Universidade Federal do Rio Grande do Sul
Instituto de Ciências Básicas da Saúde
Departamento de Bioquímica
Programa de Pós-Graduação em Ciências Biológicas: Bioquímica

TESE DE DOUTORADO

MODULAÇÕES ENERGÉTICAS CEREBRAIS PERMITEM A MANUTENÇÃO DE CRISES EPILÉPTICAS PROLONGADAS

Ben Hur Marins Mussulini

Porto Alegre, RS, Brasil

Março de 2017

Universidade Federal do Rio Grande do Sul

Instituto de Ciências Básicas da Saúde
Departamento de Bioquímica
Programa de Pós-Graduação em Ciências Biológicas: Bioquímica

TESE DE DOUTORADO

MODULAÇÕES ENERGÉTICAS CEREBRAIS PERMITEM A MANUTENÇÃO DE CRISES EPILÉPTICAS PROLONGADAS

Doutorando

Ben Hur Marins Mussulini

Orientador

Prof. Dr. Diogo Losch de Oliveira

Tese apresentada no Programa de Pós-Graduação em Ciências Biológicas: Bioquímica

Requisito à obtenção do título de doutor em bioquímica.

Porto Alegre, Março de 2017

CIP - Catalogação na Publicação

Mussulini, Ben Hur Marins
MODULAÇÕES ENERGÉTICAS CEREBRAIS PERMITEM A MANUTENÇÃO DE CRISES EPILÉPTICAS PROLONGADAS / Ben Hur Marins Mussulini. -- 2017.
161 f.

Orientador: Diogo Losch de Oliveira.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Instituto de Ciências Básicas da Saúde, Programa de Pós-Graduação em Ciências Biológicas: Bioquímica, Porto Alegre, BR-RS, 2017.

1. Peixe-zebra. 2. Epilepsia. 3. Metabolismo Cerebral. 4. Estresse Oxidativo. 5. Mitocôndria. I. de Oliveira, Diogo Losch, orient. II. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

Esta tese é dedicada ao apoio, amor, e sabedoria do eterno professor, doutor e IC

Renato Dutra Dias.

"Tudo o que é afirmado sem provas,

pode ser negado sem elas".

(Euclídes)

"A melhor maneira que o homem dispõe para se

aperfeiçoar, é aproximar-se de Deus".

(Pitágoras)

"Ninguém poderá jamais aperfeiçoar-se, se não tiver

o mundo como mestre. A experiência se adiquire na prática".

(William Shakespeare)

"Quem pensa pouco,

Se equivoca muito".

(Leonardo Da Vinci)

"Não vos lamenteis inutilmente,

mas maravilhai-vos com o princípio da transitoriedade

e dele aprendei a vacuidade da vida humana.

Não alimenteis vãos desejos de que

as coisas mutáveis se tornem imutáveis."

(Buda)

AGRADECIMENTOS

Agradeço a minha família pela formação de meu caráter. A minha namorada Suelen Baggio pelo apoio nesse momento extremamente estressante. Aos meus Irmãos por me ouvirem e me deixarem desabafar mesmo sem entender sobre o que eu estava dizendo, eles sempre se fizeram presentes e auxiliaram meus pensamentos a se equilibrarem.

Sou grato ao meu orientador Diogo Losch de Oliveira por me ensinar o preço e a responsabilidade da liberdade e por se preocupar com meu amadurecimento como pesquisador e como pessoa. Mesmo com orçamentos limitados, sempre fui guiado a fazer perguntas científicas importantes e pensar com o lado mais biológico da minha formação biomédica. Essa forma de orientação me levou a fazer perguntas básicas, de forma simples e mostrar que o início de uma linha de pesquisa faz parte de um processo longo.

Agradeço aos meus co-orientadores não formais. Eduardo Pacheco Rico, por me permitir dar continuidade a seu pós-doutorado com mudanças drásticas de enfoque científico, mas chegando a resultados importantes e expressivos. Denis Rosemberg, por ter me ensinado que a felicidade de espírito é a motivação e não a finalidade. Fábio Klamt, por ter apostado em um modelo animal "alternativo" e ter me deixado explorar minhas ideias em uma metodologia pouco conhecida em nosso laboratório. Diogo Onofre Gomes de Souza, pelo coração, amizade, carinho, preocupação e ajuda em momentos delicados.

Agradeço a minha primeira orientadora Suzana Wofchuk por me dar a primeira oportunidade de entrar no mundo científico. Aos colegas de laboratório e demais colaboradores entre eles as professoras Angela Wyse, Ana Battastini e Carla Bonan pelas portas sempre abertas de seu grupo de pesquisa para interações, reagentes e troca intelectual. Um agradecimento especial à professora Maria Elisa Calcagnotto e ao professor Renato Dutra Dias por sempre se mostrarem a disposição de ajudar. Aos alunos Adriana Vizzuette, Ivi Juliana Bisttrot, Lúcia von Mengden Meirelles, Luana Moro e Gabriela Lazzarotto pelo auxílio imensurável a esta tese.

Por fim, aos órgãos de fomento CAPES, CNPq, FAPERGS e a Universidade Federal do Rio Grande do Sul pelo ensino de alta qualidade.

Sumário

Parte I

Parte I

Resumo

Epilepsia é uma desordem neurológica que afeta o sistema nervoso central, predispondo o paciente a crises recorrentes, as quais apresentam uma alta demanda energética cerebral, e que culminam na depleção dos níveis de glicose cerebrais conforme a crise epiléptica progride de aguda (até 5 min) à prolongada (de 5 min até algumas horas). A presente tese mapeou os diferentes modelos de crises e síndromes epilépticas com enfoque em peixe-zebra, no intuito de selecionar o melhor modelo em nosso universo experimental para investigar quais outros substratos energéticos poderiam ser utilizados pelo cérebro frente ao hipometabolismo da glicose em crises epilépticas prolongadas induzidas por pentilenotetrazol. Hipotetizou-se um ambiente produtor de peróxido de hidrogênio como agente modulatório do metabolismo energético neste tipo de crise epiléptica. Para tanto se caracterizou o protocolo de respirometria de alta resolução em dissociado cerebral de peixe-zebra adulto. Os peixes foram expostos a pentilenotetrazol por diferentes tempos. Detectou-se um desacoplamento entre o metabolismo da glicose e o consumo de O_2 para produção de ATP em crises epilépticas prolongadas de 20 min. Neste momento, testou-se o impacto dos seguintes substratos energéticos sobre o consumo de O_2 para produção de ATP: L-glutamato, L-glutamina, L-lactato, e β-hidroxibutirato. Também foi avaliado o sistema pró/antioxidante em amostras de cérebro de peixe-zebra adulto submetido a crises epilépticas prolongadas por 20 min (CEUA – 28043). Os resultados indicam o uso do L-glutamato e da L-glutamina como substratos energéticos para a manutenção de crises epilépticas prolongadas, e um ambiente favorável à produção de peróxido de hidrogênio, pela redução da atividade do Complexo I mitocondrial, pelo aumento da atividade das enzimas superóxido dismutase e glutationa peroxidase, e pelo aumento da oxidação de diclorofluoresceína. A literatura aponta para uma inibição da glicerol-3-fosfato-desidrogenase e piruvato-cinase, e uma ativação da glicose-6-fosfato-desidrogenase por aumento de peróxido de hidrogênio, o que culmina na diminuição da utilização da glicose como substrato energético. A completa oxidação do glutamato na presença de baixos níveis de piruvato ocorre via saída do malato da matriz mitocondrial e sua conversão a piruvato pela enzima málica. Ambas as enzimas produtoras de Fosfato de dinucleótido de nicotinamida e adenina reduzida citadas acimas apresentam atividade aumentada no modelo de convulsão abordado. Portanto, o metabolismo glutamatérgico é fundamental para a manutenção energética, e para a atividade de defesas antioxidantes em momentos de crises epilépticas prolongadas induzidas por pentilenotetrazol em peixe zebra adulto.

Palavras-chave: Peixe-zebra; Epilepsia; Pentilenotetrazol; Mitocôndria; Metabolismo Cerebral; Estresse Oxidativo.

Abstract

Epilepsy is a brain disorder, which promotes the predisposition to events of high energy expenditure known as epileptic seizure. As epileptic seizure progress form acute (until 5 min of duration) to prolonged (above 5 min of duration), lower is the concentration of glucose in the brain. This thesis mapped all models of zebrafish epileptic seizure and epileptic syndrome to choose the best model in our experimental conditions to evaluate the impact of other substrates under glucose brain hypometabolism related to prolonged epileptic seizure induced by pentylenetetrazole. It was hypothesized that an environment with high concentrations of hydrogen peroxide could be connecting with the fast metabolic modulation in this model. To do so, the high-resolution respirometry protocol for zebrafish brain dissociated was characterized. Fish were exposed to pentylenetetrazole by different duration. There was a decoupling between glucose brain metabolism and O_2 consumption to ATP synthesis after 20 min of exposure to pentylenetetrazole. At this moment, the impact of the following substrates were measured under O_2 consume to ATP synthesis: L-glutamate, L-glutamine, L-lactate, and β-hydroxybutyrate. The redox balanced was evaluated as well (CEUA – 28043). Data indicate L-glutamate and L-glutamine as the main energy substrate to maintain prolonged epileptic seizure. There was an environment prone to hydrogen peroxide, because mitochondrial complex I activity was impered, and the enzymes superoxide dismutase and glutamine peroxidase were activity, as well as the oxidation of diclorofluoresceine was increased. Hydrogen peroxide activates glucose-6-phosphate-dehydrogenase, and inhibits glycerol-3-phosphate-dehydroganase as well as piruvate kinase. Therefore the glucose metabolism would be modulated to antioxidant defense instead of glycolysis. In low pyruvate concentrations, malate from glutamate is transported to the cytosol and converted to pyruvate and Nicotinamide adenine dinucleotide phosphate. The activity of glucose-6-phosphate-dehydrogenase and citosolic malic enzyme were increased. Therefore, glutamate metabolismo is imperative to energy maintain prolonged epileptic seizure and to antioxidante defense to avoid further damage.

Palavras-chave: Zebrafish; Epilepsy; Pentylenetetrazole; Mitochondria; Brain Metabolism; Oxidative Stress.

Lista de Abreviaturas

α-KG: α-cetoglutarato

β-HB: β-hidroxibutirato

ΔG^0: Energia livre de Gibbs

AST: Aspartato-amino-transferase

ATM: Ataxia telangiectasia cinase

ATP: Adenosina trifosfato

CA: Ácido Caínico

CAT: Catalase

CO_2: Dióxido de carbono

CoA: $Coenzima_A$

Complexo I: NADH-ubiquinona-oxidorredutase

Complexo II: Succinato-ubiquinona-oxidorredutase

Complexo III: Complexo Citocromo *bc1*

Complexo IV: Citocromo *c* oxidase

Complexo V: F1-Fo-ATP sintase

DNA: Ácido desoxirribonucleico

EAAT: Transportado de aminoácidos excitatórios

F-1,6-BisP: Frutose 1,6-Bisfosfato

$FADH_2$: Dinucleótido de flavina e adenina reduzida

FCCP: Carbonil cianida-p-trifluorometoxifenilhidrazona

FDG: Fluordesoxiglicose

Fe-S: Ligação ferro-enxofre

G-1-P: Glicose-1-fosfato

G-6-P: Glicose-6-fosfato

G6PDH: Glicose-6-fosfato-desidrogenase

GABA: Ácido gama-aminobutírico

$GABA_A$: Receptor ionotrópico $GABA_A$

GAPDH: Glicerol-3-fosfato-desidrogenase

GDH: Glutamato-desidrogenase

GLS: Glutaminase

GLUT: Transportador de Glicose

Gpx: Glutationa peroxidase

GR: Glutationa redutase

GSH: Glutationa

GSSG: Glutationa oxidada

H^+-*Leak*: Vazamento de prótons

H_2O: Água

H_2O_2: Peróxido de Hidrogênio

H_Z: Hertz

IM: Membrana interna mitocondrial

I.P.: Intraperitonial

KCN: Cianeto de potássio

LDH: Lactato Desidrogenase

MCT: Transportador de

ME: Enzima málica

mtDNA: Ácido desoxirribonucleico mitocondrial

NOX: Espécies reativas de óxido nítrico

Na^+/K^+ ATPase: Sódio Potássio ATPase

NADH: Dinucleótido de nicotinamida e adenina reduzida

NADPH: Fosfato de dinucleótido de nicotinamida e adenina reduzida

O_2: Oxigênio

$O_2^{\cdot-}$: Superóxido

Olig: Oligomicina

OXPHOS: Fosforilação oxidativa

PC: Piruvato carboxilase

PK: Piruvato cinase

Prx: Peroxirredoxina

PTZ: Pentilenotetrazol

Q: $Coenzima_Q$

RNA: Ácido ribonucleico

ROS: Espécies reativas de oxigênio

ROX: Consumo de oxigênio residual

SE: *Status Epilepticus*

SDH: Succinato-desidrogenase

siRNA: Ácido ribonucleico de interferência

SNC: Sistema Nervoso Central

SOD: Superóxido-dismutase

STE: Sistema Transportador de Eletrons

TRT: Coteúdo de tiol reduzido total

I. Introdução

I.1. Epilepsia

Epilepsia é uma desordem do sistema nervoso central (SNC), caracterizada pela predisposição a crises epilépticas recorrentes que podem levar a alterações neurobiológicas, cognitivas, psicológicas e sociais (Fisher, 2015). Aproximadamente 15,5 bilhões de dólares são gastos anualmente nos Estados Unidos da América com o tratamento de pacientes epilépticos, e estima-se uma prevalência de 50 milhões de pacientes no mundo e 2,4 milhões de novos casos ao ano (Theodore *et al.*, 2006). De 30 a 50 novos casos a cada 100.000 habitantes ocorrem em países desenvolvidos, número este que pode ser de duas a três vezes maior em países subdesenvolvidos (Ndoye *et al.*, 2005), local onde se encontram em torno de 80% dos pacientes epiléticos (Dua *et al.*, 2006; Hongoro e Normand, 2006). A duração e frequência de crises epilépticas não são uma constante (Fisher *et al.*, 2014). Crises epilépticas são divididas em dois grupos (Berg e Millichap, 2013): crise epiléptica focal, na qual uma fração do SNC origina e mantêm a crise epiléptica; e crise epiléptica generalizada, na qual ambos os hemisférios apresentam hiperatividade. Crise epiléptica tônico-clônica ("grande mal"), ou espasmódica são exemplos do segundo grupo (Panayiotopoulos, 2011; Fisher, 2014), caso em que o paciente alterna episódios de tonia e extenção muscular com contrações musculares involuntárias. Esta desordem também é classificada mediante presença ou ausência de etiologia definida, sendo intitulada como sintomática ou criptogênica, respectivamente (Engel, 2011; Shorvon, 2011). Infelizmente, 60% dos pacientes não possuem etiologia definida e classificados como criptogênicos, 30% são refratários aos tratamentos farmacológicos (Xiao *et al.*, 2015), e 75% dos pacientes epilépticos que habitam países em desenvolvimento não recebem o tratamento adequado ("falha terapêutica") (Nwani *et al.*, 2013). Portanto, a procura por novos tratamentos para solucionar a refratariedade e a falha terapêutica (Newton e Garcia, 2012) e um maior esclarecimento etiológico das epilepsias são foco constante de estratégias inovadoras de pesquisa, como por exemplo, a procura de novos modelos animais para mimetizar esta desordem.

I.2. *Danio rerio*

A utilização do modelo animal peixe-zebra (*Danio rerio*) é uma inovação na pesquisa básica que contribuiu para solucionar hipóteses científicas. Estudos desenvolvidos na década de oitenta (Streisinger *et al.*, 1981; Chakrabarti *et al.*, 1983; Streisinger, 1983; Walker e Streisinger, 1983; Stewart *et al.*, 2014) e noventa (Vennstrom e Lagerkrantz, 1995) levaram pesquisadores a mapear o genoma desta espécie, o qual apresenta 70%

de ortologia com o genoma humano (Howe *et al.*, 2013). Entre as características dessa espécie para a pesquisa biomédica encontram-se alta homologia fisiológica e genética com humanos. O SNC desse organismo apresenta alta similaridade morfológica e funcional com mamíferos (Gerlai, 2011; Lopes Da Fonseca *et al.*, 2013; Suen *et al.*, 2013), os circuitos neurocomportamentais mapeados por p-ERK indicam a presença de 294 regiões anatómicas encefálicas as quais são subdivididas em prosencéfalo, mesencéfalo e rombencéfalo, sem a presença de neocortex como os mamíferos (Randlett *et al.*, 2015). Por ser um animal de pequeno porte, a varredura farmacológica de novos compostos apresenta custo-benefício expressivo ao comparar-se com roedores, além de possuir uma barreira hemato-encefálica com similar permeabilidade a macromoléculas quando comparado a mamíferos (Jeong *et al.*, 2008; Eliceiri *et al.*, 2011). Paradigmas comportamentais adaptados a essa espécie permitem estudos neuropsicológicos complexos (Gerlai, 2010; 2011). Ferramentas moleculares permitem a visualização *in vivo* da atividade neuronal e de redes neurais frente a estímulos em tempo real (Tian *et al.*, 2009; Akerboom *et al.*, 2012). Por fim, a diluição em água ou injeção intraperitonial (i.p.) de compostos reduzem os custos da pesquisa (Alfaro *et al.*, 2011). Frente a essas características, pesquisadores utilizam este modelo animal para mimetizar desordens que afetam o SNC (Kalueff *et al.*, 2014).

I.3. Peixe-zebra como modelo animal para o estudo de crises e síndromes epilépticas

Compostos pró-convulsivos que afetam o balanço entre os sistemas excitatórios e inibitórios do SNC são utilizados para o desenvolvimento de modelos não genéticos de crises epilépticas (Zhou e Danbolt, 2014; Leke e Schousboe, 2016). O composto pentilenotetrazol (PTZ) inibe os receptores ionotrópicos do tipo $GABA_A$ reduzindo a capacidade do sistema inibitório, resultando em hiperexcitabilidade cerebral e em crises epilépticas agudas ou prolongadas. O PTZ é o agente pró-convulsivo mais utilizado na indústria farmacêutica para o desenvolvimento de novas terapias e foi o primeiro modelo de crise epiléptica a ser estudado em peixe-zebra (Baraban *et al.*, 2005). Uma vez que o peixe-zebra apresentou perfil comportamental e eletrofisiológico de crises epilépticas, novos modelos foram desenvolvidos nesta espécie, entre eles: ácido domoico (Tiedeken e Ramsdell, 2007), pilocarpina (Eddins *et al.*, 2010), febre (Hunt *et al.*, 2012), gingotoxina (Hashiguchi *et al.*, 2015), alliglicina (Leclercq *et al.*, 2015) e ácido caínico (CA) (Alfaro *et al.*, 2011). Entre os modelos genéticos encontram-se: Angelman (Hortopan *et al.*, 2010), Lowe (Ramirez *et al.*, 2012) e Dravet (Baraban *et al.*, 2013). O modelo animal ideal deve apresentar os mecanismos causais da patologia (validação de construto), o perfil fenotípico da crise epiléptica (validação de face) e a resposta anticonvulsiva frente a tratamentos clínicos já validados (validação de predição) (Grone e Baraban, 2015). Apesar do modelo de crise epiléptica prolongada

induzida por PTZ em peixe-zebra apresentar validações de face e predição, o mesmo carece de validações de construto, uma vez que os mecanismos relacionados a predisposições de crises epilépticas espontâneas são pouco abordadas, por exemplo, a bioenergética cerebral, a qual alterara o balanço entre neurotransmissores excitatórios e inibitórios do SNC.

I.4. Metabolismo de sinapses excitatórias

A glicose é captada pelo SNC através de transportadores de glicose (GLUTs) presentes entre a barreira hemato-encefálica, os astrócitos (GLUT1), e os neurônios (GLUT3) (Pan e Kastin, 2016). A glicólise ocorre no citosol e é a primeira etapa da oxidação da glicose resultando na produção de ATP, NADH e piruvato (Hyder *et al.*, 2006). Os astrócitos apresentam uma baixa oxidação de piruvato e alta oxidação de glicose comparada aos neurônios (Ivanov *et al.*, 2014). O acúmulo de NADH e piruvato no citosol astrocitário eleva a síntese de lactato o qual é exportado para o espaço extracelular e captado pelos neurônios por intermédio de transportadores de ácidos monocarboxílicos (MCT), MCT 1 e MCT 2, respectivamente. Uma vez no citosol neuronal, o lactato será convertido a piruvato e oxidado na matriz mitocondrial (Pellerin e Magistretti, 2012), podendo ser completamente oxidado a CO_2, H_2O e utilizado para a síntese de ATP (Jha *et al.*, 2012). Durante o processo de oxidação do piruvato, α-cetoglutarato pode ser convertido a glutamato e exportado ao citosol para síntese de proteínas, glutationa e vesiculado como neurotransmissor (Whitelaw e Robinson, 2013). Após a despolarização neuronal, o glutamato é liberado na fenda sináptica atuando sobre receptores pós-sinápticos e retirado da fenda sináptica por transportadores de glutamato (EAATs), astrocitários majoritariamente (Danbolt *et al.*, 2016). Esse processo consome 75% da energia do SNC (Nehlig e Coles, 2007) e representa 80% da neurotransmissão cortical (Sibson *et al.*, 1998). Portanto, a reciclagem do glutamato é fundamental para reduzir o custo energético anaplerótico (Schousboe *et al.*, 2014). O glutamato é convertido à glutamina nos astrócitos, a qual é exportada para o espaço extracelular, captada pelos neurônios e reconvertida a glutamato (Parpura *et al.*, 2016), o qual é oxidado ou re-vesiculado (Mckenna, 2007). Desta maneira a glicose está diretamente vinculada a um harmonioso balanço entre anabolismo e catabolismo de outras moléculas presentes no SNC, com atividade excitatória e energética (Mergenthaler *et al.*, 2013) (**Figura 1**).

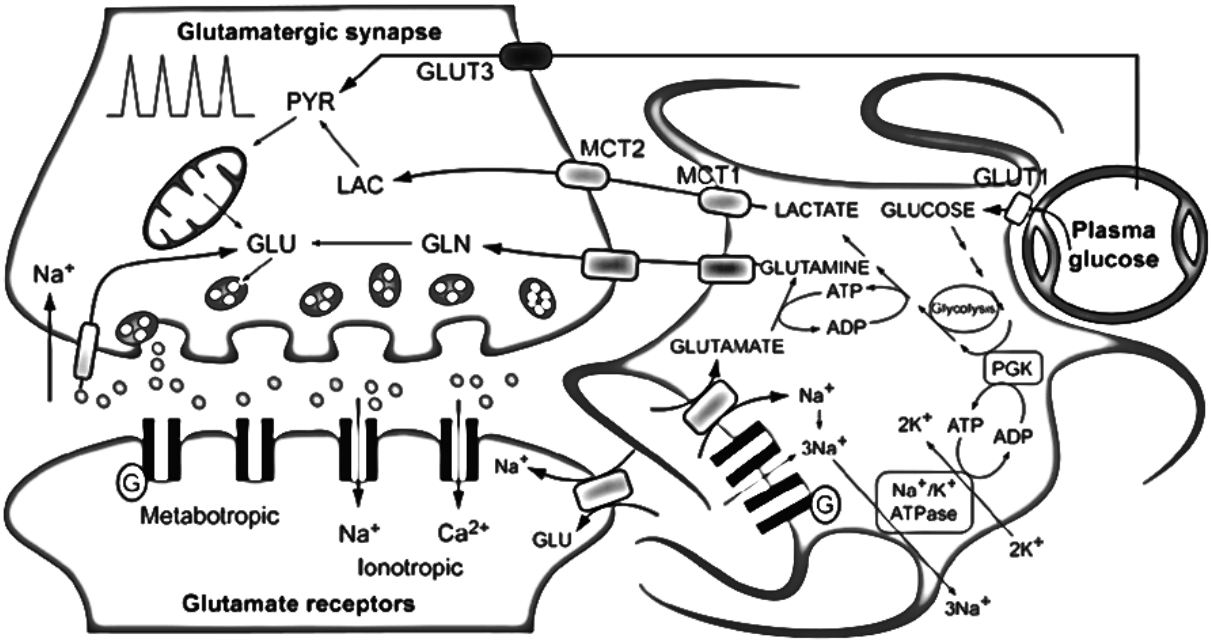

Figura 1. Bioenergética da sinapse tripartite excitatória. Representado da direita para a esquerda um vaso sanguíneo, o astrócito, e uma sinapse glutamatérgica. A glicose é transportada do vaso sanguíneo para as células cerebrais pela atividade de transportadores de glicose (GLUTs), sendo o GLUT1 e o GLUT3 os transportadores astrocitários e neuronais, respectivamente. No astrócito, a glicose é oxidada a piruvato no processo denominado glicólise. O excesso de piruvato não oxidado na mitocôndria é convertido a lactato, o qual é exportado para o meio extracelular pelo transportador de ácidos monocarboxilatos (MCT1). O neurônio captará tanto glicose (GLU3) quanto lactato (MCT2) e converterá ambos a piruvato, o qual será oxidado na mitocôndria. Durante o processo de oxidação do piruvato, o α-cetoglutarato poderá ser convertido a glutamato e exportado ao citosol neuronal, no qual será internalizado em vesículas para utilização como neurotransmissor. Mediante despolarização, as vesículas contendo glutamato fundem-se à membrana plasmática liberando este transmissor na fenda sináptica. O glutamato atua em receptores no neurônio pós-sináptico e a sinalização é terminada mediante tamponamento e captação de glutamato pela atividade de transportadores presentes nos neurônios e nos astrócitos, sendo esta última célula responsável por 90% da captação de glutamato cerebral. Este transporte se dá por cotransporte glutamato-Na^+-H^+. Para equilibrar o gradiente iônico celular, a Na^+/K^+ ATPase é ativada, consumindo 50% do ATP cerebral, produzido nos astrócitos majoritariamente pela glicólise. Após o glutamato ser captado pelos astrócitos, no citosol celular há a conversão desta molécula a glutamina, processo o qual consome ATP. A glutamina é exportada ao meio extracelular e captada pelos neurônios e convertida a glutamato. Seja por ação da glutaminase, seja pela captação, os neurônios glutamatérgicos reciclam o glutamato, reduzindo os custos anapleróticos do sistema de neurotransmissão. Na+: Sódico, K+: Potássio, Na+/K+ ATPase: Transportador sódio potássio ATPase, ATP: Adenosina trifosfato (modificada de (Kandel *et al.*, 2013)).

I.5. Outros substratos energéticos são oxidados no SNC além de glicose

O ciclo de Krebs ocorre na matriz mitocondrial a partir da síntese de citrato pela atividade da enzima citrato-sintase, cujos substratos são acetil-CoA e oxaloacetato. Após uma cadeia de eventos coordenados haveria a produção de NADH, $FADH_2$, CO_2 e ATP, reciclando de forma cíclica o oxaloacetato inicial. Entretanto, essa representação simplista do ciclo de Krebs não justifica o balanço molecular encontrado em diferentes estados alimentares e patológicos. Contudo, ao estudar a cinética molecular das enzimas que compõem o ciclo do ácido cítrico, percebe-se que o mesmo pode ser fragmentado em duas etapas. Existe um ciclo A que se inicia na oxidação do α-cetoglutarato e finda na síntese de malato, e existe o ciclo B que parte da redução do oxaloacetato

à oxidação de isocitrato. O ciclo A ocorre de três a cinco vezes mais rápido comparado ao ciclo B (**Figura 2**), o que propicia cineticamente a utilização de α-cetoglutarato como substrato energético.

O glutamato pode ser o substrato de diferentes rotas metabólicas vinculadas ao consumo ou síntese de ATP (Yelamanchi *et al.*, 2016). Após a captação de glutamina, a mesma pode ser convertida no citosol à glutamato, pela atividade da glutaminase (GLS), e ser transportado à matriz mitocôndria para ser oxidado pela ação das enzimas glutamato-desidrogenase (GDH) e aspartato-aminotransferase (AST), tendo como produto o α-cetoglutarato (Mckenna *et al.*, 2016). Estudos recentes utilizando técnicas de siRNA indicam uma colaboração maior da enzima GDH para a oxidação do glutamato quando comparado a colaboração da enzima AST (Yu *et al.*, 1982; Karaca *et al.*, 2015; Nissen *et al.*, 2015; Schousboe, 2017). Caso acetil-CoA não seja um fator limitante do ciclo de Krebs, a oxidação de glutamato via GDH permite utilizar o esqueleto de carbono desta molécula para fins anapleróticos, maximizando a eficiência de oxidação mitocondrial (**Figura 2**). Para que acetil-CoA não seja um fator limitante, outras duas vias metabólicas são fundamentais. A primeira ocorre tanto no estado alimentado, quanto no jejum e foi previamente representado na **Figura 1**. Decorrente da glicólise no astrócito, os níveis de lactato se elevam, sendo transportados para o espaço extracelular, captado pelo neurônio e convertido a piruvado pela enzima lactato-desidrogenase (LDH), fornecendo piruvato, por conseguinte, acetil-CoA ao ciclo de Krebs (Cruz *et al.*, 2012). Já em casos de jejum, o metabolismo hepático de moléculas de ácidos-graxos elevam os níveis de acetil-CoA na matriz mitocondrial de células deste órgão por um processo intitulado β-oxidação (Houten *et al.*, 2016). Os acetil-CoA disponíveis na matriz mitocondrial poderão ser oxidados, mas seu excesso pode ser convertido a acetoacetato pela atividade da enzima acetoacetato-descarboxilase ou β-hidroxibutirato pela ação da enzima β-hidroxibutirato-desidrogenase (BDH) consumindo NADH nesta reação (Wust *et al.*, 2017). Os corpos cetônicos acetatoacetato e β-hidroxibutirato são liberados na corrente sanguínea a passam a barreira hemato-encefálica, sendo reconvertidos a acetil-CoA no SNC (Egan e D'agostino, 2016). Uma vez que a β-oxidação produz NADH em excesso, os corpos cetônicos presentes no plasma tendem a apresentar uma maior presença de β-hidroxibutirato, o qual a ser convertido a acetil-CoA libera NADH para o metabolismo energético (Gorman, L. *et al.*, 2016). A **Figura 2** representa o impacto do lactato e dos corpos cetônicos sobre a capacidade de oxidação máxima mitocondrial.

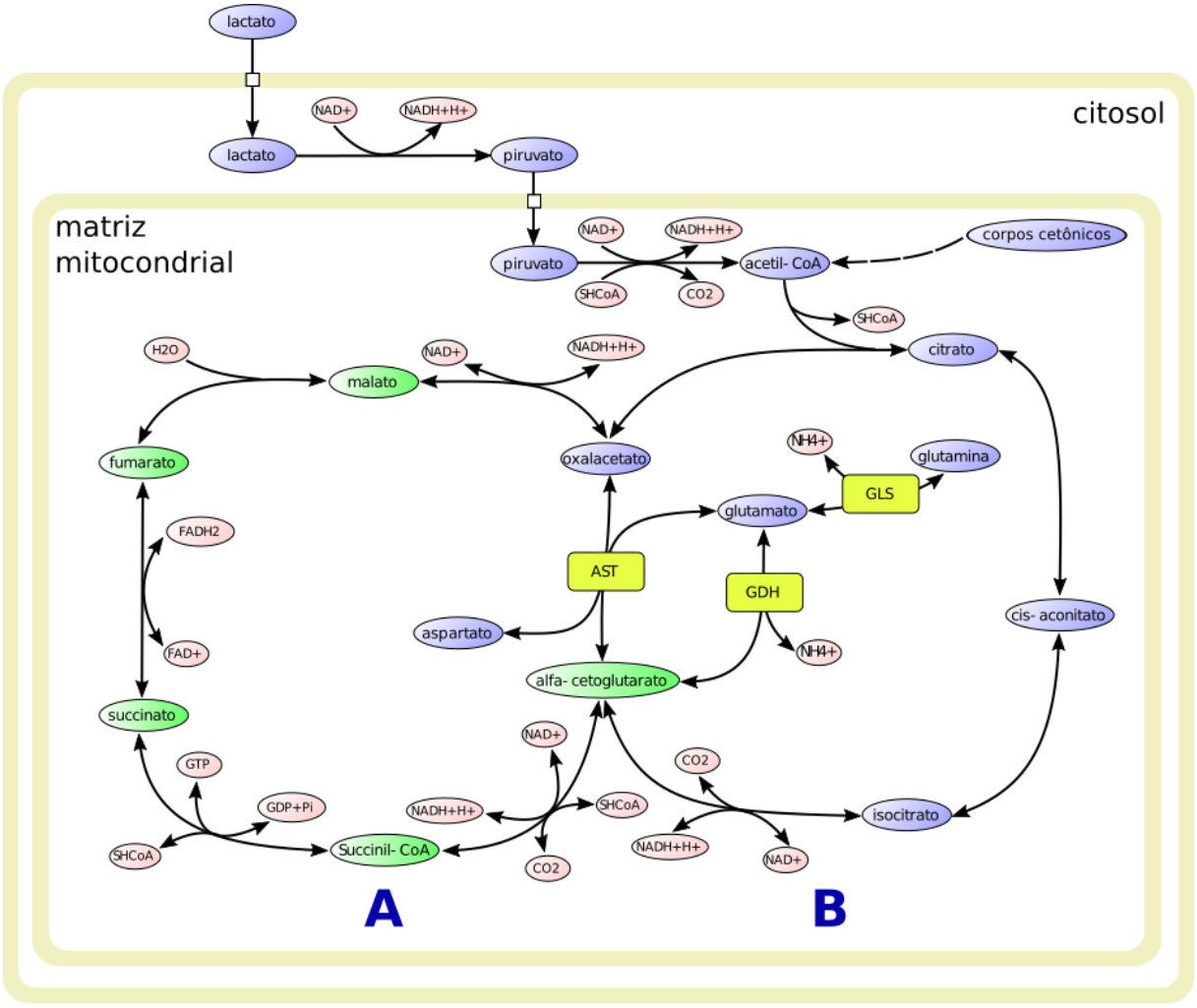

Figura 2. Eficiência máxima do ciclo de Krebs em células do SNC. Acima encontra-se a representação cinética do ciclo do ácido tricaboxílico (TCA). A cinética de reações do ciclo A é de 3 a 5 vezes maior comparada a cinética do ciclo B. Os esqueletos de carbono provenientes de piruvato, corpos cetônicos, glutamato e glutamina alimentam o TCA. Enzimas importantes para a compreensão da proposta desta tese encontram-se em Amarelo: glutamato desidrogenase (GDH); glutaminase (GLS); aspartato aminotransferase (AST). Em preto as vias enzimáticas reversíveis e não reversíveis do TCA. Os ciclos A e B encontram-se em cinética máxima de atividade (Nissen *et al.*, 2015), sendo abastecidos por esqueletos de carbonos provenientes da síntese de acetil-CoA e síntese de α-cetoglutarato (Yudkoff *et al.*, 1994; Panov *et al.*, 2009). A glutamina convertida a glutamate pela GLS (Marquez *et al.*, 2016), o lactato convertido a piruvato pela enzima LDH (Valvona *et al.*, 2016), e o β-hidroxibutirato é convertido a acetil-CoA pela atividade da enzima BDH (Achanta e Rae, 2017) (figura feita por Ben Hur Marins Mussulini).

Durante quadros hipoglicêmicos, a atividade da AST na oxidação de glutamato é imperativa (Skytt *et al.*, 2012). Essa forma de oxidação truncada inicia pela transaminação do glutamato a α-cetoglutarato pela atividade da AST consumindo níveis de oxalacetato e permitindo que o ciclo A permaneça ativo, uma vez que o processo final de oxidação do glutamato será a formação de uma nova molécula de oxaloacetato (Parpura *et al.*, 2017) (**Figura 3**). Em contra partida o prejuízo energético frente à completa oxidação do glutamato limita a capacidade energética da célula. Neste sentido, é provável que fisiologicamente tanto o ciclo A quanto B estejam

funcionando e ao mesmo tempo essa representação da oxidação do glutamato seja utilizada para fins anabólicos, e em astrócitos seja dependente da enzima piruvato-carboxilase (PC) (Brekke *et al.*, 2016).

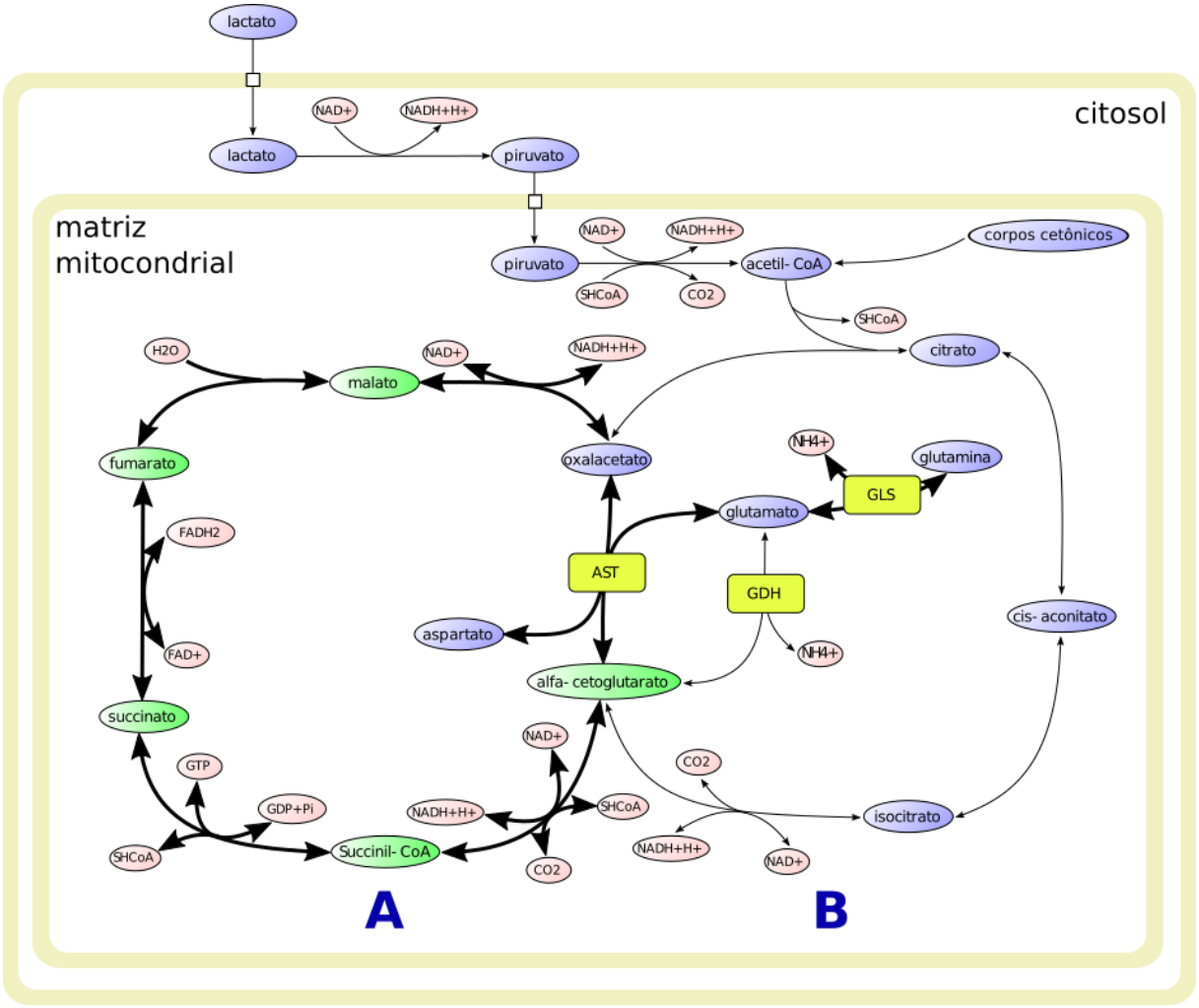

Figura 3. Oxidação truncada do glutamato em SNC. Acima encontra-se a representação cinética do ciclo do ácido tricaboxílico (TCA). A cinética de reações do ciclo A é de 3 a 5 vezes maior comparada a cinética do ciclo B. Os esqueletos de carbono provenientes de glutamato e glutamina alimentam o TCA. Enzimas importantes para a compreensão da proposta desta tese encontram-se em amarelo: glutamato desidrogenase (GDH); glutaminase (GLS); aspartato aminotransferase (AST). Em preto as vias enzimáticas reversíveis e não reversíveis do TCA. Setas tênues de coloração preta indicam atividade enzimática reduzida. O ciclo A encontra-se com cinética máxima de atividade (Nissen *et al.*, 2015), sendo abastecido por esqueletos de carbonos provenientes da síntese de α-cetoglutarato (glutamato) (Yudkoff *et al.*, 1994; Panov *et al.*, 2009). O glutamato é utilizado como fonte energética no ciclo truncado mediante consumo de oxaloacetato pela atividade da AST. Nos astrócitos a atividade da piruvato carboxilase (PC) seria o suficiente para repor os esqueletos de carbono do oxaloacetato, contudo esta enzima não encontra-se em neurônios, reduzindo a contribuição energética do glutamato para o TCA (Parpura *et al.*, 2017) (figura feita por Ben Hur Marins Mussulini).

