UNIVERSIDADE FEDERAL DO PARÁ
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM FARMACOLOGIA E BIOQUÍMICA

JULIANNE ELBA CUNHA AZEVEDO

INTOXICAÇÃO POR CAFEÍNA: PADRÕES COMPORTAMENTAIS E ELETROCORTICOGRÁFICOS EM RATOS WISTAR

BELÉM-PA

2023

UNIVERSIDADE FEDERAL DO PARÁ
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM FARMACOLOGIA E BIOQUÍMICA

INTOXICAÇÃO POR CAFEÍNA: PADRÕES COMPORTAMENTAIS E ELETROCORTICOGRÁFICOS EM RATOS WISTAR

Autor (a): JULIANNE ELBA CUNHA AZEVEDO

Orientador (a): Prof. Dr. MOISÉS HAMOY

Documento de Dissertação apresentada ao Programa de Pós-graduação em Farmacologia e Bioquímica do Instituto de Ciências Biológicas da Universidade Federal do Pará como requisito para a obtenção do título de Mestre em Farmacologia e Bioquímica.

BELÉM-PA

2023

DEDICATÓRIA/AGRADECIMENTOS

Toda honra e glória seja dada ao Senhor Jesus, sem Ele nada conseguiria fazer, pois foi Ele que guiou meus passos e permitiu que tudo viesse acontecer. Dedico ao Senhor Jesus esse título de mestre, pois Ele é o Senhor da minha vida.

Agradeço a minha família, especialmente meu pai, José Ribamar Duarte Azevedo, minha mãe (in memoriam), Maria do Socorro Cunha Azevedo, pelo apoio, carinho e ensinamentos, minha mãe foi o exemplo de mulher para mim, pois me ensinou que nunca devemos desistir dos nossos objetivos e que temos que acreditar em nós mesmos sempre.

Ao meu orientador Moisés Hamoy por ter acreditado em mim e me dado à oportunidade de ser sua orientanda, mesmo com todas as dificuldades que ocorreram ao longo do caminho, o professor Moisés, com sua paciência e atenção, me ajudou a conquistar a primeira etapa para realizar meu sonho, ser professora universitária, obrigado pelos seus ensinamentos, orientações, dedicação e apoio.

À Vanessa Joia pelo apoio e auxílio que me forneceu no período final da publicação do artigo, naquelas tardes na Universidade onde nossas mentes “fervilhavam” para concluir as solicitações do periódico, sua ajuda foi fundamental.

RESUMO

A cafeína é uma substância psicoativa utilizada mundialmente. O presente estudo analisa o comportamento relacionado à convulsão e os padrões eletrocorticográficos (ECoG) observados em ratos após uma dose tóxica de cafeína (150 mg/kg; intraperitoneal). Sessenta e três ratos foram divididos em três experimentos: 1- Descrição do comportamento associado à convulsão induzida por cafeína; 2- Comparação dos padrões eletrocorticográficos induzidos por cafeína e pentilenotetrazol e 3- Avaliação da resposta eletrocorticográfica às drogas antiepilépticas (diazepam, fenitoína e fenobarbital). A análise comportamental demonstrou convulsões tônico-clônicas com perda do reflexo postural e latência de 365,8 s após a administração da cafeína. As alterações induzidas pela cafeína no ECoG foram consistentes com o desenvolvimento de convulsões com evolução rápida e potencial de explosão consistente com os padrões comportamentais observados durante a convulsão induzida por cafeína. O ECoG das ondas cerebrais variou significativamente entre as convulsões causadas por cafeína e pentilenotetrazol. As forças cerebrais predominantes observadas durante as convulsões foram as oscilações da banda beta. As convulsões induzidas por cafeína foram resistentes à tentativa de controle com fenitoína e fenobarbital, mas responderam bem ao diazepam, o que é consistente com um estudo de pilocarpina, que mostrou que o diazepam tem efeitos anticonvulsivantes. Esses achados são importantes para o desenvolvimento de tratamentos eficazes para a intoxicação por cafeína, em particular para indivíduos com baixo limiar convulsivo.

Palavras-chave: Cafeína, convulsões, eletrocorticografia, intoxicação, toxicologia.

ABSTRACT

Caffeine is a psychoactive substance used worldwide. The present study analyzes the seizure-related behavior and electrocorticographic (ECoG) patterns observed in rats following of a toxic dose of caffeine (150 mg/kg; intraperitoneal). Sixty-three rats were divided into three experiments: 1-Behavior's Description associated with caffeine-induced convulsion; 2- Comparison of the electrocorticographic patterns induced by caffeine and pentylenetetrazole, and 3- Assessment of the electrocorticographic response to antiepileptic drugs (diazepam, phenytoin, and phenobarbital). The behavioral analysis demonstrated tonic-clonic seizures with a loss of postural reflex and a latency of 365.8 s after the caffeine's administration. Caffeine-induced changes in the ECoG were consistent with the development of seizures with rapid evolution and burst potential consistent with the behavioral patterns observed during the caffeine-induced seizure. The ECoG of the brainwaves varied significantly between the seizures caused by caffeine and pentylenetetrazole. The predominant brain forces observed during the seizures were beta-band oscillations. The caffeine-induced seizures were resistant to attempted control with phenytoin and phenobarbital, but responded well to diazepam, which is consistent with a study of Pilocarpine, which showed that diazepam has anticonvulsant effects. These findings are important for the development of effective treatments for caffeine intoxication, in particular for individuals with a low seizure threshold.

Keywords: Caffeine, seizures, electrocorticography, intoxication, toxicology.

LISTA DE ILUSTRAÇÕES

LISTA DE ABREVIATURAS E SIGLAS

- **SNC** - sistema nervoso central
- **mg -** miligrama
- **Kg -** quilograma
- **ECoG –** eletrocorticográfico
- **LPS -** lipopolissacarídeo
- **iv. -** intravenoso
- **EEG -** eletroencefalográfico
- **PTZ -** pentilenotretazol
- **UFPA -** Universidade federal do Pará
- **CONCEA -** Conselho nacional de controle de experimentação animal
- **CEPAE** - Comitê de ética em pesquisa com uso de animais
- **n. -** número
- **ip. -** intraperitoneal
- **DZP -** diazepam
- **PBT -** fernobabital
- **PHT -** fenitoína
- **SP -** São Paulo
- **MG -** Minas Gerais
- **EUA -** Estado Unidos da América
- **mm -** milímetros
- **DP -** desvio-padrão
- **PSD -** densidade espectral de potência

SUMÁRIO

1 VISÃO INTEGRADORA DO PROBLEMA

A estrutura molecular da cafeína pertence a um grupo de xantinas trimetiladas que incluem seus compostos intimamente relacionados: teobromina (presente no cacau) e teofilina (presente no chá). (TAVARES; SAKATA, 2012)

As xantinas têm em comum uma série de propriedades, em particular a capacidade de relaxar a musculatura lisa; estimular o sistema nervoso central e promover diurese. (PAUWELS et al 2001)

A cafeína é uma substância psicoativa muito utilizada em todo o mundo, com efeitos em inúmeras funções fisiológicas, incluindo resistência física, humor, sono e dor. Nos Estados Unidos, por exemplo, 85% da população adulta tem uma ingestão média diária de 135 mg de cafeína (chegando a 188 mg/dia em indivíduos de 35 a 49 anos), este composto, pode ser encontrado em cafés, chás, energéticos, refrigerantes diversos e cacau (ULLRICH et al., 2015; PONG et al., 2015). A quantidade de cafeína presente em uma xícara de café, uma das principais fontes, geralmente está entre 47 e 134mg, porém, pode variar de acordo com a espécie de planta, tipo de grão, localização geográfica e práticas culturais. (FREDHOLM et al, 1999)

Após a ingestão oral a cafeína é completamente absorvida e amplamente distribuída, atravessando facilmente barreiras tão apertadas quanto a barreira hematoencefálica. No cérebro, a cafeína ativa a liberação de transmissores principalmente excitatórios e atua como um antagonista não seletivo da adenosina para os receptores A1 e A2. (FISONE et al., 2004) Ambos os ligantes, cafeína e adenosina, apresentam alta similaridade de suas estruturas químicas. (HUANG et al., 2005) Eles podem afetar a liberação de neurotransmissores como a acetilcolina, dopamina, noradrenalina, ácido gama-aminobutírico e serotonina, o que melhora o humor, (ZHANG, 2001) estimula o organismo, melhora a concentração e elimina a fadiga física. (SMITH, 2002)

Nos últimos anos, o risco de intoxicação por cafeína aumentou devido à maior disponibilidade de analgésicos, medicamentos estimulantes do sistema nervoso central (SNC) e suplementos alimentares em lojas de varejo, lojas de alimentos saudáveis e mercados eletrônicos. (CAPPELLETTI et al., 2018)

Entre os efeitos colaterais da cafeína estão inclusos: cefaleia, insônia, taquicardia, sintomas dispépticos, relaxamento do músculo liso e estimulação do

músculo cardíaco, que ocorrem em baixas doses (cerca de 200mg para adultos). No entanto, doses elevadas, superiores a 400mg/dia parecem conduzir a sintomas de ansiedade, náuseas, irritabilidade e nervosismo, a dose oral letal para adultos foi estimada em cerca de 10g (150 a 200mg/kg) por pessoa, em crianças, a ingestão de 35mg/kg é capaz de levar à toxicidade moderada. (ALTERMANN et al, 2008; KENT, 2014).

Embora a afinidade da cafeína para os receptores de adenosina independa da idade, a suscetibilidade à ação da cafeína é maior em crianças e adolescentes, pois existem diferenças quanto à eliminação dessa substância do organismo, uma vez que a principal enzima responsável pela metabolização da cafeína, CYP1A2, possui uma atividade diferente com a idade. Os adolescentes são mais sensíveis à toxicidade por consumo excessivo de cafeína, uma vez que o metabolismo da cafeína diminui durante essa fase devido à elevada progressão natural do hormônio do crescimento, que aumenta o risco de efeitos tóxicos de cafeína nesta população. (TEMPLE et al, 2009) Outros estudos apontam, no entanto, o contrário, sugerindo que crianças sejam menos sensíveis à cafeína em relação aos adultos, exceto em altas doses. Um estudo feito com seis jovens e seis idosos revelou maior sensibilidade nos idosos, que manifestaram mais efeitos que as crianças. (NEHLING et al, 1992)

Ainda com relação à sensibilidade à cafeína, foram observadas algumas diferenças entre os efeitos da cafeína entre homens e mulheres, sugerindo-se a existência de 41 distúrbios do sono mais acentuados nas mulheres do que nos homens, associados aos efeitos estimulantes do consumo de café. (GASPAR, 2014)

Estudos em humanos e em animais sugerem que a cafeína pode induzir convulsão quando em doses tóxicas ou após períodos prolongados de ingestão, em qualquer indivíduo, mas principalmente àqueles com baixo limiar de convulsão. (KALFMAN et al. 2003; VAN KOERT et al, 2018)

Tratando-se de crises convulsivas provocadas por cafeína, deve-se considerar que a ação desta substância sobre neurotransmissores e sua influência na atividade cerebral influenciam na eficácia de determinados fármacos.

Os resultados indicam que a cafeína, em uma dose relativamente alta de 200mg/kg, reduziu a potência protetora do fenitoína, fenobarbital e diazepam,

enquanto a potência do VPA permaneceu inalterada (CHROSCINSKA-KRAWCZYK, et al 2011; KULKARNI, et al 1991)

Dada a significativa presença dessa substância no cotidiano dos indivíduos, muitos estudos já expuseram alguns dos seus possíveis efeitos colaterais. Dentre esses, a cafeína como indutora de episódios convulsivos é um dos mais graves desfechos possíveis sugeridos, e um dos menos explorados. Apesar da importância, a literatura conta com limitado conteúdo sobre o tema, então, estudos como este são necessários para aprofundar o conhecimento e aprimorar a conduta terapêutica frente a convulsões induzidas por cafeína.

Nesse contexto, o presente estudo analisou o comportamento relacionado à convulsão e os padrões eletrocorticográficos (ECoG) em ratos Wistar após a administração de uma dose tóxica de cafeína, como também avaliou a resposta a drogas antiepilépticas clássicas (diazepam, fenitoína e fenobarbital) após convulsão induzida por cafeína.

1.1 Efeitos da cafeína na atividade elétrica cerebral

A cafeína exerce grande potencial sobre o SNC, de modo a estimular principalmente o córtex, aumentando a vigilância e diminuindo a sensação de cansaço. Estudos eletrofisiológicos em ratos mostraram que a atividade elétrica é estimulada pela administração intravenosa de 100mg/kg de cafeína. Em gatos, uma dose de 10mg/kg de cafeína produz uma ativação elétrica cortical semelhante à atividade registrada durante o despertar fisiológico ou à uma atividade produzida pela estimulação direta da formação reticular, uma estrutura que desempenha um importante papel na vigilância e no despertar. (NEHLING et al, 1992)

Vários mecanismos foram recomendados para esclarecer os efeitos neuroprotetores da cafeína, abrangendo a modulação da excitotoxicidade glutamatérgica e neuroinflamação via receptores de adenosina (CHEN et al., 2013; REN; CHEN, 2020). A cafeína pode promover uma ação anti-neuroinflamatória em várias condições patológicas. O fornecimento de cafeína (injeção intraperitoneal diária) diminui a ativação da microglia induzida por lipopolissacarídeo (LPS) em três regiões do hipocampo, de maneira dependente da dose (BROTHERS et al., 2010; REN; CHEN, 2020) e anular a neuroinflamação promovida por LPS e a disfunção sináptica em cérebros de camundongos adultos (REN; CHEN, 2020)

As metilxantinas também são capazes de elevar a excitabilidade observada in vitro no hipocampo de ratos e ativar o ritmo theta do eletroencefalograma no hipocampo de coelhos. Foi observado, ainda, que altas doses de cafeína provocam modificações elétricas no hipocampo semelhantes às que são registradas no eletroencefalograma durante crises generalizadas. O potencial efeito estimulante que a cafeína exerce sobre o hipocampo mostra a importância do sistema límbico no desenvolvimento dos efeitos convulsivos e ansiogênicos das metilxantinas. (POPOLI et al, 1987)

Ao mesmo tempo em que a cafeína aumenta a atividade elétrica cortical, induz uma diminuição significativa da atividade elétrica em neurônios talâmicos, mesmo em pequenas doses (0,1mg/kg i.v). Da mesma forma, a administração iontoforética direta (técnica de transferência de drogas mediada por fluxo elétrico) de cafeína diminui a atividade elétrica espontânea de neurônios no núcleo caudado do rato, uma vez que a metilxantina parece capaz de ativar a via nigroestriatal estimulando a liberação de dopamina pelas terminações nervosas nigroestriatais. (NEHLING et al, 1992)

1.2 Cafeína e eletrocorticograma

O conceito de Eletroencefalograma (EEG), eletro-(referente ao registro de sinais elétricos vindos do cérebro) encéfalo-(referente a sinais emitidos da cabeça) e grama (ou grafia que significa escrita ou desenho) surgiram com Richard Caton (1842 − 1926), cientista Inglês que fez a observação do primeiro sinal elétrico proveniente do escalpo humano em 1875. (SANEI; CHAMBERS, 2007; SILVA, 2005; SILVA, 2017)

O registro eletroencefalográfico (EEG) é uma ferramenta alternativa utilizada para avaliar a atividade elétrica cerebral por meio da soma dos potenciais pós-sinápticos gerados por células piramidais no córtex cerebral geradas pelo fluxo de íons. (BUZSÁKI, et al., 2012) Com isso, se apresenta como uma técnica eficiente para medir o distúrbio da atividade elétrica em modelos animais. (VAN SON et al., 2018; VOICULESCU et al., 2015)

Atualmente, EEGs são realizados tanto de forma invasiva quanto não invasiva, com diversas aplicações e diferentes equipamentos, que possuem ferramentas de processamento de sinais no estado da arte, eletrodos específicos

para as tarefas necessárias e capacidade de armazenamento para realizar exames de longa duração. (SILVA, 2005; SANEI; CHAMBERS, 2007; SILVA, 2017)

Dentre as técnicas para medição da atividade cerebral há a técnica de registro eletrocorticográfico que é realizada com o posicionamento de sensores na superfície do córtex. A atividade registrada é proveniente de populações de neurônios próximos aos eletrodos. Esta técnica de registro combina alta resolução temporal e espacial. No entanto, é uma técnica invasiva, com a necessidade de procedimentos cirúrgicos.

Um estudo feito em ratos avaliou registros eletrocorticográficos após o consumo de cafeína e observou um aumento da amplitude de todas as ondas cerebrais sugerindo que a cafeína ou qualquer de seus metabólitos pode afetar direta ou indiretamente as vias envolvidas na geração desses ritmos, podendo instigar as funções cognitivas associadas às oscilações cerebrais legitimando o fato de que a cafeína age como um estimulante do sistema nervoso central. (FERRÉ, 2016; CABRAL et al, 2021)

1.3 Cafeína e convulsões

Pesquisas mostram que o consumo crônico de cafeína pode ocasionar em dependência física e tolerância aos seus efeitos centrais. (NEHLIG et al. 1992; BERNSTEIN et al. 2002; CHROSCINSKA-KRAWCZYK et al 2011) Dentre as complicações da overdose a mais perigosa é a atividade convulsiva. Observou-se em estudos que a cafeína pode diminuir o limiar convulsivo em modelos experimentais de epilepsia ou induzir convulsões em roedores quando fornecida em doses acima de 400mg/kg. (CUTRUFO et al. 1992; CZUCZWAR et al. 1990; CHROSCINSKA-KRAWCZYK et al 2011)

Estudo com animais demonstraram que a cafeína influencia a excitabilidade cerebral e desencadeia convulsões seguidas por encefalopatia em ratos. Em um estudo, várias doses de cafeína foram administradas durante observações comportamentais e registros de eletroencefalografia (EEG). Doses de 100 e 200 mg/kg de cafeína causaram dilatação pupilar, inquietação e reflexos intermitentes, enquanto no EEG, foram observadas rajadas de pontas ou ondas agudas, semelhantes ao padrão EEG visto durante crises epilépticas. Em doses acima de 150 mg/kg, ocorreram crises generalizadas; doses de 300 mg/kg e acima, evoluíram

para status epileticus fatal nos ratos; o mesmo fenômeno é visto em gatos, cães, porquinhos-da-índia, ratos e coelhos (VAN KOERT et al, 2018)

Em outro estudo, foi evidenciada a maior suscetibilidade a convulsões associada à cafeína, ratos pré-tratados com cafeína (100mg/kg) foram, em seguida, tratados com pentilenotetrazol (PTZ) (30mg/kg), que é um modelo animal de crises focais, crises generalizadas tônico-clônicas e crises de ausência. Comparado com os controles (animais tratados somente com PTZ), o grupo cuja cafeína foi administrada teve mais convulsões e um pico contínuo nas gravações de EEG. Crises severas foram observadas em 12/15 ratos no grupo cafeína, mas não nos controles. (CUTRUFO et al, 1992)

Uma pesquisa feita com anticonvulsivantes e cafeína, demonstrou que a mesma apresentou efeitos convulsivantes após a administração do pentilenotetrazol a 20 mg/kg. (TCHEKALAROVA et al, 2009; CHROSCINSKA-KRAWCZYK et al, 2011) Outro estudo buscou analisar os efeitos epileptogênicos da cafeína e outros metilxantinas em fatias de hipocampo e corroboraram que a ação específica das metilxantinas está correlacionado com suas afinidades com receptores de adenosina. (MORAIDIS; BINGMAN, 1994; CHROSCINSKA-KRAWCZYK et al 2011)

Em alguns estudos, cujo objetivo era observar a resposta a anticonvulsivantes em crises convulsivas causadas pela cafeína, ratos foram tratados com cafeína e com uma das seguintes drogas: carbamazepina, fenitoína, fenobarbital, ácido valpróico, felbamato, oxcarbazepina, lamotrigina, tiagabina, gabapentina e topiramato. Observou-se que injeções de cafeína aumentaram significativamente a quantidade de fenobarbital, carbamazepina, fenitoína, topiramato, gabapentina, ácido valpróico e felbamato necessários para proteger 50% dos ratos contra convulsões, enquanto nenhuma alteração foi observada com oxcarbazepina, lamotrigina e tiagabina. (VAN KOERT et al, 2018).

