electronic mail is intended to streamline the revision process and subsequent publication of the articles.

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**ORGANIZATION OF THE ELECTRONIC FILE:** All parts of the manuscript must be included in a single file, which must be organized with the cover page first, then the text, AND THE references followed by figures (with captions) and at the end, tables and charts (with captions).

**COVER PAGE:** The cover page must include:

a) type of article (original article, review or update)

b) full title in Portuguese, English and Spanish, with up to 120 characters. The title must be concise but informative

c) full name of each author (without abbreviations); and the institution to which each one belongs

d) place where the work was carried out

e) name, address, telephone number, and email address of the author responsible for correspondence

**ABSTRACT:** The Abstract must be structured in the case of original articles, and must clearly present the study objectives, with historical data, methods, results, and the main conclusions. It must be written Portuguese, English and Spanish, and should not exceed 200 words.

**DESCRIPTORS:** Must contain at least three key words in Portuguese based on the Health Sciences Descriptors (DeCS) -http://decs.bireme.br. In English, submit keywords based on the Medical Subject Headings (MeSH) - http://www.nlm.nih.gov/mesh/meshhome.html, at least three and at most six citations.

**INTRODUCTION:** Present the subject and purpose of the study, and provide citations, without giving an external review of the subject.

**MATERIAL AND METHOD:** Describe the experiment (quantity and quality) and the procedures in sufficient detail to allow other researchers to reproduce the results, or to continue the study. When reporting experiments involving human and subjects, indicate whether the procedures have complied with the rules of the Ethics Committee on Experiments involving Human Beings of the institution where the research was conducted, or if it is in accordance with the 1996 Declaration of Helsinki and Animal Experimentation Ethics, respectively. Accurately identify all drugs and chemicals used, including generic names, doses and administration routes. Do not use patient names, initials, or hospital records.

Provide references for the establishment of statistical procedures.

**RESULTS:** Present the results in logical sequence in the text, using tables and Illustrations. Do not repeat all the data contained in the tables and/or illustrations in the text. Emphasize or summarize only the important discoveries in the text.

**DISCUSSION:** Emphasize new and important aspects of the study. Previously published methods should be compared with the current methods, so that the results are not repeated.

**CONCLUSION:** Must be clear and concise and establish a connection between the conclusion and the study objectives. Avoid conclusions not based on data.

**ACKNOWLEDGEMENT:** Addressed to persons who have collaborated intellectually but whose contribution does not constitute co-authorship, or those who have provided material support.

**REFERENCES**: Quote up to about 20 references, restricted to the bibliography essential to the content of the article. Number references consecutively in the order in which they are mentioned in the text, using superscript Arabic numerals, in the following format: (Reduction of functions of the terminal plate.[1]) Give the names of the first three authors, followed by et al.

Journal titles should abbreviated, according to the Index Medicus.

**a)** Articles: Author(s). Title of the article. Title of the Journal. year; volume: first-last age E.g. Campbell CJ. The healing of cartilage defects. Clin Orthop Res Report. 1969;(64):45-63.

**b)** Books: Author(s) or editor(s). Title of the book. Edition, if not the first. Translator(s), if applicable. Place of publication: publisher, year. E.g. Diener HC, Wilkinson M, editors. Drug-induced headache. 2nd ed. New York: Spriger-Verlag; 1996.

**c)** Chapters of books: Author(s) of the chapter. Title of chapter. Editor(s) of the book and other data on this, as for the previous item. E.g. Chapman MW, Olson SA. Open fractures. In: Rockwood CA, Green DP. Fractures in adults. 4th ed. Philadelphia: Lippincott-Raven; 1996. p. 305-52.

**d)** Summaries: Author(s). Title, followed by (summary). Journal year; volume (supplement and its number, if applicable): page(s) E.g. Enzensberger W, Fisher PA. Metronome in Parkinson's Disease (abstract). Lancet. 1996 ;34:1337.

**e)** Personal communications should only be mentioned in the text in parentheses.

**f)** Thesis: Author, title level (master's, doctorate etc.), city: institution; year. E.g. Kaplan SJ. Post-hospital home health care: the elderly's access and utilization (dissertation). St. Louis: Washington University; 1995.

**g)** Electronic Material: Title of the document, internet address, date of access. E.g. Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis. (Online) 1995 Jan-Mar [cited 1996 Jun 5];1(1):[24 screens]. Available from:URL:http://www.cdc.gov/ncidod/EID/eid.htm

**TABLES:** Tables should be numbered in the order in which they appear in the text, with Arabic numerals. Each table must have a title and, if necessary, an explanatory caption. Charts and tables should be sent through the original files (e.g. Excel).

**FIGURES (photographs/illustrations/graphics):** Figures should be presented on separate pages and numbered sequentially, in Arabic numerals, in the order in which they appear in the text. To avoid problems that could compromise the standard of the journal, the material sent must meet the following parameters: all figures, photographs and illustrations must have graphics of adequate quality (300 dpi resolution) and must have a title and caption. In all cases, the files must have .tif extension and/or jpg. Files will be also accepted with .xls (Excel), .eps, or .psd extensions for illustrations featuring curves (graphs, drawings and diagrams). The figures include all illustrations, such as photographs, drawings, maps, graphs, etc., and should be numbered consecutively, in Arabic numerals. Figures in black and white will be reproduced free of charge, but the reserves the right to set a reasonable limit on their number.

**CAPTIONS:** Type captions in double space, accompanying the respective figures (graphics, photographs and illustrations). Each caption should be numbered in Arabic numerals, corresponding to each figure, in the order in which they are cited in the work.

**ABBREVIATIONS AND ACRONYMS:** Must be preceded by the full name when cited for the first time in the text. In the footer of the figures and tables, the meanings of abbreviations, symbols, and other signs should be given, and the source: place with the research was carried out should be stated. If the illustrations have already been published, they should be accompanied by written permission of the author or editor, showing the reference source where it was published.

**REPRODUCTION:** Only the Journal of Epilepsy and Clinical Neurophysiology may authorize the reproduction of the articles contained therein. Cases of omission will be resolved by the Editorial Board.

**SUBMISSION OF ARTICLES:** From January 2015 articles should be sent for submission to the Atha Comunicação e Editora (A/C Ana Carolina de Assis) - Rua Machado Bittencourt, 190 – 4º andar - CEP: 04044-903 – São Paulo/SP, Brazil Tel: +55 11 5087-9502 / Fax: +55 11 5579 5308 or by email to revistajecn@outlook.com

Revisão mais recente: Março de 2015

A Revista Journal of Epilepsy and Clinical Neurophysiology (JECN) é o Órgão Oficial da Liga Brasileira de Epilepsia, cujo propósito é publicar artigos científico-tecnológicos originais sobre epilepsia e neurofisiologia clínica, resultante de pesquisas clínicas e experimentais, eticamente desenvolvidas e aprovadas. Os volumes são publicados anualmente, com edições trimestrais em março, junho, setembro e dezembro de cada ano. Os artigos submetidos devem ser inéditos e concisos, redigidos em inglês, português ou espanhol. O texto deverá ser preparado de acordo com as normas técnicas e enviados pelo sistema de gerenciamento de publicações. Os artigos para serem aprovados são submetidos à avaliação de uma comissão de revisores (peer review) que recebem o texto de forma anônima e decidem por sua publicação, sugerem modificações, requisitam esclarecimentos aos autores e efetuam recomendações ao Editor Chefe. Os conceitos e declarações contidos nos trabalhos são de total responsabilidade dos autores. A Journal of Epilepsy and Clinical Neurophysiology segue na íntegra a tendência internacional do estilo Vancouver, disponível (www.icmje.org.br). Desde já agradecemos a colaboração dos autores no atendimento às instruções citadas.

## FORMATAÇÃO DE ARTIGOS

**LIMITES POR TIPO DE PUBLICAÇÃO (Extensão):** Os critérios abaixo delineados devem ser observados para cada tipo de publicação. A contagem eletrônica de palavras deve incluir: a página inicial e o texto.

| Tipo de Artigo | Resumo | Número de Palavras | Referências | Figuras | Tabelas |
|---|---|---|---|---|---|
| Original | Estruturado com até 250 palavras | 6.000 Excluindo o resumo, referências, tabelas e figuras | 45 | 10 | 6 |
| Atualização / Revisão Relato de Caso | Não é estruturado com até 250 palavras | 6.000 Excluindo o resumo, referências, tabelas e figuras | 60 | 3 | 2 |
| Editorial | 0 | 500 | 5 | 0 | 0 |

**PREPARAÇÃO DE MANUSCRITO:** A Journal of Epilepsy and Clinical Neurophysiology recebe para publicação os seguintes tipos de manuscritos: Artigo Original, Artigo de Atualização e Revisão, Relato de Caso. Os manuscritos enviados deverão estar em padrão PC com arquivos em *Word*, espaço duplo, com margem larga, devendo o autor inserir carta assinada, autorizando sua publicação, declarando que o mesmo é inédito e que não foi, ou está sendo submetido à publicação em outro periódico. Certifique-se de que o manuscrito se conforma inteiramente às instruções.

**ENSAIOS CLÍNICOS:** O periódico Journal of Epilepsy and Clinical Neurophysiology apoia as políticas para registro de ensaios clínicos da Organização Mundial de Saúde (OMS) e do Comitê Internacional de Editores de Diários Médicos (ICMJE), reconhecendo a importância dessas iniciativas para o registro e divulgação internacional de informação sobre estudos clínicos, em acesso aberto. Sendo assim, somente serão aceitos para publicação, os artigos de pesquisas clínicas que tenham recebido um número de identificação em um dos Registros de Ensaios Clínicos validados pelos critérios estabelecidos pela OMS e ICMJE. Os endereços para esses registros estão disponíveis a partir do site do ICMJE (www.icmje.org). O número de identificação deve ser declarado no texto.

**CONFLITO DE INTERESSES:** Conforme exigências do Comitê Internacional de Editores de Diários Médicos (ICMJE), grupo Vancouver e resolução do Conselho Federal de Medicina nº 1595/2000 os autores têm a responsabilidade de reconhecer e declarar conflitos de interesse financeiros e outros (comercial, pessoal, político, etc.) envolvidos no desenvolvimento do trabalho apresentado para publicação. Devem declarar e podem agradecer no manuscrito todo o apoio financeiro ao trabalho, bem como outras ligações para o seu desenvolvimento.

**CORREÇÃO DE PROVAS GRÁFICAS:** Logo que prontas, as provas gráficas em formato eletrônico serão enviadas, por e-mail, para o autor responsável pelo artigo. Os autores deverão devolver, também por e-mail, a prova gráfica com as devidas correções em, no máximo, 48 horas após o seu recebimento. O envio e o retorno das provas gráficas por correio eletrônico visa agilizar o processo de revisão e posterior publicação das mesmas.

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**ORGANIZAÇÃO DO ARQUIVO ELETRÔNICO:** Todas as partes do manuscrito devem ser incluídas em um único arquivo. O mesmo deverá ser organizado com a página de rosto, em primeiro lugar, o texto, referências seguido pelas figuras (com legendas) e ao final, as tabelas e quadros (com legendas).

**PÁGINA DE ROSTO:** A página de rosto deve conter:

a) o tipo do artigo (artigo original, de revisão ou atualização);

b) o título completo em português, inglês e espanhol com até 120 caracteres deve ser conciso, porém informativo;

c) o nome completo de cada autor (sem abreviações); e a instituição a que pertence cada um deles;

d) o local onde o trabalho foi desenvolvido;

e) nome, endereço, telefone e e-mail do autor responsável para correspondência.

**RESUMO:** O Resumo deve ser estruturado em caso de artigo original e deve apresentar os objetivos do estudo com clareza, dados históricos, métodos, resultados e as principais conclusões em português, inglês e espanhol, não devendo ultrapassar 200 palavras.

**DESCRITORES:** Deve conter no mínimo três palavras chaves baseadas nos Descritores de Ciências da Saúde (DeCS) -http://decs.bireme.br. No inglês, apresentar keywords baseados noMedical Subject Headings (MeSH) - http://www.nlm.nih.gov/mesh/meshhome.html, no mínimo três e no máximo seis citações.

**INTRODUÇÃO:** Deve apresentar o assunto e objetivo do estudo, oferecer citações sem fazer uma revisão externa da matéria.

**MATERIAL E MÉTODO:** Deve descrever o experimento (quantidade e qualidade) e os procedimentos em detalhes suficientes que permitam a outros pesquisadores reproduzirem os resultados ou darem continuidade ao estudo. Ao relatar experimentos sobre temas humanos e animais, indicar se os procedimentos seguiram as normas do Comitê Ético sobre Experiências Humanas da Instituição, na qual a pesquisa foi realizada ou de acordo com a declaração de Helsinki de 1995 e Animal Experimentation Ethics, respectivamente. Identificar precisamente todas as drogas e substâncias químicas usadas, incluindo os nomes genéricos, dosagens e formas de administração. Não usar nomes dos pacientes, iniciais, ou registros de hospitais. Oferecer referências para o estabelecimento de procedimentos estatísticos.