Na presença de níveis reduzidos de piruvato intracelulares, seja por alterações glicolíticas, seja por uma falha no sistema de intercâmbio de lactato entre astrócito e neurônio (**Figura 1**), a completa oxidação do glutamato estará sujeita à atividade da enzima málica (Sonnewald, 2014) (**Figura 4**). Uma vez que o ΔG^0 da enzima malato desidrogenase é positivo, e dependente do ΔG^0 negativo da enzima citrato sintase, os níveis de

acetil-CoA estarão reduzidos, haverá um acúmulo de malato na matriz mitocondrial. Neste sentido, malato será exportado ao citosol e clivado a piruvato pela enzima málica (ME), permitindo a completa oxidação do glutamato. Este processo produz 25 mol de ATP por mol de glutamato ao comparar-se com o ciclo truncado, o qual produz 10 mol de ATP por mol de glutamato. Além disso, os baixos níveis de oxaloacetato na matriz mitocondrial neuronal, não inibirão a succinato desidrogenase (SDH), permitindo a continuidade da oxidação.

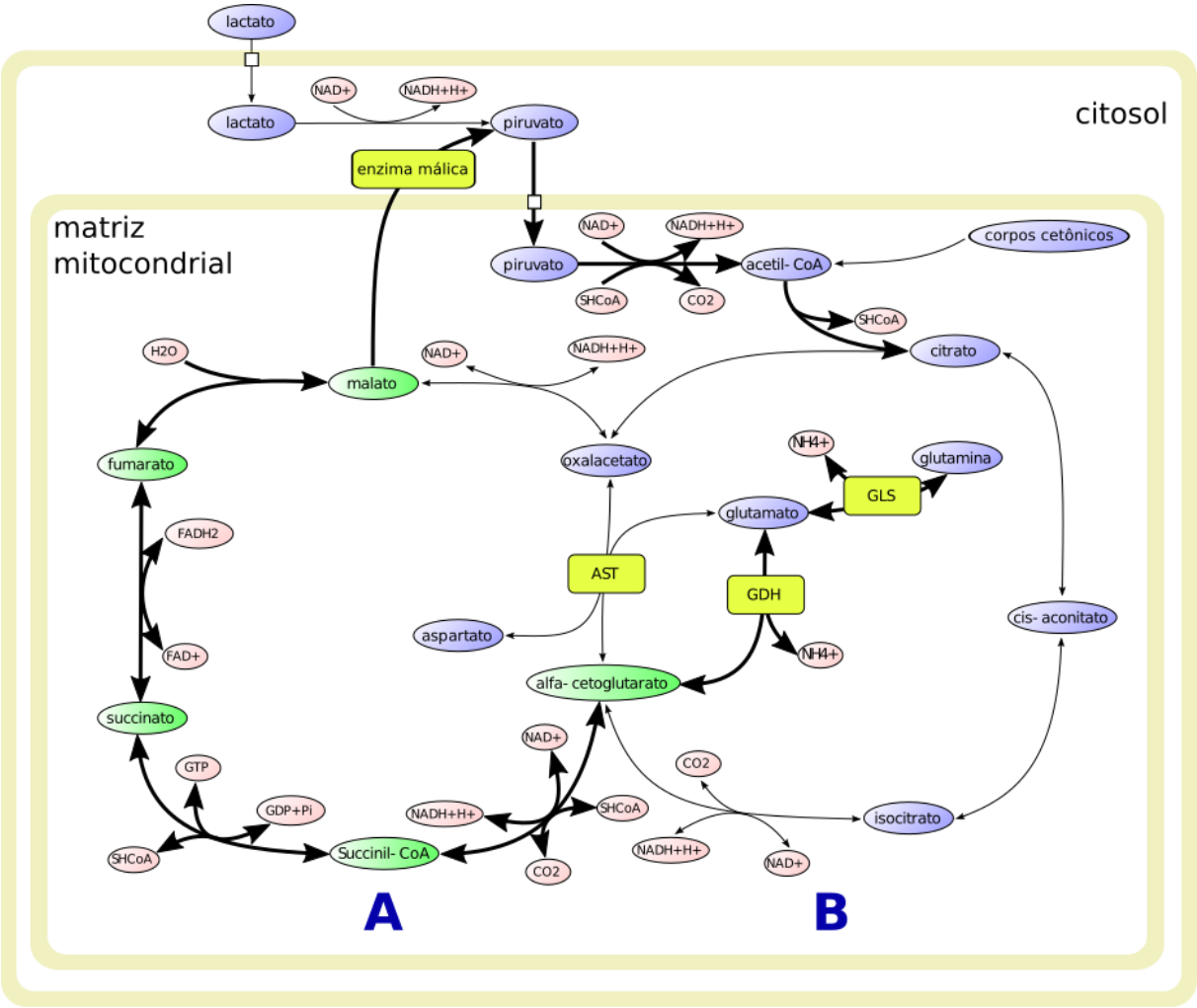

Figura 4. Completa oxidação do glutamato em SNC. Acima encontra-se a representação cinética do ciclo do ácido tricaboxílico (TCA). A cinética de reações do ciclo A é de 3 a 5 vezes maior comparada a cinética do ciclo B. Os esqueletos de carbono provenientes de glutamato e glutamina alimentam o TCA. Enzimas importantes para a compreensão da proposta desta tese encontram-se em amarelo: glutamato desidrogenase (GDH); glutaminase (GLS); aspartato aminotransferase (AST). Em preto as vias enzimáticas reversíveis e não reversíveis do TCA. Setas tênues de coloração preta indicam atividade enzimática reduzida. O ciclo A encontra-se com cinética máxima de atividade (Nissen *et al.*, 2015), sendo abastecido por esqueletos de carbonos provenientes da síntese de α-cetoglutarato (glutamato). Para que os cinco carbonos do glutamato sejam convertidos a cinco moléculas de CO_2, o glutamato é metabolizado pela atividade da GDH e o malato proveniente da oxidação do ciclo A é exportado ao citosol. Uma vez fora da matriz mitocondrial, o malato é convertido a piruvato pela atividade da enzima málica (ME) citosólica. O piruvato então adentra a matriz mitocondrial, completando a oxidação do glutamato pelo TCA (Parpura *et al.*, 2017) (figura feita por Ben Hur Marins Mussulini).

I.6. Incongruência energética de crises epilépticas prolongadas

Apesar de representar apenas 2% da massa corporal, o cérebro consome 20% do oxigênio (O_2) e 25% da glicose diária (Belanger *et al.*, 2011). Para concluir os processos fisiológicos basais da atividade cerebral em uma frequência de despolarização de 4 Hz estima-se um consumo de 30 a 50 μg ATP/g/min (Hardie *et al.*, 2012). Ao avaliar a frequência de despolarização neuronal em caso de crises epilépticas, a mesma pode elevar-se a 30 Hz (Truccolo *et al.*, 2011), esperando-se um aumento do consumo de ATP (Gorman, G. S. *et al.*, 2016). O metabolismo cerebral está relacionado ao controle de crises epilépticas e processos epileptogênicos (Rowley *et al.*, 2015). Por exemplo, a glicólise pode estar acentuada durante atividade cerebral ictal, momento eletrofisiológico de hiperatividade, e apresentar uma redução na atividade dessa via metabólica durante momentos interictais (Stafstrom *et al.*, 2008), como resultado de uma diminuição bioenergética mitocondrial (Lee *et al.*, 2012). Crises epilépticas são um sintoma comum em pacientes afetados por doenças mitocondriais (Liang *et al.*, 2012), associadas a alterações no sistema transportador de elétrons (STE) (Tenney *et al.*, 2014). Por outro lado, a glicose não seria o principal substrato energético para a manutenção de crises epilépticas prolongadas, tendo em vista a capacidade glicolítica restrita dos neurônios (Zsurka e Kunz, 2015). Além disso, pacientes afetados pela síndrome de disfunção dos transportadores de glicose apresentam crises epilépticas, mesmo com mitocôndrias saudáveis (Larsen *et al.*, 2015). Pacientes epilépticos refratários quando avaliados por emissão de pósitrom por 18F-fluorodesoxiglicose apresentam um hipometabolismo glicolítico ipslateral ao foco epiléptico (Hodolic *et al.*, 2016). Durante crises epilépticas prolongadas há redução dos níveis de glicose encefálicos conforme a crise epiléptica tende a se estender (Theodore *et al.*, 2004). Deste modo, o papel de outros substratos energéticos deve ser vital à manutenção de crises epilépticas prolongadas (Aas *et al.*, 1993; Mckenna, 2012; Mcnally e Hartman, 2012).

Após um potencial de ação, as concentrações de glutamato elevam-se na fenda sináptica a concentrações de até 1 mM e após 10 ms retorna a 20 nM (Dzubay e Jahr, 1999; Katagiri *et al.*, 2001). A captação de glutamato ocorre contra gradiente valendo-se da atividade da bomba Na^+/K^+ ATPase (Danbolt, 2001), a qual consome 50% do ATP cerebral (Danbolt *et al.*, 2016) (**Figura 1**). Em um estudo recente de nosso grupo de pesquisa a captação de glutamato foi avaliada após a indução de *status epilepticus* (SE) por Li-Cl-pilocarpina em fatias de hipocampo de ratos jovens (De Oliveira *et al.*, 2011). As fatias de hipocampo foram expostas a duas concentrações de glutamato para o experimento de captação deste neurotransmissor, a primeira concentração foi de 1 μM e a segunda concentração foi de 100 μM. A captação de glutamato frente a primeira concentração encontrou-se diminuída, contudo, frente à segunda concentração testada, a captação de glutamato

não apresentou alterações quando comparado ao controle. A captação (Zimmer *et al.*, 2017) e oxidação (Schousboe, 2017) de glutamato modulam a captação de glicose. Além disso, a completa oxidação de glicose pode ocorrer mesmo em um momento de hipometabolismo da glicose (Parpura *et al.*, 2017). Estaria o glutamato em altas concentrações na fenda sináptica sendo captado para manter energeticamente a crise epiléptica prolongada (Schousboe *et al.*, 2011; Sarikaya, 2015; Hodolic *et al.*, 2016)?

Além do glutamato, outros substratos energéticos estão presentes no SNC. Estudos *in vitro* que avaliam o impacto metabólico da glutamina utilizam uma janela de concentrações entre 0,1 e 0,5 mM (Simpson e Sherrard, 1969). O lactato extracelular pode aumentar de 1 a 10 mM em episódios de crises epilépticas prolongadas (Orringer *et al.*, 1977); níveis de corpos cetônicos na concentração de 0,7 mM são encontrados em SNC de pacientes epilépticos sujeitos a dieta cetogênica (Seymour *et al.*, 1999). Essas mudanças metabólicas devem ser um forte indício de uma mudança de preferência de substrato energético do SNC frente a crises epilépticas prolongadas.

I.7. Mitocôndria e *Danio rerio*

A mitocôndria é resultado, provavelmente, da simbiose entre uma α-protobacteria e um eucarioto há 1,5 bilhões de anos (Steele *et al.*, 2014). O grande avanço eucariótico deve-se a esse motivo, uma vez que as novas capacidades bioenergéticas moleculares possibilitaram o custo da síntese de moléculas mais complexas, como ácidos nucleicos e lipídeos, aumentando a probabilidade do surgimento de seres multicelulares (Lane e Martin, 2010). Visto como a usina energética celular por sintetizar altas concentrações de ATP, por um processo chamado de fosforilação oxidativa (oxphos), a mitocôndria possui seu próprio material genético, o qual ao decorrer da evolução migrou em parte para o núcleo eucariótico (Gray, 2012). O DNA mitocondrial (mtDNA) é uma constante nos vertebrados e é capaz de codificar 22 RNAs de transferência, 2 RNAs ribossomais e 13 proteínas componentes do STE (Anderson *et al.*, 1981). A mitocôndria é fundamental para processos anabólicos (Rygiel *et al.*, 2016), detoxificantes (Yoshimi *et al.*, 2016), sinalizatórios (Mills e O'neill, 2016), tamponantes (Del Arco *et al.*, 2016), apoptóticos (Zhao *et al.*, 2016) e catabólicos (Iommarini *et al.*, 2017), os quais podem ser associados com a formação de espécies reativas de oxigênio (ROS) resultante de disfunções no oxphos (Yu *et al.*, 2017) (**Figura 5)**. O mtDNA do peixe-zebra se assemelha ao humano em termos genéticos e funcionais (Broughton *et al.*, 2001). A utilização do peixe-zebra para estudar patologias mitocondriais é de grande relevância (Sasagawa *et al.*, 2016) e a continua validação de construto envolvendo modelos patológicos nesta

espécie é imprescindível para o aumento do impacto translacional desse modelo animal (A roadmap for precision medicine in the epilepsies, 2015).

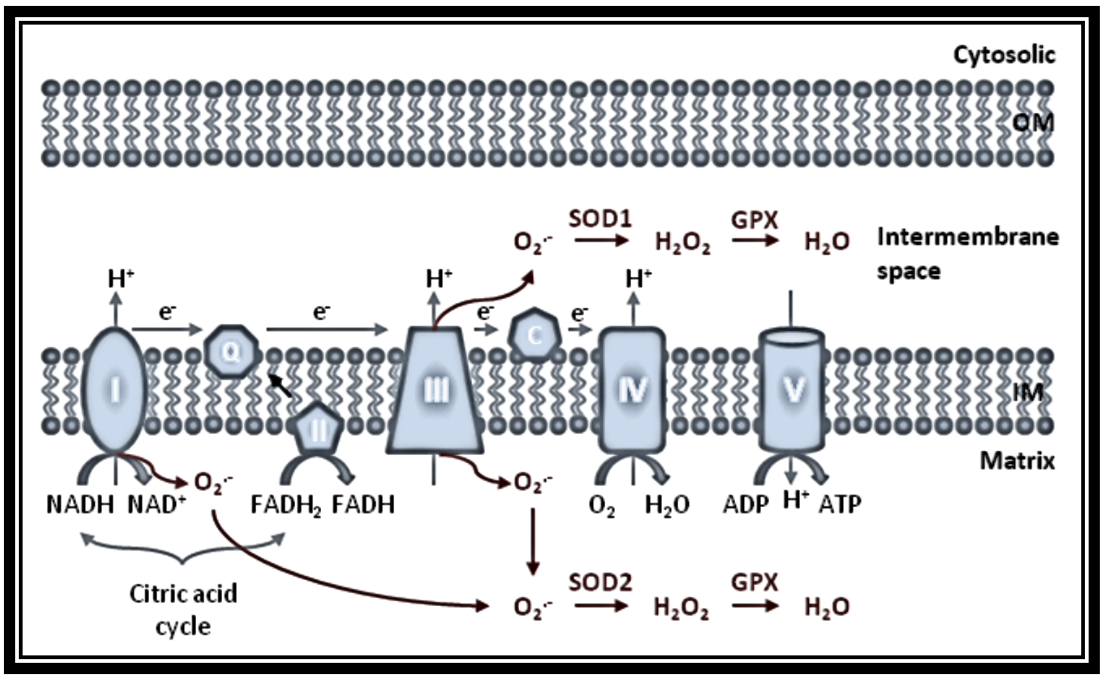

Figura 5. Contribuição do Sistema Transportador de Elétrons (STE) para a produção de espécies reativas de oxigênio (ROS). Da esquerda para a direita na membrana interna mitocondrial (IM), estão representados: Complexo I (I); coenzima-Q (Q); Complexo II (II); Complexo III (III); Citocromo C (C); ATPsintase (V); prótn (H^+); elétron (e^-). Tanto o I quanto o III contribuem para a produção de superóxido ($O_2^{\cdot-}$), o qual é detoxificado pelas enzimas superoxidodismutases (SODs), ao converte-lo em peróxido de hidrogênio (H_2O_2) no espaço intermembrana (SOD1 presente entre IM e membrana citosólica (CM)) e na matriz mitocondrial (SOD2). Por sua vez o H_2O_2 será convertido a H_2O pela enzima glutationa peroxidase (Gpx). O STE é alimentado de e- provenientes do NADH e $FADH_2$ produzidos no TCA, e convertidos a NAD^+ e FADH pelos I e II, respectivamente. O gradiente de H^+ no espaço intermembrana é formado pela atividade do I, III e IV, o qual é consumido pela atividade de V, utilizando a força protomotriz para a síntese de ATP. Para manter a força protomotriz, os H^+ que retornam a matriz mitocondrial são convertidos a água (H_2O), pela ação do IV com consumo de oxigênio (O_2), (modificado de (Zhuo *et al.*, 2012)).

Entre as diferentes técnicas em potencial para elucidar o impacto de diferentes substratos energéticos sobre o metabolismo cerebral encontra-se a respirometria de alta resolução. O aparelho Oxygraph-2K Oroboros® permite inferir o consumo de O_2 em tempo real da amostra em relação às seguintes variáveis: atividade dos Complexos I-IV; atividade da ATPsintase; consumo de O_2 máximo; H^+-*Leak*; capacidade de reserva; consumo de O_2 residual (ROX); e o impacto de substratos energéticos sobre o metabolismo celular (Davuluri *et al.*, 2016). Em suma, o equipamento disponibiliza um microambiente de temperatura, pressão e concentração de O_2 controlados, frente à utilização de um meio de incubação específico. Caso o preparado celular da amostra seja de dissociados cerebrais em um meio contendo apenas glicose, será possível inferir o quanto a amostra utiliza deste substrato para a produção de ATP acoplado ao consumo de O_2. O mesmo raciocínio é feito ao adicionar outros substratos energéticos durante o experimento (**Figura 6**).

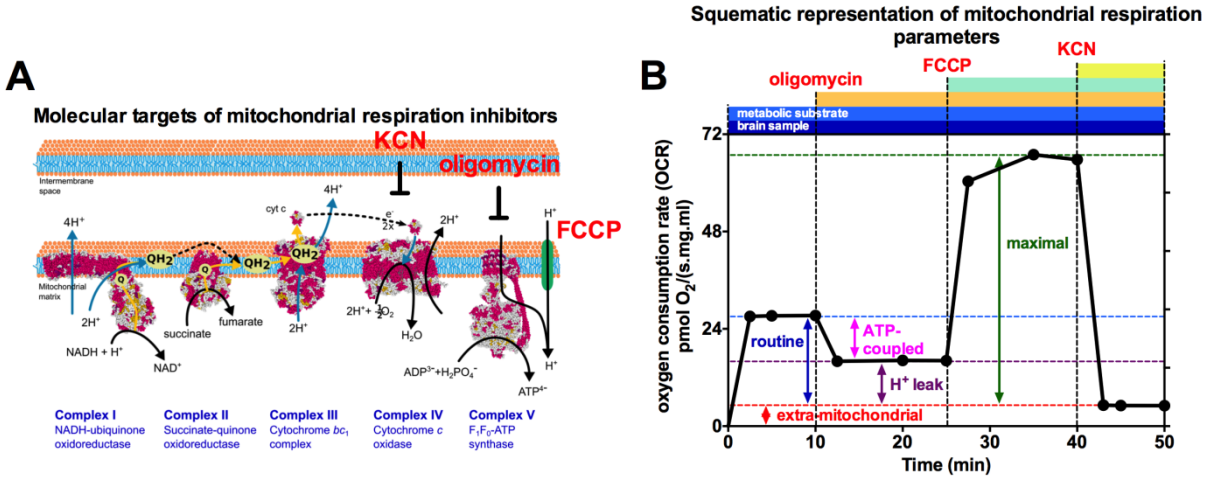

Figura 6. Impacto da modulação farmacológica sobre o consumo de O_2. (**A**) A estrutura de cada complexo respiratório presente na membrana interna mitocondrial foi obtida conforme descrito: NADH-ubquinona oxiredutase (complexo I) do organismo *Thermus thermophilus* (PDB ID 4HEA), succinato-quinona oxiredutase (complexo II) do organismo *Escherichia coli* (PDB ID 2WDV), complexo citocromo bc_1 (complexo III) do organismo *Bos taurus* (PDB ID 1PP9), citocromo *c* oxidase (complexo IV) do organismo *Bos taurus* (PDB ID 3AG4), F_1F_O-ATPsintase (complexo V) do organismo *Escherichia coli* (PDB ID 5T4Q), e citocromo *c* do organismo *Eqqus caballus* (PDB ID 1HRC). Oligomicina (Olig) é o inibidor do complexo V, carbonil cianido-p-trifluoromethoxifenilhidrazona (FCCP) é um desaclopador da membrana interna mitocondrial, cianeto de potássio (KCN) é o inibidor do complexo IV. (**B**) Taxa de consumo de oxigênio (OCR) é avaliado antes e depois da adição dos fármacos descritos em **A**. A adição de cada composto está detalhado na parte superior do gráfico. O consumo de O_2 de rotina está representado em azul, o consumo de O_2 acoplado a síntese de ATP está representado em rosa, o consumo de O_2 máximo está representado em verde, o consumo de O_2 acoplado ao vazamento de H^+ está representado em roxo, e o consumo de O_2 residual (ROX) está representado em vermelho (figura feita por Diogo Losch de Oliveira).

I.8. Efeito Janus das mudanças metabólicas do glutamato

A completa oxidação do glutamato com reduzidas concentrações de piruvato intracelular pode ser prejudicial à fisiologia celular (Panov *et al.*, 2009) (**Figura 4**). A redução dos níveis de oxaloacetato na matriz mitocondrial resultante deste processo de oxidação pode elevar o ROS, uma vez que a SDH não sofrerá inibição por oxaloacetato (Zeylemaker *et al.*, 1969). A SDH é uma fração do complexo II mitocondrial, o potencial acúmulo de elétrons neste complexo pode levar a um processo chamado de transporte reverso de elétrons (Scialo *et al.*, 2016), no qual os elétrons migram do complexo 2 para o complexo 1, propiciando a reação de Fenton (Mittler, 2017). O resultado é o aumento da atividade das enzimas SOD e da Gpx, uma vez que os níveis de catalase (CAT) no SNC são irrisórios (Sani *et al.*, 2006; Gabryel *et al.*, 2016). Para que a glutationa (GSH) seja eficiente como defesa antioxidante, sua reciclagem pela atividade da enzima glutationa reductase (GR) é fundamental, a qual consome NADPH (Massarsky *et al.*, 2017). O H_2O_2 é capaz de modular a glicólise, aumentando indiretamente a atividade da glicose-6-fosfato-desidrogenase (G6PDH) e inibindo as enzimas glicerol-3-fosfato-desidrogenase (GAPDH) e piruvato-cinase (PK) (**Figura 7**). Logo, a redução do aporte de

glicose como uma moeda metabólica para produção de ATP, no intuito de acelerar a via das pentoses-6-fosfato, seria fundamental para a produção de NADPH e redução de ROS (Lloret *et al.*, 2016).

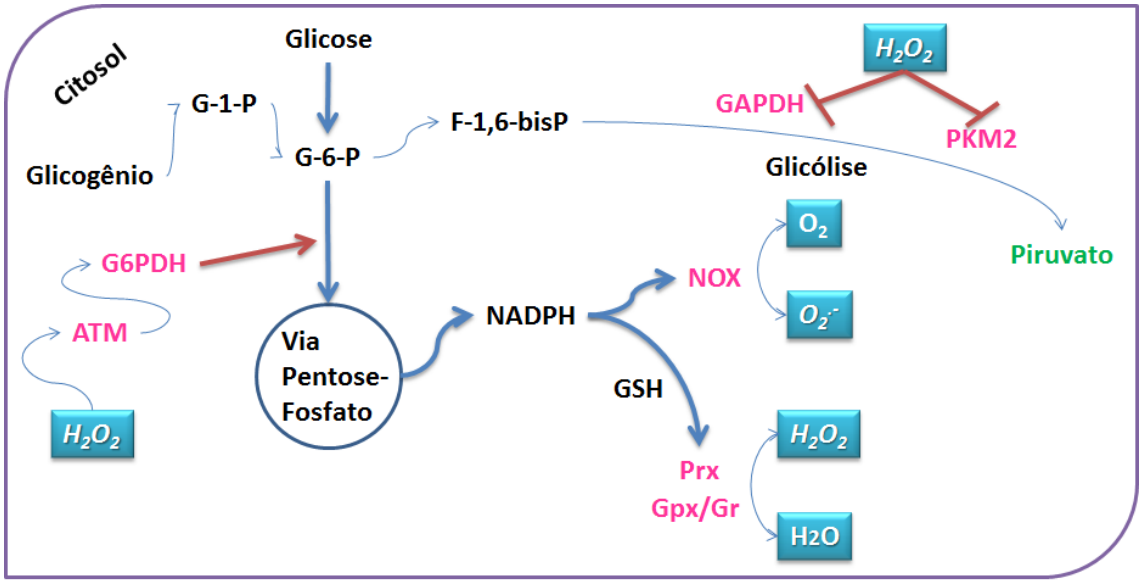

Figura 7. Regulação da glicólise por ação do peróxido de hidrogênio (H_2O_2). Representado em roxo um citosol celular genérico. Com o aumento da presença de espécies reativas de O_2 (ROS) a concentração intracelular de H_2O_2 eleva-se causando dano ao DNA. A enzima Ataxia telangiectasia cinase (ATM) presente no núcleo celular, é exportada ao citosol mediante dano oxidativo do DNA. A ATM ativa a glicose-6-fosfato-desidrogenase (G6PDH), a qual desvia a glicose-6-fosfato (G-6-P) da glicólise para a via das pentose-fosfato, aumentando a produção de NADPH, para maior eficiência de sistemas antioxidantes como por exemplo o balanço de glutationa (GSH) e o balanço de atividade enzimático da glutationa peroxidase (Gpx) e glutationa redutase (Gr), peroxidoxina (Prx) e NADPH oxidase (NOX). Concomitante, a oxidação promovida pelo H_2O_2 sobre as enzimas gliceraldeido-3-fosfato-desidrogenase (GAPDH) e piruvato-cinase (PKM2), inibe a atividade de ambas enzimas resultando em uma redução da atividade glicolítica celular (modificado de (Shiloh e Ziv, 2013; Kang *et al.*, 2015)).

I.9. Hipótese

Perante o desequilíbrio redox que há em crises epilépticas, o papel do metabolismo da glicose conectado a via das pentoses-fosfato, a redução do aporte desta molécula, e o aumento no consumo de ATP para a manutenção do evento epiléptico, hipotetizou-se a possibilidade do SNC central utilizar outras moléculas presentes no mesmo como fonte majoritária de produção de ATP ao invés da Glicose.

I.9. Objetivo Geral

Investigar a bioenergética cerebral do modelo de crises epilépticas prolongadas induzida por pentilenotetrazol em peixe-zebra adulto.

I.10. Objetivos Específicos

- Delimitar os diferentes modelos de crises e síndromes epilépticas em peixe-zebra.
- Avaliar a contribuição da D-glicose, L-glutamato, L-glutamina, L-lactato, e β-hidroxibutirato sobre a manutenção metabólica mitocondrial cerebral pela medida de consumo de O_2 para a produção de ATP em crises epilépticas prolongadas induzidas por pentilenotetrazol em peixe-zebra adulto.
- Avaliar o balanço redox de cérebro de peixe-zebra adulto, exposto a pentilenotetrazol 10 mM por 20 min.

Parte II

II.1. Capítulo I

Zebrafish as an Animal Model to Study Epileptic Seizures and Epileptic Syndromes

Ben Hur Marins Mussulini*, Suelen Baggio, Luana Moro, Renato Dutra Dias, Maria Elisa Calcagnotto, Eduardo Pacheco Rico and Diogo Losch de Oliveira*

Revisão publicada no periódico: Current Psychopharmacology, 2016, 5, 000-000.

Tema:

Revisão das dificuldades atuais enfrentadas na área terapêutica relacionada à epilepsia e como utilizar as potencialidades do peixe–zebra para superar os altos índices de epilepsias criptogênicas, falhas terapêuticas, refratariedades e comorbidades comportamentais associadas à epilepsia.

Principal conclusão:

Apesar dos avanços em utilizar o peixe-zebra como modelo animal para o estudo das crises epilépticas e síndromes epilépticas, uma grande parcela da literatura científica foca em realizar validações de face e predição, sem estudar os mecanismos patológicos de cada modelo em peixe-zebra dificultando um comparativo translacional e ressaltando a importância de novos trabalhos focando validações de construto.

Contribuição à formação do aluno:

Escrever um artigo de revisão expande a compreensão do modelo animal além de potencializar a capacidade do aluno em defender pontos de vistas de forma crítica.

Objetivo:

Selecionar o modelo de crises epiléptica prolongada em peixe-zebra para investigar as mudanças na bioenergética cerebral que potencialmente contribuiriam para a manutenção deste evento.

Send Orders for Reprints to reprints@benthamscience.ae

Current Psychopharmacology, **2016,** *5,* **000-000**

REVIEW ARTICLE

Zebrafish as an Animal Model to Study Epileptic Seizures and Epileptic Syndromes

Ben Hur Marins Mussulini[1*], Suelen Baggio[1], Luana Moro[1], Renato Dutra Dias[1], Maria Elisa Calcagnotto[2], Eduardo Pacheco Rico[3] and Diogo Losch de Oliveira[1,*]

[1]Laboratory of Cellular Neurochemistry - Universidade Federal do Rio Grande do Sul, Programa de Pós-Graduação em Ciências Biológicas - Bioquímica, Instituto de Ciências Básicas da Saúde, Departamento de Bioquímica, Porto Alegre, RS, Brazil; [2]Laboratório de Neurofisiologia e Neuroquímica da Excitabilidade Neuronal e Plasticidade Sináptica - Universidade Federal do Rio Grande do Sul, Programa de Pós-Graduação em Ciências Biológicas - Bioquímica, Instituto de Ciências Básicas da Saúde, Departamento de Bioquímica, Porto Alegre, RS, Brazil and [3]Laboratório de Sinalização Neural e Psicofarmacologia - Universidade do Extremo Sul Catarinense, Programa de Pós-Graduação em Ciências da Saúde, Criciúma, SC, Brazil

Diogo Losch de Oliveira

Ben Hur Marins Mussulini

ARTICLE HISTORY

Received: February 24, 2016
Revised: May 25, 2016
Accepted: May 31, 2016

DOI: 10.2174/2211556005666160615090734

Abstract: ***Background:*** Epilepsy is a chronic disorder of the brain. Globally, an estimated 2.4 million people are diagnosed with epilepsy each year. Recent studies have shown that 70% of epileptic patients can be successfully treated with anti-epileptic drugs. Unfortunately, 30% of the patients still suffer from intractable epilepsy, a problem that represents a difficult scientific challenge. Crucial genetic research and high throughput drug screening are combined together to search for treatment for refractory seizures.

Goals. Development of new animal models amenable to genetic manipulation and drug screening, e.g. the zebrafish (*Danio rerio*), is an important goal and represents a promising step. In this review, we summarize general aspects of epilepsy and the advances through history, and emphasize the importance of the zebrafish as an animal model representing new research strategies leading to proper treatment of human epilepsy. We also describe the advances in research using both the larvae and adult zebrafish, and discuss the barriers which zebrafish must overcome to become a better animal model for a variety of neurological disorders, including epileptic seizures and epilepsy. **Conclusion.** Even though zebrafish larvae seem to be more attractive as a tool, adult zebrafish are also very valuable, as they can be used to study the impact of genetic manipulation and drugs on behavioral changes after seizures.

Keywords: Zebrafish, Epileptic Seizures, Epilepsy, Genetic, Comorbidity, Drug-screening.

1. EPILEPSY: GENERAL ASPECTS AND EPIDEMIOLOGY

Epilepsy is a chronic brain disorder which is characterized by an enduring predisposition to generate epileptic seizures and by the neurobiological, cognitive, psychological and social consequences of this condition [1]. By definition, epilepsy requires the occurrence of at least one epileptic seizure, which is an outcome from excessive and hypersynchronous electrical discharges in a group of neurons from one or more regions of the brain. Epileptic seizures range from short to prolonged and severe seizures, from less than 1 per year to several per day [2]. Epileptic seizures are classified into two major groups, in terms of their origin within the brain [3]. The first group is represented by focal epileptic seizures, in which only a portion of the brain is affected. This type of seizure can be subdivided to simple focal seizure vs.

*Address correspondence to these author at the Laboratory of Cellular Neurochemistry - Universidade Federal do Rio Grande do Sul, Programa de Pós-Graduação em Ciências Biológicas-Bioquímica, Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Rua Ramiro Barcelos 2600 – Anexo, Zip code: 90035-003, Porto Alegre – Brazil; Tel: + 55 51 3308 5555 Ext. 5556; E-mails: ben_hurmussulini@yahoo.com.br or losch@ufrgs.br

2211-5560/16 $58.00+.00

dyscognitive focal seizure. Simple focal seizure is characterized by slightly shifted emotions and/or moods, involuntary jerking and twitching in body parts, and unusual sensory experiences, such as seeing flashing lights, without loss of consciousness. Dyscognitive focal seizures are associated with loss of consciousness or awareness during seizure manifestation. The second group, called generalized epileptic seizures, affects both sides of the brain and it is frequently subdivided into six subgroups. Absence seizures cause brief unawareness of surroundings and actions, staring blankly until the seizure is over with possible repetitive body movement. In the past, this type of seizure is also called "petit mal". Atonic seizures cause loss of muscle tonus leading to a sudden fall or collapse. Clonic seizures lead to rhythmic, repeated jerking movements. Myoclonic seizure cause sudden jerking movements or twitches. Tonic seizures are characterized by muscular tonus increased, often leading to the patient to fall to the ground. Finally, tonic-clonic seizures, also called as "grand mal" seizures, lead to loss of consciousness, and violent tonic and clonic movements of entire body, associated with loss of sphincter control [4, 5].

Epilepsy with a known cause is called symptomatic or structural/metabolic epilepsy. The causes of symptomatic epilepsy could be brain damage from prenatal or perinatal injuries (e.g. a hipoxia or trauma during birth, low birth weight), congenital abnormalities with associated brain malformations, severe head injury, stroke, or numerous CNS disorders (e.g. meningitis, encephalitis, neurocysticercosis, and brain tumor), respectively [2, 6]. Last, the most common type of epilepsy, which affects 6 out of 10 people with epilepsy, is called cryptogenic epilepsy.

According to the World Health Organization (WHO), approximately US$ 15.5 billion is spent in the United States of America each year to treat and care for epileptic patients. It is estimated that 50 million people around the world suffer from this disorder, and 2.4 million new cases are diagnosed each year [7]. In high-income countries, annual new cases are between 30 and 50 per 100 000 people in the general population. In developing countries, the prevalence of the disease can be up to two times higher [8]. This may be due to the increased risk of endemic conditions such as malaria or neurocysticercosis, higher incidence of traffic-accidents, birth-related complications, poor medical infrastructure, few preventative health programs and low accessibility to care. As a result, 80% of epileptic patients live in developing countries [9].

More than 60% of patients respond positively to the first anti-epileptic drug prescribed, and almost all patients will become seizure-free after treatment. Two to five years after diagnosis, 50 percent of patients will be able to withdraw the anti-epileptic medication [10]. Recent studies have shown that 70% of children and adults with epilepsy can be successfully treated with anti-epileptic drugs (AEDs). Unfortunately, 30% of epileptic patients still suffer from intractable epilepsy, or develop intolerability to the available drugs [11]. Furthermore, 75% of epileptic patients who live in developing countries do not receive correct treatment, a problem known as the "therapeutic gap" [12]. Epilepsy has significant economic implications in terms of health-care needs, premature death and lost work productivity [13]. Therefore, search for new therapies to treat the 30% of refractory patients and solve therapeutic gap are important goals [14]. Accordingly to WHO, there are simple, cost-effective ways to treat epilepsy in resource-poor settings, thereby significantly reducing treatment gaps [15].

Another important point about epilepsy is prevention. Around half of people who suffer head injury will develop epilepsy. Therefore, one of the most effective prevention is to use adequate gear when riding a bike, respect transit rules, never drive under the effect of drugs, and reduce risk factors for stroke, like obesity [16]. Adequate perinatal care can reduce new cases of epilepsy. The use of drugs and other methods to decrease body temperature of a child under fever can also reduce the chance of febrile seizures. Central nervous system infections are common causes of epilepsy in tropical areas [17, 18, 19]. Unfortunately, cryptogenic epilepsy, which affects 60% of all patients, has no clear etiology. Thus, research must be done to develop new therapies and to unveil the mechanism behind idiopathic epilepsy.

2. ZEBRAFISH: GENERAL ASPECTS

Production of clones of homozygous diploid zebrafish *(Danio rerio)* introduced zebrafish to the modern science [20, 21]. In 1981, George Streisinger *et al.* were able to generate clones of homozygous fish from individual homozygotes, which facilitated genetic analyses of this verte-

brate, including the use of mutagenesis [22, 23, 24]. Subsequently, Christiane Nüsslein-Volhard published a study describing 1200 mutant zebrafish isolated in a large-scale screen [55]; and Howe *et al*. (2013) performed genome sequencing of zebrafish and correlated it to the human genome [56]. This small aquatic vertebrate has become popular in biomedical research. It is a vertebrate species with high physiological and genetic homology to humans. Its advantages include easy genetic manipulation, and similarity of its central nervous system (CNS) morphology and function to those of higher order vertebrates [27-31]. In addition, zebrafish presents a tight junction-based blood–brain barrier similar to that of higher vertebrates, with substantial macromolecule permeability, which makes this model an attractive organism for high throughput screenings and drug discovery. In addition, zebrafish possess rapid development and a relatively long lifespan [32, 33]. Therefore, they may represent an ideal species for medium- and high-throughput screens for genetic mutations and small molecules [34-37]. The close parallels between mammalian and zebrafish behavioral paradigms further increase the interest for this species for neuroscience research.

Transparency and external development of embryos and larvae allow gene expression visualization by using fluorescent probes and reporter genes [38]. The increase of molecular tools available for high-resolution live-imaging has recently been expanded to include genetically encoded fluorescent calcium indicator GCaMP proteins, which can reveal the spatio-temporal activities of excitable cells such as neurons, in intact, living zebrafish [39-42]. In addition, chemicals can be added to the holding water of the fish, which readily facilitates pharmacological interventions. All these characteristics motivated researchers to model human brain disorders using zebrafish [43].

3. ZEBRAFISH AS AN ANIMAL MODEL TO STUDY EPILEPTIC SEIZURES AND EPILEPSY SYNDROMES

The study *Genes controlling and mediating locomotion behavior of the zebrafish embryo and larva* was the first work describing behavioral alterations in zebrafish that correlated with epileptic seizures [44]. The study identified zebrafish with motor deficits and uncovered an array of mutations in potential epilepsy-linked genes [45]. After this pioneering work, several other studies have been performed for modeling genetic and non-genetic causes of epileptic seizure and epilepsy in zebrafish.

3.1. Non-Genetic Models of Epileptic Seizures

Non-genetic models of epileptic seizures have been important through history of the discovery and development of antiepileptic drugs. Usually, non-genetic models focus on the modulation of the equilibrium between excitatory and inhibitory neurotransmitter systems, i.e. increasing the activity of glutamatergic neurotransmission [46] or by decreasing the GABAergic neurotransmission [47]. In this context, the first study describing a model of epileptic seizure in larvae zebrafish used this paradigm. Pentylenetetrazole (PTZ) was the first non-genetic model for zebrafish [48]. More recently, other zebrafish seizure models were developed, such as domoic acid [49], pilocarpine [50], fever [51], and kainic acid (KA) [52]. Despite the great importance of each study, all of them evaluated epileptic seizures, but not epilepsy. Bellow we describe these non-genetic models of epileptic seizure for zebrafish.

3.1.1. Pentylenetetrazole

The first work evaluating epileptic seizures in zebrafish was published by Baraban *et al*. [48]. These authors evaluated the behavioral, electrophysiological and molecular changes that occur in larvae zebrafish by exposing it in a range of PTZ ($GABA_A$ receptor antagonist) concentrations (2.5 – 15 mM). They detected a behavioral profile similar to seizures described in three distinct stages in humans. Initially, fish dramatically increased their swim activity (Stage I), which was followed by a rapid "whirlpool-like" circling swim behavior (Stage II), and subsequent culmination of a series of brief clonus-like convulsions leading to a loss of posture, e.g. fish falling to one side and remaining immobile for 1–3 s (Stage III). Behavioral profile and latency to each behavioral measure was dose-dependent and only 75% of the animals presented stage III when immersed in 15 mM concentration of PTZ. Using a CCD camera and a video tracking software, the authors were able to observe these stages and correlated them with distance travelled. Furthermore, they have shown important changes in c-Fos expression in the brain of PTZ treated animals, and also analyzed temporal

changes in electrographic activity during seizure [48].