1.4 Objetivos

1.4.1GERAL

Estudar os efeitos da cafeína sobre o sistema nervoso central para a caracterização de padrão convulsivo.

1.4.2 ESPECÍFICOS

- Caracterizar alterações comportamentais de animais em convulsão ocasionada pela aplicação intraperitoneal de uma dose tóxica de cafeína.
- Caracterizar registros eletrocorticográficos de animais em convulsão ocasionada pela aplicação intraperitoneal de cafeína, analisando as forças cerebrais (delta, theta, alfa, beta e gama).
- Avaliar a eficácia de drogas anticonvulsivantes no controle de crises convulsivas causadas pela cafeína.

2. ARTIGO: INTOXICAÇÃO POR CAFEÍNA: PADRÕES COMPORTAMENTAIS E ELETROCORTICOGRÁFICOS EM RATOS WISTAR

Julianne Elba Cunha Azevedo [1*], Alex Luiz Menezes da Silva [1], Luana Rodrigues Vieira [1], Chirlene Pinheiro Nascimento [1], Rafaela Garcia Pereira[1], Sofia de França Rodrigues[1], Akira Otake Hamoy[1], Vanessa Joia de Mello[1], Daniella Bastos de Araújo [1], Luiz André Luz Barbas [2], Maria Elena Crespo Lopez [3], Dielly Catrina Favacho Lopes [4], Moisés Hamoy[1]

[1] Laboratory of Pharmacology and Toxicology of Natural Products, Institute of Biological Sciences, Federal University of Pará, UFPA, Belém, Pará, Brazil

[2] Tropical Species Aquaculture Laboratory, Federal Institute of Education Science and Technology of Pará - Campus Castanhal, Castanhal, PA, Brazil

[3] Molecular Pharmacology Laboratory. Federal University of Pará, UFPA, Belém, Pará, Brazil

[4] Experimental Neuropathology Laboratory, Institute of Biological Sciences, Federal University of Pará

Belem, Pará, Brazil

*Corresponding author

Julianne Elba Cunha Azevedo, Laboratory of Pharmacology and Toxicology of Natural Products, Institute of Biological Sciences Federal University of Pará, Belém, Pará, Brazil, Guamá, Belém - PA, 66075-110 – Brazil. Email: julianne.azevedo@ics.ufpa.br Tel: +55 (91) 3201-8064.

Abstract

Caffeine is a psychoactive substance used worldwide. The present study analyzes the seizure-related behavior and electrocorticographic (ECoG) patterns observed in Wistar rats following the administration of a toxic dose of caffeine (150 mg/kg; intraperitoneal). Sixty-three rats were divided into three experiments: 1- Description of the behavior associated with caffeine-induced convulsion; 2- Comparison of the electrocorticographic patterns induced by caffeine and pentylenetetrazole (PTZ), and 3- Assessment of the electrocorticographic response to antiepileptic drugs (diazepam, phenytoin, and phenobarbital). The behavioral analysis demonstrated tonic-clonic seizures with a loss of postural reflex and a latency of 365.8 seconds after the administration of the caffeine. Caffeine-induced changes in the ECoG were consistent with the development of seizures with rapid evolution and burst potential consistent with the behavioral patterns observed during the caffeine-induced seizure. The ECoG of the brainwaves varied significantly between the seizures caused by caffeine and PTZ. The predominant brain forces observed during the seizures were beta-band oscillations. The caffeine-induced seizures were resistant to attempted control with phenytoin and phenobarbital, but responded well to diazepam, which is consistent with a study of Pilocarpine, which showed that diazepam has anticonvulsant effects. These findings are important for the development of effective treatments for caffeine intoxication, in particular for individuals with a low seizure threshold.

Keywords: caffeine, seizures, electrocorticography, intoxication, toxicology.

2.2 Introduction

Caffeine, a purine-like molecule (1,3,7-trimethylxanthine), is the most commonly-used psychostimulant in Western countries (Ullrich et al., 2015; Xu et al., 2015). In the United States, for example, 85% of the adult population has an average daily intake of 135 mg of caffeine (reaching 188 mg/day in the 35-49 age group). Caffeine can be found in coffee, tea, energy drinks, soft drinks, and cocoa (Pong et al., 2015; Ullrich et al., 2015). Xanthine like caffeine share a number of properties, including the ability to relax smooth muscles, stimulate the central nervous system, and promote diuresis (Bueno, 2003; Pauwels et al., 2001). However, high caffeine intake can have

a range of side effects, and a general limit of 400 mg of caffeine per day is recommended for adults, given personal variations in metabolism and sensitivity to this compound (Dam et al., 2020).

When ingested orally, caffeine is absorbed completely and distributed widely, crossing even the tightly-closed blood-brain barrier with ease. In the brain, caffeine activates primarily the release of excitatory transmitters and acts as a non-selective adenosine antagonist for the A1 and A2 receptors (Fisone et al., 2004). Both these ligands – caffeine and adenosine – are highly similar in their chemical structure (Huang et al., 2005). They may affect the release of neurotransmitters such as acetylcholine, dopamine, noradrenaline, gamma-aminobutyric acid, and serotonin, which improves mood (Zhang, 2001), stimulates the body, improves concentration, and eliminates physical fatigue (Smith, 2002).

In recent years, the risk of caffeine intoxication has increased dramatically due to the inclusion of this substance in a growing number of medications, such as pain relievers and stimulants of the Central Nervous System, and dietary supplements, which are readily available from retailers, including online markets (Cappelletti et al., 2018).

A number of studies have shown that caffeine may trigger seizures in both normal and epileptic individuals after the ingestion of a toxic dose (Banner and Czijka., 1980; Mueller and Solow., 1982; Cohen et al., 1992; Paech., 1996; Iyadurai and Chung., 2007; Röggla and Moser., 2007; Babu et al., 2011) or prolonged periods of intake (Kaufman and Sachdeo., 2003; Bonilha and Li., 2004 Maiga et al., 2011; Mackow et al., 2016). This occurs in particular in individuals that have a low seizure threshold (Kalfman et al., 2003; Van Koert et al., 2018).

The dangers of caffeine are related to its ample diffusion, which results in a high, partially-conscious consumption pattern, due to the difficulty of determining the amount ingested. This results in an inability to predict specific effects in terms of the processes triggered by caffeine – even at a “safe” dose – which may mask underlying cardiovascular conditions (Cappelletti et al., 2018).

High doses, of over 400 mg/day, appear to induce anxiety, nausea, irritability, and nervousness. The lethal oral dose for an adult has been estimated to be 10 g (approximately 150–200 mg/kg). In children, the ingestion of 35 mg/kg can lead to moderate toxicity (Altermann et al., 2008).

Electroencephalographic recordings (EEG) are an alternative tool for the assessment of the electrical activity of the brain through the sum of the postsynaptic potentials generated by the pyramidal cells of the cerebral cortex through ionic flow (Buzáki et al., 2012). This technique has proven to be a highly effective procedure for the measurement of the disturbance of electrical activity in animal models (Van Son et al., 2018; Voiculescu et al., 2015).

In this context, the present study analyzed seizure-related behavior and electrocorticographic (ECoG) patterns in Wistar rats following the administration of a toxic dose of caffeine. It also evaluates the response to classical antiepileptic drugs (diazepam, phenytoin and phenobarbital) following caffeine-induced seizure.

2.2. Materials and Methods

2.2.1 ANIMALS

Sixty-three adult male Wistar rats (250–280 g) were obtained from the Central Animal Facility of the Federal University of Pará (UFPA) and kept in the experimental vivarium of the UFPA Laboratory of Pharmacology and Toxicology of Natural Products. The animals were housed at a controlled temperature of 23–25ºC with a 12-h light-dark cycle, and *ad libitum* access to food and water.

The present study was conducted in accordance with the precepts of the Brazilian legislation for animal experimentation and the ethical principles of the National Council for the Control of Animal Experimentation (CONCEA, Brazil). Prior to initiating the study, it was approved by the UFPA Committee on Ethics in Research on the Use of Animals (CEPAE-UFPA: # 4662260819).

2.2.2 EXPERIMENTAL DESIGN

The present study was based on three separate experiments, with n = 9 animals per experimental group. The first experiment described the convulsive behavior induced by a toxic dose of caffeine (150 mg/kg; intraperitoneal [ip]), including the latency of the onset of the convulsions, in this experiment will be identified morphographic elements that was repeated in the records during the convulsions caused by caffeine. The second experiment recorded the electrocorticographic (ECoG) patterns of the rats following the administration of caffeine and compared the results with the ECoGs of rats that received pentylenetetrazole (PTZ, positive control of induced seizure, 60

mg/kg, ip) and a control group (saline solution in volume equivalent to the dose of caffeine applied). Each of these groups consisting of nine animals, which underwent surgery for the implantation of the electrodes.

In the third experiment, the animals were also divided into three groups of nine animals, in which electrodes were implanted surgically. Each group was treated with a different anticonvulsant drug, 60 seconds after the administration of the caffeine, either (a) diazepam (DZP, 10 mg/kg, intravenous [iv]), (b) phenobarbital (PBT, 10 mg/kg, iv), or (c) phenytoin (PHT, 10 mg/kg, iv). These doses are within the therapeutic range, and each group received only one of the drugs prior to the collection of the ECoG recordings.

Overall, 63 animals were used in the present study, considering that each experimental group consisted of nine animals. Experiment 1 had only a single group (n = 9 animals), whereas experiments 2 and 3 each involved three groups nine animals, with a total of 27 animals in each case.

2.2.3 CHEMICALS

Ketamine hydrochloride (König laboratory, Santana de Parnaíba-SP, Brazil), xylazine hydrochloride (Vallée laboratory, Montes Claros-MG, Brazil), and lidocaine (Hipolabor laboratory, Sabará-MG, Brazil) were used for the surgical implantation of the electrodes. Caffeine and pentylenetetrazole were obtained from Sigma (USA). Phenobarbital (Aventis-Pharma, Ribeirão Preto-SP, Brazil), phenytoin, and diazepam (União Química, Embu-Guaçu-SP, Brazil) were selected as the anticonvulsant drugs.

2.2.4 DESCRIPTION OF THE SEIZURE-RELATED BEHAVIOR

The behavior associated with the caffeine-induced seizure was described based on the observation of animals that received a 150 mg/kg dose of caffeine intraperitoneally (ip). After the administration of the caffeine, the latency of the onset of each type of convulsive behavior was recorded in the following sequence: (a) Erection of the vibrissae and retraction of the pin; (b) Immobility with deep and rhythmic breathing; (c) Excitability and motor incoordination; (d) Tonic spasms of the forelimbs; (e) Tonic-clonic seizures with transient loss of the postural reflex, and (f) Tonic-clonic seizures with a loss of the postural reflex. The convulsive condition evolved rapidly, which justifies the adoption of a 10-minute sampling interval for this

experiment. The data were pooled for the nine animals of there groups, for the presentation of the mean and standard deviation the latency of each behavior.

2.2.5 ELECTROCORTICOGRAPHY AND DATA ANALYSIS

The electrodes were implanted surgically at bregma coordinate -0.96, 1 mm to the side of each hemisphere represented by the motor cortex (Paxinos 2005). Five days after surgery, the electrodes were connected to a data acquisition system consisting of a high-impedance amplifier (Grass Technologies, P511), monitored by an oscilloscope (Protek, 6510). The data were digitized continuously at a rate of 1 KHz by a computer equipped with a data acquisition board (National Instruments, Austin, TX), and stored on a hard disk for processing using specialized software (LabVIEW express). The recording electrode was located on the right side of the hemisphere and the reference electrode on the left side (Estumano et al., 2019). All experimental procedures were conducted in a Faraday cage.

A tool was constructed using the Python programming language (version 2.7) for the analysis of the signals obtained during the experiments. The Numpy and Scipy libraries were used for mathematical processing and the Matplolib library for graphics. The graphical interface was developed using the PyQt4 library.

The records were analyzed at frequencied of up to 50 Hz. The bands were analyzed following Jalilifar et al. (2017) and Hamoy et al. (2018) , who classify the bands as Delta (1–4 Hz), Theta (4–8 Hz), Alpha (8–12 Hz), Beta (12–28), and Gamma, at 28–40 Hz (Hamoy et al., 2018; De Melo et al., 2020, Oliveira et al., 2020).

2.2.6 STATISTICAL ANALYSIS

The results are presented as the mean value ± standard deviation (SD). The normality of the variances was verified by the Kolmogorov-Smirnov test and the homogeneity by the Levene test. The groups were compared using a one-way Analysis of Variance (ANOVA), followed by Tukey's *post hoc* test, whenever appropriate. All the analyses were run in GraphPad® Prism 6, and considered a $p < 0.05$ significance level.

2.3. Results

2.3.1 BEHAVIORAL FEATURES OF CAFFEINE-INDUCED SEIZURES

The behavioral test determined the latency of the evolution of seizures following the administration of caffeine. A rapid evolution to the most intense seizure was observed. It is important to note here that, while the latency refers to the initial onset of the seizure (presented here as a mean value for the individual, with its respective variation), the behavior may be repeated during subsequent seizures. The first manifestation of a change in behavior is the erection of the vibrissae and retraction of the pinnae (ears), with a mean latency of 40.78 ± 10.12 seconds, followed by immobility with deep and rhythmic breathing (mean latency = 104.6 ± 11.93 seconds). The subsequent behavioral change is the onset of motor excitability and incoordination (mean latency = 149.2 ± 19.39 seconds), followed by tonic spasms of the forelimbs (mean latency = 179.6 ± 15.35 seconds), which are a focal seizure with secondary generalization. These shifts are followed by tonic-clonic seizure with a transient loss of the postural reflex (230.1 ± 28.68 seconds) and tonic-clonic seizure with the loss of the postural reflex, with a mean latency of 365.8 ± 33.93 seconds. As shown below, electrocorticographic changes were also triggered by the caffeine, with their onset coinciding with the latency of the different behavioral components, as presented here.

2.3.2 ELECTROCORTICOGRAPHY OF THE CONTROL GROUP

The electrocorticographic records of the control group revealed higher power at low frequencies (0–10 Hz), that is, the delta (1–4 Hz), theta (4–8 Hz), and alpha (8–12 Hz) brainwaves, with reduced amplitude variation. This indicates the synchronization of the brain between the capture of the signal by the reference electrode (left hemisphere) and the recording electrode, in the right hemisphere (Figure 1A-D).

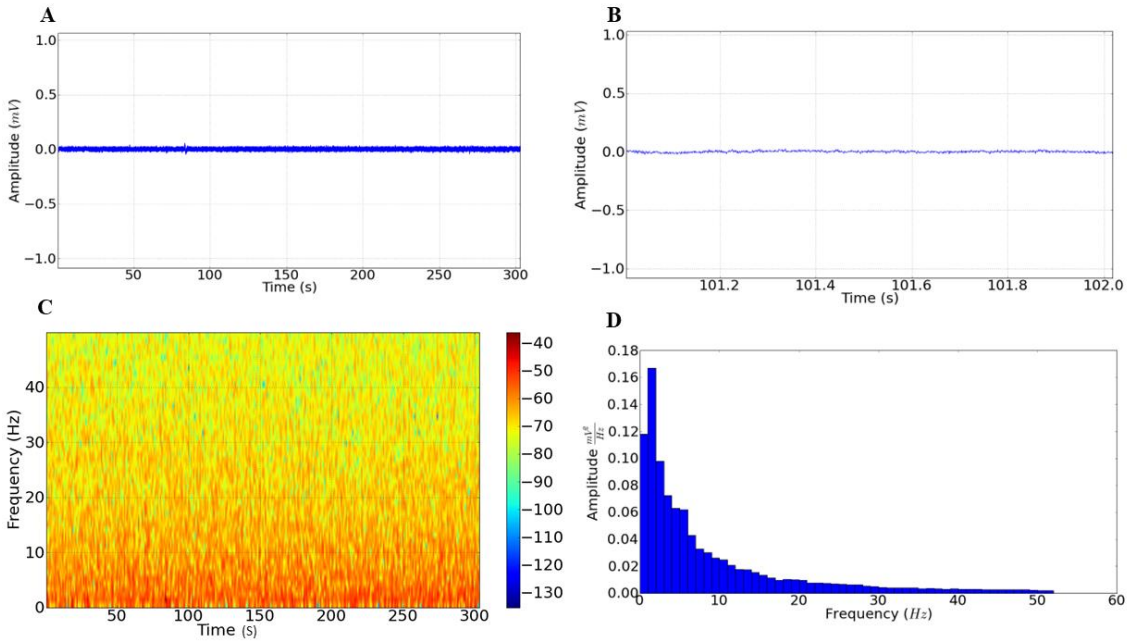

Figure 1. Electrocorticographic records of the control animals (*n* = 9) obtained during the present study: (A) Electrocorticographic trace (5 minutes) obtained from the right hemisphere, using the left hemisphere as a reference. (B) Baseline magnification over 1 second. (C) Frequency spectrogram showing higher signal power density at low frequencies (1–10 Hz). (D) Frequency histogram of one of the individuals of there control group, showing the concentration of power between 1 Hz and 10 Hz.

2.3.3 THE ECOG TRACES OF CAFFEINE-INDUCED SEIZURES HAVE THREE REPEATED PATTERNS

The caffeine-induced shifts in the ECoG were compatible with the observed development of the seizures, including their rapid evolution and the potential for bursts of increased amplitude, shown by the red arrow (Figure 2A). The timing of the ECoG responses is consistent with that of the behavioral shifts observed during the observation of the caffeine-induced seizure, including increased excitability and tonic forelimb spasms (observed at approximately 180 seconds). Caffeine-induced seizures increased brain wave oscillations, in particular in the 0–50 Hz frequency range (Figure 2B-C).

The ECoG trace of the caffeine group presented repeated patterns that are identified here as SP1, SP2 and SP3 (Figure 2D-F), which have amplitudes varying periods, but are invariably repeated. Each observed pattern contributed to the potency observed during caffeine-induced seizures.

