UNIVERSIDADE FEDERAL DE ALAGOAS
INSTITUTO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

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EPILEPSIA DO LOBO TEMPORAL: IMPACTO DA DESREGULAÇÃO CIRCADIANA E ALTERAÇÕES METABÓLICAS EM MODELO EXPERIMENTAL

MACEIÓ - AL
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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ciências da Saúde da Universidade Federal de Alagoas, como requisito parcial para obtenção do grau de Doutor em Ciências da Saúde.

Orientador(a): Prof. Dr. Daniel Leite Goes Gitaí

MACEIÓ - AL
2024

Catalogação na fonte
Universidade Federal de Alagoas
Biblioteca Central
Divisão de Tratamento Técnico
Bibliotecária: Girlaine da Silva Santos – CRB-4 – 1127

S237e Santos, Gabriele Fernanda da Conceição.
Epilepsia do lobo temporal: impacto da desregulação circadiana e alterações metabólicas em modelo experimental / Gabriele Fernanda da Conceição Santos. – 2025.
82 f.: il.: color.

Orientador: Daniel Leite Goes Gitaí.
Tese (Doutorado em Ciências da Saúde) - Universidade Federal de Alagoas, Instituto de Ciências Biológicas e da Saúde. Maceió, 2025.

Bibliografia: f. 74-82.

1. Epilepsia do lobo temporal. 2. Ritmos circadianos. 3. Metabolômica. 4. Epilepsia. 5. Neurodegeneração. I. Título.

CDU: 616.853

DEDICATÓRIA

A Deus que me concedeu a capacidade e força necessárias para
a concretização deste objetivo.

AGRADECIMENTOS

A Deus, que foi a força que me sustentou e me deu coragem para continuar minha jornada acadêmica. Só estou concluindo esta etapa da minha vida graças ao seu infinito amor.

Agradeço ao Professor Daniel Gitai pela oportunidade de realizar este trabalho sob sua orientação. Seu conhecimento profundo, visão científica, paciência e apoio constante foram fundamentais para o desenvolvimento deste projeto. Sou imensamente grato pela confiança e pela orientação ao longo de toda essa jornada acadêmica.

Também agradeço ao Programa de Pós-Graduação em Ciências da Saúde da UFAL, à coordenação do programa e aos funcionários do ICBS pela gentileza ao longo de todo o processo.

Aos amigos e colegas do Laboratório de Biologia Celular e Molecular (LBCM) — Larissa Almeida, Manoela Souza, Pedro, Myka, Junior, Carmem, Jordana, Delma, Karla e Diogo. Minha sincera gratidão pela ajuda durante todo o período de experimentos. A contribuições de todos vocês foram essenciais para o andamento dos trabalhos. Aos queridos amigos Alessandro e Roberta, minha sincera gratidão por estarem ao meu lado nos feriados e noite, dedicando seu tempo e apoio para que eu pudesse realizar meus experimentos.

Agradeço aos ratos utilizados nos experimentos deste projeto. Reconheço a importância de sua contribuição para o avanço do conhecimento científico, e sou grata pela participação que permitiu a realização dos estudos, com o devido respeito à sua utilização nas pesquisas.

Aos laboratórios parceiros LAIFE e NAPRMN, obrigada pelas colaborações. Agradeço à Universidade Federal de Alagoas (UFAL) pela infraestrutura e pelo ambiente acadêmico que proporcionaram para o desenvolvimento deste trabalho.

Aos meus pais Maria Bernadete e Amaro José pelo amor, paciencia, incentivo, ppreocupação e por sempre estarem presentes na minha vida.

Minha gratidão a todos que contribuíram de forma direta ou indireta para a realização deste trabalho.

RESUMO

A epilepsia do lobo temporal (ELT) é uma condição neurológica caracterizada por crises recorrentes e espontâneas (CREs). Evidências sugerem uma relação bidirecional entre a ELT e os ritmos circadianos, com padrões circadianos influenciando a suscetibilidade às crises e as próprias crises interferindo em outras oscilações biológicas. Além disso, estudos indicam que a gravidade das crises está associada a alterações metabólicas, que têm recebido atenção como potenciais biomarcadores devido à complexidade do diagnóstico e manejo da epilepsia. Esta tese está estruturada em dois capítulos que abordam diferentes aspectos da ELT. O primeiro capítulo explora os efeitos da desregulação circadiana, induzida pela exposição à luz constante, no processo epileptogênico. Utilizando o modelo de lítio-pilocarpina, ratos Wistar foram expostos à luz constante (grupo experimental) ou ao ciclo claro-escuro (grupo controle) e monitorados continuamente após o status epiléptico (SE). O estudo avaliou a frequência e gravidade das crises, a ritmicidade da atividade locomotora espontânea (ALE), a neurodegeneração e a expressão de genes implicados na epileptogênese. Os resultados demonstraram que a exposição à luz constante antes do SE aumentou o limiar para a indução do SE, reduziu a gravidade das crises, atenuou a perda neuronal e modulou a expressão dos transcritos Npy e Gad65. No entanto, nenhum efeito foi observado nas CREs durante a fase crônica. O segundo capítulo investiga o perfil metabólico das águas fecais de ratos submetidos ao SE, com o objetivo de identificar potenciais biomarcadores associados à epileptogênese. Amostras fecais de cinco grupos experimentais: Naïve, Pós-SE, SRS (epilético), NS (resistente ao SE) e NSRS (sem crises na fase crônica), foram analisadas por espectroscopia de Ressonância Magnética Nuclear (RMN) e processadas com métodos estatísticos como PCA, PLS-DA, OPLS-DA e ANOVA. A análise identificou 24 metabólitos importantes, incluindo níveis elevados de glutamato e glutamina no grupo SRS, redução de butirato no grupo Pós-SE e aumento de aminoácidos de cadeia ramificada (valina, isoleucina e leucina) nos grupos NS e NSRS. As análises de PCA e PLS-DA confirmam a separação entre os grupos, com uma variância total de 65%, evidenciando maior distinção entre os grupos naïve, SRS e Pós-SE, enquanto os grupos NS e NSRS apresentam padrões metabólicos semelhante. Esses resultados indicam que os mecanismos de resistência envolvem respostas adaptativas distintas daquelas observadas nos animais naïve e nos que sofreram crises, sugerindo a presença de processos biológicos específicos que necessitam de investigação mais aprofundada. Esses achados fornecem novas perspectivas sobre as alterações metabólicas associadas à ELT, com potenciais implicações para o diagnóstico e o desenvolvimento de terapias.