Berghmans *et al.* detected that the zebrafish PTZ induced-seizure model was highly sensitive to drugs that affect the brain by distinct mechanisms. Therefore, it was suggested that zebrafish could be used for primary screening of potential anti-seizure drugs. In order to perform the first AED high-throughput screen in zebrafish, they used a multi-well plate with different drugs in each well containing a zebrafish. Different from rodents, which each model respond to few AEDs, the zebrafish PTZ model was shown to be responsive to almost all known first-line AED drugs. Unfortunately, many of the drugs tested are able to induce sedative effects, and the analyses were only extended to locomotor activity measurements [53]. Afrikanova *et al*. (2013) evaluated the effect of distinct AED on zebrafish PTZ model by using electrographic measurements associated with behavioral analysis. This was the first study to define a proper toxicological evaluation of the compounds [54].

Unfortunately, to our knowledge, no study has been done using pro-convulsibe drugs in association with PTZ. This type of experiment would elucidate whether distance travelled is a good parameter to measure seizure Stage III. The definition of stage III includes length of immobility associated with seizure. Thus, if a compound induces more seizures and at the same time increases the length of immobility it could be misjudged as an anti-seizure compound. For example, Baxendale *et al*. (2012) performed a concentration–response curve of PTZ for larvae zebrafish from 1.25 to 80 mM. Animals immersed in the highest concentration presented a similar distance travelled as the control group [55]. Thus, new investigations aiming to clarify whether the distance travelled is a good measure for AED activity must be performed.

Kim *et al*. [56] were able to induce three consecutive seizures by immersing adult zebrafish in PTZ at 10 mM for 30 min, and measured memory. The animals presented a decline of learning ability only after the third seizure. The first description of behavioral seizure phenotype correlated with endocrine changes was performed in the same year. It suggested a critical window of seizure evaluation at 20 min. The seizure was induced by caffeine (250mg/L; 1.3mM), PTZ (1.5g/L; 11.0mM) and picrotoxin (100mg/L; 0.17mM). The animals exhibited seizures similar to those seen in larvae (hyperactivity bouts, tonic movements, circular and corkscrew swimming). Furthermore, the authors showed increased whole-body cortisol levels after seizure, suggesting the possibility to study stress effects at postictal moment [57].In 2012, the same research group showed that animals immersed in 11 mM of PTZ displayed high expression of c-*Fos* in the brain [58]. At almost the same time, another important article was published. Connecting cognitive impairments and epilepsy, Lee *et al.* [59] published the first study reversing the decline of memory after seizure by pretreatment with valproic acid. They confirmed that in that model heat-shock protein 70 (hsp70) mRNA was overexpressed after seizure similarly to what has been found in rodent models. In the next year, neurochemical and electrophysiological studies started. Siebel *et al*. [60] performed a study showing the impact of seizure on purinergic system signaling using a range of PTZ concentrations from 5 mM to 15 mM during 20 min of exposure. Based on this concentration and time window, Pineda *et al*. [61] published the first EEG study of adult zebrafish using the PTZ seizure model. Here we draw attention to the study "*Recording the adult zebrafish cerebral field potential during pentylenetetrazole seizures",* particularly to figure 5, which shows a correlation between time to seizure onset and the PTZ concentration exposure curve. On one hand, the EEG profile showed electrographic seizure by increased brain activity after 3 min of animals exposed to 15 mM of PTZ. On the other hand, animals exposed to 7.5 mM concentration of the drug took almost 10 min to exhibit electrographic seizure activity. Thus, even though few studies claim to have performed AED screening in adult zebrafish, none performed a proper protocol of PTZ-induced seizure. Gupta *et al*. [62], showed that fish immersed in PTZ at 6 mM exhibited seizure onset around 6 min after PTZ exposure, which does not follow EEG description by Pineda *et al*., [61]. Siebel *et al*. [63], suggest rampamycin as a potential AED, but PTZ-induced seizure began within 4 min when 7.5 mM concentration of the drug was employed. In the same study, larval zebrafish were used to test the hypothesis. Unfortunately, Baraban *et al*. [48] found no seizures score III in larvae exposed to PTZ at this concentration. This may be due to over simplification of seizure-like behavior analysis in adult zebrafish. As adult zebrafish present a fully developed cen-

tral nervous system, as well as body anatomy, it is hoped that more detailed and sophisticated analyses may be performed.

Could the seizure stages of adult zebrafish be misjudged if the larval zebrafish is used to measure it? To answer this question, we needed to evaluate whether adult zebrafish exposed to PTZ displays a detailed seizure score. Mussulini *et al*., [64] showed that animals exposed to 5 to 15 mM of PTZ presented a seizure similar to what was observed in response to KA exposure [42] as follows: stage 0, short swimming mainly in the bottom of the tank; stage I, increased swimming activity and high frequency of opercula movement; stage II, burst swimming, left and right movements, and erratic movements; stage III, circular movements; stage IV, clonic-seizure-like behavior (abnormal whole-body rhythmic muscular clonus); stage V, fall to the bottom of the tank, tonic-seizure like behavior (sinking to the bottom of the tank, loss of body posture, and principally by tonic extension of the body); stage VI, death. Concentrations bellow 10 mM of PTZ presented a high variably of latency to seizure onset. Animals exposed to 5 mM of PTZ did not present immobility neither fell to the bottom of the tank after clonic-seizure like behavior, suggesting that larval stage III happens in two separate stages in the adult. Animals exposed to 10 mM presented a seizure profile similar to those observed by Pineda *et al*. [61]. In addition, PTZ brain concentration was similar to rodent PTZ model and mortality ratio was around 30% within 72 h after seizure. Therefore, for future studies the concentration of 10 mM of PTZ appears to be adequate to study seizures as well as to investigate the anticonvulsant action of selected drugs in adult zebrafish. Pagnussat *et al*. [65] have evaluated the anxiety levels and scototaxis in adult zebrafish subjected to PTZ-induced seizures (10 mM of PTZ). The authors observed a similar latency to seizure as that demonstrated by Mussulini *et al*. [64]. Moreover, the study showed that when animals were exposed to PTZ in groups of 3 (triplets) the latency to reach the stage IV was higher when compared to a single animal.

3.1.2. Domoic Acid Epileptic Seizure Model

Domoic acid (DA) is a neurotoxin produced by diatoms of the genus *Pseudonitzschia* that targets the limbic system to induce tonic–clonic seizures. Tiedeken *et al*. [49] published a study evaluating the effects of DA in zebrafish larvae. Embryos were microinjected with DA at concentrations ranging from 0.12 to 1.26 ng/mg egg weight. Seven days later, the larval animals were treated with PTZ to evaluate their sensitivity to the chemical convulsant. *In ovo* 0.4 ng/mg DA exposure reduced the latency for the first PTZ seizure in larval fish and increased the severity of seizures, which was determined by seizure staging and behavioral parameters. The seizure score used in this work was the same as described by Baraban *et al*. [48]. This approach demonstrates that *in ovo* exposure to DA may reduce the threshold to chemically induced seizures in larval fish and increase the severity of seizure. There are many published studies about DA exposure in zebrafish larvae, but the main focus was not epilepsy. In this context, Lefebvre *et al*. [67] published a study showing the impact of intracoelomic injection of DA on adult zebrafish, which resulted in a behavioral seizure profile similar to that of larval zebrafish. Authors performed a microarray analysis and observed a transcriptional profile similar to that associated with neuronal apoptosis following a putative activation of protective pathways.

3.1.3. Kainic Acid Epileptic Seizure Model

It is well established in rodent models that KA induces seizures and epilepsy through overstimulation of the excitatory system. However, the first study involving adult zebrafish was performed only in 2011. Alfaro *et al*. [42] described a new seizure profile in adult zebrafish by injecting KA intraperitonially (1-8 mg/Kg). The animals presented the following responses: Stage I, immobility and hyperventilation of the animal; Stage II, whirlpool-like swimming behavior; Stage III, rapid movements from right to left; Stage IV, abnormal and *spasmodic muscular contractions*; Stage V, rapid whole-body clonus-like convulsions; Stage VI, sinking to the bottom of the tank and *spasms* for several minutes; Stage VII, death. Seizure profile and lethality indicates that 6 mg/Kg is an adequate dose in this model. Even though the temporal profiles of seizure behavioral manifestation induced by PTZ present a different sequence of score from KA model all manifestations are similar. It indicates similar brain regions were activated in a temporally different manner, suggesting the importance of electrophysiology studies involving neurocircuitry to elucidate the trigger mechanism of seizure in zebrafish. In the same study, this research group showed that the neurotransmission pathway involving the induction of epileptic seizure was conserved between

zebrafish and rodents, because animals pretreated with antagonists of the glutamatergic system (MK-801, DNQX) were able to block the seizure behavior manifestation. The innovation of the study regarding the use of a new epileptic model using zebrafish is as important as the use of compounds injected intraperitonially. The possibility to avoid drug getting in contact with gills, eyes and skin connected to the reduced amount of new compound tests elevates the use of adult zebrafish to drug screening making the fish more reliable with rodent models and even less expensive.

In the last few years, non-pharmacological treatment strategies to manage refractory epilepsies are increasingly being considered, including the use of diet [68]. Thus, in 2012, the same research group evaluated the impact of the polyunsaturated fatty acid docosahexaenoic (DHA) diet supplementation on seizure sensibility evoked in adult zebrafish [69]. The results showed a reduction in the severity of seizure, and suggested the possibility of using zebrafish for compound screening during their entire life. Thus, new toxicological experiments could be designed to lower refractoriness.

In order to evaluate the effects of early-life-induced seizures on the seizure susceptibility at adulthood, Menezes *et al*. [70] have evaluated the impact of an early life KA exposure (500 μM for 30 min) on seizure tolerance after 2 months. Animals exposed to KA at 24 and 360 hpf presented no alteration in the seizure profile at 2 months later when animals were injected with 6 mg/Kg of KA. On the other hand, animals exposed to KA 196 hpf presented a higher tolerance to seizure when injected with 6 mg/Kg of KA 2 months later. Although the research question was simple, the study emphasized the use of zebrafish as an animal model for the investigation of the impact of early epileptic seizure on later development, and established a time and dose window to work with larval zebrafish. We hope to see new articles evaluating the neurocognitive changes of such alteration, as well as the impact of seizure during development on adulthood by letting the animals live for 6 mpf or more time.

Castell *et al*. [71] exposed eggs of zebrafish during 72 h to KA 100 μM in order to evaluate the neuroprotective effects of a polyphenolic extract from olive pit. Although the study suffered from lack of adequate controls, the authors have observed neurochemical alterations involving the cholinergic system (similar to those seen in the KA rodent model), which instigates many researchers to use the model to study Alzheimer's disease [72].

3.1.4. Pilocarpine Epileptic Seizure Model

Treatment with high-dose of pilocarpine, a cholinergic muscarinic agonist, induces seizures in rodents following systemic or intracerebral administration. In 2011, Vermoesen *et al*. [40] published a study about the epileptic seizure model induced by pilocarpine in zebrafish larvae. Larvae exposed to 30 mM pilocarpine presented subtle convulsive behaviors such as lurching/head banging, head-to-tail undulations, increased mouth movements, tremor, body contortions, and loss of posture. The objective of the study was to test antidepressants as potential anti-seizure compounds. Citalopram, bupropion, or reboxetine were tested and presented such effect. In 2013, Baraban [73] published a study with a simple method to record extracellular field potentials in the larval zebrafish forebrain. The method provides a robust *in vivo* read-out of seizure-like activity during exposure of larvae to 40 mM pilocarpine. Large-amplitude multi-spike burst discharge in these samples was evoked. Lopes *et al*. [74] published a study in 2016 evaluating c-Fos expression 1 day after zebrafish larvae were exposed to 60 mM pilocarpine. c-Fos was overexpressed similarly to what was found by Baraban *et al* [48]. However, no brain regions were evaluated. Even though this model has been tested from perspectives of behavioral and electrophysiological phenotypes and overexpression of c-Fos, it is important to note that the above-mentioned studies used three different pilocarpine concentrations, which suggests that a new study to evaluate these differences with a detailed dose response analysis is required.

3.1.5. Ginkgotoxin Epileptic Seizure Model

Ginkgotoxin (4-O-methylpyridoxine) is a neurotoxin naturally occurring in *Ginkgo biloba*. Various supplements from this plant are available in Japan, and widely used for alleviating medical conditions [75]. However, overuse or accidental ingestion of *Ginkgo biloba* has been reported to induce epileptic seizures, unconsciousness and irritability due to an overdose of ginkgotoxin. To investigate its effects, Lee *et al*. [76] exposed zebrafish larvae to 3 and 5 dpf to ginkgotoxin 0.2, 0.5, 1 mM for 2 hours. The behavioral profile was characterized by the following: stage 0, no or low

swimming activity; stage I, mildly increased swimming activity; stage II, whole body convulsion and misshaped, were observed similar to Baraban *et al* [48]. The toxin did not affect larvae development and morphology, however, ginkgotoxin-induced malformation of optic stalk and spinal cord neurons. GABA and Pyridoxal-5-phosphate were able attenuate the effects of Ginkgotoxin, but not the effects of PTZ. This study is an example of how zebrafish can be used to expand our knowledge of epilepsy etiology.

3.1.6. Allylglycine Epileptic Seizure Model

(D,L)-allylglycine (AG, 2-amino-4-pentenoic acid) interferes with the synthesis of GABA via a mixed-mechanism including the inhibition of glutamate decarboxilase, leading to decreased GABA levels and increased glutamine concentrations in the brain [77]. Leclercq *et al*. [78] hypothesized that zebrafish larvae could present a complex seizure behavior if immersed in (D,L)-allylglycine. The larvae were placed individually in the wells of a 96-well plate; ten larvae were used per treatment group. Allylglycine was used in the concentration range of 30–300 mM; AEDs were tested at their respective maximal tolerated concentrations (MTCs): Valproate (VPA-0.5 mM), Topiramate (TPM-200 μM), Diazepam (DZP-16 μM), Levetiracetam (LEV-10 mM), and Phenobarbital (PHT-100 μM). There was a similar seizure behavioral profile as defined by Baraban *et al*. [48], which started 2 h after the incubation with Allylglycine. Full seizure scores were observed after 8 h of exposure. Electrophysiological discharges indicated neuronal hyperactivity after 90 – 120 min of exposure with full brain behavior activity from 187 min to 480 min. Furthermore, after 90 min of exposure brain GABA levels decreased to 70% of the original concentration. Both Valproate and diazepam were able to present anti-seizure effects. The only missing point in the study was the absence of c-Fos expression analysis, which would have enabled the authors to compare their results with those of other epileptic seizure models.

3.1.7. Fever Epileptic Seizure Model

Febrile seizures are triggered by a rise in body temperature (i.e., hyperthermia or fever) in the absence of CNS infection or metabolic disorder. Hunt *et al*. [51] hypothesized that activation of thermo sensitive TRPV channels in response to an increase in brain temperature might promote abnormal neuronal excitability and the subsequent exacerbation of excitability via increased glutamate-mediated synaptic transmission resulting in febrile seizures. To test it, zebrafish larvae were exposed to temperature fluctuation. Hyperthermia (HT)-induced seizures were initially associated with "ictal-like" multi-spike, large amplitude, long-duration burst discharges (>1 s) that progressed to include shorter, small amplitude, high frequency events detected by patch clamp analysis. The waveform was similar to PTZ, 4-aminopyridine or linopirdine and it had duration between 2 and 7 min. Quantitative PCR was used to assess the developmental expression profile for TRPV channels and glutamate receptor subunits, because TRPV channels are closely connected to Ca^{+2} flux and NMDA receptor activity [79]. Acute HT-induced seizures appear to involve increased activities of both TRPV4 channels and post-synaptic NMDA-type glutamate receptors, quantified by both RT-PCR and pharmacologically. GABA (1 mM) or the GABA re-uptake inhibitors nipecotic acid (1 mM) and NO-711 (100 μM) did not alter HT-induced seizure duration. RN-1734 (0.5–1 mM), a TRPV4 channel antagonist, produced a significant reduction of HT seizure activity; capsazapine (100 μM), a TRPV1 channel antagonist had no effect. MK-801 (1 mM), a competitive NMDA receptor antagonist, or ifenprodil (1 mM), a NR1 and NR2B specific NMDA receptor antagonist, significantly reduced HT-induced seizure duration. This latter study emphasized the use of NMDA receptor antagonist to control febrile seizures, and explored the correlation between temperature and an excitatory neurotransmitter system. Although the above-mentioned study has been well designed, the zebrafish larvae epileptiform activity was detected between 25-28° C. This result opposes with the international recommendations for housing and husbandry zebrafish, which recommends a temperature of 28 °C for breeding and raising fish. Furthermore, the epileptiform activity was increased under 28°C and the higher activity occurs around 33°C. Thus, more research regarding this model must been performed, and an analysis seizure-related behavior phenotypes should be added [80].

3.2. Genetic Models of Epileptic Seizures and Epilepsy Syndromes

Teng *et al*. [81] published the first study using morpholinos in zebrafish to study genetic condi-

tions of epilepsy. This study and subsequent ones including those on Angelman syndrome [82], Lowe syndrome[83] and Dravet syndrome[84] began to reinforce the potential of zebrafish not only to study genetic aspects of epilepsy, but also as a model for drug screening, which would be quite difficult in rodent models [85]. The continued high-throughput mutagenesis research efforts can lead us to additional epilepsy phenotypes resulted from clinically relevant genes [86, 87]. The morpholino approach is effective during early development and allows rapid manipulation of genes related to epilepsy, including Kcnq92, Lgi1 [81] and Chd2 [88]. Bellow we describe genetic models of epileptic seizure and epilepsy syndromes using zebrafish.

3.2.1. Knockdown of Lgi1a Gene

LGI1 gene mutations are related to a genetic disorder described as autosomal dominant partial epilepsy with auditory features- or autosomal dominant lateral temporal lobe epilepsy [89]. LGI1 encodes a secreted protein which contains a leucine-rich repeat (LRR) domain flanked by cysteine clusters at the N-terminal end and a beta-propeller repeat in the C-terminal region [90].

Teng *et al*. [81], employed 'morpholinos' (MO), modified antisense oligonucleotides, and used them to knock down expression of lgi1a generating the first model of genetically linked epilepsy in zebrafish. Two MO-targeting strategies were designed: MO-E3 to create aberrant mRNA processing by interfering with splicing within the gene. This generates a non-functional protein by interfering with splicing and MO-ATG to interfere with translation by targeting the lgi1a initiation codon. Fish injected with high doses (3 ng) of MO-E3-showed a variety of hyperactive phenotypes, which consisted predominantly of an erratic swimming behavior typified by a tight circling motion and/or jerky directional swimming, similar to early stages of PTZ-induced seizures. In parallel, 3 ng of MO-ATG resulted in morphants displaying an even more intense hyper-activity, although 40% of these fish died by 2–3 dpf. In contrast, 1 ng of MO-ATG did not induce death, but it also did not cause behavioral/developmental abnormalities. Activity was measured over a 2h period of PTZ exposure (2.5 mM), and the results showed that average activity increased in MO-E3 morphants when compared to other groups. As important as the phenotype, the study evaluated apoptosis of brain cells, suggesting the potential of the model to study neuroprotection.

3.2.2. Mind bomb Mutant Zebrafish

Disruption of E3 ubiquitin ligase activity, leads to a failure in Notch signaling, excessive numbers of neurons, and depletion of neural progenitor cells [91]. Because developmental brain abnormalities are recognized as an important feature of childhood neurological disorders such as epilepsy and autism [92], Hortophan *et al*. [82] determined whether zebrafish mutants for E3 ubiquitin ligase would display epilepsy phenotype. To do so, adult zebrafish mutant lines (mibhi904; mibta52b; tcphi3564; hdachi1618; dtlhi447) were obtained from the Zebrafish International Resource Center (ZIRC). Offspring from these mutants were sorted at 2 or 3 dpf based on morphology. Recurrent spontaneous multi-spike bursts were observed in 93% of mibhi904 mutants. Prolonged burst discharges were similar in waveform to those classified as "ictal-like" following exposure to 15 mM PTZ. Abnormal electrical discharge was not observed in gap-free recordings from tcphi3564 mutants between 3 and 6 dpf or hdachi1618 mutants at 3 dpf. Identifiable offspring from mibta52b or dtlhi447 mutants were not viable beyond 2 dpf. Behavioral changes were analyzed in mibhi904 mutants. Mutant zebrafish larvae showed stage I and Stage 3 described by Baraban *et al*. [48]. The study identified a collection of gene transcripts that may be responsible for the abnormal electrical discharge and epileptic activities observed.

3.2.3. Lowe Syndrome

Lowe syndrome is caused by mutation of gene encoding OCRL1, a type II inositol polyphosphate 5-phosphatase responsible for defects in the central nervous system, eyes and kidneys [93]. Until 2011, the mechanisms by which loss of OCRL1 leads to the phenotypic manifestations of Lowe syndrome were unclear, in part, because of the lack of an animal model that would recapitulate the disease phenotype [94]. Therefore Ramirez *et al*. [83] proposed a new animal model to study Lowe syndrome. The authors, introduced a point mutation by PCR using site-directed mutagenesis Quick change method, and then measured susceptibility to heat-induced seizures in zebrafish larvae. OCRL1-deficient zebrafish embryos were more susceptible to seizures. Electrophysiological recordings taken from the forebrain of OCRL1 mutant and control embry-

os revealed that while the temperature for seizure initiation was the same for control and mutant embryos, the OCRL1 mutants had significantly longer seizure duration than the control. Spontaneous seizure activity was not observed over the 45 min duration of the electrophysiological recordings. Deficiency of OCRL1, which was enriched in the brain, leads to neurological defects similar to those reported in Lowe syndrome patients. In OCRL1 deficient embryos, Akt/PKB signaling was reduced and there was both increased apoptosis and reduced proliferation, most strikingly in the neural tissue. Furthermore, mortality rate for the mutant in the first 3 weeks of life was 60%, while WT control group showed mortality rate of 20%. The study indicated a novel role for OCRL1 in neural development, and supported a model whereby dysregulation of phosphoinositide metabolism and clathrin-mediated membrane traffic leads to the neurological symptoms of Lowe syndrome. The contribution of the study to the field was remarkable; it finally connected the hypothesis of OCRL1 to Lowe syndrome through the use of a proper animal model, which was made possible by the ease of genetic manipulation in zebrafish. Because of potential contradiction involving seizure evoked by temperature, it would be important to replicate the study using other pro-seizure compounds.

3.2.4. Dravet Syndrome

Mutations in SCN1A gene are associated with generalized epilepsy with febrile seizures plus a more severe disorder known as Dravet syndrome (DS) [95]. SCN1A gene encodes the voltage gated sodium channel alpha-subunit NaV1.1 [96]. In zebrafish, the voltage gated sodium channel family consists of four sets of duplicated genes: scn1Laa and scn1Lab, scn4aa and scn4ab, scn5Laa and scn5Lab, and scn8aa and scn8ab, and scn1Lab, a gene that shares 77% identity with human SCN1A, and is expressed in the CNS. A zebrafish mutant for this gene was discovered previously in a chemical mutagenesis screen using the optokinetic response as an assay. These types of screens are based on inducing random point mutations using the alkylating agent N-ethyl-N-nitrosourea; the resulting mutations are typically loss-of-function and recessive. Then, reverse-transcriptase and quantitative (q) PCR revealed a decrease in mRNA expression for scn1Lab in mutant larvae at 3, 5 and 7 days post fertilization. The target of Baraban *et al*. [84] study was to characterize scn1Lab mutants at the molecular and behavioral levels, and to evaluate if the mutants exhibited spontaneous drug-resistant seizures and then used them in a novel high-throughput screening programme to identify compounds that ameliorate the epilepsy phenotype. Forebrain extracellular field recordings from paralyzed and agar-immobilized scn1Lab mutants showed frequent brief interictal-like bursts and large-amplitude, long duration, ictal-like events starting at 3 dpf and progressively becoming more prominent between 4 and 7 dpf. Mutants had elevated levels of swim activity and exhibited unprovoked seizure-like behavior consisting of whole-body convulsions and rapid undirected movement starting at 4 dpf. This behavior is similar to what is classified as Stage III seizure in larvae exposed to PTZ [48].

Spontaneous electrographic seizures were also recorded after application of different drugs (all at 1 mM concentration). Epileptiform event frequency (interictal- and ictal-like discharges) and the fractional time spent seizing in scn1Lab mutants were reduced by valproate, diazepam, potassium bromide and stiripentol, respectively. Burst durations were not significantly changed for any of these drug exposures, even though the anti-seizure effect of these drugs, the use of 1 mM is extremely high. Furthermore, first-line AEDs were not able to attenuate the seizures. Also, ketogenic diet started at 4dpf for 48h reduced seizure-like behavior and the forebrain records showed suppression of burst activity. Therefore, in this study we have the presence of a classical point in epilepsy, e.g. recurrent-seizure, and refractoriness, allowing zebrafish to be used to overcome the great problem of epilepsy field research. Besides that, a screen of 320 compounds identified a compound called clemizole that inhibits convulsive behaviors and electrographic seizures, a US Food and Drug Administration (FDA)-approved compound, which could only be tested by zebrafish characteristics.

To increase the potential use of scn1a mutant zebrafish larvae, 1000 compounds (drug library) were tested to evaluate their potential in unprovoked seizure events [97]. Moreover, other two recently suggested compounds for DS treatment (huperzine A and fenfluramine) were also screened. Animals had their behavior analyzed and EEG recorded (field recordings were obtained from forebrain structures). In the analysis of locomotor seizure behavior, two tested compounds reduced 44% the threshold for inhibition of seizure. Among the 1012 compounds screened, only 20 (or

1.97%) were found to significantly inhibit spontaneous seizure behavior in scn1Lab mutants.

Among these drugs only four were classified as positive nontoxic (visible heartbeat or movement response to stimulation) and these four compounds discussed below were further tested using an electrophysiology approach. A suppression of epileptiform electrographic discharge activity was noted in mutants exposed to dimethadione. Norfloxacin, theobromine, and cytarabine were false positives. Finally, in the locomotion assay, huperzine A failed to significantly alter scn1Lab seizure behavior at any concentration tested. In contrast, huperzine A was effective at 1 mM in the acute PTZ assay. In the locomotion assay, fenfluramine significantly reduced mutant mean swim velocity at concentrations between 100 and 500 uM; 1 mM fenfluramine was toxic in the scn1Lab and PTZ assays. The fenfluramine-treated scn1Lab mutant exhibited a suppression of spontaneous electrographic seizure discharge to levels similar to controls at 500 uM, but only a partial reduction in electrographic activity at 250 uM.

This study emphasized the use of EEG techniques even though the behavior approaches presented positive results. Although significant changes in behavior were detected, detection of such changes using video-tracking remains an issue for drug screening. Even though distance travelled has been shown to be closely related to EEG epileptic seizure activity [54], the seizure behaviors, including tremor events and all stages used to characterize larval epilepsy have not been possible to automatically record by any software. Therefore, a study screening more than 1000 compounds and selecting just some potential candidates could underestimate the effect of many other drugs.

3.2.5. CHD2 Mutation Sharing Features with Dravet Syndrome

After a whole-exome sequencing in nine Dravet-Syndrome-affected individuals, a heterozygous de novo mutation was revealed in CHD2 (encoding chromodomain helicase DNA binding protein 2) by Suls *et al*. [88]. In order to establish evidence of the implication of CHD2 in the development of epilepsy, CHD2 was knocked down in zebrafish by using targeted morpholino (MO) antisense oligomers (called E2I2-MO).

E2I2-MO showed morphological and behavioral alterations when compared to Control-MO larvae. Chd2 E2I2- MO showed abnormal motor patterns with frequent whirlpool-like movements. Occasionally, larvae also presented pectoral-fin and jaw twitching and whole-body trembling. Field-potential recordings were assessed on larval brain to confirm whether this behavior could have been a result of seizure activity. Chd2 E2I2-MO-injected larvae displayed ictal-like discharges and this spiking pattern was similar to pre-ictal discharges observed in immature hippocampi of a mouse model of temporal lobe epilepsy [98]. The study provided evidence that de novo loss-of-function mutations in CHD2 are connected to epileptic encephalopathy and generalized seizures.

3.2.6. Prickle1a Mutation Increases Seizure Sensibility

A genetic screening in epilepsy patients identified mutations in the PRICKLE locus (PK1 and PK2), suggesting an association of PK with epilepsy. Mei *et al*. [99], used the zebrafish larvae as a model and characterized pk1a function in drug induced seizures. In order to investigate the role of abnormal pk1a forms in the developing nervous system, they also explored novel aspects of pk1a function in neurite outgrowth in the retina and evaluated biochemical properties of epilepsy-related mutant forms. Morpholino was used to knockdown pk1a in zebrafish. In response to exposure to the seizure-inducing drug PTZ, pk1a morphants showed significantly higher level of activity compared with control morphants. Valproic acid (VPA), an antiepileptic drug, was able to suppress the increased motility in pk1a mutant. The pk1a knockdown induced inner plexiform layer defects, confirmed by an increase of ubiquitylation, which is probably independent of proteosomal degradation. Although no EEG data were obtained to confirm epileptic seizure behavior, the study correlates the role of pk1a gene for epilepsy outcomes and offers a model of further drug screening to reduce refractoriness under this syndrome.

3.2.7. EAST Syndrome

EAST syndrome is a severe disorder characterized by infantile-onset epilepsy, debilitating ataxia, sensorineural deafness and a salt-wasting tubulopathy, and it is caused by malfunction of potassium channel [100]. The KCNJ10 is expressed in the distal tubule of the kidney in humans and mice [101], in glial cells of the cerebral cortex and cere-

bellar cortex, in the inner ear, and in satellite cells of the auditory nerve [102].

First, using bioinformatics tools, Mahmood *et al*. [103] found an orthologous gene of human KCNJ10 in the zebrafish genome, referred to as kcnj10a. Subsequently, kcnj10a was cloned from RNA of 120hpf fish, clones were sequenced, and to study the function of this gene, heterologous expression was performed in *Xenopus* oocytes and barium-sensitive, inwardly rectifying and K+-selective currents were analyzed. The authors concluded that kcnj10a closely resembled human KCNJ10. Subsequently, these authors designed antisense morpholino oligonucleotides (MO). Fish injected with 0.5-2 ng of either MO, but not a control MO, displayed abnormal movements and showed statistically significant increase of frequency of spontaneous contractions. This increase behavior of the fish returned to normal levels when they were co-injected with kcnj10a (0.5 ng) with normal human WT KCNJ10 cRNA (50 pg). Whereas cRNA containing the human R65P mutation associated with EAST syndrome, was not able to control morphant spontaneous contractions. In addition to an increased frequency of spontaneous contractions at 30 hpf, which could indicate that neurons are hyperexcitable, several other abnormal movement phenotypes were detected in kcnj10a morphants at 120 hpf. In an experiment that measured touch-evoked escape response, morphants were found to exhibit circling locomotion with frequent 'loops' around their vertical axis. Furthermore, swimming appeared labored and morphants struggled to maintain an upright posture and performed excessive fin movements that did not accompany locomotion. They also showed abnormal facial movements. All these behaviors were interpreted as ataxia. On occasions, larvae would have a burst of speed, usually in one direction so they would continue to try to swim forward even when they hit the wall of the dish, followed by a sudden and complete loss of posture, and this behavior was described previously in larvae presenting seizure [48]. Also, kcnj10a morphants has a reduced swim speed compared to WT or p53 morphant larvae (a MO control for toxicity).

To examine if these locomotion defects could be due to morphological defects in the nervous system, 120 hpf larvae were examined by using anti-acetylated α-tubulin immunohistochemistry. No defects in any part of the central or peripheral nervous system of kcnj10a morphants were found, suggesting that the locomotion defects were due to physiological defects caused by loss of Kcnj10a. Channel functionality was further examined by expressing ZF Kcnj10a in *Xenopus* oocytes. Barium-sensitive, inwardly rectifying and K^+-selective currents were observed, which closely resembled those of human KCNJ10. The only missing aspect was EEG confirmation of epileptic seizures, a missing piece that was obtained in a subsequent study.

In order to develop and validate a reliable method for stable long-term recording of EEG activity in zebrafish, which would be less prone to artifacts than current invasive techniques, Zdebik *et al*. [104], used antisense morpholino oligonucleotides (MO) to knock-down kcnj10 in zebrafish larvae. Electrophysiological recording was made by placing a single glass electrode on the skin overlying the optic tectum of kcnj10a morphant or PTZ-treated fish. Also, some antiepileptic drugs were added to the surrounding agarose-embedded fish, including diazepam and pentobarbitone to test the methodology. Fish showed spontaneous contractions at 30 hpf, consistent with epileptic seizures. At 120 hpf, a rapid increase in locomotion was observed which was accompanied by reduced ability to change direction, followed by a loss-of-posture in kcnj10a morphant, similarly to what was observed in the PTZ- zebrafish seizure model. Electroencephalogram recordings with the kcnj10a morphant at 120 hpf showed similar activity to fish treated with PTZ, although this activity found in kcnj10a was less pronounced than in PTZ-treated fish. Antiepileptic treatment with pentobarbitone effectively suppressed the dominating seizure activity. In contrast, diazepam was not able to control seizure KCNJ10 zebrafish knock-down. The study was of great importance to prove the presence of epileptic seizure activity in this model of spontaneous seizure detectable using electrophysiological methods. Furthermore, it also provided a new technique with which one can analyze EEG in zebrafish without injuring the animal. Thus, this method may allow subsequent tests using the same animal, which makes this approach more acceptable from the perspective of ethical use of animals in research. We hope to see subsequent studies using more pro-seizure drugs and AEDs to increase the value of this technique, and the potential use of it on adult zebrafish.

4. PERSPECTIVES AND FUTURE CHALLENGES

Epilepsy is characterized as a group of disorders with many different clinical manifestations, such as behavioral alterations, electrographic signatures, pharmacological profiles and histological abnormalities. People with distinct etiologies who suffer from epilepsy can display a similar seizure symptomatology; this is why epilepsy is called a spectrum disorder, and not a unitary disease. The epilepsies are classified in distinct subcategories and different animal models are used to study each condition in particular. Thus, to study epilepsy, the animal model must recapitulate the causal mechanism(s) (construct validity), the phenotypic features (face validity) and the treatment responses seen clinically (predictive validity) in the human condition.

At least 50% of adults with epilepsy have one or more comorbidity in life [105]. People with epilepsy exhibited significantly higher chances for social phobia, agoraphobia, generalized anxiety disorder, depression, and suicidality compared with the population without epilepsy [106]. For all of these disorders, at least one experimental model paradigm has been already created with adult zebrafish. Anxiety disorders are extensively explored using adult zebrafish [107]. The first study was conducted by Levin *et al*. [108]. Animals exposed to a new aquarium present stereotyped diving behaviors, thigmotaxis, reduction of top exploration, increased homebase formation [109], increased erratic behaviors and freezing/immobility, elevated whole body cortisol levels [110] and brain c-fos expression [111]. The zebrafish is a social animal, and many studies have been performed investigating the shoaling behavior in this species. Shoaling behavior is highly explored in response to acute stress (novelty, predator, or alarm pheromone exposure), which induces changes in zebrafish shoals, including tightening the shoals, as well as increased thigmotaxis and bottom dwelling. This behavior is related to fear responses, which can be explored by distinct protocols such as, Light–dark box (avoidance of white compartment [112]); Predator fish exposure (increase escape behavior [113]); and Alarm pheromone exposure (elevated whole body cortisol [114]). Furthermore, depression [115] could be induced in zebrafish by unpredictable chronic stress [116], sleep deprivation [117] and restrain stress [118]. These behaviors are highly sensitive to anxiolytic and anxiogenic agents [119, 120]. Therefore, the close parallels between mammalian and adult zebrafish behavioral paradigms suggest the potential use of this fish to screening drugs that may be applied to treat seizures and also its associated comorbidities [121].

To reach this goal, zebrafish must overcome another barrier. The postictal state has not been properly characterized and described in zebrafish. Since some focal or generalized epileptic seizures merge cognitive, behavioral and sensorimotor impairments into the postictal state [122], it is important to describe it for all models of epileptic seizures and epilepsy described in this review. Likely, the obstacle lies in the difficulties to perform long-term *in vivo* electrophysiological studies in this model. By matching the EEG recording with alteration of behavior, it may be possible to establish the fundamental time window of manifestations that allows researchers to investigate critical points of epileptic seizures, such as postictal period. To be able to perform this characterization using zebrafish, new technological approaches must be developed [123]. New technologies developed for rodents and birds could be adapted to zebrafish. A removable miniature microdrive-headstage waterproof assembly for extracellular recordings of single unit activity in swimming mice already has been proposed [124], and could be adapted for zebrafish. Another possibility is the use of wireless technology. Charng *et al*. [125] developed a tool to perform conscious wireless electroretinogram (ERG) and visual evoked potential (VEP) recording, a method that requires surgical implants and allows researchers to record stable and repeatable signals over at least 1 month. This new technology could be also used to clearly define each seizure behavior stage/score, crucial to screening drug experiments.

For zebrafish larvae, increased travelled distance has been used as measurement of seizure activity [48]. However, this parameter presents some misinterpretation since animals exposed to proconvulsive drugs may present long periods of immobility. Moreover, the sedative effect of many putative AED molecules may also misevaluated in larval zebrafish seizure models. In this case, the motion tracking systems to date available are the limitation since these systems are not able to automatically detect seizure stages/scores in zebrafish larvae. It will be necessary to develop

algorithms that can automatically measure seizure related behavioral responses and correlate them to electrophysiological measures in a freely swimming fish for proper future conclusions, and to overcome motion tracking limitations.

CONCLUSION

The impact of zebrafish as a model to study epileptic seizures and epilepsy is irrefutable. Zebrafish are not only used for drug screening, but also for discovering new etiologies, describing mechanisms and for the investigation of the comorbidities associated with epilepsy. We need new technologies to record brain activity in freely swimming animals to study epileptogenesis and to define postictal time lapse to work with neuropsychiatric comorbidities after seizure in zebrafish. The zebrafish seems to be a powerful experimental tool for the modeling of many disorders including epilepsy, and as any other animal model, the zebrafish too demands new expertise to maximize its applicability.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGMENTS

This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS), and Financiadora de Estados e Projetos (FINEP). We thank Professor Robert Gerlai for his useful comments.

LIST OF ABBREVIATIONS

ADHD	=	Attention Deficit Hyperactivity Disorder
AED	=	Anti-epileptic Drug
(D,L)-allylglycine	=	AG, 2-amino-4-pentenoic acid
AKT	=	Protein Kinase B
Ca^{+2}	=	Calcium
CHD2	=	Chromodomain Helicase DNA Binding Protein 2
CNS	=	Central Nervous System
DA	=	Domoic Acid
DS	=	Dravet Syndrome
DZP	=	Diazepam
EAST	=	Epilepsy Ataxia Sensorineural-deafness Tubulopathy
EEG	=	Electroencephalogram
ERG	=	Electroretinogram
EVP	=	Visual Evoked Potential
FDA	=	US Food and Drug Administration
GABA	=	Gamma-Amino Butyric Acid
$GABA_A$	=	Gamma-Amino Butyric Acid Receptor A
HT	=	Hyperthermia
K^+	=	Potassium
KA	=	Kainic Acid
LEV	=	Levetiracetam
LRR	=	Leucine-rich Repeat
MCTs	=	Maximal Tolerated Concentrations
MO	=	Morpholino
NaV1.1	=	Voltage Gated Sodium Channel 1.1
NMDA	=	N-metil D-Aspartate
OCRL1	=	Type II Inositol Polyphosphate 5-phosphatase
p53	=	53 kDa protein (apoptosis signaling)
PCR	=	Polymerase Chain Reaction
PHT	=	Phenobarbital
PK	=	PRICKLE 1a
PTZ	=	Pentylenetetrazole

TPM	=	Topiramate
TRP	=	Thermo Sensitive Channels
VPA	=	Valproate

REFERENCES

[1] Fisher RS. Redefining epilepsy. CurrOpin Neurol. 2015 Apr;28(2):130-5

[2] Fisher RS, Acevedo C, Arzimanoglou A, *et al*.ILAE official report: a practical clinical definition of epilepsy. Epilepsia. 2014 Apr;55(4):475-82.

[3] Berg AT, Millichap JJ. The 2010 revised classification of seizures and epilepsy. Continuum (MinneapMinn). 2013 Jun;19(3 Epilepsy):571-97.

[4] Fisher RS. Final comments on the process: ILAE definition of epilepsy. Epilepsia. 2014 Apr;55(4):492-3.

