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

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# Registro Da Atividade Do Hipocampo E Impacto Da Privação Do Sono E Do Exercício Físico Sobre A Potência Estimulante De Doses Convulsivas De Cafeína

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**Orientador: Prof. Dr. Moisés Hamoy**

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# RESUMO

O nosso trabalho se divide em três momentos. O primeiro tem por objetivo analisar as alterações eletrocorticográficas observadas no hipocampo de ratos Wistar submetidos a doses agudas de cafeína, para registrar a atividade convulsiva e avaliar o efeito de drogas anticonvulsivantes como fenitoína, diazepam e fenobarbital e em um segundo momento é investigar como a privação de sono afeta as propriedades estimulantes da cafeína em camundongos Swiss. O presente estudo avaliou as alterações eletrocorticográficas observadas no hipocampo de ratos Wistar submetidos a doses agudas de cafeína (150mg/kg i.p), o que representa uma dose tóxica de cafeína correspondente a uma ingestão aguda estimada de mais de 12 xícaras de café para registrar sua atividade convulsiva. Nossos resultados mostraram, pela primeira vez, que a administração de altas doses de cafeína (150mg/kg i.p.) em ratos causou um aumento na distribuição espectral de potência em todas as bandas de frequência e sugeriu o aparecimento de períodos de picos ictais e interictais no eletrocorticograma (ECog). Também demonstramos que os anticonvulsivantes fenitoína, diazepam e fenobarbital têm uma resposta satisfatória quando associados à cafeína. Já neste segundo trabalho, é sabido que o sono é essencial para o bem-estar físico e emocional. Foram usados 90 camundongos suíços machos, divididos em 10 grupos (n=9) com e sem privação de sono por 24 horas. Cada grupo recebeu diferentes doses de cafeína (5, 10, 15 e 20 mg/kg) ou solução salina. Os registros de ECoG foram feitos após a administração das substâncias. Os registros de ECoG dos grupos tratados com cafeína mostraram variações na amplitude e na frequência das ondas cerebrais, dependendo da dose administrada. Foi observado um aumento na potência linear total e nas oscilações delta e teta nos grupos tratados com cafeína em comparação com o grupo de controle. A privação do sono diminui a eficácia da cafeína em camundongos suíços, conforme evidenciado pelas alterações nos registros de ECoG. E por fim, neste terceiro momento sabe-se que o uso de substâncias ergogênicas, como a cafeína, ganhou destaque na melhoria do desempenho físico e cognitivo. O objetivo deste estudo foi avaliar os efeitos da administração pós-exercício de diferentes doses de cafeína (10, 20 e 30 mg/kg) sobre a atividade cortical de camundongos suíços por meio de eletrocorticografia (ECoG). Um total de 72 camundongos suíços machos foram submetidos a exercícios de natação forçada, seguidos pela administração intraperitoneal de cafeína. Os registros de ECoG analisaram as oscilações em várias bandas de frequência (delta, teta, alfa, beta e gama) para investigar o impacto neurofisiológico da cafeína no contexto pós-exercício. Os resultados demonstraram que doses mais altas de cafeína, especialmente 30 mg/kg, aumentaram significativamente as oscilações corticais nas bandas beta e gama, sugerindo maior excitabilidade neuronal. Além disso, os animais tratados com essa dose mais alta apresentaram atividade semelhante à ictal, indicando um possível risco de eventos convulsivos. Essas descobertas destacam uma relação dependente da dose entre a cafeína e a atividade cortical, enfatizando a necessidade de cautela no uso de altas doses de cafeína em contextos esportivos devido aos possíveis efeitos adversos no sistema nervoso central.



**Palavras-chave:** Cafeína, convulsão, eletrocorticografia, hipocampo, privação de sono, cafeína, eletrocorticograma, EcoG.

# ABSTRACT

Our study is divided into three phases. The first phase aims to analyze the electrocorticographic changes observed in the hippocampus of Wistar rats subjected to acute doses of caffeine, to record convulsive activity and evaluate the effect of anticonvulsant drugs such as phenytoin, diazepam, and phenobarbital. This study evaluated the electrocorticographic changes observed in the hippocampus of Wistar rats subjected to acute doses of caffeine (150 mg/kg i.p.), which represents a toxic dose of caffeine corresponding to an estimated acute intake of more than 12 cups of coffee, to record its convulsive activity. Our results showed, for the first time, that the administration of high doses of caffeine (150 mg/kg i.p.) in rats caused an increase in spectral power distribution across all frequency bands and suggested the appearance of ictal and interictal spikes in the electrocorticogram (ECoG). We also demonstrated that the anticonvulsants phenytoin, diazepam, and phenobarbital have a satisfactory response when associated with caffeine. In this second phase, it is known that sleep is essential for physical and emotional well-being. A total of 90 male Swiss mice were used, divided into 10 groups (n=9) with and without sleep deprivation for 24 hours. Each group received different doses of caffeine (5, 10, 15, and 20 mg/kg) or saline solution. ECoG recordings were made after the administration of the substances. ECoG recordings from the caffeine-treated groups showed variations in the amplitude and frequency of brain waves, depending on the administered dose. An increase in total linear power and delta and theta oscillations was observed in the caffeine-treated groups compared to the control group. Sleep deprivation decreases the effectiveness of caffeine in Swiss mice, as evidenced by changes in the ECoG recordings. Finally, in this third phase, it is known that the use of ergogenic substances, such as caffeine, has gained prominence in improving physical and cognitive performance. The objective of this study was to evaluate the effects of post-exercise administration of different doses of caffeine (10, 20, and 30 mg/kg) on cortical activity in Swiss mice using electrocorticography (ECoG). A total of 72 male Swiss mice were subjected to forced swimming exercises, followed by intraperitoneal administration of caffeine. The ECoG recordings analyzed oscillations in various frequency bands (delta, theta, alpha, beta, and gamma) to investigate the neurophysiological impact of caffeine in the post-exercise context. The results demonstrated that higher doses of caffeine, especially 30 mg/kg, significantly increased cortical oscillations in the beta and gamma bands, suggesting increased neuronal excitability. Additionally, animals treated with this higher dose exhibited ictal-like activity, indicating a possible risk of convulsive events. These findings highlight a dose-dependent relationship between caffeine and cortical activity, emphasizing the need for caution when using high doses of caffeine in athletic contexts due to potential adverse effects on the central nervous system.



**Keywords:** Caffeine, seizure, electrocorticography, hippocampus, sleep deprivation, caffeine, electrocorticogram, EcoG.

*“Verei em Ti tão profundo amor*
*Que irá minha alma inundar*
*Sentirei de Ti uma paz tão maior*
*Meus medos irá dissipar”*

*Guilherme Kerr – Estar Contigo*

## LISTA DE ILUSTRAÇÕES

## Lista de Tabelas

## LISTA DE SIGLAS E SÍMBOLOS

| | |
|---|---|
| SNC | Sistema Nervoso Central |
| ECoG | Eletrocorticográficas |
| OMS | Organização Mundial da Saúde |
| Mg/Kg | Miligrama por Kilograma |
| I.P. | Intraperitoneal |
| N | Número |
| Fig. | Figura |

# SUMÁRIO

**Agradecimentos**

Eu sempre aprendi que tudo que a gente conta é como se estivéssemos contando uma história, e, como todas as histórias, a minha tem personagens importantes. Personagens esses que me trouxeram até aqui.

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gentil e amável. As minhas colaboradoras tão gentis que tornam meus dias alegres, Dani, Clarissa, Thaysa, Luana, Priscille, vocês são presentes divinos. Ao meu orientador, professor Moises Hamoy, ou seria José? Haha, que é a eterna figurinha do zeca pagodinho dizendo é nós e que durante esse período de doutoramento se tornou mais do que um mentor, mas um amigo que divide as risadas, as tribulações e nos trata como filhos. Eu talvez nunca consiga agradecer tamanha instrução, paciência e dedicação. Isso, como já disse, não é um adeus, é só um até logo de quem quer uma semana de férias haha, então não me tire do rol de funcionários e colaboradores.

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Nós conseguimos. Sim, nós, porque uma andorinha não faz verão sozinha e eu sei que ao meu lado existem pessoas que tornaram o dia de hoje possível.

A todos os presentes, muito obrigada. A jornada não teria sido a mesma sem vocês.

1. Introdução

A cafeína é uma substância psicoativa amplamente consumida ao redor do mundo, presente em diversas plantas, em bebidas como café, chá, refrigerantes, bebidas energéticas e até mesmo em alguns alimentos e medicamentos, com uso documentado desde o período paleolítico (Barone & Roberts, 1996; Cappelletti et al., 2015). O café é a bebida mais conhecida no mundo inteiro e a mais popular, perdendo apenas para a água. Estima-se que cerca de 1,6 bilhões de xícaras de café são consumidas diariamente no mundo inteiro (Cappelletti et al., 2015). Já em países ocidentais, estima-se que aproxidamente cerca de 90% da população seja consumidora ativa de café, com um consumo diário em aproximadamente de 200mg/dia em uma pesquisa realizada de 2009-2010 (Fulgoni et al., 2010).

Os primeiros avanços científicos sobre esse tema ocorreram em 1911/1912, quando Harry Levi Hollingworth publicou uma série de estudos investigando os efeitos da cafeína no comportamento, incluindo a qualidade do sono. Hollingworth foi pioneiro ao empregar um desenho experimental duplo-cego com controle por placebo, uma metodologia que ainda hoje é considerada o "padrão ouro" em estudos farmacológicos. No estudo, 16 participantes foram submetidos a um rigoroso protocolo de 40 dias em um laboratório dedicado. Apesar de observar variações significativas entre os indivíduos nas queixas de distúrbios do sono, ele concluiu que doses de cafeína superiores a "6 grãos" prejudicavam o sono na maioria dos participantes (Hollingworth, 1912).

Do ponto de vista químico, a cafeína é um alcaloide pertencente à família das metilxantinas, que inclui também a teofilina e a teobromina (Biaggioni et al., 1991; Reichert et al., 2022). Sua fórmula molecular é $C_8H_{10}N_4O_2$, e ela atua principalmente como um estimulante do sistema nervoso central. A ação estimulante da cafeína se dá, em grande parte, pela sua capacidade de bloquear os receptores de adenosina no cérebro. A adenosina é um neurotransmissor inibitório que promove o sono e o relaxamento ao se ligar a seus receptores específicos. Ao bloquear esses receptores, a cafeína impede a ação sedativa da adenosina, resultando em um aumento na liberação de outros neurotransmissores excitatórios, como a dopamina e a norepinefrina. Isso culmina em uma maior sensação de alerta, redução da fadiga e melhoria temporária na capacidade de concentração e desempenho cognitivo (Evans et al., 2024).

O impacto da cafeína no organismo humano vai além do sistema nervoso central. Ela possui efeitos variados no sistema cardiovascular, podendo levar a um aumento temporário da pressão arterial e da frequência cardíaca (van Dam et al., 2023; Turnbull et al., 2017; Ding et al., 2015). Em doses moderadas, esses efeitos são geralmente bem tolerados pela maioria dos indivíduos saudáveis. Contudo, em pessoas com certas condições cardiovasculares, o consumo excessivo de cafeína pode apresentar riscos. A cafeína também afeta o metabolismo, promovendo a lipólise, que é a quebra de gorduras armazenadas no tecido adiposo, e aumentando a termogênese, o que pode contribuir para a

perda de peso em certos contextos. Além disso, ela influencia o desempenho físico, atuando como um ergogênico ao aumentar a resistência e reduzir a percepção de esforço durante exercícios físicos intensos (van Dam et al., 2023) (Fig. 1).

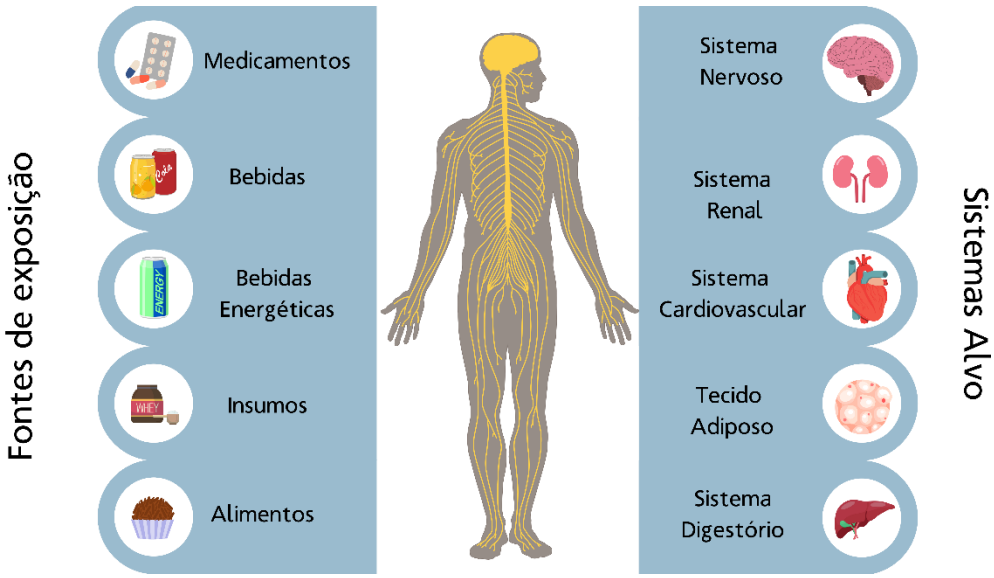


**Fig. 1:** Fontes comuns de cafeína, como café, chá, refrigerantes e energéticos, são representadas ao lado dos principais órgãos-alvo afetados pelo consumo da substância. A cafeína impacta diretamente o cérebro, onde atua como estimulante, o coração, aumentando a frequência cardíaca, o fígado, onde é metabolizada, e os rins, afetando a diurese. A imagem ilustra a distribuição e os efeitos da cafeína no sistema nervoso central, cardiovascular, e nos sistemas renal e hepático.

Contudo, a relação entre cafeína e saúde é complexa e multifacetada. O consumo crônico e em altas doses pode levar ao desenvolvimento de tolerância, dependência e síndrome de abstinência, caracterizada por sintomas como dores de cabeça, fadiga, irritabilidade e depressão leve quando a ingestão de cafeína é abruptamente reduzida ou interrompida (Guest et al., 2021). Esses efeitos adversos são resultado de adaptações neurofisiológicas que ocorrem no cérebro em resposta à exposição prolongada à cafeína. Há também um ramo crescente de pesquisa que investiga a associação entre o consumo de cafeína e a prevenção de doenças neurodegenerativas, como a doença de Alzheimer e o Parkinson (Larsson et al., 2022). Estudos epidemiológicos sugerem que consumidores regulares de café têm um risco reduzido de desenvolver essas doenças, possivelmente devido às propriedades antioxidantes da cafeína e outros compostos presentes no café, bem como sua capacidade de modular a neuroinflamação e a homeostase do cálcio neuronal (Woolf et al., 2023; Zhang et al., 2024).

Em suma, a cafeína é uma substância de efeitos vastos e variados, que atua em múltiplos sistemas fisiológicos. Embora seu consumo moderado seja geralmente considerado seguro e até benéfico em alguns contextos, é crucial que seu uso seja equilibrado e que se leve em consideração as peculiaridades de cada indivíduo para evitar potenciais efeitos adversos. A compreensão dos mecanismos de ação da cafeína continua a se expandir, revelando tanto suas vantagens quanto suas limitações no contexto da saúde humana.

## 1.1. Farmacologia da cafeína

A farmacologia da cafeína envolve uma série de processos complexos que afetam diversos sistemas fisiológicos no corpo humano. A cafeína, ou 1,3,7-trimetilxantina, é um alcaloide metilxantina que age predominantemente como um estimulante do sistema nervoso central (SNC) (Reichert et al., 2022; Ingiosi et al., 2020). Sua estrutura química permite que ela atravesse facilmente a barreira hematoencefálica, o que facilita seus efeitos psicoativos. A cafeína é rapidamente absorvida no trato gastrointestinal, com níveis plasmáticos de pico geralmente alcançados entre 30 a 60 minutos após a ingestão. Além de seu antagonismo aos receptores de adenosina, a cafeína também aumenta os níveis de ciclo AMP (adenosina monofosfato cíclico) intracelular ao inibir a enzima fosfodiesterase. Isso leva a uma amplificação dos sinais mediados por cAMP em várias células, contribuindo para seus efeitos estimulantes. A cafeína também pode influenciar a liberação de cálcio dos estoques intracelulares, afetando ainda mais a contratilidade muscular e a excitabilidade neuronal (Evans et al., 2024; Ferré, 2008; Fisone, 2004) (Fig. 2).

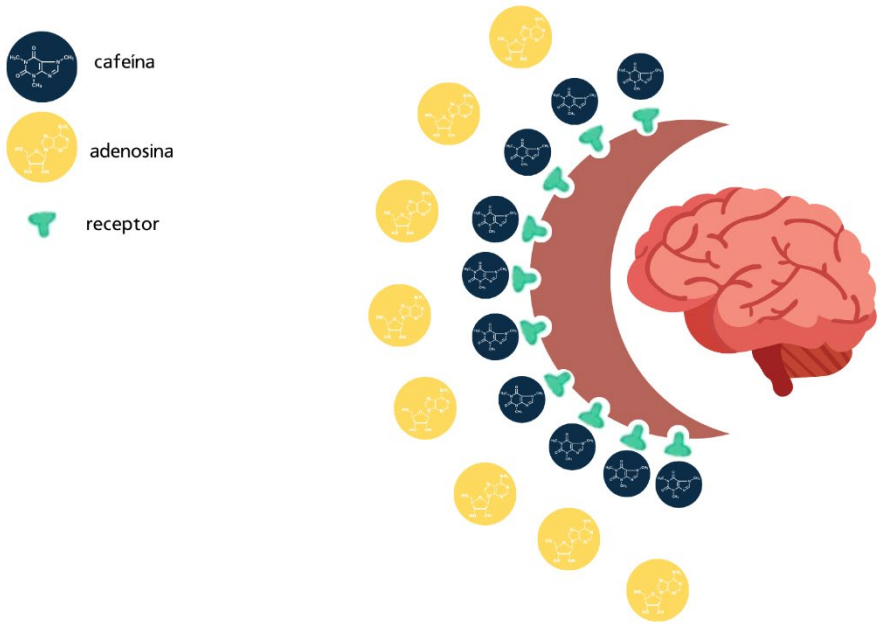


**Fig.2:** A cafeína exerce seus efeitos estimulantes no cérebro ao bloquear os receptores de adenosina, uma substância neuromoduladora que promove o relaxamento e o sono. Normalmente, a adenosina se liga aos seus receptores, diminuindo a atividade neuronal e induzindo a sensação de cansaço. No entanto, ao competir com a adenosina, a cafeína impede essa ligação, resultando em maior estado de alerta e excitação cerebral. Esta imagem ilustra como a cafeína interfere na ação da adenosina, destacando os receptores no cérebro que regulam a sensação de sonolência e o ciclo sono-vigília.

Após a ingestão, a cafeína é rapidamente absorvida no estômago e no intestino delgado. Sua biodisponibilidade é alta, geralmente em torno de 99%, e ela é amplamente distribuída por todo o corpo (Cappelletti et al., 2015; Barcelos et al., 2020). A meia-vida da cafeína varia consideravelmente entre os indivíduos, com uma média de 3 a 5 horas em adultos saudáveis. No entanto, essa meia-

vida pode ser influenciada por vários fatores, incluindo idade, gravidez, função hepática, uso de contraceptivos orais e variações genéticas no metabolismo. A cafeína é metabolizada no fígado, principalmente pelo sistema enzimático do citocromo P450, especificamente a enzima CYP1A2. O metabolismo hepático da cafeína resulta em três principais metabólitos: paraxantina, teobromina e teofilina, cada um dos quais possui propriedades farmacológicas próprias. A excreção da cafeína e seus metabólitos é realizada principalmente pelos rins (Barcelos et al., 2020; Newton et al., 1981).

Os efeitos fisiológicos da cafeína são extensos. No SNC, além de aumentar o estado de alerta e reduzir a sensação de fadiga, a cafeína pode melhorar o desempenho cognitivo em tarefas que exigem atenção sustentada e velocidade de processamento (Sanchis et al., 2020). Ela também tem efeitos positivos no humor e pode reduzir os sintomas de depressão leve a moderada. No sistema cardiovascular, a cafeína pode aumentar a pressão arterial e a frequência cardíaca, embora esses efeitos tendam a diminuir com o uso regular devido ao desenvolvimento de tolerância (Ding et al., 2015). No metabolismo, a cafeína promove a lipólise, aumentando a concentração de ácidos graxos livres no sangue e favorecendo a utilização de gorduras como fonte de energia. Ela também pode aumentar a termogênese, contribuindo para o gasto energético total. No entanto, os efeitos metabólicos da cafeína podem variar amplamente entre os indivíduos (Kennedy et al., 2022).

O uso regular de cafeína leva ao desenvolvimento de tolerância, que é caracterizada por uma diminuição nos efeitos fisiológicos e psicológicos da substância com o tempo. A tolerância resulta de adaptações neurofisiológicas, incluindo a upregulation dos receptores de adenosina no cérebro. Quando a ingestão de cafeína é interrompida, esses mecanismos adaptativos podem levar à síndrome de abstinência, que inclui sintomas como dores de cabeça, fadiga, irritabilidade e depressão leve. A cafeína pode interagir com várias outras substâncias e medicamentos. Por exemplo, o uso concomitante de cafeína e certos inibidores da CYP1A2, como a fluvoxamina, pode aumentar os níveis plasmáticos de cafeína e potencializar seus efeitos. Da mesma forma, o tabagismo pode induzir a atividade da CYP1A2, reduzindo a meia-vida da cafeína. A cafeína também pode potencializar os efeitos de outros estimulantes do SNC e contrapor-se aos efeitos de medicamentos sedativos (Cornelis et al., 2016).

A cafeína aumenta a liberação de dopamina, particularmente no núcleo accumbens, uma região do cérebro associada ao prazer e à recompensa (Ferré et al., 2016). Este efeito pode contribuir para a sensação de bem-estar e o reforço positivo associado ao consumo de cafeína. Ao aumentar os níveis de norepinefrina, a cafeína promove a ativação do sistema nervoso simpático, o que resulta em uma série de respostas fisiológicas, incluindo aumento da frequência cardíaca, pressão arterial e mobilização de energia (Turgeon et al., 202). A cafeína pode também aumentar a liberação de glutamato, um neurotransmissor excitatório que desempenha um papel crucial na plasticidade sináptica e na memória. Este aumento pode contribuir para a melhoria temporária das funções

cognitivas (Mikami et al., 2015). A cafeína pode inibir indiretamente a atividade do ácido gama-aminobutírico (GABA), um neurotransmissor inibitório que promove a calma e o relaxamento. A redução da atividade do GABA contribui para os efeitos estimulantes da cafeína.

A compreensão detalhada da farmacologia da cafeína é essencial para seu uso clínico e a avaliação de seus riscos e benefícios. Em doses moderadas, a cafeína é geralmente segura para a maioria dos indivíduos e pode oferecer vários benefícios, incluindo melhorias no desempenho cognitivo e físico. No entanto, doses elevadas ou o uso em indivíduos sensíveis podem levar a efeitos adversos significativos, como ansiedade, insônia, taquicardia e aumento do risco de distúrbios cardiovasculares. Desta forma, a cafeína é uma substância multifacetada com uma ampla gama de efeitos farmacológicos. Seu uso seguro e eficaz requer uma compreensão dos mecanismos subjacentes de sua ação, suas interações com outros medicamentos e as variações individuais na resposta à cafeína. A pesquisa contínua sobre os efeitos da cafeína e suas aplicações clínicas continua a ser uma área de grande interesse e importância na farmacologia moderna.

### 1.2. Cafeína e metabolismo

A cafeína é um estimulante do sistema nervoso central (SNC) que promove a secreção hormonal e altera parâmetros bioquímicos e fisiológicos. Sua rápida absorção pelo trato gastrointestinal permite ampla distribuição no corpo, incluindo a travessia da barreira hematoencefálica devido às suas propriedades lipofílicas. No entanto, essa absorção pode ser influenciada por fatores como pH, via de administração e características químicas. A concentração de cafeína no fluido extracelular é similar à sanguínea, e o metabolismo da cafeína culmina em sua eliminação renal. A meia-vida da cafeína varia de acordo com a dose, sendo menor em doses baixas (2,5 a 10 horas) e mais longa em doses maiores.

A cafeína exerce efeitos em vários tecidos, como o SNC, músculos esqueléticos, cardiovascular, renal e pulmonar. Seu metabolismo está relacionado à atividade da enzima hepática CYP1A2, responsável pela transformação da cafeína em teobromina, paraxantina e teofilina. A coadministração de medicamentos, como antibióticos e antidepressivos, pode interferir nesse metabolismo. Alterações no metabolismo da cafeína foram observadas em casos de disfunção hepática, no período neonatal e durante a gravidez. Os efeitos biológicos da cafeína estão relacionados principalmente à sua ação antagonista nos receptores de adenosina, inibição das fosfodiesterases e mobilização de cálcio.