Embora os capítulos abordem questões distintas devido a adaptações metodológicas impostas pela pandemia de COVID-19, eles se complementam ao avançar na compreensão da ELT sob perspectivas circadianas e metabólicas. Estudos adicionais são necessários para traduzir esses achados para a prática clínica.

Palavras-chave: Epilepsia do lobo temporal, ritmos circadianos, metabolômica, luz constante, biomarcadores, status epiléptico, neurodegeneração.

ABSTRACT

Temporal lobe epilepsy (TLE) is a neurological disorder characterized by spontaneous recurrent seizures (SRS). Evidence suggests a bidirectional relationship between TLE and circadian rhythms, with circadian patterns influencing seizure susceptibility and seizures potentially disrupting other biological oscillations. Moreover, studies indicate that seizure severity is associated with alterations in metabolites, which have gained attention as potential biomarkers for the complex diagnosis and management of epilepsy. This thesis is structured into two chapters addressing distinct aspects of TLE. The first chapter explores the effects of circadian rhythm disruption, induced by constant light exposure, on the epileptogenic process. Using a lithium-pilocarpine model, Wistar rats were exposed to constant light (experimental group) or a light-dark cycle (control group) and continuously video-monitored post-status epilepticus (SE). The study evaluated seizure frequency and severity, spontaneous locomotor activity rhythms (SLA), neurodegeneration, and the expression of genes implicated in epileptogenesis. Results demonstrated that constant light exposure prior to SE increased the threshold for SE induction, reduced seizure severity, attenuated neuronal loss, and modulated the expression of Npy and Gad65 transcripts. However, no effects were observed on SRS during the chronic phase. The second chapter investigates the metabolic profile of fecal water in rats subjected to SE, aiming to identify potential biomarkers associated with epileptogenesis. Fecal samples from five experimental groups: Naïve, Post-SE, SRS (epileptic), NS (SE-resistant) and NSRS (without seizures in the chronic phase), were analyzed by Nuclear Magnetic Resonance (NMR) spectroscopy and processed with statistical methods such as PCA, PLS-DA, OPLS-DA and ANOVA. The analysis identified 24 important metabolites, including elevated levels of glutamate and glutamine in the SRS group, reduced butyrate in the Post-SE group and increased branched-chain amino acids (valine, isoleucine and leucine) in the NS and NSRS groups. PCA and PLS-DA analyses confirm the separation between the groups, with a total variance of 65%, evidencing greater distinction between the Naïve, SRS and Post-SE groups, while the NS and NSRS groups present similar metabolic patterns. These results indicate that the resistance mechanisms involve adaptive responses distinct from those observed in naïve animals and those that suffered crises, suggesting the presence of specific biological processes that require further investigation. These findings provide novel insights into the metabolic alterations associated with TLE, with potential implications for diagnosis and therapeutic development. Although the chapters address distinct questions due to

methodological adaptations during the COVID-19 pandemic, they complement each other by advancing our understanding of TLE from both circadian and metabolic perspectives. Further studies are needed to translate these findings into clinical practice.

Keywords: Temporal lobe epilepsy, circadian rhythms, metabolomics, constant light, biomarkers, status epilepticus, neurodegeneration.

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LISTA DE ABREVIATURAS

BDNF	Fator neurotrófico derivado do cérebro
Bmal	Brain and muscle Arnt-like protein-1
Clock	Circadian locomotor output cycle kaput
CREs	Crises recorrentes e espontâneas
Cry1	Cryptochrome Circadian Regulator 1
Cry2	Cryptochrome Circadian Regulator 2
DAEs	Drogas Antiepilépticas
EH	Esclerose hipocampal
ELT	Epilepsia do lobo temporal
ELTM	Epilepsia do lobo temporal mesial
ELTN	Epilepsia do lobo temporal neocortical
FAEs	Fármacos Antiepiléticos
GC-MS	Cromatografia gasosa acoplada à espectrometria de massas
GFAP	Proteína fibrilar ácida da glia
ILAE	Liga Internacional contra Epilepsia.
LCR	Líquido cefalorraquidiano
NS	Não crises
NSQ	Núcleos supraquiasmáticos
NSRS	Sem Crises Recorrentes e Espontâneas
NYP	Neuropeptídeo Y
OMS	Organização Mundial de Saúde
Per1	Period Circadian Regulator 1
Per2	Period Circadian Regulator 2
PILO	Pilocarpina
RMN	Ressonância magnética nuclear
SE	Status Epilepticus
SNC	Sistema Nervoso Central
SRS	Crises Recorrentes e Espontâneas
SUDEP	Morte súbita inesperada na epilepsia
TNF-a	Fator de necrose tumoral
TRH	Trato retino-hipotalâmico

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1 APRESENTAÇÃO

Esta tese está organizada em dois capítulos experimentais, cada um abordando aspectos distintos da epilepsia do lobo temporal (ELT). Ambos compartilham o objetivo de contribuir para a compreensão dos mecanismos subjacentes à epilepsia, embora enfoquem questões específicas devido a desafios metodológicos enfrentados durante sua execução.

No primeiro capítulo, investigamos os efeitos da luz constante, como modelo de desregulação circadiana, sobre as fases aguda e crônica da epileptogênese induzida pelo status epiléptico (SE). Este estudo teve como objetivo explorar como a modulação ambiental, por meio da luz, influencia o processo epileptogênico, avaliando desfechos como a gravidade das crises, a neurodegeneração e a atividade locomotora. A escolha deste modelo decorreu do interesse em aprofundar o papel dos ritmos circadianos na regulação da excitabilidade neuronal e no desenvolvimento da epilepsia. É importante ressaltar que os resultados exibidos no artigo 1 referentes as Figuras 5 e 6 integram estudos previamente realizados pelo nosso grupo de pesquisa (Marques, 2018; Pereira, 2020).

Já o segundo capítulo concentra-se na análise metabolômica de águas fecais em ratos submetidos ao modelo de SE, utilizando espectroscopia de ressonância magnética nuclear (RMN). Este estudo foi projetado para identificar metabólitos associados ao processo epileptogênico, com foco na busca por biomarcadores que possam contribuir para o diagnóstico e monitoramento da ELT. A metabolômica foi escolhida como abordagem devido à sua capacidade de oferecer uma visão abrangente das alterações metabólicas relacionadas à epilepsia.