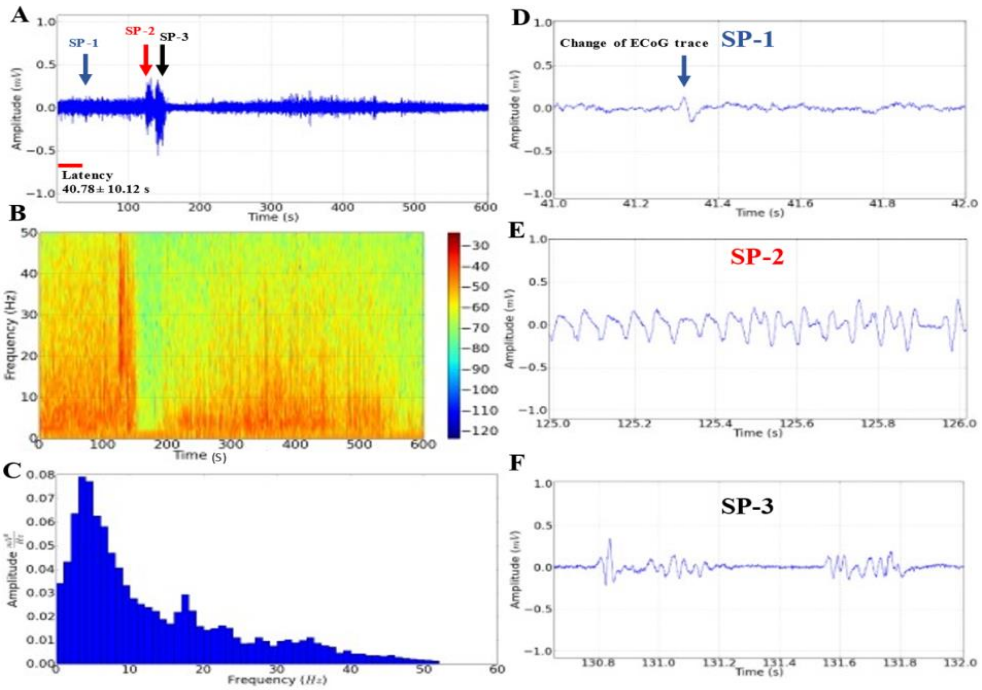

Figure 2. Electrocorticographic records of caffeine-induced seizures in Wistar rats, with a duration of 10 minutes (*n* = 9): (A) ECoG trace showing the identification points for SP1 (blue arrow), SP2 (red arrow), SP3 (black arrow), and the general latency (red line) of the seizure; (B) Frequency spectrogram following the administration of the caffeine; (C) Frequency histogram of an individual of the caffeine group showing the concentration of power between 1 Hz and 50 Hz (total of 600 seconds); the ECoG trace patterns recorded following the administration of caffeine in (D) SP1 (the blue arrow indicates the shift in the trace following the administration of caffeine), (E) SP2; (F) SP3.

2.3.4 CHANGES IN THE ECOG PROFILE CAUSED BY PENTYLENETETRAZOLE- AND CAFFEINE-INDUCED SEIZURES

Caffeine-induced changes in the ECoG trace were compared with the model of seizures induced by pentylenetetrazole, or PTZ (Figure 3), which shows changes in the ECoG trace and amplitude oscillations consistent consistent with seizure (Figure 3A, B). The frequency spectrogram indicates an increase in the power distribution at frequencies of 1–50 Hz (Figure 3C), while the histogram of amplitudes (Figure 3D) indicates a peak at 3–8 Hz. These patterns were quite distinct from those observed in the caffeine-induced seizures (Figure 2).

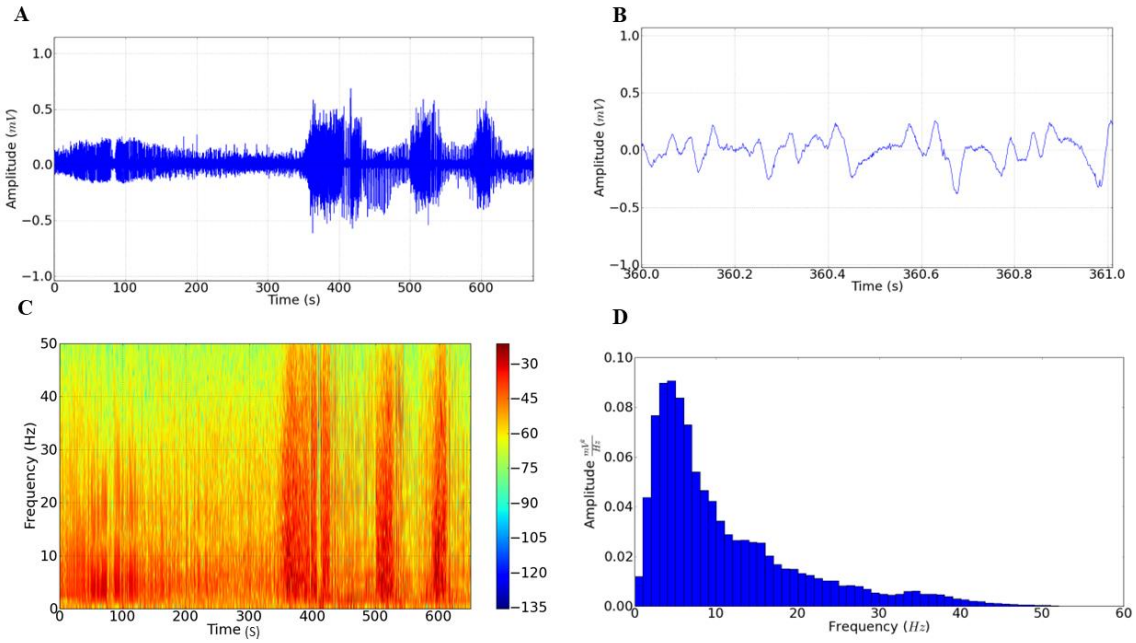

Figure 3 – Electrocorticographic records (*n* = 9) of: (A) the EcoG signal following seizure induced by Pentylenetetrazole (PTZ), over a 10-minute sample; (B) Magnification of the PTZ-altered ECoG trace at 1 second; (C) Frequency spectrogram following the PTZ-induced seizure; (D) Histogram (one column per Hz) of the power concentration (1–50 Hz) of an individual of the PTZ group.

The spectral decomposition of the ECoG records of the delta, theta, alpha, beta, and gamma waves reveals more evident differences in PTZ-induced seizures (Figure 4A) in comparison with the caffeine-induced seizures. At frequencies of 1–40 Hz (Figure 4B), the control group had a mean power of 0.04663 ± 0.01435 $mV^2/Hz \times 10^{-3}$, which was significantly lower ($F_{(2, 24)} = 95.32$, $p < 0.0001$) than either the PTZ (1.732 ± 0.3311 $mV^2/Hz \times 10^{-3}$) or the caffeine group (0.8064 ± 0.3034 $mV^2/Hz \times 10^{-3}$). This demonstrates a significant increase in the potency of the ECoG of both PTZ and caffeine (Figure 4B), in comparison with the control group.

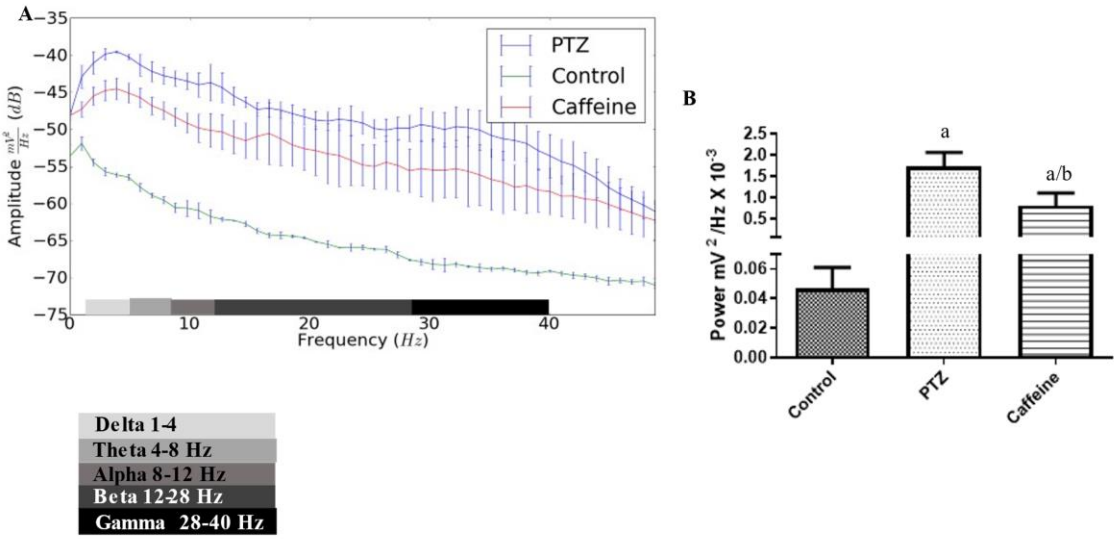

Figure 4. (A) Power Spectral Density (PSD) following the administration of caffeine and pentylenetetrazole, in comparison with the control group. The shaded horizontal lines indicate the frequency bands (delta, theta, alpha, beta, and gamma). (B) Mean amplitude recorded during seizures induced by caffeine and pentylenetetrazole in comparison with the control group. [(a)] indicates statistical difference for the control group; [(b)] indicates statistical difference for the caffeine group. $p < 0.0001$ ($n = 9$).

2.3.5 CHANGES IN BRAINWAVE OSCILLATIONS CAUSED BY CAFFEINE AND THE SP2 AND SP3 TRACE PATTERNS

The plot of the Power Spectral Density (PSD) indicates increased amplitudes in the SP2 and SP3 firing patterns in comparison with the caffeine-induced seizure (Figure 5A). The mean delta oscillation (1–4 Hz) in the control group was 0.01266 ± 0.0009746 mV^2 / Hz x 10^{-3}, which is significantly lower than the caffeine group (mean delta = 0.08804 ± 0.006953 mV^2 / Hz x 10^{-3}), which indicates increase in delta oscillations under caffeine intoxication ($p<0.0001$).

The SP2 firing pattern presented a mean value of 0.06474 ± 0.009471 mV^2 / Hz x 10^{-3}, and was not significantly from the mean delta of the caffeine group (Figure 5B). However, the SP3 was significantly higher ($F_{(3, 32)} = 272.5$; $p<0.0001$), at 0.1317 ± 0.01363 mV^2 / Hz x 10^{-3}.

In the case of the theta band (Figure 5C), the mean oscillation of the control group was 0.07722 ± 0.002454 mV^2 / Hz x 10^{-3}, significant lower ($F_{(2.32)} = 38.89$; $p <$

0.0001) than the caffeine group (0.1436 ± 0.04022 mV ^{2} / Hz x 10^{-3}), SP2 (0.1580 ± 0.01175 mV ^{2} / Hz x 10^{-3}) and SP3 (0.1823 ± 0.01055 mV^{2}/ Hz x 10^{-3}). Significant variation was also found among treatments in the alpha frequencies (Figure 5D), with the control group presenting the lowest mean (0.004028 ± 0.01055 mV ^{2} / Hz x 10^{-3}), which was significantly lower ($F_{(3.32)}$ = 414.4; p<0.0001) than that recorded for the caffeine group (0.06105 ± 0.008481 mV ^{2} / Hz x 10^{-3}). The highest power was recorded for the SP2 group (0.1669 ± 0.01882 mV ^{2} / Hz x 10^{-3}), which was statistically similar to the SP3 group (0.1557 ± 0.01013 mV ^{2} / Hz x 10^{-3}).

The beta band presented the greatest amplitude of oscillation during seizures (Figure 5E), with the SP2 group returning a mean value of 1.698 ± 0.3228 mV ^{2} / Hz x 10^{-3}, the highest power recorded in any group, and significantly higher ($F_{(3.32)}$ = 168.9; p < 0.0001) than the SP3 (0.8805 ± 0.1050 mV ^{2} / Hz x 10^{-3}), caffeine (0.2429 ± 0.08299 mV ^{2} / Hz x 10^{-3}), and control groups (0.006465 ± 0.0003215 mV ^{2} / Hz x 10^{-3}). The gamma oscillations (Figure 5F) also had the greatest amplitude in SP2, with a mean of 1.343 ± 0.1839 mV ^{2} / Hz x 10^{-3}, which was significantly higher ($F_{(3.32)}$ = 288.9; p < 0.0001) than the SP3 (0.8776 ± 0.08613 mV ^{2} / Hz x 10^{-3}) , control (0.03728 ± 0.003775 mV ^{2} / Hz x 10^{-3}), and caffeine groups (0.2289 ± 0.06078 mV ^{2} / Hz x 10^{-3}).

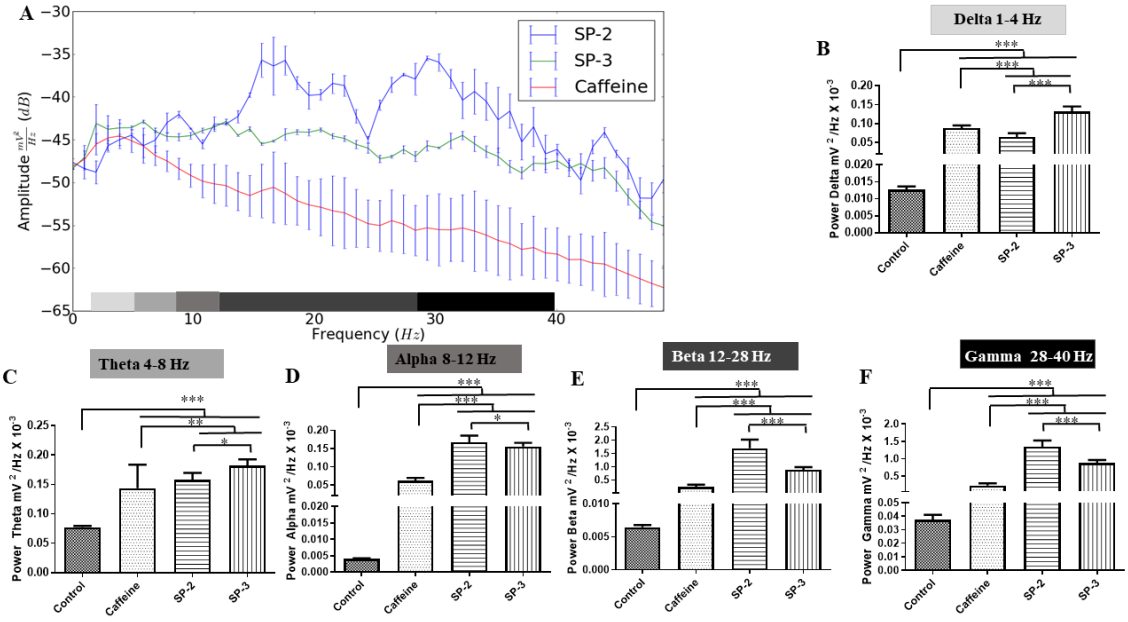

Figure 5: (A) Power Spectral Density (PSD) recorded following the administration of caffeine, comparing the SP2 and SP3 patterns with their respective brain oscillations (1–50 Hz); (B) mean delta power (1–4 Hz) during caffeine-induced seizures and in

the SP2 and SP3 patterns; (C) mean theta power (4–8 Hz) during caffeine-induced seizures and in SP2 and SP3; (D) mean alpha power (8–12 Hz) during caffeine-induced seizures and in SP2 and SP3; (E) mean beta power (12–28 Hz) during caffeine-induced seizures and in SP2 and SP3; (F) mean gamma power (28–40 Hz) during caffeine-induced seizures and in SP2 and SP3. *** *p<0.0001; **p<0.001; *p<0.05.* (*n* = 9).

2.3.6 EFFECTIVENESS OF ANTICONVULSANTS FOR THE CONTROL OF CAFFEINE-INDUCED SEIZURES

In the control group, the amplitude of the ECoG trace (0.04663 ± 0.06078 mV 2 /Hz x 10-3) was significantly lower (Figure 6D) than that of the caffeine group (0.8064 ± 0 .3034 mV 2 /Hz x 10-3) (p<0.0001).The phenobarbital group (Figure 6A) had a mean amplitude of 0.5524 ± 0.1144 mV 2 /Hz x 10-3, which was significantly lower than that of the caffeine group (p<0.05) but still significantly higher than the control group (p<0.0001) (Figure 6D). Treatment with phenytoin was even less effective (Figure 6B), with an amplitude of 0.7556 ± 0.1992 mV2/Hz x 10-3, significantly higher than the control group (p<0.0001), but not different from the caffeine group (Figure 6D). Overall, diazepam was the most effective treatment (Figure 6C), with a mean amplitude of 0.2947 ± 0.04515 mV 2 / Hz x 10-3 (Figure 6D) in comparison with phenobarbital and phenytoin (p<0.0001).showed lower recording potency than caffeine and phenytoin (p<0.0001), phenobarbital (p<0.05) and higher than the control group (p<0.05).

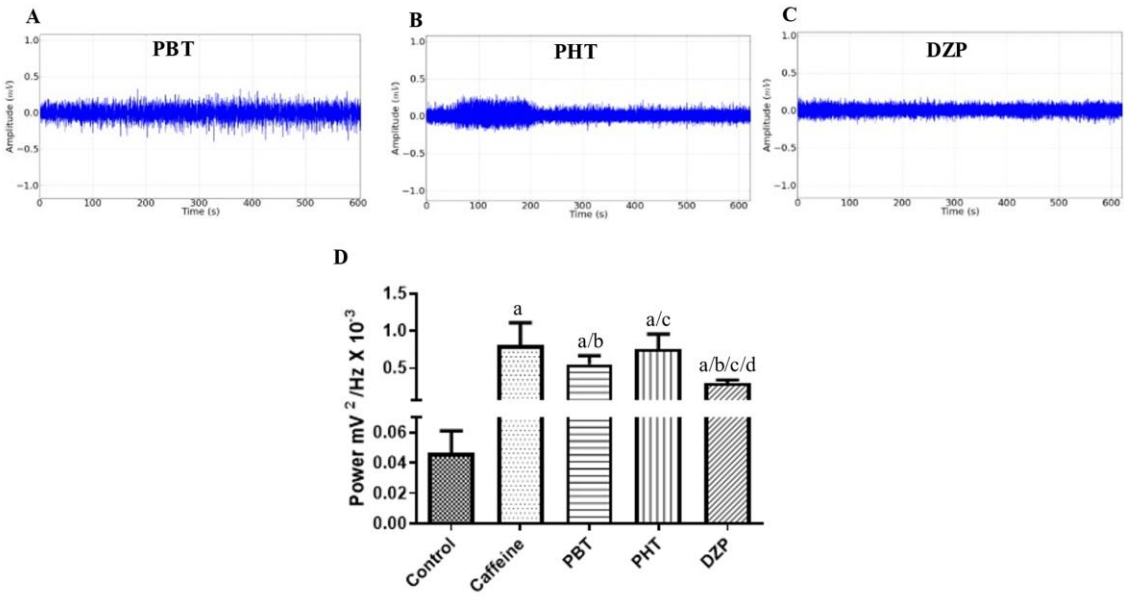

Figure 6 - Electrocorticographic (ECoG) traces demonstrating the action of (A) phenobarbital (PBT); (B) Phenytoin (PHT), and (C) Diazepam (DZP) when applied in an attempt to control caffeine-induced seizures (600 s). (D) Mean amplitude recorded in the control and caffeine groups, and in the groups in which the caffeine-induced seizures treated with phenobarbital (PBT), phenytoin (PHT), and diazepam (DZP). [(a)] indicates statistical difference for the control group; [(b)] indicates statistical difference for the caffeine group; [(c)] indicates statistical difference for the phenobarbital group; [(d)] indicates statistical difference for the phenytoin group. (*n* = 9).

2.4. Discussion

The pharmacological effects of caffeine include stimulation of the central nervous system and the heart, which normally occur at plasma concentrations of at least 15 mg/L. Common features of caffeine intoxication, also known as “caffeinism” (a state of toxicity caused by excessive caffeine consumption), may include anxiety, restlessness, insomnia, gastrointestinal disturbances, tremors, and psychomotor agitation (Cappelletti et al., 2018). The present study analyzed the seizure-related behavior and electrocorticographic (ECoG) patterns of Wistar rats following the administration of a toxic dose of caffeine, as well as evaluating the response to the anticonvulsant drugs diazepam, phenytoin and phenobarbital. This study demonstrated, for the first time, the behavioral and electrocorticographic changes caused by a toxic dose of caffeine. While factors such as the pharmacokinetic

differences between humans and rodents must be taken into account, the design of the experiment presented here provides important insights into the effects of high doses of caffeine on the brain. Following the administration of caffeine, the condition of the individual evolved rapidly to the most intense convulsion, with a latency of approximately 300 seconds to the onset of tonic-clonic crisis with the loss of the postural reflex.