**RESULTADOS:** Apresentar os resultados em sequência lógica do texto, usando tabelas e ilustrações. Não repetir no texto todos os dados constantes das tabelas e ou ilustrações. No texto, enfatizar ou resumir somente as descobertas importantes.

**DISCUSSÃO:** Enfatizar novos e importantes aspectos do estudo. Os métodos publicados anteriormente devem ser comparados com o atual para que os resultados não sejam repetidos.

**CONCLUSÃO:** Deve ser clara e concisa e estabelecer uma ligação entre a conclusão e os objetivos do estudo. Evitar conclusões não baseadas em dados.

**AGRADECIMENTOS:** Dirigidos a pessoas que tenham colaborado intelectualmente, mas cuja contribuição não justifica coautoria, ou para aquelas que tenham provido apoio material.

**REFERÊNCIAS:** Citar até cerca de 20 referências, restritas á bibliografia essencial ao conteúdo do artigo. Numerar as referências de forma consecutiva de acordo com a ordem em que forem mencionadas pela primeira vez no texto, utilizando-se números arábicos sobrescritos, no seguinte formato: (Redução das funções da placa terminal.[1]) Incluir os três primeiros autores seguidos de et al.

Os títulos de periódicos deverão ser abreviados de acordo com o Index Medicus.

a) Artigos: Autor(es). Título do artigo. Título do Periódico. ano; volume: página inicial - final

Ex.: Campbell CJ. The healing of cartilage deffects. Clin Orthop Relat Res. 1969;(64):45-63.

b) Livros: Autor(es) ou editor(es). Título do livro. Edição, se não for a primeira. Tradutor(es), se for o caso. Local de publicação: editora; ano. Ex.: Diener HC, Wilkinson M, editors. Drug-induced headache. 2nd ed. New York: Spriger-Verlag; 1996.

c) Capítulos de livros: Autor(es) do capítulo. Título do capítulo Editor(es) do livro e demais dados sobre este, conforme o item anterior. Ex.: Chapman MW, Olson SA. Open fractures. In: Rockwood CA, Green DP. Fractures in adults. 4th ed. Philadelphia: Lippincott-Raven; 1996. p. 305-52.

d) Resumos: Autor(es). Título, seguido de [abstract]. Periódico ano; volume (suplemento e seu número, se for o caso): página(s) Ex.: Enzensberger W, Fisher PA. Metronome in Parkinson's disease [abstract]. Lancet. 1996;34:1337.

e) Comunicações pessoais só devem ser mencionadas no texto entre parênteses

f) Tese: Autor, título nível (mestrado, doutorado etc.), cidade: instituição; ano. Ex.: Kaplan SJ. Post-hospital home health care: the elderley's access and utilization [dissertation]. St. Louis: Washington University; 1995.

g) Material eletrônico: Título do documento, endereço na internet, data do acesso. Ex: Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis. [online] 1995 Jan-Mar [cited 1996 Jun 5];1(1):[24 screens]. Available from:URL:http://www.cdc.gov/ncidod/EID/eid.htm

**TABELAS:** As tabelas devem ser numeradas por ordem de aparecimento no texto com números arábicos. Cada tabela deve ter um

título e, se necessário, uma legenda explicativa. Os quadros e tabelas deverão ser enviados através dos arquivos originais (p.e. Excel).

**FIGURAS (fotografias/ilustrações/gráficos):** As figuras devem ser apresentadas em páginas separadas e numeradas sequencialmente, em algarismos arábicos, conforme a ordem de aparecimento no texto. Para evitar problemas que comprometam o padrão da revista, o envio do material deve obedecer aos seguintes parâmetros: todas as figuras, fotografias e ilustrações devem ter qualidade gráfica adequada (300 dpi de resolução) e apresentar título e legenda.Em todos os casos, os arquivos devem ter extensão.tif e/ou jpg. Também são aceitos arquivos com extensão.xls (Excel),.eps, .psd para ilustrações em curva (gráficos, desenhos e esquemas).. As figuras incluem todas as ilustrações, tais como fotografias, desenhos, mapas, gráficos, etc, e devem ser numeradas consecutivamente em algarismos arábicos. Figuras em preto e branco serão reproduzidas gratuitamente, mas o editor reserva o direito de estabelecer o limite razoável.

**LEGENDAS:** Digitar as legendas usando espaço duplo, acompanhando as respectivas figuras (gráficos, fotografias e ilustrações). Cada legenda deve ser numerada em algarismos arábicos, correspondendo a cada figura, e na ordem em que foram citadas no trabalho.

**ABREVIATURAS E SIGLAS:** Devem ser precedidas do nome completo quando citadas pela primeira vez no texto. No rodapé das figuras e tabelas deve ser discriminado o significado das abreviaturas, símbolos, outros sinais e informada fonte: local onde a pesquisa foi realizada. Se as ilustrações já tiverem sido publicadas, deverão vir acompanhadas de autorização por escrito do autor ou editor, constando a fonte de referência onde foi publicada.

**REPRODUÇÃO:** Somente a Journal of Epilepsy and Clinical Neurophysiology poderá autorizar a reprodução dos artigos nelas contidos. Os casos omissos serão resolvidos pela Corpo Editorial.

**SUBMISSÃO DE ARTIGOS:** A partir de janeiro de 2015 os artigos deverão ser enviados para Submissão para a Atha Comunicação e Editora (A/C Ana Carolina de Assis) - Rua Machado Bittencourt, 190 – 4º andar - CEP: 04044-903 – São Paulo/SP, Brasil Tel: +55 11 5087-9502 / Fax: +55 11 5579 5308 ou via email para revistajecn@outlook.com

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Revisión más reciente: Marzo 2015

La Revista Journal of Epilepsy and Clinical Neurophysiology es el Órgano Oficial de la Liga Brasileña de Epilepsia, cuyo propósito es publicar artículos científico-tecnológicos originales sobre epilepsia y neurofisiología clínica, resultante de investigaciones clínicas y experimentales, éticamente desarrolladas y aprobadas. Los volúmenes son publicados anualmente, con ediciones trimestrales en marzo, junio, setiembre y diciembre de cada año. Los artículos sometidos deben ser inéditos y concisos, redactados en inglés, portugués o español. El texto deberá ser preparado de acuerdo con las normas técnicas y enviados por el sistema de gestión de publicaciones. Los artículos, para ser aprobados, son sometidos a la evaluación de una comisión de revisores (peer review) que reciben el texto de forma anónima y deciden por su publicación, sugieren modificaciones, requisitan clarificaciones a los autores y le efectúan recomendaciones al Editor Jefe. Los conceptos y declaraciones contenidas en los trabajos son de total responsabilidad de los autores. La Revista Journal of Epilepsy and Clinical Neurophysiology sigue integralmente la tendencia internacional del estilo Vancouver, disponible en (www.icmje.org.br). Desde ya agradecemos la colaboración de los autores en la atención a las instrucciones citadas.

## FORMATO DE ARTÍCULOS

**LÍMITES POR TIPO DE PUBLICACIÓN (Extensión):** Deben ser observados los criterios abajo delineados para cada tipo de publicación. El conteo electrónico de palabras debe incluir: la página inicial y texto.

| Tipo de Artículo | Resumen | Número de Palabras | Referencias | Figuras | Tablas |
|---|---|---|---|---|---|
| Original | Estructurado con hasta 250 palabras | 6.000 Excluyendo el resumen, referencias, tablas y figuras | 45 | 10 | 6 |
| Actualización / Revisión Relato de Caso | No es estructurado con hasta 250 palabras | 6.000 Excluyendo el resumen, referencias, tablas y figuras | 60 | 3 | 2 |
| Editorial | 0 | 500 | 5 | 0 | 0 |

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**Original Article**/Artigo Original/Artículo Original

# BETA-TUBULIN EXPRESSION IN THE HIPPOCAMPUS OF PATIENTS WITH MESIAL TEMPORAL LOBE EPILEPSY

## *EXPRESSÃO DE BETA-TUBULINA NO HIPOCAMPO DE PACIENTES COM EPILEPSIA DO LOBO TEMPORAL MESIAL*

## *EXPRESIÓN DE BETA-TUBULINA EN EL HIPOCAMPO DE LOS PACIENTES CON EPILEPSIA DEL LÓBULO TEMPORAL MESIAL*

Mariana Raquel Monteiro[1], Ludmyla Kandratavicius[1], Jose Eduardo Peixoto-Santos[1], Renata Caldo Scandiuzzi[1], Carlos Gilberto Carlotti Júnior[2], João Alberto Assirati Júnior[2], João Pereira Leite[1]

## ABSTRACT

Introduction: The neuronal loss and abnormal mossy fibers sprouting are frequently observed in patients with mesial temporal lobe epilepsy (MTLE). Beta-tubulin, a cytoskeleton protein, is critical for the maintenance of the neuritic structure. Objective: Considering the axonal reorganization in patients with MTLE, our objective was to analyze the beta-tubulin expression in the hippocampus of these patients. Methods: We evaluated the hippocampus of 38 MTLE patients and seven control cases. Histological sections were submitted to neo-Timm histochemistry to evaluate the sprouting of mossy fiber, and to immunohistochemistry for neuronal density evaluation (NeuN) and beta-tubulin expression. Results: The MTLE group showed lower neuronal density than the control group in the granular layer (GL), hilus, CA4, CA3, CA1, and presubiculum. The MTLE group showed higher gray value on the neo-Timm staining when compared to the control group in GL, IML, and outer molecular layer (OML), and sprouting of thicker mossy fibers in the IML. When compared to the control group, group MTLE showed higher beta-tubulin expression in GL and lower expression in CA3 region. The aberrant sprouting of mossy fibers correlated inversely with the beta-tubulin expression in several subfields of the hippocampal formation. Conclusions: The differential expression of beta-tubulin in the regions CA3 and GL of the MTLE group, as well as its correlation with neuronal loss and the mossy fiber sprouting, suggests a possible role of this protein in the neuropathological changes that occur in the hippocampus in chronic cases of MTLE.

**Keywords:** Temporal lobe epilepsy; Cytoskeleton; Tubulin; Hippocampus.

## RESUMO

*Introdução: A perda neuronal e o brotamento anormal de fibras musgosas são observados com frequência em pacientes com epilepsia do lobo temporal mesial (ELTM). A beta-tubulina, uma proteína do citoesqueleto, é essencial para a manutenção da estrutura neurítica. Objetivo: Considerando a reorganização axonal nos pacientes com ELTM, nosso objetivo foi analisar a expressão de beta-tubulina no hipocampo desses pacientes. Métodos: Foram avaliados 38 hipocampos de pacientes com ELTM e sete casos controle. Cortes histológicos foram submetidos à histoquímica de neo-Timm para avaliação do neobrotamento de fibras musgosas e à imuno-histoquímica para avaliações da densidade neuronal (NeuN) e da expressão de beta-tubulina. Resultados: O grupo ELTM apresentou menor densidade neuronal do que o grupo controle na camada granular (CG), hilo, CA4, CA3, CA1 e no pré-subículo. O grupo ELTM apresentou maior valor de cinza na coloração neo-Timm com relação ao grupo controle na CG, CMI e camada molecular externa (CME) e neobrotamento mais espesso de fibras musgosas na CMI. O grupo ELTM apresentou maior expressão de beta-tubulina na CG e menor expressão na região de CA3, quando comparado ao grupo controle. O neobrotamento aberrante de fibras musgosas correlacionou-se inversamente com a expressão de beta-tubulina em diversos subcampos da formação hipocampal. Conclusões: A expressão diferencial da beta-tubulina nas regiões da CA3 e CG do grupo ELTM, assim como suas correlações com a perda neuronal e o neobrotamento de fibras musgosas sugerem uma possível participação dessa proteína nas alterações neuropatológicas que ocorrem no hipocampo nos casos crônicos de ELTM.*

***Descritores:*** *Epilepsia do lobo temporal; Citoesqueleto; Tubulina; Hipocampo.*

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## RESUMEN

*Introducción: La pérdida neuronal y la brotación anormal de fibras musgosas se observan con frecuencia en los pacientes con epilepsia del lóbulo temporal mesial (ELTM). La beta-tubulina, una proteína del citoesqueleto, es crítica para el mantenimiento de la estructura neurítica. Objetivo: Teniendo en cuenta la reorganización axonal en pacientes con ELTM, nuestro objetivo fue analizar la expresión de beta-tubulina en el hipocampo de estos pacientes. Métodos: Se evaluó el hipocampo de 38 pacientes con ELTM y siete casos de control. Cortes histológicos fueron sometidos a la histoquímica neo-Timm para evaluar la brotación de fibras musgosas, y a inmunohistoquímica para la evaluación de la densidad neuronal (NeuN) y la expresión de beta-tubulina. Resultados: El grupo ELTM mostró una menor densidad neuronal que el grupo control en la capa granular (CG), hilo, CA4, CA3, CA1 y pré-subículo. El grupo ELTM mostró mayor valor de gris en la tinción neo-Timm en comparación con el grupo control en CG, CMI y en la capa externa molecular (CME), y la brotación de fibras musgosas más gruesas en la CMI. El grupo ELTM mostró una mayor expresión de beta-tubulina en CG y expresión más baja en la región CA3, cuando se compara con el grupo control. La brotación aberrante de fibras musgosa está inversamente correlacionada con la expresión de beta-tubulina en varios subcampos de la formación del hipocampo. Conclusiones: La expresión diferencial de beta-tubulina en las regiones CA3 y CG del grupo ELTM, así como su correlación con la pérdida neuronal y el surgimiento de fibras musgosas, sugiere un posible papel de esta proteína en los cambios neuropatológicos que se producen en el hipocampo en los casos crónicos de ELTM.*


***Descriptores:*** *Epilepsia del lóbulo temporal; Citoesqueleto; Tubulina; Hipocampo.*

## INTRODUCTION

Mesial temporal lobe epilepsy (MTLE) is the most common form of drug-resistant epilepsy in adults. Hippocampal sclerosis is often found in MTLE and is characterized by neuronal loss, gliosis, and mossy fiber sprouting[1-3]. The specific neuronal loss in hilus and CA3 subfields s intrinsically associated with sprouting of the mossy fiber to the molecular layers of *fascia dentata*[4]. This rearrangement in the neuronal circuitry is believed to contribute to hippocampal hyperexcitability[5,6]. Studies in animal models and MTLE patients indicate the participation of the cytoskeleton in mossy fiber sprouting[7-10].