A decisão de abordar temas distintos na tese reflete desafios impostos pela descontinuidade no acesso a animais de experimentação durante o período da pandemia de COVID-19. A interrupção no ciclo experimental e a impossibilidade de manter a continuidade dos mesmos grupos de animais exigiram adaptações metodológicas. Assim, optou-se por explorar dois temas independentes, mas complementares, aproveitando dados obtidos de forma robusta dentro das limitações impostas.

Embora os capítulos tratem de questões diferentes, eles se conectam pelo objetivo comum de ampliar o entendimento sobre a epilepsia, investigando fatores circadianos e metabólicos que, isoladamente, oferecem perspectivas únicas e relevantes. Essa abordagem diversificada também reflete a flexibilidade necessária para conduzir pesquisa

de alta qualidade em um cenário adverso, garantindo que os estudos realizados contribuam significativamente para o avanço do conhecimento sobre a ELT.

2 INTRODUÇÃO

Os ritmos circadianos são ritmos biológicos com um ciclo de aproximadamente 24 horas, desempenhando um papel crucial no controle de diversos processos biológicos. Em mamíferos, esses ritmos são gerados endogenamente pelo núcleo supraquiasmáticos (NSQ), conhecido como relógio central biológico, entretanto os ritmos podem ser sincronizados por fatores ambientais, como a luz, que ajuda o corpo a distinguir entre o dia e a noite, regulando assim o ritmo circadiano (Xu et al., 2020).

Estudos indicam que a desregulação dos sinais ambientais pode interferir no ritmo circadiano, como ocorre com a exposição excessiva à luz, contribuindo para o surgimento ou agravamento de doenças, como a epilepsia do lobo temporal (ELT). Os efeitos da luz constante na epilepsia são complexos e podem variar de acordo com fatores como tempo, intensidade e duração da exposição. Pesquisas recentes mostram que as crises epilépticas seguem padrões diurnos e circadianos, sugerindo que a regulação dos ritmos biológicos desempenha um papel importante na epilepsia (Spencer et al., 2016; Gitai et al., 2019).

O diagnóstico e tratamento da epilepsia representam um grande desafio clínico devido à sua complexidade e à resistência dos pacientes aos tratamentos farmacológicos, o que tem impulsionado a busca por biomarcadores específicos para a doença (Engel, Pitkänen, 2019; Godoi et al., 2022).

A metabolômica tem se destacado como uma ferramenta para definir biomarcadores relacionados ao prognóstico, diagnóstico e monitoramento da eficácia terapêutica, pois oferece uma visão abrangente das alterações metabólicas em um sistema. Com destaque para alguns metabólitos de baixa massa molecular, cujas concentrações têm sido associadas à gravidade das crises (Deutour et al., 2018; Canuto et al., 2018; Godoi et al., 2020).

Visto que as CREs apresentam um padrão temporal diurno e a luz é um importante sincronizador do ritmo circadiano, como a desregulação circadiana induzida por luz constante afeta as fases aguda e crônica da epileptogênese em termos de gravidade das crises, neurodegeneração e atividade locomotora?

E, sabendo que a caracterização de metabólitos pode fornecer informações cruciais sobre os mecanismos biológicos envolvidos, como ela pode contribuir para a identificação de biomarcadores na epilepsia? Essas questões nortearam este estudo.

Assim, ao investigar os efeitos da luz constante sobre a epileptogênese e analisar o perfil metabolômico relacionado à epilepsia, este trabalho busca preencher lacunas importantes no entendimento dos mecanismos circadianos e metabólicos envolvidos

nessa condição. A realização desses estudos de forma complementar permite não apenas compreender como fatores ambientais e biológicos influenciam a progressão da epilepsia, mas também identificar possíveis biomarcadores que possam contribuir para avanços no diagnóstico e tratamento.

3. REFERENCIAL TEORICO

3.1 Epilepsia

Na epilepsia, o cérebro apresenta uma predisposição permanente para gerar crises epilépticas, com graves consequências neurobiológicas, cognitivas, psicológicas e sociais (FISHER et al., 2014). As crises epilépticas são manifestações motoras e/ou sensoriais de natureza espontânea e autolimitada (Costa et al., 2020), que indicam um desequilíbrio entre os sistemas inibitórios e excitatórios, marcado por uma atividade excessiva ou hipersincrônica dos neurônios no Sistema Nervoso Central (SNC) (Marchin et al., 2014).

De acordo com a *International League Against Epilepsy* (ILAE), em 2017, as crises epilépticas são classificadas em dois tipos principais: focais e generalizadas, com base na localização do foco epileptogênico. Nas crises generalizadas, as descargas elétricas se originam simultaneamente em ambos os hemisférios cerebrais, enquanto nas crises focais o início da crise é restrito a uma sub-região específica do encéfalo. As crises focais podem ser classificadas como complexas, quando há comprometimento da consciência, ou simples, quando não há (Scheffer et al., 2017).

Por outro lado, a classificação da epilepsia (e não das crises) considera a investigação clínica do perfil das crises, estudos eletroencefalográficos e de neuroimagem, além de pesquisas sobre a etiologia da doença (Fisher et al., 2017).

As epilepsias de etiologia estrutural são aquelas que têm como causa lesões identificáveis no cérebro. Elas podem ser subdivididas em duas categorias principais: as adquiridas e as genéticas. As epilepsias adquiridas resultam de um insulto inicial ao sistema nervoso central, que pode incluir eventos como trauma craniano, acidente vascular cerebral (AVC), infecções ou episódios de estado epiléptico (SE). Já as epilepsias genéticas são provocadas por mutações que podem resultar em ganho ou perda de função de genes específicos, levando a alterações na excitabilidade neuronal (Fisher et al., 2014; Costa et al., 2020).

O exemplo mais comum de epilepsia com etiologia estrutural é a Epilepsia do Lobo Temporal (ELT), que tem sido foco de estudos do nosso grupo de pesquisa devido à sua importância clínica, evidenciada pela gravidade, alta incidência e, principalmente, refratariedade ao tratamento medicamentoso (ILAE, 2017).

A ELT é caracterizada pela ocorrência de crises que se originam em estruturas neocorticais ou mesiais, dando origem aos subtipos: epilepsia do lobo temporal mesial (ELTM) e epilepsia do lobo temporal neocortical (ELTN) (Henning et al., 2023).