The caffeine-induced changes in the ECoG were consistent with the development of the seizures, primarily in terms of their rapid evolution (Figure 2A). There was also a clear difference in the ECoG trace of the brainwaves of the PTZ seizures in comparison with the seizures caused by caffeine (Figure 4B). The anticonvulsant drug diazepam was far more effective for the control of caffeine-induced seizures than either phenytoin or fenobarbital (Figure 6D).

2.4.1 BEHAVIORAL AND ELECTROCORTICOGRAPHIC CHANGES: THE CHARACTERISTICS OF THE CAFFEINE-INDUCED CONVULSION COMPARED WITH OTHER MODELS

The comparison of the timing of the behavioral changes and shifts in the ECoG trace shows that the two records largely overlap (Figure 2A). The electrocorticographic responses are thus consistent with the behavioral patterns recorded during the caffeine-induced seizure, including increased excitability, such as tonic forelimb spasms (observed at 179.6 ± 15.35 seconds from the onset of the seizure). Extremely high doses of caffeine are known to increase brain excitability and trigger seizures and encephalopathy in animals, and caffeine is thus widely used as an animal model of seizure (Bauer and Sander, 2019, Chu N., 1981). The tonic clonic evolution of the caffeine-induced seizures was consistent with the findings of Van Koert et al. (2018). The seizure threshold may be lowered when caffeine is used as a thermogenic or consumed in excess (Babu et al. 2011; Bonilha & Li, 2004; Iyadurai & Chung, 2007). In comparison with the behavioral pattern observed in PTZ-induced seizures, analyzed extensively by Cutrufo et al. (1992), Luttjohann et al. (2009), and Goto et al (1983), the common features include the bristling of the vibrissae, followed by a sudden stop and subsequent excitability, both initiating focally, with secondary generalization.

Experimental convulsive models have not often used caffeine as an chemoconvulsant and the ECoG traces are distinct from those observed for PTZ

(Figures 2, 3 and 4). In experimental models that have used PTZ to induce seizures Goto et al. (1983), Bauer & Sander, (2019), and Yavuz et al. (2019) found that the combined use of caffeine permitted a reduction in the dose of PTZ, acting synergistically to lower the threshold of suceptibility and facilitate the onset of the seizure. Seizures induced by caffeine presented a standard pattern with the highest ECoG potency being identified for SP2 (Figure 2).

Following the administration of pilocarpine, the rats presented facial automatisms associated with moderate salivation, akinesia, and generalized tremors (Turski et al., 1987; Hoexter et al., 2005). Caffeine-induced excitability has many behavioral features that are similar to the experimental pilocarpine model, albeit with reduced latency. Hoexter et al. (2005) found that association with caffeine did not influence the intensity of the seizures in the pilocarpine or kainic acid models.

2.4.2 ELECTROCORTICOGRAPHIC CHANGES: ECOG PATTERNS AND BRAIN FORCES

The changes observed in the ECoG trace confirm the convulsive potential of caffeine (Van Koert et al., 2018; Nehlig et al., 1992). The predominant trace patterns were SP2 and SP3 (figure 2), in which the brain oscillations with the greatest power were the alpha, beta, and gamma bands.

The SP2 pattern had the greatest potency, and was the principal change observed during the seizure induced by caffeine. The oscillations in the beta frequency band (12–28 Hz) were the most powerful (Figure 5). Nehlig et al. (1992) identified beta as the band activated most intensively following the administration of caffeine, regardless of whether the dose is toxic or not. There is also a predominance of beta waves in PTZ seizures (Luttjohann et al., 2009), and this is the principal feature of epileptic seizures in general (Van Son et al., 2018). Hamoy et al (2018) also observed a predominance of beta oscillations in seizures induced by cunaniol.

The observed oscillation patterns varied in the other low-frequency bands, that is, the theta (4–8 Hz), alpha (8–12 Hz), and gamma bands (28–40 Hz). The theta band is characteristic of low-conscious states (Korotkova et al. 2018; Crivelli-Decker et al. 2018), in which the SP3 pattern is the most prominent. In the case of the alpha band, which is associated with processes of relaxation and imagination (Minami & Amano, 2017; Ahn et al., 2019), no statistical difference was found between the SP2 and SP3 patterns. In the gamma band, by contrast, which is correlated with visual, tactile and

auditory stimuli (Merkel et al., 2018; Meier et al., 2020), there was a predominance of SP2 (Figure 5). The predominant oscillation shifts from pattern SP2 to SP3. In SP2, the predominant power is in the beta wave band, while in SP3, it is in the delta range, with the power alternating during the convulsive condition, depending on the observed pattern.

2.4.3 EVALUATION OF THE ANTICONVULSANT DRUGS FOR THE CONTROL OF CAFFEINE-INDUCED SEIZURES

Diazepam was the anticonvulsant drug that presented the most effective control of caffeine-induced seizures, in comparison with Phenobarbital and Phenytoin. The electrocorticogram of the group pre-treated with benzodiazepine indicated a decrease in the intensity of the convulsive condition in comparison with the caffeine group, with a mean value closest to that of the negative control group. Even so, the ECoG potency was not reduced completely, despite causing an improvement in the intensity of the cerebral asynchrony during the convulsion. Marangos et al. (1981), Chweh et al. (1986), and Van et al, (2018) tested diazepam for the control of caffeine-induced seizures, although it was not found to be effective. The results of the present study indicate that brain activity did not decrease to baseline levels following the application of diazepam.

Diazepam presented good results for the control of convulsive conditions induced experimentally by cunaniol (Costa et al. 2006; Hamoy et al. 2018), and in the control of the convulsive phase observed during intoxication with oleandrin (De Melo et al. 2020). In general, diazepam is the most effective drug used for the control of seizures caused by chemoconvulsants. The effects of caffeine on the Central Nervous System go beyond the simple antagonism of the adenosine receptor (Bauer & Sander 2019; Esmaile & Heidare 2019; Nehling et al. 1992; Popoli et al. 1987), and may include the antagonism of benzodiazepine receptors, but in this case, the binding is weak, which may be related to the better response of diazepam, although brain activity is not reduced completely (Figure 6).

A number of previous studies (Morrissett et al., 1987; Suchomelova et al., 2015; Töllner et al., 2016; Rossi et al., 2017) have demonstrated the protective action of Diazepam against the anticonvulsant effects of Pilocarpine. This action is based on the fact that the drug does not permit the spread of convulsive discharges. In

caffeine-induced seizures, diazepam reduced the power of the ECoG trace, although it remained above baseline levels.

Jankiewicz et al. (2007) found that caffeine reduced the effects of the anticonvulsants phenobarbital and phenytoin against electroshock-induced seizures in mice. Despite being based on a different model, the results of the present study found no evidence of any control of the caffeine-induced seizures by phenytoin, and the brain potential recorded by the ECoG trace was still significantly above the baseline level following the administration of phenobarbital (Figure 6).

While phenytoin is known to increase caffeine clearance and reduced its half-life by nearly 50%. (Bauer and Sander, 2019; Wietholtz et al, 1989), the ECoG traces indicated that neither this drug nor phenobarbital was effective for the control of caffeine-induced seizures (Figure 6). Previous studies have shown that caffeine lowers the threshold for PTZ-induced seizures and modifies the response to anticonvulsants, carbamazepine, phenytoin, and valproate (Luszczki et al., 2006; Töllner et al, 2016), which is similar to the findings of the present study (Figure 6). Given this, the consumption of caffeine may interfere with the treatment of epileptic patients.

Chrościńska-Krawczyk et al. (2011) found that caffeine reduces the protective effects of traditional antiepileptic drugs (carbamazepine, phenobarbital, phenytoin, and valproate) as well as the newer compound, topiramate, against electroconvulsiveness in mice. This indicates that methylxanthines should be taken with caution in patients with a low seizure threshold.

2.5. Conclusions

Convulsions caused by caffeine are less potent than those triggered by pentylenetetrazole. The analysis of the electrocorticographic traces of animals in seizure found three distinct patterns. The highest oscillation in the recording pattern was observed in the beta wave band, which is consistent with other convulsive models. Caffeine-induced seizures are resistant to phenytoin and phenobarbital but respond well to diazepam. These findings provide important insights for the treatment of caffeine intoxication, in particular for individuals with a low seizure threshold.

2.6 References

Ahn S, Mellin JM, Alagapan S, Alexander ML, Gilmore JH, Jarskog LF, Fröhlich F. (2019) Targeting reduced neural oscillations in patients with schizophrenia by transcranial alternating current stimulation. Neuroimaging. 1 ;186: 126-136. https://doi.org/10.1016/j.neuroimage.2018.10.056 .

Altermann, MA, Dias, SC, Luiz, MV, Navarro F. (2008). The influence of caffeine as an ergogenic resource in physical exercise: its action and side effects. Brazilian Journal of Sports Nutrition, v.2, n.10, 225-239.

Babu KM, Zuckerman MD, Cherkes JK, Nrcc TC, Hack JB (2011). First seizure after using ENERGY for 5 hours. Pediatr Emerg Care 27:539–40. https://doi.org/10.1097/pec.0b013e31821dc72b

Banner W, Czaijka P (1980). Acute caffeine overdose in the newborn. Am J Dis Child 134:495–8. https://doi.org/10.1001/archpedi.1980.02130170045015

Bauer PR, Sander JW. (2019) Caffeine use by people with epilepsy : the myths and the evidence . Curr Neurol Neurosci Rep. 14;19(6):32. doi: 10.1007/s11910-019-0948-5.

Bonilha L, Li LM (2004). Heavy coffee drinking and epilepsy. Apprehension 13: 284-5. https://doi.org/10.1016/S1059-1311(03)00079-7

Bueno, MAS, (2003). The current role of methylxanthines (aminophylline and theophylline) in respiratory diseases. Hospital Israelita Albert Einstein. Sao Paulo.

Buzsáki G, Anastassiou CA, Koch C. (2012). The origin of extracellular fields and currents - EEG, ECoG, LFP and spikes. Nat Rev Neurosci. 13(6):407–420. doi:10.1038/nrn3241.

Cappelletti S, Piacentino D, Fineschi V, Frati P, Cipolloni L, Aromatario M. (2018) Caffeine-related deaths: form of deaths and risk categories. Nutrients, 10, 611; doi: 10.3390 / nu10050611.

Chrościńska-Krawczyk M., Jargiełło-Baszak M., Wałek M., Tylus B., Czuczwar SJ (2011) Caffeine and the anticonvulsant potency of antiepileptic drugs: experimental and clinical data. Pharmacol Rep. 63(1):12-8. doi: 10.1016/s1734-1140(11)70394-2.

Chu N. (1981) Convulsions induced by caffeine and aminophylline. Epilepsy.; 22:85-94

Chweh AY, Ulloque RA, Swinyard EA (1986) Benzodiazepine inhibition of [3H]flunitrazepam binding and caffeine-induced seizures in mice. Eur J Pharmacol. 122(2):161-5. doi: 10.1016/0014-2999(86)90098-1.

Cohen SM, Laurito CE, Jo Curran M., (1992) Grand mal seizure in a postpartum patient after intravenous infusion of caffeine sodium benzoate to treat persistent headache. J Clin Anesth 4:48–51. https://doi.org/10.1016/0952-8180(92)90121-g

Coast AE , Rocha FF, Torres MLB, Souccar C., De Lima TC M., Lapa . AJ, Lima-Landman . MT (2006). Behavioral effects of a neurotoxic compound isolated from Clibadium Surinamense L (Asteraceae). Neurotoxicol Teratol. 28(3):349-53. doi: 10.1016/j.ntt.2006.01.010.

Crivelli-Decker J, Hsieh LT, Clarke A, Ranganath C. (2018). Theta oscillations promote temporal sequence learning. *Neurobiol Learn* Mem.153(Pt A):92-103. doi:10.1016/j.nlm.2018.05.001

Cutrufo C, Bortot L, Giachetti A, Manzini S (1992). Differential effects of various xanthines on pentylenetetrazol-induced seizures in rats: an EEG and behavioral study. Eur J Pharmacol. 222(1):1-6. doi:10.1016/0014-2999(92)90454-c.

Dam , RMV, Hu , FB , Willett , WC, (2020) Coffee, Caffeine and Health. N Engl J Med. 383(4):369-378. DOI: 10.1056/NEJMra1816604 .

De Melo BS, Morais BP, Ferreira Sá VSS, Lourinho FD, Toda IPSP, Do Nascimento JLM, Marques DN, da Silva MCF, Cardoso GTM, Barbas LAL, Torres MF, Muto NA, de Mello VJ, Hamoy M (2020). Behavioral, electrocorticographic and electromyographic changes induced by the ethanolic extract of Nerium oleander: Evaluation of anticonvulsant therapy. Neurotoxicology. 78:21-28. https://doi.org/10.1016/j.neuro.2020.02.001

Esmaili Z, Heydari A. (2019) Effect of acute caffeine administration on PTZ-induced seizure threshold in mice: Involvement of adenosine receptors and NO-cGMP signaling pathway. Epilepsy Res. 2019 January; 149:1-8. doi: 10.1016/j.eplepsyres.2018.10.013.

Fisone G, Borgkvist A, Usiello A. (2004). Caffeine as a psychomotor stimulant: mechanism of action. Cell Mol Life Sci 61:857-872. https://doi.org/10.1007/s00018-003-3269-3

Goto M., Morishita S., Fukuda H. (1983) Anticonvulsant action of diazepam in mice pretreated with caffeine. *J Pharmacobiodyn*. 6(9):654-659. doi:10.1248/bpb1978.6.654

Hamoy, M., Batista, LS, Melo, VJ, Gomes, WL, Farias, RAF, Batista, PS, (2018). Seizures elicited by cunaniol: characterization of behavior and electroencephalographic analyses. Toxicol Appl Pharmacol. 360, 193-200.

Hoexter MQ, Rosa PS, Tufik S., Mello LE (2005). Consequences of prolonged caffeine administration and its withdrawal on pilocarpine and kainate-induced seizures in rats. Epilepsy Set; 46(9):1401-6. doi: 10.1111/j.1528-1167.2005.63904.x.

Huang ZL, Qu WM, Eguchi N, Chen JF, Schwarzschild MA, Fredholm BB, Urade Y, Hayaishi O. (2005). A2A, but not A1, adenosine receptors mediate the arousal effect of caffeine. Nat Neurosci 8:858-859. https://doi.org/10.1038/nn1491

Iyadurai SJP, Chung SS, (2007) New-onset seizures in adults: possible association with consumption of popular energy drinks. Epilepsy Behavior 10:504-8. https://doi.org/10.1016/j.yebeh.2007.01.009

Jalilifar M, Yadollahpour A, Moazedi AA, Ghotbeddin Z., (2017). Quantitative extracellular EEG assessments to classify specific features of the major phases of seizure acquisition based on the Rat kindling model. *Neurosci Lett*. 656:144-151. doi:10.1016/j.neulet.2017.07.034.

Jankiewicz K., Chrościńska-Krawczyk M., Błaszczyk B., Czuczwar S J. (2007). Caffeine and antiepileptic drugs: experimental and clinical data**.** Przegl Lek. 64(11):965-7. PMID: 18409414

Kaufman, K., Sachdeo, R., (2003). Caffeinated beverages and decreased seizure control. Seizure. 12, 519-21. https://doi.org/10.1016/S1059-1311(03)00048-7

Korotkova T, Ponomarenko A, Monaghan CK, Poulter SL, Cacucci F, Wills T, Hasselmo ME, Lever C. (2018). Reconciling the different faces of the hippocampal

teat : The role of the teat oscillations in cognitive, emotional and innate behaviors. Neurosci Biobehav Rev. doi: 10.1016/j.neubiorev.2017.09.004.

Luszczki JJ, Zuchora . M., Sawicka KM, Kozińska J., Czuczwar JS (2006) Acute exposure to caffeine diminishes the anticonvulsant action of ethosuximide, but not that of clonazepam, phenobarbital, and valproate against pentetrazole-induced seizures in mice. Pharmacol Rep. 58(5):652-9 PMID: 17085857

Lüttjohann A, Fabene PF, van Luijtelaar G (2009). A revised Racine scale for PTZ-induced seizures in rats. Physiological Behavior. 2009; 98(5): 579-586. doi : 10.1016 / j.physbeh.2009.09.005 .

Mackow MJ, Krishnan B, Bingaman WE, Najm IM, Alexopoulos AV, Nair DR. (2016). Increased caffeine intake leads to aggravation of electrocorticographic epileptiform discharges recorded with a responsive neurostimulation device. Clin Neurofisiol 127:2341-2

Maiga D, Seyni H, Sidikou A, Azouma A. (2012). Seizures in users of tramadol and caffeine: about 8 cases and review of the literature. Pan Afr Med J 13:24. https://www.ncbi.nlm.nih.gov/pubmed/23308329 .

Marangos PJ, Martino AM, Paul SM, Skolnick P. (1981) Benzodiazepines and inosine antagonize caffeine-induced seizures. Psychopharmacology (Berl). 72(3):269-73. doi: 10.1007/BF00431829.

Meier MA, Lemercier CE, Kulisch C, Kiss B, Lendvai B, Adham N, Gerevich Z. (2020) The novel antipsychotic cariprazine stabilizes gamma oscillations in rat hippocampal slices. Br J Pharmacol. 177(7):1622-1634. doi: 10.1111/bph.14923. Epub.

Merkel N, Wibral M, Bland G, Singer W. (2018). Endogenously generated gamma band oscillations in the early visual cortex: a neurofeedback study. Hmm Brain Mapp. 39(9):3487-3502. doi: 10.1002/hbm.24189.

Minami S, Amano K. (2017). Perceived Illusory Jitter in the Frequency of Alpha Oscillations. Curr Biol. (15):2344-2351.e4. doi: 10.1016/j.cub.2017.06.033.

Morrisett RA, Jope RS, Snead OC (1987). Drug effects on initiation and maintenance of status epilepticus induced by pilocarpine administration in lithium-pretreated rats. *Exp* Neurol.97(1):193-200. doi:10.1016/0014-4886(87)90293-7

Mueller SM, Solow EB (1982). Seizures associated with a new combination of stimulant pills. Ann Neurol 11:322. https://doi.org/10.1002/ana.410110316

Nehlig A, Daval JL, Debry G (1992) Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. *Res Brain Res Brain Rev* . 17(2):139-170. doi:10.1016/0165-0173(92)90012-b.

Oliveira F, Rodrigues K, Hamoy M, Sarquis IR, Hamoy A, Lopez ME, Ferreira I, Macchi BM, Nascimento L (2020) Fatty acid amides synthesized from Andiroba oil (Carapa guianensis Aublet.) Show anticonvulsant action with GABA-A receptor modulation in mice: a putative therapeutic option. J.Pharmaceuticals (Basel). 10;13(3):43. doi: 10.3390/ph13030043.

Paech M (1996). Unexpected postpartum seizures associated with caffeine-treated post-dural puncture headache. Int J Obstet Anesth 5:43–6. https://doi.org/10.1016/s0959-289x(96)80073-2

Pauwels RA, Buist AS, Calverley PM, Jenkins CR, Hurd SS (2001). Global strategy for diagnosis, management and prevention. J Respir Crit Care Med. 163, 1256-76. https://doi.org/10.1164/ajrccm.163.5.2101039

Pong NY, or TCT, Ip NY., (2015). Plant alkaloids as drugs lead to Alzheimer's disease.Neurochem Int. 89:260 - 270 https://doi.org/10.1016/j.neuint.2015.07.018

Popoli P, Sagratella S, Scotti de Carolis A. (1987). An EEG and behavioral study on the excitatory properties of caffeine in rabbits. *Arch Int Pharmacodyn Ther* . 290(1):5-15.