[5] Panayiotopoulos CP. The new ILAE report on terminology and concepts for organization of epileptic seizures: a clinician's critical view and contribution. Epilepsia. 2011 Dec;52(12):2155-60.

[6] Shorvon SD.The etiologic classification of epilepsy. Epilepsia, 52(6):1052–1057, 2011

[7] Theodore WH, Spencer SS, Wiebe S, *et al*. Epilepsy in North America: a report prepared under the auspices of the global campaign against epilepsy, the International Bureau for Epilepsy, the International League Against Epilepsy, and the World Health Organization. Epilepsia. 2006 Oct;47(10):1700-22.

[8] WHO, IBE & ILAE. (2005) Atlas: epilepsy care in the world 2005. World Health Organization, Geneva.

[9] WHO. (2006) Neurological disorders: public health challenges. WHO Press, Geneva, Switzerland.

[10] Glauser T, Ben-Menachem E, Bourgeois B, *et al*. ILAE treatment guidelines: evidence-based analysis of antiepileptic drug efficacy and effectiveness as initial monotherapy for epileptic seizures and syndromes. Epilepsia. 2006 Jul;47(7):1094-120.

[11] Xiao F, An D, Chen S, *et al*. Clinical and Electroencephalographic (EEG) Features Associated With Refractoriness in Benign Childhood Epilepsy With Centrotemporal Spikes. J Child Neurol. 2015 Oct;30(12):1591-7.

[12] Nwani PO, Nwosu MC, Enwereji KO, *et al*. Epilepsy treatment gap: prevalence and associated factors in Southeast Nigeria. ActaNeurol Scand. 2013 Aug;128(2):83-90.

[13] World Bank. (2006) Disease control priorities in developing countries. Oxford University Press &The World Bank, New York, NY.

[14] Newton CR, Garcia HH. Epilepsy in poor regions of the world. Lancet. 2012 Sep 29;380(9848):1193-201.

[15] Megiddo I, Colson A, Chisholm D, *et al.* Health and economic benefits of public financing of epilepsy treatment in India: An agent-based simulation model. Epilepsia. 2016 Jan 14. epi.13294.

[16] Kanner AM. Management of psychiatric and neurological comorbidities in epilepsy. Nat Rev Neurol. 2016 Jan 18.

[17] Boivin MJ, Kakooza AM, Warf BC, *et al.* Reducing neurodevelopmental disorders and disability through research and interventions. Nature. 2015 Nov 19;527(7578):S155-60.

[18] Devinsky O, Spruill T, Thurman D, Friedman D. Recognizing and preventing epilepsy-related mortality. Neurology. 2015 Dec 16.pii: 10.1212/WNL.0000000000002253.

[19] Price P, Kobau R, Buelow J, Austin J, Lowenberg K. Improving understanding, promoting social inclusion, and fostering empowerment related to epilepsy: Epilepsy Foundation public awareness campaigns--2001 through 2013.Epilepsy Behav. 2015 Mar;44:239-44.

[20] Stewart AM, Braubach O, Spitsbergen J, Gerlai R, Kalueff AV. Zebrafish models for translational neuroscience research: from tank to bedside. Trends Neurosci. 2014 May;37(5):264-78.

[21] Streisinger G, Walker C, Dower N, Knauber D, Singer F. Production of clones of homozygous diploid zebra fish (Brachydaniorerio). Nature. 1981 May 28;291(5813):293-6.

[22] Streisinger G. Extrapolations from species to species and from various cell types in assessing risks from chemical mutagens. Mutat Res. 1983 Jan;114(1):93-105.

[23] Chakrabarti S, Streisinger G, Singer F, Walker C. Frequency of gamma-Ray Induced Specific Locus and Recessive Lethal Mutations in Mature Germ Cells of the Zebrafish, BRACHYDANIO RERIO. Genetics. 1983 Jan;103(1):109-23.

[24] Walker C, Streisinger G. Induction of Mutations by gamma-Rays in Pregonial Germ Cells of Zebrafish Embryos. Genetics. 1983 Jan;103(1):125-36.

[25] MLA style: "Christiane Nüsslein-Volhard - Biographical". Nobelprize.org. Nobel Media AB 2014. Web. 27 Jan 2016. http://www.nobelprize.org/nobel_prizes/medicine/laureates/1995/nusslein-volhard-io.html

[26] Howe K, Clark MD, Torroja CF, *et al.* The zebrafish reference genome sequence and its relationship to the human genome. Nature. 2013 496(7446):498-503.

[27] Lopes da Fonseca, T. *et al.* The zebrafish homologue of Parkinson's disease ATP13A2 is essential for embryonic survival. Brain Res. Bull. (2013) 90, 118–126.

[28] Suen, M.F. *et al.* Assessments of the effects of nicotine and ketamine using tyrosine hydroxylase-green fluorescent protein transgenic zebrafish as biosensors. Biosens. Bioelectron. (2013) 42, 177–185.

[29] Gerlai, R. A small fish with a big future: zebrafish in behavioral neuroscience. Rev. Neurosci. (2011) 22, 3–4.

[30] Gerlai, R. Zebrafish antipredatory responses: a future for translational research? Behav. Brain Res. (2010) 207, 223–231.

[31] Gerlai, R. High-throughput behavioral screens: the first step towards finding genes involved in vertebrate brain function using zebrafish. (2010) Molecules 15, 2609–2622.

[32] Eliceiri BP, Gonzalez AM, Baird A. Zebrafish model of the blood-brain barrier: morphological and permeability studies. Methods MolBiol (2011) 686: 371–378.

[33] Jeong JY, Kwon HB, Ahn JC, Kang D, Kwon SH, *et al.* Functional and developmental analysis of the blood-brain barrier in zebrafish. Brain Res Bull (2008) 75: 619–628.

[34] Laggner, C. *et al.* (2012) Chemical informatics and target identification in a zebrafish phenotypic screen. Nat. Chem. Biol. 8, 144–146

[35] Kokel, D. and Peterson, R.T. (2011) Using the zebrafish photomotor response for psychotropic drug screening. Methods Cell Biol. 105, 517–524

[36] Kokel, D. and Peterson, R.T. (2008) Chemobehaviouralphenomics and behaviour-based psychiatric drug discovery in the zebrafish. Brief. Funct. Genomic Proteomic 7, 483–490

[37] Ali, S. *et al.* (2012) Behavioral profiling of zebrafish embryos exposed to a panel of 60 water-soluble compounds. Behav. Brain Res. 228, 272–283

[38] Cunliffe VT. Building a zebrafish toolkit for investigating the pathobiology of epilepsy and identifying new treatments for epileptic seizures. J Neurosci Methods. 2015 Jul 26.pii: S0165-0270(15)00269-1.

[39] Tian L, Hires SA, Mao T, Huber D, Chiappe ME, Chalasani SH, *et al.* Imaging neural activity in worms, flies and mice with improved GCaMP calcium indicators. Nat Methods 2009;6:875–81.

[40] Akerboom J, Chen TW, Wardill TJ, Tian L, Marvin JS, Mutlu S, *et al.* Optimization of a GCaMP calcium indicator for neural activity imaging. J Neurosci 2012;32: 13819–40.

[41] Baxendale S, Holdsworth CJ, Meza Santascoy PL, Harrison MR, Fox J, Parkin CA, *et al.* Identification of compounds with anti-convulsant properties in a zebrafish model of epileptic seizures. Dis Models Mech 2012;5:773–84.

[42] Cunliffe VT, Baines RA, Giachello CN. Epilepsy research methods update: Understanding the causes of epileptic seizures and identifying new treatments using non-mammalian model organisms. Seizure. 2015 Jan;24:44-51.

[43] Kalueff AV, Stewart AM, Gerlai R. Zebrafish as an emerging model for studying complex brain disorders. Trends Pharmacol Sci. 2014 Feb;35(2):63-75.

[44] Granato, M. *et al.* Genes controlling and mediating locomotion behavior of the zebrafish embryo and larva. Development 1996 123, 399–413.

[45] Muto, A. *et al.* Forward genetic analysis of visual behavior in zebrafish. PLoS Genet. 1, e66 (2005).

[46] Zhou Y & Danbolt NC. Glutamate as a neurotransmitter in the healthy brain. J Neural Transm (Vienna). 2014 Aug;121(8):799-81.

[47] Schousboe A, Madsen KK, Barker-Haliski ML, White HS. The GABA synapse as a target for antiepileptic drugs: a historical overview focused on GABA transporters. Neurochem Res. 2014 Oct;39(10):1980-7.

[48] Baraban SC, Taylor MR, Castro PA, Baier H. Pentylenetetrazole induced changes in zebrafish behavior, neural activity and c-fos expression. Neuroscience. 2005;131(3):759-68.

[49] Tiedeken JA, Ramsdell JS. Embryonic exposure to domoic Acid increases the susceptibility of zebrafish larvae to the chemical convulsant pentylenetetrazole. Environ Health Perspect. 2007 Nov;115(11):1547-52.

[50] Vermoesen K, Serruys AS, Loyens E, *et al.* Assessment of the convulsant liability of antidepressants using zebrafish and mouse seizure models. Epilepsy Behav. 2011 Nov;22(3):450-60.

[51] Hunt RF, Hortopan GA, Gillespie A, Baraban SC. A novel zebrafish model of hyperthermia-induced seizures reveals a role for TRPV4 channels and NMDA-type glutamate receptors. Exp Neurol. 2012 Sep;237(1):199-206.

[52] Alfaro JM, Ripoll-Gómez J, Burgos JS.Kainate administered to adult zebrafish causes seizures similar to those in rodent models. Eur J Neurosci. 2011 Apr;33(7):1252-5.

[53] Berghmans S, Hunt J, Roach A, Goldsmith P. Zebrafish offer the potential for a primary screen to identify a wide variety of potential anticonvulsants. Epilepsy Res. 2007 Jun;75(1):18-28. Epub 2007 May 7.

[54] Afrikanova T, Serruys AS, Buenafe OE, *et al.* Validation of the zebrafish pentylenetetrazol seizure model: locomotor versus electrographic responses to antiepileptic drugs. PLoS One. 2013;8(1):e54166.

[55] Baxendale S, Holdsworth CJ, Meza Santoscoy PL, *et al.* Identification of compounds with anti-convulsant properties in a zebrafish model of epileptic seizures. Dis Model Mech. 2012 Nov;5(6):773-84.

[56] Kim Y, Lee Y, Hansol Lee, Jung MW & Lee C. Impaired avoidance learning and increased hsp70 mRNA expression in pentylenetetrazol-treated zebrafish. 2010 Nov 13(3)275-281.

[57] Wong K, Stewart A, Gilder T, *et al.* Modeling seizure-related behavioral and endocrine phenotypes in adult zebrafish. Brain Res. 2010 Aug 12;1348:209-15.

[58] Stewart AM, Desmond D, Kyzar E, *et al.* Perspectives of zebrafish models of epilepsy: what, how and where next? Brain Res Bull. 2012 Feb 10;87(2-3):135-43.

[59] Lee Y, Kim D, Kim YH, Lee H, Lee CJ. Improvement of pentylenetetrazol-induced learning deficits by valproic acid in the adult zebrafish. Eur J Pharmacol. 2010 Sep 25;643(2-3):225-31.

[60] Siebel AM, Piato AL, Capiotti KM, *et al.* PTZ-induced seizures inhibit adenosine deamination in adult zebrafish brain membranes. Brain Res Bull. 2011 Nov 25;86(5-6):385-9.

[61] Pineda R, Beattie CE, Hall CW. Recording the adult zebrafish cerebral field potential during pentylenetetrazole seizures. J Neurosci Methods. 2011 Aug 30;200(1):20-8.

[62] Gupta P, Khobragade SB, Shingatgeri VM.Effect of Various Antiepileptic Drugs in Zebrafish PTZ-Seizure Model. Indian J Pharm Sci. 2014 Mar;76(2):157-63.

[63] Siebel AM, Menezes FP, da Costa Schaefer I, Petersen BD, Bonan CD. Rapamycin suppresses PTZ-induced seizures at different developmental stages of zebrafish. PharmacolBiochemBehav. 2015 Dec;139 Pt B:163-8.

[64] Mussulini BH, Leite CE, Zenki KC, *et al.* Seizures induced by pentylenetetrazole in the adult zebrafish: a detailed behavioral characterization. PLoS One. 2013;8(1):e54515.

[65] Pagnussat N, Piato AL, Schaefer IC, *et al.* One for all and all for one: the importance of shoaling on behavioral and stress responses in zebrafish. Zebrafish. 2013 Sep;10(3):338-42.

[66] Lange M., Neuzeret F., Fabreges B., *et al.* Inter-individual and inter-strain variations in zebrafish locomotor ontogeny.PLoS One. 2013 Aug 9;8(8):e70172.[116] Grone BP, Baraban SC. Animal models in epilepsy research: legacies and new directions. Nat Neurosci. 2015 Mar;18(3):339-43.

[67] Lefebvre KA, Tilton SC, Bammler TK, *et al*. Gene expression profiles in zebrafish brain after acute exposure to domoic acid at symptomatic and asymptomatic doses. Toxicol Sci. 2009 Jan;107(1):65-77.

[68] Araya-Quintanilla F, Celis-Rosati A, Rodriguez-Leiva C, Silva-Navarro C, Silva-Pinto Y, Toro-Jeria B. Effectiveness of a ketogenic diet in children with refractory epilepsy: a systematic review. Rev Neurol. 2016 May 16;62(10):439-48.

[69] Sierra S, Alfaro JM, Sánchez S, Burgos JS. Administration of docosahexaenoic acid before birth and until aging decreases kainate-induced seizures in adult zebrafish. Brain Res Bull. 2012 Aug 1;88(5):467-70.

[70] Menezes FP, Rico EP, Da Silva RS. Tolerance to seizure induced by kainic acid is produced in a specific period of zebrafish development. ProgNeuropsychopharmacolBiol Psychiatry. 2014 Dec 3;55:109-12.

[71] Castell E, Galindo C, Montell L, Segura E, Baeza MM, GuillénV.Effect on zebrafish neurodevelopment and neuroprotection of a polyphenolic extract olive seeds. Nutr Hosp. 2014 Aug 1;30(2):338-42.

[72] Santana S, Rico EP, Burgos JS. Can zebrafish be used as animal model to study Alzheimer's disease? Am J Neurodegener Dis. 2012;1(1):32-48.

[73] Baraban SC. Forebrain electrophysiological recording in larval zebrafish. J Vis Exp. 2013 Jan 24;(71). pii: 50104. doi: 10.3791/50104.

[74] Lopes MW, Sapio MR, Leal RB, Fricker LD. Knockdown of Carboxypeptidase A6 in Zebrafish Larvae Reduces Response to Seizure-Inducing Drugs and Causes Changes in the Level of mRNAs Encoding Signaling Molecules. PLoS One. 2016 Apr 6;11(4):e0152905.

[75] Hashiguchi M, Ohta Y, Shimizu M, Maruyama J, Mochizuki M. Meta-analysis of the efficacy and safety of Ginkgo biloba extract for the treatment of dementia. J Pharm Health Care Sci. 2015 Apr 10;1:14.

[76] Lee GH, Sung SY, Chang WN *et al.* Zebrafish larvae exposed to ginkgotoxin exhibit seizure-like behavior that is relieved by pyridoxal-5'-phosphate, GABA and anti-epileptic drugs. Dis Model Mech. 2012 Nov;5(6):785-95.

[77] Fisher SK, Davies WE. The effect of the convulsant allylglycine (2-amino-4-pentenoic acid) on the activity of glutamic acid decarboxylase and the concentration of GABA in different regions of guinea pig brain. Biochem Pharmacol, 25 (1976), pp. 1881–1885

[78] Leclercq K, Afrikanova T, Langlois M, *et al.* Cross-species pharmacological characterization of the allylglycine seizure model in mice and larval zebrafish. Epilepsy Behav. 2015 Apr;45:53-63.

[79] Bhaskaran MD, Smith BN. Effects of TRPV1 activation on synaptic excitation in the dentate gyrus of a mouse model of temporal lobe epilepsy. Exp. Neurol. 2010 223, 529–536.

[80] Marsh ED. Fishing for the mechanisms causing febrile seizures: employing a novel model to uncover the physiological generators of seizures with fever. Exp Neurol. 2013 Feb;240:108-11.

[81] Teng, Y. *et al.* Knockdown of zebrafish Lgi1a results in abnormal development, brain defects and a seizure-like behavioral phenotype. Hum. Mol. Genet. 19, 4409–4420 (2010).

[82] Hortopan GA, Dinday MT, Baraban SC. Spontaneous seizures and altered gene expression in GABA signaling pathways in a mind bomb mutant zebrafish. J Neurosci. 2010 Oct 13;30(41):13718-28.

[83] Ramirez, I.B. *et al.* Impaired neural development in a zebrafish model for Lowe syndrome. Hum. Mol. Genet. 21, 1744–1759 (2012).

[84] Baraban, S.C., Dinday, M.T. &Hortopan, G.A. Drug screening in Scn1a zebrafish mutant identifies clemizole as a potential Dravet syndrome treatment. Nat. Commun. 4, 2410 (2013).

[85] Dinday, M.T., Baraban, S.C. Large-Scale Phenotype-Based Antiepileptic Drug Screening in a Zebrafish Model of Dravet Syndrome(1,2,3).

[86] Kettleborough, R.N. *et al.* A systematic genome-wide analysis of zebrafish proteincoding gene function.Nature 496, 494–497 (2013).eNeuro. 2015 Aug 31;2(4). pii: ENEURO.0068-15.2015.

[87] Moens, C.B., Donn, T.M., Wolf-Saxon, E.R. & Ma, T.P. Reverse genetics in zebrafish by TILLING. Brief. Funct. Genomic. Proteomic. 7, 454–459 (2008).

[88] Suls, A. *et al.* De novo loss-of-function mutations in CHD2 cause a fever-sensitive myoclonic epileptic encephalopathy sharing features with Dravet syndrome. Am. J. Hum. Genet. 93, 967–975 (2013).

[89] Poza, J.J., Sa´enz, A., Martı´nez-Gil, A., *et al.* Autosomal dominant lateral temporal epilepsy: clinical and genetic study of a large Basque pedigree linked to chromosome 10q. Ann. Neurol. 1999 45, 182–188.

[90] Head, K., Gong, S., Joseph, S., *et al*. The expression pattern of the LGI1 gene in tissues and organs from BAC transgenic mice demonstrate a neuronal and glial expression pattern as well as other distinct cell types in the adult animal. Mamm. Genome. 2007 18, 328–337.

[91] de la Pompa JL, Wakeham A, Correia KM, *et al*. Conservation of the Notch signalling pathway in mammalian neurogenesis. Development. 1997 124:1139 –1148.

[92] Clayton-Smith J, Laan L. Angelman syndrome: a review of the clinical and genetic aspects. J Med Genet. 2003 40:87–95.

[93] Lowe, M. Structure and function of the Lowe syndrome protein OCRL1. Traffic. 2005 6, 711–719.

[94] Devuyst, O. and Thakker, R.V. Dent's disease. Orphanet J. Rare Dis. 2010 5, 28.

[95] Bender, A. C. *et al.* SCN1A mutations in Dravet syndrome: impact of interneuron dysfunction on neural networks and cognitive outcome. Epilepsy Behav. 2012 23, 177–186.

[96] Catterall, W. A., Kalume, F. & Oakley, J. C. NaV1.1 channels and epilepsy. J. Physiol. 588, 1849–1859 (2010).

[97] Large-Scale Phenotype-Based Antiepileptic Drug Screening in a Zebrafish Model of Dravet Syndrome(1,2,3). Dinday MT, Baraban SC. eNeuro. 2015 Aug 31;2(4).

[98] Derchansky, M, Jahromi, SS, Mamani, M, *et al*. Transition to seizures in the isolated immature mouse hippocampus: a switch from dominant phasic inhibition to dominant phasic excitation. J. Physiol. 2008 586, 477–494.

[99] Mei X, Wu S, Bassuk AG, Slusarski DC. Mechanisms of prickle1a function in zebrafish epilepsy and retinal neurogenesis. Dis Model Mech. 2013 May;6(3):679-88.

[100] Bockenhauer, D, Feather, S, Stanescu, HC, *et al*. Epilepsy, ataxia, Disease Models & Mechanisms D MM 660 dmm.biologists.org RESEARCH ARTICLE Zebrafish model of EAST syndrome sensorineural deafness, tubulopathy, and KCNJ10 mutations. N. Engl. J. Med. 2009 360, 1960-1970.

[101] Bleich, M. Membrane physiology – bridging the gap between medical disciplines. N. Engl. J. Med. 2009 360, 2012-2014.

[102] Reichold, M, Zdebik, A A, Lieberer, E, *et al*. KCNJ10 gene mutations causing EAST syndrome (epilepsy, ataxia, sensorineural deafness, and tubulopathy) disrupt channel function. Proc. Natl. Acad. Sci. USA. 2010 107, 14490-14495.

[103] Mahmood F1, Mozere M, Zdebik AA, *et al*. Generation and validation of a zebrafish model of EAST (epilepsy, ataxia, sensorineural deafness and tubulopathy) syndrome. Dis Model Mech. 2013 May;6(3):652-60.

[104] Zdebik AA, Mahmood F, Stanescu HC, Kleta R, Bockenhauer D, Russell C. Epilepsy in kcnj10 morphant zebrafish assessed with a novel method for long-term EEG recordings. PLoS One. 2013 Nov 14;8(11):e79765.

[105] Grone BP, Baraban SC. Animal models in epilepsy research: legacies and new directions. Nat Neurosci. 2015 Mar;18(3):339-43.

[106] Keezer MR, Sisodiya SM, Sander JW. Comorbidities of epilepsy: current concepts and future perspectives. Lancet Neurol. 2016 Jan;15(1):106-15.

[107] Lin JJ, Mula M, Hermann BP. Uncovering the neurobehavioural comorbidities of epilepsy over the lifespan. Lancet. 2012 Sep 29;380(9848):1180-92.

[108] Stewart A, Gaikwad S, Kyzar E, Green J, Roth A, Kalueff AV. Modeling anxiety using adult zebrafish: a conceptual review. Neuropharmacology. 2012 Jan;62(1):135-43.

[109] Levin ED, Bencan Z, Cerutti DT. Anxiolytic effects of nicotine in zebrafish. Physiol Behav. 2007 Jan 30;90(1):54-8.

[110] Ibrahim M, Mussulini BH, Moro L, *et al*. Anxiolytic effects of diphenyl diselenide on adult zebrafish in a novelty paradigm. Prog Neuropsychopharmacol Biol Psychiatry. 2014 Oct 3;54:187-94.

[111] Abreu MS, Giacomini AC, Koakoski G, Piato AL, Barcellos LJ. Evaluating "anxiety" and social behavior in jundiá (*Rhamdia quelen*). Physiol Behav. 2016 Jun 1;160:59-65.

[112] Cachat J, Kyzar EJ, Collins C, *et al*. Unique and potent effects of acute ibogaine on zebrafish: the developing utility of novel aquatic models for hallucinogenic drug research. Behav Brain Res. 2013 Jan 1;236(1):258-69.

[113] Maximino C, Marques de Brito T, Dias CA, Gouveia A Jr, Morato S. Scototaxis as anxiety-like behavior in fish. Nat Protoc. 2010 Feb;5(2):209-16.

[114] Luca, R.M. and Gerlai, R. In search of optimal fear inducing stimuli: differential behavioral responses to computer animated images in zebrafish. Behav. Brain Res. 2012 226, 66–76

[115] Speedie, N. and Gerlai, R. Alarm substance induced behavioral responses in zebrafish (Danio rerio). Behav. Brain Res. 2008 188, 168–177

[116] Nguyen M, Stewart AM, Kalueff AV. Aquatic blues: modeling depression and antidepressant action in zebrafish.

ProgNeuropsychopharmacolBiol Psychiatry. 2014 Dec 3;55:26-39.

[117] Piato, A.L. *et al*. Unpredictable chronic stress model in zebrafish (Danio rerio): behavioral and physiological responses. Prog. Neuropsychopharmacol. Biol. Psychiatry. 2011, 35, 561–567.

[118] Singh, A. *et al*. Involvement of the a1-adrenoceptor in sleepwaking and sleep loss-induced anxiety behavior in zebrafish. Neuroscience. 2013, 245, 136–147.

[119] Piato, A.L. *et al*. (2011) Acute restraint stress in zebrafish: behavioral parameters and purinergic signaling. Neurochem. Res. 2011, 36, 1876–1886.

[120] Stewart A, Wu N, Cachat J, *et al*. Pharmacological modulation of anxiety-like phenotypes in adult zebrafish behavioral models. Prog Neuropsychopharmacol Biol Psychiatry. 2011 Aug 1;35(6):1421-31.

[121] Maximino C, da Silva AW, Gouveia A Jr, Herculano AM. Pharmacological analysis of zebrafish (*Danio rerio*) scototaxis. Prog Neuropsychopharmacol Biol Psychiatry. 2011 Mar 30;35(2):624-31.

[122] Fisher RS, Engel JJ Jr. Definition of the postictal state: when does it start and end? Epilepsy Behav. 2010 Oct;19(2):100-4.

[123] Racine RJ. Modification of seizure activity by electrical stimulation. II. Motor seizure. Electroencephalogr Clin Neurophysiol. 1972 Mar;32(3):281-94.

[124] Korshunov VA, Averkin RG. A method of extracellular recording of neuronal activity in swimming mice. J Neurosci Methods. 2007 Sep 30;165(2):244-50.

[125] Charng J, Nguyen CT, He Z, *et al*. Conscious wireless electroretinogram and visual evoked potentials in rats. PLoS One. 2013 Sep 12;8(9):e74172.

II.2. Capítulo II

Brain Metabolic Preference Shifts Under Prolonged Epileptic Seizure Episodes in Adult Zebrafish

Ben Hur Marins Mussulini*, Gabriela Lazzarotto, Ivi Juliana Bristot, Renato Dutra Dias, Fabio Klamt, Diogo Losch de Oliveira*.

Artigo submetido ao periódico Journal of Cerebral Blood Flow and Metabolism.

Tema:

Elucidar o papel da glicose e de outros substratos energéticos na manutenção de crises epilépticas prolongadas utilizando o modelo animal de imersão do peixe-zebra em pentilenotetrazol por até 20 min pela medida de consumo de O_2 para síntese de ATP.

Principal conclusão:

Após 20 min de crise epiléptica prolongada o cérebro de peixe-zebra apresenta uma redução na utilização de glicose acoplada ao consumo de O_2 à síntese de ATP. Em contra-partida, o L-glutamato passa a suprir energeticamente as crises epilépticas prolongadas pela medida de consumo de O_2 acoplado à síntese de ATP. Apesar da L-glutamina desempenhar papel similar, a mesma encontra-se reduzida em SNC de pacientes epilépticos. como substrato energético. O β-hydroxybutirato foi capaz de atenuar o efeito frente a uma utilização aguda. Portanto o L-glutamato é o principal substrato energético cerebral de crises epilépticas prolongadas induzidas por pentilenotetrazol em peixe-zebra adulto.

Contribuição à formação do aluno:

Treinamento na metodologia de respirometria de alta resolução.

Objetivo:

Avaliar a contribuição da D-glicose, L-glutamato, L-glutamina, L-lactato, e β-hidroxibutirato sobre a manutenção metabólica mitocondrial cerebral pela medida de consumo de O_2 para a produção de ATP em crises epilépticas prolongadas induzidas por pentilenotetrazol em peixe-zebra adulto.

II.3. Capítulo III

Glucose-6-phosphate Dehydrogenase and Citosolic Malic Enzyme Are Important to Antioxidant Defense During Prolonged Epileptic Seizure Induced by Pentylenetetrazole in Adult Zebrafish.

Ben Hur Marins Mussulini*, Lúcia Von Mengden, Gabriela Lazzarotto, Emerson Santos, Suelen Baggio, Ivi Juliana Bristot, Renato Dutra Dias, Maria Elisa calcagnotto, Fabio Klamt, Diogo Losch de Oliveira*

Artigo será submetido ao periódico Free Radical Biology & Medicine.

Tema:

Investigar se ocorre um ambiente favorável à produção de H_2O_2, e se o mesmo está vinculado a modulações que poderiam explicar a alteração metabólica cerebral de glicose para outros substratos energéticos e o impacto desta mudança sobre o sistema de defesa antioxidante em crises epilépticas prolongadas.

Principal conclusão:

Após 20 min de crise epiléptica ocorre um aumento no ROX, diminuição na atividade do complexo I do STE e aumento da atividade da SOD indicando um ambiente favorável à produção de H_2O_2. Para combater o estresse oxidativo (aumento de TBARS, redução de tióis totais, redução de potencial antioxidante total e aumento de oxidação de DCF), nota-se a redução dos níveis de GSH e aumento da atividade da Gpx sem alteração na atividade da GR, necessitando um aumento dos níveis de NADPH citosólicos. A glicose é desviada para a via das pentoses-fosfato e a oxidação completa do L-glutamato eleva a atividade da ME citosólica.

Contribuição à formação do aluno:

Aprendizado de técnicas relevantes à análise de estresse oxidativo.

Objetivo:

Avaliar o balanço redox de cérebro de peixe-zebra adulto, exposto a pentilenotetrazol 10 mM por 20 min, para elucidar uma mudança bioenergética drástica em um curto espaço de tempo.

Parte III

III.1. Discussão

Tendo em vista a escala evolutiva dos vertebrados, a alta conservação e função mitocondrial nestes grupos e a importância desta organela frente a quadros patológicos, nosso objetivo foi validar a bioenergética cerebral do modelo de crise epiléptica prolongada induzida por PTZ em peixe-zebra, por intermédio da técnica de respirometria de alta resolução em amostra de cérebro (Kalueff *et al.*, 2014), seguindo as normas do livro *"Mitochondrial pathways and respiratory control: An introduction to OXPHOS analysis"*(Gnaiger, 2014).

Perante o desequilíbrio redox que há em crises epilépticas, o papel do metabolismo da glicose conectado a via das pentoses-fosfato, a redução do aporte desta molécula, e o aumento no consumo de ATP para a manutenção do evento epiléptico, hipotetizou-se a possibilidade do SNC central utilizar outras moléculas presentes no mesmo como fonte majoritária de produção de ATP ao invés da Glicose. Uma vez que o metabolismo glicolítico é imprescindível à avaliação do objetivo desta tese, a integridade celular foi um ponto crítico (Brand e Nicholls, 2011). Inicialmente, optou-se por uma dissociação enzimática por tripsina, contudo danos à membrana celular e alteração do funcionamento de transportadores de substratos pode ser resultante deste processo de digestão da matriz extracelular (Huang *et al.*, 2010). Outro ponto negativo ao adotar um processo de dissociação celular é o tempo de preparação de amostra. Por utilizarmos um modelo de crise epiléptica prolongada aguda, o tempo de preparação de amostra poderia superar o tempo de crise epiléptica o que poderia resultar em erros de interpretações de dados (Brown *et al.*, 2008). Além disso, os passos de centrifugação de amostra com mudanças bruscas de temperatura podem alterar o perfil metabólico da amostra (Haukaas *et al.*, 2016). Portanto, optou-se por uma técnica de dissociação celular mecânica clássica. Realizou-se o protocolo de homogeneização mecânica para evitar futuras "praticidades custosas". Utilizou-se três controles de integridade celular o azul de tripam, a atividade da LDH no sobrenadante, e o aumento no consumo de O_2 ao expor a amostra a succinato. Apenas tumores cerebrais apresentam transportadores de succinato na membrana celular em SNC (Pajor, 2006; Zhunussova *et al.*, 2015), portanto qualquer alteração no consumo de oxigênio poderia ser um indicativo de redução de integridade de membrana após a preparação celular (Dunkley *et al.*, 2008). Tanto o método de dissociação mecância, quanto o enzimático apresentaram resultados semelhantes. Contudo o primeiro método leva a uma menor perda de tecido durante o processo. Uma vez que havia perda de tecido durante a preparação, realizamos uma curva de tecido por consumo de O_2, observando linearidade entre 1 e 3 cérebros utilizados na preparação do dissociado celular. Além disso, trabalhar com 8 mg de tecido (2 cérebros) aumentou a replicabilidade experimental e por isso optou-se por essa quantidade de tecido em cada passo a seguir.

Para mimetizar o ambiente extracelular cerebral utilizou-se a solução salina balanceado de Hanks com adição de HEPES-Na^+ para evitar potencial acidificação promovida pelos substratos a serem testados. Apesar de oligomicina (Olig) 8 μg/mL reduzir drasticamente o consumo de O_2, essa variação poderia ser um indicativo de dano mitocondrial por toxicidade promovida pela Olig e não da inibição completa da ATPsintase (Gnaiger, 2014). Portanto, optou-se por utilizar Olig 4 μg/mL. O ionóforo FCCP possui uma faixa restrita entre dose máxima e efeito tóxico mitocondrial, adicionou-se o composto à amostra em concentrações contínuas de 0,05 μM até atingir a concentração ideal de 0,25 μM. Por fim, preparou-se KCN sempre próximo ao fim do experimento, cuja concentração foi 1 mM (Pesta e Gnaiger, 2012).

Estudos de crises epilépticas, com utilização de peixe-zebra como modelo animal, focam no perfil fenotípico da crise, expressão de c-*Fos* e registros eletrofisiológicos (Grone e Baraban, 2015). Este cenário começou a mudar com um estudo utilizando larvas de peixe-zebra geneticamente modificadas para expressar a síndrome epiléptica de Dravet (Baraban *et al.*, 2013). O metabolismo energético destes animais apresentou uma redução da atividade glicolítica e não alteração metabólica mitocondrial quando expostos ao PTZ (Kumar *et al.*, 2016). No estudo em questão, utilizou-se o equipamento Seahorse® e larvas vivas para inferir os resultados. Portanto, a modulação metabólica foi global, não respondendo nosso objetivo. Decorrente da revisão sistemática dos modelos de crises e síndromes epilépticas e da limitação de importação de animais geneticamente modificados, as escolhas ficaram restritas a modelos químicos de indução. O CA é um agente pró-convulsivo por ter papel agonista ao receptor ionotrópico glutamatérgico do tipo cainato. Peixe-zebras injetados i.p. com CA apresentam crises epilépticas prolongadas que podem durar entre algumas horas e alguns dias. Este modelo não possui caracterização eletrofisiológica, portanto, delimitar uma janela de tempo de estudo com o objetivo proposto poderia acarretar em uma alta variabilidade nos dados. Além disso, um dos substratos a serem testados seria o L-glutamato, uma vez que ainda há muito debate sobre o impacto dessa molécula sobre o metabolismo da perspectiva de sinalização celular, utilizar o modelo de CA poderia culminar em uma resposta ambígua (Bhangoo e Swanson, 2013). Até o momento, não foi descrito nenhum modelo de crises epilépticas prolongadas induzidas por pilocarpina em peixe-zebra adulto. Por experiência prática com o modelo de crise epiléptica prolongada induzida por PTZ (Mussulini *et al.*, 2013), optou-se por este modelo para inferir se havia um hipo ou hipermetabolismo da glicose acoplado ao consumo de O_2 à produção de ATP em amostras de dissociado celular cerebral. Este modelo já foi caracterizado da perspectiva fenotípica (Wong *et al.*, 2010; Mussulini *et al.*, 2013; Mussulini B.H., 2016), genética (Stewart *et al.*, 2012) e eletrofisiológica (Pineda *et al.*, 2011). Utilizou-se a concentração de PTZ 10 mM para indução de crises epilépticas decorrente da replicabilidade experimental. Os

peixes apresentaram crises tônico-clônicas após 2,5 min de imersão, variabilidade entre escore convulstivos até 5 min de imersão, e crises tônico-clônicas até 20 min de imersão. Mesmo após a retirada dos animais do PTZ a crise epiléptica persiste por até 40 min (Pineda *et al.*, 2011). Este é o tempo limite de exposição com mortalidade aceitável, uma vez que este é um parâmetro importante para varredura de fármacos anticonvulsivos, e já foi demonstrado que a eficiência de novos fármacos pode ser testada com uma faixa de mortalidade entre 25% e 35% após a indução de crises epilépticas (Goodman *et al.*, 1953). O peixe retorna a apresentar apenas escore zero de crise epiléptica 1 h após a retirada do animal da solução de PTZ. Baseado em trabalho prévio de nosso laboratório optou-se como tempo final 3 h após a retirada do animal da solução de PTZ (Mussulini *et al.*, 2013).

Tanto o hipometabolismo quanto o hipermetabolismo de glicose acoplado ao consumo de O_2 foram detectados. Após 20 min de imersão do animal em PTZ 10 mM, o dissociado cerebral apresentou uma redução no consumo de O_2 acoplado à síntese de ATP, quando apenas glicose foi o substrato energético fornecido a amostra. Estudos utilizam D-glicose 5.5 mM como fonte energética em seus tampões, não sendo um fator limitante à atividade cerebral (Liesa e Shirihai, 2013). Em contrapartida, 1h após a retirada dos animais da solução de PTZ 10 mM o dissociado celular cerebral apresentou um aumento no consumo de O_2 acoplado à síntese de ATP, quando apenas glicose foi o substrato energético fornecido a amostra. Experimentos realizados *in vitro* sugerem que, durante a crise epiléptica prolongada, a glicólise pode encontrar-se diminuída favorecendo a via das pentoses-fosfato, e 24 h após o momento de hiperexcitabilidade há um aumento na utilizando de glicose como substrato energético o que pode ser associado à neurodegeneração (Rodriguez-Rodriguez *et al.*, 2012). O peixe-zebra apresenta alta plasticidade cerebral após danos, então uma janela de tempo menor que reflita os achados *in vitro* não é uma surpresa (Baumgart *et al.*, 2012; Schmidt *et al.*, 2014).

Apesar do hipermetabolismo da glicose acoplado ao consumo de O_2 à síntese de ATP ser foco de etiologias epilépticas (Sarikaya, 2015), esta alteração metabólica ocorre em um momento de ausência da manifestação comportamental da crise epiléptica prolongada induzida por PTZ em peixe-zebra (Mussulini *et al.*, 2013). Este tipo de modulação sugere o papel do sistema purinérgico na inibição da crise epiléptica (Cieslak *et al.*, 2017), contudo a falta de estudos avaliando a presença de ATP e seus produtos de hidrólise na fenda sináptica neste modelo de crise epiléptica limita uma discussão aprofundada (Siebel *et al.*, 2011; Siebel *et al.*, 2015). Por outro lado, o hipometabolismo da glicose acoplado ao consumo de O_2 à síntese de ATP, após 20 min de crises epilépticas prolongadas, fomenta o debate das incongruências energéticas para o funcionamento do SNC em um momento de hiperexcitabilidade. Para atingir o objetivo proposto pela tese, ao estabilizar a respiração de rotina, em amostras de dissociados celulares de cérebros de peixe zebra expostos à água ou ao PTZ

por 20 min, adicionaram-se ao oroboros® os substratos apresentados a seguir e avaliou-se o consumo de O_2 à síntese de ATP em cada situação na presença de glicose.

A respiração de rotina aumentou ao adicionar-se L-glutamato 1mM a amostra controle. O L-glutamato é um neurotransmissor excitatório do SNC, permitindo o influxo de Ca^{+2} nos neurônios e astrócitos presentes na amostra e acelerando o ciclo de Krebs, elevando a respiração de rotina (Nedergaard *et al.*, 2002). A despolarização celular e captação de glutamato é um sinal para mitocôndrias deslocarem-se do corpo celular para a região sináptica e acelerar o metabolismo deste aminoácido a α-cetoglutato (Ward *et al.*, 2000; Chang *et al.*, 2006; Mckenna *et al.*, 2016; Robinson e Jackson, 2016). Apesar da concentração de L-glutamato 1 mM ser considerada excitotóxica em estudos *in vitro*, a mesma se apresenta na fenda sináptica em eventos de despolarização celular (Danbolt *et al.*, 2016). O mesmo impacto não foi detectado sobre a respiração de rotina em amostras de dissociados celulares de cérebros de peixe-zebra imersos em PTZ por 20 min**,** contudo, ambas concentrações testadas elevaram o O_2 para produção de ATP a níveis de controle (Karaca *et al.*, 2015).

L-glutamina conecta sistemas de detoxificação, neurotransmissão, metabolismo entérico e gliconeogênese (Stumvoll *et al.*, 1999), síntese de GABA (Behar e Rothman, 2001), reciclagem de glutamato, reações de transaminações e defesas antioxidantes (Newsholme *et al.*, 2003). Decorrente de todas essas funções, a oxidação de glutamina ocorre apenas em concentrações elevadas deste substrato energético (Schousboe *et al.*, 1993; Tani *et al.*, 2014). Dissociados celulares de peixe-zebra imersos em PTZ por 20 min apresentaram similar consumo de O_2 para a síntese de ATP comparada ao controle quando L-glutamina 0,5 mM foi adicionado como substrato energético ao experimento. Durante processos epileptogênicos em roedores e humanos, há uma redução progressiva dos níveis cerebrais de glutamina e da atividade da glutamina-sintetase (Bidmon *et al.*, 2008). Mediante a redução na eficiência do ciclo glutamato-glutamina, a probabilidade de o glutamato retornar ao neurônio por intermédio da fenda sináptica é alta, potencializando a hiperexcitabilidade (Eid *et al.*, 2016). Portanto, as alterações metabólicas para manter as crises epilépticas prolongadas deve ser estudada focando seu impacto sobre os processos neurodegenerativos (Bryant *et al.*, 2009).