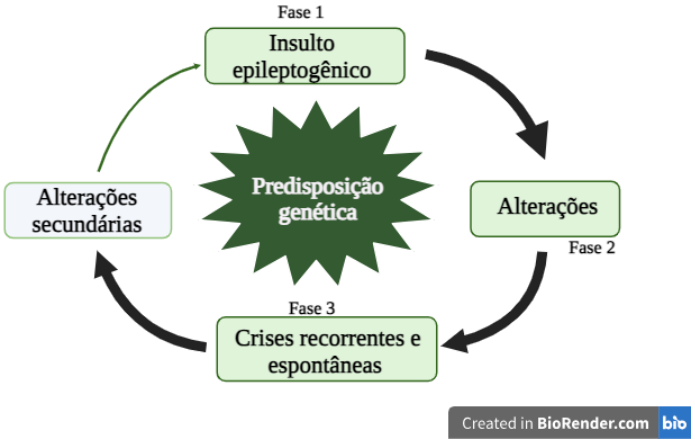
A epilepsia do lobo temporal mesial (TLEM) é a mais frequente em adultos. Cerca de 40% dos pacientes com TLEM permanecem com crises, mesmo após receberem a terapia médica ideal (Engel, 2016; Englot & Chang, 2014).

Pacientes com ELTM apresentam crises focais complexas ou simples, iniciadas em estruturas mesiais do lobo temporal, frequentemente precedidas por auras (Englot et al., 2020), e que podem durar de alguns segundos até 1-2 minutos antes da perda de consciência (Blair, 2012). As auras são componentes das crises que ocorrem antes da perda de consciência, e os pacientes podem se lembrar desses sintomas da aura posteriormente (Henning et al., 2023)

A esclerose hipocampal (EH) é uma das patologias mais comuns em pacientes com ELTM refratária ao tratamento farmacológico (Tom, 2014). A EH é caracterizada pela perda celular nos subcampos CA1, CA3 e hilo do hipocampo, gliose e dispersão de células granulares do giro denteado (Fernandes, 2013; Walker, 2015;). Além disso, a EH faz parte de um conjunto de alterações que ocorrem durante a epileptogênese e pode ser desencadeada por um insulto ao sistema nervoso central (Lévesque et al., 2021)

A epileptogênese é o processo no qual um cérebro normal se transforma progressivamente em um cérebro epilético, ou seja, aquele com uma predisposição permanente para gerar crises recorrentes e espontâneas (CREs) (Pitkänen et al., 2015; Engel et al., 2020). Esse processo ocorre em três fases: a primeira fase que se inicia mediante a um insulto inicial, também denominado insulto epileptogênico, que pode ser causado por injúria cerebral decorrente de convulsão febril, traumatismo, acidente vascular encefálico ou status epilético (SE); período de latência, no qual não há manifestações de crises, mas é marcado por mudanças estruturais significativas em toda a rede neural, que a nível celular podemos destacar alterações como neurodegeneração, neurogênese, brotamento axonal, lesões axonais e de mielina, remodelação dendrítica, diferentes tipos de gliose, invasão de células inflamatórias, dano à barreira hematoencefálica, angiogênese, modificações na composição da matriz extracelular, possível acúmulo de substâncias (como ferro e cálcio) e canalopatias adquiridas (Fu et al., 2022; Liu et al., 2024). Como consequência desse processo, o indivíduo começa a apresentar as crises recorrentes e espontâneas (CREs), caracterizando a fase crônica (Pitkanen et al., 2015). As CREs, por sua vez, podem atuar como um novo distúrbio epileptogênico, causando alterações secundárias que levam a um efeito progressivo das mudanças estruturais no cérebro epilético (Figura 1).

Figura 1 – Processo epileptogênico



Fonte: Elaborado pela autora (2024).

Genes que apresentam alterações transcricionais em resposta a diferentes insultos epileptogênicos são utilizados como marcadores de epileptogênese. A GFAP (Proteína fibrilar ácida da glia), um filamento intermediário específico de astrócitos, tem seus níveis estão aumentados durante a epileptogênese. Essa superexpressão é um marcador biológico da astrogliose reativa, presente ao longo desse processo (Brenner et al., 2021).

Outros genes superexpressos na epileptogênese, que atuam como marcadores são o TNF-α (Fator de Necrose Tumoral), uma importante citocina pró-inflamatória (Khaboushan et al., 2022); o Neuropeptídeo Y (NPY), que está associado a diversos processos neurológicoa, como dor, excitabilidade e neurogênese (Cattaneo et al., 2021) e o Fator Neurotrófico Derivado do Cérebro (BDNF), também associado à excitabilidade neuronal e neurogênese (Liu et al., 2024).

Apesar do conhecimento do processo epileptogênico, a exploração das vias moleculares e morfofuncionais envolvidas não é totalmente compreendida. Para atingir este objetivo, modelos de teste apropriados devem ser utilizados para explicar os mecanismos envolvidos nestes processos.

3.1 Modelos experimentais

Há diferentes tipos de modelos experimentais para indução da ELT, sendo os mais utilizados aqueles que se baseiam na estimulação elétrica ou química de estruturas límbicas, como o hipocampo e a amígdala. A estimulação química é feita com substâncias como o ácido caínico e a pilocarpina (PILO), que induzem convulsões autossustentáveis, aumentando a neurotransmissão colinérgica por meio da inibição GABAérgica ou da

estimulação glutamatérgica (Reddy; Kuruba, 2013). A epileptogênese induzida por PILO culmina na conversão de um cérebro normal para um cérebro epiléptico (Lévesque et al., 2021) e em associação de cloreto de lítio com a PILO, algumas horas antes da administração da PILO, ajuda a potencializar os efeitos da droga (Jingjing et al., 2019) fazendo com que uma dose menor de pilocarpina seja utilizada (Kovalenko et al., 2022) o que gera, consequentemente, redução da mortalidade dos animais.

Este modelo é amplamente utilizado para estudar o SE agudo e a ELT crônica, que surge semanas após o SE experimental. A epilepsia induzida pela pilocarpina simula características clínicas e fisiopatológicas da TLE humana, como esclerose hipocampal, brotamento de fibras musgosas, dispersão celular no giro dentado e gliose. Além disso, reproduz danos neuronais graves nas estruturas mesiais do cérebro (Kovalenko et al., 2022; Lévesque et al., 2016)

3.3 Tratamento

O tratamento da epilepsia geralmente se dá por meio do uso de fármacos antiepilépticos (FAEs), como ácido valproico, benzodiazepínicos, carbamazepina, fenitoína e fenobarbital (Kanner et al., 2022). No entanto, existe uma limitação significativa nesse tratamento, 30%–40% dos pacientes com epilepsia apresentam resistência aos medicamentos (Engel, 2019). Além disso, pacientes resistentes ao tratamento apresentam um risco elevado de SUDEP (morte súbita e inesperada em epilepsia), que é de duas a dez vezes maior em comparação à população geral (Fattorusso et al., 2021).