Röggla G, Moser B. (2007). Convulsions in a nightclub. Lancet 2007 ;370:220 . https://doi.org/10.1016/S0140-6736(07)61121-0

Rossi A, Murta V, Auzmendi J, Ramos AJ (2017). Early treatment with gabapentin during the latency period raises the seizure threshold, reduces microglial activation and macrophage infiltration in the lithium m-pilocarpine model of epilepsy . Pharmaceuticals (Basel). 28;10(4):93. doi: 10.3390/ph10040093.PMID: 29182533

Sagratella S., Niglio T., Scotti de Carolis A (1985) An investigation into the anticonvulsant mechanism of action of ketamine and phencyclidine on seizures due

to cortical application of penicillin in rabbits. Pharmacol Res Commun. 17(8):773-86. doi: 10.1016/0031-6989(85)90092-x.

Smith A., (2002) Effects of caffeine on human behavior. Food Chem Toxicol; 40:1243– 1255. https://doi.org/10.1016/S0278-6915(02)00096-0

Suchomelova L, Lopez-Meraz ML, Niquet J, Kubova H, Wasterlain CG (2015) Hyperthermia exacerbates status epilepticus-induced epileptogenesis and neuronal loss in immature rats. Neuroscience. 1; 305:209-24. doi: 10.1016/j.neuroscience.2015.08.006

For llner K, Twele F, Löscher W. (2016) Evaluation of the pentylenetetrazole seizure threshold test in epileptic mice as a surrogate model for drug testing against pharmacoresistant seizures. Epilepsy behavior. 57(Pt A): 95-104. doi: 10.1016/j.yebeh.2016.01.032.

Turski WA, Cavalheiro EA, Coimbra C, da Penha Berzaghi M, Ikonomidou-Turski C, Turski L. (1987) Only a few antiepileptic drugs prevent pilocarpine-induced seizures. *Res. brain* _ 434(3):281-305. doi:10.1016/0165-0173(87)90002-6

Ullrich S, de Vries Y, Kühn S, Repantis D, Dresler M, Ohla K. Feeling smart., (2015) Effects of caffeine and glucose on cognition, mood and self-judgment. Physiological Behavior. ;151:629 - 637. https://doi.org/10.1016/j.physbeh.2015.08.028

Van Koert, R., Bauer, P., Schuitema, I., Sander, J., Visser, G., (2018). Caffeine and seizures: A systematic review and quantitative analysis. Epilepsy and Behavior. 80, 37-4. https://doi.org/10.1016/j.yebeh.2017.11.003 .

Van Son, D., Schalbroeck, R., Angelidis, A., van der Wee, NJA, van der Does, W., & Putman, P. (2018). Acute effects of caffeine on selective threat attention: moderation by anxiety and EEG theta/beta ratio. Biological Psychology, 136, 100-110. https://doi: 10.1016 / j.biopsycho.2018.05.006

Voiculescu M, Segarceanu A, Negutu M, Ghita I, Fulga I, Coman AO (2015). The effect of caffeine on brain asymmetry in rats. J. Med Life.8(4):476-82. https://www.ncbi.nlm.nih.gov/pubmed/26664474

Wietholtz H, Zysset T, Kreiten K, Kohl D, Bichsel R, Matern S. (1989) Effect of phenytoin, carbamazepine and valproic acid on caffeine metabolism. Eur J Clin Pharmacol. ; 36: 401-6 .

Xu F, Liu P, Pekar JJ, Lu H. (2015) Does acute caffeine intake alter brain metabolism in young adults? Neuroimaging. 110C:39 - 47. https://doi.org/10.1016/j.neuroimage.2015.01.046

Yavuz M, Albayrak N, Özgür M, Gülçebi İdriz Oğlu M, Çavdar S, Onat F.(2019) The effect of prenatal and postnatal caffeine exposure on pentilentetrazole-induced seizures in non-epileptic and epileptic offspring. Neurosci Lett. 20; 713:134504. doi: 10.1016/j.neulet.2019.134504.

Zhang WY., (2001) A risk-benefit assessment of caffeine as an analgesic adjuvant. Drug Saf 24:1127-1142. https://doi.org/10.2165/00002018-200124150-00004 .

3. CONCLUSÕES INTEGRADORAS

As convulsões causadas pela cafeína são menos potentes do que as desencadeadas pelo pentilenotetrazol. A análise dos traços eletrocorticográficos dos animais em convulsão encontrou três padrões distintos. A maior oscilação no padrão de registro foi observada na banda de onda beta, o que é consistente com outros modelos convulsivos. As convulsões induzidas por cafeína são resistentes à fenitoína e ao fenobarbital, mas respondem bem ao diazepam. Esses achados fornecem informações importantes para o tratamento da intoxicação por cafeína, em particular para indivíduos com baixo limiar convulsivo.

4. REFERÊNCIAS

ALTERMANN, MA, DIAS, SC, LUIZ, MV, NAVARRO, F. A influência da cafeína como recurso ergogênico no exercício físico: sua ação e efeitos colaterais. Revista Brasileira de Nutrição Esportiva v. 2, n. 10, p. 225-239, 2008. ISSN 1981-9927.

BERNSTEIN, G. et al. Caffeine dependence in teenagers. Drug and Alcohol Dependence, v. 66, p. 1–6, mar, 2002. doi.10.1016/s0376-8716(01)00181-8

BROTHERS, H. et al. Caffeine attenuates lipopolysaccharide-induced neuroinflammation. Neurosci. Lett.,v 480, n. 2, p. 97-100, agu, 2010. doi.10.1016 / j.neulet.2010.06.013.

BUZSÁKI, G., ANASTASSIOU, CA, KOCH, C. A origem dos campos extracelulares e correntes - EEG, ECoG, LFP e picos. Nat. Rev. Neurosci. 13 (6), 407–420, 2012. doi.org/10.1038/nrn3241.

CABRAL, D. et al. Characterization of electrocorticographic, electromyographic and electrocardiographic recordings after the use of caffeine in Wistar rats. Einstein, v. 19, p. 1-10, nov., 2021. doi. org/10.31744/ einstein_journal/ 2021AO6417

CAPPELLETTI, S., PIACENTINO, D., FINESCHI, V., FRATI, P., CIPOLLONI, L., AROMATARIO, M.. Mortes relacionadas à cafeína: forma de mortes e categorias de risco. Nutrientes 10, 611, 2018. Doi 10.3390/nu10050611.

CHEN, J.; ELTZSCHIG, H.; FREDHOLM, B. Adenosine receptors as drug targets — what are the challenges? Nature Reviews Drug Discovery, v.12, p. 265–286, mar, 2013. doi. 10.1038 / nrd3955

CHROŚCIŃSKA-KRAWCZYK, M. et al. Caffeine and the anticonvulsant potency of antiepileptic drugs: experimental and clinical data. Pharmacological Reports, v.63, p.12-18, jan/feb, 2011. doi.10.1016/s1734-1140(11)70394-2.

CUTRUFO, C. et al. Differential effects of various xanthines on pentylenetetrazole-induced seizures in rats: an EEG and behavioural study. European Journal of Pharmacology, v. 222, n. 3, p. 1-6, 1992. doi.org/10.1016/0014-2999(92)90454-C

CZUCZWAR, S. et al. Influence of Different Methylxanthines on the Anticonvulsant Action of Common Antiepileptic Drugs in Mice. Epilepsy, v.31, p. 318-323, may/jun, 1990. doi.org/10.1111/j.1528-1157.1990.tb05382.x

FERRÉ, S. Mechanisms of the psychostimulant effects of caffeine: implications for substance use disorders. Psychopharmacology, v. 233, n. 10, p. 1963-1979, 2016. doi 10.1007/s00213-016-4212-2.

FISONE, G.; BORGKVIST, A.; USIELLO, A. Caffeine as a psychomotor stimulant: mechanism of action. Cell Mol Life Sci v.61, p.857-872. 2004 doi.org/10.1007/s00018-003-3269-3

FREDHOLM, B. et al. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacological reviews, v. 51, n. 1, p. 83-133.1999. https://pubmed.ncbi.nlm.nih.gov/10049999/

GASPAR, S. Avaliação do risco da exposição a substâncias estimulantes (cafeína, taurina e glucuronolactona) em adolescentes do distrito de Lisboa. Dissertação (Mestrado em Segurança Alimentar) - Faculdade de Farmácia, Universidade de Coimbra. 2014.

HUANG, Z. et al. Adenosine A2A, but not A1, receptors mediate the arousal effect of caffeine. Nature Neuroscience, v.8, p.858-859, 2005. Doi.org/10.1038/nn1491

KAUFMAN, K., SACHDEO, R. Bebidas com cafeína e diminuição do controle de convulsões. Apreensão 12, 519-521, 2003. doi.org/10.1016/S1059-1311(03)00048-7.

KENT R. O. (Org.) Manual de toxicologia clínica. 6. ed. Porto Alegre: McGraw-Hill, 2014.

KULKARNI, C.; JOSEPH, T.; DAVID, J. Influence of adenosine receptor antagonists, aminophylline and caffeine, on the convulsive protective capacity of antiepileptic drugs in rats. Indian Journal of Experimental Biology, v.29, n.8, p.751-754, 1991. PMID: 1769717

MORAIDIS, I.; BINGMAN, D. Epileptogenic actions of xanthines in relation to their affinities for adenosine A receptors in CA3 neurons of hippocampal slices (guinea pig). Brain Research, v.640, p. 140-145, 1994. doi.org/10.1016/0006-8993(94)91868-6

NEHLIG, A., DAVAL, JL, DEBRY, G. Cafeína e o sistema nervoso central: mecanismos de ação, efeitos bioquímicos, metabólicos e psicoestimulantes. Res Brain Res Brain Rev 17 (2), 139–170, 1992. https://doi.org/10.1016/0165-0173(92)90012-b.

PAUWELS, R. et al. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) Workshop Summary. American Journal of Respiratory and Critical Care Medicine, v.163, 5th ed, p.1256-1276, apr, 2001. doi: 10.1164/ajrccm.163.5.2101039

PONG, Y. et al. Plant alkaloids as drug leads for Alzheimer's disease Neurochem Int. v.89, p.260-270, aug, 2015. doi.org/10.1016/j.neuint.2015.07.018

POPOLI, P; SAGRATELLA, S; SCOTTI DE CAROLIS, A. An EEG and behavioural study on the excitatory properties of caffeine in rabbits. Archives Internationales de Pharmacodynamie et de Therapie, v.290, p.5-15, nov, 1987. PMID: 2895610.

REN, X.; CHEN, J. Caffeine and Parkinson's Disease: Multiple Benefits and Emerging Mechanisms Front. Neurosci. v.14, dec, 2020. doi.org/10.3389/fnins.2020.602697

SANEI, S.; CHAMBERS, J. A. Eeg signal processing. In: . [S.l.]: John Wiley & Sons, Inc., 2007.

SILVA, C. Processamento de Sinais de EEG para Classificação de Tarefas Motoras em Sistemas de Interface Cérebro-Máquina. 2017. 189 p. Tese - Universidade Federal de Santa Catarina, Florianópolis (SC), 2017.

SILVA, E. N. F. L. da. Eletroencephalography: basic principles, clinical applications, and related fields. [S.l.]: Lippincott Williams and Wilkins, 2005.

SMITH, A. Efeitos da cafeína no comportamento humano. Food Chem Toxicol; 40: 1243-1255, 2002. doi.org/10.1016/S0278-6915(02)00096-0

ULLRICH, S. et al. Efeitos da cafeína e glicose na cognição, humor e autojulgamento. Comportamento Fisiol.; 151: 629-637, 2015 doi.org/10.1016/j.physbeh.2015.08.028

TAVARES, C., SAKATA, R. K. Cafeína para o tratamento de dor. Rev Bras Anestesiol ; 62: 3: 387-401, 2012.

TCHEKALAROVA, J.; KUBOVA, H.; PAVEL, E. Postnatal caffeine treatment affects differently two pentylenetetrazol seizure models in rats. Seizure. Sep;18(7):463-9, 2009 doi: 10.1016/j.seizure.2009.04.002.

TEMPLE, J. L. et al. Sex differences in reinforcing value of caffeinated beverages in adolescents. Behavioural pharmacology, v. 20, n. 8, p. 731-741, 2009. DOI: 10.1097/FBP.0b013e328333b27c

VAN KOERT, R., BAUER, P., SCHUITEMA, I., SANDER, J., VISSER, G. Cafeína e convulsões: uma revisão sistemática e análise quantitativa. Epilepsia Comportament, 2018. doi.org/ 10.1016/j.yebeh.2017.11.003, 37-4

VAN SON, D. et al. Efeitos agudos da cafeína na atenção seletiva à ameaça: moderação pela ansiedade e relação teta/beta do EEG. Psicologia Biológica, 136, 100-110, 2018. doi: 10.1016 / j.biopsycho.2018.05.006

VOICULESCU, M. et al. O efeito da cafeína na assimetria cerebral em ratos. J. Med Life.8 (4): 476-82, 2015. PMID: 26664474

ZHANG, W. Uma avaliação de risco-benefício da cafeína como adjuvante analgésico. Drug Saf 24: 1127-1142, 2001. doi.org/10.2165/00002018-200124150-00004.

Dados Internacionais de Catalogação na Publicação (CIP) de acordo com ISBD
Sistema de Bibliotecas da Universidade Federal do Pará
Gerada automaticamente pelo módulo Ficat, mediante os dados fornecidos pelo(a) autor(a)

C972i Cunha Azevedo, Julianne Elba.
Intoxicação por cafeína: Padrões comportamentais e eletrocorticográficos em ratos Wistar / Julianne Elba Cunha Azevedo. — 2023.
47 f. : il. color.

Orientador(a): Prof. Dr. Moisés Hamoy
Dissertação (Mestrado) - Universidade Federal do Pará, Instituto de Ciências Biológicas, Programa de Pós-graduação em Farmacologia e Bioquímica, Belém, 2023.

1. cafeína. 2. convulsões. 3. eletrocorticografia. 4. intoxicação. 5. toxicologia. I. Título.

CDD 615.9

Food and Chemical Toxicology 170 (2022) 113452

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Caffeine intoxication: Behavioral and electrocorticographic patterns in Wistar rats

Julianne Elba Cunha Azevedo [a,*], Alex Luiz Menezes da Silva [a], Luana Rodrigues Vieira [a], Chirlene Pinheiro Nascimento [a], Rafaela Garcia Pereira [a], Sofia de França Rodrigues [a], Akira Otake Hamoy [a], Vanessa Joia de Mello [a], Daniella Bastos de Araújo [a], Luis André Luz Barbas [b], Maria Elena Crespo Lopez [c], Dielly Catrina Favacho Lopes [d], Moisés Hamoy [a]

[a] Laboratory of Pharmacology and Toxicology of Natural Products, Institute of Biological Sciences, Federal University of Pará, UFPA, Belém, Pará, Brazil
[b] Tropical Species Aquaculture Laboratory, Federal Institute of Education Science and Technology of Pará - Campus Castanhal, Castanhal, PA, Brazil
[c] Molecular Pharmacology Laboratory. Federal University of Pará, UFPA, Belém, Pará, Brazil
[d] Experimental Neuropathology Laboratory, Institute of Biological Sciences, Federal University of Pará, Belem, Pará, Brazil

ARTICLE INFO

Handling Editor: Dr. Jose Luis Domingo

Keywords:
Caffeine
Seizures
Electrocorticography
Intoxication
Toxicology

ABSTRACT

Caffeine is a psychoactive substance used worldwide. The present study analyzes the seizure-related behavior and electrocorticographic (ECoG) patterns observed in rats following of a toxic dose of caffeine (150 mg/kg; intraperitoneal). Sixty-three rats were divided into three experiments: 1-Behavior's Description associated with caffeine-induced convulsion; 2- Comparison of the electrocorticographic patterns induced by caffeine and pentylenetetrazole, and 3- Assessment of the electrocorticographic response to antiepileptic drugs (diazepam, phenytoin, and phenobarbital). The behavioral analysis demonstrated tonic-clonic seizures with a loss of postural reflex and a latency of 365.8 s after the caffeine's administration. Caffeine-induced changes in the ECoG were consistent with the development of seizures with rapid evolution and burst potential consistent with the behavioral patterns observed during the caffeine-induced seizure. The ECoG of the brainwaves varied significantly between the seizures caused by caffeine and pentylenetetrazole. The predominant brain forces observed during the seizures were beta-band oscillations. The caffeine-induced seizures were resistant to attempted control with phenytoin and phenobarbital, but responded well to diazepam, which is consistent with a study of Pilocarpine, which showed that diazepam has anticonvulsant effects. These findings are important for the development of effective treatments for caffeine intoxication, in particular for individuals with a low seizure threshold.

1. Introduction

Caffeine, a purine-like molecule (1,3,7-trimethylxanthine), is the most commonly-used psychostimulant in Western countries (Ullrich et al., 2015; Xu et al., 2015). In the United States, for example, 85% of the adult population has an average daily intake of 135 mg of caffeine (reaching 188 mg/day in the 35–49 age group). Caffeine can be found in coffee, tea, energy drinks, soft drinks, and cocoa (Pong et al., 2015; Ullrich et al., 2015). Xanthine like caffeine share a number of properties, including the ability to relax smooth muscles, stimulate the central nervous system, and promote diuresis (Bueno, 2003; Pauwels et al., 2001). However, high caffeine intake can have a range of side effects, and a general limit of 400 mg of caffeine per day is recommended for adults, given personal variations in metabolism and sensitivity to this compound (Dam et al., 2020).

When ingested orally, caffeine is absorbed completely and distributed widely, crossing even the tightly-closed blood-brain barrier with ease. In the brain, caffeine activates primarily the release of excitatory transmitters and acts as a non-selective adenosine antagonist for the A1 and A2 receptors (Fisone et al., 2004). Both these ligands – caffeine and adenosine – are highly similar in their chemical structure (Huang et al., 2005). They may affect the release of neurotransmitters such as

* Corresponding author. Laboratory of Pharmacology and Toxicology of Natural Products, Institute of Biological Sciences Federal University of Pará, Belém, Pará, Brazil, GUá, Belém, PA, 66075-110, Brazil.
E-mail address: julianne.azevedo@ics.ufpa.br (J.E.C. Azevedo).

https://doi.org/10.1016/j.fct.2022.113452
Received 24 May 2022; Received in revised form 6 September 2022; Accepted 28 September 2022
Available online 14 October 2022

acetylcholine, dopamine, noradrenaline, gamma-aminobutyric acid, and serotonin, which improves mood (Zhang, 2001), stimulates the body, improves concentration, and eliminates physical fatigue (Smith, 2002).

In recent years, the risk of caffeine intoxication has increased dramatically due to the inclusion of this substance in a growing number of medications, such as pain relievers and stimulants of the Central Nervous System, and dietary supplements, which are readily available from retailers, including online markets (Cappelletti et al., 2018).

A number of studies have shown that caffeine may trigger seizures in both normal and epileptic individuals after the ingestion of a toxic dose (Banner and Czaijka, 1980; Mueller and Solow., 1982; Cohen et al., 1992; Paech, 1996; Iyadurai and Chung., 2007; Röggla and Moser., 2007; Babu et al., 2011) or prolonged periods of intake (Kaufman and Sachdeo., 2003; Bonilha and Li., 2004 Maiga et al., 2012; Mackow et al., 2016). This occurs in particular in individuals that have a low seizure threshold (Kaufman and Sachdeo, 2003; Van Koert et al., 2018).

The dangers of caffeine are related to its ample diffusion, which results in a high, partially-conscious consumption pattern, due to the difficulty of determining the amount ingested. This results in an inability to predict specific effects in terms of the processes triggered by caffeine – even at a "safe" dose – which may mask underlying cardiovascular conditions (Cappelletti et al., 2018).

High doses, of over 400 mg/day, appear to induce anxiety, nausea, irritability, and nervousness. The lethal oral dose for an adult has been estimated to be 10 g (approximately 150–200 mg/kg). In children, the ingestion of 35 mg/kg can lead to moderate toxicity (Altermann et al., 2008).