O metabolismo cerebral depende de altas concentrações de lactato extracelular (Pellerin, 2008), encontrando-se na faixa de 1 mM neste ambiente (Machler *et al.*, 2016). Durante crises epilépticas prolongadas essa concentração pode elevar-se a 10 mM ou mais (Dulac *et al.*, 2014), a qual pode apresentar um efeito tamponante de Ca^{+2} (Ohbuchi *et al.*, 2010), e reduzindo a frequência de despolarização celular (Bozzo *et al.*, 2013). Apesar da "propaganda" negativa sobre o lactato, o mesmo foi capaz de elevar o consumo de O_2 para a produção de ATP em amostras de peixe-zebra imersos em PTZ por 20 min, similar ao que ocorre em modelos de

acidente vascular cerebral (Laird *et al.*, 2013). Seja pela ação quelante de Ca^{+2}, seja por um aumento no H^+*Leak* o lactato não foi capaz de elevar tal parâmetro a níveis de controle. Uma vez que haja uma redução na utilização de glicose como substrato energético acoplado ao consumo de O_2 para a produção de ATP no modelo de crise epiléptica prolongada utilizado neste estudo, em nenhum momento inferiu-se a atividade da glicólise, assim como o aumento de lactato pode ser resultante da atividade periférica em um momento de crise tônico-clônico prolongada (Lipka e Bulow, 2003). O metabolismo do lactato depende da enzima LDH, a qual é citosólica e reforça a integridade celular do protocolo proposto.

A dieta cetogênica é utilizada como tratamento para pacientes refratários e sua eficiência já foi observada no modelo de indução de status epilépticos por injeção i.p. de ácido caínico e síndrome de Dravet utilizando o peixe-zebra como modelo animal (Sierra *et al.*, 2012; Kumar *et al.*, 2016). A exposição aguda do β-hidroxibutirato levou ao aumento do consumo de O_2 para a síntese de ATP, porém não a nível de controle. Em contra partida, outros parâmetros de homeostase mitocondrial, como consumo de O_2 máxima da amostra, foi restaurado a níveis de controle (Gano *et al.*, 2014). Entretando, o impacto do β-hidroxibutirato 0,7 mM sobre o controle sugere a necessidade de futuros estudos, principalmente em relação à acetilação de enzimas mitocondriais (Juge *et al.*, 2010; Kim *et al.*, 2015).

Peixe-zebras imersos na solução de PTZ 10 mM por 20 min apresenta uma redução no consumo de O_2 para a síntese de ATP se apenas glicose for o substrato energético disponível em amostras de dissociado celular cerebral. L-glutamato, L-glutamina, L-lactato e β-hidroxibutirato, foram capazes de reverter este parâmetro de forma total ou parcial. Redução na velocidade máxima de consumo de O_2 pode ser um indicativo de dano mitocondrial (Brand e Nicholls, 2011), contudo esse parâmetro é dependente da força protomotriz mitocondrial, uma vez que é avaliada pelo retorno dos H^+ do espaço entre membranas para a matriz mitocondrial. Em um primeiro momento o resultado referente a este parâmetro poderia ser um indício de dano mitocondrial, contudo, frente a adição de outros substratos energéticos o mesmo parâmetro voltou a apresentar atividade em níveis de controle. Portanto, a redução no consumo do O_2 máximo aparenta ser um resultado da redução de substratos energéticos, decorrente de uma redução do metabolismo da glicose, o que leva a uma redução dos níveis de NADH e $FADH_2$ comprometendo a força protomotriz e reduzindo o consumo de O_2 para a produção de ATP. Frente a substratos energéticos oxidados na matriz mitocondrial, ou que é oxidado a piruvato esse parâmetro foi reestabelecido, indicando uma mudança nos padrões energéticos para sustentar a crise epiléptica prolongada sem dano aparente mitocondrial, o que ocorre em casos de exercício físico intenso e atividade mitocondrial muscular (Moghetti *et al.*, 2016). Uma vez que o KCN pode comprometer outras enzimas que utilizam O_2 como substrato

energético (Patriarca *et al.*, 1971), neste estudo não foi abordado nenhum tipo de discussão referente ao consumo de oxigênio residual e ao ROS. Portanto, a utilização de um protocolo de inibição dos Complexos I e III seria o mais apropriado para avaliar o ROX.

Frente a alterações metabólicas expressivas em um curto espaço de tempo, os dados dessa tese apontam para uma alteração de função enzimática por variação do balanço NAD^+/NADH (Allmann e Bringaud, 2017), do balanço FAD^+/$FADH_2$ (Venneti e Thompson, 2017), do balanço ADP/ATP (Saunier *et al.*, 2016) e por aumento de H_2O_2 (Peters *et al.*, 2016). Da perspectiva experimental, avaliar o balanço energético também seria uma proposta de difícil interpretação, uma vez que a hiperexcitabilidade cerebral eleva o *turnover* destas moléculas (Dona *et al.*, 2016). Uma vez que o estresse oxidativo já foi avaliado nesta espécie, da perspectiva de enzimologia clássica (Pereira *et al.*, 2016), optou-se por esta proposta para elucidar a modulação metabólica proposta por esta tese.

A atividade do complexo I encontra-se alterado em patologias associadas à neurodegeneração (Schapira, 1998) como, Parkinson (Bose e Beal, 2016), Alzheimer (Onyango *et al.*, 2017), e epilepsia (Frey *et al.*, 2017). Por ser um sítio com presença de ligações Fe-S, um desequilíbrio na função deste complexo é alvo de estudos que avaliam o impacto de ROS sobre as patologias mencionadas (Pitkanen e Robinson, 1996). Detectou-se uma redução de 33% da atividade do complexo I e aumento de 50% no ROX comparado ao controle no modelo de crise epiléptica prolongada induzida por PTZ em peixe-zebra. Similar a modelos roedores de *SE* ocorre a ativação do sistema antioxidante por intermédio do aumento da atividade da SOD (Mori *et al.*, 1991). Macromoléculas são alvos de oxidação do $O_2^{\cdot -}$, formando ligações irreversíveis, portanto a ativação da SOD e a conversão dessa espécie de oxigênio a H_2O_2 reduz o risco de ROS e possibilita a ativação de outras defesas antioxidantes (Demaurex e Schwarzlander, 2016). Apesar de mais estável ao comparar-se com o $O_2^{\cdot -}$, o H_2O_2 pode ser clivado de forma espontânea a radicais hidroxilas e aumentar os riscos de ROS se o sistema antioxidante não estiver em eficiência plena (Brand, 2016). Em nível periférico a enzima responsável por detoxificar H_2O_2 é a CAT, entretanto a expressão desta enzima em nível de SNC encontra-se reduzida ou nula (Craig e Housley, 2016), basta comparar a atividade basal da SOD e da CAT. Portanto, a defesa antioxidante cerebral depende do sistema de detoxificação via atividade da Gpx e dos níveis de GSH (Rae e Williams, 2016). Nossos dados indicam uma redução dos níveis de GSH, similar ao observado em pacientes (Mueller *et al.*, 2001). Uma vez que houve um ambiente favorável a produção de H_2O_2 esperava-se uma redução drástica dos níveis de GSH mediante o aumento expressivo da atividade da GPx, o que não foi observado sugerindo uma reciclagem eficiente de GSH (Bellissimo *et al.*, 2001). Em contra partida, não se observou alteração da atividade

da enzima GR, similar a outros modelos de crises epilépticas prolongadas (Lei *et al.*, 2016). Outro motivo para uma não alteração da GR pode ser em parte pela estratégia metodológica escolhida, a qual consiste em adicionar NADPH e GSSG em excesso ao sistema de incubação. Caso controle e tratado possuam os mesmos níveis de expressão enzimática frente a mesma concentração de substratos enzimáticos, a formação de produto será a mesma, não significando que ambos os grupos encontravam-se em mesmas condições de GSSG e NADPH intracelulares.

Em contrapartida, a atividade elevada da enzima Gpx dependeria de elevados níveis de NADPH intracelulares (Brown *et al.*, 2014). Portanto, avaliou-se a atividade da G6PDH e ME citosólica. Em condições favoráveis à produção de H_2O_2 como a observada em crise epiléptica prolongadas induzidas por PTZ em peixe-zebra adulto ocorre um aumento no ROX, diminuição na atividade do complexo I do STE e aumento da atividade da SOD indicando um ambiente favorável à produção de H_2O_2. Para combater o estresse oxidativo (aumento de TBARS, redução de tióis totais, redução de potencial antioxidante total e aumento de oxidação de DCF), ocorre a ativação da enzima G6PDH, o que resulta no desvio da glicólise à via das pentoses-fosfato no intuito de maximizar a produção de NADPH (Kang *et al.*, 2015; Lee-Young *et al.*, 2016). Essa enzima apresentou um aumento de atividade na faixa de 30% ao comparar-se com o controle após a crise epiléptica prolongada induzida por PTZ em amostras de cérebro de peixe-zebra. Essa enzima apresenta um aumento de atividade na faixa de 35% ao comparar-se com o controle após a crise epiléptica prolongada induzida por PTZ em amostras de cérebro de peixe-zebra. Ambas enzimas desempenham papel fundamental na sobrevivência neuronal em episódios de hiperexcitabilidade e são novos alvos de pesquisa para o desenvolvimento de terapias farmacológicas (Bolanos *et al.*, 2008; Hadera *et al.*, 2016). Mesmo com a ativação do sistema antioxidante e da ativação dessas enzimas metabólicas detectamos dano oxidativo. O sistema antioxidante é ativo em um intervalo de tempo posterior ao aumento de ROS (Birben *et al.*, 2012). É provável que no momento escolhido para avaliação do estresse oxidativo, as defesas antioxidantes estejam sendo ativadas após a presença de um dano inicial, o que corresponde ao aumento de ROX, aumento de ROS, redução do potencial antioxidante total da amostra, aumento de dano lipídico e redução de grupamentos tiois totais (TRT). A presença de estresse oxidativo promove a ativação de defesas antioxidantes de forma direta (SOD, Gpx, GSH), e por forma indireta modula o metabolismo (**Figura 8)**.

Apesar de resultados contundentes na literatura apontarem uma relação entre o aumento de glicose em SNC e aumento de atividade cerebral, esse conceito pode estar equivocado (D'amico e Kowalska, 2014; Greve *et al.*, 2016; Reid *et al.*, 2016; Stoessl, 2017). Estudos apontam para um aumento na marcação de FDG em focos

epilépticos durante crises (Joo *et al.*, 2015; Sarikaya, 2015), técnica utilizada para mapear regiões cerebrais operáveis de pacientes epilépticos refratários (Willmann *et al.*, 2007). O que essa tese indica é que, mesmo perante tais achados, um fator não está necessariamente vinculado a outro em termos de produção de ATP para a manutenção de crises epilépticas prolongadas por parte da atividade mitocondrial. Avalia-se o astrócito como principal célula de acesso da glicose ao SNC (Jurcovicova, 2014), sendo a oxidação da glicose nessa célula um processo vital para o acúmulo de lactato extracelular, o qual será o principal substrato energético celular (Machler *et al.*, 2016). A glicose captada pelos neurônios via GLUT3 é utilizada para funções como síntese de ribonucleotídeos (Hay, 2016), NADPH (Amorini *et al.*, 2016), neurotransmissores (Volkow *et al.*, 2017), glicoproteínas (Garcia-Ayllon *et al.*, 2017), moléculas precursoras de glutationa (Pacold *et al.*, 2016), aminoácidos (Amorini *et al.*, 2017), entre outras funções (Langhans *et al.*, 2016). Esperar que o neurônio consuma glicose como fonte energética é inviável para a manutenção de células com expectativa de vida de décadas. É tão inesperado, que processos de neurodegeneração são acompanhados de mudanças energéticas, nas quais essas células passam a dar preferência à utilização de glicose como substrato energético (Divakaruni *et al.*, 2017). Uma vez que o único substrato energético a disposição da amostra seja glicose é de se esperar que a amostra como um todo a utilize como substrato energético acoplado ao consumo de O_2 para síntese de ATP, fato este que não se altera nos 5 min iniciais de crises epilépticas induzidas por PTZ em peixe-zebra adulto. Em um momento de desequilíbrio do sistema redox, onde o aumento de NADPH, e até mesmo de moléculas utilizadas para a síntese de GSH, há uma desvinculação na utilização de glicose acoplada ao consumo de O_2 para síntese de ATP. O cenário aponta para a possível utilização de glutamato e glutamina, ambos percursores de α-KG, contudo, estudos envolvendo espectrometria de massa indicam que glutamina só seria utilizada como fonte energética em casos de excesso molecular (Zhu *et al.*, 2017), essa seria a principal diferença ao comparar-se os resultados das amostras cerebrais de peixe-zebra expostos ao PTZ por 20 min, na presença de glicose + glutamato 0,1 mM e glicose + glutamina 0,1 mM. Uma vez que a amostra apresenta todos os tipos celulares cerebrais, e que o astrócito tende a produzir lactato ao invés de consumi-lo (Dienel, 2014), pode estar subestimando o papel desta célula e por isso não elevar essa resposta a níveis de controle, o que ambiciona novos estudos para validar a bioenergética de diferentes tipos celulares do SNC de peixe-zebra (Jourdain *et al.*, 2016). Em termos de metabolismo do β-HB, o mesmo encontra-se em baixas concentrações plasmáticas, mesmo ao decorrer de crises epilépticas prolongadas (Paoli *et al.*, 2013), portanto o resultado esperado é a não alteração no parâmetro de consumo de O_2 para síntese de ATP visto ao expor a amostra à β-HB 0,1 mM. Em termos de desequilíbrio redox é complicado definir casualidade. A hiperexcitabilidade em si pode levar a este

desequilíbrio, contudo, a oxidação completa do glutamato em um momento de declínio de piruvato intracelular pode contribuir para uma aceleração da atividade do complexo II e para o transporte reverso de elétrons para o complexo I, propiciando o aumento de $O_2^{\cdot -}$ ativando SOD, elevando os níveis de H_2O_2, levando a um consumo de GSH e ativação da Gpx, culminando na necessidade de NADPH ativando G6PDH e ME no processo. Apenas com futuros estudos será possível detalhar o que é causa e o que é consequência das mudanças metabólicas necessárias para manter as crises epilépticas prolongadas.

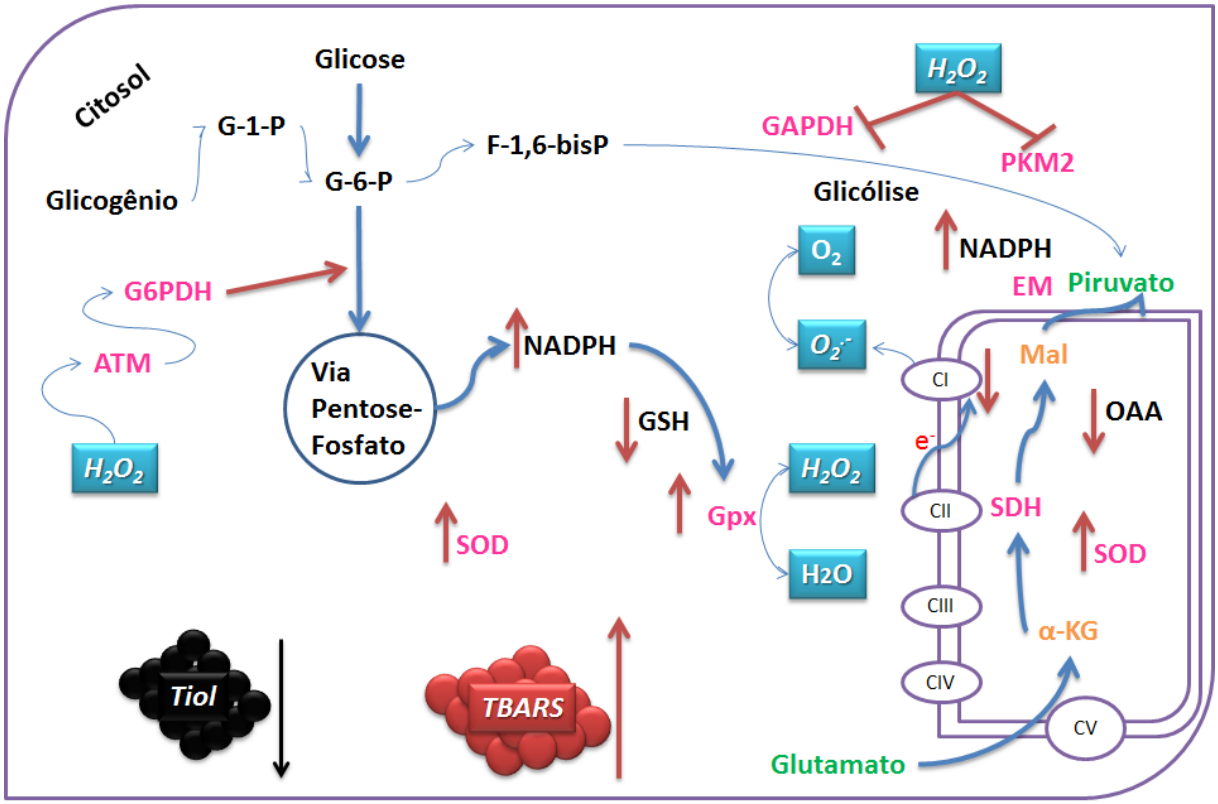

Figura 8. Modulação do metabolismo cerebral promovido por espécies reativas de oxigênio contribuem para a mudança do padrão energético de crises epilépticas prolongadas. Representado em roxo um citosol celular genérico. Também em roxo no canto direito inferior encontra-se uma mitocôndria com sua membrana interna e externa e os cinco complexos do STE: complexo I (CI), complexo II (CII), complexo III (CIII), complexo IV (CIV), complexo V (CV). Em laranja, inserido na mitocôndria, encontra-se um resumo do ciclo B do TCA. Em verde, substratos energéticos que adentram o TCA. Em rosa encontra-se todos os processos enzimáticos discutidos nesta tese. Com o aumento da presença de espécies reativas de O_2 (ROS) a concentração intracelular de H_2O_2 eleva-se causando dano ao DNA. A enzima Ataxia telangiectasia cinase (ATM) presente no núcleo celular, é exportada ao citosol mediante dano oxidativo do DNA. A ATM ativa a glicose-6-fosfato-desidrogenase (G6PDH), a qual desvia a glicose-6-fosfato (G-6-P) da glicólise para a via das pentose-fosfato, aumentando a produção de NADPH, para maior eficiência de sistemas antioxidantes como por exemplo o balanço de glutationa (GSH) e o balanço de atividade enzimática da glutationa peroxidase (Gpx). Concomitante, a oxidação promovida pelo H_2O_2 sobre as enzimas gliceraldeido-3-fosfato-desidrogenase (GAPDH) e piruvato-cinase (PKM2), inibe a atividade de ambas resultando em uma redução da atividade glicolítica celular. Decorrente da restrição energética, o sistema nervoso central passa a utilizar outras fontes energéticas, como o L-glutamato. A oxidação completa do glutamato na presença de piruvato em baixas concentrações reduz a concentração de oxaloacetato mitocondrial, a succinato desidrogenase passa a trabalhar em sua eficiência máxima, o que promove o transporte reverso de elétrons do complexo II para o complexo I aumentando a produção de superóxido e diminuindo a atividade do último, elevando a atividade da SOD. Para evitar os efeitos deletérios desta espécie reativa de oxigênio, a glutationa peroxidase é ativada consumindo níveis intracelulares da NADPH que são repostos pela via das pentoses fosfato e pela ativadade da enzima málica citosólica no processo de oxidação completa do glutamato, ambas atividades aumentadas neste momento. Apesar da modulação, ocorre dano oxidativo por aumento de lipoperoxidação (TBARS) e oxidação de grupamentos tióis em proteínas (Tiol). Uma vez que o ciclo se estabelece devido a uma necessidade energética ele se retroalimenta. Dados desta tese somados a imagem modificada de (Kang *et al.*, 2015).

III.2. Conclusão

Modulações energéticas cerebrais permitem a manutenção de crises epilépticas prolongadas induzidas por pentilenotetrazol em peixe-zebra adulto, a qual promove um desbalanço no sistema Redox celular que retroalimenta a necessidade da utilização de L-glutamato, L-glutamina, L-lactato e β-hidorxibutirato, uma vez que o metabolismo da D-glicose é imperativo para o combate ao estresse oxidativo.

III.3. Perspectivas

Esta tese é o início de uma nova linha de pesquisa em nosso laboratório. Para poder chegar a melhores conclusões de nossos achados, três novos projetos terão início em março de 2017. O primeiro projeto aperfeiçoará o protocolo de dissociação celular. O segundo projeto visará avaliar o impacto sinalizatório do glutamato sobre o metabolismo energético. O terceiro projeto caracterizará a detecção de níveis de H_2O_2 pelo equipamento oxygraph 2K® e modulará farmacologicamente a atividade das enzimas glicose-6-fosfato-desidrogenase e enzima-málica citosólica para confirmação dos achados desta tese.

III.4. Referências

AAS, J. E. et al. Redistribution of glutamate and glutamine in slices of human neocortex exposed to combined hypoxia and glucose deprivation in vitro. **J Cereb Blood Flow Metab,** v. 13, n. 3, p. 503-15, May 1993. ISSN 0271-678X (Print)

0271-678X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/8097518 >.

ACHANTA, L. B.; RAE, C. D. beta-Hydroxybutyrate in the Brain: One Molecule, Multiple Mechanisms. **Neurochem Res,** v. 42, n. 1, p. 35-49, Jan 2017. ISSN 1573-6903 (Electronic)

0364-3190 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27826689 >.

AKERBOOM, J. et al. Optimization of a GCaMP calcium indicator for neural activity imaging. **J Neurosci,** v. 32, n. 40, p. 13819-40, Oct 03 2012. ISSN 1529-2401 (Electronic)

0270-6474 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23035093 >.

ALFARO, J. M.; RIPOLL-GOMEZ, J.; BURGOS, J. S. Kainate administered to adult zebrafish causes seizures similar to those in rodent models. **Eur J Neurosci,** v. 33, n. 7, p. 1252-5, Apr 2011. ISSN 1460-9568 (Electronic)

0953-816X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21375600 >.

ALLMANN, S.; BRINGAUD, F. Glycosomes: A comprehensive view of their metabolic roles in T. brucei. **Int J Biochem Cell Biol,** v. 85, p. 85-90, Feb 06 2017. ISSN 1878-5875 (Electronic)

1357-2725 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/28179189 >.

AMORINI, A. M. et al. Metabolic, enzymatic and gene involvement in cerebral glucose dysmetabolism after traumatic brain injury. **Biochim Biophys Acta,** v. 1862, n. 4, p. 679-87, Apr 2016. ISSN 0006-3002 (Print)

0006-3002 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26844378 >.

_____. Severity of experimental traumatic brain injury modulates changes in concentrations of cerebral free amino acids. **J Cell Mol Med,** v. 21, n. 3, p. 530-542, Mar 2017. ISSN 1582-4934 (Electronic)

1582-1838 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27696676 >.

ANDERSON, S. et al. Sequence and organization of the human mitochondrial genome. **Nature,** v. 290, n. 5806, p. 457-65, Apr 09 1981. ISSN 0028-0836 (Print)

0028-0836 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/7219534 >.

BARABAN, S. C.; DINDAY, M. T.; HORTOPAN, G. A. Drug screening in Scn1a zebrafish mutant identifies clemizole as a potential Dravet syndrome treatment. **Nat Commun,** v. 4, p. 2410, 2013. ISSN 2041-1723 (Electronic)

2041-1723 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24002024 >.

BARABAN, S. C. et al. Pentylenetetrazole induced changes in zebrafish behavior, neural activity and c-fos expression. **Neuroscience,** v. 131, n. 3, p. 759-68, 2005. ISSN 0306-4522 (Print)

0306-4522 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/15730879 >.

BAUMGART, E. V. et al. Stab wound injury of the zebrafish telencephalon: a model for comparative analysis of reactive gliosis. **Glia,** v. 60, n. 3, p. 343-57, Mar 2012. ISSN 1098-1136 (Electronic)

0894-1491 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22105794 >.

BEHAR, K. L.; ROTHMAN, D. L. In vivo nuclear magnetic resonance studies of glutamate-gamma-aminobutyric acid-glutamine cycling in rodent and human cortex: the central role of glutamine. **J Nutr,** v. 131, n. 9 Suppl, p. 2498S-504S; discussion 2523S-4S, Sep 2001. ISSN 0022-3166 (Print)

0022-3166 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11533301 >.

BELANGER, M.; ALLAMAN, I.; MAGISTRETTI, P. J. Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation. **Cell Metab,** v. 14, n. 6, p. 724-38, Dec 7 2011. ISSN 1932-7420 (Electronic)

1550-4131 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22152301 >.

BELLISSIMO, M. I. et al. Superoxide dismutase, glutathione peroxidase activities and the hydroperoxide concentration are modified in the hippocampus of epileptic rats. **Epilepsy Res,** v. 46, n. 2, p. 121-8, Aug 2001. ISSN 0920-1211 (Print)

0920-1211 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11463513 >.

BERG, A. T.; MILLICHAP, J. J. The 2010 revised classification of seizures and epilepsy. **Continuum (Minneap Minn),** v. 19, n. 3 Epilepsy, p. 571-97, Jun 2013. ISSN 1538-6899 (Electronic)

1080-2371 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23739099 >.

BHANGOO, S. K.; SWANSON, G. T. Kainate receptor signaling in pain pathways. **Mol Pharmacol,** v. 83, n. 2, p. 307-15, Feb 2013. ISSN 1521-0111 (Electronic)

0026-895X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23095167 >.

BIDMON, H. J. et al. Glutamine synthetase becomes nitrated and its activity is reduced during repetitive seizure activity in the pentylentetrazole model of epilepsy. **Epilepsia,** v. 49, n. 10, p. 1733-48, Oct 2008. ISSN 1528-1167 (Electronic)

0013-9580 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18479397 >.

BIRBEN, E. et al. Oxidative stress and antioxidant defense. **World Allergy Organ J,** v. 5, n. 1, p. 9-19, Jan 2012. ISSN 1939-4551 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23268465 >.

BOLANOS, J. P. et al. Regulation of glycolysis and pentose-phosphate pathway by nitric oxide: impact on neuronal survival. **Biochim Biophys Acta,** v. 1777, n. 7-8, p. 789-93, Jul-Aug 2008. ISSN 0006-3002 (Print)

0006-3002 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18455501 >.

BOSE, A.; BEAL, M. F. Mitochondrial dysfunction in Parkinson's disease. **J Neurochem,** v. 139 Suppl 1, p. 216-231, Oct 2016. ISSN 1471-4159 (Electronic)

0022-3042 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27546335 >.

BOZZO, L.; PUYAL, J.; CHATTON, J. Y. Lactate modulates the activity of primary cortical neurons through a receptor-mediated pathway. **PLoS One,** v. 8, n. 8, p. e71721, 2013. ISSN 1932-6203 (Electronic)

1932-6203 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23951229 >.

BRAND, M. D. Mitochondrial generation of superoxide and hydrogen peroxide as the source of mitochondrial redox signaling. **Free Radic Biol Med,** v. 100, p. 14-31, Nov 2016. ISSN 1873-4596 (Electronic)

0891-5849 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27085844 >.

BRAND, M. D.; NICHOLLS, D. G. Assessing mitochondrial dysfunction in cells. **Biochem J,** v. 435, n. 2, p. 297-312, Apr 15 2011. ISSN 1470-8728 (Electronic)

0264-6021 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21726199 >.

BREKKE, E. et al. Anaplerosis for Glutamate Synthesis in the Neonate and in Adulthood. **Adv Neurobiol,** v. 13, p. 43-58, 2016. ISSN 2190-5215 (Print). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27885626 >.

BROUGHTON, R. E.; MILAM, J. E.; ROE, B. A. The complete sequence of the zebrafish (Danio rerio) mitochondrial genome and evolutionary patterns in vertebrate mitochondrial DNA. **Genome Res,** v. 11, n. 11, p. 1958-67, Nov 2001. ISSN 1088-9051 (Print)

1088-9051 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11691861 >.

BROWN, N. C.; ANDREAZZA, A. C.; YOUNG, L. T. An updated meta-analysis of oxidative stress markers in bipolar disorder. **Psychiatry Res,** v. 218, n. 1-2, p. 61-8, Aug 15 2014. ISSN 1872-7123 (Electronic)

0165-1781 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24794031 >.

BROWN, S. A.; SIMPSON, A. J.; SIMPSON, M. J. Evaluation of sample preparation methods for nuclear magnetic resonance metabolic profiling studies with Eisenia fetida. **Environ Toxicol Chem,** v. 27, n. 4, p. 828-36, Apr 2008. ISSN 0730-7268 (Print)

0730-7268 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18333692 >.

BRYANT, A. S. et al. Maintenance of thalamic epileptiform activity depends on the astrocytic glutamate-glutamine cycle. **J Neurophysiol,** v. 102, n. 5, p. 2880-8, Nov 2009. ISSN 1522-1598 (Electronic)

0022-3077 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19741104 >.

CHAKRABARTI, S. et al. Frequency of gamma-Ray Induced Specific Locus and Recessive Lethal Mutations in Mature Germ Cells of the Zebrafish, BRACHYDANIO RERIO. **Genetics,** v. 103, n. 1, p. 109-23, Jan 1983. ISSN 0016-6731 (Print)

0016-6731 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17246098 >.

CHANG, D. T.; HONICK, A. S.; REYNOLDS, I. J. Mitochondrial trafficking to synapses in cultured primary cortical neurons. **J Neurosci,** v. 26, n. 26, p. 7035-45, Jun 28 2006. ISSN 1529-2401 (Electronic)

0270-6474 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16807333 >.

CIESLAK, M.; WOJTCZAK, A.; KOMOSZYNSKI, M. Role of the purinergic signaling in epilepsy. **Pharmacol Rep,** v. 69, n. 1, p. 130-138, Feb 2017. ISSN 1734-1140 (Print)

1734-1140 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27915186 >.

CRAIG, A. J.; HOUSLEY, G. D. Evaluation of Gene Therapy as an Intervention Strategy to Treat Brain Injury from Stroke. **Front Mol Neurosci,** v. 9, p. 34, 2016. ISSN 1662-5099 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27252622 >.

CRUZ, R. S. et al. Intracellular shuttle: the lactate aerobic metabolism. **ScientificWorldJournal,** v. 2012, p. 420984, 2012. ISSN 1537-744X (Electronic)

1537-744X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22593684 >.

D'AMICO, A.; KOWALSKA, T. Paradoxal metabolic flare detected by 18F-fluorodeoxyglucose positron emission tomography in a patient with metastatic breast cancer treated with aromatase inhibitor and biphosphonate. **Indian J Nucl Med,** v. 29, n. 1, p. 34-7, Jan 2014. ISSN 0972-3919 (Print)

0974-0244 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24591781 >.

DANBOLT, N. C. Glutamate uptake. **Prog Neurobiol,** v. 65, n. 1, p. 1-105, Sep 2001. ISSN 0301-0082 (Print)

0301-0082 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11369436 >.

DANBOLT, N. C.; FURNESS, D. N.; ZHOU, Y. Neuronal vs glial glutamate uptake: Resolving the conundrum. **Neurochem Int,** v. 98, p. 29-45, Sep 2016. ISSN 1872-9754 (Electronic)

0197-0186 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27235987 >.

DAVULURI, G. et al. Inactivation of 3-hydroxybutyrate dehydrogenase 2 delays zebrafish erythroid maturation by conferring premature mitophagy. **Proc Natl Acad Sci U S A,** v. 113, n. 11, p. E1460-9, Mar 15 2016. ISSN 1091-6490 (Electronic)

0027-8424 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26929344 >.

DE OLIVEIRA, D. L. et al. Early life LiCl-pilocarpine-induced status epilepticus reduces acutely hippocampal glutamate uptake and Na+/K+ ATPase activity. **Brain Res,** v. 1369, p. 167-72, Jan 19 2011. ISSN 1872-6240 (Electronic)

0006-8993 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20977896 >.

DEL ARCO, A. et al. Calcium regulation of mitochondrial carriers. **Biochim Biophys Acta,** v. 1863, n. 10, p. 2413-21, Oct 2016. ISSN 0006-3002 (Print)

0006-3002 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27033520 >.

DEMAUREX, N.; SCHWARZLANDER, M. Mitochondrial Flashes: Dump Superoxide and Dance with Protons Now. **Antioxid Redox Signal,** v. 25, n. 9, p. 550-1, Sep 20 2016. ISSN 1557-7716 (Electronic)

1523-0864 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27400996 >.

DIENEL, G. A. Lactate shuttling and lactate use as fuel after traumatic brain injury: metabolic considerations. **J Cereb Blood Flow Metab,** v. 34, n. 11, p. 1736-48, Nov 2014. ISSN 1559-7016 (Electronic)

0271-678X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25204393 >.

DIVAKARUNI, A. S. et al. Inhibition of the mitochondrial pyruvate carrier protects from excitotoxic neuronal death. **J Cell Biol**, Mar 02 2017. ISSN 1540-8140 (Electronic)

0021-9525 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/28254829 >.

DONA, F. et al. Variations of ATP and its metabolites in the hippocampus of rats subjected to pilocarpine-induced temporal lobe epilepsy. **Purinergic Signal,** v. 12, n. 2, p. 295-302, Jun 2016. ISSN 1573-9546 (Electronic)

1573-9538 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26939579 >.

DUA, T. et al. Epilepsy Care in the World: results of an ILAE/IBE/WHO Global Campaign Against Epilepsy survey. **Epilepsia,** v. 47, n. 7, p. 1225-31, Jul 2006. ISSN 0013-9580 (Print)

0013-9580 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16886987 >.

DULAC, O. et al. Occasional seizures, epilepsy, and inborn errors of metabolism. **Lancet Neurol,** v. 13, n. 7, p. 727-39, Jul 2014. ISSN 1474-4465 (Electronic)

1474-4422 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24943345 >.

DUNKLEY, P. R.; JARVIE, P. E.; ROBINSON, P. J. A rapid Percoll gradient procedure for preparation of synaptosomes. **Nat Protoc,** v. 3, n. 11, p. 1718-28, 2008. ISSN 1750-2799 (Electronic)

1750-2799 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18927557 >.

DZUBAY, J. A.; JAHR, C. E. The concentration of synaptically released glutamate outside of the climbing fiber-Purkinje cell synaptic cleft. **J Neurosci,** v. 19, n. 13, p. 5265-74, Jul 1 1999. ISSN 0270-6474 (Print)

0270-6474 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10377338 >.

EDDINS, D. et al. Zebrafish provide a sensitive model of persisting neurobehavioral effects of developmental chlorpyrifos exposure: comparison with nicotine and pilocarpine effects and relationship to dopamine deficits. **Neurotoxicol Teratol,** v. 32, n. 1, p. 99-108, Jan-Feb 2010. ISSN 1872-9738 (Electronic)

0892-0362 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19268529 >.

EGAN, B.; D'AGOSTINO, D. P. Fueling Performance: Ketones Enter the Mix. **Cell Metab,** v. 24, n. 3, p. 373-5, Sep 13 2016. ISSN 1932-7420 (Electronic)

1550-4131 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27626197 >.

EID, T. et al. The Glutamate-Glutamine Cycle in Epilepsy. **Adv Neurobiol,** v. 13, p. 351-400, 2016. ISSN 2190-5215 (Print). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27885637 >.

ELICEIRI, B. P.; GONZALEZ, A. M.; BAIRD, A. Zebrafish model of the blood-brain barrier: morphological and permeability studies. **Methods Mol Biol,** v. 686, p. 371-8, 2011. ISSN 1940-6029 (Electronic)

1064-3745 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21082382 >.

ENGEL, J., JR. The etiologic classification of epilepsy. **Epilepsia,** v. 52, n. 6, p. 1195-7; discussion 1205-9, Jun 2011. ISSN 1528-1167 (Electronic)

0013-9580 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21671927 >.

FISHER, R. S. Final comments on the process: ILAE definition of epilepsy. **Epilepsia,** v. 55, n. 4, p. 492-3, Apr 2014. ISSN 1528-1167 (Electronic)

0013-9580 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24731068 >.

FISHER, R. S. Redefining epilepsy. **Curr Opin Neurol,** v. 28, n. 2, p. 130-5, Apr 2015. ISSN 1473-6551 (Electronic)

1350-7540 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25734953 >.

FISHER, R. S. et al. ILAE official report: a practical clinical definition of epilepsy. **Epilepsia,** v. 55, n. 4, p. 475-82, Apr 2014. ISSN 1528-1167 (Electronic)

0013-9580 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24730690 >.

FREY, S. et al. The addition of ketone bodies alleviates mitochondrial dysfunction by restoring complex I assembly in a MELAS cellular model. **Biochim Biophys Acta,** v. 1863, n. 1, p. 284-291, Jan 2017. ISSN 0006-3002 (Print)

0006-3002 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27815040 >.

GABRYEL, B. et al. Superoxide dismutase 1 and glutathione peroxidase 1 are involved in the protective effect of sulodexide on vascular endothelial cells exposed to oxygen-glucose deprivation. **Microvasc Res,** v. 103, p. 26-35, Jan 2016. ISSN 1095-9319 (Electronic)

0026-2862 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26477504 >.

GANO, L. B.; PATEL, M.; RHO, J. M. Ketogenic diets, mitochondria, and neurological diseases. **J Lipid Res,** v. 55, n. 11, p. 2211-28, Nov 2014. ISSN 1539-7262 (Electronic)

0022-2275 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24847102 >.

GARCIA-AYLLON, M. S. et al. HNK-1 Carrier Glycoproteins Are Decreased in the Alzheimer's Disease Brain. **Mol Neurobiol,** v. 54, n. 1, p. 188-199, Jan 2017. ISSN 1559-1182 (Electronic)

0893-7648 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26738850 >.

GERLAI, R. High-throughput behavioral screens: the first step towards finding genes involved in vertebrate brain function using zebrafish. **Molecules,** v. 15, n. 4, p. 2609-22, Apr 12 2010. ISSN 1420-3049 (Electronic)

1420-3049 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20428068 >.

GERLAI, R. A small fish with a big future: zebrafish in behavioral neuroscience. **Rev Neurosci,** v. 22, n. 1, p. 3-4, 2011. ISSN 0334-1763 (Print)

0334-1763 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21615256 >.

GNAIGER, E. **Mitochondrial pathways and respiratory control. An introduction to OXPHOS analysis.** Innsbruck: OROBOROS MiPNet Publications, 2014.

GOODMAN, L. S. et al. Comparison of maximal seizures evoked by pentylenetetrazol (metrazol) and electroshock in mice, and their modification by anticonvulsants. **J Pharmacol Exp Ther,** v. 108, n. 2, p. 168-76, Jun 1953. ISSN 0022-3565 (Print)

0022-3565 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/13062087 >.

GORMAN, G. S. et al. Mitochondrial diseases. **Nat Rev Dis Primers,** v. 2, p. 16080, Oct 20 2016. ISSN 2056-676X (Electronic)

2056-676X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27775730 >.

GORMAN, L. et al. Serum Beta Hydroxybutyrate Concentrations in Cats with Chronic Kidney Disease, Hyperthyroidism, or Hepatic Lipidosis. **J Vet Intern Med,** v. 30, n. 2, p. 611-6, Mar-Apr 2016. ISSN 1939-1676 (Electronic)

0891-6640 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26773702 >.

GRAY, M. W. Mitochondrial evolution. **Cold Spring Harb Perspect Biol,** v. 4, n. 9, p. a011403, Sep 01 2012. ISSN 1943-0264 (Electronic)

1943-0264 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22952398 >.

GREVE, D. N. et al. Different partial volume correction methods lead to different conclusions: An (18)F-FDG-PET study of aging. **Neuroimage,** v. 132, p. 334-43, May 15 2016. ISSN 1095-9572 (Electronic)

1053-8119 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26915497 >.

GRONE, B. P.; BARABAN, S. C. Animal models in epilepsy research: legacies and new directions. **Nat Neurosci,** v. 18, n. 3, p. 339-43, Mar 2015. ISSN 1546-1726 (Electronic)

1097-6256 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25710835 >.