Para esses pacientes, uma alternativa terapêutica é a ressecção cirúrgica das estruturas epileptogênicas (Ahmad et al., 2020). Porém, esse procedimento pode causar efeitos adversos, como comprometimento cognitivo, problemas de memória e transtornos neuropsiquiátricos (Amaral et al., 2014; Yang et al., 2016; Escudeiro et al., 2017). Outras opções terapêuticas alternativas como a dieta cetogênica (Vaccarezza; Silva, 2015), a neuroestimulação (Laxer et al., 2014) e mudanças no estilo de vida podem ajudar no controle parcial das crises.

Evidências sugerem uma interação entre a ELT e o sistema circadiano, uma vez que os padrões de crises oscilatórias, embora imprevisíveis, refletem os ritmos circadianos (Xu et al., 2020; Spencer et al., 2016; Nieuwenhuyse et al., 2015).

3.4 Ritmo circadiano

Os ritmos biológicos são eventos cíclicos, recorrentes e periódicos (Moura, 2014) Alguns ritmos são influenciados diretamente pelos ciclos ambientais, enquanto outros são gerados internamente, podendo se ajustar a essas variações ambientais (Silva, 2017). Franz Halberg classificou os ritmos biológicos em três tipos principais: infradianos, com períodos acima de 28 horas; ultradianos com período inferior a 20 horas; e circadianos, com ciclos próximos de 24 horas (Moura, 2014).

Dentre esses, os ritmos circadianos são os mais bem estudados na cronobiologia, ciência que estuda os fenômenos biológicos que se repetem com certa frequência, que podem ou não estar sincronizados com ciclos ambientais. O sistema de relógio circadiano é essencial para a manutenção dos ritmos fisiológicos e comportamentais, garantindo que esses processos sejam alinhados aos ciclos ambientais, como o claro/escuro (Silva, 2017; Matos et al., 2018).

Os ritmos biológicos são sistematicamente regulados pelos núcleos supraquiasmáticos (NSQ), localizados na base do hipotálamo, conhecidos como o relógio biológico central. Contudo, outras estruturas cerebrais e órgãos periféricos, como o fígado, rins, hipocampo, corpo estriado e cerebelo, também possuem ritmos autônomos e funcionam como relógios periféricos (Figura 2) (Fonken et al., 2014).

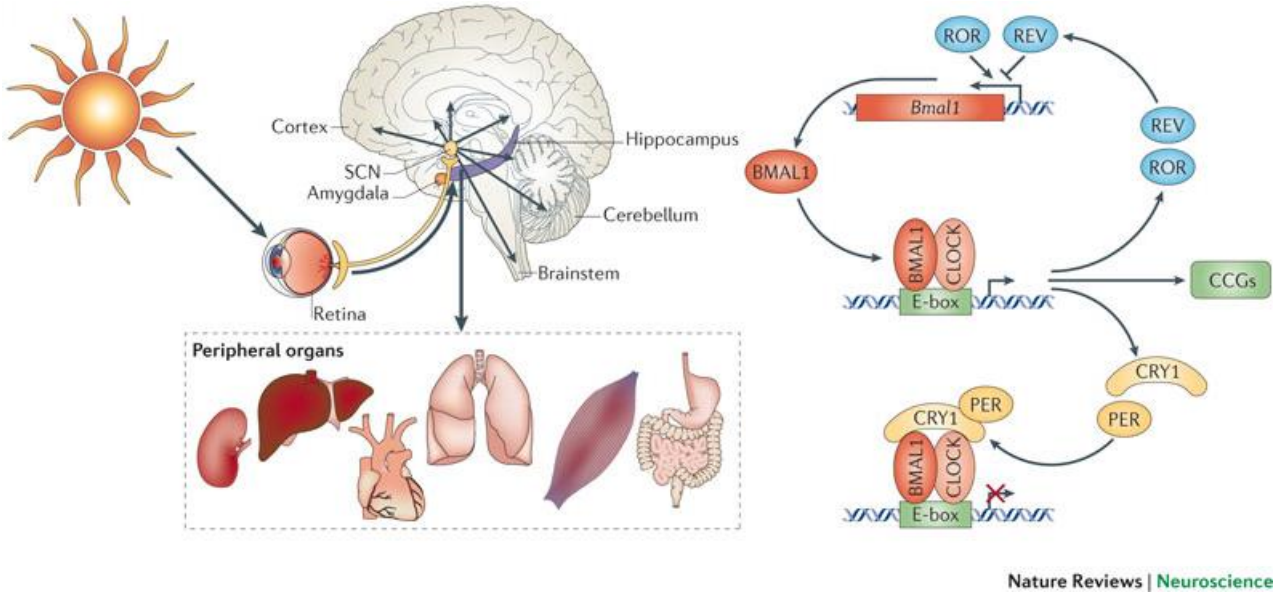
A nível molecular, os mecanismos subjacentes à geração de ritmo, baseia-se em alças de retroalimentação, que estão interconectadas pela regulação da transcrição e tradução de genes centrais do relógio (Coomans; Ramkiso Ensing; Meijer, 2015). Além desta auto-regulação, esses genes sincronizam os osciladores que estão distribuídos nos tecidos periféricos, mantendo-os sincronizados ao organismo (Gitaí et al., 2019). A regulação inicia através da expressão dos genes Bmal1 e Clock, que ao serem traduzidos em proteínas, formam um heterodímero (CLOCK:BMAL1), promovendo a transcrição de genes alvos, incluindo genes da família Citocromo (Cry1 e Cry2) e Período (Per1, Per2 e Per3) (Bolcinger; Schibler, 2014; Coomans; Ramkisoensing; Meijer, 2015). Como o mecanismo é disposto de uma maquinaria de retroalimentação, proteínas PER e CRY são repressoras do heterodímero CLOCK:BMAL1, no qual são as proteínas responsáveis pela ativação da maquinaria do relógio molecular (Figura 2) (Partch; Green; Takahashi, 2014). Os genes do relógio regulam a expressão de milhares de genes alvos que, por conseguinte, apresentam expressão e função rítmicas.