A cafeína e seus metabólitos, devido à sua semelhança estrutural com a purina, interagem com os receptores de adenosina, influenciando o metabolismo celular e a inflamação. A cafeína pode suprimir os efeitos da adenosina em baixas doses, agravando a inflamação, enquanto em altas doses reduz danos inflamatórios. Ela também modula o metabolismo de lipídios e glicose, estimulando a lipólise e o metabolismo de gorduras por meio da ativação de catecolaminas e inibição de fosfodiesterases. No entanto, a cafeína pode

aumentar a glicemia e afetar a homeostase da glicose, alterando o metabolismo de glicogênio e o cálcio intracelular, o que afeta neurotransmissores e processos celulares.

O oxigênio ($O_2$) é essencial para a vida, mas sua redução produz radicais livres que podem causar estresse oxidativo, danificando lipídios, proteínas e ácidos nucleicos. Embora as espécies reativas de oxigênio (ROS) em níveis baixos sejam importantes para funções celulares, seu excesso está ligado à inflamação e a várias doenças crônicas, como diabetes, câncer e doenças neurodegenerativas. No sistema nervoso central (SNC), o estresse oxidativo e a inflamação exacerbam condições como Alzheimer, Parkinson e lesão cerebral traumática, devido à alta vulnerabilidade do cérebro. Estratégias terapêuticas antioxidantes e anti-inflamatórias, como o café, têm mostrado potencial neuroprotetor. A literatura atual revela que, além de seus efeitos psicoativos, a cafeína afeta os sistemas endócrino, cardiovascular, respiratório, renal e gastrointestinal. Seus efeitos variam com a dose e podem influenciar condições como hipertensão. A cafeína apresenta propriedades antioxidantes, reduzindo o estresse oxidativo e a peroxidação lipídica, além de melhorar o estado redox em diversos tecidos, como o cérebro e fígado. Estudos mostram que a cafeína atenua marcadores pró-inflamatórios e o acúmulo de β-amiloide no hipocampo, ajudando a combater inflamações e neurodegeneração, como no Alzheimer, por meio da modulação de vias inflamatórias, como a NF-κB.

Evidências recentes sugerem que a cafeína e seus metabólitos atuam em vias de sinalização redox e inflamatórias. A produção excessiva de espécies reativas de oxigênio (ROS) ativa o fator de transcrição NF-κB, responsável pela expressão de citocinas inflamatórias, como IL-1β e TNF-α. A via de ativação do NF-κB é mediada por receptores celulares estimulados por ROS e inflamação. Embora o mecanismo de ativação por ROS ainda não seja totalmente claro, sabe-se que o NF-κB pode tanto induzir como proteger contra o estresse oxidativo.

Como já dito anteriormente, a cafeína, por sua vez, é um antagonista dos receptores de adenosina, e essa ação pode modular a produção de citocinas inflamatórias, dependendo da dose. Em doses baixas, a cafeína inibe a produção de citocinas pró-inflamatórias, enquanto em doses altas pode aumentar a inflamação. Além disso, há indícios de que a cafeína modula o NF-κB em células microgliais, contribuindo para seus efeitos anti-inflamatórios. O processo inflamatório e o estresse oxidativo são estreitamente relacionados, e compostos antioxidantes, como a cafeína, podem ter efeitos benéficos em modular essas respostas inflamatórias.

### 1.3. A cafeína e a privação de sono

O sono é um estado fisiológico complexo e essencial para a saúde e o bem-estar humanos. Ele desempenha um papel crítico em diversas funções biológicas, incluindo a regulação do humor, a consolidação da memória, a

recuperação física e a manutenção do sistema imunológico. O sono é dividido em dois estados principais: o sono de movimento rápido dos olhos (REM, do inglês Rapid Eye Movement) e o sono não-REM (NREM), que por sua vez é subdividido em quatro estágios distintos (Peever et al., 2017). Cada um desses estágios desempenha funções específicas e é regulado por complexos mecanismos neurofisiológicos. Durante o sono NREM, o corpo passa por uma série de processos reparadores. Nos estágios 3 e 4 do sono NREM, conhecidos como sono de ondas lentas, ocorre a liberação de hormônios de crescimento e a reparação dos tecidos. É também durante esses estágios que o cérebro processa e consolida informações, transformando experiências recentes em memórias de longo prazo. O sono REM, por outro lado, é crucial para a regulação emocional e a cognição. Durante esse estágio, ocorrem os sonhos mais vívidos, e o cérebro processa emoções e experiências, ajudando a manter a estabilidade emocional (Buysse et al., 1991; Barbato et al., 2021) (Tabela 1).

| **Sono Não REM** | |
|---|---|
| NREM 1 | Sono leve, em que ainda estamos cochilando e podemos despertar com qualquer barulho |
| NREM 2 | Sono leve intermediário, em que o batimento cardíaco e a respiração diminuem, os músculos relaxam mais, a temperatura corporal cai e o movimento dos olhos cessa |
| NREM 3 | Sono profundo, que ocorre principalmente durante a primeira metade da noite e é fundamental para o descanso do corpo |
| | |
| **Sono REM** | |
| O sono REM (Rapid Eye Movement) é uma fase do ciclo do sono caracterizada por movimentos rápidos dos olhos, intensa atividade cerebral, e a ocorrência de sonhos vívidos. Durante essa fase, o cérebro está quase tão ativo quanto quando estamos acordados, mas o corpo permanece praticamente paralisado devido à inibição motora que impede movimentos voluntários. O sono REM desempenha um papel crucial na consolidação da memória, no aprendizado e na regulação emocional. | |

Tabela 1: Tabela ilustrando as diferentes fases do ciclo do sono, que incluem o sono não-REM (NREM) e o sono REM. As fases NREM estão divididas em três estágios: o sono leve (estágios 1 e 2), que corresponde ao início do adormecimento e relaxamento corporal, e o sono profundo (estágio 3), essencial para a restauração física. O sono REM, caracterizado por movimentos rápidos dos olhos e intensa atividade cerebral, é fundamental para a consolidação da memória e o processamento emocional. A tabela descreve as principais características de cada fase, como a atividade cerebral, o tônus muscular, a frequência cardíaca e a ocorrência de sonhos.

A privação de sono, definida como a insuficiência crônica ou aguda de sono, tem efeitos deletérios em quase todos os aspectos da saúde humana (Borbély et al., 1981; Schiller et al., 2021; Sang et al., 2023). A curto prazo, a privação de sono pode levar a prejuízos cognitivos significativos, incluindo

diminuição da atenção, redução do tempo de reação, dificuldades de aprendizado e problemas de memória (Konduru et al., 2021). Esses efeitos podem ser particularmente perigosos em atividades que exigem vigilância constante, como dirigir ou operar máquinas pesadas. A longo prazo, a privação crônica de sono está associada a uma série de problemas de saúde. Estudos epidemiológicos indicam que a falta de sono crônica está correlacionada com um aumento no risco de várias doenças, incluindo hipertensão, doenças cardiovasculares, diabetes tipo 2, obesidade e certos tipos de câncer. Isso se deve, em parte, à disfunção metabólica induzida pela privação de sono, que inclui a resistência à insulina e o aumento do apetite por alimentos ricos em calorias (Benington et al., 1995).

Durante o sono, o cérebro passa por diferentes fases, cada uma delas caracterizada por tipos específicos de ondas cerebrais que podem ser observadas em um eletroencefalograma (EEG) (Girardeau et al., 2021; Lewis et al., 2021). Essas ondas variam em frequência e amplitude, refletindo os níveis de atividade cerebral em cada fase. As ondas Beta (12-30 Hz) estão presentes principalmente quando estamos acordados e em estado de alerta ou foco mental. No entanto, durante o sono REM, as ondas betas também aparecem, pois o cérebro está quase tão ativo quanto no estado de vigília (Fernandez et al., 2020). Essa fase está associada a sonhos vívidos e movimentos oculares rápidos. Já as ondas Alfa (8-12 Hz) são típicas durante o relaxamento e a transição entre a vigília e o sono, especialmente no estágio 1 do sono não-REM (NREM). Essas ondas indicam um estado de relaxamento, quando estamos com os olhos fechados, mas ainda conscientes. Após essas ondas, ocorrem as ondas Teta (4-8 Hz) que ocorrem durante os estágios 1 e 2 do sono NREM, que correspondem ao início do sono leve. Elas são associadas à transição entre o estado de vigília e o sono mais profundo, refletindo um estado de consciência reduzida e relaxamento mais profundo (Lafortune et al., 2014).

Além disso, temos os fusos de sono e complexos K que aparecem no estágio 2 do sono NREM. Fusos de sono são breves explosões de atividade rápida (12-16 Hz), enquanto os complexos K são grandes ondas lentas e de alta amplitude. Ambos são importantes para a consolidação da memória e a estabilidade do sono. Após isso, temos as ondas Delta (0.5-4 Hz) são típicas do estágio 3 do sono NREM, também conhecido como sono profundo ou de ondas lentas (sono delta). Essas ondas são de baixa frequência e alta amplitude, indicativas de um estado de sono profundo e restaurador. O sono delta é fundamental para a recuperação física e regeneração celular. Essas ondas cerebrais variam conforme o ciclo do sono progride, alternando entre fases de sono leve, profundo e REM, cada uma desempenhando um papel crucial no descanso e nas funções cognitivas e corporais (Girardeau et al., 2021; Lewis et al., 2021) (Tabela 2).

| **ESTÁGIOS DO SONO** | |
|---|---|
| Vigília | |
| Estágio 1 do sono não REM | Ondas teta |
| Estágio 2 do sono | Ondas teta+ complexo k + fuso |

| | |
|---|---|
| Estágio 3 do sono | Ondas delta |
| Estágio 4 do sono | Ondas delta atingem maior amplitude e menor frequência |
| **Sono REM** | |
| Ondas beta e gama | Estado de vigília |

**Tabela 2:** Estágios do Sono e suas Correspondentes Ondas Cerebrais. Esta tabela apresenta uma visão geral dos diferentes estágios do sono, incluindo o sono leve, sono profundo e sono REM, e as respectivas ondas cerebrais associadas a cada fase, destacando as características eletrofisiológicas que definem cada estágio e suas implicações para a saúde e bem-estar.

A interação da cafeína com o sono e a privação de sono é um campo de estudo complexo que envolve a compreensão de mecanismos neuroquímicos, fisiológicos e comportamentais (Gardiner et al., 2023). A cafeína, sendo um estimulante do sistema nervoso central (SNC), exerce efeitos significativos na arquitetura do sono e no estado de vigília, influenciando tanto a qualidade quanto a quantidade de sono (Temple et al., 2017). Essa interação é mediada principalmente pelo antagonismo da cafeína aos receptores de adenosina no cérebro, mas envolve também uma série de outros processos neurobiológicos. A ingestão de cafeína, especialmente nas horas que precedem o período de sono, pode aumentar a latência do sono, que é o tempo necessário para adormecer. Isso ocorre porque a cafeína impede a ação sedativa da adenosina, mantendo o cérebro em um estado mais alerta. A cafeína pode alterar a arquitetura do sono, reduzindo a quantidade de sono de ondas lentas (NREM estágios 3 e 4), que é crucial para a recuperação física e consolidação da memória (Drake et al., 2013). Ela também pode diminuir o tempo total de sono REM, que é importante para a regulação emocional e processos cognitivos.

Além dos efeitos físicos, a privação de sono tem um impacto profundo na saúde mental. A falta crônica de sono está fortemente associada ao desenvolvimento de transtornos de humor, como depressão e ansiedade (Reynolds et al., 2010; Krause et al., 2017; Sang et al., 2023). A privação de sono afeta os neurotransmissores e os circuitos neurais envolvidos na regulação do humor, o que pode levar a alterações emocionais significativas e à exacerbação de sintomas psiquiátricos. Há também evidências de que a privação de sono pode precipitar episódios de mania em indivíduos com transtorno bipolar. A privação de sono também afeta o sistema imunológico (Schiller et al., 2021). A falta de sono crônica resulta em uma resposta imunológica prejudicada, aumentando a suscetibilidade a infecções e reduzindo a eficácia das vacinas. Estudos mostram que indivíduos privados de sono apresentam uma menor produção de citocinas, proteínas que desempenham um papel crucial na resposta imunológica, e uma redução na atividade das células naturais killer (NK), que são essenciais para a defesa contra vírus e tumores (Vaccaro et al., 2020; Clark et al., 2014; Everson et al., 2014).

A regulação do sono é mediada por uma interação complexa entre processos homeostáticos e circadianos (Borbély et al., 2016; Daan et al., 1984; Allada et al., 2017; Baranwal et al., 2023). O processo homeostático refere-se à necessidade acumulada de sono, que aumenta quanto mais tempo uma pessoa permanece acordada. O processo circadiano é regulado pelo núcleo

supraquiasmático (SCN) do hipotálamo, que sincroniza o ciclo sono-vigília com o ambiente externo, principalmente através da exposição à luz (Baranwal et al., 2023). A disrupção desses processos, como ocorre com trabalhadores em turnos noturnos ou indivíduos com jet lag, pode resultar em dificuldades para adormecer, manutenção do sono e qualidade do sono. O sono é um componente essencial e multifacetado da saúde humana, influenciando uma ampla gama de processos fisiológicos e psicológicos (Matenchuk et al., 2020). A privação de sono, seja aguda ou crônica, tem efeitos profundos e negativos em quase todos os aspectos do funcionamento humano. A compreensão da importância do sono e a adoção de hábitos saudáveis de sono são cruciais para a promoção da saúde e do bem-estar geral. A pesquisa contínua sobre os mecanismos do sono e as consequências da privação de sono é fundamental para desenvolver estratégias eficazes para mitigar os impactos negativos da falta de sono e melhorar a qualidade de vida.

### 1.4. Efeitos Fisiológicos e Comportamentais

A cafeína é amplamente utilizada para combater a sonolência e aumentar o estado de alerta. Isso é especialmente útil em situações que requerem atenção sustentada, como dirigir ou estudar. Estudos mostram que a cafeína pode melhorar o desempenho em tarefas que exigem atenção, tempo de reação e processamento de informações. No entanto, esses efeitos variam de acordo com a dose e a sensibilidade individual (Gardiner et al., 2023). A capacidade da cafeína de reduzir a sensação de fadiga a torna popular entre trabalhadores em turnos, estudantes e atletas. Ela ajuda a prolongar a vigília e melhorar o desempenho físico em atividades de resistência. A cafeína pode melhorar o humor e reduzir os sintomas de depressão leve, provavelmente devido ao aumento da dopamina e norepinefrina no cérebro. No entanto, o consumo excessivo pode levar a ansiedade, nervosismo e irritabilidade (Fang et al., 2023).

O uso regular de cafeína leva ao desenvolvimento de tolerância, o que significa que doses maiores são necessárias para obter os mesmos efeitos. Isso ocorre devido à adaptação dos receptores de adenosina, que aumentam em número (upregulation) para compensar o bloqueio contínuo pela cafeína. Além disso, a cafeína pode causar dependência física. A retirada abrupta pode resultar em sintomas de abstinência, como dores de cabeça, fadiga, irritabilidade e humor deprimido. Os efeitos a longo prazo do consumo regular de cafeína no sistema nervoso central ainda são objeto de pesquisa. Embora a cafeína em doses moderadas seja geralmente considerada segura para a maioria das pessoas, há preocupações sobre seus efeitos em indivíduos com certos transtornos neurológicos ou psiquiátricos. Por exemplo, pessoas com transtornos de ansiedade podem experimentar exacerbação dos sintomas com a ingestão de cafeína (Bashkatova et al., 2023).

Além disso, a cafeína pode ter um impacto no sono, mesmo quando consumida horas antes de dormir. Ela pode reduzir a qualidade e a quantidade do sono, afetando o desempenho cognitivo e o humor no dia seguinte. O sono

inadequado, por sua vez, está associado a uma série de problemas de saúde, incluindo comprometimento da função imunológica, aumento do risco de doenças cardiovasculares e diminuição da capacidade de aprendizagem e memória.

### 1.5. Cafeína e o exercício físico

A cafeína tem sido amplamente utilizada como um agente ergogênico, ou seja, uma substância que melhora o desempenho físico, especialmente em atividades de resistência e alta intensidade. Seu efeito benéfico no treinamento físico ocorre através de vários mecanismos no corpo, envolvendo tanto o sistema nervoso central quanto processos fisiológicos periféricos. A cafeína atua no sistema nervoso central (SNC) bloqueando os receptores de adenosina, o que reduz a sensação de cansaço e fadiga durante o exercício. Ao reduzir a percepção subjetiva de esforço, os atletas conseguem realizar atividades físicas por mais tempo e com maior intensidade. Esse efeito é especialmente útil em exercícios de longa duração, como corridas de resistência e ciclismo.

A cafeína estimula a liberação de adrenalina (epinefrina), um hormônio que prepara o corpo para situações de estresse ou esforço físico. A adrenalina acelera o ritmo cardíaco, aumenta o fluxo sanguíneo para os músculos e melhora a capacidade de resposta muscular, aumentando o rendimento durante treinos intensos. Isso proporciona um impulso extra em exercícios anaeróbicos e de força. A cafeína aumenta a mobilização de ácidos graxos livres no sangue, promovendo o uso de gordura como fonte de energia durante o exercício. Isso preserva as reservas de glicogênio muscular, retardando a fadiga e permitindo que os atletas mantenham o desempenho por períodos mais longos. Esse mecanismo é particularmente importante em atividades de endurance, onde a depleção de glicogênio pode ser um fator limitante.

A cafeína também pode afetar diretamente a função muscular, aumentando a capacidade de contração e força dos músculos. Ela facilita a liberação de cálcio no retículo sarcoplasmático das fibras musculares, o que melhora a capacidade dos músculos de contrair com mais força e eficiência. Isso é benéfico em exercícios de força, como levantamento de peso e treinamento de resistência. Ao atuar no SNC, a cafeína aumenta a atenção, o estado de alerta e o foco, o que pode melhorar a coordenação motora e a precisão durante o exercício. Esse efeito é vantajoso em esportes que exigem concentração e habilidades motoras finas, como tênis ou ginástica, onde o desempenho cognitivo é tão importante quanto o físico. Estudos indicam que a cafeína pode melhorar o consumo máximo de oxigênio (VO2 máx.), um dos principais indicadores de desempenho aeróbico. Isso significa que os atletas podem usar o oxigênio de forma mais eficiente durante o exercício, aumentando a capacidade de trabalho em atividades de resistência, como corridas e natação. A cafeína também tem efeitos analgésicos, que ajudam a reduzir a percepção de dor muscular durante e após o exercício. Isso permite que os atletas mantenham a intensidade do treino por mais tempo, mesmo diante de desconforto físico, como ocorre em sessões de treinamento intensas ou em provas de endurance. Esses múltiplos efeitos da cafeína contribuem para o aumento do desempenho atlético em

diferentes modalidades esportivas, tornando-a uma das substâncias mais estudadas e utilizadas como complemento pré-treino

### 1.6. Privação de sono, exercício físico e cafeína: uma combinação preocupante

A privação de sono tem efeitos adversos significativos sobre a saúde, comprometendo funções cognitivas, emocionais e fisiológicas. Quando combinada com o exercício físico, que é uma forma de estresse fisiológico, a privação de sono pode exacerbar os efeitos negativos, levando a um aumento do risco de lesões, diminuição da capacidade de recuperação e deterioração do desempenho físico (Hirshkowitz et al., 2015; Gardiner et al., 2023; Medic et al., 2017). A introdução da cafeína nesse cenário pode agravar ainda mais esses problemas, ao invés de mitigá-los. Como já foi dito anteriormente, a cafeína é um antagonista dos receptores de adenosina, uma substância que promove a sonolência e regula o ciclo sono-vigília. Embora a cafeína possa temporariamente aumentar o estado de alerta em indivíduos privados de sono, seu uso prolongado ou em altas doses pode mascarar a necessidade fisiológica de sono, levando a um "déficit de sono" ainda maior. Isso pode resultar em um ciclo vicioso, onde o indivíduo continua a usar cafeína para combater a sonolência, enquanto a qualidade do sono continua a deteriorar-se (Irish et al., 2015; Hillman et al., 2013).

Além disso, a privação de sono crônica associada ao uso frequente de cafeína pode levar à fadiga mental e física acumulada, prejudicando o tempo de reação, a capacidade de tomada de decisão e a coordenação motora. Esses efeitos são particularmente preocupantes em indivíduos que realizam exercícios físicos, uma vez que o comprometimento cognitivo pode aumentar o risco de acidentes e lesões (Medic et al., 2017). O uso de cafeína durante a privação de sono e o exercício físico pode também ter implicações cardiovasculares adversas (Chieng et al., 2022). A cafeína é conhecida por aumentar a frequência cardíaca e a pressão arterial, efeitos que são exacerbados durante o exercício físico devido ao aumento natural da demanda cardiovascular. Em indivíduos privados de sono, que já podem apresentar disfunção autonômica e aumento do estresse oxidativo, esses efeitos podem sobrecarregar ainda mais o sistema cardiovascular, aumentando o risco de eventos adversos, como arritmias cardíacas e hipertensão (Zheng et al., 2022).

A recuperação após o exercício físico é crucial para a adaptação e o crescimento muscular. O sono desempenha um papel fundamental nesse processo, facilitando a reparação tecidual, a síntese proteica e a regulação hormonal. O uso de cafeína em um estado de privação de sono pode interferir na qualidade do sono subsequente, prejudicando a recuperação e, a longo prazo, comprometendo o desempenho físico e o ganho de massa muscular (Gardiner et al., 2023).

Além disso, a cafeína pode causar desidratação devido ao seu efeito diurético, o que é particularmente problemático durante e após o exercício físico,

onde a manutenção do equilíbrio hídrico é crucial para a performance e a recuperação. A desidratação pode aumentar o risco de cãibras musculares, fadiga e, em casos graves, levar a problemas como a rabdomiólise (Reyes et al., 2018; Tinawi et al., 2022).

Embora a cafeína possa oferecer benefícios em termos de aumento de alerta e desempenho físico em situações normais, seu uso em indivíduos privados de sono que estão realizando exercício físico apresenta riscos significativos. Esses incluem aumento do risco cardiovascular, comprometimento da recuperação, desidratação e deterioração do desempenho físico a longo prazo. Portanto, é essencial que o uso de cafeína seja cuidadosamente monitorado nesses contextos, e que a prioridade seja dada à recuperação adequada do sono e à manutenção de hábitos de vida saudáveis para otimizar a saúde e o desempenho físico.

## 2. Objetivos

**Objetivo Geral:** Analisar as alterações eletrocorticográficas no hipocampo e o impacto da privação do sono e do pós-treino sobre a potência estimulante da cafeína.

**Objetivos Específicos:**

- Testar doses baixas e moderadas de cafeína em ratos wistar.
- Analisar o efeito da cafeína na privação de sono.
- Analisar o efeito da cafeína no exercício físico pós-treino.

## 3. Hipóteses

3.1. A privação do sono e o exercício físico afetam a atividade do hipocampo, alterando a resposta neural à cafeína. A privação do sono pode levar a uma diminuição da atividade hipocampal, enquanto o exercício pode aumentar essa atividade.

3.2. O efeito da cafeína sobre a atividade hipocampal será mais pronunciado em indivíduos que experienciaram privação do sono em comparação com aqueles que se exercitaram, sugerindo uma interação entre os dois fatores que modula a resposta à cafeína.

3.3. A privação do sono aumenta a sensibilidade à cafeína, resultando em uma resposta convulsiva mais forte, enquanto o exercício físico pode atenuar essa sensibilidade.

3.4. As ondas cerebrais registradas no hipocampo durante a privação do sono e o exercício físico mostram padrões distintos que correlacionam com a eficácia da cafeína, afetando sua potência estimulante.

3.5. A privação do sono e o exercício físico têm efeitos distintos sobre o comportamento e o desempenho cognitivo, mediando a resposta a doses convulsivas de cafeína.

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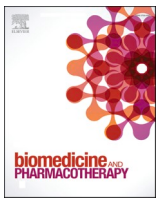

# Recording of hippocampal activity on the effect of convulsant doses of caffeine

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ABSTRACT

Seizures occur when there is a hyper-excitation of the outer layer of the brain, with subsequent excessive synchrony in a group of neurons. According to the World Health Organization (WHO), an estimated 50 million people are affected by this disease, a third of whom are resistant to the treatments available on the market. Caffeine (1,3,7-trimethylxanthine), which belongs to the purine alkaloid family, is the most widely consumed psychoactive drug in the world. It is ingested by people through drinks containing this substance, such as coffee, and as an adjuvant in analgesic therapy with non-steroidal antiflammatory drugs. The present study evaluated the electrocorticographic changes observed in the hippocampus of Wistar rats subjected to acute doses of caffeine (150 mg/kg i.p), which represents a toxic dose of caffeine corresponding to an estimated acute intake of more than 12 cups of coffee to record its convulsant activity. Our results showed, for the first time, that the administration of high doses of caffeine (150 mg/kg i.p.) in rats caused an increase in the spectral distribution of power in all frequency bands and suggested the appearance of periods of ictal and interictal peaks in the electrocorticogram (ECog). We have also shown that the anticonvulsants phenytoin, diazepam and phenobarbital have a satisfactory response when associated with caffeine.