The cytoskeleton is a highly dynamic structure that participates in plasticity process, cellular transport, cell morphology, and in the development and stabilization of axons and dendrites[11,12]. Changes in the cytoskeleton can impair neuronal performance[13]. Microtubules are an important component of the cytoskeleton, participating in chromosome segregation, axonal transport, and neuronal polarity[11,14]. Since beta-tubulin protein is an essential element of the microtubules, our objective was to analyze beta-tubulin expression in MTLE patients.

## MATERIALS AND METHODS

### Patients

We analyzed hippocampi from 38 patients with MTLE, submitted to epilepsy surgery at the Ribeirao Preto Epilepsy Surgery Program. Tissue collection and processing were conducted according to a protocol approved by our institution's Research Ethics Board (process HCRP 093/2008). For comparison, we used seven control hippocampi from necropsy.

Inclusion criteria for MTLE were: (I) seizure semiology consistent with MTLE; (II) pre-surgical investigation confirming the seizure onset zone in the temporal lobe; (III) anterior and mesial temporal interictal spikes on EEG; (IV) no lesions other than uni- or bilateral hippocampal atrophy on high-resolution magnetic resonance imaging scans; (V) clinical histopathological examination compatible with HS; and (VI) no evidence of dual pathology identifiable by any of the assessment methods described (clinical, electrophysiology, neuroimaging, and histopathology). Exclusion criteria were: (I) focal neurological abnormalities on physical examination; (II) generalized or extra-temporal EEG spikes; and (III) marked cognitive impairment indicating dysfunction[15].

For the control group, inclusion criteria were: (I) age at death between 18 and 60 years; (II) *post mortem* time ≤ 10 hours. The exclusion criteria were: (I) History of neurological disease; (II) Brain pathology present at necropsy evaluation; and (III) *post mortem* time > 10 hours.

### Tissue collection and hippocampal tissue processing

The hippocampal tissue was collected at the surgery center or autopsy room and sectioned into 1 cm thick coronal blocks. For the immunohistochemistries, blocks were fixed in formaldehyde, dehydrated, and paraffin-embedded. Blocks for neo-Timm histochemistry were fixed in glutaraldehyde with sodium sulfite, cryoprotected, and frozen.

### Neo-Timm histochemistry and Immunohistochemistry

For neo-Timm staining[1], sections were processed in batches containing at least one HS and two controls cases (for standardization). Slices were immersed in a developer solution (10 mL 50% Arabic gum, 30 mL of citric acid 1.3 M and sodium citrate 0.9 M, 90 mL of hydroquinone 0.5 M, and 1.5 mL of silver nitrate 17%) for 40 min (light staining) or 50 min (dark staining). Sections were washed, dried, dehydrated, xylene-cleared, and mounted in Krystalon (EM Science, Gibbstown, USA).

Immunohistochemistry was performed according to previously published protocols[2], with antibodies against NeuN (Chemicon-Millipore, Billerica, MA, USA) and beta-tubulin protein (Santa Cruz Biotechnology, Santa Cruz, CA, USA), diluted at 1:1000 and 1:25, respectively. After the revelation, sections were dehydrated, cleared with xylene, and mounted with Krystalon.

### Neuron count and neo-Timm quantification

The hippocampal subfields were subdivided according to Lorente de No's classification[16], and included: inner (IML) and outer molecular layer of *fascia dentata* (OML); granular layer of fascia dentate (GL), hilus, CA4, CA3, CA2, CA1, prosubiculum, subiculum, parasubiculum, and layer III of entorhinal cortex. Mossy fiber sprouting was evaluated in neo-Timm

stained sections in the hilus, granular layer, IML, and OML. Neuronal counting was performed in sections submitted to NeuN immunohistochemistry, in GL, hilus, CA4, CA3, CA2, CA1, prosubiculum, subiculum, parasubiculum, and layer III of entorhinal cortex. Neuron density was estimated according to Abercrombie's method[17]. Beta-tubulin expression was evaluated as an immunoreactive area for beta-tubulin antigen, following published protocols[18-20].

All measurements were done in Image J analysis system (NIH, USA, public domain). The statistical analyzes were realized by SigmaPlot (version 11) program. We used test t for comparison between MTLE and control groups. Statistical significance was set at $p < 0.05$.

## RESULTS

### Clinical Findings

The clinical variables of MTLE and control groups are presented in Table 1. The age of the control group was higher than MTLE group ($p = 0.048$, Fisher's exact test). The two groups of patients had no differences in gender or collected side.

### Histological evaluation

MTLE group had reduced neuron density in GL, hilus, CA4, CA3, CA1, and prosubiculum when compared to control ($p \leq 0.009$; Figure 1). There were no differences between control and MTLE in CA2, subiculum, parasubiculum, and entorhinal cortex.

Neo-Timm staining was significantly higher in GL, IML, and OML of MTLE group, compared to controls ($p < 0.001$; Figure 2). There was no difference between MTLE and control groups in the hilus. The length of mossy fiber sprouting in the IML of MTLE was of 222.02 ± 76.58 $\mu$m, whereas control cases had no mossy fiber sprouting in this region (Figure 2 D).

Beta-tubulin expression was seen in cell bodies, axons, and dendrites. MTLE patients had increased immunoreactive area for beta-tubulin in GL and decreased in CA3 when compared to the control group ($p < 0.005$; Figure 3).

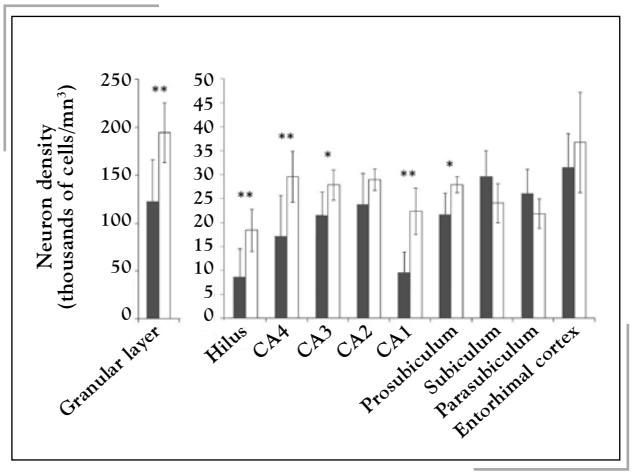


**Figure 1.** Neuron density in human hippocampal formation. The MTLE group (black bars) had lower neuron densities than the control group (white bars) in GL, hilus, CA4, CA3, CA1 e prosubiculum. * $p < 0.05$; ** $p < 0.001$.

**Table 1.** Clinical variables from MTLE and control groups.

| Clinical variable | | MTLE | Control | Statistics |
|---|---|---|---|---|
| Gender (n) | Male | 17 | 4 | No difference |
| | Female | 21 | 3 | |
| IPI (n) | Present | 15 | n.a. | n.a. |
| | Absent | 22 | n.a. | n.a. |
| Age of first seizure (years) | | 6.9 ±7.9 | n.a. | n.a. |
| Age of recurrent seizures (years) | | 11.5 ±7.5 | n.a. | n.a. |
| Type of seizure (n) | CPS | 13 | n.a. | n.a. |
| | CPSG | 25 | n.a. | n.a. |
| Seizure Frequency (Seizures by month) | | 15 ±19.8 | n.a. | n.a. |
| Hand dominance (n) | Right | 35 | n.a. | n.a. |
| | Left | 2 | n.a. | |
| | Bilateral | 1 | n.a. | |
| Verbal memory tasks (n) | Average or above | 9 | n.a. | n.a. |
| | Below average | 23 | n.a. | |
| Non-verbal memory tasks (n) | Average or above | 10 | n.a. | n.a. |
| | Below average | 22 | n.a. | |
| Full-scale IQ | | 84.2 ±11.3 | n.a. | n.a. |
| Years at school | | 5.7 ±4.2 | n.a. | n.a. |
| Age at procedure* (years) | | 38.7 ±7.1 | 48.1 ±18.9 | $p = 0.048$ |
| Epilepsy duration (years) | | 27.5 ±10.1 | n.a. | n.a. |
| Collected side (n) | Right | 19 | 3 | No difference |
| | Left | 19 | 4 | |

Values presented as mean ± standard deviation. IPI: initial precipitant insult; CPS: complex partial seizure; CPSG: complex partial seizure with secondary generalization; HS: hippocampal sclerosis; IQ: intelligence quotient; n = number of cases; n.a.: not applicable; *Age at surgery for MTLE and at death for control cases.

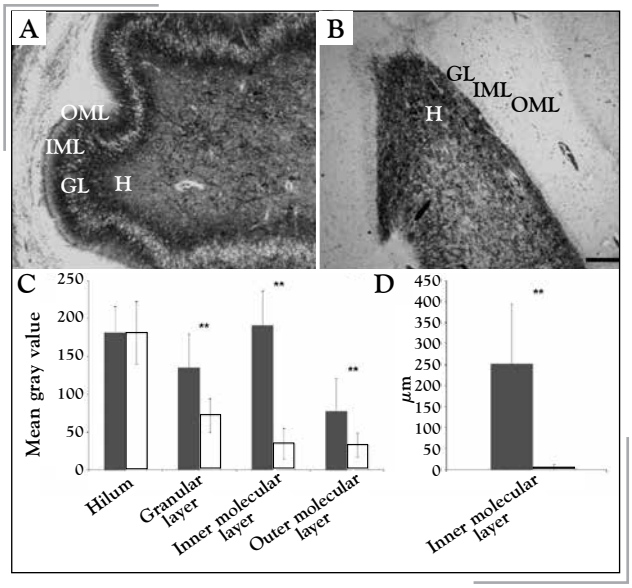


**Figure 2.** Mossy fiber sprouting. Illustrative image of neo-Timm histochemistry in MTLE (A) and control (B) patients GL: granular layer; H: hilus; IML: inner molecular layer and OML: outer molecular layer. C: Gray value graph of MTLE (black bars) and control (white bars) groups. D: Length of mossy fiber sprouting graph in MTLE and control groups. Asterisks: p<0.001. Bar (A-B): 200 $\mu$m.

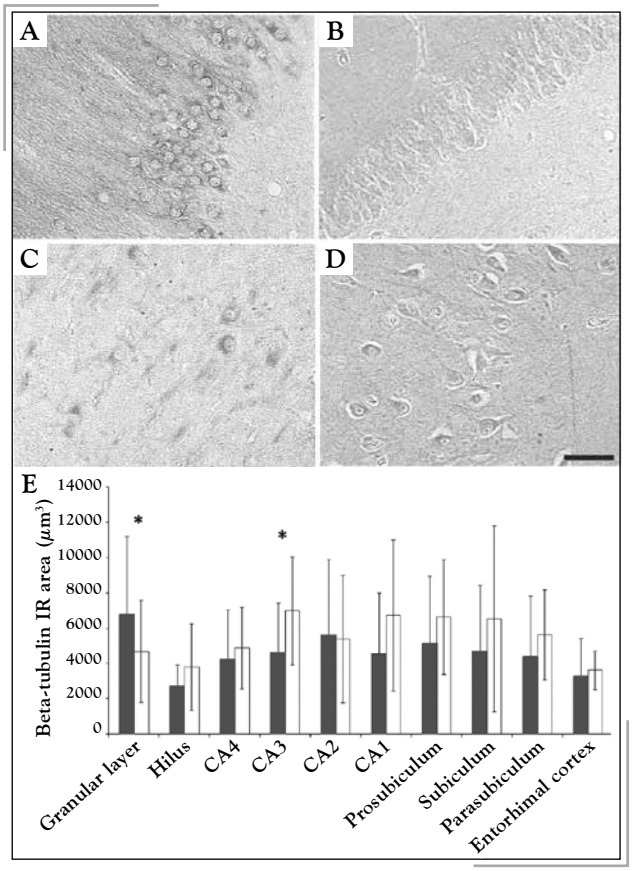


**Figure 3.** Beta-tubulin expression in hippocampal subfields of MTLE and control cases. In granular layer, the MTLE group (A and E) had higher beta-tubulin expression than the control group (B and E). In CA3 the MTLE group (C and E) had less beta-tubulin expression than the control group (D and E). The black bars in E represent MTLE group and white bars represent the control group. * p < 0.05. Bar (A-D) = 50 $\mu$m.