Electroencephalographic recordings (EEG) are an alternative tool for the assessment of the electrical activity of the brain through the sum of the postsynaptic potentials generated by the pyramidal cells of the cerebral cortex through ionic flow (Buzsáki et al., 2012). This technique has proven to be a highly effective procedure for the measurement of the disturbance of electrical activity in animal models (Van Son et al., 2018; Voiculescu et al., 2015).

In this context, the present study analyzed seizure-related behavior and electrocorticographic (ECoG) patterns in Wistar rats following the administration of a toxic dose of caffeine. It also evaluates the response to classical antiepileptic drugs (diazepam, phenytoin and phenobarbital) following caffeine-induced seizure.

2. Materials and methods

2.1. Animals

Sixty-three adult male Wistar rats (250–280 g) were obtained from the Central Animal Facility of the Federal University of Pará (UFPA) and kept in the experimental vivarium of the UFPA Laboratory of Pharmacology and Toxicology of Natural Products. The animals were housed at a controlled temperature of 23–25 °C with a 12-h light-dark cycle, and *ad libitum* access to food and water.

The present study was conducted in accordance with the precepts of the Brazilian legislation for animal experimentation and the ethical principles of the National Council for the Control of Animal Experimentation (CONCEA, Brazil). Prior to initiating the study, it was approved by the UFPA Committee on Ethics in Research on the Use of Animals (CEPAE-UFPA: # 4662260819).

2.2. Experimental design

The present study was based on three separate experiments, with n = 9 animals per experimental group. The first experiment described the convulsive behavior induced by a toxic dose of caffeine (150 mg/kg; intraperitoneal [ip]), including the latency of the onset of the convulsions, in this experiment will be identified morphographic elements that was repeated in the records during the convulsions caused by caffeine. The second experiment recorded the electrocorticographic (ECoG) patterns of the rats following the administration of caffeine and compared the results with the ECoGs of rats that received pentylenetetrazole (PTZ, positive control of induced seizure, 60 mg/kg, ip) and a control group (saline solution in volume equivalent to the dose of caffeine applied). Each of these groups consisting of nine animals, which underwent surgery for the implantation of the electrodes.

In the third experiment, the animals were also divided into three groups of nine animals, in which electrodes were implanted surgically. Each group was treated with a different anticonvulsant drug, 60 s after the administration of the caffeine, either (a) diazepam (DZP, 10 mg/kg, intravenous [iv]), (b) phenobarbital (PBT, 10 mg/kg, iv), or (c) phenytoin (PHT, 10 mg/kg, iv). These doses are within the therapeutic range, and each group received only one of the drugs prior to the collection of the ECoG recordings.

Overall, 63 animals were used in the present study, considering that each experimental group consisted of nine animals. Experiment 1 had only a single group ($n = 9$ animals), whereas experiments 2 and 3 each involved three groups nine animals, with a total of 27 animals in each case.

2.3. Chemicals

Ketamine hydrochloride (König laboratory, Santana de Parnaíba-SP, Brazil), xylazine hydrochloride (Vallée laboratory, Montes Claros-MG, Brazil), and lidocaine (Hipolabor laboratory, Sabará-MG, Brazil) were used for the surgical implantation of the electrodes. Caffeine and pentylenetetrazole were obtained from Sigma (USA). Phenobarbital (Aventis-Pharma, Ribeirão Preto-SP, Brazil), phenytoin, and diazepam (União Química, Embu-Guaçu-SP, Brazil) were selected as the anticonvulsant drugs.

2.4. Description of the seizure-related behavior

The behavior associated with the caffeine-induced seizure was described based on the observation of animals that received a 150 mg/kg dose of caffeine intraperitoneally (ip). After the administration of the caffeine, the latency of the onset of each type of convulsive behavior was recorded in the following sequence: (a) Erection of the vibrissae and retraction of the pin; (b) Immobility with deep and rhythmic breathing; (c) Excitability and motor incoordination; (d) Tonic spasms of the forelimbs; (e) Tonic-clonic seizures with transient loss of the postural reflex, and (f) Tonic-clonic seizures with a loss of the postural reflex. The convulsive condition evolved rapidly, which justifies the adoption of a 10-min sampling interval for this experiment. The data were pooled for the nine animals of there groups, for the presentation of the mean and standard deviation the latency of each behavior.

2.5. Electrocorticography and data analysis

The electrodes were implanted surgically at bregma coordinate −0.96, 1 mm to the side of each hemisphere represented by the motor cortex (Paxinos and Watson, 2005). Five days after surgery, the electrodes were connected to a data acquisition system consisting of a high-impedance amplifier (Grass Technologies, P511), monitored by an oscilloscope (Protek, 6510). The data were digitized continuously at a rate of 1 KHz by a computer equipped with a data acquisition board (National Instruments, Austin, TX), and stored on a hard disk for processing using specialized software (LabVIEW express). The recording electrode was located on the right side of the hemisphere and the reference electrode on the left side. All experimental procedures were conducted in a Faraday cage.

A tool was constructed using the Python programming language (version 2.7) for the analysis of the signals obtained during the experiments. The Numpy and Scipy libraries were used for mathematical processing and the Matplolib library for graphics. The graphical

interface was developed using the PyQt4 library.

The records were analyzed at frequencied of up to 50 Hz. The bands were analyzed following Jalilifar et al. (2017) and Hamoy et al. (2018), who classify the bands as Delta (1–4 Hz), Theta (4–8 Hz), Alpha (8–12 Hz), Beta (12–28), and Gamma, at 28–40 Hz (Hamoy et al., 2018; De Melo et al., 2020; Oliveira et al., 2020).

2.6. Statistical analysis

The results are presented as the mean value ± standard deviation (SD). The normality of the variances was verified by the Kolmogorov-Smirnov test and the homogeneity by the Levene test. The groups were compared using a one-way Analysis of Variance (ANOVA), followed by Tukey's *post hoc* test, whenever appropriate. All the analyses were run in GraphPad® Prism 6, and considered a $p < 0.05$ significance level.

3. Results

3.1. Behavioral features of caffeine-induced seizures

The behavioral test determined the latency of the evolution of seizures following the administration of caffeine. A rapid evolution to the most intense seizure was observed. It is important to note here that, while the latency refers to the initial onset of the seizure (presented here as a mean value for the individual, with its respective variation), the behavior may be repeated during subsequent seizures. The first manifestation of a change in behavior is the erection of the vibrissae and retraction of the pinnae (ears), with a mean latency of 40.78 ± 10.12 s, followed by immobility with deep and rhythmic breathing (mean latency = 104.6 ± 11.93 s). The subsequent behavioral change is the onset of motor excitability and incoordination (mean latency = 149.2 ± 19.39 s), followed by tonic spasms of the forelimbs (mean latency = 179.6 ± 15.35 s), which are a focal seizure with secondary generalization. These shifts are followed by tonic-clonic seizure with a transient loss of the postural reflex (230.1 ± 28.68 s) and tonic-clonic seizure with the loss of the postural reflex, with a mean latency of 365.8 ± 33.93 s. As shown below, electrocorticographic changes were also triggered by the caffeine, with their onset coinciding with the latency of the different behavioral components, as presented here.

3.2. Electrocorticography of the control group

The electrocorticographic records of the control group revealed higher power at low frequencies (0–10 Hz), that is, the delta (1–4 Hz), theta (4–8 Hz), and alpha (8–12 Hz) brainwaves, with reduced amplitude variation. This indicates the synchronization of the brain between the capture of the signal by the reference electrode (left hemisphere) and the recording electrode, in the right hemisphere (Fig. 1A–D).

3.3. The ECoG traces of caffeine-induced seizures have three repeated patterns

The caffeine-induced shifts in the ECoG were compatible with the observed development of the seizures, including their rapid evolution and the potential for bursts of increased amplitude, shown by the red arrow (Fig. 2A). The timing of the ECoG responses is consistent with that of the behavioral shifts observed during the observation of the caffeine-induced seizure, including increased excitability and tonic forelimb spasms (observed at approximately 180 s). Caffeine-induced seizures increased brain wave oscillations, in particular in the 0–50 Hz frequency range (Fig. 2B and C).

The ECoG trace of the caffeine group presented repeated patterns that are identified here as SP1, SP2 and SP3 (Fig. 2D–F), which have amplitudes varying periods, but are invariably repeated. Each observed pattern contributed to the potency observed during caffeine-induced seizures.

3.4. Changes in the ECoG profile caused by pentylenetetrazole- and caffeine-induced seizures

Caffeine-induced changes in the ECoG trace were compared with the model of seizures induced by pentylenetetrazole, or PTZ (Fig. 3), which shows changes in the ECoG trace and amplitude oscillations consistent

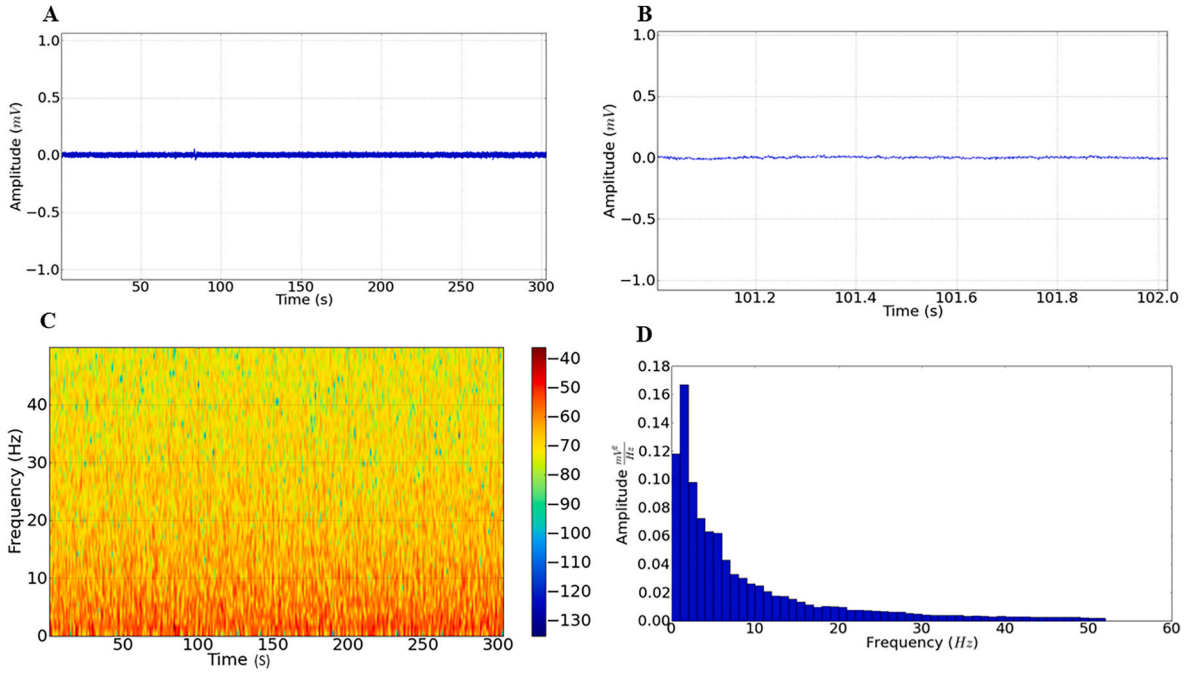

Fig. 1. Electrocorticographic records of the control animals ($n = 9$) obtained during the present study: (A) Electrocorticographic trace (5 min) obtained from the right hemisphere, using the left hemisphere as a reference. (B) Baseline magnification over 1 s. (C) Frequency spectrogram showing higher signal power density at low frequencies (1–10 Hz). (D) Frequency histogram of one of the individuals of there control group, showing the concentration of power between 1 Hz and 10 Hz.

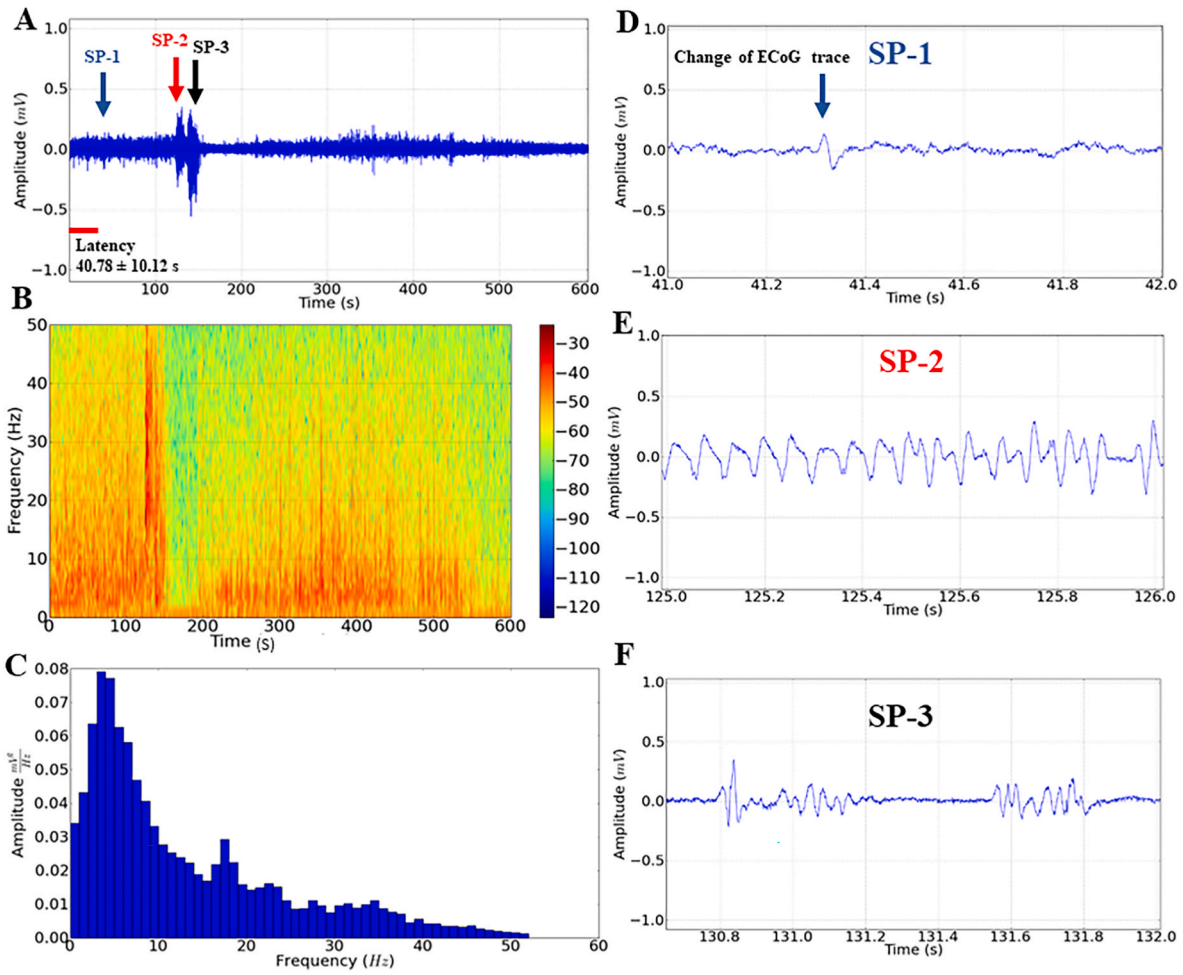

Fig. 2. Electrocorticographic records of caffeine-induced seizures in Wistar rats, with a duration of 10 min ($n = 9$): (A) ECoG trace showing the identification points for SP1 (blue arrow), SP2 (red arrow), SP3 (black arrow), and the general latency (red line) of the seizure; (B) Frequency spectrogram following the administration of the caffeine; (C) Frequency histogram of an individual of the caffeine group showing the concentration of power between 1 Hz and 50 Hz (total of 600 s); the ECoG trace patterns recorded following the administration of caffeine in (D) SP1 (the blue arrow indicates the shift in the trace following the administration of caffeine), (E) SP2; (F) SP3. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

consistent with seizure (Fig. 3A and B). The frequency spectrogram indicates an increase in the power distribution at frequencies of 1–50 Hz (Fig. 3C), while the histogram of amplitudes (Fig. 3D) indicates a peak at 3–8 Hz. These patterns were quite distinct from those observed in the caffeine-induced seizures (Fig. 2).

The spectral decomposition of the ECoG records of the delta, theta, alpha, beta, and gamma waves reveals more evident differences in PTZ-induced seizures (Fig. 4A) in comparison with the caffeine-induced seizures. At frequencies of 1–40 Hz (Fig. 4B), the control group had a mean power of 0.04663 ± 0.01435 mV ^{2}/Hz x 10^{-3}, which was significantly lower ($F_{(2, 24)} = 95.32$, $p < 0.0001$) than either the PTZ (1.732 ± 0.3311 mV ^{2}/Hz x 10^{-3}) or the caffeine group (0.8064 ± 0.3034 mV ^{2}/Hz x 10^{-3}). This demonstrates a significant increase in the potency of the ECoG of both PTZ and caffeine (Fig. 4B), in comparison with the control group.

3.5. Changes in brainwave oscillations caused by caffeine and the SP2 and SP3 trace patterns

The plot of the Power Spectral Density (PSD) indicates increased amplitudes in the SP2 and SP3 firing patterns in comparison with the caffeine-induced seizure (Fig. 5A). The mean delta oscillation (1–4 Hz) in the control group was 0.01266 ± 0.0009746 mV ^{2}/Hz x 10^{-3}, which is significantly lower than the caffeine group (mean delta = 0.08804 ± 0.006953 mV ^{2}/Hz x 10^{-3}), which indicates increase in delta oscillations under caffeine intoxication ($p < 0.0001$).

The SP2 firing pattern presented a mean value of 0.06474 ± 0.009471 mV ^{2}/Hz x 10^{-3}, and was not significantly from the mean delta of the caffeine group (Fig. 5B). However, the SP3 was significantly higher ($F_{(3, 32)} = 272.5$; $p < 0.0001$), at 0.1317 ± 0.01363 mV ^{2}/Hz x 10^{-3}.

In the case of the theta band (Fig. 5C), the mean oscillation of the control group was 0.07722 ± 0.002454 mV ^{2}/Hz x 10^{-3}, significant lower ($F_{(2.32)} = 38.89$; $p < 0.0001$) than the caffeine group (0.1436 ± 0.04022 mV ^{2}/Hz x 10^{-3}), SP2 (0.1580 ± 0.01175 mV ^{2}/Hz x 10^{-3}) and SP3 (0.1823 ± 0.01055 mV^2/Hz x 10^{-3}). Significant variation was also found among treatments in the alpha frequencies (Fig. 5D), with the control group presenting the lowest mean (0.004028 ± 0.01055 mV ^{2}/Hz x 10^{-3}), which was significantly lower ($F_{(3.32)} = 414.4$; $p < 0.0001$) than that recorded for the caffeine group (0.06105 ± 0.008481 mV ^{2}/Hz x 10^{-3}). The highest power was recorded for the SP2 group (0.1669 ± 0.01882 mV ^{2}/Hz x 10^{-3}), which was statistically similar to the SP3 group (0.1557 ± 0.01013 mV ^{2}/Hz x 10^{-3}).