## 1. Introduction

A seizure is known to cause an imbalance in the propagation of the electrical activity of hyperexcitable cortical neurons, with subsequent excessive synchrony within a neuronal ensemble [1]. This term is defined electrographically as epileptiform discharges averaging more than 2.5 Hz for at least 10 s or a pattern with a defined evolution and duration of at least 10 s [2]. Acute symptomatic seizures have an average incidence rate of 29–39 per 100,000 per year [3] and can be associated with serious outcomes [4]. In this sense, it is known that there are drugs that can induce a convulsive state [5,6] and studies show that substances such as caffeine are a pro-convulsive agent [7–10] when used in toxic doses, such as the dose suggested by this study, which is equivalent to a dose of more than 12 cups of coffee [11,12].

Caffeine (1,3,7-trimethylxanthine), which belongs to the purine alkaloid family, is the most widely consumed psychoactive drug in the world [13,14] and can be found in various drinks, but especially in coffee, which is one of the most widely consumed drinks in the world and where 90 % of the intake of this substance takes place [14–16]. It is also used as an analgesic adjuvant therapy with non-steroidal anti-inflammatory drugs (NSAIDs) [17–19]. The widespread use of caffeine is explained by the improvement in mood, vigilance, and alertness [20]. In recent years, its use has increased even more due to its benefits for memory, concentration, and physical performance [21]. On the other hand, the side effects of prolonged or excessive caffeine consumption on sleep, migraine, intraocular pressure, pregnant women, children, and adolescents, as well as the central nervous system and digestive system have been reported [22,23].

Structurally like adenosine, it acts as a mixed competitive antagonist of the A1 and A2A adenosine receptors. This molecular similarity allows

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caffeine to bind to adenosine binding sites on neural receptors, interfering with their normal activity. As a result, caffeine exerts stimulating effects, increasing wakefulness and decreasing the perception of fatigue, and is widely consumed in various cultures around the world (See Fig. 1). This compound is a non-selective antagonist of adenosine receptors (ARs), an inhibitory neuromodulator that has four G protein-coupled receptors: A1, A2A, A2B and A3 [24,25], but its action is especially on A1 and A2A receptors [26,27] which is why it is a potent stimulant of the Central Nervous System (CNS) [28,29]. It is also easily absorbed in the gastrointestinal tract [30] and can cross the biological membrane and the blood-brain barrier due to its lipophilic properties [31].

The chronic use of caffeine is related to a lower risk of developing neurodegenerative diseases such as Alzheimer's [32] through mechanisms such as preventing the production of β-amyloid (Aβ) [33,34]. Furthermore, in some experimental model's caffeine has played a role in preventing the loss of dopaminergic neurons and the development of the motor symptoms of Parkinson's disease by inactivating adenosine A receptors [35].

It is worth noting that anticonvulsants such as diazepam, phenytoin and phenobarbital are used to treat seizures [36,37]. Its mechanisms of action is a positive allosteric modulators of different GABA-A receptors [38]. However, Gasior et al., 1996 [39] demonstrated that the combination of high doses of caffeine with anticonvulsants reduced the protective action of the anticonvulsant. Furthermore, caffeine can reduce the effect of diazepam on the central nervous system [40] and can act as a phenobarbital antagonist [41].

Therefore, this study aims to analyze the electrocorticographic changes observed in the hippocampus of Wistar rats submitted to acute doses of caffeine, to record convulsant activity and the effect of anticonvulsant drugs - phenytoin, diazepam, and phenobarbital.

## 2. Methodology

### 2.1. Animals

For this study we used 45 adults male Wistar rats weighing between 200 and 220 g. They were obtained from the animal house of the Federal University of Pará and housed in the Laboratory of Pharmacology and Toxicology of Natural Products under conditions of free access to food and water, constant room temperature (23–25°C) and a 12-hour light-dark cycle. The animals were housed individually in cages. The research was conducted following the principles of the national legislation governing the use and breeding of animals for experimentation and the Ethical Principles of the National Council for the Control of Animal Experimentation (CONCEA) and was approved by CEUA UFPA under no. 2675110219 (ID 001142).

### 2.2. Chemical substances

The following chemical substances were used for this study: Ketamine Hydrochloride, obtained from the Köing Laboratory (Santana de Parnaíba, SP, Brazil); Xylazine Hydrochloride, obtained from the Vallée Laboratory (Montes Claros, MG, Brazil); Lidocaine, a local anesthetic, obtained from the Hipolabor Laboratory (Sabará, MG, Brazil), for electrode implantation; caffeine obtained from SIGMA (USA); Phenytoin, Diazepam and Phenobarbital from the Cristália laboratory.

### 2.3. Experiment 1

#### 2.3.1. Experimental groups

The animals were divided into the following experimental groups: a) Control Group (n=9): received an injection of saline solution in a volume of 0.5 ml intreperitoneally (i.p.); b) Caffeine Group: this group received anhydrous caffeine 150 mg/kg i.p. after which recordings will be acquired in the hippocampus region lasting 600 s to evaluate

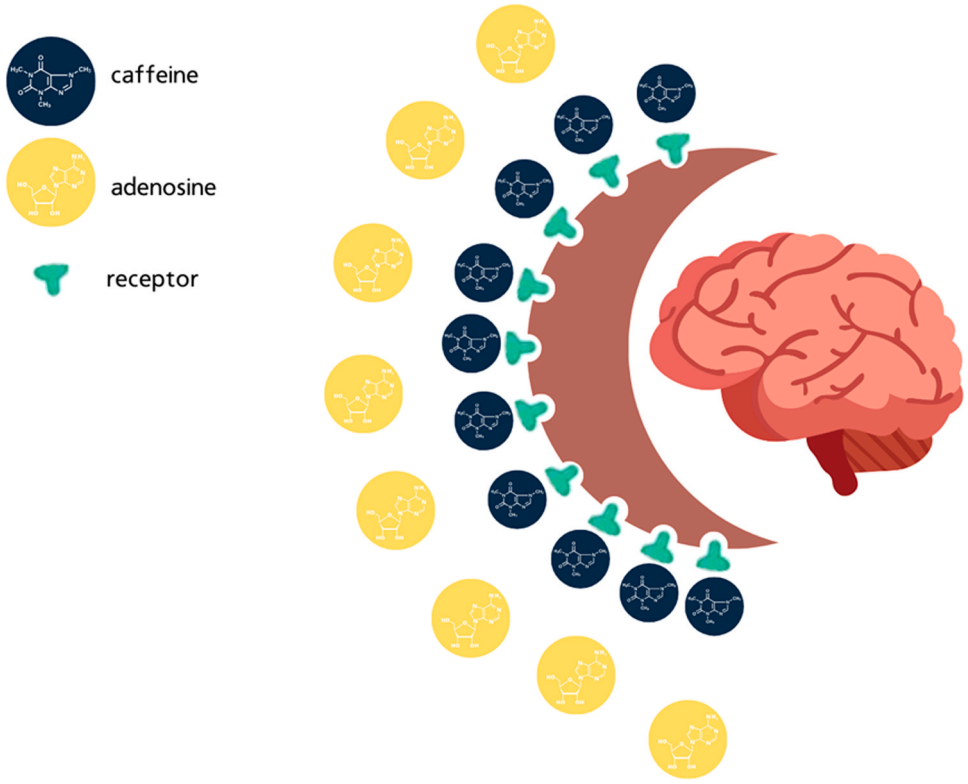


**Fig. 1.** Interaction of the caffeine molecule with the adenosine receptor. Caffeine acts as a competitive antagonist of adenosine receptors, particularly the A1 and A2A subtypes.

hippocampal activity and identify the patterns of tracings caused by high doses of caffeine.

### 2.4. Experiment 2

#### 2.4.1. Action of anticonvulsants

The following groups were compared to evaluate the control of deflagrations that cause changes in the hippocampus recordings: a) Control group received saline solution i.p.; b) Received caffeine at a dose of 150 mg/kg i.p.; c) Group received caffeine (150 mg/kg i.p.) and phenytoin (10 mg/kg. i. p.) concomitantly. p.) concomitantly, then the hippocampal recordings were taken for 600 s.; d) group received caffeine (150 mg/kg i.p.) and Diazepam (10 mg/kg i.p.) concomitantly and e) caffeine (150 mg/kg i.p.) and Phenobarbital (10 mg/kg i.p.) group, all groups were recorded for a period of 10 min with (n=9).

### 2.5. Electrophysiology

#### 2.5.1. Recording the rat's hippocampal activity

The electrodes used to obtain the recording were implanted at the coordinates AP = 3.4 mm, ml = ± 2.0 mm DV = −2.0 mm [42] (Fig. 1). The electrode was made from a 0.2 mm diameter nickel/chrome alloy and insulated with liquid insulation. On the fourth day after surgery, the experiment began to acquire hippocampal recordings to assess activity after caffeine (150 mg/kg i.p.). 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 continuously digitized at a rate of 1 kHz by a computer equipped with a data acquisition board (National Instruments, Austin, TX), stored on a hard disk and processed using specialized software (LabVIEW express). The recording electrode was located on the right side of the hemisphere and the reference electrode on the left. The entire experiment was carried out inside a Faraday cage.

### 2.6. Electrophysiological data analysis

To analyze the acquired signals, a program was built using the Python programming language version 2.7. The Numpy and Scipy libraries were used for mathematical processing and the Matplotlib library for graphics. The graphical interface was developed using the PyQt4 library. The recordings were analyzed up to 40 Hz, and the bands were analyzed according to [43,44], corresponding to Delta (1–4 Hz), Theta (4–8 Hz), Alpha (8–12 Hz), Beta (12−28) and Gamma (28–40 Hz). During the evaluation of brain oscillations, the Delta-Alpha coupling and the Beta-Gamma coupling were analyzed.

### 2.7. Statistical analysis

The data was analyzed by comparing mean values. ANOVA analysis of variance was applied, followed by Tukey's test. The level of significance considered was *p < 0.05, ** p<0.01 and *** p<0.001. The data was analyzed using GraphPad Prism®5 softwares.

## 3. Results

The hippocampus recording showed regularity and stability during acquisition of the control recording, with amplitude below 0.05 mV. The spectrogram showed greater energy intensity below 5 Hz, with a more

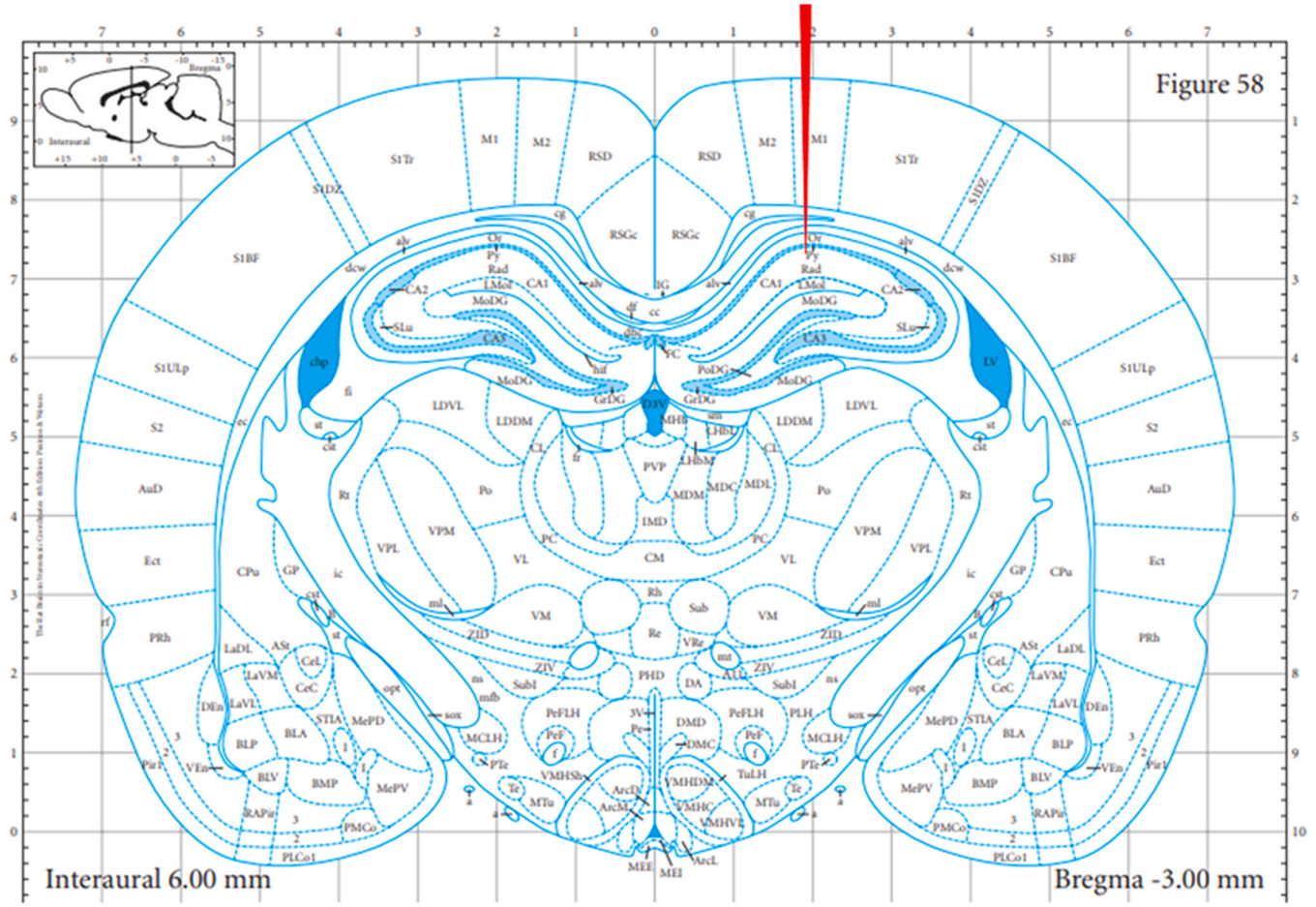


**Fig. 2.** Schematic representation of the electrode used to acquire recordings in the hippocampus, the area accessed was CA1 (Paxinos and Watson, 2012).

intense frequency distribution below 20 Hz (Figure A). During the hippocampal recording after caffeine application, there was a change in the tracing which made it possible to evaluate phases related to high-frequency, high-amplitude potential release and low-frequency, high-amplitude single shots with an average amplitude of 0.4 mV (Figure B Center). The spectrogram shows a distribution of energy during the potential release throughout the 40 Hz frequency range, with a decrease in energy during the period of single shots (Fig. 2 B Left).

The spectral distribution of power showed an increase in all frequency bands during the ictal and interictal-like spike periods when compared to the power of the control group, in which case caffeine triggered greater force in oscillations up to 40 Hz (Fig. 3 A). When comparing the total power of the caffeine recording with the ictal and interictal-like spike periods, it can be seen that the ictal period presents greater power and is responsible for the increase in maximum peak of the recording, being greater than that of caffeine where these periods are interspersed with periods with lower power represented by the inertial-like spike periods (Fig. 3B). In the linear power graph, the control group had a lower average power than the other groups. The caffeine group showed lower linear power than the mean power of the ictal period and lower than the interictal-like spike periods. The ictal group had higher linear power compared to the other groups, and the ILP group had higher low-frequency linear power than the control group (Fig. 3 C).

For delta oscillations, the control group (0.02866 ± 0.0030 mV2/Hz x 10–3) was lower than the other groups: the caffeine group (0.2914 ± 0.019 mV2/Hz x 10–3), the ictal period (0.5509 ± 0.0746 mV2/Hz x 10–3) and the interictal-like spike period (0.1576 ± 0.0323 mV2/Hz x 10–3). The caffeine group had lower mean linear power than the ictal period and higher than the interictal-like spike period. The highest delta power was recorded during the ictal period (Fig. 4 A).

For the theta oscillations (4–8 Hz), which are very prevalent in the hippocampus, the control group had a proportional mean that was more distant than the caffeine groups (the control group had a lower mean linear power than the other groups (0. 01937 ± 0.00307 mV2/Hz x 10–3), caffeine group (0.42288 ± 0.0226 mV2/Hz x 10–3), ictal period (0.899 ± 0.1959 mV2/Hz x 10–3) and interictal period (0.1540 ± 0.04123 mV2/Hz x 10–3). The period with the highest theta energy level was during the ictal period, which was higher than the other groups (Fig. 4 B).

For alpha oscillations, it was observed that the control group had a mean of 0.01112 ± 0.00076 mV2/Hz x 10–3, which was lower than the other groups: caffeine group 0.265 ± 0.02423 mV2/Hz x 10–3, ictal period 0.4931 ± 0.0872 mV2/Hz x 10–3 and interictal period -like spike 0. 106 ± 0.0268 mV2/Hz x 10–3. 106 ± 0.0268 mV2/Hz x 10–3. The average power for the total caffeine recording had an energy level maximized by the ictal period and decreased by the interictal period, which generated power oscillation during the recording (Fig. 4 C).

For beta oscillations, the control group (0.01320 ± 0.002236 mV2/Hz x 10–3) was lower than the other groups: the caffeine group (0.4058 ± 0.0688 mV2/Hz x 10–3), the ictal period (0.7554 ± 0.1552 mV2/Hz x 10–3) and the interictal-like spike period (0.2046 ± 0.03022 mV2/Hz x 10–3). The caffeine group had lower mean linear power than the ictal period and higher than the interictal-like spike period. The greatest power in beta oscillations was recorded during the ictal period (Fig. 4 D).

The oscillations in the gamma range presented for the control group (0.005028 ± 0.0006125 mV2/Hz x 10–3) were lower than the other groups: caffeine group (0.05531±0.01087 mV2/Hz x 10–3), ictal period (0.07314 ± 0.013382 mV2/Hz x 10–3) and interictal-like spike period (0.02824 ± 0.005743 mV2/Hz x 10–3). The caffeine group had lower mean linear power than the ictal period and higher than the interictal-like spike period. The highest power intensity was observed in the gamma oscillations in the ictal period (Fig. 4 E).

Recording of the hippocampus during the use of anticonvulsants to control seizures triggered by caffeine showed that animals treated with phenytoin, diazepam and phenobarbital showed a reduction in the

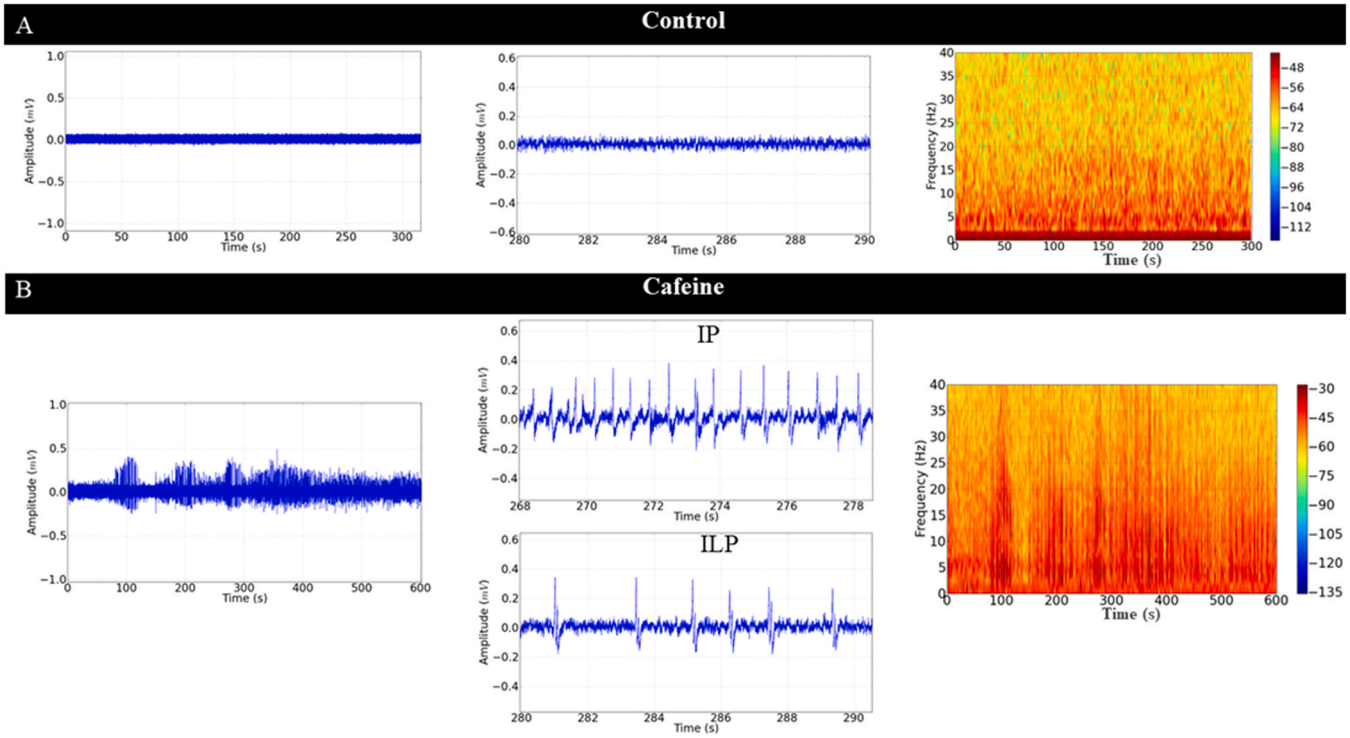


**Fig. 3.** Hippocampus recording lasting 300 s, showing activity in area CA1 (A left), amplification of 10-second recordings (280–290 s) (A center), spectrogram of energy distribution with amplitude up to 40 Hz (A right); Recordings from the CA1 hippocampus after caffeine use lasting 600 s (B on the left), amplification of recordings in salvo of potentials (top) and single shots (bottom) (B in the center) and spectrogram of energy distribution during the action of caffeine in the hippocampus (B on the right).

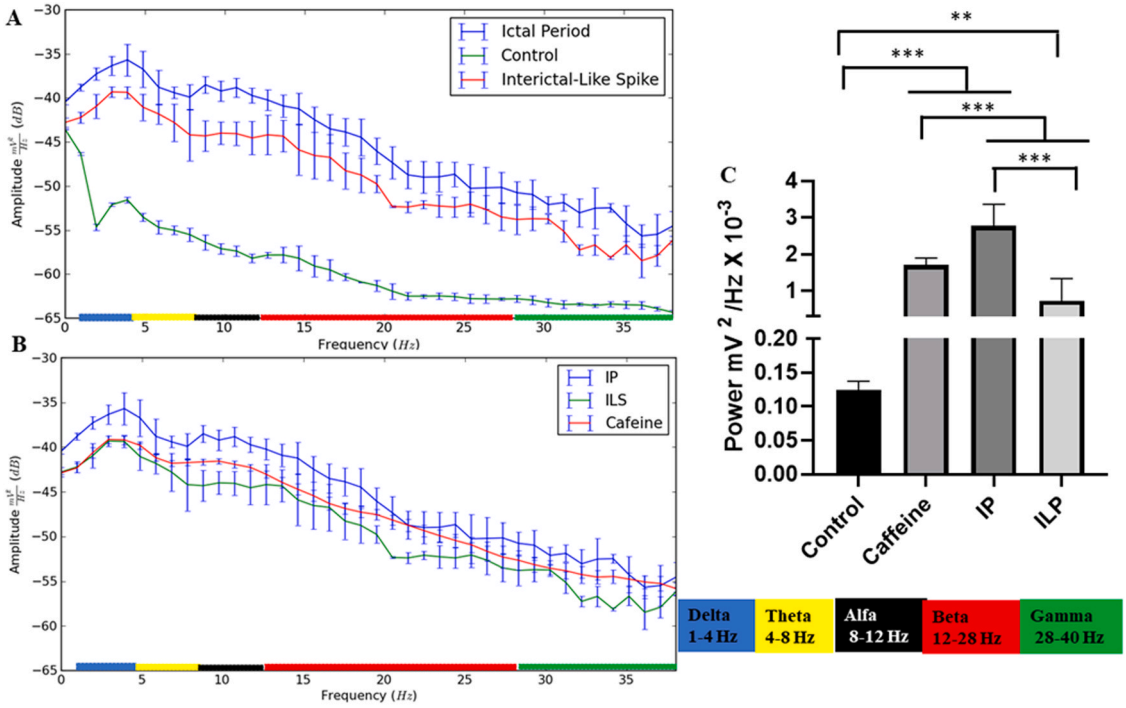


**Fig. 4.** Spectral power distribution graph (40 Hz) indicating the amplitudes for the frequencies of the oscillations found in the hippocampus of rats during the convulsive action provoked by caffeine, comparing the powers in the frequencies between the ictal period (IP), Iterictal-like spike (ILS) and the recording power for the control group (A), Spectral power distribution, indicating the distribution of mean powers in each frequency up to 40 Hz for the ictal, interictal-like spike and caffeine groups (B); Graph of the distribution of mean power for the groups (C). The colors indicate the frequency bands found in the rats' hippocampus with the following indications: delta 1–4 Hz marked in blue; Alpha 4–8 Hz marked in yellow; Alpha 8–12 Hz marked in black; Beta 12–28 Hz marked in red, and Gamma 28–40 Hz marked in green. After ANOVA, followed by Tukey's test, *p<0.05, **p<0.01 and ***p<0.001, (n=9).

amplitude of the tracing with morphographic elements indicating good seizure control, which can be confirmed in the tracing and spectrograms in Figures A, B and C. In the linear power graph, the control group (0.1246 ± 0.0127 mV2/Hz x 10–3) had a similar mean to the caffeine Diazepam-treated group (0.1076 ± 0.04604 mV2/Hz x 10–3) (p= 0.9946). These groups were smaller than the caffeine (1.713 ± 0.1859 mV2/Hz x 10–3), caffeine phenytoin (0.4766 ± 0.0476 mV2/Hz x 10–3) and caffeine phenobarbital (0.2812 ± 0.04594 mV2/Hz x 10–3) groups. The caffeine group showed greater linear power than the other groups (Fig. 5 D).