### Correlations

In the MTLE group, we found positive correlations between beta-tubulin expression in the entorhinal cortex and CA1 (p = 0.002; r = 0.71) and subiculum (p = 0.005; r = 0.70). Neuron density and beta-tubulin expression correlated positively in the subiculum (p = 0.03; r = 0.45). Neo-Timm gray values in IML showed a negative correlation with beta-tubulin expression in CA4 (p = 0.04; r = -0.65) and gray values in OML showed negative correlation with beta-tubulin expression in the entorhinal cortex (p = 0.05 e r = -0.76). The expression of beta-tubulin in the hilus correlated negatively with the length of GL (p = 0.03; r = -0.65) and the length of IML (p = 0.0005; r = -0.87). In the control group, we found only a positive correlation between age and beta-tubulin expression in hilus (p = 0.04; r = 0.75).

## DISCUSSION

The MTLE group had lower neuronal density than the control group in GL, hilus, CA4, CA3, CA2, CA1, and the prosubiculum. This pattern of cell loss corresponds to the classical HS (HS type 1 according to the new ILAE classification) described in the literature[21-25]. The CA2 and the subiculum showed preserved neuronal density[26,27]. We found a positive correlation between the neuronal density and beta-tubulin expression in subiculum, suggesting a preservation of both neuron density and cell morphology. The positive correlation between the beta-tubulin expression in CA1 and the entorhinal cortex might indicate the preservation of the alvear pathway, a fiber pathway of entorhinal axons that make synapses with CA1 neurons[28].

Mossy fiber sprouting and reorganization of axon collaterals are important histopathological changes observed in MTLE1[23,29]. After intense neuronal loss in hilus, CA4, and CA3 regions, the mossy fibers reorganize toward the molecular layers of fascia dentata, resulting in new synaptic terminals with dendrites of local interneurons and also granule cells[29]. We found increased neo-Timm staining in IML and OML, indicating mossy fiber sprouting into the molecular layers of fascia dentata. Furthermore, we saw that the lower the beta-tubulin expression in the hilus and CA4, the higher the degree of mossy fiber sprouting in the molecular layer, corroborating the association between neuron loss and axonal reorganization in MTLE.

The decreased beta-tubulin expression in CA3 of MTLE patients might be related to neuronal loss and degenerative changes that occur in this region. Neuropathological studies in patients and animal models of MTLE showed significant neuronal degeneration and decreased dendritic arborization in CA3 subfield[7,30-32]. In the MTLE group, we found increased beta-tubulin expression in GL, even with neuronal loss in this region. The increased beta-tubulin expression in the GL can be related to the mossy fiber sprouting seen in our MTLE patients.

In summary, our data indicate changes in beta-tubulin expression in the hippocampus of MTLE patients. We found correlations between beta-tubulin expression, neuronal loss, and mossy fiber sprouting. Our study suggests that changes in beta-tubulin expression could be an indicative of neuron loss and mossy fiber sprouting.

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**Original Article**/Artigo Original/Artículo Original

# EPILEPSY AND EEG ABNORMALITIES IN CHILDREN WITH AUTISM SPECTRUM DISORDER

## *EPILEPSIA E ANORMALIDADES ELETROENCEFALOGRÁFICAS EM CRIANÇAS COM TRANSTORNO NO ESPECTRO DO AUTISMO*

## *EPILEPSIA Y ANORMALIDADES ELECTROENCEFALOGRÁFICAS EN NIÑOS CON TRASTORNO EN EL ESPECTRO DEL AUTISMO*

Marília Barbosa de Matos[1], Angélica Luciana Nau[2], Gabriela Foresti Fezer[2], Bianca Simone Zeigelboim[3], Paulo Breno Noronha Liberalesso[1,3]

### ABSTRACT

Introduction: Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental disorder characterized by impaired communication and social interaction, and by restricted and repetitive behavior. Children with ASD are more likely to have seizures than children with normal neurological development. Objective: Analyze the incidence of seizures and EEG abnormalities in a cohort of 63 patients with ASD. Methods: Children with autism were included in the study, which calculated the incidence of epilepsy and analyzed the main abnormalities in the EEG. All the patients were evaluated by the same physician, and underwent EEG and MRI of the brain. Results: A total of 63 patients were included between January 2010 and January 2015; 23 (36.51%) female and 40 (63.49%) male; ages at diagnosis ranged from 17 to 58 months (35.97 ± 11.77 months); in 16 (25.4%) patients the MRI was reported to be abnormal. All the patients with autism and epilepsy had abnormal EEGs; 11 (17.4%) had a diagnosis of epilepsy (n=7; 63.6% female and n=4; 36.4% male); and the mean age at diagnosis of epilepsy was 33.7 ± 4.3 months. Conclusion: Our findings suggest that in patients with autism, epilepsy rates are higher than in the general population, but there is no unique pattern of discharge in the EEG.

**Keywords:** Autism spectrum disorder, Epilepsy, Electroencephalogram.

### RESUMO

*Introdução: O transtorno do espectro do autismo (TEA) é um distúrbio de desenvolvimento neural heterogêneo caracterizado por comunicação e interação social deficientes e por comportamento restrito e repetitivo. As crianças com TEA têm maior probabilidade de ter convulsões do que as crianças com desenvolvimento neurológico normal. Objetivo: Analisar a incidência de convulsões e anormalidades eletroencefalográficas em uma coorte de 63 pacientes com TEA. Métodos: Foram incluídas no estudo, crianças com autismo, a incidência de epilepsia foi calculada e as principais anomalias do EEG foram analisadas. Todos os pacientes foram avaliados pelo mesmo médico e foram submetidas a EEG e RM do cérebro. Resultados: De janeiro de 2010 a janeiro de 2015, foram incluídos 63 pacientes, 23 (36,51%) do sexo feminino e 40 (63,49%) do sexo masculino; a idade ao diagnóstico variou de 17 a 58 meses (35,97 ± 11,77 meses); em 16 (25,4%) pacientes o laudo da RM relatou anormalidade. Todos os pacientes com autismo e epilepsia tinham uma anormalidade no EEG, 11 (17,4%) tinham diagnóstico de epilepsia (n = 7; 63,6% meninas e n = 4; 36,4% meninos); a média de idade ao diagnóstico de epilepsia foi 33,7 ± 4,3 meses. Conclusão: Nossos achados sugerem que em pacientes com autismo, as taxas de epilepsia ainda são mais altas do que as da população de risco geral e não existe um padrão único de descarga no EEG.*

***Descritores:*** *Transtorno Autístico; Epilepsia; Eletroencefalograma.*

### RESUMEN

*Introducción: El trastorno del espectro del autismo (TEA) es un disturbio de desarrollo neural heterogéneo caracterizado por comunicación e interacción social deficientes y por comportamiento restringido y repetitivo. Los niños con TEA tienen mayor probabilidad de tener convulsiones que los niños con desarrollo neurológico normal. Objetivo: Analizar la incidencia de convulsiones y anormalidades electroence-*

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*falográficas en una cohorte de 63 pacientes con TEA. Métodos: Fueron incluidos en el estudio niños con autismo, la incidencia de epilepsia fue calculada y las principales anomalías del EEG fueron analizadas. Todos los pacientes fueron evaluados por el mismo médico y fueron sometidos a EEG y RM del cerebro. Resultados: De enero de 2010 a enero de 2015, fueron incluidos 63 pacientes, 23 (36,51%) del sexo femenino y 40 (63,49%) del sexo masculino; la edad en el momento del diagnóstico varió de 17 a 58 meses (35,97 ± 11,77 meses); en 16 (25,4%) pacientes el laudo de la RM relató anormalidad. Todos los pacientes con autismo y epilepsia tenían una anormalidad en el EEG, 11 (17,4%) tenían diagnóstico de epilepsia (n = 7; 63,6% niñas y n = 4; 36,4% niños); el promedio de edad en el momento del diagnóstico de epilepsia fue 33,7 ± 4,3 meses. Conclusión: Nuestros hallazgos sugieren que en pacientes con autismo, las tasas de epilepsia aún son más altas que las de la población de riesgo general y no existe un estándar único de descarga en el EEG.*

***Descriptores:*** *Trastorno Autístico; Epilepsia; Electroencefalograma.*

## INTRODUCTION

Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental disorder characterized by impaired communication and social interaction and by restricted and repetitive behavior[1]. The diagnosis of ASD is based on clinical criteria; however, some neuroimaging and neurophysiological tests are often performed to establish the etiology and severity of the disease[2,3]. There are some neurological conditions related to ASD, and among those, epilepsy is the best-known comorbidity. Epilepsy is reported to occur in 5 to 46% of individuals with ASD, which exceeds the prevalence of epilepsy in general population (0.7-1%)[2,4,5].

The increased prevalence of epilepsy and/or EEG abnormality in patients with ASD reinforce the belief that these patients have some basal neurologic condition, but this association is not completely understood[4,5]. There are no seizure type or eletroencephalography (EEG) patterns specifically associated with ASD. Some studies have been already reported complex partial seizures (with or without secondarily generalized), absence, and generalized tonic-clonic seizures. Recognition of different EEG types or patterns can be very useful as evidence of cortical dysfunction in ASD, and may reveal some sort of brain damage[5]. The aim of our research is to analyze the occurrence of epilepsy and the main EEG abnormalities in children with ASD.

## METHODS

We retrospectively reviewed the medical records of 63 children with ASD and epilepsy, admitted to the Department of Pediatric Neurology of Pequeno Principe Hospital, Curitiba, Brazil, between January 2010 and January 2015, evaluating them for epilepsy diagnosis. In order to be included in study the participants had to have a diagnosis of ASD based on DSM-V[3] clinical criteria established by and expert clinical evaluation. For the purpose of this study, the definition of epilepsy adopted was the occurrence of at least one epileptic seizure and a brain disorder characterized by persistent predisposition of the brain to generate seizures and by neurobiological, cognitive, psychological and social consequences of this condition[6].

All patients were evaluated by the same physician, and performed EEG MRI of the brain. The EEG tests were performed in digital equipment Neuropmap EEG-40i, Neurofax Nihon Kohden EEG-1200 or EEG Brain Wave II, lasting as a minimum 30 minutes. The electrodes were placed according to the International 10-20 System of Electrode Placement (this international system is based on the relationship between the location of an electrode on the scalp and the underlying area of cerebral cortex).

Clinical variables included gender, age of autism diagnosis, age of epilepsy onset, maternal age at pregnancy, perinatal factors (such asphyxia), family history of neurological disease, EEG pattern, MRI findings, and pharmacological treatment. The systematic analysis of EEG abnormalities considered: (a) focal pattern - up to three independent and well-delineated epileptogenic focus; (b) multifocal pattern - more than three independent epileptogenic focus; and (c) generalized pattern - synchronous discharges in large areas of two hemispheres brain.

Data were analyzed using descriptive statistics. The local Ethics Committee on Research Involving Human Subjects (number registration - 44905015.0.0000.0097) approved all aspects of this research.

## RESULTS

### Patients characteristics and neuroimaging

From January 2010 to January 2015, 63 patients with ASD were included in the study, 23 (36.51%) female and 40 (63.49%) male. The age at diagnosis ranged from 17 to 58 months (35.97 ± 11.77 months). Maternal age at pregnancy ranged from 21.4 to 58 years (30.03 ± 4.27 years). Prolonged labor and perinatal asphyxia occurred in 5 (7.94%) and premature birth in 6 (9.52%) patients. Family history of neurological disease was reported in 10 (15.87%) patients - 4 siblings with delayed speech development, 2 siblings with neuropsychomotor development delay, 1 sibling with autism, 1 cousin with autism, 1 cousin with delayed speech development and 1 cousin with Down syndrome (DS). All patients included in the study underwent MRI examination of the brain. In sixteen (25.4%) patients the MRI was reported to be abnormal. All patients with ASD and epilepsy had an abnormality of EEG.