HADERA, M. G. et al. Modification of Astrocyte Metabolism as an Approach to the Treatment of Epilepsy: Triheptanoin and Acetyl-L-Carnitine. **Neurochem Res,** v. 41, n. 1-2, p. 86-95, Feb 2016. ISSN 1573-6903 (Electronic)

0364-3190 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26433381 >.

HARDIE, D. G.; ROSS, F. A.; HAWLEY, S. A. AMPK: a nutrient and energy sensor that maintains energy homeostasis. **Nat Rev Mol Cell Biol,** v. 13, n. 4, p. 251-62, Mar 22 2012. ISSN 1471-0080 (Electronic)

1471-0072 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22436748 >.

HASHIGUCHI, M. et al. Meta-analysis of the efficacy and safety of Ginkgo biloba extract for the treatment of dementia. **J Pharm Health Care Sci,** v. 1, p. 14, 2015. ISSN 2055-0294 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26819725 >.

HAUKAAS, T. H. et al. Impact of Freezing Delay Time on Tissue Samples for Metabolomic Studies. **Front Oncol,** v. 6, p. 17, 2016. ISSN 2234-943X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26858940 >.

HAY, N. Reprogramming glucose metabolism in cancer: can it be exploited for cancer therapy? **Nat Rev Cancer,** v. 16, n. 10, p. 635-49, Oct 2016. ISSN 1474-1768 (Electronic)

1474-175X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27634447 >.

HODOLIC, M.; TOPAKIAN, R.; PICHLER, R. (18)F-fluorodeoxyglucose and (18)F-flumazenil positron emission tomography in patients with refractory epilepsy. **Radiol Oncol,** v. 50, n. 3, p. 247-53, Sep 1 2016. ISSN 1318-2099 (Print)

1318-2099 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27679539 >.

HONGORO, C.; NORMAND, C. Health Workers: Building and Motivating the Workforce. 2006. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21250311 >.

HORTOPAN, G. A.; DINDAY, M. T.; BARABAN, S. C. Spontaneous seizures and altered gene expression in GABA signaling pathways in a mind bomb mutant zebrafish. **J Neurosci,** v. 30, n. 41, p. 13718-28, Oct 13 2010. ISSN 1529-2401 (Electronic)

0270-6474 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20943912 >.

HOUTEN, S. M. et al. The Biochemistry and Physiology of Mitochondrial Fatty Acid beta-Oxidation and Its Genetic Disorders. **Annu Rev Physiol,** v. 78, p. 23-44, 2016. ISSN 1545-1585 (Electronic)

0066-4278 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26474213 >.

HOWE, K. et al. The zebrafish reference genome sequence and its relationship to the human genome. **Nature,** v. 496, n. 7446, p. 498-503, Apr 25 2013. ISSN 1476-4687 (Electronic)

0028-0836 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23594743 >.

HUANG, H. L. et al. Trypsin-induced proteome alteration during cell subculture in mammalian cells. **J Biomed Sci,** v. 17, p. 36, May 11 2010. ISSN 1423-0127 (Electronic)

1021-7770 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20459778 >.

HUNT, R. F. et al. A novel zebrafish model of hyperthermia-induced seizures reveals a role for TRPV4 channels and NMDA-type glutamate receptors. **Exp Neurol,** v. 237, n. 1, p. 199-206, Sep 2012. ISSN 1090-2430 (Electronic)

0014-4886 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22735490 >.

HYDER, F. et al. Neuronal-glial glucose oxidation and glutamatergic-GABAergic function. **J Cereb Blood Flow Metab,** v. 26, n. 7, p. 865-77, Jul 2006. ISSN 0271-678X (Print)

0271-678X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16407855 >.

IOMMARINI, L. et al. Mitochondrial metabolism and energy sensing in tumor progression. **Biochim Biophys Acta**, Feb 14 2017. ISSN 0006-3002 (Print)

0006-3002 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/28213331 >.

IVANOV, A. I. et al. Glycolysis and oxidative phosphorylation in neurons and astrocytes during network activity in hippocampal slices. **J Cereb Blood Flow Metab,** v. 34, n. 3, p. 397-407, Mar 2014. ISSN 1559-7016 (Electronic)

0271-678X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24326389 >.

JEONG, J. Y. et al. Functional and developmental analysis of the blood-brain barrier in zebrafish. **Brain Res Bull,** v. 75, n. 5, p. 619-28, Mar 28 2008. ISSN 0361-9230 (Print)

0361-9230 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18355638 >.

JHA, M. K.; JEON, S.; SUK, K. Pyruvate Dehydrogenase Kinases in the Nervous System: Their Principal Functions in Neuronal-glial Metabolic Interaction and Neuro-metabolic Disorders. **Curr Neuropharmacol,** v. 10, n. 4, p. 393-403, Dec 2012. ISSN 1570-159X (Print)

1570-159X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23730261 >.

JOO, E. Y. et al. Functional neuroimaging findings in patients with lateral and mesio-lateral temporal lobe epilepsy; FDG-PET and ictal SPECT studies. **J Neurol,** v. 262, n. 5, p. 1120-9, May 2015. ISSN 1432-1459 (Electronic)

0340-5354 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25794857 >.

JOURDAIN, P. et al. L-Lactate protects neurons against excitotoxicity: implication of an ATP-mediated signaling cascade. **Sci Rep,** v. 6, p. 21250, Feb 19 2016. ISSN 2045-2322 (Electronic)

2045-2322 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26893204 >.

JUGE, N. et al. Metabolic control of vesicular glutamate transport and release. **Neuron,** v. 68, n. 1, p. 99-112, Oct 6 2010. ISSN 1097-4199 (Electronic)

0896-6273 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20920794 >.

JURCOVICOVA, J. Glucose transport in brain - effect of inflammation. **Endocr Regul,** v. 48, n. 1, p. 35-48, Jan 2014. ISSN 1210-0668 (Print)

1210-0668 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24524374 >.

KALUEFF, A. V.; STEWART, A. M.; GERLAI, R. Zebrafish as an emerging model for studying complex brain disorders. **Trends Pharmacol Sci,** v. 35, n. 2, p. 63-75, Feb 2014. ISSN 1873-3735 (Electronic)

0165-6147 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24412421 >.

KANDEL, E. R. et al. **Principles of neural science**. 5th. New York, NY ; London: McGraw-Hill, 2013. l, 1709 p. ISBN 9780071390118.

KANG, S. W.; LEE, S.; LEE, E. K. ROS and energy metabolism in cancer cells: alliance for fast growth. **Arch Pharm Res,** v. 38, n. 3, p. 338-45, Mar 2015. ISSN 0253-6269 (Print)

0253-6269 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25599615 >.

KARACA, M. et al. GDH-Dependent Glutamate Oxidation in the Brain Dictates Peripheral Energy Substrate Distribution. **Cell Rep,** v. 13, n. 2, p. 365-75, Oct 13 2015. ISSN 2211-1247 (Electronic). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26440896 >.

KATAGIRI, H.; TANAKA, K.; MANABE, T. Requirement of appropriate glutamate concentrations in the synaptic cleft for hippocampal LTP induction. **Eur J Neurosci,** v. 14, n. 3, p. 547-53, Aug 2001. ISSN 0953-816X (Print)

0953-816X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11553304 >.

KIM, D. Y. et al. Ketone bodies mediate antiseizure effects through mitochondrial permeability transition. **Ann Neurol,** v. 78, n. 1, p. 77-87, Jul 2015. ISSN 1531-8249 (Electronic)

0364-5134 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25899847 >.

KUMAR, M. G. et al. Altered Glycolysis and Mitochondrial Respiration in a Zebrafish Model of Dravet Syndrome. **eNeuro,** v. 3, n. 2, Mar-Apr 2016. ISSN 2373-2822 (Electronic)

2373-2822 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27066534 >.

LAIRD, M. D. et al. Augmentation of normal and glutamate-impaired neuronal respiratory capacity by exogenous alternative biofuels. **Transl Stroke Res,** v. 4, n. 6, p. 643-51, Dec 2013. ISSN 1868-601X (Electronic)

1868-4483 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24323418 >.

LANE, N.; MARTIN, W. The energetics of genome complexity. **Nature,** v. 467, n. 7318, p. 929-34, Oct 21 2010. ISSN 1476-4687 (Electronic)

0028-0836 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20962839 >.

LANGHANS, W.; BERTHOUD, H. R.; WESTERTERP-PLANTENGA, M. Introduction to 'All roads take to the brain: neural control of energy homeostasis in health and disease'. **Int J Obes (Lond),** v. 40, n. 2, p. 191-2, Feb 2016. ISSN 1476-5497 (Electronic)

0307-0565 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26857268 >.

LARSEN, J. et al. The role of SLC2A1 mutations in myoclonic astatic epilepsy and absence epilepsy, and the estimated frequency of GLUT1 deficiency syndrome. **Epilepsia,** v. 56, n. 12, p. e203-8, Dec 2015. ISSN 1528-1167 (Electronic)

0013-9580 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26537434 >.

LECLERCQ, K. et al. Cross-species pharmacological characterization of the allylglycine seizure model in mice and larval zebrafish. **Epilepsy Behav,** v. 45, p. 53-63, Apr 2015. ISSN 1525-5069 (Electronic)

1525-5050 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25845493 >.

LEE-YOUNG, R. S. et al. Glucose-6-phosphate dehydrogenase contributes to the regulation of glucose uptake in skeletal muscle. **Mol Metab,** v. 5, n. 11, p. 1083-1091, Nov 2016. ISSN 2212-8778 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27818934 >.

LEE, E. M. et al. Changes in glucose metabolism and metabolites during the epileptogenic process in the lithium-pilocarpine model of epilepsy. **Epilepsia,** v. 53, n. 5, p. 860-9, May 2012. ISSN 1528-1167 (Electronic)

0013-9580 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22429025 >.

LEI, X. G. et al. Paradoxical Roles of Antioxidant Enzymes: Basic Mechanisms and Health Implications. **Physiol Rev,** v. 96, n. 1, p. 307-64, Jan 2016. ISSN 1522-1210 (Electronic)

0031-9333 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26681794 >.

LEKE, R.; SCHOUSBOE, A. The Glutamine Transporters and Their Role in the Glutamate/GABA-Glutamine Cycle. **Adv Neurobiol,** v. 13, p. 223-257, 2016. ISSN 2190-5215 (Print). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27885631 >.

LIANG, L. P. et al. Mitochondrial oxidative stress and epilepsy in SOD2 deficient mice: attenuation by a lipophilic metalloporphyrin. **Neurobiol Dis,** v. 45, n. 3, p. 1068-76, Mar 2012. ISSN 1095-953X (Electronic)

0969-9961 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22200564 >.

LIESA, M.; SHIRIHAI, O. S. Mitochondrial dynamics in the regulation of nutrient utilization and energy expenditure. **Cell Metab,** v. 17, n. 4, p. 491-506, Apr 02 2013. ISSN 1932-7420 (Electronic)

1550-4131 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23562075 >.

LIPKA, K.; BULOW, H. H. Lactic acidosis following convulsions. **Acta Anaesthesiol Scand,** v. 47, n. 5, p. 616-8, May 2003. ISSN 0001-5172 (Print)

0001-5172 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12699523 >.

LIU, L. et al. Malic enzyme tracers reveal hypoxia-induced switch in adipocyte NADPH pathway usage. **Nat Chem Biol,** v. 12, n. 5, p. 345-52, May 2016. ISSN 1552-4469 (Electronic)

1552-4450 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26999781 >.

LLORET, A. et al. Reductive Stress: A New Concept in Alzheimer's Disease. **Curr Alzheimer Res,** v. 13, n. 2, p. 206-11, 2016. ISSN 1875-5828 (Electronic)

1567-2050 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26391042 >.

LOPES DA FONSECA, T. et al. The zebrafish homologue of Parkinson's disease ATP13A2 is essential for embryonic survival. **Brain Res Bull,** v. 90, p. 118-26, Jan 2013. ISSN 1873-2747 (Electronic)

0361-9230 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23123961 >.

MACHLER, P. et al. In Vivo Evidence for a Lactate Gradient from Astrocytes to Neurons. **Cell Metab,** v. 23, n. 1, p. 94-102, Jan 12 2016. ISSN 1932-7420 (Electronic)

1550-4131 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26698914 >.

MARQUEZ, J. et al. Glutamate and Brain Glutaminases in Drug Addiction. **Neurochem Res**, Dec 23 2016. ISSN 1573-6903 (Electronic)

0364-3190 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/28012058 >.

MASSARSKY, A.; KOZAL, J. S.; DI GIULIO, R. T. Glutathione and zebrafish: Old assays to address a current issue. **Chemosphere,** v. 168, p. 707-715, Feb 2017. ISSN 1879-1298 (Electronic)

0045-6535 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27836271 >.

MCKENNA, M. C. The glutamate-glutamine cycle is not stoichiometric: fates of glutamate in brain. **J Neurosci Res,** v. 85, n. 15, p. 3347-58, Nov 15 2007. ISSN 0360-4012 (Print)

0360-4012 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17847118 >.

MCKENNA, M. C. Substrate competition studies demonstrate oxidative metabolism of glucose, glutamate, glutamine, lactate and 3-hydroxybutyrate in cortical astrocytes from rat brain. **Neurochem Res,** v. 37, n. 11, p. 2613-26, Nov 2012. ISSN 1573-6903 (Electronic)

0364-3190 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23079895 >.

MCKENNA, M. C. et al. Glutamate oxidation in astrocytes: Roles of glutamate dehydrogenase and aminotransferases. **J Neurosci Res,** v. 94, n. 12, p. 1561-1571, Dec 2016. ISSN 1097-4547 (Electronic)

0360-4012 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27629247 >.

MCNALLY, M. A.; HARTMAN, A. L. Ketone bodies in epilepsy. **J Neurochem,** v. 121, n. 1, p. 28-35, Apr 2012. ISSN 1471-4159 (Electronic)

0022-3042 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22268909 >.

MERGENTHALER, P. et al. Sugar for the brain: the role of glucose in physiological and pathological brain function. **Trends Neurosci,** v. 36, n. 10, p. 587-97, Oct 2013. ISSN 1878-108X (Electronic)

0166-2236 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23968694 >.

MILLS, E. L.; O'NEILL, L. A. Reprogramming mitochondrial metabolism in macrophages as an anti-inflammatory signal. **Eur J Immunol,** v. 46, n. 1, p. 13-21, Jan 2016. ISSN 1521-4141 (Electronic)

0014-2980 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26643360 >.

MITTLER, R. ROS Are Good. **Trends Plant Sci,** v. 22, n. 1, p. 11-19, Jan 2017. ISSN 1878-4372 (Electronic)

1360-1385 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27666517 >.

MOGHETTI, P. et al. Metabolic Effects of Exercise. **Front Horm Res,** v. 47, p. 44-57, 2016. ISSN 1662-3762 (Electronic)

0301-3073 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27348753 >.

MORI, N. et al. Increased activity of superoxide dismutase in kindled brain and suppression of kindled seizure following intra-amygdaloid injection of superoxide dismutase in rats. **Brain Res,** v. 557, n. 1-2, p. 313-5, Aug 23 1991. ISSN 0006-8993 (Print)

0006-8993 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/1747763 >.

MUELLER, S. G. et al. Brain glutathione levels in patients with epilepsy measured by in vivo (1)H-MRS. **Neurology,** v. 57, n. 8, p. 1422-7, Oct 23 2001. ISSN 0028-3878 (Print)

0028-3878 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11673583 >.

MUSSULINI B.H., B. S., MORO L., DIAS R.D., CALCAGNOTTO M.E., RICO E.P., AND DE OLIVEIRA D.L. Zebrafish as an Animal Model to Study Epileptic Seizures and Epileptic Syndromes. **Current Psychopharmacology,** v. 5, n. 2, p. 194-210, 2016. ISSN 2211-5560. Disponível em: < http://www.eurekaselect.com/143308/article >.

MUSSULINI, B. H. et al. Seizures induced by pentylenetetrazole in the adult zebrafish: a detailed behavioral characterization. **PLoS One,** v. 8, n. 1, p. e54515, 2013. ISSN 1932-6203 (Electronic)

1932-6203 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23349914 >.

NDOYE, N. F. et al. Prevalence of epilepsy its treatment gap and knowledge, attitude and practice of its population in sub-urban Senegal an ILAE/IBE/WHO study. **Seizure,** v. 14, n. 2, p. 106-11, Mar 2005. ISSN 1059-1311 (Print)

1059-1311 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/15694563 >.

NEDERGAARD, M.; TAKANO, T.; HANSEN, A. J. Beyond the role of glutamate as a neurotransmitter. **Nat Rev Neurosci,** v. 3, n. 9, p. 748-55, Sep 2002. ISSN 1471-003X (Print)

1471-003X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12209123 >.

NEHLIG, A.; COLES, J. A. Cellular pathways of energy metabolism in the brain: is glucose used by neurons or astrocytes? **Glia,** v. 55, n. 12, p. 1238-50, Sep 2007. ISSN 0894-1491 (Print)

0894-1491 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17659529 >.

NEWSHOLME, P. et al. Glutamine and glutamate--their central role in cell metabolism and function. **Cell Biochem Funct,** v. 21, n. 1, p. 1-9, Mar 2003. ISSN 0263-6484 (Print)

0263-6484 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12579515 >.

NEWTON, C. R.; GARCIA, H. H. Epilepsy in poor regions of the world. **Lancet,** v. 380, n. 9848, p. 1193-201, Sep 29 2012. ISSN 1474-547X (Electronic)

0140-6736 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23021288 >.

NISSEN, J. D. et al. Dysfunctional TCA-Cycle Metabolism in Glutamate Dehydrogenase Deficient Astrocytes. **Glia,** v. 63, n. 12, p. 2313-26, Dec 2015. ISSN 1098-1136 (Electronic)

0894-1491 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26221781 >.

NWANI, P. O. et al. Epilepsy treatment gap: prevalence and associated factors in Southeast Nigeria. **Acta Neurol Scand,** v. 128, n. 2, p. 83-90, Aug 2013. ISSN 1600-0404 (Electronic)

0001-6314 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23425044 >.

OHBUCHI, T. et al. Acid-sensing ion channels in rat hypothalamic vasopressin neurons of the supraoptic nucleus. **J Physiol,** v. 588, n. Pt 12, p. 2147-62, Jun 15 2010. ISSN 1469-7793 (Electronic)

0022-3751 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20442265 >.

ONYANGO, I. G.; KHAN, S. M.; BENNETT, J. P., JR. Mitochondria in the pathophysiology of Alzheimer's and Parkinson's diseases. **Front Biosci (Landmark Ed),** v. 22, p. 854-872, Jan 01 2017. ISSN 1093-4715 (Electronic)

1093-4715 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27814651 >.

ORRINGER, C. E. et al. Natural history of lactic acidosis after grand-mal seizures. A model for the study of an anion-gap acidosis not associated with hyperkalemia. **N Engl J Med,** v. 297, n. 15, p. 796-9, Oct 13 1977. ISSN 0028-4793 (Print)

0028-4793 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19702 >.

PACOLD, M. E. et al. A PHGDH inhibitor reveals coordination of serine synthesis and one-carbon unit fate. **Nat Chem Biol,** v. 12, n. 6, p. 452-8, Jun 2016. ISSN 1552-4469 (Electronic)

1552-4450 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27110680 >.

PAJOR, A. M. Molecular properties of the SLC13 family of dicarboxylate and sulfate transporters. **Pflugers Arch,** v. 451, n. 5, p. 597-605, Feb 2006. ISSN 0031-6768 (Print)

0031-6768 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16211368 >.

PAN, W.; KASTIN, A. J. The Blood-Brain Barrier: Regulatory Roles in Wakefulness and Sleep. **Neuroscientist**, Mar 11 2016. ISSN 1089-4098 (Electronic)

1073-8584 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26969345 >.

PANAYIOTOPOULOS, C. P. The new ILAE report on terminology and concepts for organization of epileptic seizures: a clinician's critical view and contribution. **Epilepsia,** v. 52, n. 12, p. 2155-60, Dec 2011. ISSN 1528-1167 (Electronic)

0013-9580 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22004554 >.

PANOV, A. et al. The neuromediator glutamate, through specific substrate interactions, enhances mitochondrial ATP production and reactive oxygen species generation in nonsynaptic brain mitochondria. **J Biol Chem,** v. 284, n. 21, p. 14448-56, May 22 2009. ISSN 0021-9258 (Print)

0021-9258 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19304986 >.

PAOLI, A. et al. Beyond weight loss: a review of the therapeutic uses of very-low-carbohydrate (ketogenic) diets. **Eur J Clin Nutr,** v. 67, n. 8, p. 789-96, Aug 2013. ISSN 1476-5640 (Electronic)

0954-3007 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23801097 >.

PARPURA, V. et al. Glutamate and ATP at the Interface Between Signaling and Metabolism in Astroglia: Examples from Pathology. **Neurochem Res**, Feb 25 2016. ISSN 1573-6903 (Electronic)

0364-3190 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26915104 >.

_____. Glutamate and ATP at the Interface Between Signaling and Metabolism in Astroglia: Examples from Pathology. **Neurochem Res,** v. 42, n. 1, p. 19-34, Jan 2017. ISSN 1573-6903 (Electronic)

0364-3190 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26915104 >.

PATRIARCA, P. et al. Enzymatic basis of metabolic stimulation in leucocytes during phagocytosis: the role of activated NADPH oxidase. **Arch Biochem Biophys,** v. 145, n. 1, p. 255-62, Jul 1971. ISSN 0003-9861 (Print)

0003-9861 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/4399354 >.

PELLERIN, L. Brain energetics (thought needs food). **Curr Opin Clin Nutr Metab Care,** v. 11, n. 6, p. 701-5, Nov 2008. ISSN 1363-1950 (Print)

1363-1950 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18971641 >.

PELLERIN, L.; MAGISTRETTI, P. J. Sweet sixteen for ANLS. **J Cereb Blood Flow Metab,** v. 32, n. 7, p. 1152-66, Jul 2012. ISSN 1559-7016 (Electronic)

0271-678X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22027938 >.

PEREIRA, T. C.; CAMPOS, M. M.; BOGO, M. R. Copper toxicology, oxidative stress and inflammation using zebrafish as experimental model. **J Appl Toxicol,** v. 36, n. 7, p. 876-85, Jul 2016. ISSN 1099-1263 (Electronic)

0260-437X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26888422 >.

PESTA, D.; GNAIGER, E. High-resolution respirometry: OXPHOS protocols for human cells and permeabilized fibers from small biopsies of human muscle. **Methods Mol Biol,** v. 810, p. 25-58, 2012. ISSN 1940-6029 (Electronic)

1064-3745 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22057559 >.

PETERS, S. et al. Hydrogen peroxide modulates energy metabolism and oxidative stress in cultures of permanent human Muller cells MIO-M1. **J Biophotonics**, Nov 29 2016. ISSN 1864-0648 (Electronic)

1864-063X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27896951 >.

PINEDA, R.; BEATTIE, C. E.; HALL, C. W. Recording the adult zebrafish cerebral field potential during pentylenetetrazole seizures. **J Neurosci Methods,** v. 200, n. 1, p. 20-8, Aug 30 2011. ISSN 1872-678X (Electronic)

0165-0270 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21689682 >.

PITKANEN, S.; ROBINSON, B. H. Mitochondrial complex I deficiency leads to increased production of superoxide radicals and induction of superoxide dismutase. **J Clin Invest,** v. 98, n. 2, p. 345-51, Jul 15 1996. ISSN 0021-9738 (Print)

0021-9738 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/8755643 >.

RAE, C. D.; WILLIAMS, S. R. Glutathione in the human brain: Review of its roles and measurement by magnetic resonance spectroscopy. **Anal Biochem**, Dec 26 2016. ISSN 1096-0309 (Electronic)

0003-2697 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/28034792 >.

RAMIREZ, I. B. et al. Impaired neural development in a zebrafish model for Lowe syndrome. **Hum Mol Genet,** v. 21, n. 8, p. 1744-59, Apr 15 2012. ISSN 1460-2083 (Electronic)

0964-6906 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22210625 >.

RANDLETT, O. et al. Whole-brain activity mapping onto a zebrafish brain atlas. **Nat Methods,** v. 12, n. 11, p. 1039-46, Nov 2015. ISSN 1548-7105 (Electronic)

1548-7091 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26778924 >.

REID, W. C. et al. Neurobehavioral Abnormalities in the HIV-1 Transgenic Rat Do Not Correspond to Neuronal Hypometabolism on 18F-FDG-PET. **PLoS One,** v. 11, n. 3, p. e0152265, 2016. ISSN 1932-6203 (Electronic)

1932-6203 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27010205 >.

A roadmap for precision medicine in the epilepsies. **Lancet Neurol,** v. 14, n. 12, p. 1219-28, Dec 2015. ISSN 1474-4465 (Electronic)

1474-4422 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26416172 >.

ROBINSON, M. B.; JACKSON, J. G. Astroglial glutamate transporters coordinate excitatory signaling and brain energetics. **Neurochem Int,** v. 98, p. 56-71, Sep 2016. ISSN 1872-9754 (Electronic)

0197-0186 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27013346 >.

RODRIGUEZ-RODRIGUEZ, P. et al. Excitotoxic stimulus stabilizes PFKFB3 causing pentose-phosphate pathway to glycolysis switch and neurodegeneration. **Cell Death Differ,** v. 19, n. 10, p. 1582-9, Oct 2012. ISSN 1476-5403 (Electronic)

1350-9047 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22421967 >.

ROWLEY, S. et al. Mitochondrial respiration deficits driven by reactive oxygen species in experimental temporal lobe epilepsy. **Neurobiol Dis,** v. 75, p. 151-8, Mar 2015. ISSN 1095-953X (Electronic)

0969-9961 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25600213 >.

RYGIEL, K. A.; PICARD, M.; TURNBULL, D. M. The ageing neuromuscular system and sarcopenia: a mitochondrial perspective. **J Physiol,** v. 594, n. 16, p. 4499-512, Aug 15 2016. ISSN 1469-7793 (Electronic)

0022-3751 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26921061 >.

SANI, M. et al. Catalase activity and rhythmic patterns in mouse brain, kidney and liver. **Comp Biochem Physiol B Biochem Mol Biol,** v. 145, n. 3-4, p. 331-7, Nov-Dec 2006. ISSN 1096-4959 (Print)

1096-4959 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17045502 >.

SARIKAYA, I. PET studies in epilepsy. **Am J Nucl Med Mol Imaging,** v. 5, n. 5, p. 416-30, 2015. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26550535 >.

SASAGAWA, S. et al. In Vivo Detection of Mitochondrial Dysfunction Induced by Clinical Drugs and Disease-Associated Genes Using a Novel Dye ZMJ214 in Zebrafish. **ACS Chem Biol,** v. 11, n. 2, p. 381-8, Feb 19 2016. ISSN 1554-8937 (Electronic)

1554-8929 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26630578 >.

SAUNIER, E.; BENELLI, C.; BORTOLI, S. The pyruvate dehydrogenase complex in cancer: An old metabolic gatekeeper regulated by new pathways and pharmacological agents. **Int J Cancer,** v. 138, n. 4, p. 809-17, Feb 15 2016. ISSN 1097-0215 (Electronic)

0020-7136 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25868605 >.

SCHAPIRA, A. H. Human complex I defects in neurodegenerative diseases. **Biochim Biophys Acta,** v. 1364, n. 2, p. 261-70, May 06 1998. ISSN 0006-3002 (Print)

0006-3002 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/9593927 >.

SCHMIDT, R. et al. Stab wound injury of the zebrafish adult telencephalon: a method to investigate vertebrate brain neurogenesis and regeneration. **J Vis Exp**, n. 90, p. e51753, Aug 04 2014. ISSN 1940-087X (Electronic)

1940-087X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25146302 >.

SCHOUSBOE, A. A Tribute to Mary C. McKenna: Glutamate as Energy Substrate and Neurotransmitter-Functional Interaction Between Neurons and Astrocytes. **Neurochem Res,** v. 42, n. 1, p. 4-9, Jan 2017. ISSN 1573-6903 (Electronic)

0364-3190 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26721512 >.

SCHOUSBOE, A. et al. Glutamate metabolism in the brain focusing on astrocytes. **Adv Neurobiol,** v. 11, p. 13-30, 2014. ISSN 2190-5215 (Print). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25236722 >.

SCHOUSBOE, A. et al. Neuron-glia interactions in glutamatergic neurotransmission: roles of oxidative and glycolytic adenosine triphosphate as energy source. **J Neurosci Res,** v. 89, n. 12, p. 1926-34, Dec 2011. ISSN 1097-4547 (Electronic)

0360-4012 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21919035 >.

SCHOUSBOE, A. et al. Glutamate and glutamine metabolism and compartmentation in astrocytes. **Dev Neurosci,** v. 15, n. 3-5, p. 359-66, 1993. ISSN 0378-5866 (Print)

0378-5866 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/7805590 >.

SCIALO, F. et al. Mitochondrial ROS Produced via Reverse Electron Transport Extend Animal Lifespan. **Cell Metab,** v. 23, n. 4, p. 725-34, Apr 12 2016. ISSN 1932-7420 (Electronic)

1550-4131 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27076081 >.

SEYMOUR, K. J. et al. Identification of cerebral acetone by 1H-MRS in patients with epilepsy controlled by ketogenic diet. **MAGMA,** v. 8, n. 1, p. 33-42, Mar 1999. ISSN 0968-5243 (Print)

0968-5243 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10383091 >.

SHILOH, Y.; ZIV, Y. The ATM protein kinase: regulating the cellular response to genotoxic stress, and more. **Nat Rev Mol Cell Biol,** v. 14, n. 4, p. 197-210, Apr 2013. ISSN 1471-0080 (Electronic)

1471-0072 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23486281 >.

SHORVON, S. D. The etiologic classification of epilepsy. **Epilepsia,** v. 52, n. 6, p. 1052-7, Jun 2011. ISSN 1528-1167 (Electronic)

0013-9580 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21449936 >.

SIBSON, N. R. et al. Functional energy metabolism: in vivo 13C-NMR spectroscopy evidence for coupling of cerebral glucose consumption and glutamatergic neuronalactivity. **Dev Neurosci,** v. 20, n. 4-5, p. 321-30, 1998. ISSN 0378-5866 (Print)

0378-5866 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/9778568 >.

SIEBEL, A. M. et al. Role of adenosine signaling on pentylenetetrazole-induced seizures in zebrafish. **Zebrafish,** v. 12, n. 2, p. 127-36, Apr 2015. ISSN 1557-8542 (Electronic)

1545-8547 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25560904 >.

SIEBEL, A. M. et al. PTZ-induced seizures inhibit adenosine deamination in adult zebrafish brain membranes. **Brain Res Bull,** v. 86, n. 5-6, p. 385-9, Nov 25 2011. ISSN 1873-2747 (Electronic)

0361-9230 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21907764 >.

SIERRA, S. et al. Administration of docosahexaenoic acid before birth and until aging decreases kainate-induced seizures in adult zebrafish. **Brain Res Bull,** v. 88, n. 5, p. 467-70, Aug 01 2012. ISSN 1873-2747 (Electronic)

0361-9230 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22542883 >.

SIMPSON, D. P.; SHERRARD, D. J. Regulation of glutamine metabolism in vitro by bicarbonate ion and pH. **J Clin Invest,** v. 48, n. 6, p. 1088-96, Jun 1969. ISSN 0021-9738 (Print)

0021-9738 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/5771190 >.

SKYTT, D. M. et al. siRNA knock down of glutamate dehydrogenase in astrocytes affects glutamate metabolism leading to extensive accumulation of the neuroactive amino acids glutamate and aspartate. **Neurochem Int,** v. 61, n. 4, p. 490-7, Sep 2012. ISSN 1872-9754 (Electronic)

0197-0186 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22542772 >.

SONNEWALD, U. Glutamate synthesis has to be matched by its degradation - where do all the carbons go? **J Neurochem,** v. 131, n. 4, p. 399-406, Nov 2014. ISSN 1471-4159 (Electronic)

0022-3042 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24989463 >.

STAFSTROM, C. E.; ROOPRA, A.; SUTULA, T. P. Seizure suppression via glycolysis inhibition with 2-deoxy-D-glucose (2DG). **Epilepsia,** v. 49 Suppl 8, p. 97-100, Nov 2008. ISSN 1528-1167 (Electronic)

0013-9580 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19049601 >.

STEELE, S. L.; PRYKHOZHIJ, S. V.; BERMAN, J. N. Zebrafish as a model system for mitochondrial biology and diseases. **Transl Res,** v. 163, n. 2, p. 79-98, Feb 2014. ISSN 1878-1810 (Electronic)

1878-1810 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24055494 >.

STEWART, A. M. et al. Zebrafish models for translational neuroscience research: from tank to bedside. **Trends Neurosci,** v. 37, n. 5, p. 264-78, May 2014. ISSN 1878-108X (Electronic)

0166-2236 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24726051 >.

STEWART, A. M. et al. Perspectives of zebrafish models of epilepsy: what, how and where next? **Brain Res Bull,** v. 87, n. 2-3, p. 135-43, Feb 10 2012. ISSN 1873-2747 (Electronic)

0361-9230 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22155548 >.

STOESSL, A. J. Glucose utilization: still in the synapse. **Nat Neurosci,** v. 20, n. 3, p. 382-384, Feb 23 2017. ISSN 1546-1726 (Electronic)

1097-6256 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/28230843 >.

STREISINGER, G. Extrapolations from species to species and from various cell types in assessing risks from chemical mutagens. **Mutat Res,** v. 114, n. 1, p. 93-105, Jan 1983. ISSN 0027-5107 (Print)

0027-5107 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/6828046 >.

STREISINGER, G. et al. Production of clones of homozygous diploid zebra fish (Brachydanio rerio). **Nature,** v. 291, n. 5813, p. 293-6, May 28 1981. ISSN 0028-0836 (Print)

0028-0836 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/7248006 >.

STUMVOLL, M. et al. Role of glutamine in human carbohydrate metabolism in kidney and other tissues. **Kidney Int,** v. 55, n. 3, p. 778-92, Mar 1999. ISSN 0085-2538 (Print)

0085-2538 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10027916 >.

SUEN, M. F. et al. Assessments of the effects of nicotine and ketamine using tyrosine hydroxylase-green fluorescent protein transgenic zebrafish as biosensors. **Biosens Bioelectron,** v. 42, p. 177-85, Apr 15 2013. ISSN 1873-4235 (Electronic)

0956-5663 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23202349 >.

TANI, H. et al. A local glutamate-glutamine cycle sustains synaptic excitatory transmitter release. **Neuron,** v. 81, n. 4, p. 888-900, Feb 19 2014. ISSN 1097-4199 (Electronic)

0896-6273 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24559677 >.

TENNEY, J. R. et al. Cerebral glucose hypometabolism is associated with mitochondrial dysfunction in patients with intractable epilepsy and cortical dysplasia. **Epilepsia,** v. 55, n. 9, p. 1415-22, Sep 2014. ISSN 1528-1167 (Electronic)

0013-9580 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25053176 >.

THEODORE, W. H. et al. Epilepsy duration, febrile seizures, and cerebral glucose metabolism. **Epilepsia,** v. 45, n. 3, p. 276-9, Mar 2004. ISSN 0013-9580 (Print)

0013-9580 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/15009230 >.

THEODORE, W. H. et al. Epilepsy in North America: a report prepared under the auspices of the global campaign against epilepsy, the International Bureau for Epilepsy, the International League Against Epilepsy, and the World Health Organization. **Epilepsia,** v. 47, n. 10, p. 1700-22, Oct 2006. ISSN 0013-9580 (Print)

0013-9580 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17054693 >.

TIAN, L. et al. Imaging neural activity in worms, flies and mice with improved GCaMP calcium indicators. **Nat Methods,** v. 6, n. 12, p. 875-81, Dec 2009. ISSN 1548-7105 (Electronic)

1548-7091 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19898485 >.

TIEDEKEN, J. A.; RAMSDELL, J. S. Embryonic exposure to domoic Acid increases the susceptibility of zebrafish larvae to the chemical convulsant pentylenetetrazole. **Environ Health Perspect,** v. 115, n. 11, p. 1547-52, Nov 2007. ISSN 0091-6765 (Print)

0091-6765 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18007982 >.

TRUCCOLO, W. et al. Single-neuron dynamics in human focal epilepsy. **Nat Neurosci,** v. 14, n. 5, p. 635-41, May 2011. ISSN 1546-1726 (Electronic)

1097-6256 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21441925 >.

VALVONA, C. J. et al. The Regulation and Function of Lactate Dehydrogenase A: Therapeutic Potential in Brain Tumor. **Brain Pathol,** v. 26, n. 1, p. 3-17, Jan 2016. ISSN 1750-3639 (Electronic)

1015-6305 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26269128 >.

VENNETI, S.; THOMPSON, C. B. Metabolic Reprogramming in Brain Tumors. **Annu Rev Pathol,** v. 12, p. 515-545, Jan 24 2017. ISSN 1553-4014 (Electronic)

1553-4006 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/28068482 >.

VENNSTROM, B.; LAGERKRANTZ, H. [The 1995 Nobel Prize in medicine or physiology--awarded Edward B. Lewis, Christiane Nusslein-Volhard and Eric Wieschaus]. **Ugeskr Laeger,** v. 157, n. 50, p. 6999-702, Dec 11 1995. ISSN 0041-5782 (Print)

0041-5782 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/8545917 >.

VOLKOW, N. D. et al. Neurochemical and metabolic effects of acute and chronic alcohol in the human brain: Studies with positron emission tomography. **Neuropharmacology**, Jan 18 2017. ISSN 1873-7064 (Electronic)

0028-3908 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/28108358 >.

WALKER, C.; STREISINGER, G. Induction of Mutations by gamma-Rays in Pregonial Germ Cells of Zebrafish Embryos. **Genetics,** v. 103, n. 1, p. 125-36, Jan 1983. ISSN 0016-6731 (Print)

0016-6731 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17246099 >.

WARD, M. W. et al. Mitochondrial membrane potential and glutamate excitotoxicity in cultured cerebellar granule cells. **J Neurosci,** v. 20, n. 19, p. 7208-19, Oct 1 2000. ISSN 1529-2401 (Electronic)

0270-6474 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11007877 >.

WHITELAW, B. S.; ROBINSON, M. B. Inhibitors of glutamate dehydrogenase block sodium-dependent glutamate uptake in rat brain membranes. **Front Endocrinol (Lausanne),** v. 4, p. 123, 2013. ISSN 1664-2392 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24062726 >.

WILLMANN, O. et al. The contribution of 18F-FDG PET in preoperative epilepsy surgery evaluation for patients with temporal lobe epilepsy A meta-analysis. **Seizure,** v. 16, n. 6, p. 509-20, Sep 2007. ISSN 1059-1311 (Print)

1059-1311 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17532231 >.

WONG, K. et al. Modeling seizure-related behavioral and endocrine phenotypes in adult zebrafish. **Brain Res,** v. 1348, p. 209-15, Aug 12 2010. ISSN 1872-6240 (Electronic)

0006-8993 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20547142 >.

WUST, R. C. et al. Ketones and inborn errors of metabolism: old friends revisited. **J Inherit Metab Dis,** v. 40, n. 1, p. 3-4, Jan 2017. ISSN 1573-2665 (Electronic)

0141-8955 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27830424 >.

XIAO, F. et al. Clinical and Electroencephalographic (EEG) Features Associated With Refractoriness in Benign Childhood Epilepsy With Centrotemporal Spikes. **J Child Neurol,** v. 30, n. 12, p. 1591-7, Oct 2015. ISSN 1708-8283 (Electronic)

0883-0738 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25792427 >.

YELAMANCHI, S. D. et al. A pathway map of glutamate metabolism. **J Cell Commun Signal,** v. 10, n. 1, p. 69-75, Mar 2016. ISSN 1873-9601 (Print)

1873-9601 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26635200 >.

YOSHIMI, N. et al. Blood metabolomics analysis identifies abnormalities in the citric acid cycle, urea cycle, and amino acid metabolism in bipolar disorder. **BBA Clin,** v. 5, p. 151-8, Jun 2016. ISSN 2214-6474 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27114925 >.

YU, A. C.; SCHOUSBOE, A.; HERTZ, L. Metabolic fate of 14C-labeled glutamate in astrocytes in primary cultures. **J Neurochem,** v. 39, n. 4, p. 954-60, Oct 1982. ISSN 0022-3042 (Print)

0022-3042 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/6126524 >.

YU, L. et al. Modeling the Genetic Regulation of Cancer Metabolism: Interplay Between Glycolysis and Oxidative Phosphorylation. **Cancer Res**, Feb 15 2017. ISSN 1538-7445 (Electronic)

0008-5472 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/28202516 >.

YUDKOFF, M. et al. Tricarboxylic acid cycle in rat brain synaptosomes. Fluxes and interactions with aspartate aminotransferase and malate/aspartate shuttle. **J Biol Chem,** v. 269, n. 44, p. 27414-20, Nov 04 1994. ISSN 0021-9258 (Print)

0021-9258 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/7961653 >.