The power recorded in the hippocampus in the delta oscillations decreased after the use of anticonvulsants. Thus, for the control group, the mean (0.02886 ± 0.00308 mV2/Hz x 10–3) was similar to that of the caffeine Diazepam group (0.02773 ± 0.0034 mV2/Hz x 10–3) (p= 0.997). However, they were lower than the caffeine group (0.29114 ± 0.1904 mV2/Hz x 10–3), phenytoin caffeine (0.108 ± 0.00789 mV2/Hz x 10–3) and phenobarbital caffeine (0.07372 ± 0.01007 mV2/Hz x 10–3). The caffeine group showed greater linear power than the other groups (Fig. 6A).

The linear power graph showed that the theta oscillations were the most difficult to control in the hippocampus, so the control group was smaller than the other groups (0.01937 ± 0. 00307 mV2/Hz x 10–3), the caffeine group (0.4228 ± 0.02266 mV2/Hz x 10–3), caffeine phenytoin (0.1420 ± 0.01851 mV2/Hz x 10–3), caffeine diazepam (0.03829 ± 0.000501 mV2/Hz x 10–3) and caffeine phenobarbital (0.1270 ± 0.01205 mV2/Hz x 10–3). However, there was a decrease in power intensity in theta oscillations (Fig. 6 B).

The power recorded in the hippocampus in the alpha oscillations decreased after the use of anticonvulsants. Thus, for the control group, the mean (0.01112 ± 0.00076 mV2/Hz x 10–3) was like that of the caffeine Diazepam group (0.001643 ± 0.00312 mV2/Hz x 10–3) (p= 0.8705). However, they were lower than the caffeine group (0.2654 ± 0.02423 mV2/Hz x 10–3), phenytoin caffeine (0.0498 ± 0.007612 mV2/Hz x 10–3) and phenobarbital caffeine (0.03246 ± 0.00559 mV2/Hz x 10–3). The caffeine group showed greater linear power than the other groups (Fig. 6C).

The power recorded in the hippocampus in the beta oscillations decreased after the use of anticonvulsants; thus, for the control group, the mean (0.01320 ± 0. 002236 mV2/Hz x 10–3) had a similar mean to the groups treated with caffeine diazepam (0.02129 ± 0.00679 mV2/Hz x 10–3) (p= 0.9814) and the caffeine phenobarbital group (0.03925 ± 0.005413 mV2/Hz x 10–3) (p= 0.4045). The diazepam caffeine group and phenobarbital caffeine group were similar (p= 0.739). The other groups were higher, the caffeine group (0.4058 ± 0.06882 mV2/Hz x 10–3) and the phenytoin caffeine group (0.1089 ± 0.001731 mV2/Hz x 10–3). The caffeine group showed greater linear power than the other groups (Fig. 6D).

The power recorded in the hippocampus in the gamma oscillations decreased after the use of anticonvulsants. Thus, for the control group, the mean (0.005028 ± 0.0006125 mV2/Hz x 10–3) was similar to that of the caffeine Diazepam group (0.006415 ± 0.001342 mV2/Hz x 10–3) (p= 0.9792). However, they were lower than the caffeine group (0.05531 ± 0.01087 mV2/Hz x 10–3), phenytoin caffeine (0.0269 ± 0.00365 mV2/Hz x 10–3) and phenobarbital caffeine (0.0131 ± 0.001101 mV2/Hz x 10–3). The caffeine group showed greater linear power than the other groups (Fig. 6E).

## 4. Discussion

In this study, we demonstrated for the first time that the administration of high doses of caffeine (150 mg/kg i.p.) in rats showed an increase in the spectral distribution of power in all frequency bands and suggested the appearance of an ictal period and interictal-like spike in the electrocorticogram (ECog). We also demonstrated that anticonvulsants have a satisfactory response when associated with caffeine. Our results corroborate several studies that show an association between

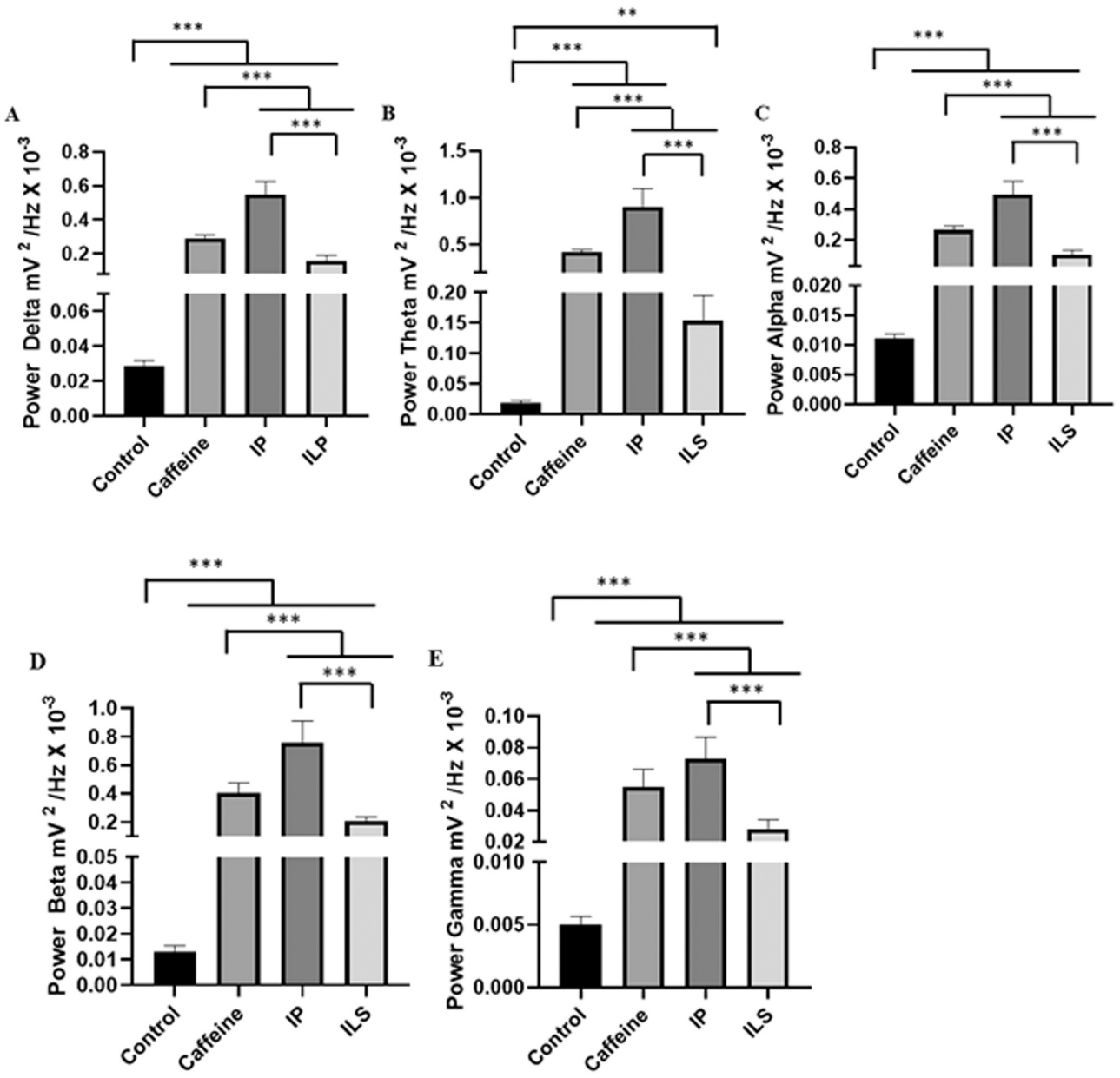


**Fig. 5.** Evaluation of brain oscillations after caffeine administration with evaluation of the ictal period and interictal-like spike period: Variation in hippocampal oscillations in delta (1–4 Hz) (A); Variations found in hippocampal oscillations in Theta (4–8 Hz) (B); Variations in hippocampal oscillations in Alpha (8–12 Hz) (C); Variation in oscillations in Beta (12–28 Hz) (D) and Variations found in oscillations in Gamma (28–40 Hz) (E). After ANOVA followed by Tukey's test. (*p<0.05, **p< 0.01 and ***p<0.001, n = 9).

high doses of caffeine and the occurrence of seizures [45–50].

The normal daily consumption of caffeine varies among individuals, with many typically consuming between 100 and 400 mg per day, roughly equivalent to 1–4 cups of coffee [14]. Higher doses can pose an increased risk of adverse effects, including seizures, particularly in individuals predisposed to epilepsy. Caffeine's potential to trigger seizures at elevated doses is attributed to its action as a central nervous system stimulant and its effects on adenosine receptors, which regulate neuronal excitability.

In terms of restrictions, individuals with a history of epilepsy or susceptibility to seizures should exercise caution regarding caffeine consumption and may need to limit intake. It is recommended that such individuals consult a physician to establish safe limits for caffeine consumption. The specific role of caffeine in the current number of epilepsy patients is a complex and multifaceted issue warranting further investigation. While some studies suggest caffeine may have anticonvulsant properties under certain circumstances, its potential to induce seizures at high doses necessitates careful monitoring when used in epileptic patients. Additionally, exploring the relationship between caffeine consumption and the incidence or control of seizures in epilepsy patients could yield significant insights for clinical practice and public health recommendations. The pharmacological effects of caffeine include stimulation of the CNS and the heart, which occur at plasma concentrations of 15 mg/L. Some common features of caffeine intoxication can include anxiety, restlessness, insomnia, gastrointestinal disturbances, tremors, and psychomotor agitation [51].

Caffeine's mechanism of action involves nonspecific antagonization of adenosine receptors, mainly A1 and A2A receptors [52]. A1 receptors, coupled to GI/o proteins, activate the suppression of adenylyl cyclase, inhibit the production of cyclic adenosine 3′,5′-monophosphate (cAMP) and suppress glutamatergic presynaptic transmission [53], through the inhibition of voltage-dependent Ca 2+ channels [54,55].

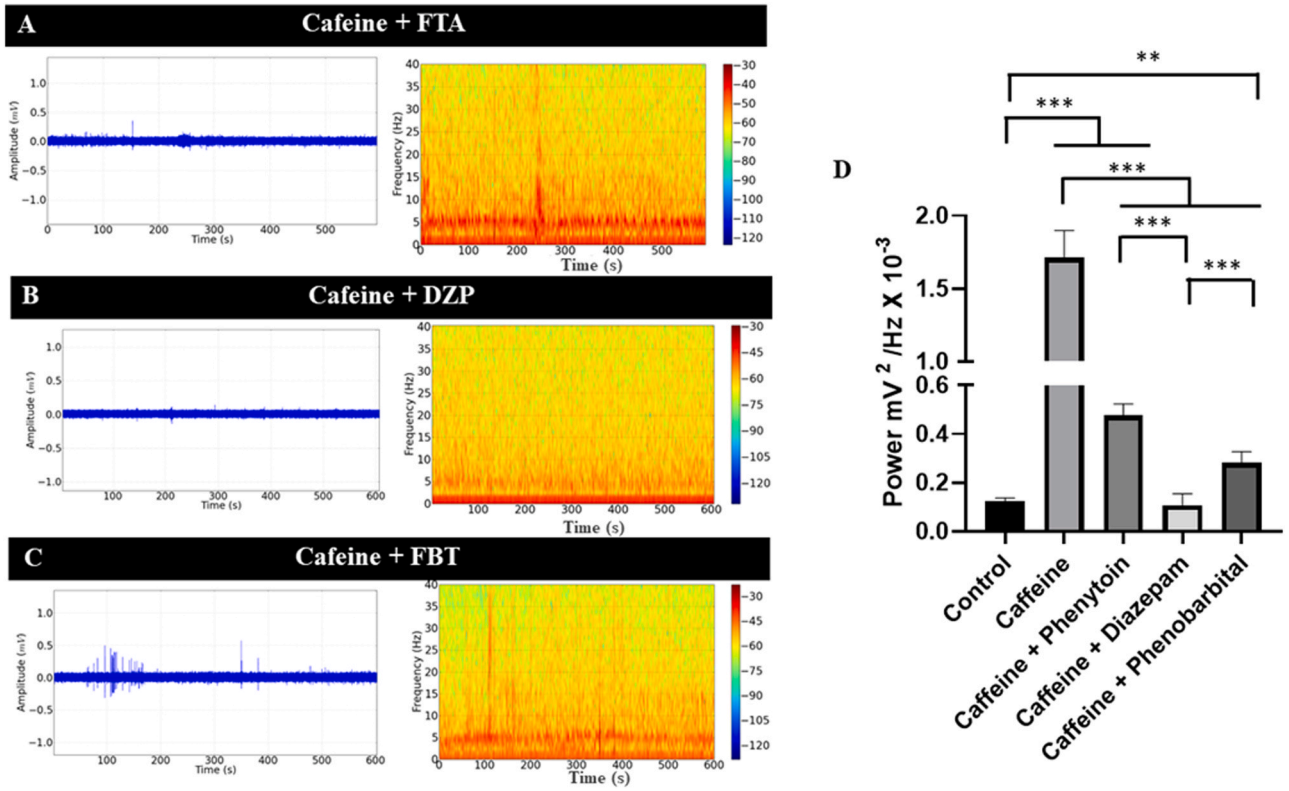


**Fig. 6.** 600 s hippocampus recording showing activity in area CA1 (A to the left), power distribution spectrogram with amplitude up to 40 Hz (center) in the control of caffeine-induced seizures in the hippocampus using the following drugs: Phenytoin (A), Diazepam (B) and Phenobarbital (C). Linear power graph of frequencies up to 40 Hz, observed after the use of anticonvulsants (D). After ANOVA followed by Tukey's test. (*p<0.05, **p< 0.01 and ***p<0.001, n = 9).

In the hippocampus, A1 receptors are mainly located presynaptically [56] and their main role is to decrease the evoked release of glutamate [57], A1 receptors control the recruitment of aminomethylphosphonic acid (AMPA) and N-methyl D-Aspartate (NMDA) receptors [58], which are involved in the initiation and establishment of epileptic seizures [59]. The inhibition of tonic GABA A currents has been reported in post-synaptic neurons located in pyramidal cells present in the hippocampus, which regulates homeostasis without impairing GABAergic synaptic inhibition [60].

A2A receptors are normalizes CNS hyperactivity [24] because they are coupled to Gs/olf proteins and act in the production of cAMP, which activates protein kinase A (PKA) and opens Ca 2+ channels [61]. These receptors have the function of facilitating glutamate release and long-term potentiation at synapses in the hippocampus [62]. They also facilitate synaptic NMDA currents in the hippocampus, which are responsible for synaptic plasticity and neurodegeneration [63,64]. It is worth noting that they are most widely distributed in the corpus striatum, nucleus accumbens and olfactory tubercle, with a small concentration in the hippocampus [65,66].

Some studies suggest that the antagonism of A1R receptors leads to neuronal excitation and that of A2A to neuronal inhibition [24,52, 67–70], but they do not consider the effects caused by the integration of two receptors. Yang et al., 2007 [71] demonstrated that 3,7-dimethyl-1--propargylxanthine (DMPX), a xanthine compound, selective antagonist of A2A and A1 receptors in the ratio of 3/1 obtained pro-convulsant effects. In addition, epileptogenic alterations during the evolution of kindling caused by A2A receptors can be reduced by genetic ablation or pharmacological inhibition, which indicates that they are not related to the onset of seizure activity, but to its aggravation [24,62,72]. As caffeine is a non-selective adenosine antagonist, this effect was also observed in our results, as caffeine administration caused high and low frequency firing.

These mechanisms contribute to the caffeine response, the administration of 150 mg/kg i.p. in our study was sufficient for the appearance of the ictal period and interictal-like spike characteristic of a seizure also described by Azevedo, 2022 [47], which suggests a decrease in the seizure threshold described in the literature [9,11]. Benzodiazepines are known for their anticonvulsant effect, acting through the modulation of GABAergic receptors in the brain, which results in a reduction in neuronal excitability. When administered in conjunction with caffeine, which can increase neuronal excitability through its effects on adenosine receptors, benzodiazepines can neutralize or reduce the risk of caffeine-induced seizures.

Our study with caffeine suggests that this substance causes the appearance of this rhythmic pattern in rats because it was possible to analyze and compare different amplitudes and oscillations in the hippocampus of these animals since the power spectral distribution showed an increase in all frequency bands during the ictal period and interictal-like spike periods, a fact commonly observed in studies that take into account epileptic models in humans [73]. This situation becomes even more worrying when we consider that there are studies proposing that caffeine-induced seizures can cause post-ictal - post-seizure - hypoxia and consequently death, since responses at brain level depend intrinsically on the supply of oxygen by the blood, but during the seizure vasoconstriction is noted, which prevents important organs such as the brain from being irrigated [45].

In this study, we used acute dose of caffeine in rats and its effects on the hippocampus of these animals, noting that other studies in the scientific community that used acute doses of caffeine suggest a significant drop in the partial pressure of oxygen in the hippocampus in the pre-convulsion period caused by the action of caffeine or its metabolites [74]. It is also important to say that in this research we focused on the action of caffeine in the hippocampus of rats, in the CA1 area, and we observed a great excitation of the neurons of this area given the increase in frequency in the electroencephalogram, other studies previously carried out by the scientific community also sought to analyze the effect

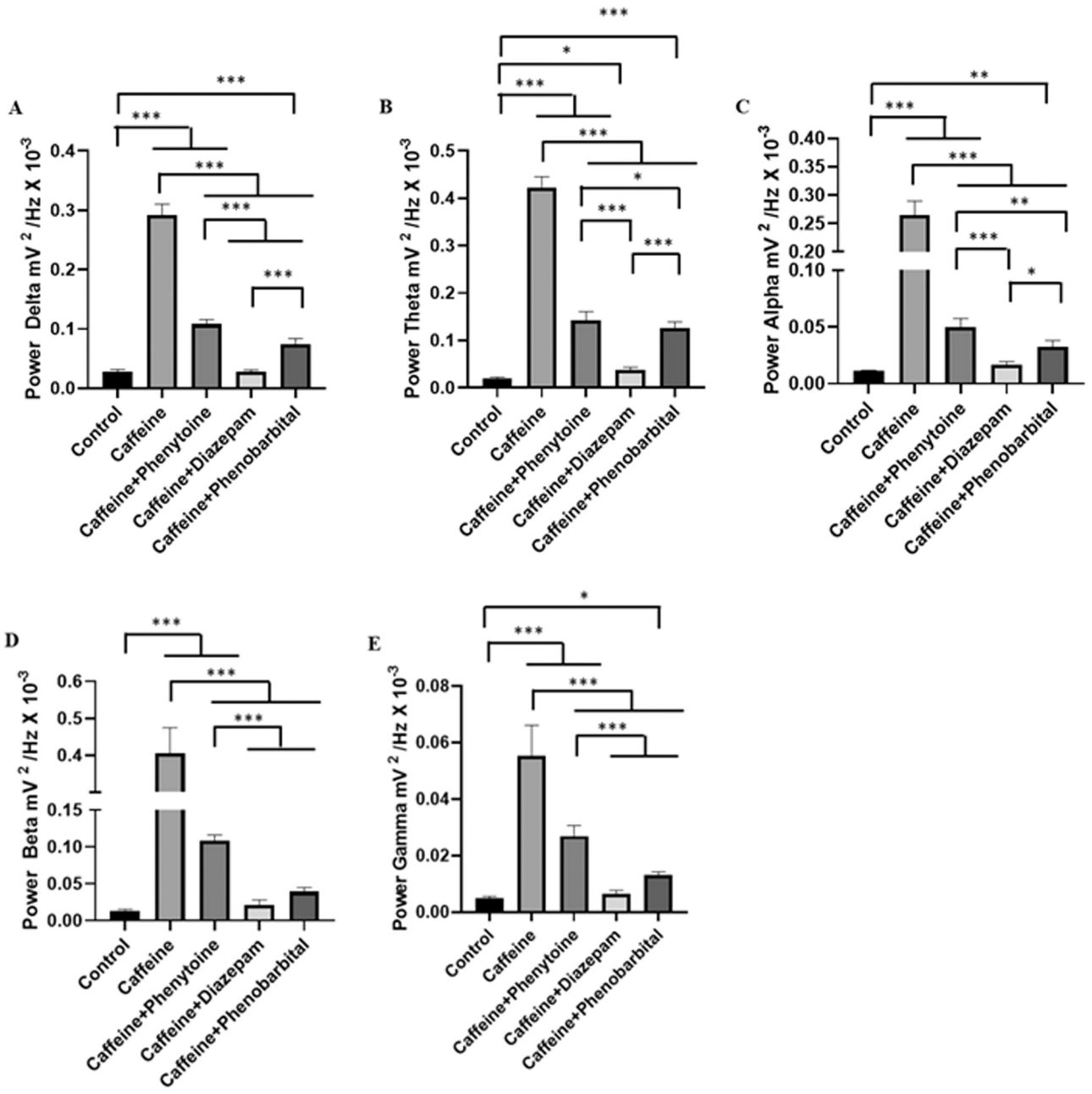


**Fig. 7.** Evaluation of brain oscillations after administration of anticonvulsants to control seizures triggered by caffeine with evaluation of the ictal period and interictal-like spike period: Variation in hippocampal oscillations in delta (1–4 Hz) (A); Variations found in oscillations in Theta (4–8 Hz) (B); Variations in oscillations in Alpha (8–12 Hz) (C); Variation in oscillations in Beta (12–28 Hz) (D) and Variations found in oscillations in Gamma (28–40 Hz) (E). After ANOVA followed by Tukey's test. (*p<0.05, **p< 0.01 and ***p<0.001, n = 9).

of caffeine and the excitability generated by it in this region, but used a different methodology which was the decapitation of the rats to study the desired area [75].

The hippocampus expresses high levels of A1 receptors and low levels of A2A receptors [76–78]. Caffeine acts on the hippocampus, affecting learning and memory in a dose-dependent manner. Studies have shown that low doses of caffeine made the Morris water maze efficient in action retention and retrieval [79], while high doses of caffeine disrupted fear conditioning [80].

## Ethics declarations

All procedures were approved by the ethics committee (CEUA/UFPA N). All the experiments were carried out using the ARRIVE checklist.

## Financial Disclosure

None.

## Non-financial Disclosure

None

## CRediT authorship contribution statement

**Murilo Farias dos Santos:** Methodology. **Maria Klara Otake Hamoy:** Methodology, Formal analysis. **Luana Vasconcelos de Souza:** Writing – original draft. **Moisés Hamoy:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis,

Data curation, Conceptualization. **Daniella Bastos de Araújo:** Writing – review & editing, Writing – original draft, Conceptualization. **Gabriela Brito Barbosa:** Methodology. **Raíssa Vieira de Souza:** Methodology. **Felipe Kiyoshi Yoshino:** Formal analysis. **Yris da Silva Deiga:** Methodology. **Gloria Calandrini de Amorim:** Methodology. **Anthony Lucas Gurgel do Amaral:** Methodology. **Priscille Fidelis Pacheco Hartcopff:** Writing – original draft. **Luciana Eiró-Quirino:** Writing – review & editing, Writing – original draft, Investigation. **Rodrigo Gonçalves dos Santos:** Methodology. **Laís Helena Baptista Amóras:** Methodology.

## Declaration of Competing Interest

None

## Data Availability

Data will be made available on request.