### Prevalence and epilepsy classification

Of 63 individuals,[7] (17.4%) had epilepsy diagnosis (n=7; 63.6% female and n=4; 36.4% male). The mean age at diagnosis of epilepsy was 33.7 ± 4.3 months. We obtained EEG reports for all the 63 patients, as part of their clinical follow-up. The EEG information for 52 (82.54%) participants without clinical seizures did not report epileptiform discharge, but in 50% of these patients showed a mild to moderatly disorganized background activity. For the eleven participants with clinical epilepsy, EEG data supported the diagnosis and was useful in the classification

of seizure type: 1 patient (9.1%) had spike and polispike generalized discharges; 1 patient (9.1%) had sharp wave discharges in the left middle temporal lobe; 3 patients (27.3%) had multifocal sharp waves; 1 patient (9.1%) had sharp wave discharges in the left anterior and middle temporal lobe; 1 patient (9.1%) had spike-wave generalized discharges with irregular morphology and multifocal sharp waves; 1 patient (9.1%) had sharp wave discharges in the left frontal lobe and left anterior temporal lobe; 1 patient (9.1%) had sharp wave discharges in the right frontal lobe and right anterior temporal lobe. In the other two patients the EEG was reported only with mild to moderatly disorganized background activity (no epileptiform discharges).

### Clinical treatment

Antiepileptic drugs and/or antipsychotic drugs, such as sodium valproate, lamotrigine, pericyazine, risperidone, clonazepam and clobazam were being prescribed for eighteen patients (28.57%). The majority of them (55.5%; n=10) were receiving only one drug, while 22.2% (n=4) of the patients were under a treatment with two drugs and the other 22.2% (n=4) were using three drugs.

## DISCUSSION

Epilepsy is considered a well-known significant comorbidity associated with ASD, and it causes important disability to individuals.

The mean age of epilepsy diagnosis in this study was 33.7 ± 4.3 months. Epilepsy in ASD has two peaks of presentation, one in early childhood and the other in adolescence, which is believed to be more common[5,8]. In a retrospective British study, 150 individuals with autism were evaluated for onset of epilepsy, and revealed that in the majority of cases, seizures first began after the age of 10 years, consistently with the prevalence founded in other studies, which is 22% to 38% in adolescents and young adults with ASD[4]. The prevalence of epilepsy in individuals with ASD founded in our sample was 17.4%, consistently with previous data, although it is been reported a wide range (5-46%) in epilepsy frequency in those patients[9,10]. This variability has been credited to the heterogeneity of samples with respect to age, sex, comorbidity, intellectual disability, ASD fenotypes and epilepsy diagnose criteria[7]. The founded epilepsy prevalence associated to autism in our sample was not as high as others studies, probably because we included only younger children. It can be hypothesized that after a few years, at adolescence, some of those patients without seizures present with onset of epilepsy, increasing those numbers.

Regarding the gender, the autism sample was formed mainly for males, thus, the association with epilepsy was more prevalente in female (63.6%). It is believed that female gender is more associated with epilepsy[4,11]. A meta-analysis found the male to female ratio in autism with epilepsy was close to 2:1 vs. 3.5:1 in autism without epilepsy[7]. Unfortunately, further information is necessary to determine the risk for epilepsy as a function of gender.

Among the eleven patients with the association between autism and epilepsy, all were associated to neurologic conditions or MRI abnormalities. Some well-known neurological comorbidities associated to ASD, as tuberous sclerosis, fragile X syndrome and DS, have a high rate of epilepsy themselves, and might be the underlying cause to the increased prevalence in non-idiopathic groups of ASD[7,12,13]. The emerging literature of structural and functional neuroimaging in autism may reveals some underlying central nervous system (CNS) abnormality(5). In tuberous sclerosis, for example, the change that affects the frontal or mesiotemporal structure (limbic system) can be the origin of an autistic phenotype[7,9]. Even though, idiopathic groups of ASD still show a considerable increase of epilepsy rate above general population (1%)[9]. That suggests a possible common pathophysiological alterations between these two conditions[14]. An altered balance between excitatory and inhibitory synapses, that contributes to epileptogenic discharges, could affect learning and social behavior[15].

The clinical diagnosis of seizure in autistic individuals can be challenging because behavior abnormalities can be attributed to either complex partial seizure and/or to the clinical characteristics of autism itself [5]. No specific seizure type were associated with autism, but there are some reports with higher prevalence of abnormality in temporal region[8,9], which is consistent with our findings (36.6% - 4/11 children). Although, many reports of background or interictal EEG changes in individuals with autism without seizures, has not being considered evidence of epilepsy, but a sign of cerebral dysfunction, which may leads to behavioral, communicative and cognitive deficits[5]. Recently, high rates of epileptiform EEG in children with autism without a history of seizures or epilepsy had been reported[9,16]. In our study, 50% of the children without clinical seizures had EEG abnormalities at the background activity, but none of them presented with epileptiform discharge.

EEG has an important role at investigation of individuals with ASD and clinical seizures, although there is not a consensus if it should be performed in all children with autism[17]. Occasionally founded discharged usually is treated only in specific cases, such as Landau-Kleffner syndrome and infantile spasm[16,18]. Experimental studies suggest that in the immature brain, interictal spikes may result in alterations in neuronal network function, impaired short and longterm potentiation, and possibly decreased neurogenesis or cell loss of specific populations[19]. Although this hypothesis is not already well established, if further investigations reveal the real causal relationship between epilepsy and autism, a substantial percentage of patients could conceivably benefit from treatment, not only because of seizures control, but also by improving behavioral, language, or cognitive disturbance[5,18].

The main limitation of our research was the small number of patients studied. However, we believe that our data can contribute to better understanding of the relationship between ASD and epilepsy.

Our findings suggest that in autism samples, epilepsy rates are still higher than the general population risk epilepsy, and there is not a unique pattern of discharge at the EEG. The ASD investigation should include an EEG as instrument for epilepsy diagnosis, especially in children with neurological associated disorders. A large systematic studies and appropriate longitudinal follow-up may better shed light on clinical aspects of the relationship between ASD and epilepsy, and could have a significant impact on outcomes for patients and their families.

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JECN Journal of Epilepsy and Clinical Neurophysiology

**Review Article**/Artigo de Revisão/Artículo de Revisión

# INFLAMMATORY REACTION IN EPILEPSY

## *REAÇÃO INFLAMATÓRIA NA EPILEPSIA*

## *REACCIÓN INFLAMATORIA EN LA EPILEPSIA*

José Eduardo Peixoto-Santos[1], Ana Paula Pinheiro Martins[1], Ludmyla Kandratavicius[1], Tonicarlo R Velasco[1], João Pereira Leite[1]

### ABSTRACT

Epilepsies are the second most common neurological disease. The pathological mechanisms of this disease are not fully understood. Several studies claim that inflammation plays a significant role both in structural and physiological changes that lead to the emergence of seizures. Although in some epilepsies, such as Rasmussen's encephalitis, the inflammation has definite importance, in several other epileptic syndromes, the participation of inflammatory reaction still lacks evidence. In such cases, the experimental models are useful for reveal how cytokines, molecules that modulate the inflammatory response, may affect seizures and how seizures may change the expression of these inflammatory molecules. Even with these works, much remains to be clarified with regard to the influence of inflammation on epileptic syndromes. The purpose of this brief review is to discuss the links between inflammatory processes, the origin of crises, and tissue damages in epilepsy.

**Keywords:** Epilepsy; Inflammation; Models, animal; Rasmussen Syndrome; Interleukins.

### RESUMO

*As epilepsias são a segunda doença neurológica mais frequentes. Os mecanismos patológicos dessa doença ainda não são completamente compreendidos. Vários trabalhos alegam que a inflamação tem um papel importante tanto nas alterações estruturais quanto fisiológicas que levam à geração de crises. Embora em alguns tipos de epilepsia, como a encefalite de Rasmussen, a inflamação tenha importância evidente, em várias outras síndromes epilépticas ainda faltam evidências para confirmar a participação da reação inflamatória. Nesses casos, os modelos experimentais são úteis para revelar como as citocinas, moléculas que modulam a resposta inflamatória, podem afetar as crises e como as crises podem alterar a expressão dessas moléculas inflamatórias. Mesmo com esses trabalhos, muito ainda precisa ser esclarecido com relação à influência da inflamação sobre as síndromes epilépticas. O objetivo desta breve revisão foi discutir as ligações entre os processos inflamatórios, a origem das crises e os danos teciduais na epilepsia.*

***Descritores:*** *Epilepsia; Inflamação; Modelos animais; Encefalite; Interleucinas.*

### RESUMEN

*Las epilepsias son la segunda enfermedad neurológica más común. Los mecanismos patológicos de esta enfermedad no se entienden completamente. Varios estudios afirman que la inflamación juega un papel importante tanto en los cambios estructurales como en los fisiológicos que conducen a la generación de las convulsiones. Aunque en algunos tipos de epilepsia, tales como la encefalitis de Rasmussen, la inflamación tiene una importancia evidente, en varios otros síndromes epilépticos todavía carecen de pruebas para confirmar la participación de la reacción inflamatoria. En estos casos, los modelos experimentales son útiles para revelar cómo las citoquinas, moléculas que modulan la respuesta inflamatoria, pueden afectar a las convulsiones y cómo las convulsiones pueden cambiar la expresión de estas moléculas inflamatorias. Incluso con estos trabajos, queda mucho por aclarar con respecto a la influencia de la inflamación en los síndromes epilépticos. El propósito de esta breve revisión es discutir los vínculos entre los procesos inflamatorios, el origen de la crisis y el daño tisular en la epilepsia.*

***Descriptores:*** *Epilepsia, Inflamación, Modelos animales, Encefalitis, Interleucinas.*

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## INTRODUCTION

Epilepsy is characterized by an enduring cerebral predisposition to seizure generation, which leads neurobiological, cognitive, psychological, and social consequences[1]. World prevalence varies between 1% and 3%, depending on the region[2]. Epilepsy etiologies can be idiopathic, symptomatic or cryptogenic[3,4].

Several pathological changes occur in the brain of epileptic patients Amongst the changes seen are reduced GABAergic neurotransmission, changes in NMDA and AMPA receptors, ionic unbalance and changes in $Ca^{2+}$-dependent intracellular signal cascades, synaptic reorganization, selective neuron death, and astrogliosis[5]. According to some studies, inflammatory reaction is an important factor for epileptogenesis and seizure generation. In this review, we aim to report the inflammatory reaction in the central nervous system, as well as human and animal model data regarding the role of several inflammatory changes in epileptogenesis, epilepsy, and seizure generation.

### Inflammation in the central nervous system

The inflammatory reaction is a characteristic response of vascularized tissues to injuries, aiming to isolate and eliminate the aggressor agent, and also to remodel the insulted tissue. An excessive reaction, however, could lead to pathophysiological changes in the tissue.

Brain inflammation has particularities, and experimental models are useful to investigate the course of inflammatory changes in the brain. Studies with experimental models have clarified the importance of T-cells in tissue protection, the contribution of adhesion molecules, cytokines, and enzymes for the recruitment of macrophages, as well as the modulatory action of glial cells on the inflammatory process[6]. However, several questions on brain inflammatory reaction remain unsolved. For instance, the mechanisms responsible for different patterns of inflammatory response in brain diseases such as multiple sclerosis and viral encephalitis[6]. Besides, only recently a study described the existence of functional lymphatic vessels in the brain [7]. Such unexpected finding indicates a probable doorway for the entrance of immune cells in the central nervous system[7].

### Inflammatory changes in epilepsy

In the last decades, the role of inflammation in the physiopathology of human epilepsy has gained attention. Several inflammatory molecules were observed in the brain after epileptic seizures[8,9]. Some syndromes, such as Rasmussen's encephalitis and West syndrome, present an important therapeutic response to steroidal anti-inflammatory drugs. In TLE, experimental models and human studies have described chronic inflammation with microgliosis, astrogliosis, and the expression of several inflammatory molecules in the epileptic focus [8,10-12]. The expression of IL-1β, NFκB and COX-2 after pilocarpine-induced status epilepticus is associated with neuron death and astroglial activation[13] Recent studies from our group have shown increased neuroinflammatory-related molecules in TLE patients[11, 12].

### Rasmussen's encephalitis

Rasmussen's encephalitis is a rare epileptic syndrome, characterized by brain inflammation that evolves to atrophy of the affected hemisphere, progressive hemiparesis, cognitive impairment, and continuous epileptic seizures[14]. It affects children in the first ten years of life and seldom starts in the adult period[15]. The main treatment for Rasmussen's encephalitis is hemispherectomy.

Histological evaluation shows chronic cortical inflammation, infiltration of T-cells, neuron loss, microglial nodules, reactive astroglia, and, in some cases, evidences of neuronophagia[16,17]. The specific etiology, however, remains unknown[17] There is evidence pointing out that a viral infection could be the etiology of the autoimmune reaction and chronic inflammation characteristics of Rasmussen's[14]. However, some ultrastructural studies have not found evidence of viral infection, autoimmune reaction or disruption of the blood-brain barrier[16].