Embora o NSQ produza ritmos circadianos de forma endógena, esses ritmos também podem ser sincronizados com sinais ambientais, chamados Zeitgebers (sinais externos) (Fonken et al., 2014; Gitaí et al., 2019).

Uma característica essencial do ritmo circadiano é sua capacidade de persistir em condições ambientais constantes, como luz contínua ou escuridão contínua. Esse comportamento, conhecido como “free-running” (corrida livre), permite que os relógios biológicos mantenham seu funcionamento em ciclos ligeiramente diferentes de 24 horas. Apesar disso, eles conseguem se sincronizar ou "ajustar" em resposta a estímulos externos. Essa habilidade possibilita que os organismos antecipem mudanças cíclicas no ambiente, mesmo na ausência de variações externas, garantindo sua adaptação por um período significativo (Videnovic et al., 2014). No entanto, qualquer disfunção na ritmicidade circadiana pode prejudicar inúmeros processos fisiológicos e comportamentais. Entre eles, destacam-se a qualidade do sono, o estado de alerta, o desempenho cognitivo, o controle motor, o metabolismo e a saúde mental (Weaver, 2016a).

Os ritmos biológicos podem sofrer dessincronização quando há desregulação dos sinais ambientais, como, por exemplo, exposição inadequada à luz (em excesso ou em falta), ou exposição contínua a esses sinais. Além disso, alterações nos genes circadianos podem provocar essa dessincronização, que muitas vezes está associada ao desenvolvimento de diversas doenças. (Gitaí et al., 2019).

Figura 2 – Organização do ritmo circadiano em mamíferos.



FONTE: Kondratova, Anna & Kondratov, Roman, 2012.

3.5 Luz como modulador do ritmo

A luz é a principal pista ambiental externa usada pelo corpo para distinguir o dia da noite e assim, sincronizar os ritmos circadianos. Essa atividade se deve provavelmente à sua capacidade de reorganizar o sistema de relógio, mesmo quando submetidos a exposições rápidas a flash (Kaladchibachi & Fernandez, 2018).

Quando a exposição à luz é desregulada os ritmos biológicos podem tornar-se dessincronizados com consequências negativas para a saúde (Bedrosian; Nelson, 2017). Um exemplo desregulação mediada pela exposição da luz é a secreção de determinados hormônios, como a melatonina, ela é secretada durante a noite e seu efeito vasodilatador faz com que a temperatura do corpo caia e outras funções fisiológicas diminuam para preparação para o sono. A luz inibe a liberação de melatonina, de modo que a alternância natural de luz e escuridão está ligada diretamente ao sistema cronobiológico do corpo (Tähkämö et al., 2018).

Estudos em animais demonstraram que mutações ou deleções dos genes do relógio acarretam em ritmos com períodos anormais ou mesmo fenótipos arrítmicos quando testados sob condições constantes. De fato, a desregulação da ritmicidade pode ter um impacto negativo e muitos processos fisiológicos e comportamentais, tais como o desempenho cognitivo, controle motor, a qualidade do sono, o estado de alerta, o metabolismo e a saúde mental (Weaver, 2016a).

A maior parte das evidências sobre os efeitos da exposição atípica à luz nas respostas afetivas provém de estudos com modelos animais, principalmente roedores, devido à facilidade de controle das condições de iluminação nesses experimentos. Uma vantagem do uso de roedores é que as espécies mais comuns em laboratório são noturnas, o que significa que a exposição à luz durante a noite ocorre em sua fase ativa e desperta (Walker et al., 2020; Aielo et al., 2020).

Outro ponto importante a considerar é a produção de melatonina pela glândula pineal. Embora humanos e roedores apresentem padrões distintos de atividade e repouso, tanto espécies noturnas quanto diurnas produzem melatonina durante a fase escura, evidenciando uma similaridade circadiana fundamental (Walker et al., 2020).

A luz desempenha um papel crucial na ritmicidade das funções biológicas, podendo desorganizar a temporização interna. Em razão dessa influência da luz nos ritmos circadianos, protocolos que utilizam luz são comumente empregados em experimentos para induzir respostas do relógio biológico. Essas alterações nos ritmos circadianos podem ser induzidas por mudanças na fase ou amplitude do ritmo, por exemplo.

Em condições de luz constante, o acoplamento entre o ambiente e os ritmos biológicos pode ser comprometido, fazendo com que o relógio biológico oscile de forma diferente ou até mesmo seja interrompido em relação ao tempo externo. Isso pode resultar em uma dessincronização interna espontânea ou transitória, ou até na interrupção da oscilação circadiana. Esse fenômeno é comparável a modelos genéticos do relógio circadiano, como mutações nos genes Bmal1 e Cry1 + Cry2 (Reppert; Weaver, 2002), ou a lesões no NSQs (Chiesa et al., 2010). Esses modelos que são frequentemente utilizados para investigar os efeitos do sistema de controle circadiano em diferentes processos biológicos, bem como em diversas patologias (Wang et al., 2023).

Além da exposição a luz à noite, os ritmos circadianos podem ser interrompidos por outra conveniência moderna, como viagens para países com fusos horários diferentes (jet lag) podem comprometer a fisiologia circadiana. A ressincronização com o novo fuso horário ocorre de forma lenta, o que frequentemente resulta em perturbações do sono e desconfortos gastrointestinais (Bedrosian; Nelson, 2017)

Estudos com humanos e modelos experimentais em animais identificaram que o jet lag social e o trabalho em turnos, caracterizados pela exposição prolongada à luz artificial durante a noite, estão associados a um aumento do risco de desenvolver doenças metabólicas crônicas, como diabetes mellitus tipo 2, além de diferentes tipos de câncer, incluindo o câncer de mama (Weaver, 2016; Wallker et al., 2020).

Outro fator importante que afeta a população humana moderna é a poluição luminosa noturna, que pode causar o desalinhamento do sistema de controle circadiano. O uso excessivo de aparelhos eletrônicos durante a fase escura do dia, por exemplo, pode induzir mudanças inadequadas na fase circadiana, resultando em efeitos agudos, como alterações no sono, no estado de alerta e nos níveis de melatonina. Estudos indicam que até mesmo baixos níveis de iluminação noturna podem reiniciar a fase circadiana e suprimir a liberação de melatonina, sugerindo que a exposição comum à luz artificial pode, de fato, afetar negativamente a função circadiana (Zeitzer et al., 2000; Gooley et al., 2011; Leite, 2021).