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## SEGUNDO CAPÍTULO DA TESE

# *Impact of Sleep Deprivation on the Stimulant Potency of Caffeine in Swiss Mice*

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## Abstract

Sleep is essential for physical and emotional well-being. Approximately 45% of the world's population suffers from sleep deprivation, resulting in reduced cognitive functions and an increased risk of accidents. Chronic sleep deprivation is a public health problem associated with cardiometabolic diseases and mental disorders. To mitigate the effects of sleep deprivation, the population uses energy drinks containing caffeine, an affordable stimulant which, although effective, has several contraindications and can lead to addiction. 90 male Swiss mice were used, divided into 10 groups (n=9) with and without 24-hour sleep deprivation. Each group received different doses of caffeine (5, 10, 15, and 20 mg/kg) or saline solution. ECoG recordings were made after administration of the substances. The ECoG recordings of the caffeine-treated groups showed variations in the amplitude and frequency of brain waves, depending on the dose administered. An increase in total linear power and delta and theta oscillations was observed in the caffeine-treated groups compared to the control group. Sleep deprivation decreases the efficacy of caffeine in Swiss mice, as evidenced by changes in ECoG recordings. This study provides important insights into the interaction between sleep deprivation and the effects of caffeine, contributing to the development of more effective strategies to manage sleep-related problems and improve cognitive performance and alertness.

**Key-words: Sleep deprivation, caffeine, electrocorticogram, EcoG**

## Introduction

Sleep is an essential component for maintaining physical and emotional well-being. The universal daily recommendation is 7 to 9 hours of sleep per day, but it is estimated that around 45% of the world's population suffers from sleep deprivation (Hirshkowitz et al., 2015; Ohayon, 2011). The literature shows that patients with short periods of sleep experience a reduction in cognitive functions, humoral imbalance and an increased risk of accidents and injuries (Medic, Wille, Hemels, 2019).

The chronicity of sleep deprivation has become a public health problem, since a high proportion of the population has been suffering from the deleterious effects of this health problem, as there is a higher prevalence in cases of cardiometabolic diseases and the appearance of mental health disorders (Morrison et al., 2022; Fang et al., 2019). In addition, the health of society is compromised, generating lower productivity and thus causing various problems in different segments (Hillman and Lack, 2013).

A viable solution widely used by the general population to solve the problem of reduced productivity and excessive tiredness caused by sleep deprivation has been the use of energy drinks containing caffeine (Heckman, Weil, Gonzalez, 2010). However, caffeine, despite appearing to be a harmless psychostimulant, precisely because it is widely accessible and unrestricted, has several contraindications in its use as well as in its quantities (Addicott, 2016). Around 80% of the world's population uses caffeine as an adjuvant to improve physical and cognitive performance and it has become a socially acceptable drug (McLellan, Caldwell, Lieberman, 2016). It is an adenosine antagonist that reduces sleep by acting on the homeostatic component of sleep-wake regulation (Fredholm, 1995).

Caffeine acts on the Central Nervous System, stimulating a reduction in the perception of fatigue and drowsiness (Landolt, 2008), which is why it is widely consumed throughout the day in response to insufficient sleep to promote a state of wakefulness (McLellan, 2016). It is an alkaloid that shares a similar structure to adenosine, which allows it to block adenosine receptors (Crippa et al, 2014). However, the use of caffeine to stimulate wakefulness can result in impaired sleep onset and subsequent sleep maintenance, potentially creating a cycle of decreased sleep and caffeine dependence (Snel and Lorist, 2011). Thus, it is understood that caffeine consumption can reduce sleep efficiency through a reduction in total sleep time (Ohayon et al, 2015).

Given the above, the adenosine molecule is widely used as an important sleep regulator, when it is in the extracellular medium, it is able to increase in various areas of the brain during the waking period, and decrease during sleep, in addition to being able to be enhanced during long periods awake (Reichert, Deboer, Landolt, 2022). Therefore, it is inferred that the sleep-inducing properties of the adenosine molecule affect or induce fatigue after prolonged wakefulness (Huang, Zhang, Qu W, 2014; Lázaro et al, 2017), by preventing the release of excitatory neurotransmitters, which results in a decrease in cortical excitability (Alstadhaug, Andreou, 2019).

More recent studies show that adenosine can alter the circadian clock and changing the relationship between this biological clock and attenuation of the homeostatic mechanisms of sleep (Reichert, Deboer and Landolt, 2022), with increased duration of

wakefulness, nocturnal awakenings, and changes between sleep stages occurring with a tendency to rebound, with a decrease in deeper sleep stages (Clark and Landolt, 2017). The concentration of adenosine in the body is controlled by complex regulatory processes that depend on the metabolic state of neurons and astrocytes. Adenosine acts to catalyze the formation and decomposition of ATP, generating a degradation product from ATP depletion in the brain. This ATP accumulates in the extracellular space and is degraded by 5'-EN into adenosine, resulting in prolonged neuronal activity during wakefulness, i.e. this adenosine is like a large homeostatic accumulator of the need to sleep (Reichert, Deboer, Landolt, 2022).

In the brain regions, A1 and A2 adenosine receptors are stimulated (Ferré, 2008), which are associated with the stimulating effects of caffeine (Fisone, Borgkvist and Usiello, 2014; Yu et al. 2009). Caffeine acts to increase dopamine neurotransmission by blocking presynaptic A receptors that modulate the release of glutamate and dopamine (Quarta et al, 2004; Ciruela et al, 2006). This accumulation of extracellular adenosine promotes sleep and disinhibits areas that promote sleep, as well as inhibiting activities in areas that promote wakefulness (Porkka-Heiskanen, Strecker, McCarley, 2000). In addition, the reduction in the somnogenic effects of adenosine explains the nocturnal awakening effects of caffeine (Huang, Zhang, Qu W, 2014).

The adenosine receptors, A1 and A2, are important for controlling sleep and wakefulness, but in the presence of pharmacological stimulation of these receptors, with specific antagonists, they can increase, as is the case with caffeine, which will take the place of adenosine, keeping the body awake and alert, giving a "false" idea of absence of tiredness and fatigue and inhibiting sleep, but the literature already shows that caffeine increases energy levels by reducing fatigue levels, thus improving cognitive and physical performance (Ferré, 2016; McLellan, Caldwell, Lieberman, 2016; Ferré et al., 2018).

The objective of our study is to investigate how lack of sleep affects the stimulant properties of caffeine. By conducting experiments on Swiss mice, we aim to understand the extent to which sleep deprivation can diminish the potency of caffeine. This research will provide insights into the interactions between sleep and the effects of caffeine, potentially contributing to the development of more effective strategies for managing sleep-related issues and improving alertness and cognitive performance.

## Methodology

*Animals*

For this study, a total of 90 heterogeneous male Swiss mice aged between 8 and 9 weeks and weighing between 25 g and 27 g were used. They were housed in the animal house of the Laboratory of Pharmacology and Toxicology of Natural Products - LFTPN / ICB/UFPA. The animals. The animals were acclimatized for 10 days before the experimental manipulation. They were kept in 50cm x 60cm x 20cm (height x width x depth) boxes with wood shavings, at a temperature set between 25-27°C, in 12-hour light/dark cycles, receiving rodent food and filtered water during the tests. Work registered with the Animal Ethics Committee (CEUA) - UFPA under ID number 6968280324.

All experiments were conducted only after the loss of reflex in each animal, ensuring that they were adequately anesthetized. We strictly adhered to the institutional guidelines and regulations of the university where the analyses were performed, as well as international standards such as the ARRIVE guidelines. This ensured that our research was conducted with the highest ethical standards, prioritizing the welfare and humane treatment of the animals involved in the study.

*Drugs*

The following chemical substances were used in the work: the anesthetic Ketamine Hydrochloride obtained from the Köing Laboratory (Santana de Parnaíba, SP, Brazil); Xylazine Hydrochloride obtained from the Vallée laboratory (Montes Claros, MG, Brazil); the local anesthetic Lidocaine obtained from the Hipolabor laboratory (Sabará, MG, Brazil) for electrode implants and 0.9% saline solution and Caffeine Anhydrous obtained from the SIGMA laboratory.

*Experimental Design*

A total of 90 animals were divided into 10 groups (n=9). Five days after electrode implantation, the groups were treated with caffeine or saline solution, followed by 10 minutes of accommodation, after which the ECoG was recorded for 180 seconds.

*Electrocorticographic recording (EcoG)*

*Groups of animals without sleep deprivation:*

For the electrocorticogram, the groups were divided as follows: a) Control group, treated with saline solution (n=9); b) Caffeine group (treated with caffeine at 5 mg/kg via i.p.) (n=9); c) Caffeine group (10mg/kg via i.p.) (n=9); d) Caffeine group (15 mg/kg via i.p.) (n=9); e) Caffeine group (20mg/kg via i.p.) (n=9).

*Groups of animals deprived of 24 hours of sleep:*

The procedure consists of placing the animals in an acrylic box measuring 30 x 20 x 19 cm, filled with a thin layer of water up to a height of 5 cm and 6 platforms 8 cm high with a diameter of 2.5 cm, in such a way that when they fall asleep, they unbalance themselves from the platform and fall into the water and wake up.

For the electrocorticogram, the groups were divided as follows: a) Control group (treated with saline solution) (n=9); b) Caffeine group (5 mg/kg via i.p.) (n=9); c) Caffeine group (10 mg/kg via i.p.) (n=9); d) Caffeine group (15 mg/kg via i.p.) (n=9); e) Caffeine group (20 mg/kg via i.p.) (n=9).

*Surgery to place electrodes*

The animals were anesthetized by intraperitoneal injection of a combination of 10% ketamine hydrochloride at a dose of 100 mg/kg and 2% xylazine hydrochloride at a dose of 10 mg/kg.) The degree of anesthetic depth was assessed by means of the pain reflex after the interdigital reflex test. In cases where the reflex was still present, anesthetic supplementation was carried out with 1/3 of the dose initially applied. After abolishing the corneal reflex and verifying the depth of anesthesia, the animals were placed in a stereotactic apparatus.

The head was then trichotomized and the local anaesthetic Lidocaine was applied to the incised skin and to the ear bars used to attach the stereotaxic, to potentiate the anaesthetic effect and reduce the effect of the pressure exerted, respectively.

Afterwards, the asepsis procedure was carried out with alcoholic iodopolivinylpyrolidone (PVPI). After surgical procedures to expose the skull, two bilateral holes were drilled in the mouse's skull with a dental drill. Silver electrodes (925) (1.0 mm diameter tip exposure) were placed in the dura mater above the occipital cortex at the coordinates of the breech - 2 mm and ± 1.0 mm lateral (Paxinus) in the region of the visual cortex. A screw was fixed into the skull, and the electrodes fixed with dental acrylic (self-curing acrylic) using the screw as a base and ground for the ECoG recordings.

*Electrocorticographic recording*

During the recordings, the animals were kept in acrylic boxes with a restricted space of 50cm x 60cm x 20cm (height x width x depth). For all treatments, the ECoG recordings followed a standard protocol: application of the drug (caffeine) and 10 minutes of accommodation, followed by 180 seconds of ECoG recording.

*Data analysis*

The recordings were obtained using a differential amplifier with high AC input impedance (Grass Technologies, Model P511) adjusted with 0.3 HZ and 0.3 KHz filtering, monitored with an oscilloscope (Protek, Model 6510) and continuously digitized at a rate of 1 KHz by a computer equipped with a data acquisition card (National Instruments, Austin, TX).

The ECoGs were characterized using a tool built in the Python programming language (version 5.0) and the Signal ® 3.0 program. These programs enabled analysis of the frequency domain of brain waves, as well as visual inspection of wave patterns.

The "Numpy" and "Scipy" libraries were used for mathematical processing, and the "matplolib" library was used to obtain graphs and plots.

A graphical interface was developed using the PyQt4 library. Spectrograms were calculated using the Hamming window with 256 points (256/1000 s).

For power spectral density - PSD, each frame was generated with an overlap of 128 points per window. For each frame, the PSD was calculated using Welch's average periodogram method.

The frequency histograms were obtained by calculating the PSD of the signal using the Hamming window with 256 non-overlapping points, resulting in a resolution of 1 Hz per bin. Each wave displayed in the PSD is an average of a set of experiments. PSDs were calculated in each group and the averages were shown by individual bins. The analyses were carried out at frequencies up to 40 Hz and divided into bands according to Jalilifar et al. (2017) into Delta (0.5 - 3.0 Hz), Theta (3.5 - 7 Hz), Alpha (8 - 12), Beta (13 - 20 Hz) and Gamma (20 - 40 Hz), for interpretation of the dynamics during the development of brain activity.

*Statistical analysis*

After checking that the assumptions of normality and homogeneity of variances were met, the Kolmogorov-Sminov and Levene tests were used, respectively. The results were submitted to descriptive statistics, such as mean and standard deviation. One-way analysis of variance (ANOVA) was used, followed by a Tukey test. A significance index of *$p<0.05$, **$p<0.01$ and ***$p<0001$ will be adopted.

*Results*

The ECoG recordings of the control group showed amplitude below 0.05 mV to 0.07 mV (Figure 1 A, left), as shown in the 1-second amplification with the background frequency in beta (Figure 1 A, center) with a spectrogram showing greater energy intensity below 10 Hz (Figure 1 A, right). The electrocorticographic recording for the group that received caffeine 5 mg/kg i.p. acutely, showed amplitude in the recording with a variation of up to 0.08 mV (Figure 1 B, right) as shown in the amplification of the recording (Figure 1 B center), the spectrogram shows energy intensity at frequencies up to 40 Hz (Figure 1 B, right). The group treated with caffeine 10 mg/kg i.p. showed tracing characteristics with an amplitude of 0.12 mV (Figure 1C, left). The treated group showed a preponderance of low-frequency oscillations (Figure 1 C, center), as the spectrogram showed a more intense distribution of power at frequencies below 20 Hz (Figure 1 C,

240 right). The electrocorticographic recordings for the group treated acutely with 15 mg/kg
241 i.p. showed amplitude in the recording with a variation of up to 0.2 mV with a change in
242 the ECoG tracing (Figure 1 D, right) with a decrease in frequency can be seen in the
243 amplification of the recording (Figure 1 D, center), the spectrogram shows energy
244 intensity below 20 Hz (Figure 1 D, right).

245 For the group treated with caffeine 20mg/kg i.p., the animals'
246 electrocorticographic tracings showed a preponderance of low frequencies (below 30 Hz).
247 The amplification of the tracing shows the slowing down of the recording with an increase
248 in amplitude up to 2mV (Figure 1 E, left and center), the spectrogram shows a greater
249 distribution of power up to 40 Hz than the other groups (Figure 1 E, right).

**Figure 1**

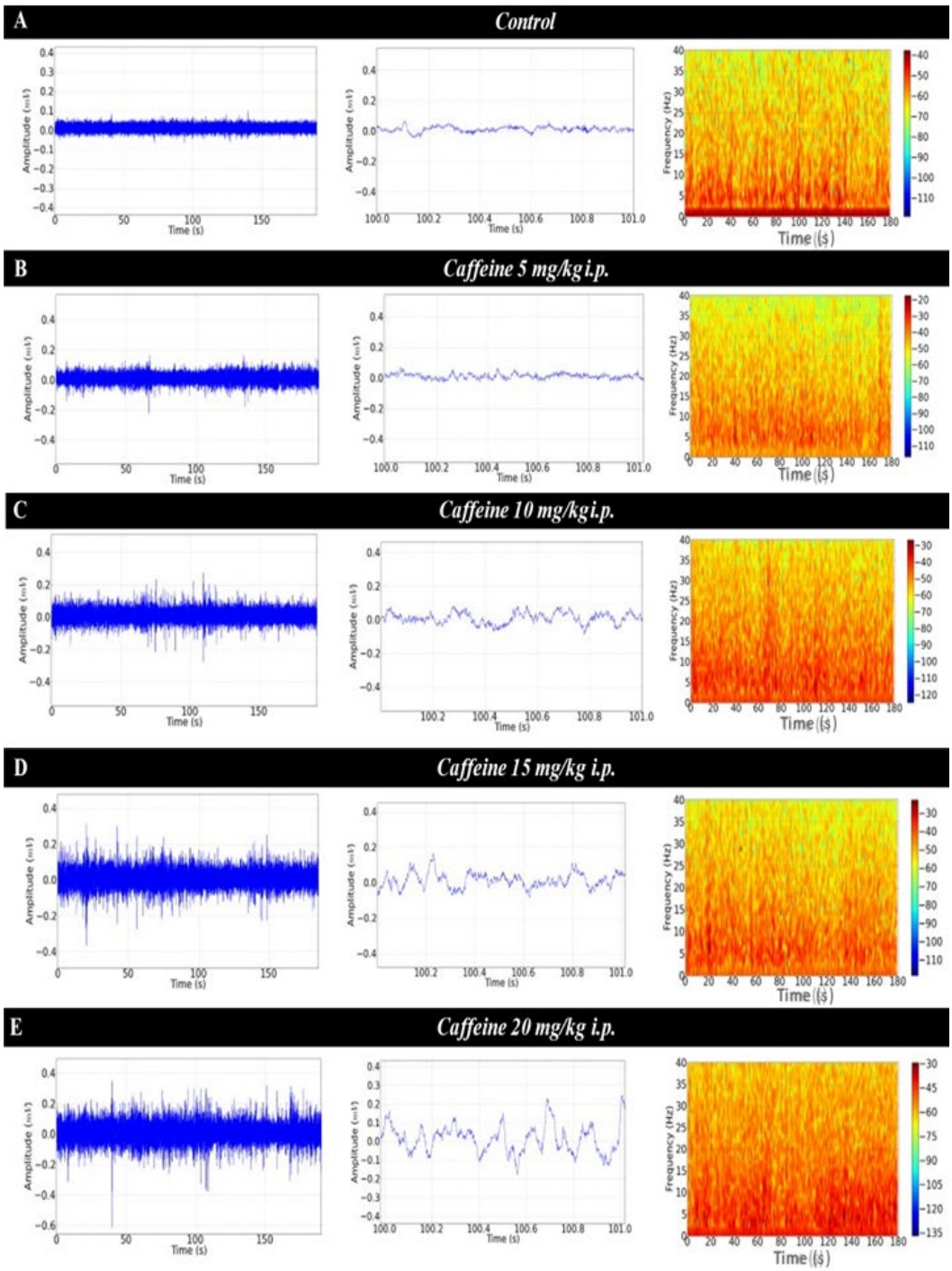


**Figure 1.** Demonstrations of 3-minute electrocorticographic (ECoG) recordings for animals treated with caffeine without sleep deprivation. ECoG tracing of the animal control group (application of 0.9% saline solution i.p.) (left), 1-second amplification of the tracing (100-101s) (center) and energy distribution spectogram (right) (A); ECoG tracing for the group of animals treated with caffeine 5mg/kg i. p. (left), amplification of the recording in 1 second (center) and respective spectogram with power distribution in frequencies up to 40 Hz (right) (B); ECoG tracing for the group treated with caffeine 10mg/kg i. p. (left), 1-second recording amplification (center) and spectogram (right) (C); ECoG tracing for the group treated with caffeine 15 mg/kg i.p. (left), 1-second recording amplification (center) and spectogram (right)

(D); ECoG tracing for the group treated with caffeine 20 mg/kg i.p. (left), 1-second recording amplification (center) and spectogram of power distribution at frequencies up to 40 Hz (right) (E).

The mean total linear power in the control group (0.2312 ± 0.035 $mV^2$ / Hz x $10^{-3}$) was lower than the caffeine-treated groups: caffeine 5 mg/kg (0.56002 ± 0.1385 $mV^2$ / Hz x $10^{-3}$), 10 mg/kg (0.7728 ± 0. 07638 $mV^2$ / Hz x $10^{-3}$), 15 mg/kg (1.104 ± 0.247 $mV^2$ / Hz x $10^{-3}$), 20 mg/kg (1.686 ± 0.3593 $mV^2$ / Hz x $10^{-3}$), all groups showed differences and the average power recorded was proportional to the dose of caffeine (Figure 2 A).

For delta oscillations (0.5 -3 Hz), the control group had a lower mean power (0.0618 ± 0.0101 $mV^2$ / Hz x $10^{-3}$) than the other groups. The groups treated with 5mg/kg (0.0947 ± 0.0198 $mV^2$ / Hz x $10^{-3}$), 10 mg/kg (0.102 ± 0.0141 $mV^2$ / Hz x $10^{-3}$) and 15 mg/kg (0.106 ± 0.0188 $mV^2$ / Hz x $10^{-3}$) were similar (p= 0.6953). The group treated with 20 mg/kg of caffeine had a higher mean linear power for delta oscillations (0.156 ± 0.0257 $mV^2$ / Hz x $10^{-3}$) (Figure 2 B).

For theta oscillations (3.5-7 Hz), the control group had a lower mean power than the other groups (0.0382 ± 0.00453 $mV^2$ / Hz x $10^{-3}$). The groups treated with 5mg/kg (0.126 ± 0.0231 $mV^2$ / Hz x $10^{-3}$) and 10 mg/kg (0.1684 ± 0.02616 $mV^2$ / Hz x $10^{-3}$) were similar (p=0.1511). The highest mean powers for theta oscillations were in the groups treated with caffeine 15 mg/kg (0.2606 ± 0.042 $mV^2$ / Hz x $10^{-3}$) and 20 mg/kg (0.349 ± 0.0657 $mV^2$ / Hz x $10^{-3}$) (Figure 2 C).

The mean power in alpha oscillations (8-12 Hz) in the control group (0.0130 ± 0.0025 $mV^2$ / Hz x $10^{-3}$) was lower than the mean power in the caffeine-treated groups. The groups treated with 5 mg/kg (0.0435 ± 0.0104 $mV^2$ / Hz x $10^{-3}$) and 10 mg/kg (0.0555 ± 0.0115 $mV^2$ / Hz x $10^{-3}$) were similar (p= 0.2940). The groups treated with 15mg/kg (0.106 ± 0.0137 $mV^2$ / Hz x $10^{-3}$) and 20mg/kg (0.127 ± 0.019 $mV^2$ / Hz x $10^{-3}$) showed the highest mean power among the groups (Figure 2 D).

The mean power in beta oscillations (13-20 Hz) in the control group (0.0111 ± 0.00136 $mV^2$ / Hz x $10^{-3}$) was similar to the group treated with 5 mg/kg (0.02872 ± 0.00616 $mV^2$ / Hz x $10^{-3}$) (p= 0.0722). The group treated with 5 mg/kg was similar to the group treated with 10mg/kg (0.0380 ± 0.00724 $mV^2$ / Hz x $10^{-3}$) (p=0.6089). The groups treated with 15 mg/kg (0.1182 ± 0.00217 $mV^2$ / Hz x $10^{-3}$) and 20 mg/kg (0.1912 ± 0.0198 $mV^2$ / Hz x $10^{-3}$) were superior to the other groups (Figure 2 E).

The mean power in gamma oscillations (20-40 Hz) in the control group (0.01058 ± 0.00219 $mV^2$ / Hz x $10^{-3}$) was similar to the group treated with 5 mg/kg (0.01281 ± 0.00187 $mV^2$ / Hz x $10^{-3}$) (p= 0.6117). The group treated with 5 mg/kg was similar to the group treated with 10mg/kg (0.0154 ± 0.0021 $mV^2$ / Hz x $10^{-3}$) (p = 0.4392). The groups treated with 15 mg/kg (0.0236 ± 0.00249 $mV^2$ / Hz x $10^{-3}$) and 20 mg/kg (0.0314 ± 0.0059 $mV^2$ / Hz x $10^{-3}$) were superior to the other groups (Figure 2 F).