Some studies have offered an alternative hypothesis for the development of Rasmussen's encephalitis. One study has proposed that anti-GluR3 autoantibodies would be generated during the humoral response to a pathogen[18]. In an unrelated event or brain injury, opening of the blood-brain barrier would expose GluR3 do autoimmune attack, triggering the autoimmune reaction and Rasmussen's onset[18]. This hypothesis was based on the reduction of seizure and cognitive improvement of children with Rasmussen and serum anti-GluR3 antibodies after plasmapheresis[18]. However, not all Rasmussen cases present with anti-GluR3 antibodies[14]. Another hypothesis proposes that granzyme B release from T-cells would promote the tissue damage[17,19]. With damage, GluR3 antigens would be released, leading to autoantibody production, which, in its turn, would promote seizures by binding glutamate receptors[14]. The presence of large groups of T cells in patients is the primary support for this model[17, 19].

Clinical characteristics of Rasmussen's differs in manifestation, pathological findings, and progression[14]. As an example, two cases with clinical and histological Rasmussen's characteristics, including hemiparesis, had no seizures[20]. It is possible that a careful evaluation and the definition of pathological degrees could improve the prognosis and provide new treatment strategies[21].

### Experimental models

Experimental models are extremely useful to evaluate the influence of inflammatory molecules in brain diseases. If maintained for a long time, inflammation can increase the tissue damage, instead of promoting healing and protection. In epilepsy, the effect of inflammatory molecules depends on the type of molecules, on the number of receptors, and on the duration of the exposure[8,22]. The impacts of inflammatory molecules also depend on the animal model, and external factors not directly related to the type of insult[8,22].

### Influence of inflammatory molecules on epilepsy models

The influence of inflammatory molecules in neuron death and seizure generation was evaluated with knockout mice, overexpression models, and by inhibitory chemical manipulations. Interleukin-1β receptor type I (IL-1R type I) deficient mice have a delayed onset of bicuculline-induced seizures when compared to wild-type mice[23]. Mice overexpressing IL-1β receptor antagonist (IL-Ra) have a lower number and duration

of seizures[23]. Furthermore, knockout mice for caspase-1, an enzyme needed for IL-1β activation, have delayed seizure onset and reduced number and duration of seizures after treatment with kainic acid (KA)[24]. Inhibition of caspase-1 in Sprague-Dawley rats delays seizure onset, and reduces the number and duration of KA-induced seizures, similar to data from knockout mice[24]. In hippocampal slices, inhibition of caspase-1 promotes a lower production of IL-1β after exposure to lipopolysaccharide (LPS)[24]. IL-1β also seems to me crucial to febrile seizures[8]. Sprague-Dawley rats injected with a combination of IL-1β and AMPA have increased seizure activity than rats treated with AMPA alone[25]. Wistar rats subjected to audiogenic amygdala kindling treated with IL-1β, on the other hand, have reduced after discharges, lower seizure severity and increased threshold to full kindling[26]. In summary, most studies indicate a proconvulsant effect of IL-1β.

Interleukin-6 (IL-6) seems to have both pro and anticonvulsant effects. For instance, knockout mice for IL-6 have a higher susceptibility to audiogenic-induced seizures, but not to maximal electroshock[27]. Reduced levels of GABA, glycine, glutamate, and glutamine were observed in these knockout mice, as well as increased aspartate levels. IL-6 knockout mice also have a higher susceptibility to KA-induced seizures, increased neuron loss, lower levels of metallothioneins I/II, higher levels of nitric oxide synthase and decreased astrogliosis[28]. Intranasal application of IL-6 increases mortality and severity of pentylenetetrazole (PTZ) induced seizures[29]. The neuromodulatory effect of IL-6 might be related to the influence of this molecule on the expression of metallothioneins, on the expression and function of adenosine receptors type A1, and over GABAergic neurotransmission[28-30].

Mutation in the High-mobility group box 1 protein (HMGB1) binding site in Toll-like receptor 4 (TLR4), a receptor important in pathogen recognition, increases latency to KA-induced seizures, whereas the binding of HMGB1 to wild-type receptors increases seizure frequency and duration[31]. Antagonists of TLR4 increase seizure latency and reduce duration and frequency of KA-induced and bicuculline-induced seizures[31]. The proconvulsant effect of HMG1 in TLR4 seems dependent of GluN2B-containing NMDA receptors since the block of this receptor subtype undo the effects of HMGB1 on KA-induced seizures[31].

### Influence of seizures on inflammatory molecules expression

After seizures, several molecules are up or down regulated[32]. Inflammatory mediators and proteins that control reactive oxygen species are increased to minimize tissue damage. Several studies have evaluated the expression of inflammatory molecules after seizures, to establish correlations between seizures and epilepsy.

Wistar rats submitted to PTZ induced seizures have higher levels of IL-1, IL-6, superoxide dismutase and catalase when compared to controls[33]. TLR4 is expressed in astrocytes and neurons in animals submitted to KA or bicuculline-induced status epilepticus. TLR4 is also expressed in hippocampi of TLE patients[31].

KA-induced status epilepticus is associated with increased levels of IL-1β, tumor necrosis factor alpha (TNF-α), IL-6, leukemia inhibitory factor (LIF) and the signal transducer glycoprotein 130 (Gp130)[34]. Studies with Sprague-Dawley rats also found increased IL-1β, TNF-α, IL-6, IL-Ra, as well as astrogliosis, after KA-induced status[35,36].

Some studies have indicated a significant crosstalk between seizures, oxidative stress and inflammation in epilepsy models. A study by inhibition of lipid peroxidation found reduced levels of IL-1β, reduced edema and increased latency to seizures[37]. Another study showed that transgenic mice overexpressing metallothioneins I/II have lower microglial activation, lower neuron loss, lower levels of IL-1, IL-6, IL-12, and TNF-α, and higher levels of IL-10, basic fibroblast growth factor (bFGF), transforming growth factor beta (TGF-β), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and glial cell-derived neurotrophic factor (GDNF)[38].

Rats treated with the nerve agent soman, a cholinesterase enzyme inhibitor, present higher levels of oxidative stress, increased neuron death, and higher IL-1β [39,40]. The antidote for soman significantly reduces IL-1β and reduces seizure-induced damage [39, 40]. Another cholinesterase inhibitor, sarin promotes increase in the expression of IL-1β, IL-6, TNF-α, and prostaglandin E2 (PGE2)[41]. Another study with sarin found that IL-1 was increased in seizing rats, but not in non-seizing rats[42].

## CONCLUDING REMARKS

Several questions arise from the studies linking epilepsy and inflammation. Not all human epilepsies have a close relation to inflammation, comparing with Rasmussen's encephalitis. Besides, more clinical studies are needed to clarify the importance of inflammatory disease in epilepsies.

One problem to be defined is the pro- and anti-inflammatory actions of several cytokines. For instance, TNF-α is both pro and anti-inflammatory, depending on the concentration and kind of receptor in which it binds[8]. The same occurs with IL-6[27,29]. Thus, a generalization is not possible. Transgenic/knockout mice often present with changes in several pathways not directly linked to the protein of interest, making it difficult to separate the effect of a single protein.

Although not all epilepsies have high inflammatory changes, the presence of gliosis and some inflammatory molecules in the epileptic focus point out that the inflammation can be important in several epilepsies.

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**Review Article**/Artigo de Revisão/Artículo de Revisión

# CLOSED-LOOP OPTOGENETIC STRATEGY IN EXPERIMENTAL EPILEPSY: HOW AFFORDABLE IS THE IMPLEMENTATION OF THIS EMERGENT TECHNIQUE?

## *ESTRATÉGIA OPTOGENÉTICA DE ALÇA FECHADA NA EPILEPSIA EXPERIMENTAL: QUÃO ACESSÍVEL É A IMPLEMENTAÇÃO DESTA TÉCNICA EMERGENTE?*

## *ESTRATEGIA OPTOGENÉTICA DE BUCLE CERRADO EN LA EPILEPSIA EXPERIMENTAL: ¿CUÁN ASEQUIBLE ES LA APLICACIÓN DE ESTA TÉCNICA EMERGENTE?*

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## ABSTRACT

To explore complex mechanisms in the brain is an expensive task, which requires a combination of technological development and theoretical advances in neurobiology. In fact, it still is extremely challenging to diagnose accurately and treat some neurological diseases like drug-resistant epilepsy. In some cases, pharmacological interventions, electrical stimulation and surgery in epilepsy can be the specific cause of cognitive impairments and/or psychiatric comorbidities. Therefore, developing more selective strategies to control events produced by abnormal brain activity is mandatory. Our objective was to synthesize and organize information from the literature about the fundamental concepts that support the combination of optogenetics and closed-loop strategies in experimental epilepsy. We also sought to discuss how affordable would be the implementation of these emergent techniques. For this purpose, we first reviewed the literature on the closed-loop optogenetics and its applications for experimental epilepsy. Then, in order to evaluate the feasibility of this approach, we organized the information available in the literature on the materials necessary, and their respective costs. The combination of real-time detection and optogenetics has enormous potential to produce breakthroughs in neuroscience and its use for seizure control will certainly open new possibilities for more effective treatments of epilepsy. Overall, the costs of implementing a robust system with a high temporal precision and accuracy for detection and interference in seizures are relatively small. In addition, costs can be even lower if researchers choose open source hardware tools and software. Therefore, implementation of optogenetics with strategies of closed-loop in experimental epilepsy seems to demand more joint interdisciplinary efforts and innovative scientific questions than financial resources.

**Keywords:** Neurobiology; Epilepsy; Optogenetics; Neurosciences.

## RESUMO

*Investigar mecanismos complexos no cérebro é uma tarefa dispendiosa, que requer a combinação de desenvolvimento tecnológico e avanços teóricos em neurobiologia. De fato, realizar diagnósticos e tratar apropriadamente desordens neurológicas, como epilepsia resistente ao tratamento farmacológico, ainda é um grande desafio. Em alguns casos, as intervenções farmacológicas, a estimulação elétrica e a cirúrgica em epilepsia podem ser as próprias causadoras de prejuízos cognitivos e/ou comorbidades psiquiátricas. Portanto, é mandatório o desenvolvimento de estratégias mais seletivas para controlar eventos gerados por atividade anormal do encéfalo. Nosso objetivo foi sintetizar e organizar informações da literatura sobre os conceitos fundamentais que dão suporte à combinação de optogenética e estratégias de alça fechada em epilepsia experimental. Além disso, objetivamos discutir o quão financeiramente acessível seria a implementação dessas novas técnicas. Para isso, primeiramente revisamos a literatura sobre optogenética e estratégias de alça fechada e suas aplicações para epilepsia experimental. Em seguida, com o objetivo de avaliar quão acessível seria essa abordagem, organizamos a informação disponível na literatura sobre os materiais necessários e seus respectivos custos. A combinação de detecção em tempo real e optogenética tem um potencial enorme para produzir avanços*

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*em neurociências e seu uso para o controle de crises certamente abrirá novas possibilidades para tratamentos mais eficientes da epilepsia. Em geral, os custos para a implementação de um sistema robusto, com alta precisão temporal e acurácia para detecção e interferência em crises são relativamente pequenos. Além disso, eles podem ser ainda menores se os pesquisadores optarem por ferramentas de hardware e software de fonte aberta. Portanto, a implementação da optogenética com estratégia de alça fechada em epilepsia experimental parece demandar mais esforços interdisciplinares conjuntos e perguntas científicas inovadoras do que recursos financeiros.*

***Descritores:*** *Neurobiologia; Epilepsia; Optogenética; Neurociências.*

## RESUMEN

*Investigar los mecanismos complejos en el cerebro es una tarea costosa, que requiere una combinación de desarrollo tecnológico y los avances teóricos en la neurobiología. De hecho, todavía es um gran desafio diagnosticar con precisión y tratar apropriadamente trastornos neurológicos como la epilepsia resistente al tratamiento farmacológico. En algunos casos, las intervenciones farmacológicas, la estimulación eléctrica y la cirugía pueden ser por sí mismas la causa de los deterioros cognitivos y/o comorbilidades psiquiátricas. Por esta razon, es obligatorio el desarrollo de estrategias más selectivas para controlar los eventos producidos por la actividad cerebral anormal. Nuestro objetivo fue sintetizar y organizar la información de la literatura acerca de los conceptos fundamentales que soportan la combinación de la optogenética y estrategias de bucle cerrado en la epilepsia experimental. Además, tratamos de discutir cuán asequible sería la implementación de estas nuevas técnicas. Para ello, primero hemos revisado la literatura sobre la optogenética y las estrategias de bucle cerrado y sus aplicaciones en la epilepsia experimental. Luego, con el fin de evaluar cómo sería este enfoque económico, organizamos la información disponible en la literatura sobre los materiales requeridos y sus costos. La combinación de la detección en tiempo real y la optogenética tiene un enorme potencial para producir avances en la neurociencia y su uso para control de las crisis epilépticas sin duda abrirá nuevas posiblidades para tratamientos más eficaces de la epilepsia. Generalmente, los costos de implementación de un sistema robusto con una alta precisión temporal y la exactitud de detección y de interfencia en las convulsiones son relativamente pequeños. Además, los costos pueden ser incluso más bajos si los pesquisadores eligierenherramientas de hardware y software de código abierto y libre acceso. Por lo tanto, la aplicación de la optogenética con la estrategia de bucle cerrado en la epilepsia experimental parece exigir más esfuerzos interdisciplinarios conjuntos y preguntas científicas innovadoras que recursos financieros.*

***Descriptores:*** *Neurobiología; Epilepsia; Optogenética; Neurociencias.*

## INTRODUCTION

Every cubic millimeter of our brain has hundreds of millions of neurons connected by trillions of synapses that work with the temporal precision of milliseconds. Of course, such complexity conveys to scientists an atmosphere of high motivation to unveil, at least partially, this intriguing and mysterious universe. On the other hand, this journey is quite demanding and requires a combination of technological development and theoretical advances in neurobiology. In the last 15 years, relevant tools were developed to investigate structural, molecular and functional aspects of intact circuits in the brain[1-5]. Despite this, to accurately diagnose and treat some neurological diseases, like drug-resistant epilepsy, is still extremely challenging. In fact, there is an enormous concern on how to overcome the lack of temporal and cell-type specificity of the current therapies in epilepsy. Pharmacological treatments, electrical deep brain stimulations or, in some patients with difficult-to-control seizures, surgical interventions can be causes of cognitive deficits and/or psychiatric comorbidities[6]. In this context, is not surprising that the epilepsy researchers have confirmed such enthusiasm regarding the development of more specific techniques for interventions in the central nervous system, such as Optogenetics[7]. Additionally, researchers have been implementing closed-loop strategies to perform electrical deep brain stimulation, transcranial magnetic stimulation or optogenetics, conditioned to the detection of a seizure or an abnormal oscillatory pattern. Closed-loop optogenetic strategy in experimental models of epilepsy has recently been validated, suggesting exciting future therapeutic avenues. In the present review, we sought to synthesize and organize information on the fundamental concepts that support the use of this strategy in experimental epilepsy and, discuss how affordable the implementation of this emergent technique would be.