The beta band presented the greatest amplitude of oscillation during seizures (Fig. 5E), with the SP2 group returning a mean value of 1.698 ± 0.3228 mV ^{2}/Hz x 10^{-3}, the highest power recorded in any group, and significantly higher ($F_{(3.32)} = 168.9$; $p < 0.0001$) than the SP3 (0.8805 ± 0.1050 mV ^{2}/Hz x 10^{-3}), caffeine (0.2429 ± 0.08299 mV ^{2}/Hz x

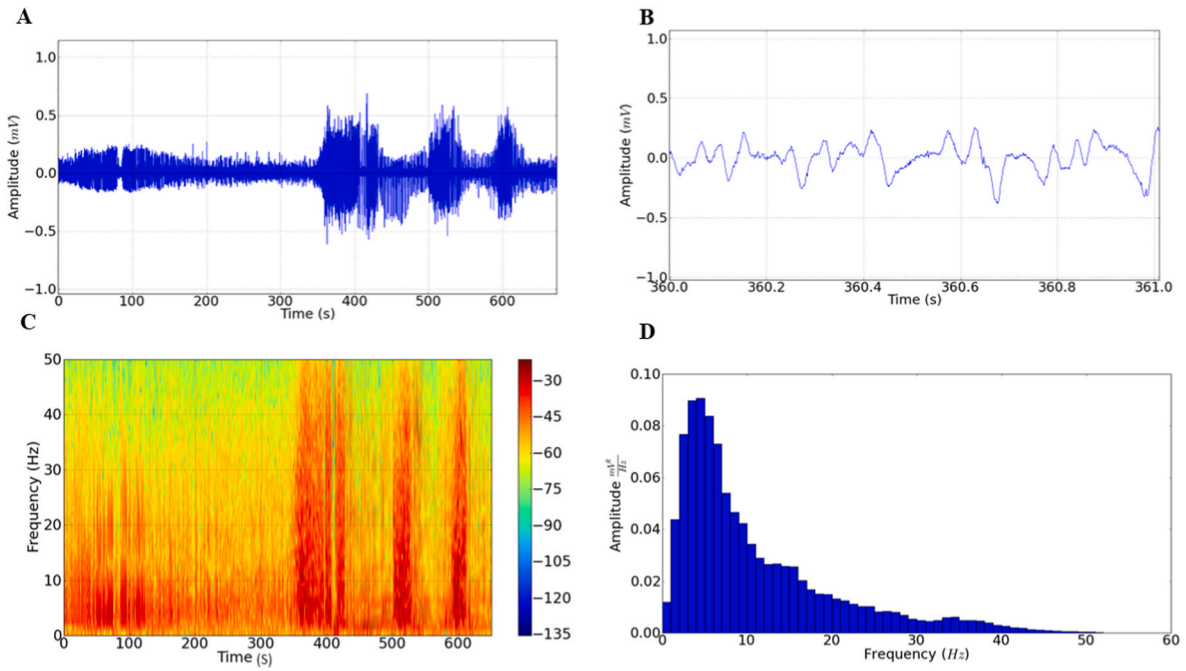

Fig. 3. Electrocorticographic records ($n = 9$) of: (A) the EcoG signal following seizure induced by Pentylenetetrazole (PTZ), over a 10-min sample; (B) Magnification of the PTZ-altered ECoG trace at 1 s; (C) Frequency spectrogram following the PTZ-induced seizure; (D) Histogram (one column per Hz) of the power concentration (1–50 Hz) of an individual of the PTZ group.

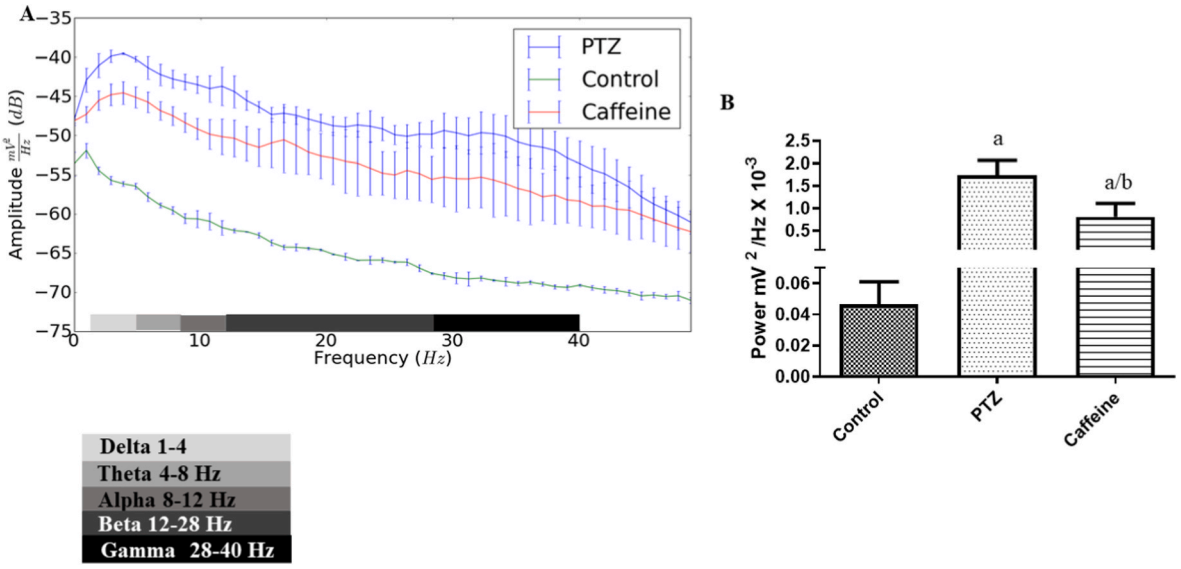

Fig. 4. (A) Power Spectral Density (PSD) following the administration of caffeine and pentylenetetrazole, in comparison with the control group. The shaded horizontal lines indicate the frequency bands (delta, theta, alpha, beta, and gamma). (B) Mean amplitude recorded during seizures induced by caffeine and pentylenetetrazole in comparison with the control group. [(a)] indicates statistical difference for the control group; [(b)] indicates statistical difference for the caffeine group. $p <$ 0.0001 ($n = 9$).

10^{-3}), and control groups (0.006465 ± 0.0003215 mV ^{2}/Hz x 10^{-3}). The gamma oscillations (Fig. 5F) also had the greatest amplitude in SP2, with a mean of 1.343 ± 0.1839 mV ^{2}/Hz x 10^{-3}, which was significantly higher ($F_{(3.32)} = 288.9$; $p < 0.0001$) than the SP3 (0.8776 ± 0.08613 mV ^{2}/Hz x 10^{-3}), control (0.03728 ± 0.003775 mV ^{2}/Hz x 10^{-3}), and caffeine groups (0.2289 ± 0.06078 mV ^{2}/Hz x 10^{-3}).

3.6. Effectiveness of anticonvulsants for the control of caffeine-induced seizures

In the control group, the amplitude of the ECoG trace (0.04663 ± 0.06078 mV 2/Hz x 10-3) was significantly lower (Fig. 6D) than that of the caffeine group (0.8064 ± 0 0.3034 mV 2/Hz x 10-3) ($p < 0.0001$). The phenobarbital group (Fig. 6A) had a mean amplitude of 0.5524 ± 0.1144 mV 2/Hz x 10-3, which was significantly lower than that of the caffeine group ($p < 0.05$) but still significantly higher than the control group ($p < 0.0001$) (Fig. 6D). Treatment with phenytoin was even less

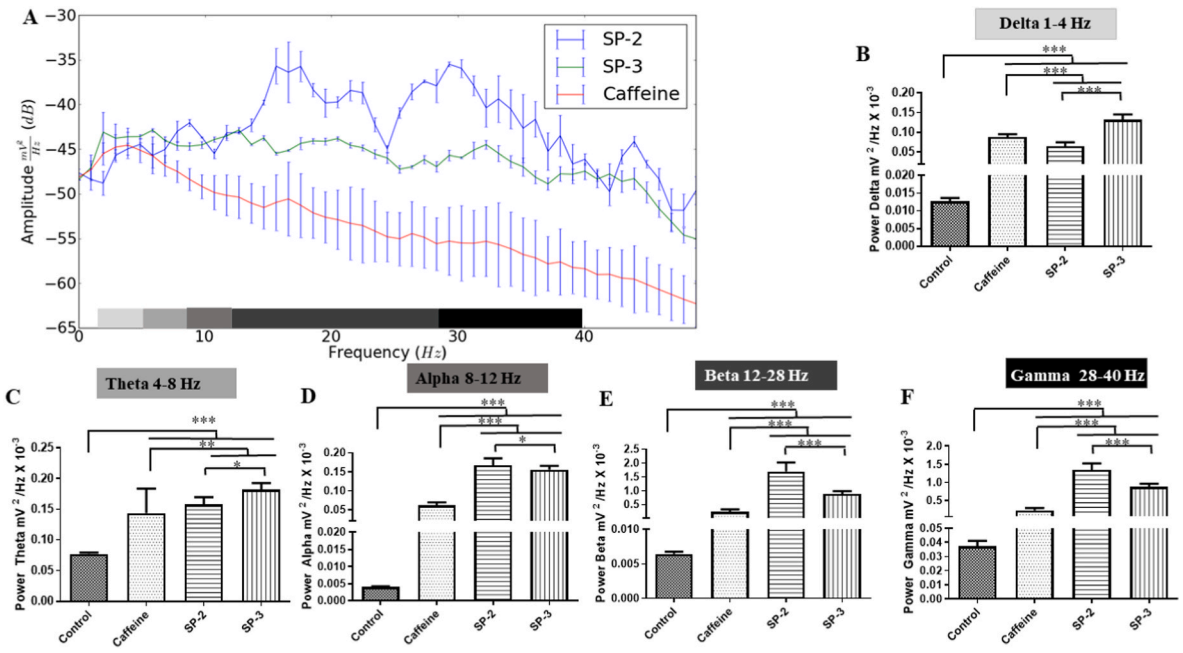

Fig. 5. (A) Power Spectral Density (PSD) recorded following the administration of caffeine, comparing the SP2 and SP3 patterns with their respective brain oscillations (1–50 Hz); (B) mean delta power (1–4 Hz) during caffeine-induced seizures and in the SP2 and SP3 patterns; (C) mean theta power (4–8 Hz) during caffeine-induced seizures and in SP2 and SP3; (D) mean alpha power (8–12 Hz) during caffeine-induced seizures and in SP2 and SP3; (E) mean beta power (12–28 Hz) during caffeine-induced seizures and in SP2 and SP3; (F) mean gamma power (28–40 Hz) during caffeine-induced seizures and in SP2 and SP3. ***$p < 0.0001$; **$p < 0.001$; *$p < 0.05$. ($n = 9$).

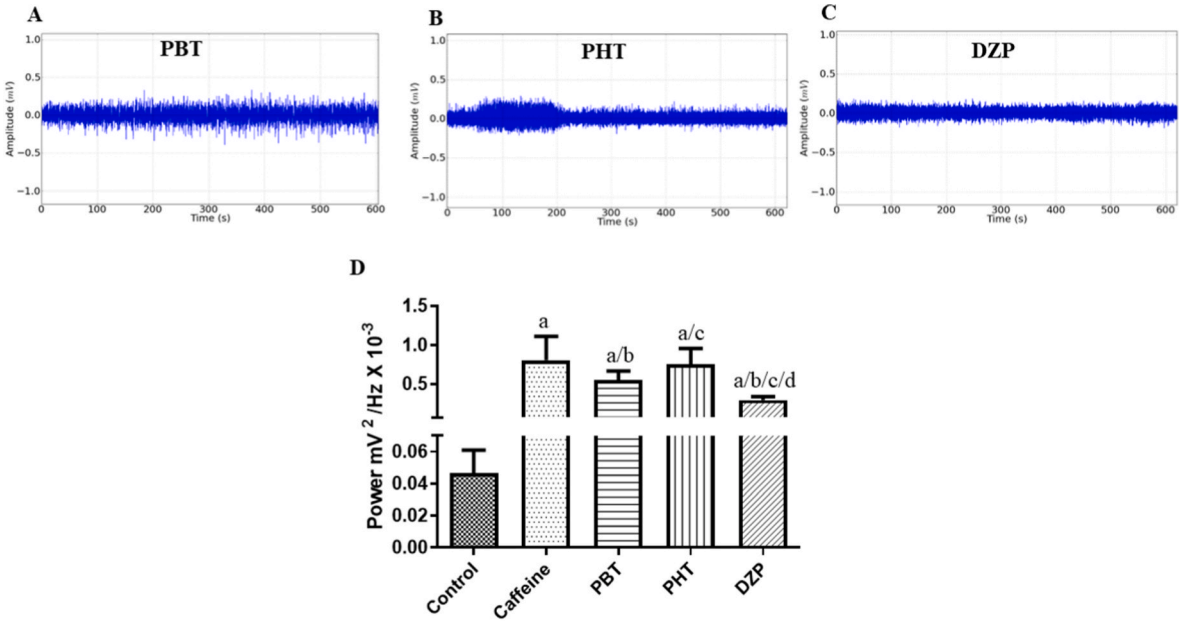

Fig. 6. Electrocorticographic (ECoG) traces demonstrating the action of (A) phenobarbital (PBT); (B) Phenytoin (PHT), and (C) Diazepam (DZP) when applied in an attempt to control caffeine-induced seizures (600 s). (D) Mean amplitude recorded in the control and caffeine groups, and in the groups in which the caffeine-induced seizures treated with phenobarbital (PBT), phenytoin (PHT), and diazepam (DZP). [(a)] indicates statistical difference for the control group; [(b)] indicates statistical difference for the caffeine group; [(c)] indicates statistical difference for the phenobarbital group; [(d)] indicates statistical difference for the phenytoin group. ($n = 9$).

effective (Fig. 6B), with an amplitude of 0.7556 ± 0.1992 mV2/Hz x 10-3, significantly higher than the control group ($p < 0.0001$), but not different from the caffeine group (Fig. 6D). Overall, diazepam was the most effective treatment (Fig. 6C), with a mean amplitude of 0.2947 ± 0.04515 mV 2/Hz x 10-3 (Fig. 6D) in comparison with phenobarbital and phenytoin ($p < 0.0001$).showed lower recording potency than caffeine and phenytoin ($p < 0.0001$), phenobarbital ($p < 0.05$) and higher than the control group ($p < 0.05$).

4. Discussion

The pharmacological effects of caffeine include stimulation of the central nervous system and the heart, which normally occur at plasma concentrations of at least 15 mg/L. Common features of caffeine

intoxication, also known as "caffeinism" (a state of toxicity caused by excessive caffeine consumption), may include anxiety, restlessness, insomnia, gastrointestinal disturbances, tremors, and psychomotor agitation (Cappelletti et al., 2018). The present study analyzed the seizure-related behavior and electrocorticographic (ECoG) patterns of Wistar rats following the administration of a toxic dose of caffeine, as well as evaluating the response to the anticonvulsant drugs diazepam, phenytoin and phenobarbital. This study demonstrated, for the first time, the behavioral and electrocorticographic changes caused by a toxic dose of caffeine. While factors such as the pharmacokinetic differences between humans and rodents must be taken into account, the design of the experiment presented here provides important insights into the effects of high doses of caffeine on the brain. Following the administration of caffeine, the condition of the individual evolved rapidly to the most intense convulsion, with a latency of approximately 300 s to the onset of tonic-clonic crisis with the loss of the postural reflex.

The caffeine-induced changes in the ECoG were consistent with the development of the seizures, primarily in terms of their rapid evolution (Fig. 2A). There was also a clear difference in the ECoG trace of the brainwaves of the PTZ seizures in comparison with the seizures caused by caffeine (Fig. 4B). The anticonvulsant drug diazepam was far more effective for the control of caffeine-induced seizures than either phenytoin or fenobarbital (Fig. 6D).

4.1. Behavioral and electrocorticographic changes: the characteristics of the caffeine-induced convulsion compared with other models

The comparison of the timing of the behavioral changes and shifts in the ECoG trace shows that the two records largely overlap (Fig. 2A). The electrocorticographic responses are thus consistent with the behavioral patterns recorded during the caffeine-induced seizure, including increased excitability, such as tonic forelimb spasms (observed at 179.6 $\pm$ 15.35 s from the onset of the seizure). Extremely high doses of caffeine are known to increase brain excitability and trigger seizures and encephalopathy in animals, and caffeine is thus widely used as an animal model of seizure (Bauer and Sander, 2019; Chu N., 1981). The tonic clonic evolution of the caffeine-induced seizures was consistent with the findings of Van Koert et al. (2018). The seizure threshold may be lowered when caffeine is used as a thermogenic or consumed in excess (Babu et al., 2011; Bonilha and Li, 2004; Iyadurai and Chung, 2007). In comparison with the behavioral pattern observed in PTZ-induced seizures, analyzed extensively by Cutrufo et al. (1992), Lüttjohann et al. (2009), and Goto et al. (1983), the common features include the bristling of the vibrissae, followed by a sudden stop and subsequent excitability, both initiating focally, with secondary generalization.

Experimental convulsive models have not often used caffeine as an chemoconvulsant and the ECoG traces are distinct from those observed for PTZ (Figs. 2–4). In experimental models that have used PTZ to induce seizures Goto et al. (1983), Bauer & Sander (2019), and Yavuz et al. (2019) found that the combined use of caffeine permitted a reduction in the dose of PTZ, acting synergistically to lower the threshold of susceptibility and facilitate the onset of the seizure. Seizures induced by caffeine presented a standard pattern with the highest ECoG potency being identified for SP2 (Fig. 2).

Following the administration of pilocarpine, the rats presented facial automatisms associated with moderate salivation, akinesia, and generalized tremors (Turski et al., 1987; Hoexter et al., 2005). Caffeine-induced excitability has many behavioral features that are similar to the experimental pilocarpine model, albeit with reduced latency. Hoexter et al. (2005) found that association with caffeine did not influence the intensity of the seizures in the pilocarpine or kainic acid models.

4.2. Electrocorticographic changes: ECoG patterns and brain forces

The changes observed in the ECoG trace confirm the convulsive potential of caffeine (Van Koert et al., 2018; Nehlig et al., 1992). The predominant trace patterns were SP2 and SP3 (Fig. 2), in which the brain oscillations with the greatest power were the alpha, beta, and gamma bands.

The SP2 pattern had the greatest potency, and was the principal change observed during the seizure induced by caffeine. The oscillations in the beta frequency band (12–28 Hz) were the most powerful (Fig. 5). Nehlig et al. (1992) identified beta as the band activated most intensively following the administration of caffeine, regardless of whether the dose is toxic or not. There is also a predominance of beta waves in PTZ seizures (Lüttjohann et al., 2009), and this is the principal feature of epileptic seizures in general (Van Son et al., 2018). Hamoy et al. (2018) also observed a predominance of beta oscillations in seizures induced by cunaniol.

The observed oscillation patterns varied in the other low-frequency bands, that is, the theta (4–8 Hz), alpha (8–12 Hz), and gamma bands (28–40 Hz). The theta band is characteristic of low-conscious states (Korotkova et al., 2018; Crivelli-Decker et al., 2018), in which the SP3 pattern is the most prominent. In the case of the alpha band, which is associated with processes of relaxation and imagination (Minami and Amano, 2017; Ahn et al., 2019), no statistical difference was found between the SP2 and SP3 patterns. In the gamma band, by contrast, which is correlated with visual, tactile and auditory stimuli (Merkel et al., 2018; Meier et al., 2020), there was a predominance of SP2 (Fig. 5). The predominant oscillation shifts from pattern SP2 to SP3. In SP2, the predominant power is in the beta wave band, while in SP3, it is in the delta range, with the power alternating during the convulsive condition, depending on the observed pattern.

4.3. Evaluation of the anticonvulsant drugs for the control of caffeine-induced seizures

Diazepam was the anticonvulsant drug that presented the most effective control of caffeine-induced seizures, in comparison with Phenobarbital and Phenytoin. The electrocorticogram of the group pretreated with benzodiazepine indicated a decrease in the intensity of the convulsive condition in comparison with the caffeine group, with a mean value closest to that of the negative control group. Even so, the ECoG potency was not reduced completely, despite causing an improvement in the intensity of the cerebral asynchrony during the convulsion. Marangos et al. (1981), Chweh et al. (1986), and Van Son et al. (2018) tested diazepam for the control of caffeine-induced seizures, although it was not found to be effective. The results of the present study indicate that brain activity did not decrease to baseline levels following the application of diazepam.