**Figure 2**

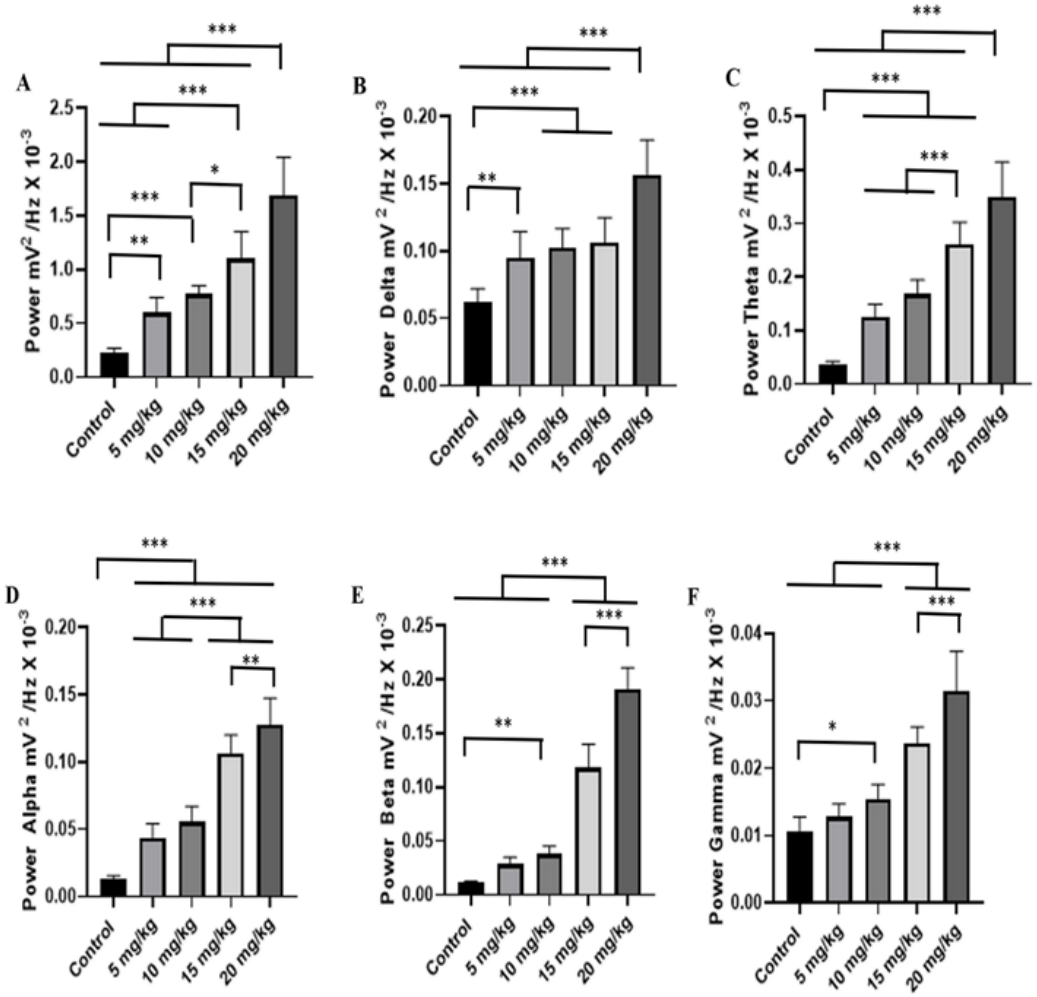


**Figure 2.** Graph of linear power distribution in the frequencies up to 40 Hz in the ECoG recordings between the non-sleep deprived groups with different caffeine treatments (A); Average linear power between the groups with frequency in the delta oscillations (0.5- 3 Hz) (B); Average linear power distribution in the theta oscillations (3.5- 7 Hz) for the groups (C). 5- 3 Hz) (B); Average power distribution in theta frequency (3.5- 7 Hz) for the groups (C); Graph of linear power distribution in the recordings between the groups in alpha oscillations (8-12 Hz) (D); Average power distribution of the

groups in beta frequency (13-20 Hz) ( E); Graph of linear power distribution between the groups in gamma frequency (20-40 Hz) (F). (After ANOVA followed by Tukey, * $p<0.05$ ** $p<0.01$ ***$p<0.001$, n=9).

For the group of animals deprived of sleep for 24 hours, an increase in amplitude to 0.08 mV was observed in the ECoG due to the capture of acute vertex waves (Figure 3 A, left), demonstrated in the 1-second amplification with a preponderance of the background frequency in Alpha (Figure 3 A, center), the frequency spectrogram shows a greater distribution of energy between the frequencies up to 15 Hz (Figure 3 A, right). The group treated with caffeine 5 mg/kg i.p. and deprived of sleep showed ECoG tracing amplitude of 0.06 mV with a preponderance of low frequency rhythms (Figure 3 B, left), amplification of the recording shows Beta background oscillations (13-20 Hz) (Figure 3 B, center), the spectrogram shows power distribution below 15 Hz (Figure 3 B, right). The group treated with caffeine 10 mg/kg i.p. and sleep deprivation showed slowing of the tracing with a preponderance of frequencies between delta, theta, alpha, a considerable gain in the powers represented by the frequencies from 1 to 20 Hz (Figure 3 C left), characteristics of slowing of the tracing (Figure 3 C, center), the spectrogram showed energy intensity below 20 Hz (Figure 3 C right). The group treated with caffeine 15 mg/kg i.p. and sleep deprivation showed an increase in the power of the tracing in beta with less preponderance for the delta, theta and alpha frequencies, a considerable gain in the powers represented by the frequencies from 13 to 20 Hz (Figure 3 D left), with a tracing amplitude of 0.1 mV (Figure 3 D, center), the spectrogram showed energy intensity below 20 Hz (Figure 3 D right).

For the sleep-deprived group that received caffeine 20 mg/kg i.p., the animals' electrocorticographic tracings showed a preponderance of frequencies below 30 Hz (delta, theta, alpha and beta), but with an increase in gamma. The amplification of the tracing shows slowing and irregularities in the recording (Figure 3 E, left and center), the spectrogram shows a greater distribution of power up to 40 Hz than the other groups (Figure 3 E, right).

Figure 3

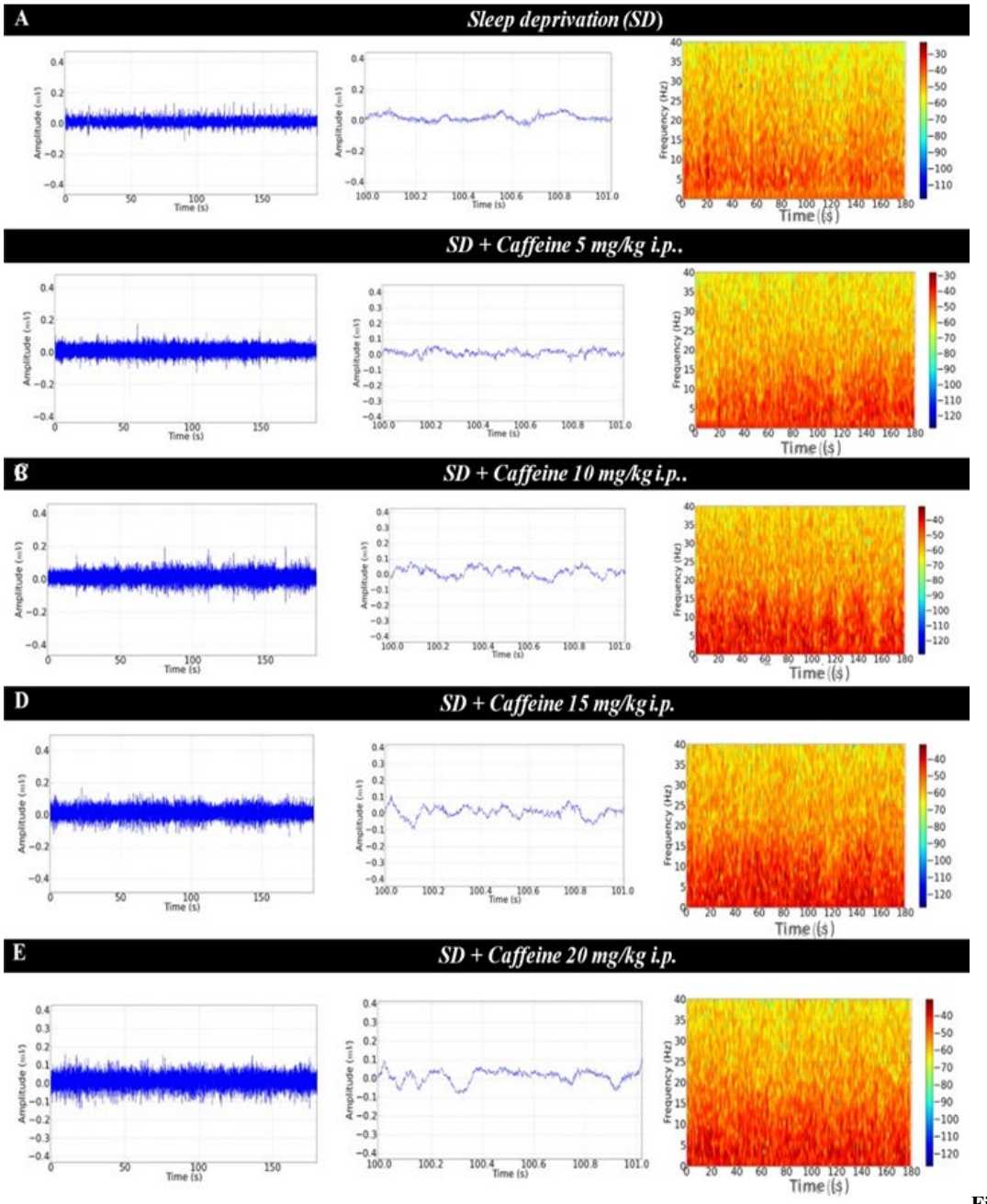


**Figure 3 -** Demonstrations of 3-minute electrocorticographic (ECoG) recordings for animals treated with caffeine after 24 hours of sleep deprivation. ECoG tracing of the sleep-deprived animal control group (application of 0.9% saline solution i.p.) (left), 1-second amplification of the tracing (100-101s) (center) and energy distribution spectogram (right) (A); ECoG tracing for the group of sleep-deprived animals treated with caffeine 5mg/kg i. p. (left), amplification of the recording in 1 second (center) and respective spectogram with power distribution in frequencies up to 40 Hz (right) (B); ECoG tracing for the sleep deprived group treated with caffeine 10mg/kg i. p. (left), 1-second recording amplification (center) and spectogram (right) (C); ECoG tracing for the sleep deprived group treated with caffeine 15 mg/kg i.p. (left), 1-second recording amplification (center) and spectogram (right) (D); ECoG tracing for the sleep deprived group treated with caffeine 20 mg/kg i.p. (left), 1-second recording amplification (center) and spectogram of power distribution in frequencies up to 40 Hz (right) (E).

The mean total linear power in the control group for the sleep-deprived animals (0.353 ± 0.0447 $mV^2$ / Hz x $10^{-3}$) was similar to the groups treated with caffeine 5mg/kg (0.362 ± 0.06198 $mV^2$ / Hz x $10^{-3}$) and 10 mg/kg (0.442 ± 0.122 $mV^2$ / Hz x $10^{-3}$) (p= 0.532). The groups treated with 10 mg/kg and 15 mg/kg (0.581 ± 0.1565 $mV^2$ / Hz x $10^{-3}$) were similar (p= 0.1052). The group treated with 20 mg/kg had a higher mean power than the other groups (figure 4 A).

For delta oscillations, the control group (0.0859 ± 0.0858 $mV^2$ / Hz x $10^{-3}$) was similar to the groups treated with caffeine 5 mg/kg (0.085 ± 0.0109 $mV^2$ / Hz x $10^{-3}$), 10 mg/kg (0.0863 ± 0.0119 $mV^2$ / Hz x $10^{-3}$) and 15 mg/kg (0.098 ± 0.0114 $mV^2$ / Hz x $10^{-3}$) (p= 0.1964). The group treated with caffeine 20 mg/kg (0.1198 ± 0.0116 $mV^2$ / Hz x $10^{-3}$) had higher power than the other groups (Figure 4 B).

For theta oscillations, the control group had a lower mean power than the other groups (0.0676 ± 0.0167 $mV^2$ / Hz x $10^{-3}$). The groups treated with 5mg/kg (0.144 ± 0.0339 $mV^2$ / Hz x $10^{-3}$) was lower than the groups treated with 10 mg/kg (0.193 ± 0.0205 $mV^2$ / Hz x $10^{-3}$) and 15 mg/kg (0.187 ± 0.0121 $mV^2$ / Hz x $10^{-3}$). The groups treated with 10 mg/kg and 15 mg/kg were similar (p= 0.9893). The group treated with caffeine 20 mg/kg (0.305 ± 0.032 $mV^2$ / Hz x $10^{-3}$) was superior to the other groups (Figure 4 C).

The mean power in the alpha oscillations of the control group (0.0411 ± 0.00795 $mV^2$ / Hz x $10^{-3}$) was lower than the mean power of the caffeine-treated groups. The groups treated with 5 mg/kg (0.0758 ± 0.0161 $mV^2$ / Hz x $10^{-3}$) and 10 mg/kg (0.0820 ± 0.0137 $mV^2$ / Hz x $10^{-3}$) were similar (p= 0.8995). The groups treated with 15mg/kg (0.1067 ± 0.0203 $mV^2$ / Hz x $10^{-3}$) and 20mg/kg (0.134 ± 0.0121 $mV^2$ / Hz x $10^{-3}$) showed the highest mean power among the groups (Figure 4 D).

The mean power in beta oscillations in the sleep-tested control group (0.0166 ± 0.00428 $mV^2$ / Hz x $10^{-3}$) was similar to the 5mg/kg group (0.0267 ± 0.00448 $mV^2$ / Hz x $10^{-3}$) (p= 0.6453). The group treated with 5 mg/kg was similar to the group treated with 10 mg/kg (0.0393 ± 0.0103 $mV^2$ / Hz x $10^{-3}$) (p=0.4370). The groups treated with 15 mg/kg (0.0787 ± 0.0154 $mV^2$ / Hz x $10^{-3}$) and 20 mg/kg (0.2014 ± 0.02879 $mV^2$ / Hz x $10^{-3}$) were superior to the other groups (Figure 4 E).

The mean power in gamma oscillations for the sleep-deprived control group (0.01305 ± 0.001872 $mV^2$ / Hz x $10^{-3}$) was similar to the group treated with 5 mg/kg

(0.0132 ± 0.00122 mV² / Hz x 10-³) and the group treated with 10mg/kg (0.0141 ± 0.00164 mV² / Hz x 10-³) (p= 0.7874). The group treated with 15 mg/kg (0.0161 ± 0.00182 mV² / Hz x 10-³) was similar to the group treated with 10 mg/kg (p= 0.2076). The group treated with 20 mg/kg (0.0209 ± 0.00296 mV² / Hz x 10-³) was superior to the other groups (Figure 4 F ).

**Figure 4**

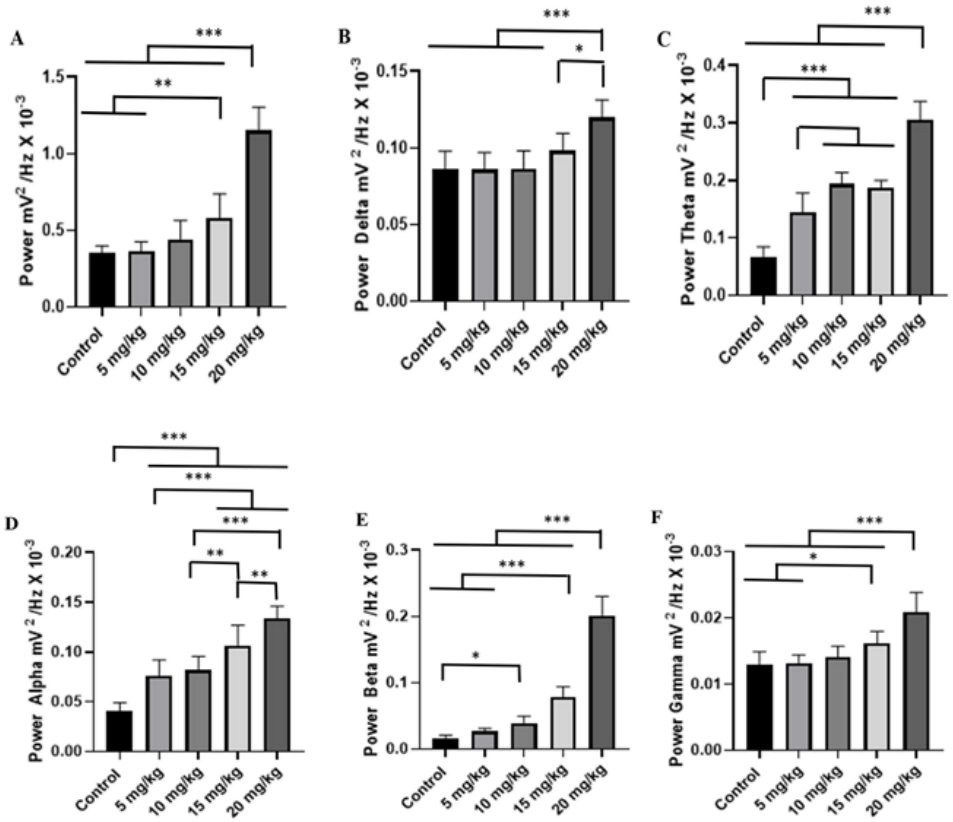


**Figure 4 -** Graph of linear power distribution in the frequencies up to 40 Hz in the ECoG recordings between the sleep deprived groups submitted to different caffeine treatments (A); Average linear power distribution between the groups in the frequency of delta oscillations (0. 5- 3 Hz) (B); Average linear power distribution in the frequency of theta oscillations (3.5- 7 Hz) (C). 5- 3 Hz) (B); Average power distribution in theta frequency (3.5- 7 Hz) (C); Graph of linear power distribution in the recordings between the groups in alpha oscillations (8-12 Hz) (D); Average power distribution of the groups in beta frequency (13-20 Hz) (E); Graph of linear power distribution between the groups in gamma frequency (20-40 Hz) (F). (After ANOVA followed by Tukey, * $p<0.05$ ** $p<0.01$ ***$p<0.001$, n=9).

The relationship between the groups without sleep deprivation and those with sleep deprivation treated with different doses of caffeine showed different total power

averages, thus, when relating the power averages of the control vs SD groups (p= 0.8738), Groups treated with caffeine 5 mg/kg (p= 0.1004). The sleep-deprived group showed lower power after treatment with caffeine 10 mg/kg, 15 mg/kg and 20 mg/kg (Figure 5 A).

For delta oscillations, the sleep-deprived group was superior to the non-sleep-deprived group. During treatment with 5 mg/kg of caffeine, the groups were similar (p=0.9694). The groups treated with 10 mg/kg of caffeine were similar (p= 0.4466). The groups treated with 15mg/kg were similar (p= 0.9893) (Figure 5 B).

For theta, the control groups with sleep deprivation and without sleep deprivation were similar (p=0.645), and the similarity was repeated for the groups treated with 5 mg/kg (p=0.9711), 10 mg/kg (p=0.8208) and 20 mg/kg (p=0.1260) (Figure 5C).

For the alpha oscillations, the sleep-deprived group had a higher mean power than the control group and the groups treated with 5 mg/kg and 10 mg/kg. The groups treated with 15mg/kg (p=0.999) and 20 mg/kg (p= 0.9929) were similar (Figure 5 D).

For the oscillations in beta, the control (p=0.9985), 5 mg/kg (p=0.999), 10 mg/kg (p= 0.999) and 20 mg/kg (0.8992) groups were similar. The animals treated with 15 mg/kg in the control group without sleep deprivation were superior (Figure 5 E).

For Gamma oscillations, the control groups were similar (p=0.6540), 5 mg/kg (p=0.999), 10 mg/kg (p= 0.9879). The animals without sleep deprivation treated with 15 mg/kg and 20 mg/kg had higher gamma averages than the groups with sleep deprivation (Figure 6 E).

**Figure 5**

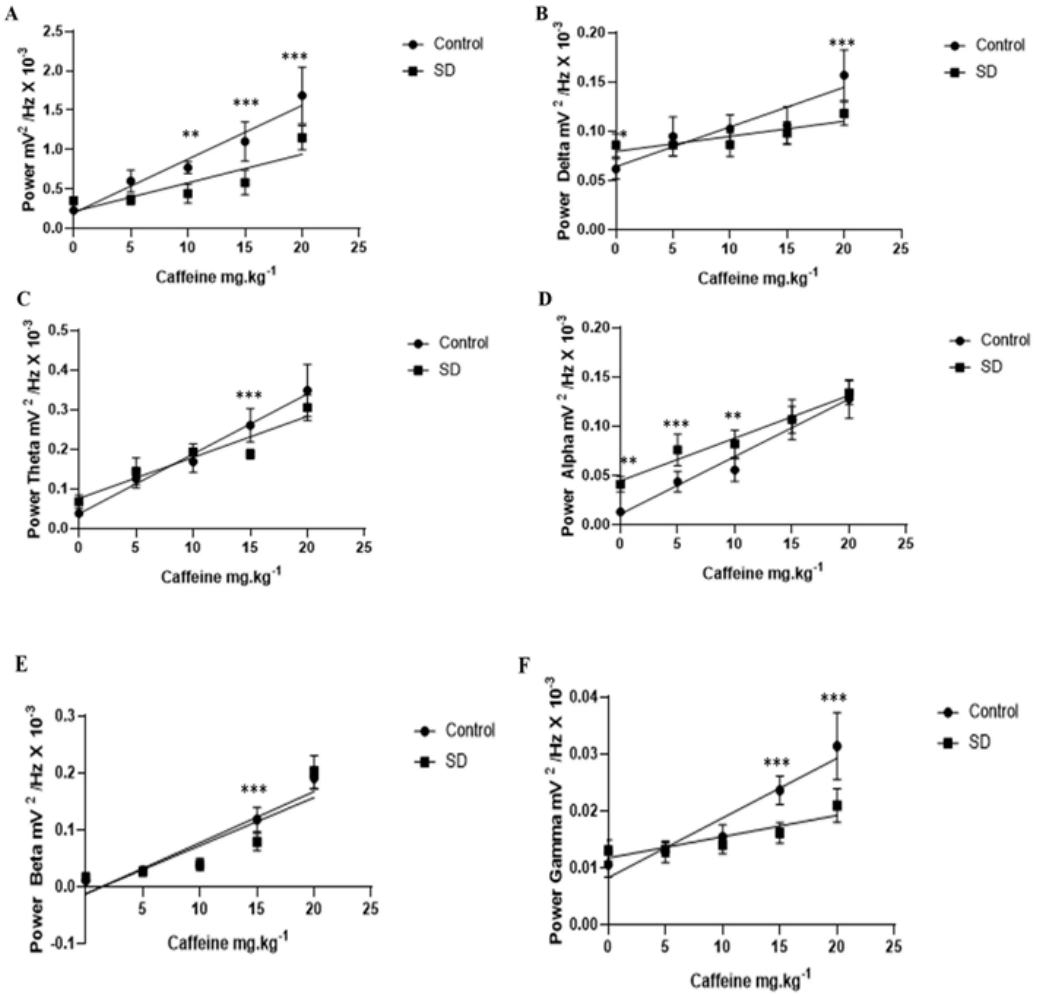


**Figure 5-** Linear regression graph of the mean power of the recordings between the groups without sleep deprivation and with sleep deprivation treated with caffeine at frequencies up to 40 Hz in the ECoG recordings (A); Linear regression of the mean power in the delta oscillations (0. 5- 3 Hz) (B); Linear regression of the mean power distribution in the theta frequency (3.5- 7 Hz) (C). 5- 3 Hz) (B); Linear regression of the mean power distribution at theta frequency (3.5- 7 Hz) (C); Linear regression of the mean power in the recordings between the groups at alpha oscillations (8-12 Hz) (D); Linear regression of the mean power of the groups at beta frequencies (13-20 Hz) (E); Linear regression of the mean gamma frequencies (20-40 Hz) (F). (After ANOVA followed by Tukey, * $p<0.05$ ** $p<0.01$ ***$p<0.001$, n=9).

*Discussion*

Caffeine is a substance naturally found in various plants, such as coffee, tea, cocoa and some varieties of soft drinks and energy drinks (Bruton et al. 2010). It belongs to the class of chemical compounds called methylxanthines and is known for its stimulating

effect on the central nervous system. It is widely consumed throughout the world due to its stimulating effects, which include increased concentration, alertness, and decreased fatigue (Morelli & Simola, 2011). These effects occur because caffeine blocks the action of a brain chemical called adenosine, which is responsible for promoting drowsiness and feelings of tiredness. By blocking adenosine, caffeine promotes an alert response in the body (Reyes & Cornelis, 2018).

This substance is also known chemically as 1,3,7-trimethylxanthine and has a very interesting chemical structure and is classified as a methylxanthine. Its molecular formula is C8H10N4O2 (Martínez-Hernández et al. 2016). Chemically speaking, caffeine's structure is based on a purine ring, which consists of two fused rings, an imidazole and a pyrimidine. This ring is a common feature in many biochemical compounds, including other xanthines and nucleotides. In addition, it has three methyl groups (CH3) attached to the purine ring. These methyl groups are responsible for giving caffeine its stimulating properties and other biological activities. It also contains two amine groups (NH2) attached to the purine ring. These amine groups contribute to caffeine's pharmacological properties, such as its ability to interact with adenosine receptors in the brain. Caffeine has a carbonyl group (C=O) and an oxygen atom (O) attached to the purine ring. These groups are important for caffeine's pharmacological activity, allowing specific interactions with proteins and receptors in the body.

In addition to its stimulating effects, caffeine can also have other effects on the body. For example, it can increase heart rate, stimulate acid production in the stomach and increase diuresis, which can lead to increased urinary frequency. Although moderate caffeine consumption is generally considered safe for most people, excessive consumption can lead to negative side effects such as nervousness, anxiety, insomnia, tremors and heart palpitations (Stavric et al. 1988). In addition, some people may be more sensitive to caffeine than others and may experience side effects even with lower doses. As with any substance, it is important to consume caffeine in moderation and to be aware of your own tolerance limits. People with certain medical conditions, such as sleep disorders, anxiety or heart problems, should exercise caution when consuming caffeine and may need to limit their intake (Verster & Koenig, 2017).

Caffeine is often used to mitigate the effects of sleep deprivation due to its ability to promote alertness and concentration. When sleep deprivation occurs, adenosine levels in the brain increase. Adenosine is a chemical that promotes sleepiness and relaxation

(Porkka-Heiskanen et al. 1999). Caffeine acts by blocking adenosine receptors, which prevents adenosine from exerting its sedative effects (Song et al, 2023). This substance can help increase alertness and vigilance, even when someone is sleep deprived. This can be particularly useful in situations where a person needs to stay awake and focused, such as during long hours of work or study (McLellan, Caldwell, Lieberman 2016).