ZEYLEMAKER, W. P.; KLAASSE, A. D.; SLATER, E. C. Studies on succinate dehydrogenase. V. Inhibition by oxaloacetate. **Biochim Biophys Acta,** v. 191, n. 2, p. 229-38, Nov 04 1969. ISSN 0006-3002 (Print)

0006-3002 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/5354260 >.

ZHAO, X. et al. Zinc oxide nanoparticles induce oxidative DNA damage and ROS-triggered mitochondria-mediated apoptosis in zebrafish embryos. **Aquat Toxicol,** v. 180, p. 56-70, Nov 2016. ISSN 1879-1514 (Electronic)

0166-445X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27658222 >.

ZHOU, Y.; DANBOLT, N. C. Glutamate as a neurotransmitter in the healthy brain. **J Neural Transm (Vienna),** v. 121, n. 8, p. 799-817, Aug 2014. ISSN 1435-1463 (Electronic)

0300-9564 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24578174 >.

ZHU, H. et al. Single-neuron identification of chemical constituents, physiological changes, and metabolism using mass spectrometry. **Proc Natl Acad Sci U S A,** v. 114, n. 10, p. 2586-2591, Mar 07 2017. ISSN 1091-6490 (Electronic)

0027-8424 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/28223513 >.

ZHUNUSSOVA, A. et al. Tumor microenvironment promotes dicarboxylic acid carrier-mediated transport of succinate to fuel prostate cancer mitochondria. **Am J Cancer Res,** v. 5, n. 5, p. 1665-79, 2015. ISSN 2156-6976 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26175936 >.

ZHUO, Y.; LUO, H.; ZHANG, K. Leber hereditary optic neuropathy and oxidative stress. **Proc Natl Acad Sci U S A,** v. 109, n. 49, p. 19882-3, Dec 04 2012. ISSN 1091-6490 (Electronic)

0027-8424 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23197830 >.

ZIMMER, E. R. et al. [18F]FDG PET signal is driven by astroglial glutamate transport. **Nat Neurosci,** v. 20, n. 3, p. 393-395, Mar 2017. ISSN 1546-1726 (Electronic)

1097-6256 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/28135241 >.

ZSURKA, G.; KUNZ, W. S. Mitochondrial dysfunction and seizures: the neuronal energy crisis. **Lancet Neurol,** v. 14, n. 9, p. 956-66, Sep 2015. ISSN 1474-4465 (Electronic)

1474-4422 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26293567 >.

Anexos

Anexo A.1.

Guia para autores do periódico Journal of Cerebral Blood Flow and Metabolism.

Share

Journal of Cerebral Blood Flow & Metabolism

2015 Impact Factor: 4.929
2015 Ranking: 41/256 in Neurosciences | 22/133 in Endocrinology & Metabolism | 14/70 in Hematology
2016 Release of Journal Citation Reports, Source: 2015 Web of Science Data

Published in Association with International Society of Cerebral Blood Flow and Metabolism

Editors-in-Chief

Martin Lauritzen, MD	Rigshospitalet - Glostrup and University of Copenhagen, Denmar
I. Mhairi Macrae	Glasgow University, UK

Other Titles in:

Clinical Neuroscience | Neurobiology | Neurology

eISSN: 15597016 | ISSN: 0271678X | Current volume: 37 | Current issue: 2 Frequency: Monthly

Download flyer Recommend to Library

Manuscript Submission Guidelines & Editorial Policies *Journal of Cerebral Blood Flow and Metabolism*

This journal is a member of the Committee on Publication Ethics.

The Journal of Cerebral Blood Flow & Metabolism (JCBFM) features international peer-reviewed contributions highlighting experimental, theoretical, and clinical aspects of brain circulation, imaging and metabolism. It is truly relevant to any physician or scientist with an interest in brain function, including neurologists, neurochemists, physiologists, pharmacologists, anesthesiologists, neuroradiologists, neurosurgeons, neuropathologists, and neuroscientists. The Journal of Cerebral Blood Flow & Metabolism (JCBFM) stands at the interface between basic and clinical neurovascular research.

PUBLICATION PAGE CHARGES (Do not apply to invited authors)
After final layout for publication, each page of an article will incur a fixed charge of:
£62 per page for members of the ISCBFM
£83 per page for non-members
This charge is fully inclusive of colour reproduction of all colour images in print, HTML and PDF formats. It covers also a proportion of the costs of processing and producing the article for publication.

OPEN ACCESS
Upon acceptance of a manuscript, authors will be charged a one-time article processing charge (APC) to be paid by the author, institution or funder. This fee covers the cost of publication and ensures that articles will be freely available in perpetuity.
- The full price article processing charge (APC) is £1,600 + VAT where applicable*
- The discounted article processing charge (APC) for ISCBFM members is £1,350+ VAT where applicable*
*If the paying author/institution is based in the European Union, to comply with European law, Value Added Tax (VAT) must be added to the APC. Providing a VAT registration number will allow an institution to avoid paying this tax, except for UK institutions. An invoice will be issued in GBP.

For further information click to 5.3 Open access and author archiving

Page charges will not apply to open access papers where the author is paying an article processing charge.

Please read the guidelines below then visit the journal's submission site https://mc.manuscriptcentral.com/jcbfm to upload your manuscript. Please note that manuscripts not conforming to these guidelines may be returned.

Only manuscripts of sufficient quality that meet the aims and scope of Journal of Cerebral Blood Flow and Metabolism will be reviewed. Papers judged by the Editors to be of insufficient general interest or otherwise inappropriate are rejected promptly without external review.

Originality: A submitted manuscript must be an original contribution not previously published (except as an abstract or preliminary report), must not be under consideration for publication elsewhere, and, if accepted, must not be published elsewhere in similar form, in any language, without the consent of Sage Publications. Each person listed as an author is expected to have participated in the study to a significant extent. Although the Editors and referees make every effort to ensure the validity of published manuscripts, the final responsibility rests with the authors, not with JCBFM, its Editors or the publisher.

1. Article types

Although the Editors and referees make every effort to ensure the validity of published manuscripts, the final responsibility rests with the authors, not with JCBFM, its Editors, or the publisher. For further details on how to layout the article please see 2.4 Journal layout.

Article Type	Word Limit	Tables/Figures
Original Articles are full-length reports of current research.	Abstract: 200 words maximum Article: 6,000 words including abstract but excluding references, tables and figures	Up to 7 in total
Review Articles (including Systematic Reviews) are comprehensive analyses of specific topics	Abstract: 200 words maximum Article: 8,000 words including abstract but excluding references, tables and figures	Up to 10 in total
Mini Review Articles should focus on a clearly defined topic of current interest, and describe recent developments in the field.	Abstract: 100 words maximum Article: 2,500 words including abstract, introduction and conclusion but excluding references, tables and figures	Up to 2 in total
Opinion Articles represent an opportunity to present particular views on a topic using the review format.	Abstract: 200 words maximum Article: 8,000 words including abstract but excluding references, tables and figures	Up to 10 in total
Brief Opinion Articles should present a particular view on a hot topic using the mini review format.	Abstract: 100 words maximum Article: 2,500 words including abstract, introduction and conclusion but excluding references, tables and figures	Up to 2 in total
Rapid Communications are intended to disseminate information as quickly as possible due to the new research and information presented.	Abstract: 200 words maximum Article: 6,000 words including abstract but excluding references, tables and figures	Up to 7 in total
Brief Communications are short research articles	Abstract: 100 words maximum Article: 2,000 words including abstract but excluding references, tables and figures	Up to 2 in total
Commentary Articles put an original article in context and expand on how the findings are relevant	Abstract: 100 words maximum Article: 1,500 words including abstract but excluding references, tables and figures	Up to 2 in total
Negative Results Reports are intended to provide a forum for data that did not substantiate the alternative hypothesis (i.e. a difference between the experiment groups), and/or did not produce published findings. Since the net effect of a Negative Result is to discourage repetition, the standards for acceptance will be highly demanding. Typically, Type II error considerations are mandatory.	Submission consists of: A) One-page Summary to appear in the print edition only: 500 words maximum including abstract but excluding references, tables and figures. Structure of Summary: Title page, abstract, keywords, summary text, disclosure of interests statement, reference list, list of figure legends if any, tables if any in editable format. Up to 2 (figures+tables) in separate image files named “summary fig X” and B) Full text paper to appear online only. In preparing this manuscript follow the guidelines for Original Articles.	Up to 2 in total in summary; up to 7 in full text paper

Back to top

2. Preparing your manuscript

2.1 Word processing formats

Preferred formats for the text and tables of your manuscript are Word DOC, RTF, XLS. LaTeX files are not accepted. The text should be double-spaced throughout and with a minimum of 3cm for left and right hand margins and 5cm at head and foot. Text should be standard 10 or 12 point.

2.2 Artwork, figures and other graphics

For guidance on the preparation of illustrations, pictures and graphs in electronic format, please visit SAGE's Manuscript Submission Guidelines.

Figures supplied in colour will appear in colour online regardless of whether or not these illustrations are reproduced in colour in the printed version. Colour reproduction of images in print is covered by the page charges for publication of a manuscript.

2.3 Supplementary material

Authors submitting supplementary information must include the following statement with the main document, before the reference list:

Supplementary material for this paper is available at: http://jcbfm.sagepub.com/content/by/supplemental-data.

This journal is able to host additional materials online (e.g. datasets, podcasts, videos, images, etc.) alongside the full-text of the article. These will be subjected to peer review alongside the article, but are not published in print.

Authors should submit supplementary information files in the final format as they are not edited and will appear online exactly as submitted. Supplementary material should be uploaded if possible as one supplementary file including figure and movie legends. Movies and extensive tables should be uploaded in separate supplementary files.

Supplementary files must be as small as possible so that they can be downloaded quickly. Images should not exceed 640 x 480 pixels. For movies we recommend 480 x 360 pixels as the maximum frame size and a frame rate of 15 frames per second. If applicable to the presentation of the information, use a 256-color palette. Please consider the use of lower specification for all of these points if the supplementary information can still be presented clearly. Our recommended maximum data rate is 150 KB/s.

2.4 Journal Layout

JCBFM conforms to the SAGE House Style, please review the guidelines on SAGE UK House Style.

Components of original articles should be in the following order:

- Title page
- Unstructured abstract
- Five key words
- Introduction
- Material and Methods
- Results
- Discussion
- Acknowledgements
- Author Contribution statement (only for original research papers, not for reviews)
- Disclosure/Conflict of Interest
- Sentence regarding supplementary information on JCBFM website, if any
- References
- Figure legends
- Tables (in editable format, either in main document or as separate main document files)
- Figures (in separate image files, not inserted in main document)

Cover letter
A cover letter to the Editors should state that the material is original research, has not been previously published and has not been submitted for publication elsewhere while under consideration of JCBFM.

Title page of manuscript
The title page should include (a) the complete manuscript title; (b) all authors' full names (listed as first name, middle initial, last name) and affiliations; (c) the name, postal address for correspondence, telephone number, and e-mail address; and (d) the sources of funding that require acknowledgment (see section 4.4 for further details). Please ensure that all forms of support are acknowledged, including pharmaceutical and industry support, on the cover page; (e) a running headline of no more than 50 characters (including spaces) should be supplied which conveys the essential message of the paper.

If authors regard it as essential to indicate that two or more co-authors are equal in status, they may be identified on the manuscript title page by an asterisk symbol with the caption "These authors contributed equally to this work" immediately under the author affiliations list.

Please note that the title, keywords and abstract are key to ensuring that readers find your article online through online search engines such as Google. Please refer to the information and guidance on how best to title your article, write your abstract and select your keywords by visiting the SAGE Journal Author Gateway for guidelines on How to Help Readers Find Your Article Online

Unstructured abstract and keywords on a separate page following title page.
The abstract should be unstructured, factual and comprehensive. The use of abbreviations and acronyms should be limited and general statements (e.g. "the significance of the results is discussed") should be avoided. Five keywords should be provided in alphabetical order below the abstract.

Materials/subjects and methods section
This section should contain sufficient detail, so that all experimental procedures can be reproduced, and include references. Methods, however, that have been published in detail elsewhere should not be described in detail. Authors should provide the name of the manufacturer and their location for any specifically named medical equipment and instruments, and all drugs should be identified by their pharmaceutical names, and by their trade name if relevant.

2.5 Reference Style

JCBFM adheres to the SAGE Vancouver reference style, please review the guidelines on SAGE Vancouver to ensure your manuscript conforms to this reference style. If you use EndNote to manage references, you can download the SAGE Vancouver output file here.

2.6 English language editing services

Authors seeking assistance with English language editing, translation, or figure and manuscript formatting to fit the journal's specifications should consider using SAGE Language Services. Visit SAGE Language Services and our SAGE Journal Author Gateway for further information.

Back to top

3. Submitting your manuscript

3.1 How to submit your manuscript

JCBFM is hosted on SAGE Track, a web based online submission and peer review system powered by ScholarOne™ Manuscripts. Visit https://mc.manuscriptcentral.com/jcbfm to login and submit your article online.

IMPORTANT: Please check whether you already have an account in the system before trying to create a new one. If you have reviewed or authored for the journal in the past year it is likely that you will have had an existing account. For further guidance on submitting your manuscript online please visit ScholarOne Online Help.

3.2 How to submit your revised manuscript

Authors submitting a revised manuscript after review are asked in the submission process to include a point-by-point response to reviewers' comments. It is imperative that your response be entered as editable text into the box provided for it. This text may in addition be uploaded as a file with embedded figures and/or tables if authors consider it necessary (File name: Author response to referees, file type: Other).

Papers revised after a decision may NOT be submitted as new manuscripts. This also applies to revisions after a reject-with-resubmission decision. Revisions submitted as new papers will be unsubmitted by the Editorial Office. If you encounter difficulties submitting your revision please contact the Editorial Office for help.

Authors should submit two copies of the revised manuscript: one clean document (file type: Main Document) and one showing highlighted changes made in response to reviewers' comments (file type: Other). Changes should be indicated using colour markings of the text. Please do not use underlining or the track changes function to highlight changes. Comment boxes are allowed. If the paper is accepted, the clean document only will be forwarded to the publisher.

3.3 Corresponding author contact details

Provide full contact details for the corresponding author including email, mailing address and telephone numbers. Academic affiliations are required for all co-authors.

3.4 Publication of Twitter Handles

JCBFM has its own Twitter account @_JCBFM and JCBFM Facebook Page. Authors and readers are invited to join the ongoing discussion on these social media pages on quality issues and overcoming the 'transitional roadblock" in the field of biomedicine.

As a way of encouraging ongoing discussion within the field, *JCBFM* authors are offered the option of providing their Twitter handle to be published alongside their name and email address within their article. This way, *JCBFM* readers who have questions or thoughts regarding your paper can tweet you directly. Providing a Twitter handle for publication is entirely optional, if you are not comfortable with *JCBFM* promoting your article along with your personal Twitter handle then please do not supply it.

By providing your personal twitter handle you agree to let *JCBFM* and SAGE Publications use it in any posts related to your journal article. You may also be contacted by other Twitter users. *JCBFM* and SAGE Publications will have no control over you or your tweets at any time. If you would like guidance on how to promote your article yourself on Twitter or other Social Media channels please visit http://www.uk.sagepub.com/journalgateway/files/using_social_media_to_promote.doc.

To include your Twitter handle within your article please provide this within the SAGE Track Submission form when prompted and on the separate title page in the format outlined below (please refrain from adding this to the manuscript itself to facilitate anonymous peer review).

As an example of how to supply this information please see the example below:

Joe Bloggs, Department of Neuroscience, University Hospital, Town, Zip code, USA.
Email: JoeBloggs@email.com
Twitter: @drjoebloggs

4. Editorial policies

4.1 Peer review

JCBFM operates a single-blind reviewing policy in which the reviewers' identities are concealed from the author. Manuscripts sent out for peer review are evaluated by at least one independent reviewer (normally two, sometimes more). Reviewers have access to all materials that may be relevant to the evaluation of the manuscript, including supplementary material for e-only publication.

You will be asked at the submission site to provide the names of five peers who could be called upon to review your manuscript, two of whom must be members of the JCBFM Editorial Board. Recommended reviewers should be experts in their fields and should be able to provide an objective assessment of the manuscript. Please be aware of any conflicts of interest when recommending reviewers. Examples of conflicts of interest include (but are not limited to) the below:

- The reviewer should have no prior knowledge of your submission
- The reviewer should not have recently collaborated with any of the authors
- Reviewer nominees from the same institution as any of the authors are not permitted

Please note that the Editors welcome and will consider recommendations made, but are not obliged to invite any recommended/opposed reviewers to assess your manuscript.

The Editors then make a decision based on the reviewers' evaluations:

- Accept, with or without editorial revisions.
- Revise, with the author addressing concerns raised by the reviewers before a final decision is reached.
- Reject, but indicate to the authors that further work might justify a resubmission.
- Reject outright, typically on grounds of specialist interest, lack of novelty, insufficient conceptual advance or major technical and/or interpretational problems.

Anonymity and Confidentiality: All details about submitted manuscripts are kept confidential and no comments are issued to outside parties or organizations about manuscripts under consideration or if they are rejected. Editors are restricted to making public comments on a published article's content and their evaluation. We do not release reviewers' identities to authors, except when reviewers specifically ask to be identified.

We ask reviewers not to identify themselves to authors without the Editors' knowledge. If they wish to reveal their identities while the manuscript is under consideration, this should be done via the Editor; if this is not practicable, we ask authors to inform the Editor as soon as possible after the reviewer has revealed his or her identity. We deplore any attempt by authors to confront reviewers or try to determine their identities. Our own policy is to neither confirm nor deny any speculation about reviewers' identities, and we encourage reviewers to adopt a similar policy.

Upon accepting an invitation to evaluate a manuscript, reviewers must keep the manuscript and associated data confidential, and not redistribute them without the journal's permission. If a reviewer asks a colleague to assist in assessing a manuscript, confidentiality must be ensured and their names must be provided to the journal with the final report.

4.2 Authorship

JCBFM encourages transparency by requiring and publishing author contributions statements in all original research articles. A statement of authors' responsibilities must be included in all manuscripts containing original research (not in reviews) above the reference list, specifying the contribution of every author, omitting none. Moreover, upon acceptance of a paper, a *JCBFM* Authorship Form must be sent by the corresponding author as an e-mail to the Editors. In this form, all authors must state their specific contributions to the manuscript and sign the form. Again, no author may be left out. If more than one form is necessary to include all authors, the corresponding author is responsible for collating and scanning all forms into one file and dispatching it to the Editorial Office.

The list of authors in the manuscript should include all those who can legitimately claim authorship. This is all those who:

1. Made a substantial contribution to the concept and design, acquisition of data or analysis and interpretation of data,
2. Drafted the article or revised it critically for important intellectual content,
3. Approved the version to be published.

Authors should meet the conditions of all of the points above, and each should have participated sufficiently in the work to take public responsibility for appropriate portions of the content. When a large, multicenter group has conducted the work, the group should identify the individuals who accept direct responsibility for the manuscript. These individuals should fully meet the criteria for authorship.
Please refer to the International Committee of Medical Journal Editors (ICMJE) authorship **guide**lines for more information on authorship.

4.3 Acknowledgements

All contributors who do not meet the criteria for authorship should be listed in an Acknowledgments section. Examples of those who might be acknowledged include a person who provided purely technical help, or a department chair that provided only general support.

4.4 Funding acknowledgements

JCBFM requires all authors to acknowledge their funding in a consistent fashion under a separate heading. Please visit the Funding Acknowledgements page on the SAGE Journal Author Gateway to confirm the format of the acknowledgment text in the event of funding, or state that: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. Please note: JCBFM requires this information at first submission and in all following versions of the manuscript.

4.5 Declaration of conflicting interests

It is the policy of *JCBFM* to require a declaration of conflicting interests from all authors enabling a statement to be carried within the paginated pages of all published articles.

Please ensure that a 'Declaration of Conflicting Interests' statement is included at the end of your manuscript, after any acknowledgements and prior to the references. If no conflict exists, please state explicitly that 'The Author(s) declare(s) that there is no conflict of interest'. If a conflict exists for some but not all authors, list the authors and conflicting interests, followed if applicable by "all other authors declare that they have no conflict of interest".

For guidance on conflict of interest statements, please see the ICMJE recommendations here.

4.6 Research ethics and patient consent

All papers reporting experiments on human subjects must state in the methods section that the relevant ethics committee or institutional review board provided (or waived) approval. The full proper name of the institution committee or review board must be provided, in addition to the approval number.

In addition, authors must provide the proper name of the ethical **guide**lines or standards that governed conduct of the study (the responsible committee on human experimentation (institutional or regional)) or the Helsinki Declaration of 1975 (and as revised in 1983).

It must be confirmed that written, informed consent was obtained from all subjects or their legal representatives. It is the author's responsibility to ensure that a patient anonymity is carefully protected. Authors should mask patients' eyes and remove patients' names from figures unless they obtain written consent from the patients or their legal guardian. Authors should be **guide**d by the ICMJE Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals. Please also refer to the ICMJE Recommendations for the Protection of Research Participants.

4.7 Clinical trials

JCBFM endorses the ICMJE requirement that clinical trials are registered in a WHO-approved public trials registry at or before the time of first patient enrolment. However, consistent with the AllTrials campaign, retrospectively registered trials will be considered if the justification for late registration is acceptable. The trial registry name and URL, and registration number must be included at the end of the abstract. Authors reporting phase II and phase III randomized controlled trials should refer to the Consolidated Standards of Reporting Trials (CONSORT) recommendations to facilitate the complete and transparent reporting of trial findings. Reports that do not conform to the CONSORT **guide**lines and Checklist may need to be revised before formal review.

4.8 Reporting guidelines

The relevant EQUATOR Network reporting **guide**lines should be followed depending on the type of study. For example, all randomized controlled trials submitted for publication should include a completed Consolidated Standards of Reporting Trials (CONSORT) flow chart as a cited figure, and a completed CONSORT checklist as a supplementary file.

For guiding principles for reporting statistical methods and results please consult the SAMPL **Guide**lines for Biomedical Journals: http://www.equator-network.org/wp-content/uploads/2013/03/SAMPL-**Guide**lines-3-13-13.pdf

For example, authors reporting tumor markers prognostic studies are encouraged to follow the REMARK **guide**lines for complete and transparent reporting.

For describing human biospecimens, we recommend referring to the BRISQ reporting **guide**lines (Biospecimen Reporting for Improved Study Quality) and ensuring at least Tier 1 characteristics are provided (doi: 10.1002/cncy.20147).

All authors reporting systematic reviews should refer to the PRISMA guidelines.

Other resources can be found at NLM's Research Reporting Guidelines and Initiatives.

4.9 Animal Experiments

For experiments involving animals (or material from animals, such as cell cultures, brain slices) submitted for publication, the following must be stated explicitly in the Material and Methods section:

- the full proper name (NOT "local" ethics committee) of the institutional or licensing committee which approved the experimental protocols and any relevant details regarding animal welfare and drug side effects;
- the name of the guidelines/regulations which governed how the experiments were CONDUCTED;
- a statement confirming that the experiments have been REPORTED following/in compliance with the ARRIVE guidelines (Animal Research: Reporting in Vivo Experiments) for how to REPORT animal experiments https://www.nc3rs.org.uk/arrive-guidelines. This means that you will be required to confirm compliance with the ARRIVE guidelines **both** in the submission site, where you will be asked to fill out a detailed check sheet, **and** in the Materials and Methods section of your manuscript.

4.10 Image integrity and standards

Images submitted with a manuscript for review should be minimally processed (for instance, to add arrows to a micrograph). Authors should retain their unprocessed data and metadata files, as Editors may request them to aid in manuscript evaluation. If unprocessed data is unavailable, manuscript evaluation may be stalled until the issue is resolved.
A certain degree of image processing is acceptable for publication, but the final image must correctly represent the original data and conform to community standards. The guidelines below will aid in accurate data presentation at the image processing level:

- Authors should list all image acquisition tools and image processing software packages used. Authors should document key image-gathering settings and processing manipulations in the Methods section.
- Images gathered at different times or from different locations should not be combined into a single image, unless it is stated that the resultant image is a product of time-averaged data or a time-lapse sequence. If juxtaposing images is essential, the borders should be clearly demarcated in the figure and described in the legend.
- Touch-up tools, such as cloning and healing tools in Photoshop, or any feature that deliberately obscures manipulations, is to be avoided.
- Processing (such as changing brightness and contrast) is appropriate only when it is applied equally across the entire image and is applied equally to controls. Contrast should not be adjusted so that data disappear. Excessive manipulations, such as processing to emphasize one region in the image at the expense of others (for example, through the use of a biased choice of threshold settings), is inappropriate, as is emphasizing experimental data relative to the control.

For gels and blots, positive and negative controls, as well as molecular size markers, should be included on each gel and blot – either in the main figure or an expanded data supplementary figure. The display of cropped gels and blots in the main paper is encouraged if it improves the clarity and conciseness of the presentation. In such cases, the cropping must be mentioned in the figure legend.

- Vertically sliced gels that juxtapose lanes that were not contiguous in the experiment must have a clear separation or a black line delineating the boundary between the gels.
- Cropped gels in the paper must retain important bands.
- Cropped blots in the body of the paper should retain at least six band widths above and below the band.
- High-contrast gels and blots are discouraged, as overexposure may mask additional bands.
- Authors should strive for exposures with grey backgrounds. Immunoblots should be surrounded by a black line to indicate the borders of the blot, if the background is faint.
- For quantitative comparisons, appropriate reagents, controls and imaging methods with linear signal ranges should be used.

Microscopy adjustments should be applied to the entire image. Threshold manipulation, expansion or contraction of signal ranges and the altering of high signals should be avoided. If 'pseudo-coloring' and nonlinear adjustments (for example 'gamma changes') are used, this must be disclosed. Adjustments of individual color channels are sometimes necessary on 'merged' images, but this should be noted in the figure legend. We encourage inclusion of the following with the final revised version of the manuscript for publication:

- In the Methods section, specify the type of equipment (microscopes/objective lenses, cameras, detectors, filter model and batch number) and acquisition software used. Although we appreciate that there is some variation between instruments, equipment settings for critical measurements should also be listed.
- The display lookup table (LUT) and the quantitative map between the LUT and the bitmap should be provided, especially when rainbow pseudo-color is used. It should be stated if the LUT is linear and covers the full range of the data.
- Processing software should be named and manipulations indicated (such as type of deconvolution, three-dimensional reconstructions, surface and volume rendering, 'gamma changes', filtering, thresholding and projection).
- Authors should state the measured resolution at which an image was acquired and any downstream processing or averaging that enhances the resolution of the image.

The data will be published in the online version of *JCBFM*, or detailed information provided in the articles on how the data can be obtained.

Examples of data types include but are not limited to: statistical data files, replication code, text files, audio files, images, videos, appendices, and additional charts and graphs necessary to understand the original research. The Editors may also grant exceptions for data that cannot legally or ethically be released. All data submitted should comply with Institutional or Ethical Review Board requirements and applicable government regulations. For further information, please contact the Editorial Office.

4.11 Availability of data and materials

An inherent principle of publication is that others should be able to replicate and build upon the authors' published claims. Therefore, a condition of publication in the JCBFM is that authors are required to make materials, data and associated protocols promptly available to readers without undue qualifications.

- Any restrictions on the availability of materials or information must be disclosed to the Editors at the time of submission.
- Any restrictions must also be disclosed in the submitted manuscript, including details of how readers can obtain materials and information.
- If materials are to be distributed by a for-profit company, this must be stated in the paper.
- Supporting data must be made available to Editors and peer-reviewers at the time of submission for the purposes of evaluating the manuscript. Peer-reviewers may be asked to comment on the terms of access to materials, methods and/or data sets; JCBFM reserves the right to refuse publication in cases where authors do not provide adequate assurances that they can comply with the journal's requirements for sharing materials.

After publication, readers who encounter refusal by the authors to comply with these policies should contact the chief Editors of the journal. In cases where Editors are unable to resolve a complaint, the journal may refer the matter to the authors' funding institution and/or publish a formal statement of correction, attached online to the publication, stating that readers have been unable to obtain necessary materials to replicate the findings. Details about how to share some specific materials, data and methods can be found in the sections below. The preferred way to share large data sets is via public repositories. Some of these repositories offer authors the option to host data associated with a manuscript confidentially, and provide anonymous access to peer-reviewers before public release.

These repositories coordinate public release of the data with the journal's publication date either advance online publication (Online First) or, through print/online publication. This option should be used when possible, but it is the authors' responsibility to communicate with the repository to ensure that public release is made promptly on the journal's Online First (or print/online) publication date. Any supporting data sets for which there is no public repository must be made available as Supplementary Information files that will be accessible on http://jcbfm.sagepub.com upon publication. In cases where it is technically impossible for such files to be provided to the journal, the authors must make the data available to Editors and peer-reviewers at submission, and directly upon request to any reader on and after the publication date, the author providing a URL or other unique identifier in the manuscript.

Authors are strongly recommended to deposit their original datasets to a recommended data repository as part of the manuscript submission process. We encourage submission of datasets to recognized repositories where possible, such as FIGSHARE (https://figshare.com/) or DRYAD (http://www.datadryad.org/).

4.12 Sharing materials

A condition of publication in *JCBFM* is that authors are required to make materials, data and associated protocols promptly available to others without undue qualifications. It is acceptable to request reasonable payment to cover costs of distribution and reagents may be made available via commercial or non-commercial third party providers. Authors reporting new chemical compounds integral to the conclusions of the paper must provide the chemical structure, synthesis and characterization of the compounds with sufficient experimental details to allow other researchers to reproduce the synthesis and characterization.

For biological materials such as mutant strains and cell lines, *JCBFM* requires authors to use established public repositories when one exists (for example, Jackson Laboratory, the European Mouse Mutant Archive (EMMA), the European Conditional Mouse Mutagenesis Program (EUCOMM), the Knockout Mouse Project (KOMP), Addgene, RIKEN Bioresource Centre, the Mutant Mouse Regional Resource Centers, American Type Culture Collection (Americas), American Type Culture Collection (Asia/Europe), UK Stem Cell Bank), and provide accession numbers in the manuscript.

Cell lines:
The distribution of human cell lines used in research should not be hindered by restrictions from donors. Researchers developing cell lines must investigate and disclose any restrictions associated with the human or other tissue they are using, particularly if someone else collected the samples, if the samples come from multiple clinical sources or if they come from several legal jurisdictions. If a scientist needs to create cell lines that might be used for as-yet-unforeseen purposes, only tissue with no restrictions should be used. Authors of papers that involve consent forms must, at time of submitting the manuscript, make Editors aware of any limits that result from those forms.

Flow cytometry:
Every manuscript that contains flow cytometry experiments should identify in the Methods section all antibody reagents by clone identifier, vendor and fluorochrome. Authors should identify the instrument and software used to collect and analyse experimental data. Axes labels for plots or graphs depicting flow cytometry data should state the marker (for example, CD4) and the axes scales (log or linear) should be clearly visible. Authors should provide numerical analysis for the number of cells analysed and the absolute numbers or percentages (with statistics stated in either the text, legend or in a supplementary table) of the relevant cell population(s) within post-sort fractions. Hints for good general practice in the description of flow cytometry experiments can be found at the MIFlowCyt Standards section of SourceForge.

For papers describing a new cell population or for which a given sorted cell population is critical to the main message imparted by the new work, authors should describe in a supplementary figure or two the full gating strategy used for the experiments described in the manuscript. A figure depicting the 'gates' used to identify sorted subsets is useful and should be provided to the referees on request. These data would include preliminary forward and side scatter gates of the starting cell population, indicating where boundaries between 'positive' and 'negative' staining cell populations are defined. For preliminary sorts that use 'cocktails' of antibodies to exclude certain cell populations, for example, lineage-minus (Lin-), the antibodies and fluorochromes that are contained in the 'cocktail' need to be specified for the 'dump' channel.

For further information about accessibility of biological data and materials, see the following: Cech, T. R. (2003), Sharing Publication-Related Data and Materials: Responsibilities of Authorship in the Life Sciences. DOI: 10.17226/10613.

Sharing data sets
A condition of publication in *JCBFM* is that authors are required to make materials, data and associated protocols promptly available to others without undue qualifications. Data sets must be made freely available to readers from the date of publication, and must be provided to Editors and peer-reviewers at submission, for the purposes of evaluating the manuscript. For the following types of data set, submission to a community-endorsed, public repository is mandatory. Accession numbers must be provided in the paper. Examples of appropriate public repositories are listed below.

DNA and protein sequences
Protein sequences: Uniprot
DNA and RNA sequences: Genbank/European Nucleotide Archive(ENA)/DDBJ, Protein DataBank, UniProt.
DNA and RNA sequencing data (traces for capillary electrophoresis and short reads for next-generation sequencing): NCBI trace and short-read archive, ENA's Sequence Read Archive.
When publishing reference genomes, the assembly must be made available in addition to the sequence reads.

This policy includes even short stretches of novel sequence information such as epitopes, functional domains, genetic markers, or haplotypes. Short novel sequences must include surrounding sequence information to provide context.

The sequences of all RNAi, antisense and morpholino probes must be included in the paper or deposited in a public database, with the accession number quoted. When an unpublished library is included in the paper, at minimum the sequences of the probes central to the conclusions of the paper must be presented.

Simple genetic polymorphisms should be submitted to dbSNP. For data linking genotyping and phenotyping information, we strongly recommend submission to dbGAP or EGA, two repositories that have mechanisms for access control for human health-related phenotypes.

Macromolecular structures
Authors of papers describing structures of biological macromolecules must provide atomic coordinates and related experimental data (structure factor amplitudes/intensities for crystal structures, or restraints for NMR structures) upon request of Editors for the purposes of evaluating the manuscript, if they are not already freely accessible in a publicly available and recognized database (for example, Protein DataBank, Uniprot, Nucleic Acids Database or Biological Magnetic Resonance Databank). Electron microscopy-derived density maps and coordinate data must be deposited in EMDB.

Where there is no public repository and if the data sets are too large to submit to the journal online, authors should either consult the journal Editorial Office for advice or provide five separate copies of these data to the Editors in an appropriate format (for example, CD or DVD) for the purposes of peer-review. If authors wish to deposit work into a Protein DataBank (PDB) please contact the Editorial Office.

Microarray data
MIAME-compliant microarray data: deposit in GEO or ArrayExpress upon submission to the journal. Data must be MIAME-compliant, as described at the MGED web site specifying microarray standards.

Crystallographic data for small molecules
Manuscripts reporting new three-dimensional structures of small molecules from crystallographic analysis should include a .cif file and a structural figure with probability ellipsoids for publication as Supplementary Information. The structure factors for each structure should also be submitted. Both the structure factors and the structural output must have been checked using the IUCR's CheckCIF routine, and a PDF copy of the output must be included at submission, together with a justification for any alerts reported. Crystallographic data for small molecules should be submitted to the Cambridge Structural Database and the deposition number referenced appropriately in the manuscript. Full access must be provided on publication.

Other datasets
In addition to the above-mentioned mandatory requirements for data submission to community-endorsed public databases, JCBFM strongly recommends deposition of other types of data sets into appropriate public repositories that are at an earlier stage of development. Examples of such repositories that facilitate sharing large data sets, some of which can offer the option of anonymous referee access to data before publication, include:

For proteomics data: PRIDE , PeptideAtlas, Tranche
For protein interaction data: IMEx consortium of databases including DIP, IntAct and MINT
For chemical compound screening and assay data: PubChem
Other databases recommended include IntAct and the Global Proteome Machine Organization.
Earth sciences databases recommended include Pangaea, the publishing network for geoscientific and environmental data; PetDB, for geochemical data of rocks on the ocean floor; and GEOROC, geochemistry of rocks from the oceans and continents.
See also: World Data Center system; National Climatic Data Center.
Astronomy and Astrophysics: NucAstroData; Plasma gate; Smithsonian/NASA astrophysics data system; SIMBAD astronomical database; UK solar system data centre.
Physics: NIST physical reference data; Hepdata reaction data.
Biology: NBII; ITIS (taxonomy); NCBI taxonomy; Species 2000; National Center for Ecological Analysis and Synthesis; Dryad.

4.13 Pre-print servers

In the case of working manuscripts or early drafts of manuscripts posted on a pre-print servers: Authors should alert the Editor when submitting their manuscript if they have posted it on a pre-print server. Authors should not post an updated version of their manuscript on the pre-print server while it is being peer reviewed for possible publication in the journal. If the article is accepted for publication, the author may re-use their work according to the journal's self-archiving policy.

Back to top

5. Publishing policies

5.1 Publishing ethics

SAGE and *JCBFM* are committed to upholding the integrity of the academic record. We encourage authors to refer to the *JCBFM* Editorial Policies Document, the Committee on Publication Ethics' International Standards for Authors and view the Publication Ethics page on the SAGE Author Gateway

5.1.1 Plagiarism

The Journal and SAGE take issues of copyright infringement, plagiarism or other breaches of best practice in publication very seriously. We seek to protect the rights of our authors and we always investigate claims of plagiarism or misuse of published articles. Equally, we seek to protect the reputation of the journal against malpractice. Submitted articles may be checked with duplication-checking software. Where an article, for example, is found to have plagiarised other work or included third-party copyright material without permission or with insufficient acknowledgement, or where the authorship of the article is contested, we reserve the right to take action including, but not limited to: publishing an erratum or corrigendum (correction); retracting the article; taking up the matter with the head of department or dean of the author's institution and/or relevant academic bodies or societies; or taking appropriate legal action.

CrossCheck is a multi-publisher initiative to screen published and submitted content for originality. The *JCBFM* uses CrossCheck to detect instances of overlapping and similar text in submitted manuscripts. To find out more visit the CrossCheck website.

5.1.2 Correction and retraction process

Content published as Online First (OF) is final and cannot be amended. The online and print versions are both part of the published record hence the original version must be preserved and changes to the paper should be made as a formal correction. If an error is noticed in an OF article, a correction should accompany the article when it publishes in print. An HTML (or full-text) version of the correction will also be created and linked to the original article. If the error is found in an article after print publication the correction will be published online and in the next available print issue. Please note the following categories of corrections to print and online versions of peer reviewed content:

- Erratum - Notification of an important error made by the journal that affects the publication record or the scientific integrity of the paper, or the reputation of the authors, or of the journal.
- Corrigendum - Notification of an important error made by the author that affects the publication record or the scientific integrity of the paper, or the reputation of the authors or the journal.
- Retraction - Notification of invalid results. All co-authors must sign a retraction specifying the error and stating briefly how the conclusions are affected.

Decisions about corrections are made by the Editor (sometimes with peer-reviewers' advice) and this sometimes involves author consultation. Requests to make corrections that do not affect the paper in a significant way or impair the reader's understanding of the contribution (a spelling mistake or grammatical error, for example) are not considered. In cases where co-authors disagree about a correction, the Editors will take advice from independent peer-reviewers and impose the appropriate correction, noting the dissenting author(s) in the text of the published version.

5.2 Contributor's Publishing Agreement

Before publication, SAGE requires the author as the rights holder to sign a Journal Contributor's Publishing Agreement. SAGE's Journal Contributor's Publishing Agreement is an exclusive licence agreement which means that the author retains copyright in the work but grants SAGE the sole and exclusive right and licence to publish for the full legal term of copyright. Exceptions may exist where an assignment of copyright is required or preferred by a

proprietor other than SAGE. In this case copyright in the work will be assigned from the author to the society. For more information please visit our Frequently Asked Questions on the SAGE Journal Author Gateway.

5.3 Open access and author archiving

For information on funding body compliance, and depositing your article in repositories, please visit SAGE Publishing Policies on our Journal Author Gateway.

JCBFM offers optional open access publishing via the SAGE Choice programme. For more information please visit the SAGE Choice website.

Page charges will not apply to open access papers where the author is paying an article processing charge.

Upon acceptance of your manuscript, you will be charged a one-time article processing charge (APC) to be paid by you, your institution or funder. This fee covers the cost of publication and ensures that your articles will be freely available in perpetuity.
- The full price article processing charge (APC) is £1,600 + VAT where applicable*
- The discounted article processing charge (APC) for ISCBFM members is £1,350+ VAT where applicable*

*If the paying author/institution is based in the European Union, to comply with European law, Value Added Tax (VAT) must be added to the APC. Providing a VAT registration number will allow an institution to avoid paying this tax, except for UK institutions. An invoice will be issued in GBP.