3.6 Luz e doenças

Em muitas patologias, os ritmos biológicos podem alterar ou estabelecer um novo padrão, provavelmente devido a adaptações no sistema circadiano endógeno. No caso da ELTm, por exemplo, embora as crises epilépticas sejam imprevisíveis, evidências sugerem que elas apresentam um padrão diário de 24 horas (Nieuwenhuyse et al., 2015; Nzwalo et al., 2016; Gitaí, 2019; Xu et al., 2020). As crises tendem a ocorrer mais

frequentemente durante o estado de vigília, e alguns estudos observaram picos de crises concentrados em um único momento do dia (unimodal) ou em dois períodos distintos (bimodal) ao longo de 24 horas (Nzwalo et al., 2016; Baud et al., 2018; Gitaí et al., 2019).

A interação entre ritmos circadianos e crises epilépticas é bidirecional. Estudos demonstraram que os zeitgebers ambientais, como a alimentação e a luz, influenciam tanto o início quanto a intensidade das crises (Cho, 2012; Mirzoev et al., 2012; Mishra et al., 2015; Khan et al., 2018; Najar et al., 2024). Em modelos experimentais de ELTM, foram observadas alterações nos padrões circadianos de atividade locomotora e temperatura corporal, tanto semanas após o SE quanto na fase crônica da epilepsia (Quigg et al., 2001; Stewart; Leung, 2003; Pitsch et al., 2017) A atividade locomotora apresentou atrasos significativos na acrofase (>4 horas) (Stewart; Leung, 2003). Além disso, os níveis de cortisol demonstraram uma correspondência com os padrões de ocorrência das crises, sugerindo uma relação estreita entre esses eventos (Van Campen et al., 2015).

Estudo realizado por nosso grupo de pesquisa, em modelo de epilepsia induzida por Lítio-PILO intraperitoneal, mostrou que os genes do relógio apresentam expressão alterada nas fases pós SE e em ratos epiléticos, mantidos ao ciclo claro-escuro (12h/12h). A expressão de Bmal1, Per1 e Per2 foi mantida em ratos epilépticos, enquanto Per3 apresentou um perfil arrítmico na fase epiléptica. Cry1 e Cry2 estavam ausentes na fase pós SE, porém seu perfil foi recuperado na fase epiléptica (Matos et al., 2018). Essas alterações nos genes do relógio podem contribuir para explicar os mecanismos que levam a alteração de ritmicidade e aparecimento de determinadas comorbidades no processo epileptogênico.

Por outro lado, em uma relação inversa, alterações no ritmo circadiano podem influenciar o processo epileptogênico. A modificação do fotoperíodo de 12h claro/12h escuro para 18h claro/6h escuro reduziu o limiar para crises clônicas induzidas por pentilenotetrazol (PTZ) (TAHSILI-FAHADAN et al., 2008). A alteração do ritmo em modelos knockout destaca a importância dos genes do oscilador central no limiar de crises e na epileptogênese. Gerstener et al. (2014), em um estudo realizado com fotoperíodo de 14h claro/10h escuro, demonstraram que, na fase escura, o limiar para que um estímulo evoque uma crise é menor. Camundongos knockout para BMAL1 apresentaram uma redução do limiar para o início das CREs tanto na fase clara quanto na escura. Esses achados sugerem que tanto a alteração do ritmo induzida pela luz quanto a deleção de BMAL1 estão associadas à ocorrência de crises epilépticas.

Nesse contexto, é essencial desenvolver ensaios em modelos de epilepsia que envolvam a alteração e/ou perda do ritmo circadiano em laboratório, utilizando protocolos

de manipulação de sincronizadores externos. Estudos com animais mantidos em livre curso, ensaios funcionais com modelos knockdown e knockout, e a aplicação de diferentes Drogas Antiepilépticas (DAEs) em distintos períodos do ritmo circadiano têm se mostrado ferramentas valiosas (Marques, 2018).

3.7 Metabólitos

Metabólitos da flora intestinal, como os ácidos graxos de cadeia curta (AGCCs) e ácidos cólicos não conjugados, também podem influenciar a expressão de genes circadianos. Em um estudo com adultos saudáveis suplementados com insulina agave, os AGCCs demonstram variações rítmicas ao longo do dia, sendo que sua concentração nas fezes é mais elevada pela manhã e diminui gradualmente ao longo do dia (Kaczmarek et al., 2017).

Metabólitos representam os produtos resultantes, sejam intermediários ou finais, do processo metabólico em uma amostra biológica (Canuto et al., 2017). Essas substâncias podem ser produzidas diretamente pelo organismo hospedeiro ou derivados de micro-organismos, xenobióticos, dieta e outras fontes exógenas (Johnson et al., 2016). O conjunto abrangente de todos os metabólitos de baixa massa molecular (até 1500 Da), presentes ou sujeitos a modificações em um sistema biológico específico, é identificado como metaboloma, sendo a metabolômica a análise abrangente e quantitativa desse (Canuto et al., 2017).

A análise metabolômica é dividida em duas abordagens principais: a metabolômica alvo, que quantifica metabólitos específicos de determinada classe ou rota metabólica, e a metabolômica global, que identifica um grande número possível de metabólitos de diferentes classes no sistema biológico em estudo. A escolha entre elas depende dos objetivos da pesquisa, enquanto a metabolômica alvo foca em compostos específicos, a global oferece uma visão abrangente das alterações metabólicas (Klassen et al., 2017).

Uma variedade de tecidos e biofluidos pode ser usada para caracterizar o metaboloma como saliva, sangue, plasma, fezes, líquido cefalorraquidiano (LCR), tecido cerebral, entre outros (Ursulino, 2018).

A ressonância magnética nuclear (RMN) e a cromatografia gasosa acoplada a espectrometria de massas (GC-MS) (Canuto et al., 2017) são métodos amplamente utilizados em estudos metabolômicos devido à sua eficácia em fornecer dados estruturais sobre diferentes classes químicas (Mamães et al., 2020). A RMN é uma técnica robusta, simples e abrangente, que permite a análise de amostras biológicas intactas, como sólidos

ou semissólidos, com pouca ou nenhuma manipulação (Lenz; Wilson, 2007; Putri et al., 2013). Suas principais vantagens incluem a necessidade de pequenas quantidades de amostra e a preservação da integridade da amostra após a análise, ainda que possa haver leve contaminação por solventes deuterados (Mamães et al., 2020).