### Optogenetics

Optogenetics is defined by the integrated use of optics and genetics to control well-defined events within specified cells of living tissue[8]. This technique is based on the use of light-sensitive proteins called opsins that comprise inhibitory channels and pumps, excitatory channels and coupled receptors called G-protein. They can be expressed in selected cell types of selected cell areas, enabling the temporally precise control of genetically defined neuronal populations[8]. The excitatory channel opsins depolarize the cell, allowing cations to pass into the cell and generating an action potential when activated by light. For example, one of the most known opsins is the light-gated proton channel, channelrhodopsin-2 (ChR2). ChR2 opens upon blue light (473 nm) stimulation to produce a large permeability for monovalent and divalent cations[9]. On the other hand, the inhibitory opsins can hyperpolarize the cell. For instance, when exposed to yellow light (570 nm), halorhodopsin (HR)[10] pumps chloride ion into the cell, causing inhibition of neuronal activity. Another example is the Archaerhodopsin (Arch)[11], which can hyperpolarize the cell by pumping out protons (H+) upon green light stimulation (532 nm). Since the characterization of the expression and functionality of ChR2, HR, and variants, an impressive number of new opsins have been engineered. This effort was primarily focused on the improvement of its expression, temporal precision and phototransduction efficiency[12]. Besides, technology for light delivery in rodents in vivo alongside electrophysiological recordings has become very sophisticated and diverse. Therefore, optogenetics is now useful for the establishment of causal relationships between the activity of specific subpopulations of brain cells and mammalian behavior[13-15].

## Optogenetic control of seizures

Once it is possible to manipulate specific populations of neurons, it becomes easier to inquiry the key networks and mechanisms involved in initiating, sustaining, propagating and terminating seizures[16,17]. Optogenetics can be used in epilepsy to activate or inhibit specific neuronal populations in a brain circuit of interest, allowing normalization of its excitatory/inhibitory balance[17]. For instance, artificially-induced epileptiform activity in the hippocampus *in vitro* can be strongly attenuated by optogenetic inhibition (yellow light activation of HR) of its excitatory and granule cells[16]. More recently, Sukhotinsky et al.[18] have demonstrated that optogenetic inhibition of hippocampal pyramidal cells is sufficient to delay electrographic and behavioral initiation of status epilepticus in the lithium-pilocarpine model of acute elicited seizures. Interestingly, optogenetic activation of interneurons has also been considered an attractive possibility of halting seizures[19]. For instance, Krook-Magnuson et al.[19] have reported that activation of parvalbumin positive neurons (PV+, a class of inhibitory interneuron) did interrupt seizures upon light application in a mouse model of temporal lobe epilepsy. Consistently, Ledri et al.[20] have reported a successful suppression of ongoing epileptiform activity in the hippocampus in vitro by a massive light-induced release of GABA from ChR2-expressing interneurons. Those are only a few examples of the enormous importance of the use of optogenetics in the experimental epilepsy research. However, determining when the interventions should start (e.g. a couple of minutes before, right before, during, just after the seizure) is still a challenge in epilepsy.

## Closed-loop feedback in experimental epilepsy

Closed-loop systems are widely applied in engineering and easily found in everyday life. A simple thermostat used in air-conditioning devices is a good example of an on-off closed-loop system. It uses the information of an output signal - the measured temperature - and the input signal - the desired temperature - to guide the conditioning system to exert control over the temperature of the environment. In other words, these systems use the error signal between output and input to drive the control over the system accurately. In neuroscience, closed-loop systems are a lot useful because they allow one to control a neural system given target conditions. In an elegant work, Bérenyi et al.[21] have demonstrated that transcranial electrical stimulation triggered by spike-and-wave discharges in a rodent model of generalized epilepsy was very efficient in reducing its epileptic activity.

It is noteworthy to mention the main differences between a closed-loop and an open-loop control. Most of the works on electrical stimulation and optogenetics to date are based on an open-loop system. In this type of approach, the information used to control the input to the system is an off-line information that may be taken from the literature or previous neural recordings. The system works without feeding back the neural effect of the stimulation[22]. On the other hand, closed-loop control comprises: (1) the effector or actuator, that delivers the information, or that drives the feed-forward information to the system; (2) the system itself, that is the target neural circuit or cells; (3) the sensor to measure the output of the system; and (4) the controller to effectively compute the error signal between input and output and select the appropriate intervention to the system (Figure 1).

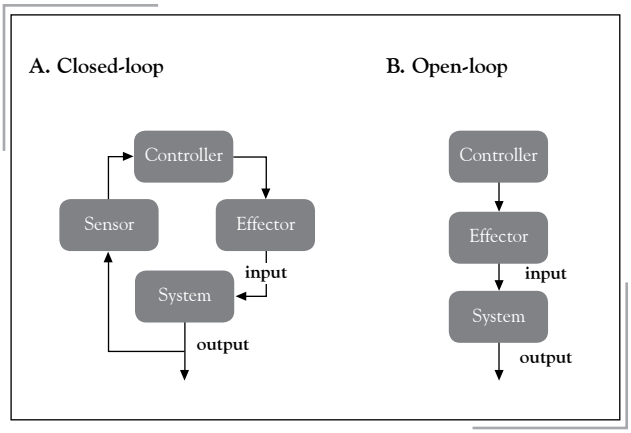


**Figure 1.** Comparison between closed-loop and open-loop systems. In closed-loop systems, the effector drives the feed-forward information to the system. The sensor sends online information regarding the output of the system to the controller to compute effectively the error signal between input and output. Then, the controller regulates the appropriate intervention to the system. In open-loop systems, the information used to control the input to the system is an off-line information that may be taken from the literature or previous neural recordings. This system works without feeding back the neural effect of the stimulation[22].

Closed-loop optogenetic strategy in experimental models of epilepsy has recently been described independently by two seminal studies from Huguenard and Soltezs laboratories[17,19].

Paz et al.[17] tested for the role of thalamus in post-stroke seizures. Also, they examined whether the thalamus could be targeted by optogenetic stimulation to interrupt seizures. They used a rodent model for induced photothrombosis that results in late epilepsy (>1 month) after stroke[23] in the right somatosensory cortex. The researchers then created a device with multiple electrodes and a chronic multisite optrode[12]. This method allows the selective illumination and registration of thalamocortical neurons while monitoring their firing during epileptic activity. To design the closed-loop system, the authors routed an EEG channel to a programmable real-time digital signal processor that calculated the EEG line length[24] and triggered laser stimulation upon crossing of a threshold. For each subject, the authors set the line length threshold for seizure detection manually at the beginning of the experiment. This approach was capable of detecting and silencing seizures within 1 s of initiation in rats with chronic implants. In summary, Paz et al.[17] have shown that selective optogenetic inhibition of thalamocortical neurons interrupted ongoing epileptic activities in thalamus and cortex, as well as the behavioral seizure.

Krook-Magnuson et al.[19] have developed a sophisticated closed-loop system to halt seizures with optogenetics. They generated a model of temporal lobe epilepsy by injecting kainate unilaterally into the dorsal hippocampus. Two weeks later, spontaneous and recurrent seizures have emerged. Then, the animals were implanted with electrodes and individual optical fibers in the hippocampus. Seizures were detected using custom software able to combine and evaluate signal power properties, spike features and frequency properties of the recorded EEG. The authors also individually tuned the system to the specific EEG signature of the animals. ChR2 or HR were expressed using Cre-lox strategy to target specifically pyramidal cells or PV+ interneurons in the hippocampus. The results indicated the closed

loop optogenetic system successfully halted more than 50% of the spontaneous and recurrent seizures by optogenetically inhibition of principal cells or by excitation of PV+ interneurons in the hippocampus upon seizure detection.

### ... and how affordable is it?

As discussed before, overall, closed-loop optogenetic technology requires: (1) light source (laser or light-emitting diode) plus optical fiber to work as the effector; (2) electrodes or optodes to probe the neural activity (e.g. seizure) in the system; (3) and an analog to digital system to transfer this information back to a controller. The computer (or controller), in turn, will run custom algorithms (usually written in MatLab, Labview, Phyton, C++) to detect specific events of interest and, with the lowest latency as possible, trigger the effector, closing the loop. Also, to make specific cell types responsible for light, it is required the use of virus vector to deliver the genes of the opsins and, in some cases, it is also necessary the use of Cre-expressing animals.

Table 1 synthesizes information on the main materials and its respective prices for an implementation of the closed loop optogenetic approach in experimental epilepsy. We also provide a more detailed list of supplies with costs estimation (Table 2); it is mainly based on Armstrong et al.[25], and the prices have been updated to 2015. Amplifier was listed twice in Table 2 mainly because A-M Systems amplifiers are cheaper and widely used, but the equipment use their own connector for input data, which can be inconvenient if the amplifier is used for a variety of experimental apparatus, while those from Neurophase (old known as Brownlee Precision) use Bayonet Neill-Concelman (BNC) connectors for it. Usually, the National Instruments (NI) digitizer board is an affordable and convenient option for closed-loop systems. The NI board digitizes the data while can simultaneously detect specific events, such as a seizure, that will activate the digital output to control the effector (e.g. laser). Besides, this board is compatible with LabVIEW, C++ programming and can be accessed by the MATLAB software through the Data Acquisition Toolbox. Among these options, LabVIEW is the user-friendliest option for investigators who are not familiar with programming. It is relatively straightforward use its graphical programming language to implement real-time event detections and to close the loop by controlling an effector. However, if the investigator wants to use a more sophisticated detection by using a high number

**Table 1.** Estimated cost for closed loop optogenetics.

| Items | Price |
|---|---|
| Differential Amplifier | $2,230.00 |
| Laser Source | $2,500.00 |
| National Instruments A/D board | $2,129.00 |
| Consumables for Optogenetics | $1,908.00 |
| Total | $8,767.00 |

**Table 2.** Equipment and consumables required for implementation of closed-loop optogenetic approach in experimental epilepsy (modified from Armstrong C, Krook-Magnuson E, Oijala M, Soltesz I. Closed-loop optogenetic intervention in mice. Nature protocols. 2013;8:1475–93).

| Company | Supply | Full name | Part Number | Cost in 2015 | Per |
|---|---|---|---|---|---|
| Thorlabs | (Patch cable) optical fiber from laser/led device to commutator | Ø200 μm, 0.39 NA, FC/PC-FC/PC Fiber Patch Cable, 2 m | M72L02 | $80.60 | each |
| | Optical fiber | 0.39 NA, Ø200 μm Core Multimode Optical Fiber, Low OH for 400 - 2200 nm, TECS Clad | FT200EMT | $1.50 | per meter |
| | Fiber Stripping Tool | Fiber Stripping Tool, Typical Cladding/Coating: 285 μm / 500 μm | T14S21 | $66.61 | each |
| | Ruby Fiber Scribe | Ruby DualScribe™ Fiber Optic Scribe | S90R | $50.50 | each |
| | Fiber Polishing Disc (Harder to use, but faster) | LC/PC Connector Polishing Disc | D50-LC | $84.10 | each |
| | Fiber Polishing Disc (Easier to use, but slower | LC/PC Ferrule Polishing Disc | D50-L | $84.50 | each |
| | Glass Polishing Plate | Glass Polishing Plate, 9.5" x 13.5" | CTG913 | $36.00 | each |
| | Polishing Sheets | 13" x 9" Aluminum Oxide Lapping (Polishing) Sheet, 0.3 μm Grit (10 Sheets) | LFG03P | $15.50 | pack of 10 |
| | | 13" x 9" Aluminum Oxide Lapping (Polishing) Sheet, 1 μm Grit (10 Sheets) | LFG1P | $13.80 | pack of 10 |
| | | 13" x 9" Aluminum Oxide Lapping (Polishing) Sheet, 3 μm Grit (10 Sheets) | LFG3P | $13.80 | pack of 10 |
| | | 13" x 9" Silicon Carbide Lapping (Polishing) Sheet, 5 μm Grit (10 Sheets) | LFG5P | $13.80 | pack of 10 |
| | Syringes for epoxy | 3 cc Empty Epoxy Syringe, Package of 10, Disposable | MS403-10 | $10.20 | pack of 10 |
| | Epoxy | Epoxy for Fiber Optic Connectors, Long Pot Life, 10 Packets | F112 | $106.00 | pack of 10 |
| | Light Power Meter | Compact Power Meter Console, Mechanical Analog & Graphics LC Display | PM100A | $906.00 | each |
| | Photodiode Power Sensor | Standard Photodiode Power Sensor, Si, 200 - 1100 nm, 50 mW | S120VC | $407.00 | each |