In addition, it can reduce the drowsiness associated with sleep deprivation, helping the individual to stay awake and alert for longer than they would normally be able to without it (Gardiner et al., 2023). Studies suggest that caffeine can improve certain aspects of cognitive performance, such as information processing speed and short-term memory, which may be useful in offsetting the negative effects of sleep deprivation on cognitive function (Paiva et al., 2022). Although caffeine can help to temporarily alleviate the effects of sleep deprivation, it is important to note that its effects are limited and temporary. Caffeine does not replace the need for adequate sleep and does not solve the underlying problems of chronic sleep deprivation.

It is important to exercise caution when using caffeine to compensate for sleep deprivation. Excessive caffeine consumption can lead to negative side effects such as nervousness, anxiety, insomnia and heart palpitations (Nehlig, 2016). In addition, caffeine can interfere with sleep quality if it is consumed too close to bedtime. Therefore, it is advisable to limit caffeine consumption and try to prioritize adequate sleep whenever possible (Gardiner et al., 2023).

There are different stages of sleep, each characterized by distinct patterns of brain activity. The brain waves generated during sleep are key to distinguishing between these stages. There is the NREM, or Non-REM, stage, and within this we can subdivide it into Stage 1 (N1), 2 (N2), 3 (N3). Stage 1 is the initial stage of sleep, where the transition between being awake and falling asleep occurs. The brain waves in this stage are predominantly theta waves, which are low amplitude and high frequency. In stage 2 (N2), brain activity decreases further, and characteristic brain wave patterns called K-complexes and sleep spindles appear. The waves continue to be predominantly theta waves but can also include short-duration delta waves. Finally, stage 3 (N3) is the deepest stage of NREM sleep, also known as slow-wave sleep. The brain waves are now predominantly delta waves, with a low frequency and high amplitude. This stage is essential for physical and mental restoration (Barbato et al., 2020).

After these stages, we have what we call the REM (Rapid Eye Movement) stage and during REM sleep, the brain displays intense activity, similar to wakefulness, even though the body's muscles are relaxed. Brain waves during REM sleep are predominantly beta waves and disorganized, with periods of theta waves and even alpha waves. The literature shows that this stage is associated with vivid dreams and is essential for memory consolidation and emotional processing. The NREM and REM phases alternate throughout the night in cycles of approximately 90 minutes, with REM sleep becoming longer as the night progresses. These cycles are important for ensuring quality, restorative sleep (Blumberg et al., 2020).

Brain waves during sleep are measured using electroencephalography (EEG), which records the electrical activity of the brain. This technique allows us to better understand sleep patterns and how they influence physical and mental health.

## Declaration of conflict of interest

The authors have no conflict of interest to declare.


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## Disclosure Statement

Financial Disclosure: none.

Non-financial Disclosure: none

## Author contributions

Luciana Eiró-Quirino, Moisés Hamoy: Conceptualization; Luciana Eiró-Quirino, Pedro Henrique de Castro Sampaio, Raíssa Vieira de Souza, Clarissa Araújo da Paz, Daniella Bastos de Araújo, Gabriela Brito Barbosa, Luana Vasconcelos de Souza: Writing- Original draft preparation; Clarissa Araújo da Paz: Methodology; Luciana Eiró-Quirino, Moisés Hamoy: Visualization, Investigation; Luciana Eiró-Quirino, , Clarissa Araújo da Paz, Daniella Bastos de Araújo, Pedro Henrique de Castro Sampaio, Raíssa Vieira de Souza: Writing- Reviewing and Editing; Moisés Hamoy: Data curation, Moisés Hamoy: Supervision, Moisés Hamoy: Software, Moisés Hamoy: Validation.

## Data availability statement

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

## Ethics declarations

All procedures were approved by the ethics committee (CEUA/UFPA- ID 6968280324). All the experiments were carried out using the ARRIVE checklist.

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## TERCEIRO CAPÍTULO DA TESE

# Characterization of the cortical activity of post-workout caffeine use in Swiss mice

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## Abstract

The use of ergogenic substances, such as caffeine, has gained prominence in improving both physical and cognitive performance. This study aimed to evaluate the effects of post-exercise administration of different doses of caffeine (10, 20, and 30 mg/kg) on the cortical activity of Swiss mice through electrocorticography (ECoG). A total of 72 male Swiss mice were subjected to forced swimming exercise, followed by intraperitoneal caffeine administration. The ECoG recordings analyzed oscillations in various frequency bands (delta, theta, alpha, beta, and gamma) to investigate the neurophysiological impact of caffeine in the post-exercise context. Results demonstrated that higher doses of caffeine, particularly 30 mg/kg, significantly increased cortical oscillations in the beta and gamma bands, suggesting enhanced neuronal excitability. Additionally, animals treated with this higher dose showed ictal-like activity, indicating a potential risk of convulsive events. These findings highlight a dose-dependent relationship between caffeine and cortical activity, emphasizing the need for caution in using high doses of caffeine in athletic contexts due to potential adverse effects on the central nervous system. Further studies are warranted to explore the long-term effects and safety of caffeine use post-exercise.

**Key-words: Caffeine; Electrocorticography; Swiss mice; Cortical activity; Post-exercise; Neuronal excitability**

## Introduction

The pursuit of improving physical and mental performance has led to the increasing use of ergogenic substances, such as caffeine and pre-workout supplements. Caffeine, a natural alkaloid found in various plants such as coffee (Coffea spp.) and tea (Camellia sinensis), is one of the most consumed psychoactive substances in the world, widely known for its stimulating effects on the central nervous system (CNS) (Guest et al., 2021). Its mechanism of action involves blocking adenosine receptors, an inhibitory neurotransmitter, which results in increased release of excitatory neurotransmitters, such as dopamine and norepinephrine, promoting greater alertness and reducing the perception of fatigue (Temple et al., 2017; Cappelletti et al., 2015).

In the sports context, caffeine has been used as an ergogenic agent due to its ability to enhance endurance exercise performance, increase muscle strength, and optimize cognition during intense activities. Studies indicate that caffeine ingestion before exercise can reduce the perception of effort, increase fatty acid oxidation, and preserve muscle glycogen, making it an appealing substance for athletes and physical activity enthusiasts (Guest et al., 2021; Talanian et al., 2016).

In addition to caffeine, pre-workout supplements have gained popularity in recent years among gym-goers and athletes from different sports disciplines. These supplements generally contain a combination of ingredients aimed at improving physical performance, such as caffeine, creatine, beta-alanine, and branched-chain amino acids (BCAAs) (Wickham et al., 2018; Saunders et al., 2023). The presence of caffeine in pre-workout supplements is of particular interest, as its synergistic effects with other components can enhance ergogenic benefits, leading to better performance in high-intensity activities (Pakulak et al., 2022; Gardiner et al., 2023).

Thus, investigating the effects of caffeine, whether alone or in combination with other ingredients found in pre-workout supplements, is essential to understand its impact on athletic performance and metabolism, as well as to provide information that can guide the safe and effective use of these substances.

The aim of this study is to investigate the effects of administering different doses of post-workout caffeine on the cortical activity of Swiss mice, through electrocorticography (ECoG). The goal is to analyze changes in brain oscillations across various frequency bands (delta, theta, alpha, beta, and gamma) and to evaluate how physical exercise, combined with caffeine, impacts cortical excitability and power distribution across different frequencies, providing insights into the neurophysiological effects of caffeine in the context of intense physical activity.

## Materials and Methods

### *Animals*

We used 72 adult male Swiss mice obtained from the Biological Sciences Institute of the Federal University of Para and kept in the experimental animal house of the Laboratory of Pharmacology and Toxicology of Natural Products. All the animals were housed under a controlled temperature of 23-25°C and a 12-hour light-dark cycle, with food and water available ad libitum. The animals were divided into the following experimental groups: a) control group; b) post-training control (SHAM); c) caffeine control 10 mg/kg i.p.; d) caffeine control 20mg/kg i.p.; e) caffeine control 30 mg/kg i.p.; f) caffeine 10mg/kg i.p. post-training, g) caffeine 20 mg/kg i.p. post-training and h) caffeine 30 mgkg i.p. post-training. Training consisted of 30 minutes of forced swimming. After the training period, the animals were treated with caffeine immediately via i.p. 10 minutes after application, the animals were connected to a high-impedance amplifier for ECoG assessment. Each group consisted of 9 individuals.

The research was conducted in accordance with the precepts of national legislation for experimentation and the Ethical Principles of the National Council for the Control of Animal Experimentation (CONCEA), approved by the Research Ethics Committee on the Use of Animals under numbering 2675110219.

***Chemical products***

The chemical products used to carry out the work: 1. Electrode implant surgery: anesthetic Ketamine Hydrochloride, obtained from the Köing Laboratory (Santana de Parnaíba, SP, Brazil); Xylazine Hydrochloride, obtained from the Vallée laboratory (Montes Claros, MG, Brazil); the local anesthetic Lidocaine, from the Hipolabor laboratory (Sabará, MG, Brazil); Caffeine (Sigma, USA).

***Electrocorticogram ECoG***

The electrodes were surgically implanted at the bregma -0.36 coordinate and 1 mm to the side of each hemisphere represented by the motor cortex (Paxios 2005). On the fifth postoperative day, 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 continuously digitized at a rate of 1 KHz by a computer equipped with a data acquisition board (National Instruments, Austin, TX), stored on a hard disk and processed using specialized software (LabVIEW express). The recording electrode was located on the right side of the hemisphere and the reference electrode on the left. The entire experiment was carried out inside a Faraday cage. The recordings were made from 7:30 am to 10:30 am.

### *Data analysis*

To analyze the acquired signals, a tool was built using the Python programming language version 2.7. 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 recordings were analyzed up to 40 Hz, and the bands were analyzed according to Jalilifar et al. (2017) and Hamoy et al. (2018), which correspond to: Delta (0.5-3 Hz), Theta (3.5-7.5 Hz), Alpha (8-12 Hz), Beta (13-20) and Gamma (20-40 Hz).

### *Statistical analysis*

The results were expressed as mean ± standard deviation (SD). Normality and homogeneity of variance were verified using the Kolmogorov-Smirnov and Levene tests, respectively. Behavioral analyses of seizures (latencies) and electrocorticographic results were performed using one-way analysis of variance (ANOVA) and Tukey's post-test. The level of significance was set at *$p<0.05$, **$p<0.01$ and ***$p<0.001$ in all cases. GraphPad® Prism 8 software was used for all analyses.

### *Results*

The ECoG recordings of the control group showed amplitude below 0.1 mV (Figure 1 A, left), as shown in the 1-second amplification (Figure 1 A, center) with spectrogram showed the highest energy intensity below 10 Hz (Figure 1 A, right). The electrocorticographic recordings for the group subjected to physical exercise for 30 minutes showed greater amplitude in the recording similar to the 0.1 mV control group ( Figure 1 B, right) as shown in the amplification of the recording ( Figure 1 B center), the spectrogram shows greater energy intensity below 30 Hz ( Figure 1 B, right). The groups treated with caffeine 10 mg/kg i.p., 20 mg/kg i.p. and 30 mg i.p. showed tracings with a preponderance of amplitude at 0.1 mV (Figure 1 C, D, E, left), with increased irregularity in the tracing varying according to the increase in dose (Figure 1 C, D, E, center), the spectrogram shows a greater distribution of power at 40 Hz with greater intensity at frequencies below 10 Hz (Figure 1 C, D, E, right). The groups subjected to 30 minutes of physical exercise and treated with caffeine 10 mg/kg i.p., 20 mg/kg i.p. and 30 mg i.p. showed tracings with amplitude above 0.1 mV (Figure 1 F, G, H, left), with increased irregularities in the tracing (Figure 1 F, G, H, center) , the spectrogram shows a greater distribution of power in the frequencies below 40 Hz (Figure 1 C, D, E, right).

Treatment with 30 mg/kg of caffeine showed irregularities in the tracing similar to the convulsive state, with a volley of potentials characterized by a peak amplitude of 0.3 to 0.4 mV, followed by a post-ictal period characterized by a decrease in activity.

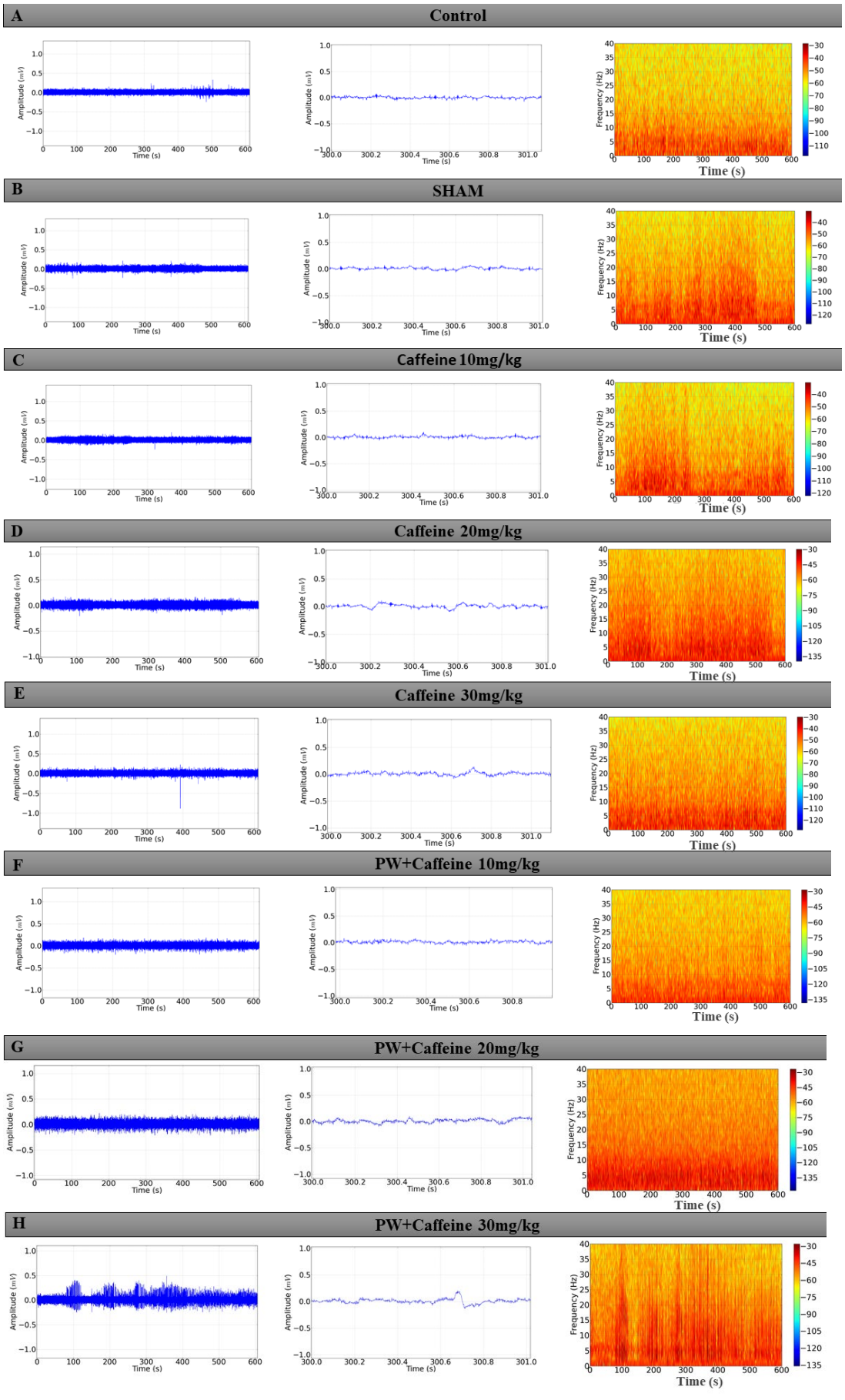

A
Control
B
SHAM
C
Caffeine 10mg/kg
D
Caffeine 20mg/kg
E
Caffeine 30mg/kg
F
PW+Caffeine 10mg/kg
G
PW+Caffeine 20mg/kg
H
PW+Caffeine 30mg/kg
Amplitude (mV)
Time (s)
Frequency (Hz)

**Figure 1.** Demonstrations of electrocorticographic (ECoG) recordings lasting 10 minutes. ECoG tracing of the groups showing amplitude and regularity of the recording (left), 1-second amplification of the tracing showing morphographic characteristics of the recording (center) and spectrogram of energy distribution at frequencies up to 40 Hz (right), for the following groups: Control (A); SHAM group (B); group treated with caffeine 10mg/kg i. p. (C); caffeine 20 mg/kg i.p. (D); caffeine 30 mg/kg i.p. (E); Post-workout group and caffeine 10mg/kg i.p (F); Post-workout group and caffeine 20mg/kg i.p (G); Post-workout group and caffeine 30mg/kg i.p (H).

The control group (0.3509 ± 0.07640 $mV^2$ / Hz x $10^{-3}$) was similar to the SHAM group ( p= 0.0592) and the group treated with caffeine 10 mg/kg ( p= 0.1536), but was lower than the other groups. The SHAM group (0.574 ± 0.1008 $mV^2$ / Hz x $10^{-3}$) was similar to the groups treated with caffeine 20 mg/kg (p=0.999) and caffeine 30 mg/kg ( p= 0.989), but was lower than the other groups. All the groups submitted to physical exercise and post-workout caffeine showed an increase in power up to 40 HZ, with a higher average than the other groups. The PW caffeine 30 mg/kg group (1.294 ± 0.2610 $mV^2$ / Hz x $10^{-3}$) was higher than the other treated groups (Figure 2A).

For delta oscillations, the control group had a mean of ( 0.1645 ± 0.03303 $mV^2$ / Hz x $10^{-3}$) and was similar to the SHAM groups ( p=0.9997); caffeine 10mg/kg ( p= 0.9999) , caffeine 20mg/kg ( p= 0.9593) , caffeine 30 mg/kg ( p= 0.3975) and PW caffeine 30 mg/kg ( p= 0.0684). The PW caffeine group ( 0.4206 ± 0.07556 $mV^2$ / Hz x $10^{-3}$ ) was higher than the other groups. The PW 10 mg/kg ( 0.2576 ± 0.04412 $mV^2$ / Hz x $10^{-3}$) and PW 30 mg/kg groups were similar ( p=0.734) ( Figure 2 B).

For theta oscillations, the control group mean (0.1015 ± 0.0159 $mV^2$ / Hz x $10^{-3}$) was similar to the SHAM group ( p= 0.5003) and the group treated with caffeine 10mg/kg ( p= 0.7319). The SHAM group ( 0.1607 ± 0.02867 $mV^2$ / Hz x $10^{-3}$ ) was similar to the groups treated with caffeine 20 mg/kg ( p= 0.5612) and treated with caffeine 30 mg/kg ( p= 0.1916) . The group treated with caffeine 20 mg/kg was similar to the PW caffeine 10 mg/kg group ( p= 0.3090). The group treated with caffeine 30 mg/kg was similar to the Pw caffeine 10 mg/kg group ( p= 0.7271). The PW caffeine 20 mg/kg group was superior to the other treated groups (figure 2 C).

For alpha activity ( 8-12 Hz), the control group's mean power (0.03399 ± 0.006531 $mV^2$ / Hz x $10^{-3}$) was similar to that of the groups treated with caffeine 10mg/kg ( p= 0.1145) and 30 mg/kg ( p= 0.3711) , but it was lower than that of the other groups. The SHAM group (0.07167 ± 0.01522 $mV^2$ / Hz x $10^{-3}$) was similar to the groups treated with caffeine 10 mg/kg (p=0.999) , 20mg/kg (p= 0.9967), 30 mg/kg (p= 0.979) and PW caffeine 10 mg/kg (p= 0.999), but was lower than the other groups. The PW caffeine 30 mg/kg group was superior to the other groups (Figure 2 D).

For beta oscillation activity, the control group (0.02788 ± 0.00586 $mV^2$ / Hz x $10^{-3}$) was similar to the SHAM group (p= 0.3672) and the group treated with 10 mg/kg caffeine (p= 0.1542), but was inferior to the other groups. The SHAM group (0.04632± 0.008391 $mV^2$ / Hz x $10^{-3}$) was similar to the groups treated with caffeine 10mg/kg (p=0.9998) , 20 mg/kg (p= 0.2831) , 30 mg/kg (p= 0.7997) and PW caffeine 10 mg/kg (p= 0.8731). The group treated with 30 mg/kg caffeine ( 0.05899± 0.00875 $mV^2$ / Hz x

10-³) was similar to the PW caffeine 10 mg/kg (p= 0.999) and PW caffeine 20 mg/kg (p= 0.3868) groups. The PW caffeine group (0.1777 ± 0.04383 mV² / Hz x 10-³) was superior to the other groups ( Figure 2 E).

For gamma oscillations, the control group ( 0.02805± 0.00627 mV² / Hz x 10-³) was similar to the SHAM ( p= 0.1391) and caffeine 10 mg/kg ( p= 0.0853) groups, but was lower than the other groups. The SHAM group (0.0395± 0.007989 mV² / Hz x 10-³) was similar to the groups treated with caffeine 10mg/kg ( p=0.999), 20mg/kg (p= 0.7160) and 30 mg/kg (p= 0.9976). The groups treated with caffeine 10mg/kg, 20mg/kg and 30mg/kg were similar ( p= 0.8343). The PW caffeine 10 mg/kg and PW caffeine 20mg/kg groups were similar ( p=0.0661). The PW caffeine 30 mg/kg group was superior to the other groups ( Figure 2 F).

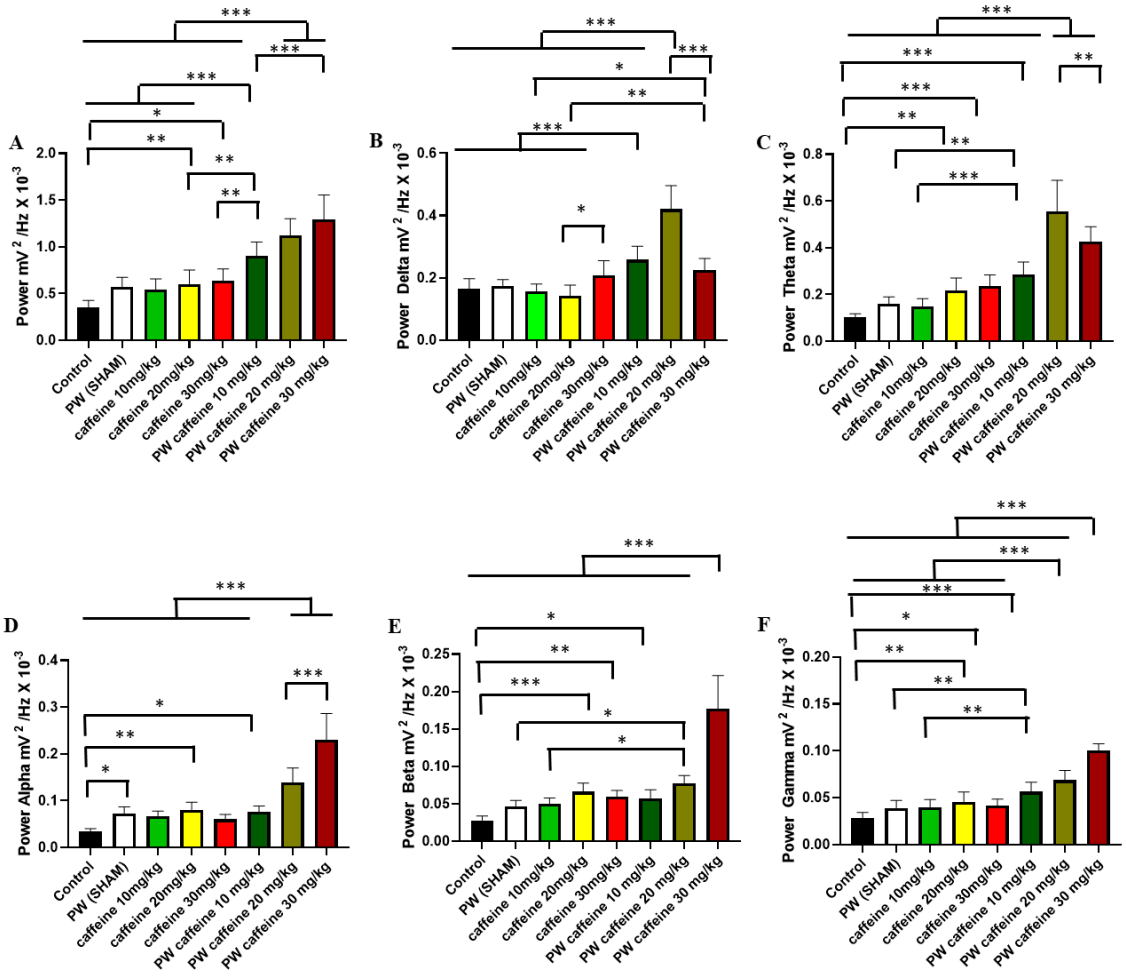



**Figure 2:** Linear power distribution graph recorded between the groups at frequencies up to 40 Hz (A); Linear power distribution graph of brain oscillations in the Delta band ( 0.5-3 Hz) (B); Linear power distribution graph of brain oscillations in Theta ( 3. 5-7.5 Hz) (C); Graph of power distribution in brain oscillations in the Alpha band ( 8-12 Hz) (D); Graph of power distribution in brain oscillations in beta ( 13-20 Hz) (E); Graph of power distribution in brain oscillations in Gamma ( 20-40 Hz) (F). (After ANOVA followed by Tukey, *p<0.05, **p<0.01 and ***p<0.001 n=9).