5.4 Permissions

Authors are responsible for obtaining permission from copyright holders for reproducing any illustrations, tables, figures or lengthy quotations previously published elsewhere. For further information including guidance on fair dealing for criticism and review, please visit our Frequently Asked Questions on the SAGE Journal Author Gateway

6. On acceptance and publication

6.1 *JCBFM* Authorship Form required

Once a manuscript is accepted, a *JCBFM* Authorship form is required in addition to the SAGE Contributor's Publishing Agreement. The corresponding author is responsible for sending a completed Authorship Form to the Editors. In this form, all authors must state their specific contributions to the manuscript and sign the form. Hand written signatures are required and no author may be left out. If all authors cannot fill out and sign the same form, the corresponding author is responsible for collecting authors' forms. They must then be compiled and scanned into one PDF file, and e-mailed to the Editorial Office. Failure to promptly return the form will delay publication.

6.2 SAGE Production

Your SAGE Production Editor will keep you informed as to your article's progress throughout the production process. Proofs will be sent by PDF to the corresponding author and should be returned promptly. The Production Editor informs authors after publication once their paper is available online.

6.3 Access to your published article

SAGE provides authors with online access to their final article.

6.4 Online First publication

Online First allows final revision articles (completed articles in queue for assignment to an upcoming issue) to be published online prior to their inclusion in a final journal issue which significantly reduces the lead time between submission and publication. For more information please visit our Online First Fact Sheet

Back to top

7. Further Information

Online First allows final revision articles (completed articles in queue for assignment to an upcoming issue) to be published online prior to their inclusion in a final journal issue which significantly reduces the lead time between submission and publication. For more information please visit our Online First Fact Sheet Any correspondence, queries or additional requests for information on the manuscript submission process should be sent to the Journal of Cerebral Blood Flow and Metabolism Editorial Office: katherine.knaust@charite.de or lene.rask.01@regionh.dk.

For inquiries related to advertising, reprints or publishing a supplement, please contact the publisher's office: Tamara.Haq@sagepub.co.uk.

Back to top

Individual Subscription, Print Only

$812.00
Buy now

Individual Subscription, Combined (Print & E-access)

$829.00
Buy now

Institutional Subscription, E-access

$1,505.00
Buy now

Institutional Subscription, Print Only

$1,639.00
Buy now

Institutional Subscription & Backfile Lease, E-access Plus Backfile (All Online Content)

$1,672.00
Buy now

Institutional Subscription, Combined (Print & E-access)

$1,672.00
Buy now

Institutional Subscription & Backfile Lease, Combined Plus Backfile (Current Volume Print & All Online Content)

$1,839.00
Buy now

Institutional Backfile Purchase, E-access (Content through 1998)

$2,333.00
Buy now

Individual, Single Print Issue

$90.00
Info

Institutional, Single Print Issue

$150.00
Info

Anexo A.2.

Guia para autores do periódico Free Radical Biology & Medicine.

FREE RADICAL BIOLOGY & MEDICINE

An official Journal of the Society for Redox Biology and Medicine
An official Journal of the Society for Free Radical Research-Europe

An Affiliate Journal of the International Society for Free Radical Research (SFRRI)

AUTHOR INFORMATION PACK

TABLE OF CONTENTS

ISSN: 0891-5849

DESCRIPTION

Free Radical Biology and Medicine is an international, interdisciplinary journal that publishes original contributions and reviews on a broad range of topics relating to **redox biology**, **signaling**, **biological chemistry** and medical implications of **free radicals**, **reactive species**, **oxidants** and **antioxidants**.

AUDIENCE

Biochemists, physiologists, pathologists, toxicologists.

IMPACT FACTOR

2015: 5.784 © Thomson Reuters Journal Citation Reports 2016

ABSTRACTING AND INDEXING

ADONIS
BIOSIS
Elsevier BIOBASE
Cambridge Scientific Abstracts
Chemical Abstracts
Current Contents
MEDLINE®
EMBASE
Science Citation Index
Toxicology Abstracts
Scopus
EMBiology

EDITORIAL BOARD

Editor in Chief

Kelvin J. A. Davies, University of Southern California, Los Angeles, California, USA

GUIDE FOR AUTHORS

Your Paper Your Way

We now differentiate between the requirements for new and revised submissions. You may choose to submit your manuscript as a single Word or PDF file to be used in the refereeing process. Only when your paper is at the revision stage, will you be requested to put your paper in to a 'correct format' for acceptance and provide the items required for the publication of your article.
To find out more, please visit the Preparation section below.

INTRODUCTION

Free Radical Biology & Medicine is an international, interdisciplinary journal that publishes original contributions and reviews on a broad range of topics relating to redox biology, signaling, biological chemistry and medical implications of free radicals, reactive species, oxidants and antioxidants.

Types of paper
Full-length research articles, Review articles, Hypothesis papers, Methods articles and Letters to the Editor.

Original Articles: Original articles are the normal medium of publication. Although there is no fixed length, articles should be as concise as possible, while providing sufficient information for the work to be repeated and for the claims of the authors to be judged by the readers.

Reviews are typically commissioned by the Review Editor. All Review Articles should be authoritative, state-of-the-art accounts of the selected research field, be of high interest, balanced and accurate. Beyond summaries of important scientific developments and ideas, authors are encouraged to identify and discuss how the field may be impacted or develop in the future, including insights that may be of significance to the scientific community. All FRBM Review Articles undergo rigorous and full peer review, in the same way as regular research papers, and publication cannot be guaranteed.

Unsolicited reviews will be considered **only** in exceptional cases and should be preceded by a letter of enquiry from the prospective author, who should be a recognized expert in the field of the proposed article. Presubmission enquires may be sent to the Editorial Office Frbmreviews@elsevier.com. Specifically, authors must provide the following in their review proposal: 1) both your own and any co-author(s) affiliation and full contact details; 2) an explanation of the current interest and significance to the broad readership of the journal, that is, compelling reasons why the review should be considered; 3) a 500-600 word summary which clearly outlines what will be discussed in the article, plus up to 20 key references that indicate the intended breadth of the proposed article (please note that references should include work published in the past 2-4 years). Please also include 1-2 summary tables highlighting developments in the field (as well as controversies) and (a) schematic(s) to illustrate key points of the article. **Only proposals that include the required information and are submitted to the Editorial Office (frbmreviews@elsevier.com) will be considered.**

Letters to the Editor: Letters to the Editor are intended to stimulate discussion and debate in areas of general concern and controversy in free radical and oxidant research, and generally reflect the personal opinions of the author(s). They should be written in a continuous style and should normally not exceed two printed pages and contain no more than one figure and table.

Critical Methods: Authors wishing to contribute a Critical Methods paper are advised first to contact the Editor, Dr. Kelvin Davies. Please e-mail the outline and abstract of the proposed manuscript to frbm@elsevier.com before uploading the submission. These papers are contributed by scientists who are leading specialists in their field of expertise, normally at the invitation of the Editors. Critical Methods papers must conform to a strict format. Abstract: In 200 words or less, state why this is the appropriate method to use, the general method type (HPLC, Elisa, enzymatic assay, etc.) and the instrumentation (UV detector, Plate reader, UV spectrophotometer, etc.). Introduction: Background of methodology. Provide example of the use of the method. If other methods are flawed, briefly explain the problem with them. Principles: Explain the chemical and/or biological basis of the method. Describe what the assay does and does not mean. For example, many molecular biology measurements provide relative changes rather than absolute values. Another example, is that the usual spectrofluorimetric determination of intracellular calcium concentration is an average among cells that does not indicate individual cells and does not account for gradients within cells. Materials: Provide a detailed list of every reagent. Include source and catalog number. Instrumentation: Describe

the required instrument(s)? For example, for spectrophotometer, a double beam spectrophotometer and for HPLC , specific detector, gradient mixer, etc. An actual model number and the vendor, etc. should be included. Protocol: Describe in detail each step. This can be divided into subsections. For example, for electrophoretic mobility assays, extraction of nuclear proteins would be a section and gel electrophoresis would be another. If there are multiple buffer solutions, each could also be a separate section. Indicate the minimum (and maximum if needed) concentration of percent change that is required for the assay to produce significant results. Indicate the timing of steps including any waiting periods. Provide representative illustrations of steps where useful. If there are steps where particular care must be paid that are not obvious (for example, making sure a sample is placed into a well below the buffer using a long thin pipette) this should be in bold lettering. Precautions should also be in bold for any steps where something can easily go wrong. Calculations and Expected Results: Describe any post protocol calculations in detail. Provide representative results. Caveats: Describe any caveats that need to be considered. Acknowledgements Section (self explanatory). Conflict of Interest: State if the authors have patents or financial interests in the protocol or instruments." **In light of the special structure and format of these sorts of papers, the 'Your Paper Your Way' submission option is not available for Critical Methods papers**.

Contact details for submission
Papers should be submitted using the *Free Radical Biology & Medicine* online submission system, http://ees.elsevier.com/frbm For questions on the submission or reviewing process, please contact the Editorial Office at frbm@elsevier.com

BEFORE YOU BEGIN

Ethics in publishing
Please see our information pages on Ethics in publishing and Ethical guidelines for journal publication.

Policy and ethics
The work described in your article must have been carried out in accordance with *The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans* http://www.wma.net/en/30publications/10policies/b3/index.html; *Uniform Requirements for manuscripts submitted to Biomedical journals* http://www.icmje.org. This must be stated at an appropriate point in the article.

Animal experiments
Where animals have been used in a study, the institutional ethical or animal welfare Authority under which the work was conducted must be stated, along with the specific authorisation reference number. Circumstances relating to animal experimentation must meet the *International Guiding Principles for Biomedical Research Involving Animals*, as issued by the Council for the International Organizations of Medical Sciences. These guidelines are obtainable from: Executive Secretary C.I.O.M.S., c/o WHO, Appia, CH-1211 Geneva 27, Switzerland, or at the following URL: http://www.cioms.ch/frame_1985_texts_of_guidelines.htm, or the *EC Directive 86/609/EEC for animal experiments* http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm.

Declaration of interest
All authors are requested to disclose any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work. More information.

Submission declaration and verification
Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis or as an electronic preprint, see 'Multiple, redundant or concurrent publication' section of our ethics policy for more information), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder. To verify originality, your article may be checked by the originality detection service CrossCheck.

Authorship
All authors should have made substantial contributions to all of the following: (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, (3) final approval of the version to be submitted.

Changes to authorship
Authors are expected to consider carefully the list and order of authors **before** submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion or rearrangement of author names in the authorship list should be made only **before** the manuscript has been accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the **corresponding author**: (a) the reason for the change in author list and (b) written confirmation (e-mail, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed.
Only in exceptional circumstances will the Editor consider the addition, deletion or rearrangement of authors **after** the manuscript has been accepted. While the Editor considers the request, publication of the manuscript will be suspended. If the manuscript has already been published in an online issue, any requests approved by the Editor will result in a corrigendum.

Article transfer service
This journal is part of our Article Transfer Service. This means that if the Editor feels your article is more suitable in one of our other participating journals, then you may be asked to consider transferring the article to one of those. If you agree, your article will be transferred automatically on your behalf with no need to reformat. Please note that your article will be reviewed again by the new journal. More information.

Copyright
Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (see more information on this). An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.

Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations. If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases.

For open access articles: Upon acceptance of an article, authors will be asked to complete an 'Exclusive License Agreement' (more information). Permitted third party reuse of open access articles is determined by the author's choice of user license.

Author rights
As an author you (or your employer or institution) have certain rights to reuse your work. More information.

Elsevier supports responsible sharing
Find out how you can share your research published in Elsevier journals.

Role of the funding source
You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement then this should be stated.

Funding body agreements and policies
Elsevier has established a number of agreements with funding bodies which allow authors to comply with their funder's open access policies. Some funding bodies will reimburse the author for the Open Access Publication Fee. Details of existing agreements are available online.

Elsevier journals comply with current NIH public access policy.

Open access

This journal offers authors a choice in publishing their research:

Open access

• Articles are freely available to both subscribers and the wider public with permitted reuse.
• An open access publication fee is payable by authors or on their behalf, e.g. by their research funder or institution.

Subscription

• Articles are made available to subscribers as well as developing countries and patient groups through our universal access programs.
• No open access publication fee payable by authors.

Regardless of how you choose to publish your article, the journal will apply the same peer review criteria and acceptance standards.

For open access articles, permitted third party (re)use is defined by the following Creative Commons user licenses:

Creative Commons Attribution (CC BY)
Lets others distribute and copy the article, create extracts, abstracts, and other revised versions, adaptations or derivative works of or from an article (such as a translation), include in a collective work (such as an anthology), text or data mine the article, even for commercial purposes, as long as they credit the author(s), do not represent the author as endorsing their adaptation of the article, and do not modify the article in such a way as to damage the author's honor or reputation.

Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND)
For non-commercial purposes, lets others distribute and copy the article, and to include in a collective work (such as an anthology), as long as they credit the author(s) and provided they do not alter or modify the article.

The open access publication fee for this journal is **USD 2600**, excluding taxes. Learn more about Elsevier's pricing policy: https://www.elsevier.com/openaccesspricing.

Green open access
Authors can share their research in a variety of different ways and Elsevier has a number of green open access options available. We recommend authors see our green open access page for further information. Authors can also self-archive their manuscripts immediately and enable public access from their institution's repository after an embargo period. This is the version that has been accepted for publication and which typically includes author-incorporated changes suggested during submission, peer review and in editor-author communications. Embargo period: For subscription articles, an appropriate amount of time is needed for journals to deliver value to subscribing customers before an article becomes freely available to the public. This is the embargo period and it begins from the date the article is formally published online in its final and fully citable form. Find out more.

This journal has an embargo period of 12 months.

Language (usage and editing services)
Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use the English Language Editing service available from Elsevier's WebShop.

Your Paper-Your Way
As part of the Your Paper-Your Way service, authors may submit a PDF version of their manuscript for use in the refereeing process. This PDF file can be in any format or lay-out that can be used by referees to evaluate your work. It should contain high enough quality figures for refereeing. References can be in any style or format, as long as the full paper title is present. After revision, at acceptance, source files of the paper, figures, tables and figure captions will then be required to produce the final published version - not before.

Submission in the traditional way is also still possible.

Submission

Submission to this journal proceeds totally online and you will be guided stepwise through the creation and uploading of your files. The system automatically converts source files to a single PDF file of the article, which is used in the peer-review process (see above). Please note that even though manuscript source files are converted to PDF files at submission for the review process, these source files are needed for further processing after acceptance. All correspondence, including notification of the Editor's decision and requests for revision, takes place by e-mail removing the need for a paper trail.

Submit your article
Please submit your article via http://ees.elsevier.com/frbm

Referees
A minimum of four suitable potential reviewers (please provide their name, email addresses, and institutional affiliation) should be provided. When compiling this list of potential reviewers please consider the following important criteria: they must be knowledgeable about the manuscript subject area; must not be from your own institution; at least two of the suggested reviewers should be from another country than the authors'; and they should not have recent (less than four years) joint publications with any of the authors. However, the final choice of reviewers is at the editors' discretion.

PREPARATION

NEW SUBMISSIONS

Submission to this journal proceeds totally online and you will be guided stepwise through the creation and uploading of your files. The system automatically converts your files to a single PDF file, which is used in the peer-review process.
As part of the Your Paper Your Way service, you may choose to submit your manuscript as a single file to be used in the refereeing process. This can be a PDF file or a Word document, in any format or lay-out that can be used by referees to evaluate your manuscript. It should contain high enough quality figures for refereeing. If you prefer to do so, you may still provide all or some of the source files at the initial submission. Please note that individual figure files larger than 10 MB must be uploaded separately.

References
There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/book title, chapter title/article title, year of publication, volume number/book chapter and the pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct.

Formatting requirements
There are no strict formatting requirements but all manuscripts must contain the essential elements needed to convey your manuscript, for example Abstract, Keywords, Introduction, Materials and Methods, Results, Conclusions, Artwork and Tables with Captions.
If your article includes any Videos and/or other Supplementary material, this should be included in your initial submission for peer review purposes.
Divide the article into clearly defined sections.

Figures and tables embedded in text
Please ensure the figures and the tables included in the single file are placed next to the relevant text in the manuscript, rather than at the bottom or the top of the file. The corresponding caption should be placed directly below the figure or table.

REVISED SUBMISSIONS

Use of word processing software
Regardless of the file format of the original submission, at revision you must provide us with an editable file of the entire article. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier). See also the section on Electronic artwork.
To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Article structure

Subdivision - unnumbered sections
Divide your article into clearly defined sections. Each subsection is given a brief heading. Each heading should appear on its own separate line. Subsections should be used as much as possible when cross-referencing text: refer to the subsection by heading as opposed to simply 'the text'.

Introduction
State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods
Provide sufficient detail to allow the work to be reproduced, with details of supplier and catalogue number when appropriate. Methods already published should be indicated by a reference: only relevant modifications should be described.

Results
Results should be clear and concise.

Discussion
This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions
The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Essential title page information

• ***Title.*** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
• ***Author names and affiliations.*** Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
• ***Corresponding author.*** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. **Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.**
• ***Present/permanent address.*** If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Abstract

A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

Graphical abstract
A Graphical abstract is mandatory for this journal. It should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership online. Authors must provide images that clearly represent the work described in the article. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more. The image should be readable at a size of 5 × 13 cm using a regular screen resolution of 96 dpi. Preferred file types: TIFF, EPS, PDF or MS Office files. You can view Example Graphical Abstracts on our information site.
Authors can make use of Elsevier's Illustration and Enhancement service to ensure the best presentation of their images also in accordance with all technical requirements: Illustration Service.

The Editors strongly encourage graphical abstracts and suggest that amongst the examples given http://www.elsevier.com/graphicalabstracts, that example 11 and 12 best reflect the sort of graphical abstract most suited to this field and journal.

Highlights
Highlights are mandatory for this journal. They consist of a short collection of bullet points that convey the core findings of the article and should be submitted in a separate editable file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point). You can view example Highlights on our information site.

Keywords
Following the abstract, list keywords for indexing. These keywords should cover precisely the contents of the submitted paper and should give readers sufficient information as to the relevance of the paper to their particular field.

Abbreviations
Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Acknowledgements
Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Formatting of funding sources
List funding sources in this standard way to facilitate compliance to funder's requirements:

Funding: This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa].

It is not necessary to include detailed descriptions on the program or type of grants and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding.

If no funding has been provided for the research, please include the following sentence:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Nomenclature and abbreviations
Stylistic details must be kept constant. For example, reactive oxygen species is abbreviated as ROS, reactive nitrogen species is abbreviated as RNS, and electron spin resonance is abbreviated either ESR or EPR(for electron paramagnetic resonance). Either can be used, but both should be given and stated as equivalent at the first mention.(This is the recommendation of the International EPR Society.) Formulas for radicals follow IUPAC recommendations and contain a superscripted (not centered) large dot that precedes a charge,if any. Thus, superoxide is represented by O2• −, not O2− •, or some other permutation.

Other examples are HO• or •OH (not OH•), RO•, ROO•/•NO2, •CH2OH, etc. In the text, names of radicals are preferred, rather than using formulas in the middle of sentences. For names of radicals, use alkoxyl, peroxyl, and hydroxyl and not alkoxy, peroxy, etc. (correct nomenclature requires the 'l' on the end of radicals, as in methyl, hydroxyl, etc.). Use tert, not t-, etc., for abbreviations. For example, CORRECT: tert-butoxyl, sec-peroxyl; INCORRECT: t-butoxy, s-peroxy.

Wherever possible, nomenclature and abbreviations should be in accordance with internationally agreed rules. When an enzyme or compound is first mentioned in the text, specification by its code number accompanied by its systematic name (as distinct from its trivial name) is requested by the Editors, but not checked for correctness.

Official names of drugs are preferred to trade names.

Standard three-letter codes for the common amino acids may be used freely and without definition, but the one-letter codes should be restricted to comparisons of long protein sequences. Similar considerations apply to nucleosides and nucleotides. Standard three-letter codes for carbohydrates and for purine and pyrimidine bases may also be used. All other abbreviations should be defined when they first appear in the text. If an extensive list of abbreviations is used, please provide an alphabetical list with definitions followed by the references at the end of the article.

Temperatures denoted by an unqualified degree symbol are assumed to be Celsius. For solution strengths, percentages should be expressed by the sign %, followed in cases of ambiguity by w/w, w/v, or v/v [e.g., 5% (w/v) means 5 g/100 ml].

All non-standard abbreviations should be defined in a footnote.

Footnotes
Footnotes should be used sparingly. Number them consecutively throughout the article. Many word processors build footnotes into the text, and this feature may be used. Should this not be the case, indicate the position of footnotes in the text and present the footnotes themselves separately at the end of the article.

Image manipulation
Whilst it is accepted that authors sometimes need to manipulate images for clarity, manipulation for purposes of deception or fraud will be seen as scientific ethical abuse and will be dealt with accordingly. For graphical images, this journal is applying the following policy: no specific feature within an image may be enhanced, obscured, moved, removed, or introduced. Adjustments of brightness, contrast, or color balance are acceptable if and as long as they do not obscure or eliminate any information present in the original. Nonlinear adjustments (e.g. changes to gamma settings) must be disclosed in the figure legend.

Electronic artwork
General points
• Make sure you use uniform lettering and sizing of your original artwork.
• Preferred fonts: Arial (or Helvetica), Times New Roman (or Times), Symbol, Courier.
• Number the illustrations according to their sequence in the text.
• Use a logical naming convention for your artwork files.
• Indicate per figure if it is a single, 1.5 or 2-column fitting image.
• For Word submissions only, you may still provide figures and their captions, and tables within a single file at the revision stage.
• Please note that individual figure files larger than 10 MB must be provided in separate source files.
A detailed guide on electronic artwork is available.
You are urged to visit this site; some excerpts from the detailed information are given here.
Formats
Regardless of the application used, when your electronic artwork is finalized, please 'save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):
EPS (or PDF): Vector drawings. Embed the font or save the text as 'graphics'.
TIFF (or JPG): Color or grayscale photographs (halftones): always use a minimum of 300 dpi.
TIFF (or JPG): Bitmapped line drawings: use a minimum of 1000 dpi.
TIFF (or JPG): Combinations bitmapped line/half-tone (color or grayscale): a minimum of 500 dpi is required.
Please do not:
• Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); the resolution is too low.
• Supply files that are too low in resolution.
• Submit graphics that are disproportionately large for the content.

Color artwork
Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF), or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color online (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in color in the printed version. **For color reproduction in print, you will receive**

information regarding the costs from Elsevier after receipt of your accepted article. Please indicate your preference for color: in print or online only. Further information on the preparation of electronic artwork.

Figure captions
Ensure that each illustration has a caption. A caption should comprise a brief title (**not** on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Tables

Please submit tables as editable text and not as images. Tables can be placed either next to the relevant text in the article, or on separate page(s) at the end. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be sparing in the use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules and shading in table cells.

References

Citation in text
Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

Web references
As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

Data references
This journal encourages you to cite underlying or relevant datasets in your manuscript by citing them in your text and including a data reference in your Reference List. Data references should include the following elements: author name(s), dataset title, data repository, version (where available), year, and global persistent identifier. Add [dataset] immediately before the reference so we can properly identify it as a data reference. The [dataset] identifier will not appear in your published article.

References in a special issue
Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Reference management software
Most Elsevier journals have their reference template available in many of the most popular reference management software products. These include all products that support Citation Style Language styles, such as Mendeley and Zotero, as well as EndNote. Using the word processor plug-ins from these products, authors only need to select the appropriate journal template when preparing their article, after which citations and bibliographies will be automatically formatted in the journal's style. If no template is yet available for this journal, please follow the format of the sample references and citations as shown in this Guide.

Users of Mendeley Desktop can easily install the reference style for this journal by clicking the following link:
http://open.mendeley.com/use-citation-style/free-radical-biology-and-medicine
When preparing your manuscript, you will then be able to select this style using the Mendeley plug-ins for Microsoft Word or LibreOffice.

Reference formatting
There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/book title, chapter title/article title, year of publication, volume number/book chapter and the pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be

applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct. If you do wish to format the references yourself they should be arranged according to the following examples:

Reference style
Text: Indicate references by number(s) in square brackets in line with the text. The actual authors can be referred to, but the reference number(s) must always be given.
Example: '..... as demonstrated [3,6]. Barnaby and Jones [8] obtained a different result ....'
List: Number the references (numbers in square brackets) in the list in the order in which they appear in the text.
Examples:
Reference to a journal publication:
[1] J. van der Geer, J.A.J. Hanraads, R.A. Lupton, The art of writing a scientific article, J. Sci. Commun. 163 (2010) 51–59.
Reference to a book:
[2] W. Strunk Jr., E.B. White, The Elements of Style, fourth ed., Longman, New York, 2000.
Reference to a chapter in an edited book:
[3] G.R. Mettam, L.B. Adams, How to prepare an electronic version of your article, in: B.S. Jones, R.Z. Smith (Eds.), Introduction to the Electronic Age, E-Publishing Inc., New York, 2009, pp. 281–304.
Reference to a website:
[4] Cancer Research UK, Cancer statistics reports for the UK. http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/, 2003 (accessed 13.03.03).
Reference to a dataset:
[dataset] [5] M. Oguro, S. Imahiro, S. Saito, T. Nakashizuka, Mortality data for Japanese oak wilt disease and surrounding forest compositions, Mendeley Data, v1, 2015. http://dx.doi.org/10.17632/xwj98nb39r.1.

Reference style
Type references double-spaced and number them consecutively in the order in which they are first mentioned in the text, not alphabetically. Cite references in the text, tables, and legends in sequential, numerical order, placing the numbers in square brackets. References cited only in tables or figure legends should be numbered in accordance with a sequence established by the first mention in the text of the particular table or figure. Journal titles are to be abbreviated according to the List of Journals Indexed in Index Medicus published by the U.S. Department of Health and Human Services. Examples of reference style are as follows:

Journal:
[1] Muller, F. L.; Lustgarten, M. S.; Jang, Y.; Richardson, A.; Van Remmen, H. Trends in oxidative aging theories. Free Radic. Biol. Med. 43:477-503; 2007.

Book:
[2] Van Faassen, E.; Vanin, A., eds. Radicals For Life: the Various Forms of nitric oxide. Amsterdam: Elsevier; 2007.

Chapter in edited book:
[3] Zuo, L.; Clanton, T. L. Detection of reactive oxygen and nitrogen species in tissues using redox-sensitive fluorescent probes. In: Sen, C. K.; Packer, L., eds. Redox cell biology and genetics, part A. Methods in enzymology, volume 352. San Diego: Academic Press; 2002: 307-325.

Abstract:
[4] Freeman, B.; Aslan, M. Tissue oxidation and nitration reactions in a mouse model and humans with sickle cell disease (abstract). Free Radic. Biol. Med. 33:S298; 2002.

Manuscripts that have been accepted for publication may be cited as "in press" in the reference list using the estimated year of publication:
[5] Aguirre, J.; Lambeth, J.D. Nox enzymes from fungus to fly to fish and what they tell us about Nox function in mammals. Free Radic. Biol. Med. In press; 2010.

Reference to a paper as "in press" implies that it has been accepted for publication. Evidence (e.g., a photocopy of the note of acceptance from the journal concerned) should accompany the submitted typescript. Papers that are "in press" should be included as a number in the text. Other papers

submitted before or simultaneously with the paper in question should be included as a number in the text and in the References section, stating the name of the journal. Copies of papers that are submitted elsewhere should be provided for inspection by the Editors. Omission of this information will delay publication and may lead to redating of a submitted manuscript. Papers presented at scientific meetings that are not available in published form should not be cited as references in the References section.

Unpublished results should not be listed in the References section. In the text they are mentioned as follows: "(Tervoort MV and Glimcher J, unpublished data)". When unpublished results are cited, the data should be provided for the Editors' information when essential for proper evaluation, or if requested.

A personal communication should be mentioned in the text as follows: "(Tervoort MV, personal communication)". Authors should not make unauthorized use of personal communications. Personal communications are not to be included in the Reference section.

Journal abbreviations source
Journal names should be abbreviated according to the List of Title Word Abbreviations.

Video

Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. In order to ensure that your video or animation material is directly usable, please provide the files in one of our recommended file formats with a preferred maximum size of 150 MB. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including ScienceDirect. Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages. Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

Supplementary material

Supplementary material such as applications, images and sound clips, can be published with your article to enhance it. Submitted supplementary items are published exactly as they are received (Excel or PowerPoint files will appear as such online). Please submit your material together with the article and supply a concise, descriptive caption for each supplementary file. If you wish to make changes to supplementary material during any stage of the process, please make sure to provide an updated file. Do not annotate any corrections on a previous version. Please switch off the 'Track Changes' option in Microsoft Office files as these will appear in the published version.

Supplementary material captions
Each supplementary material file should have a short caption which will be placed at the bottom of the article, where it can assist the reader and also be used by search engines.

RESEARCH DATA

This journal encourages and enables you to share data that supports your research publication where appropriate, and enables you to interlink the data with your published articles. Research data refers to the results of observations or experimentation that validate research findings. To facilitate reproducibility and data reuse, this journal also encourages you to share your software, code, models, algorithms, protocols, methods and other useful materials related to the project.

Below are a number of ways in which you can associate data with your article or make a statement about the availability of your data when submitting your manuscript. If you are sharing data in one of these ways, you are encouraged to cite the data in your manuscript and reference list. Please refer to the "References" section for more information about data citation. For more information on depositing, sharing and using research data and other relevant research materials, visit the research data page.

Data linking
If you have made your research data available in a data repository, you can link your article directly to the dataset. Elsevier collaborates with a number of repositories to link articles on ScienceDirect with relevant repositories, giving readers access to underlying data that give them a better understanding of the research described.

There are different ways to link your datasets to your article. When available, you can directly link your dataset to your article by providing the relevant information in the submission system. For more information, visit the database linking page.

For supported data repositories a repository banner will automatically appear next to your published article on ScienceDirect.

In addition, you can link to relevant data or entities through identifiers within the text of your manuscript, using the following format: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN).

Data in Brief
You have the option of converting any or all parts of your supplementary or additional raw data into one or multiple data articles, a new kind of article that houses and describes your data. Data articles ensure that your data is actively reviewed, curated, formatted, indexed, given a DOI and publicly available to all upon publication. You are encouraged to submit your article for *Data in Brief* as an additional item directly alongside the revised version of your manuscript. If your research article is accepted, your data article will automatically be transferred over to *Data in Brief* where it will be editorially reviewed and published in the open access data journal, *Data in Brief*. Please note an open access fee is payable for publication in *Data in Brief*. Full details can be found on the Data in Brief website. Please use this template to write your Data in Brief.

AudioSlides
The journal encourages authors to create an AudioSlides presentation with their published article. AudioSlides are brief, webinar-style presentations that are shown next to the online article on ScienceDirect. This gives authors the opportunity to summarize their research in their own words and to help readers understand what the paper is about. More information and examples are available. Authors of this journal will automatically receive an invitation e-mail to create an AudioSlides presentation after acceptance of their paper.

Interactive plots
This journal enables you to show an Interactive Plot with your article by simply submitting a data file. Full instructions.

Full Online Submission
The following list will be useful during the final checking of an article prior to sending it to the journal for review. Please consult this Guide for Authors for further details of any item.
Ensure that the following items are present:
One Author designated as corresponding Author:
• E-mail address
• Full postal address
• Telephone and fax numbers
All necessary files have been uploaded
• Keywords
• All figure captions
• All tables (including title, description, footnotes)
Further considerations
• Manuscript has been "spellchecked" and "grammar-checked"
• References are in the correct format for this journal - preferred but not essential.
• All references mentioned in the Reference list are cited in the text, and vice versa
• Permission has been obtained for use of copyrighted material from other sources (including the Web)
• Color figures are clearly marked as being intended for color reproduction on the Web (free of charge) and in print or to be reproduced in color on the Web (free of charge) and in black-and-white in print
• If only color on the Web is required, black and white versions of the figures are also supplied for printing purposes
For any further information please visit our customer support site at http://support.elsevier.com.

AFTER ACCEPTANCE

Online proof correction

Corresponding authors will receive an e-mail with a link to our online proofing system, allowing annotation and correction of proofs online. The environment is similar to MS Word: in addition to editing text, you can also comment on figures/tables and answer questions from the Copy Editor. Web-based proofing provides a faster and less error-prone process by allowing you to directly type your corrections, eliminating the potential introduction of errors.
If preferred, you can still choose to annotate and upload your edits on the PDF version. All instructions for proofing will be given in the e-mail we send to authors, including alternative methods to the online version and PDF.
We will do everything possible to get your article published quickly and accurately. Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor. It is important to ensure that all corrections are sent back to us in one communication. Please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility.

Offprints

The corresponding author will, at no cost, receive a customized Share Link providing 50 days free access to the final published version of the article on ScienceDirect. The Share Link can be used for sharing the article via any communication channel, including email and social media. For an extra charge, paper offprints can be ordered via the offprint order form which is sent once the article is accepted for publication. Both corresponding and co-authors may order offprints at any time via Elsevier's Webshop. Corresponding authors who have published their article open access do not receive a Share Link as their final published version of the article is available open access on ScienceDirect and can be shared through the article DOI link.

AUTHOR INQUIRIES

Visit the Elsevier Support Center to find the answers you need. Here you will find everything from Frequently Asked Questions to ways to get in touch.
You can also check the status of your submitted article or find out when your accepted article will be published.

Anexo A.3.

Carta da Comissão de Ética no Uso de Animais.

CARTA DE APROVAÇÃO

Comissão De Ética No Uso De Animais analisou o projeto:

Número: 28043
Título: ESTUDO DOS PARÂMETROS NEUROQUÍMICOS, COMPORTAMENTAIS, FARMACOLÓGICOS E ELETROFISIOLÓGICOS DA CONVULSÃO EM PEIXE-ZEBRA (Danio rerio) ADULTO INDUZIDA POR PENTILENOTETRAZOL

Pesquisadores:

Equipe UFRGS:

DIOGO LOSCH DE OLIVEIRA - coordenador desde 01/11/2014
MARIA ELISA CALCAGNOTTO - pesquisador desde 01/11/2014
Eduardo Pacheco Rico - pesquisador desde 01/11/2014
Sandro Daniel Cordova - Aluno de Doutorado desde 01/11/2014
Fernanda Cenci Vuaden - desde 01/11/2014
Ben Hur Marins Mussulini - Aluno de Doutorado desde 01/11/2014
KAMILA CAGLIARI ZENKI - Aluno de Mestrado desde 01/11/2014
SUELEN BAGGIO - Aluno de Mestrado desde 01/11/2014
CHAIRINI CÁSSIA THOMÉ - desde 01/11/2014
EMERSON SANTOS DA SILVA - desde 01/11/2014
NATÃ EZEQUIEL SEHN DA ROSA - desde 01/11/2014

Equipe Externa:

Carlos Eduardo Leite - pesquisador desde 01/11/2014

Comissão De Ética No Uso De Animais aprovou o mesmo , em reunião realizada em 10/11/2014 - Sala 330 - Anexo I do Prédio da Reitoria - Campus Centro/UFRGS- Av Paulo Gama, 110 - Bairro Farroupilha - Porto Alegre, em seus aspectos éticos e metolodológicos, para a utilização de 4259 peixes-zebra, de acordo com as Diretrizes e Normas Nacionais e Internacionais, especialmente a Lei 11.794 de 08 de novembro de 2008 que disciplina a criação e utilização de animais em atividades de ensino e pesquisa.

Porto Alegre, Terça-Feira, 18 de Novembro de 2014

STELA MARIS KUZE RATES
Coordenador da comissão de ética

Lista de Artigos Publicados

1. Baggio S, Mussulini BH, de Oliveira DL, Zenki K, Santos E, Rico E. Embryonic alcohol exposure promotes long-term effects on cerebral glutamate transport of adult zebrafish. Neuroscience Letters, 2017, Volume 636, pages 265-269.
2. Mussulini BH, Baggio S, Moro L, Dias RD, Calcagnotto ME, Rico EP, de Oliveira DL.Zebrafish as an Animal Model to Study Epileptic Seizures and Epileptic Syndromes. Current Psychopharmacology. 2016, Issue 2 (5) 194 – 210.
3. Schmitz F, Pierozan P, Rodrigues AF, Biasibetti H, Coelho DM, Mussulini BH, Pereira MS, Parisi MM, Barbé-Tuana F, de Oliveira DL, Vargas CR, Wyse AT. Chronic Treatment with a Clinically Relevant Dose of Methylphenidate Increases Glutamate Levels in Cerebrospinal Fluid and Impairs Glutamatergic Homeostasis in Prefrontal Cortex of Juvenile Rats. Mol Neurobiol. 2016 May;53(4):2384-96.
4. Vuaden FC, Savio LE, Rico EP, Mussulini BH, Rosemberg DB, de Oliveira DL, Bogo MR, Bonan CD, Wyse AT. Methionine Exposure Alters Glutamate Uptake and Adenine Nucleotide Hydrolysis in the Zebrafish Brain. Mol Neurobiol. 2016 Jan;53(1):200-9.
5. Braga M, Dick T, de Oliveira DL, Guerra AS, Mussulini BH, Souza DO, Rocha JB. Evaluation of zinc effect on cadmium action in lipid peroxidation and metallothionein levels in the brain. Toxicology Report. 2 (2015) 858–863.
6. Ibrahim M*, Mussulini BH*, Moro L, de Assis AM, Rosemberg DB, de Oliveira DL, Rocha JB, Schwab RS, Schneider PH, Souza DO, Rico EP. Anxiolytic effects of diphenyl diselenide on adult zebrafish in a novelty paradigm. Prog Neuropsychopharmacol Biol Psychiatry. 2014 Oct 3;54:187-94.
7. Zenki KC, Mussulini BH, Rico EP, de Oliveira DL, Rosemberg DB. Effects of ethanol and acetaldehyde in zebrafish brain structures: an *in vitro* approach on glutamate uptake and on toxicity-related parameters. Toxicol In Vitro. 2014 Aug;28(5):822-8.
8. Mussulini BH, Leite CE, Zenki KC, Moro L, Baggio S, Rico EP, Rosemberg DB, Dias RD, Souza TM, Calcagnotto ME, Campos MM, Battastini AM, de Oliveira DL. Seizures induced by pentylenetetrazole in the adult zebrafish: a detailed behavioral characterization. PLoS One. 2013;8(1):e54515. (Master degree)
9. Rosemberg DB, Braga MM, Rico EP, Loss CM, Córdova SD, Mussulini BH, Blaser RE, Leite CE, Campos MM, Dias RD, Calcagnotto ME, de Oliveira DL, Souza DO. Behavioral effects of taurine pretreatment in zebrafish acutely exposed to ethanol. Neuropharmacology. 2012 Sep;63(4):613-23.
10. Leke R, de Oliveira DL, Mussulini BH, Pereira MS, Kazlauckas V, Mazzini G, Hartmann CR, Silveira TR, Simonsen M, Bak LK, Waagepetersen HS, Keiding S, Schousboe A, Portela LV. Impairment of the organization of locomotor and exploratory behaviors in bile duct-ligated rats. PLoS One. 2012;7(5):e36322.
11. da Cunha MJ, da Cunha AA, Ferreira AG, Machado FR, Schmitz F, Lima DD, Delwing D, Mussulini BH, Wofchuk S, Netto CA, Wyse AT. Physical exercise reverses glutamate uptake and oxidative stress effects of chronic homocysteine administration in the rat. Int J Dev Neurosci. 2012 Apr;30(2):69-74.
12. Ferreira AG, da Cunha AA, Scherer EB, Machado FR, da Cunha MJ, Braga A, Mussulini BH, Moreira JD, Wofchuk S, Souza DO, Wyse AT. Evidence that hyperprolinemia alters glutamatergic homeostasis in rat brain: neuroprotector effect of guanosine. Neurochem Res. 2012 Jan;37(1):205-13. 12.
13. Rosemberg DB, Rico EP, Mussulini BH, Piato AL, Calcagnotto ME, Bonan CD, Dias RD, Blaser RE, Souza DO, de Oliveira DL. Differences in spatio-temporal behavior of zebrafish in the open tank paradigm after a short-period confinement into dark and bright environments. PLoS One. 2011 May 2;6(5):e19397.
14. Machado FR, Ferreira AG, da Cunha AA, Tagliari B, Mussulini BH, Wofchuk S, Wyse AT. Homocysteine alters glutamate uptake and Na+,K+-ATPase activity and oxidative status in rats hippocampus: protection by vitamin C. Metab Brain Dis. 2011 Mar;26(1):61-7.
15. de Oliveira DL, Bavaresco C, Mussulini BH, Fischer A, Souza DO, Wyse AT, Wofchuk S. Early life LiCl-pilocarpine-induced status epilepticus reduces acutely hippocampal glutamate uptake and Na+/K+ ATPase activity. Brain Res. 2011 Jan 19;1369:167-72.
16. Rico EP, de Oliveira DL, Rosemberg DB, Mussulini BH, Bonan CD, Dias RD, Wofchuk S, Souza DO, Bogo MR. Expression and functional analysis of Na(+)-dependent glutamate transporters from zebrafish brain. Brain Res Bull. 2010 Mar 16;81(4-5):517-23.