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| Company | Supply | Full name | Part Number | Cost in 2015 | Per |
|---|---|---|---|---|---|
| Example: CNI Laser | Blue, Amber or Red Laser | Fiber-couple DPSS Blue (wavelength 473 nm), Amber (wavelength 589 nm) or Red (wavelength 635 nm), 50 mW TTL modulation (on/off) | - | $2500.00 ** | each |
| NeuroPhase | Amplifier | Brownlee Precision 4-Channel Instrumentation Amplifier Model 410 | Model 410 | USA price / International Price $2550 / $3250 | each |
| A-M Systems | | Model 1700 Differential AC Amplifier, 110 V, 60 Hz | 690000 | $2,230 | each |
| National Instruments | 8 channel digitizer | NI USB-6221 M Series DAQ Device, BNC Term, U.S. (120 V) | 780117-01 | $2,129 | each |
| | 16 channel digitizer | NI USB-6229 M Series DAQ Device, BNC Term, U.S. (120 V) | 780116-01 | $2,661 | each |
| Dell | Computer | Dell Inspiron Desktop, Intel i7 processor, 64-bit Windows 7 Professional, 16GB memory | Inspiron Desktop | $989.98 | each |
| Tocris | kainic acid (available in 1, 10, or 50mg) | Kainic Acid, 10 mg | 0222 10 mg | $149.00 | 10 mg vial |
| USA Scientific | 5 mL pipette tips | 5 ml pipet tip, type A, racks | 1050-0700 | $51.30 | 10 racks of 50 |
| ACE Surgical Supply | Local Anaesthetic | Bupivacaine HCL - 0.5% 50ml | 011663-01 | $4.29 | each |
| Valley Vet Supply | Analgesic + antibiotic | Neo-Predef | 617RX | $17.99 | each |
| | Analgesic | Banamine (Flunixin Meglumine) Injectable Solution Veterinary 50mg/ml 100ml | 134RX | $27.95 | each |
| Kent Scientific | Curver Iris Forceps | Iris Forceps, curved, 10cm long, 1 x 2 teeth, 0.8mm tips | INS650917 | $35 | each |
| | Pointed Forceps | Tweezer #5 Dumoxel, 11cm, 0.1 x 0.06mm Tips | INS600098 | $55 | each |
| A-M Systems | Mounted Alligator Clip | Helping Hands Soldering Stand | 726200 | $9.00 | each |
| Doric Lenses | Patch cables (from commutator to animal) | Mono Fiberoptic Patchcord (zirconia ferrule connector) 30 cm long with flange | MFP_200/220/900-0.53_0.3_FC-ZF1.25(F) | $135* | each |
| | | Branching Fiberoptic Patchcord (zirconia ferrule connector) 30 cm long with flange | BFP_200/240/900-0.22_0.30m_FC-2xZF1.25(F) | $195* | each |
| | Optical Commutator | 1x1 Fiberoptic Rotatory Joint | FRJ_1x1_FC-FC | $595* | each |
| | Cannula holder for stereotax | Stereotaxic Cannula holder for 1.25 mm ferrule | SCH_1.25 | $315* | each |
| Dremel | Hand Drill | Dremel 3000-1/24 1 Attachment/24 Accessories Rotary Tool | Model 3000-1/24 | $62.06 | each |
| | Flexible shaft | Dremel 225-01 Flex Shaft Attachment | 225-01 | $29.66 | each |
| | Keyless chuck | Dremel 4486 MultiPro Keyless Chuck | 4486 | $11.78 | each |
| Fine Science Tools | Fine tipped delicate forceps | Dumont micro-blunted, atraumatic tipped forceps #5/45 | 11253-25 | $52.00 | each |
| | Small surgery scissors | Iris scissors, delicate pattern 9cm | 14060-09 | $61.00 | each |
| Fisher Scientific | Disposable scalpel | Fisherbrand Single-Use Scalpels | 089275A | $90.00 | pack of 20 |
| | Sterile q tips | Fisherbrand Polyester-tipped applicators; Sterile, 2 per envelope | 23400111 | $24.00 | pack of 200 |
| | Gloves | Microflex Evolution One Powder-Free Latex Exam Gloves, medium | 11-462-68C | $84.09 | pack of 100 |
| | Sterile gloves | 50 pair sterile size 7.5 gloves | 11-388-122E | $347.71 | pack of 50 |
| | Small petri dishes | BD Falcon Standard Disposable Petri Dishes, surface area 21.29 cm2 | 08-757-100B | $197.46 | pack of 500 |
| | Lint-free wipes | Kimwipes Delicate Task Wipers | 34155EMD | $6.25 | pack fo 280 |
| | Weigh dishes for mixing dental cement | Fisherbrand Hexagonal Polystyrene Weighing Dishes Top I.D.: 1.4 in. (3.6cm); Base I.D.: 0.9 in. (2.4cm); Depth: 0.4 in. (0.95cm) | 02-202-100 | $95.00 | pack of 500 |
| Ikea | Lamp | NOT Floor uplight/reading lamp, white, white | 301.451.29 | $9.99 | each |
| Intermatic | Light timer | Indoor plug-in timer | TN311 | $18.16 | each |

**Table 2.** Equipment and consumables required for implementation of closed-loop optogenetic approach in experimental epilepsy (modified from Armstrong C, Krook-Magnuson E, Oijala M, Soltesz I. Closed-loop optogenetic intervention in mice. Nature protocols. 2013;8:1475–93).

| Company | Supply | Full name | Part Number | Cost in 2015 | Per |
|---|---|---|---|---|---|
| Jackson Laboratory | PV Cre mice | B6;129P2-Pvalbtm1(cre)Arbr/J | 008069 | $464* | breeding pair |
| | CamKII Cre mice | B6.Cg-Tg(Camk2a-cre)T29-1Stl/J | 005359 | $464* | breeding pair |
| | Floxed ChR2 mice | B6;129S-Gt(ROSA)26Sortm32 (CAG-COP4*H134R/EYFP)Hze/J | 012569 | $296* | breeding pair |
| | Floxed eNpHR3.0 mice | B6;129S-Gt(ROSA)26Sortm39 (CAG-hop/EYFP)Hze/J | 014539 | $354* | breeding pair |
| Kientec | 225um ID ferrules (ceramic) | Zirconia Ferrule, 225 $\mu$m inner diameter | FZI-LC-225 | $3.25 | each |
| Loctite | Gel super glue | Loctite Super Glues | LOC1364076 | $12.19 | each |
| Logitech | HD USB web cam | HD Webcam C270 - USB, 3MP, 1280 x 720 | Logitech C270 960-000694 | $39.99 | each |
| McMaster-Carr | Screws for mice | 1/8" length | 91773A052 | $8.20 | pack of 100 |
| | Screwdriver that fits these screws | #0 Philips blade miniature screwdriver | 7026A18 | $5.03 | each |
| Office Max | Uninterrupted power supply | APC Back-UPS XS Series Batteryackup, BX1500G, 1500VA/865 Watt | 21880582 | $199.99 | each |
| | External hard drives | WD My Book 6TB External USB 3.0 Hard Drive With Backup, Black | 24828046 | $249.99 | each |
| Moore Medical | Suture | Coated VICRYL (polyglactin 910) Precision Point-Reverse Cutting Sutures Undyed Braided P-2 5-0 | 58723 | $217.00 | each |
| | Hydrogen Peroxide | Hydrogen Peroxide 3% 8oz | 90153 | $1.09 | each |
| Heartland Veterinary Supply and Pharmacy | Antibiotic | Baytril Injectable 2.27% 100 ml | 2900-RX | $52.95 | each |
| Santa Cruz Animal Health | Inhaled Anaesthetic | Isoflurane, 250 ml bottle | sc-363629Rx | $34.00 | each |
| Pearson Dental | Drill bit | S.S. White Carbide Bur HP #2 Pkg. of 10 | W60-0234 | $19.50 | each |
| | Liquid for dental cement | Teets C.C. Liquid (16 oz.) | C73-0076 | $25.50 | each |
| | Dental cement powder | Teets C.C. Clear Powder (1lb.) | C73-0060 | $44.95 | each |
| Plastics One | Electrical commutators | 2 channel electrical commutator with single brush | SL2C/SB | $120.24* | each |
| | Cables from commutator to amplifier | 2 channel cable with mesh covering 305 to 2 banana plugs, 100cm length | 305-491/2 W/ MESH | $36.73* | each |
| | Cables from animal to commutator | 2 channel cable with mesh covering and 2 305 connections, 35cm length | 305-305 W/ MESH | $42.11* | each |
| | Electrodes | Bipolar Electrode Unit for Small Animals; 2 channel electrode, untwisted length = 10mm | MS303/3-A/SP | $7.53* | each |
| Precision Fiber Products | Zirconia sleeve | PFP Ceramic Split Sleeve, 1.25mm ID | SM-CS125S | $0.95 | each |

*Cost in 2013; **Average price from different companies.

of parameters, LabVIEW maybe not be the best choice. For instance, Krook-Magnuson et al.[19] took advantage of a custom written MATLAB-script to combine different parameters of EEG signal to achieve a better accuracy of spontaneous seizures detection, although it will include the MATLAB license in the price, which would be with LabVIEW as well. Consequently, to achieve the lowest-cost would be a better option to use programming languages like C and Python (http://wiki.python.org.br/).

As a general rule, using open source tools for a closed loop approach will reduce costs, and increase flexibility and control of the desired application. In the last years, a considerable amount of free software programs and hardware tools were developed by research groups to use in neuroscience. For instance, we can cite Open Ephys (http://www.open-ephys.org) combined with Intan headstages (http://www.intantech.com/), to acquiring and real-time processing data, Pulse Pal (https://sites.google.com/site/pulsepalwiki/home) and Cyclops Driver (https://goo.gl/ItZlNO), to precisely drive light sources for optogenetic stimulation in closed-loop experiments using recordings and event detections with Open Ephys. Combined these hardware tools can significantly reduce the costs presented in Table 1.

So, how affordable is the implementation of this emergent technique? Despite the high temporal precision and accuracy in detecting and interfering with seizures, we can conclude the costs of the fundamental components for closed-loop system assembly are relatively small. For comparison, according the Table 1, the costs of the main materials needed for closed loop optogenetics makes approximately U$9,000 while commercially available multi-microelectrode electrophysiology systems can hardly be acquired for less than U$30,000. Regardless, costs can be even lower if the research group chooses freeware software programs and hardware tools. To facilitate the access to more detailed information on optogenetics, closed loop systems and open source tools for Brazilian researchers and students, we have made a website (openoptobrasil.wordpress.com).

## FINAL CONSIDERATIONS

Closed loop optogenetic is an emergent technique with the astonishing potential to push the neuroscience field forward. Real-time seizure detection combined with optogenetic control is now a reality and will certainly open future therapeutic avenues. The fundamental principles behind this approach are (1) the use of light and genetics to control well-defined events within specified cells of living tissue, and (2) the combination of fast real-time processing to detect events of interested from a system and, then, precisely control effectors that will act on the same system, only if the event is detected, closing the loop. Therefore, implementation of closed-loop optogenetics in experimental epilepsy seems to demand more joint interdisciplinary efforts and innovative scientific questions than money resources.

## ACKNOWLEDGMENTS

We would like to thank Ingrid Miranda Esteves for the valuable contributions to the manuscript and Renata Caldo Scandiuzzi for the excellent technical support. This work was supported by the São Paulo Research Foundation and Coordination for the Improvement of Higher Education Personnel (FAPESP/CAPES, grant # 2014/18211-0, to Cleiton Lopes Aguiar) and the National Council for Scientific and Technological Development (CNPq, grants # 476250/2013-7 and # 466995/2014-8, to João Pereira Leite), in Brazil.

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JECN *Journal of Epilepsy and Clinical Neurophysiology*

São Paulo, 2015.

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