The power spectral density graph shows that the group submitted to physical exercise and subsequently treated with caffeine 30 mg/kg i.p. showed a significant increase in oscillations from 10 to 27 Hz, which includes the beta band entirely. Based on this analysis, the beta oscillations were analyzed more specifically (Figure -3).

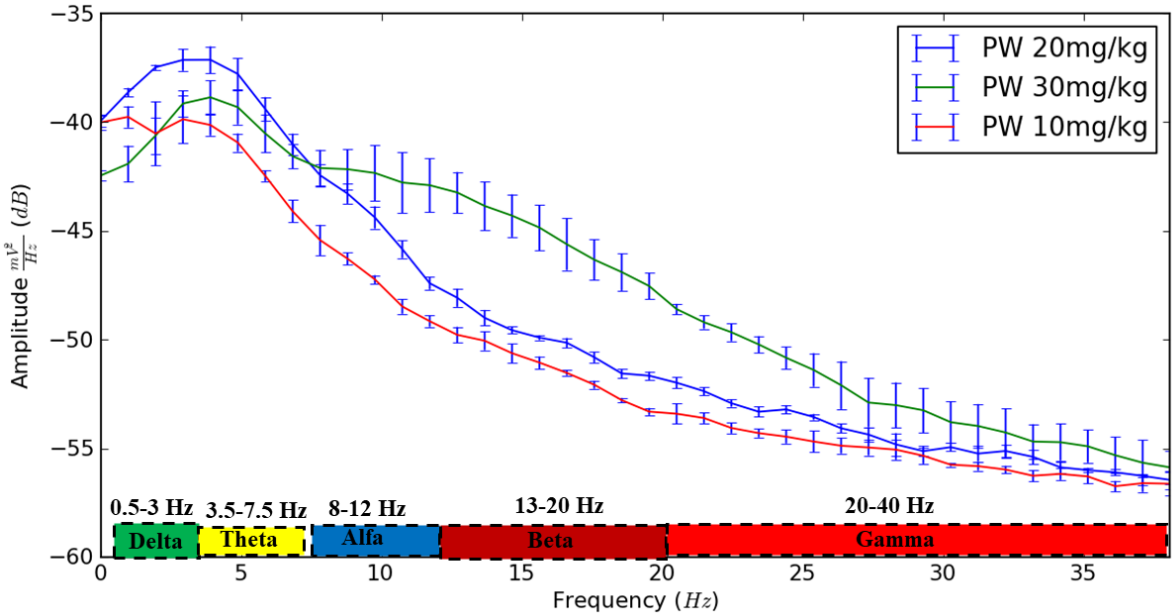
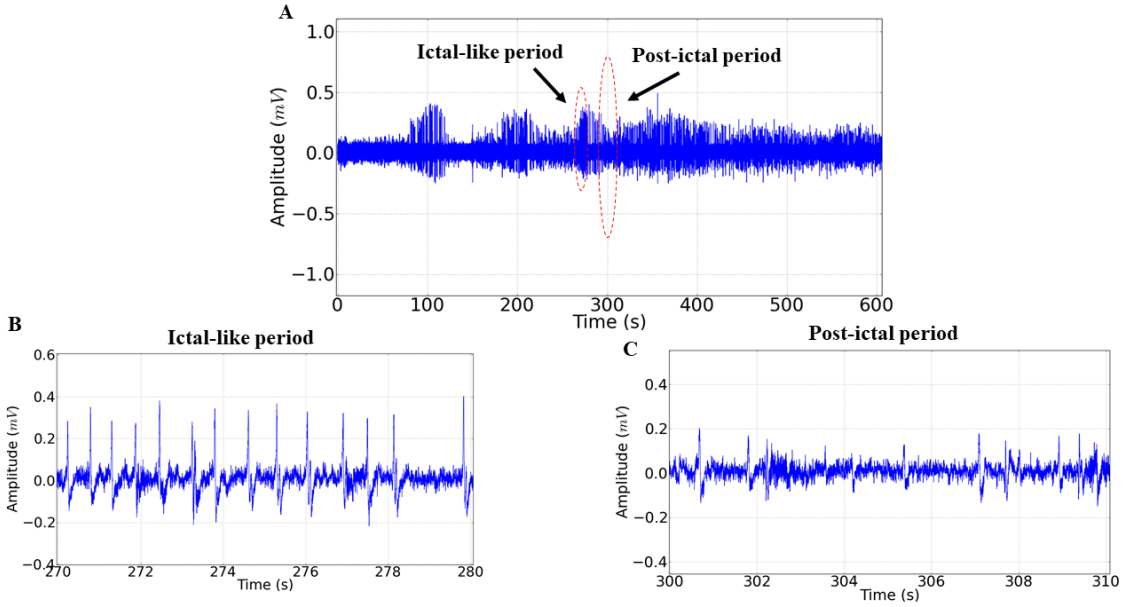



**Figure 3 -** PSD f power spectral density graph showing the variation in power for the groups subjected to physical exercise followed by caffeine at doses of 10mg/kg, 20mg/kg and 30 mg/kg, indicating the frequency bands in the delta, theta, alpha, beta and gamma oscillations.

After 30 minutes of swimming combined with 30mg/kg of caffeine, the ECoG tracings showed greater brain excitability, with the presence of repetitive firing potentials characterized by polyspikes ( Figure 4 A and B). As well as the presence of post-ictal periods characterized by a decrease in firing amplitude ( Figure 4 A and C).

During recording, the power spectral density (PSD) graph showed an increase in power when polyspikes appeared, indicating a higher level of energy during recording; however, during the post-ictal period, the level of energy was reduced when compared to the period when potentials were saved (Figure 4 D).

The oscillations in the beta frequencies were the most affected, so the evaluation of the average power during the ictal and post-ictal periods was measured. For the control group, the average was 0.02788 ± 0.00586 $mV^2$ / Hz x $10^{-3}$, similar to the SHAM group (p= 0.9020) and the group treated with 30 mg/kg of caffeine (p= 0.5044). However, they were lower than the other groups. During the ictal period, the average of (0.4277 ± 0.07653 $mV^2$ / Hz x $10^{-3}$) was higher than the other groups. During the post-ictal period ( 0.04733 ± 0.02316 $mV^2$ / Hz x $10^{-3}$) was similar to the control (p= 0.8875) , SHAM ( 0.999) and caffeine 30mg/kg ( p=0.9857) groups ( Figure 4 E).

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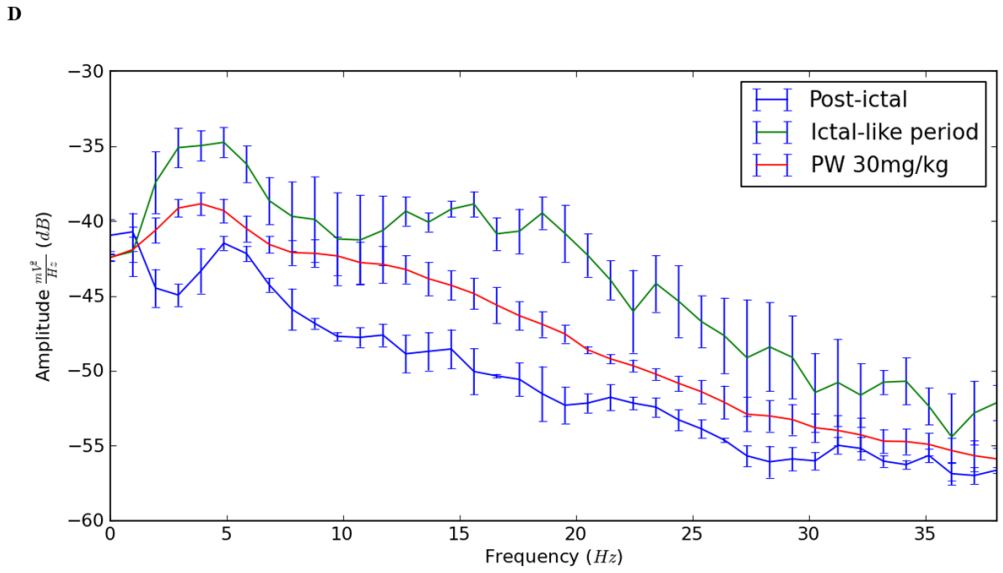


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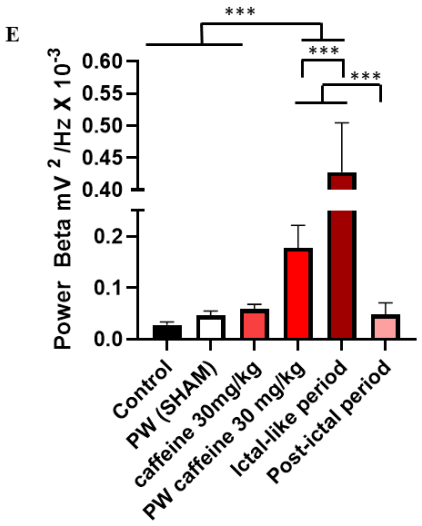


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244 **Figure 4.** Electrocorticographic (ECoG) recordings obtained from animals submitted to physical exercise followed by
245 30 mg/kg of caffeine, showing graphical elements characteristic of cerebral excitability, indicated by arrows (A);

Magnified tracing showing polyspikes characterized as ictal-like period (B), magnified tracing of the post-ictal period (C); PSD showing the distribution of emergy during the ictal and post-ictal periods (D) and linear power distribution graph indicating the difference between the ictal and post-ictal periods (E).

## Discussion

Caffeine is a natural alkaloid that primarily acts as a stimulant of the central nervous system (CNS), widely consumed for its ability to increase alertness and reduce fatigue. Its main action in the CNS occurs through the blockage of adenosine receptors, which are inhibitory neurotransmitters responsible for inducing relaxation and drowsiness. In the brain, adenosine binds to its receptors (A1 and A2A), promoting the feeling of tiredness throughout the day (Guest et al., 2021; Cappelletti et al., 2015; Soós et al., 2021). When caffeine is ingested, it competes with adenosine for these receptors, blocking them. This results in the inhibition of adenosine's sedative effects, leading to an increase in the release of excitatory neurotransmitters, such as dopamine, norepinephrine, and glutamate (Cappelletti et al., 2015; Jodra et al., 2021; Mielgo-Ayuso et al., 2019). This increase in neurotransmitters stimulates the cerebral cortex, improving attention, vigilance, and, in many cases, cognitive and physical performance. Furthermore, caffeine influences other neurochemical pathways, such as the dopaminergic system, which is involved in mood regulation, motivation, and pleasure. This may explain why caffeine consumption is also associated with feelings of well-being and mood improvement in some individuals (Ericson et al., 2017).

Other effects of caffeine on the CNS include increased cerebral metabolic rate and improved motor coordination. In moderate doses, caffeine can enhance cognitive and physical performance, but high doses can provoke adverse effects, such as anxiety, insomnia, increased heart rate, and, in extreme cases, convulsive episodes due to neuronal hyperexcitability (Mielgo-Ayuso et al., 2019). Caffeine can also modulate brainwave activity, as observed in your study with mice, where an increase in high-frequency oscillations (beta and gamma) was noted after caffeine administration. These oscillations are associated with states of alertness and higher cognition, reflecting caffeine's stimulating effect on the brain (Anas Sohail et al., 2021).

This manuscript focuses on the neurophysiological effects observed in the cortical activity of mice subjected to different doses of caffeine after physical exercise. The electrocorticographic (ECoG) results showed that caffeine administration, especially at higher doses (30 mg/kg), significantly increased brain oscillations, mainly in high-frequency bands such as beta and gamma, compared to control and SHAM groups. These oscillations are associated with increased neuronal excitation and cortical activation, suggesting that caffeine enhances brain activity after exercise. The groups that received 30 mg/kg of caffeine exhibited signs of heightened excitability, with episodes of ictal-like activity, characterized by repetitive neuronal firing and bursts of potentials, which may indicate a greater predisposition to convulsive activities with high doses of caffeine associated with intense physical effort. This finding is consistent with previous studies from our research group, indicating that caffeine at high concentrations can induce a state

of cortical hyperexcitability, exacerbating the central nervous system's response (Eiró-Quirino et al., 2024).

Additionally, lower frequencies, such as the delta and theta bands, showed little variation in the caffeine-treated groups, with the most prominent effects observed in higher frequency bands. This suggests that caffeine, after physical exercise, predominantly affects neural circuits responsible for cognitive and alertness processes, more associated with beta and gamma frequencies, rather than relaxation or sleep processes, which are normally related to lower frequencies.

The data obtained show a dose-dependent relationship of caffeine's effects, with higher doses resulting in greater power and irregularity in the electrocorticographic recordings. This finding reinforces the idea that, although caffeine may have a beneficial effect in enhancing physical and mental performance at moderate doses, its use in high doses should be approached with caution due to the risk of adverse effects on the central nervous system.

Caffeine has been widely used as an ergogenic agent, meaning a substance that improves physical performance, especially in endurance and high-intensity activities (Elosegui et al., 2022). Its beneficial effect on physical training occurs through various mechanisms in the body, involving both the central nervous system and peripheral physiological processes. Caffeine acts on the CNS by blocking adenosine receptors, which reduces the sensation of tiredness and fatigue during exercise (Jodra et al., 2020). By reducing the subjective perception of effort, athletes can perform physical activities for longer and with greater intensity. This effect is particularly useful in long-duration exercises, such as endurance running and cycling.

Caffeine stimulates the release of adrenaline (epinephrine), a hormone that prepares the body for stress or physical exertion (Imam-Fulani et al., 2022). Adrenaline increases heart rate, boosts blood flow to muscles, and enhances muscle response, improving performance during intense workouts (Stadheim et al., 2021). This provides an extra boost in anaerobic and strength exercises. Caffeine increases the mobilization of free fatty acids in the blood, promoting the use of fat as an energy source during exercise. This preserves muscle glycogen stores, delaying fatigue and allowing athletes to maintain performance for longer periods. This mechanism is particularly important in endurance activities, where glycogen depletion can be a limiting factor (Goldstein et al., 2010; Wang et al., 2022; Stadheim et al., 2021).

Caffeine can also directly affect muscle function by increasing the muscles' contraction strength and efficiency. It facilitates the release of calcium in the sarcoplasmic reticulum of muscle fibers, enhancing the muscles' ability to contract with more force. This is beneficial in strength exercises, such as weightlifting and resistance training.

By acting on the CNS, caffeine enhances attention, alertness, and focus, which can improve motor coordination and precision during exercise. This effect is advantageous in sports that require concentration and fine motor skills, such as tennis or

gymnastics, where cognitive performance is as important as physical performance. Studies indicate that caffeine can improve maximum oxygen consumption (VO2 max.), one of the key indicators of aerobic performance (Mielgo-Ayuso et al., 2019; Gonzaga et al., 2017). This means that athletes can use oxygen more efficiently during exercise, increasing work capacity in endurance activities, such as running and swimming. Caffeine also has analgesic effects, which help reduce the perception of muscle pain during and after exercise. This allows athletes to maintain training intensity for longer, even in the face of physical discomfort, as occurs in intense training sessions or endurance events (Wang et al., 2022).

These multiple effects of caffeine contribute to increased athletic performance in various sports modalities, making it one of the most studied and widely used substances as a pre-workout supplement. Therefore, this study contributes to the understanding of the mechanisms by which caffeine can affect cortical activity in the context of physical exercise, highlighting the need for further studies investigating the impact of different doses over long periods and their relationship with the safety of stimulant use in athletic activities.

## Conclusion

We conclude that post-workout caffeine administration in Swiss mice has significant effects on cortical activity, as observed in electrocorticographic (ECoG) recordings. Higher doses of caffeine, especially 30 mg/kg, induced a significant increase in high-frequency brain oscillations (beta and gamma), suggesting greater neuronal excitation. Furthermore, in conditions associated with physical exercise, caffeine showed the potential to exacerbate brain activity, with traces of hyperexcitability that may predispose animals to convulsive activity. These findings highlight the importance of considering the neurophysiological effects of caffeine, especially at high doses, and reinforce the need for caution in the use of ergogenic stimulants in contexts of intense physical exertion. Future studies are essential to better understand the safety limits and the mechanisms underlying the combined effects of exercise and caffeine on the central nervous system.

## Declaration of conflict of interest

The authors have no conflict of interest to declare.

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## Disclosure Statement

Financial Disclosure: none.

Non-financial Disclosure: none

## Author contributions

Luciana Eiró-Quirino, Moisés Hamoy: Conceptualization; Luciana Eiró-Quirino, Priscille Fidelis Pacheco Hartcopff; Bianca Pacheco Lima[1], Cibely Camille Fernandes Ferreira[1], Dayanne Raquel de Sousa Barros[1], Gabriel Varela Oliveira[1], Julianna Osíris ribeiro Lobato, Mahara de Souza Libório[1], Yara Carolina Barbosa Padilha[1], Daniella Rocha Bittencourt[1], Akira Otake Hamoy: Methodology; Luciana Eiró-Quirino, Moisés Hamoy: Visualization, Investigation; Luciana Eiró-Quirino, Luana Vasconcelos de Souza, Clarissa Araújo da Paz, Daniella Bastos de Araújo: Writing- Reviewing and Editing; Moisés Hamoy: Data curation, Moisés Hamoy: Supervision, Moisés Hamoy: Software, Moisés Hamoy: Validation.

## Data availability statement

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

## Ethics declarations

All procedures were approved by the ethics committee (CEUA/UFPA- ID 2675110219). All the experiments were carried out using the ARRIVE checklist.

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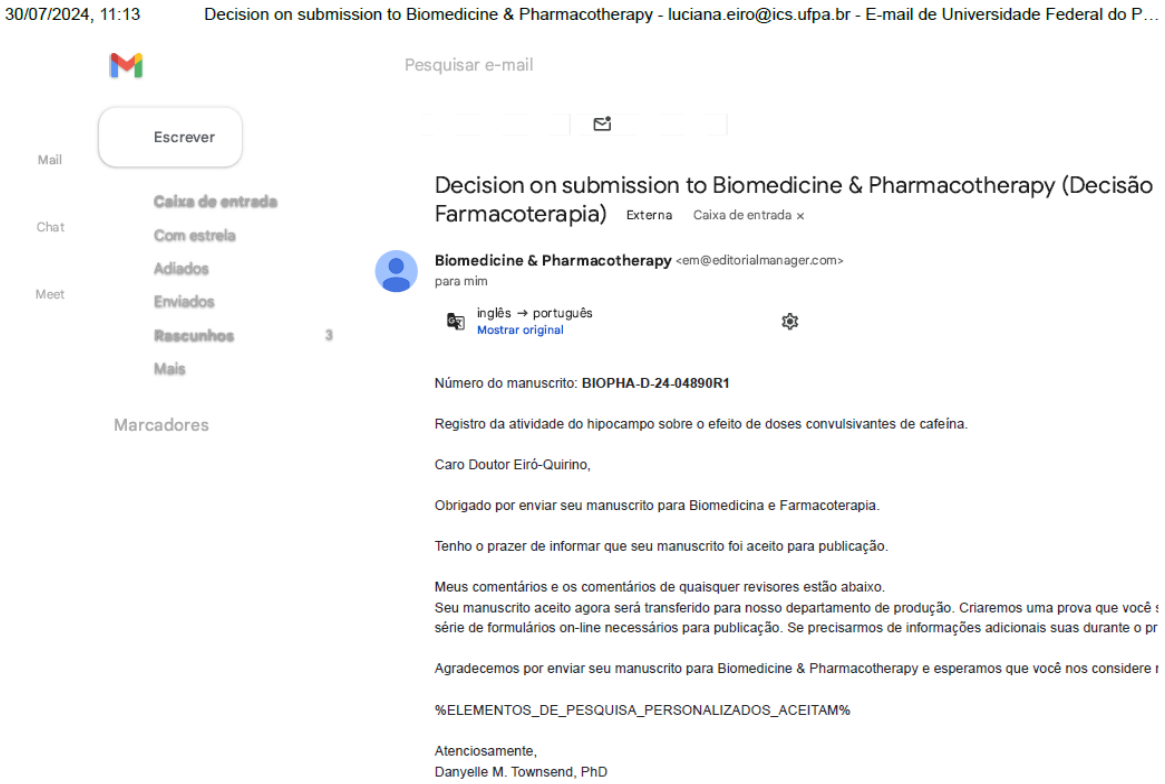

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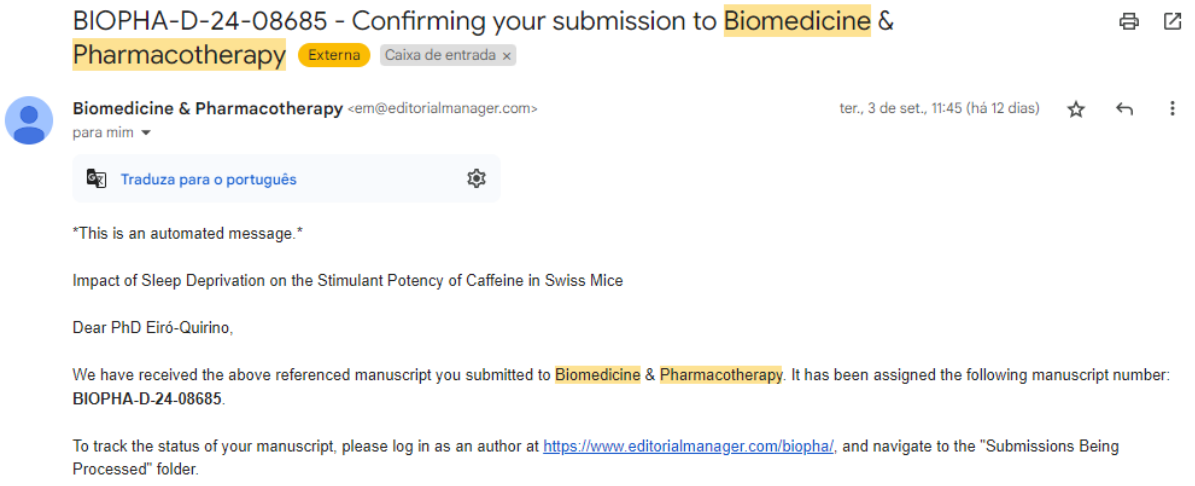

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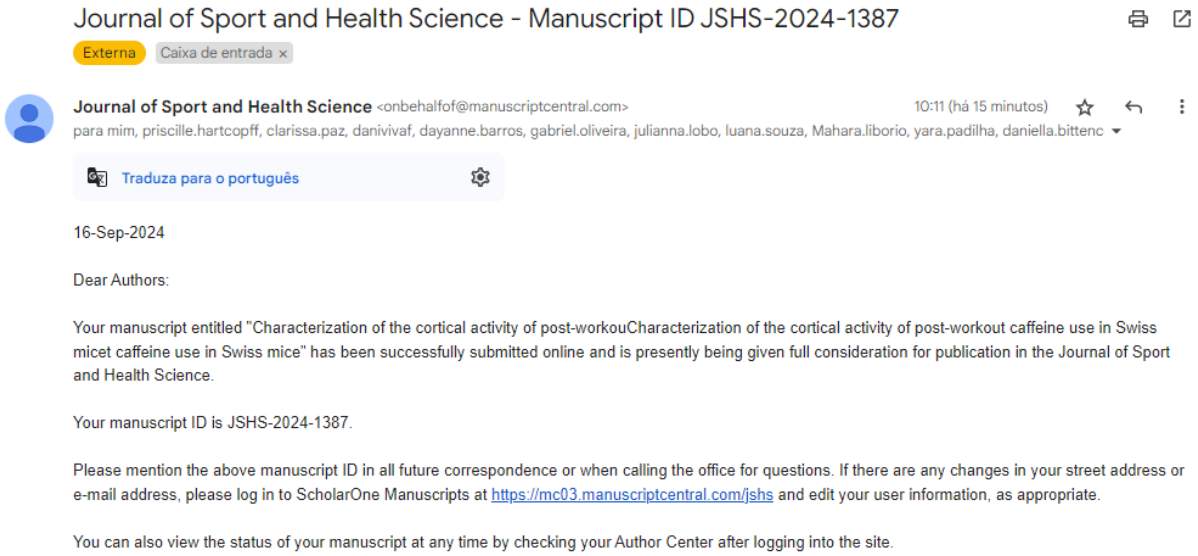

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