Diazepam presented good results for the control of convulsive conditions induced experimentally by cunaniol (Costa et al. 2006; Hamoy et al., 2018), and in the control of the convulsive phase observed during intoxication with oleandrin (De Melo et al., 2020). In general, diazepam is the most effective drug used for the control of seizures caused by chemoconvulsants. The effects of caffeine on the Central Nervous System go beyond the simple antagonism of the adenosine receptor (Bauer and Sander, 2019; Esmaili and Heydari, 2019; Nehlig et al., 1992; Popoli et al., 1987), and may include the antagonism of benzodiazepine receptors, but in this case, the binding is weak, which may be related to the better response of diazepam, although brain activity is not reduced completely (Fig. 6).

A number of previous studies (Morrisett et al., 1987; Suchomelova et al., 2015; Torllner et al., 2016; Rossi et al., 2017) have demonstrated the protective action of Diazepam against the anticonvulsant effects of Pilocarpine. This action is based on the fact that the drug does not permit the spread of convulsive discharges. In caffeine-induced seizures, diazepam reduced the power of the ECoG trace, although it remained above baseline levels.

Jankiewicz et al. (2007) found that caffeine reduced the effects of the anticonvulsants phenobarbital and phenytoin against

electroshock-induced seizures in mice. Despite being based on a different model, the results of the present study found no evidence of any control of the caffeine-induced seizures by phenytoin, and the brain potential recorded by the ECoG trace was still significantly above the baseline level following the administration of phenobarbital (Fig. 6).

While phenytoin is known to increase caffeine clearance and reduced its half-life by nearly 50%. (Bauer and Sander, 2019; Wietholtz et al., 1989), the ECoG traces indicated that neither this drug nor phenobarbital was effective for the control of caffeine-induced seizures (Fig. 6). Previous studies have shown that caffeine lowers the threshold for PTZ-induced seizures and modifies the response to anticonvulsants, carbamazepine, phenytoin, and valproate (Luszczki et al., 2006; Torllner et al., 2016), which is similar to the findings of the present study (Fig. 6). Given this, the consumption of caffeine may interfere with the treatment of epileptic patients.

Chrościńska-Krawczyk et al. (2011) found that caffeine reduces the protective effects of traditional antiepileptic drugs (carbamazepine, phenobarbital, phenytoin, and valproate) as well as the newer compound, topiramate, against electroconvulsiveness in mice. This indicates that methylxanthines should be taken with caution in patients with a low seizure threshold.

5. Conclusions

Convulsions caused by caffeine are less potent than those triggered by pentylenetetrazole. The analysis of the electrocorticographic traces of animals in seizure found three distinct patterns. The highest oscillation in the recording pattern was observed in the beta wave band, which is consistent with other convulsive models. Caffeine-induced seizures are resistant to phenytoin and phenobarbital but respond well to diazepam. These findings provide important insights for the treatment of caffeine intoxication, in particular for individuals with a low seizure threshold.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Ahn, S., Mellin, J.M., Alagapan, S., Alexander, M.L., Gilmore, J.H., Jarskog, L.F., Fröhlich, F., 2019. Targeting reduced neural oscillations in patients with schizophrenia by transcranial alternating current stimulation. Neuroimaging 1 (186), 126–136. https://doi.org/10.1016/j.neuroimage.2018.10.056.

Altermann, M.A., Dias, S.C., Luiz, M.V., Navarro, F., 2008. The influence of caffeine as an ergogenic resource in physical exercise: its action and side effects. Braz. J.Sports Nutr. 2 (10), 225–239.

Babu, K.M., Zuckerman, M.D., Cherkes, J.K., Nrcc, T.C., Hack, J.B., 2011. First seizure after using ENERGY for 5 hours. Pediatr. Emerg. Care 27, 539–540. https://doi.org/10.1097/pec.0b013e31821dc72b.

Banner, W., Czaijka, P., 1980. Acute caffeine overdose in the newborn. Am. J. Dis. Child. 134, 495–498. https://doi.org/10.1001/archpedi.1980.02130170045015.

Bauer, P.R., Sander, J.W., 2019. Caffeine use by people with epilepsy : the myths and the evidence. Curr. Neurol. Neurosci. Rep. 14;19 (6), 32. https://doi.org/10.1007/s11910-019-0948-5.

Bonilha, L., Li, L.M., 2004. Heavy coffee drinking and epilepsy. Apprehension 13, 284–285. https://doi.org/10.1016/S1059-1311(03)00079-7.

Bueno, M.A.S., 2003. The Current Role of Methylxanthines (Aminophylline and Theophylline) in Respiratory Diseases. Hospital Israelita Albert Einstein, Sao Paulo.

Buzsáki, G., Anastassiou, C.A., Koch, C., 2012. The origin of extracellular fields and currents - EEG, ECoG, LFP and spikes. Nat. Rev. Neurosci. 13 (6), 407–420. https://doi.org/10.1038/nrn3241.

Cappelletti, S., Piacentino, D., Fineschi, V., Frati, P., Cipolloni, L., Aromatario, M., 2018. Caffeine-related deaths: form of deaths and risk categories. Nutrients 10, 611, 10.3390/nu10050611.

Chrościńska-Krawczyk, M., Jargiełło-Baszak, M., Wałek, M., Tylus, B., Czuczwar, S.J., 2011. Caffeine and the anticonvulsant potency of antiepileptic drugs: experimental and clinical data. Pharmacol. Rep. 63 (1), 12–18. https://doi.org/10.1016/s1734-1140(11)70394-2.

Chu, N., 1981. Convulsions induced by caffeine and aminophylline. Epilepsy 22, 85–94.

Chweh, A.Y., Ulloque, R.A., Swinyard, E.A., 1986. Benzodiazepine inhibition of [3H] flunitrazepam binding and caffeine-induced seizures in mice. Eur. J. Pharmacol. 122 (2), 161–165. https://doi.org/10.1016/0014-2999(86)90098-1.

coast, A.E., Rocha, F.F., Torres, M.L.B., Souccar, C., De Lima, T.C.M., Lapa, A.J., Lima-Landman, M.T., 2006. Behavioral effects of a neurotoxic compound isolated from Clibadium Surinamense L (Asteraceae). Neurotoxicol. Teratol. 28 (3), 349–353. https://doi.org/10.1016/j.ntt.2006.01.010.

Cohen, S.M., Laurito, C.E., Jo Curran, M., 1992. Grand mal seizure in a postpartum patient after intravenous infusion of caffeine sodium benzoate to treat persistent headache. J. Clin. Anesth. 4, 48–51. https://doi.org/10.1016/0952-8180(92)90121-g.

Crivelli-Decker, J., Hsieh, L.T., Clarke, A., Ranganath, C., 2018. Theta oscillations promote temporal sequence learning. Neurobiol. Learn. Mem. 153 (Pt A), 92–103. https://doi.org/10.1016/j.nlm.2018.05.001.

Cutrufo, C., Bortot, L., Giachetti, A., Manzini, S., 1992. Differential effects of various xanthines on pentylenetetrazol-induced seizures in rats: an EEG and behavioral study. Eur. J. Pharmacol. 222 (1), 1–6. https://doi.org/10.1016/0014-2999(92)90454-c.

Dam, R.M.V., Hu, F.B., Willett, W.C., 2020. Coffee, caffeine and health. N. Engl. J. Med. 383 (4), 369–378. https://doi.org/10.1056/NEJMra1816604.

De Melo, B.S., Morais, B.P., Ferreira, Sá VSS., Lourinho, F.D., Toda, I.P.S.P., Do Nascimento, J.L.M., Marques, D.N., da Silva, M.C.F., Cardoso, G.T.M., Barbas, L.A.L., Torres, M.F., Muto, N.A., de Mello, V.J., Hamoy, M., 2020. Behavioral, electrocorticographic and electromyographic changes induced by the ethanolic extract of Nerium oleander: evaluation of anticonvulsant therapy. Neurotoxicology 78, 21–28. https://doi.org/10.1016/j.neuro.2020.02.001.

Esmaili, Z., Heydari, A., 2019. Effect of acute caffeine administration on PTZ-induced seizure threshold in mice: involvement of adenosine receptors and NO-cGMP signaling pathway. Epilepsy Res. 149, 1–8. https://doi.org/10.1016/j.eplepsyres.2018.10.013, 2019 January.

Fisone, G., Borgkvist, A., Usiello, A., 2004. Caffeine as a psychomotor stimulant: mechanism of action. Cell. Mol. Life Sci. 61, 857–872. https://doi.org/10.1007/s00018-003-3269-3.

Goto, M., Morishita, S., Fukuda, H., 1983. Anticonvulsant action of diazepam in mice pretreated with caffeine. J. Pharmacobio-Dyn 6 (9), 654–659. https://doi.org/10.1248/bpb1978.6.654.

Hamoy, M., Batista, L.S., Melo, V.J., Gomes, W.L., Farias, R.A.F., Batista, P.S., 2018. Seizures elicited by cunaniol: characterization of behavior and electroencephalographic analyses. Toxicol. Appl. Pharmacol. 360, 193–200.

Hoexter, M.Q., Rosa, P.S., Tufik, S., Mello, L.E., 2005. Consequences of prolonged caffeine administration and its withdrawal on pilocarpine and kainate-induced seizures in rats. Epilepsy Set 46 (9), 1401–1406. https://doi.org/10.1111/j.1528-1167.2005.63904.x.

Huang, Z.L., Qu, W.M., Eguchi, N., Chen, J.F., Schwarzschild, M.A., Fredholm, B.B., Urade, Y., Hayaishi, O., 2005. A2A, but not A1, adenosine receptors mediate the arousal effect of caffeine. Nat. Neurosci. 8, 858–859. https://doi.org/10.1038/nn1491.

Iyadurai, S.J.P., Chung, S.S., 2007. New-onset seizures in adults: possible association with consumption of popular energy drinks. Epilepsy Behav. 10, 504–508. https://doi.org/10.1016/j.yebeh.2007.01.009.

Jalilifar, M., Yadollahpour, A., Moazedi, A.A., Ghotbeddin, Z., 2017. Quantitative extracellular EEG assessments to classify specific features of the major phases of seizure acquisition based on the Rat kindling model. Neurosci. Lett. 656, 144–151. https://doi.org/10.1016/j.neulet.2017.07.034.

Jankiewicz, K., Chrościńska-Krawczyk, M., Błaszczyk, B., Czuczwar, S.J., 2007. Caffeine and antiepileptic drugs: experimental and clinical data. Przegl. Lek. 64 (11), 965–967. PMID: 18409414.

Kaufman, K., Sachdeo, R., 2003. Caffeinated beverages and decreased seizure control. Seizure 12, 519–521. https://doi.org/10.1016/S1059-1311(03)00048-7.

Korotkova, T., Ponomarenko, A., Monaghan, C.K., Poulter, S.L., Cacucci, F., Wills, T., Hasselmo, M.E., Lever, C., 2018. Reconciling the different faces of the hippocampal teat : the role of the teat oscillations in cognitive, emotional and innate behaviors. Neurosci. Biobehav. Rev. https://doi.org/10.1016/j.neubiorev.2017.09.004.

Luszczki, J.J., Zuchora, M., Sawicka, K.M., Kozińska, J., Czuczwar, J.S., 2006. Acute exposure to caffeine diminishes the anticonvulsant action of ethosuximide, but not that of clonazepam, phenobarbital, and valproate against pentetrazole-induced seizures in mice. Pharmacol. Rep. 58 (5), 652–659. PMID: 17085857.

Lüttjohann, A., Fabene, P.F., van Luijtelaar, G., 2009. A revised Racine scale for PTZ-induced seizures in rats, 2009 Physiol. Behav. 98 (5), 579–586, 10.1016/j.physbeh.2009.09.005.

Mackow, M.J., Krishnan, B., Bingaman, W.E., Najm, I.M., Alexopoulos, A.V., Nair, D.R., 2016. Increased caffeine intake leads to aggravation of electrocorticographic epileptiform discharges recorded with a responsive neurostimulation device. Clin Neurofisiol 127, 2341–2342.

Maiga, D., Seyni, H., Sidikou, A., Azouma, A., 2012. Seizures in users of tramadol and caffeine: about 8 cases and review of the literature. Pan Afr Med J 13, 24. https://www.ncbi.nlm.nih.gov/pubmed/23308329.

Marangos, P.J., Martino, A.M., Paul, S.M., Skolnick, P., 1981. Benzodiazepines and inosine antagonize caffeine-induced seizures. Psychopharmacology (Berl) 72 (3), 269–273. https://doi.org/10.1007/BF00431829.

Meier, M.A., Lemercier, C.E., Kulisch, C., Kiss, B., Lendvai, B., Adham, N., Gerevich, Z., 2020. The novel antipsychotic cariprazine stabilizes gamma oscillations in rat

hippocampal slices. Br. J. Pharmacol. 177 (7), 1622–1634. https://doi.org/10.1111/bph.14923.Epub.
Merkel, N., Wibral, M., Bland, G., Singer, W., 2018. Endogenously generated gamma band oscillations in the early visual cortex: a neurofeedback study. Hmm Brain Mapp 39 (9), 3487–3502. https://doi.org/10.1002/hbm.24189.
Minami, S., Amano, K., 2017. Perceived illusory jitter in the frequency of alpha oscillations. Curr. Biol. (15), 2344–2351. https://doi.org/10.1016/j.cub.2017.06.033 e4.
Morrisett, R.A., Jope, R.S., Snead, O.C., 1987. Drug effects on initiation and maintenance of status epilepticus induced by pilocarpine administration in lithium-pretreated rats. Exp. Neurol. 97 (1), 193–200. https://doi.org/10.1016/0014-4886(87)90293-7.
Mueller, S.M., Solow, E.B., 1982. Seizures associated with a new combination of stimulant pills. Ann. Neurol. 11, 322. https://doi.org/10.1002/ana.410110316.
Nehlig, A., Daval, J.L., Debry, G., 1992. Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. Res Brain Res Brain Rev 17 (2), 139–170. https://doi.org/10.1016/0165-0173(92)90012-b.
Oliveira, F., Rodrigues, K., Hamoy, M., Sarquis, I.R., Hamoy, A., Lopez, M.E., Ferreira, I., Macchi, B.M., Nascimento, L., 2020. Fatty acid amides synthesized from Andiroba oil (Carapa guianensis Aublet.) Show anticonvulsant action with GABA-A receptor modulation in mice: a putative therapeutic option. J.Pharmaceuticals (Basel). 10 13 (3), 43. https://doi.org/10.3390/ph13030043.
Paech, M., 1996. Unexpected postpartum seizures associated with caffeine-treated post-dural puncture headache. Int. J. Obstet. Anesth. 5, 43–46. https://doi.org/10.1016/s0959-289x(96)80073-2.
Pauwels, R.A., Buist, A.S., Calverley, P.M., Jenkins, C.R., Hurd, S.S., 2001. Global strategy for diagnosis, management and prevention. J Respir Crit Care Med 163, 1256–1276. https://doi.org/10.1164/ajrccm.163.5.2101039.
Paxinos, G., Watson, C., 2005. The rat brain in steretaxic coordinates, 5 ed. Academic Press, New York.
Pong, N.Y., or, T.C.T., Ip, N.Y., 2015. Plant alkaloids as drugs lead to Alzheimer's disease. Neurochem. Int. 89, 260–270. https://doi.org/10.1016/j.neuint.2015.07.018.
Popoli, P., Sagratella, S., Scotti de Carolis, A., 1987. An EEG and behavioral study on the excitatory properties of caffeine in rabbits. Arch. Int. Pharmacodyn. Ther. 290 (1), 5–15.
Röggla, G., Moser, B., 2007. Convulsions in a nightclub. Lancet 370, 220. https://doi.org/10.1016/S0140-6736(07)61121-0, 2007.
Rossi, A., Murta, V., Auzmendi, J., Ramos, A.J., 2017. Early treatment with gabapentin during the latency period raises the seizure threshold, reduces microglial activation and macrophage infiltration in the lithium m-pilocarpine model of epilepsy. Pharmaceuticals 28;10 (4), 93. https://doi.org/10.3390/ph10040093. PMID: 29182533.
Smith, A., 2002. Effects of caffeine on human behavior. Food Chem. Toxicol. 40, 1243–1255. https://doi.org/10.1016/S0278-6915(02)00096-0.
Suchomelova, L., Lopez-Meraz, M.L., Niquet, J., Kubova, H., Wasterlain, C.G., 2015. Hyperthermia exacerbates status epilepticus-induced epileptogenesis and neuronal loss in immature rats. Neuroscience 1 (305), 209–224. https://doi.org/10.1016/j.neuroscience.2015.08.006.
Torllner, K., Twele, F., Löscher, W., 2016. Evaluation of the pentylenetetrazole seizure threshold test in epileptic mice as a surrogate model for drug testing against pharmacoresistant seizures. Epilepsy Behav. 57 (Pt A), 95–104. https://doi.org/10.1016/j.yebeh.2016.01.032.
Turski, W.A., Cavalheiro, E.A., Coimbra, C., da Penha Berzaghi, M., Ikonomidou-Turski, C., Turski, L., 1987. Only a few antiepileptic drugs prevent pilocarpine-induced seizures. *Res. brain* _ 434 (3), 281–305. https://doi.org/10.1016/0165-0173(87)90002-6.
Ullrich, S., de Vries, Y., Kühn, S., Repantis, D., Dresler, M., Ohla, K., Feeling smart, 2015. Effects of caffeine and glucose on cognition, mood and self-judgment. Physiol. Behav. 151, 629–637. https://doi.org/10.1016/j.physbeh.2015.08.028.
Van Koert, R., Bauer, P., Schuitema, I., Sander, J., Visser, G., 2018. Caffeine and seizures: a systematic review and quantitative analysis. Epilepsy Behav. 80 https://doi.org/10.1016/j.yebeh.2017.11.003, 37-4.
Van Son, D., Schalbroeck, R., Angelidis, A., van der Wee, N.J.A., van der Does, W., Putman, P., 2018. Acute effects of caffeine on selective threat attention: moderation by anxiety and EEG theta/beta ratio. Biol. Psychol. 136, 100–110. https://doi:10.1016/j.biopsycho.2018.05.006.
Voiculescu, M., Segarceanu, A., Negutu, M., Ghita, I., Fulga, I., Coman, A.O., 2015. The effect of caffeine on brain asymmetry in rats. J. Med Life. 8 (4), 476–482. https://www.ncbi.nlm.nih.gov/pubmed/26664474.
Wietholtz, H., Zysset, T., Kreiten, K., Kohl, D., Bichsel, R., Matern, S., 1989. Effect of phenytoin, carbamazepine and valproic acid on caffeine metabolism. Eur. J. Clin. Pharmacol. 36, 401–406.
Xu, F., Liu, P., Pekar, J.J., Lu, H., 2015. Does acute caffeine intake alter brain metabolism in young adults? Neuroimaging 110C, 39–47. https://doi.org/10.1016/j.neuroimage.2015.01.046.
Yavuz, M., Albayrak, N., Özgür, M., Gülçebi İdriz Oğlu, M., Çavdar, S., Onat, F., 2019. The effect of prenatal and postnatal caffeine exposure on pentilentetrazole-induced seizures in non-epileptic and epileptic offspring. Neurosci. Lett. 20 (713), 134504 https://doi.org/10.1016/j.neulet.2019.134504.
Zhang, W.Y., 2001. A risk-benefit assessment of caffeine as an analgesic adjuvant. Drug Saf. 24, 1127–1142. https://doi.org/10.2165/00002018-200124150-00004.