Essas moléculas têm sido extensivamente estudadas em diversas condições homeostáticas e patológicas devido a alterações nas concentrações de metabólitos devido a distúrbios celulares causados por fatores genéticos, nutricionais e fisiopatológicos (Filca & Edwards, 2016; Rinschen et al., 2019).

Na área clínica, a metabolômica tem sido aplicada para definir biomarcadores relacionados ao prognóstico ou diagnóstico de uma doença ou toxicidade/eficácia de medicamentos e, ao fazê-lo, espera fornecer maior compreensão fisiopatológica da doença ou toxicidade/eficácia terapêutica. (Canuto et al., 2017; Mamães et al., 2020).

Estudos na área oncológica mostram como alterações metabólicas podem servir de apoio no diagnóstico e monitoramento de cânceres como próstata, estômago, colorretal e mama, contribuindo para avanços no manejo dessas doenças (Mamães et al., 2020).

Nos casos de ELT, a gravidade das crises foi associada a alterações em metabólitos comuns como acetato, alanina, creatina, glutamina, glicerofosfocolina, taurina e valina em hipocampo de pacientes resistentes a medicamentos (Detour et al., 2018).

Recentemente, nosso grupo de pesquisa identificou um perfil metabólico distinto em ratos epilépticos, estes incluíram níveis baixos de D-glicose e ácido L-láctico, e níveis mais elevados de ácido L-glutâmico e glicina. Os resultados indicam uma correlação entre disbiose nesses animais e metabólitos fecais, que são conhecidos por influenciar a atividade cerebral epiléptica, promovendo inflamação crônica, desequilíbrio entre excitação e inibição neural e possíveis distúrbios metabólicos (Oliveira et al., 2020).

Os metabólitos de baixo peso molecular tem recebido considerável atenção no campo da epilepsia, com potencial uso enquanto biomarcadores, devido à complexidade do diagnóstico e tratamento da doença (Martinez; Peplow, 2023).

O diagnóstico de epilepsia e crises agudas apresenta um grande desafio clínico (Beamer et al., 2021) devido às altas taxas de diagnósticos incorretos, uma vez que os sinais clínicos podem ser facilmente confundidos com distúrbios de aparência semelhante, como os ataques não epilépticos psicogênicos (Dickson et al., 2017).

Além disso, os tratamentos medicamentosos para a epilepsia concentram-se predominantemente no controle dos sintomas e na prevenção de crises convulsivas. No entanto, esses fármacos não abordam as causas subjacentes da doença, tampouco

previnem o desenvolvimento de epilepsia após uma lesão ou insulto cerebral. Diante disso, há uma necessidade premente de avanços na pesquisa e no desenvolvimento de terapias preventivas que possam não apenas modificar o curso da epileptogênese, mas também intervir de forma eficaz para mitigar o risco de surgimento da epilepsia (Liu et al., 2024).

Esse cenário tem intensificado o interesse pela descoberta e validação de novos biomarcadores específicos para o processo epileptogênico (Engel; Pitkanen, 2020; Beamer et al., 2021).

Há, portanto, uma necessidade urgente de identificar biomarcadores de epileptogênese que possam reconhecer pacientes com alto risco de desenvolver epilepsia após um possível evento desencadeador (Pitkanen et al., 2018). Isso ajudaria a selecionar adequadamente a população de sujeitos para estudos, além de fornecer biomarcadores que indiquem a eficácia das intervenções terapêuticas, evitando a necessidade de aguardar a ocorrência de crises. Para serem amplamente aplicáveis na prática clínica, os biomarcadores ideais precisam ser não invasivos. É provável que um perfil composto por múltiplos biomarcadores seja necessário para alcançar maior especificidade e sensibilidade (Engel; Pitkanen, 2020; Beamer et al., 2021).

Vale ressaltar que, em cada fase, o processo epileptogênico e a identificação de biomarcadores podem ser afetados pela composição genética, pela microbiota e pelo exposoma (Engel; Pitkanen, 2020).

A ELT representa um grande desafio na neurologia devido à sua complexidade diagnóstica, impacto funcional e qualidade de vida dos pacientes. Investigar essa condição é fundamental não apenas para compreender os mecanismos que desencadeiam as crises, mas também para identificar fatores que influenciam sua gravidade, frequência e progressão. Além disso, investigações sobre a relação da ELT com ritmos biológicos e alterações metabólicas podem abrir caminhos para novas abordagens terapêuticas e diagnósticas, especialmente ao explorar biomarcadores que facilitem intervenções mais precisas e personalizadas. Essa linha de pesquisa é de extrema importância para avançar na compreensão dos processos subjacentes à epileptogênese e para promover inovações no manejo clínico da epilepsia.

O presente trabalho foi estruturado na forma de artigo científico, visando à submissão para publicação na revista *Frontiers in Neurology*, que possui fator de impacto de 2.7. A escolha dessa revista reflete sua relevância e prestígio na área de neurologia, sendo uma plataforma reconhecida para a disseminação de pesquisas inovadoras e de alta qualidade. A estrutura e o conteúdo do artigo foram cuidadosamente elaborados para

atender aos critérios exigidos pela publicação, contribuindo para o avanço do conhecimento científico na área de epilepsia e suas implicações circadianas e metabólicas.

4 JUSTIFICATIVA

A epilepsia é uma das condições neurológicas mais prevalentes, afetando milhões de pessoas ao redor do mundo, e sua complexidade se reflete na variedade de fatores envolvidos em sua gênese e progressão (FINHER et al., 2014). Um dos aspectos que tem atraído crescente atenção é o impacto do ritmo circadiano na fisiologia da epilepsia (Whang et al., 2023). O ritmo circadiano, que regula processos biológicos em ciclos de aproximadamente 24 horas, tem um papel importante nas crises epilépticas, com alterações nesse ritmo podendo influenciar a frequência e intensidade das crises (Khan et al., 2018; Giatí et al. 2019;). A compreensão das interações entre o ritmo circadiano e a epilepsia pode abrir novas abordagens para o tratamento da doença, além de fornecer insights para o desenvolvimento de terapias mais eficazes.

Este estudo é relevante porque a epilepsia representa um grande desafio médico e social, impactando significativamente a qualidade de vida dos pacientes. Avançar no entendimento dos mecanismos subjacentes à epilepsia é essencial para criar intervenções terapêuticas e diagnósticos que possam minimizar esse impacto.

Além disso, a epilepsia tem sido associada a alterações metabólicas que podem servir como potenciais biomarcadores para diagnóstico e prognóstico da doença (Godoi et al., 2022). Nesse contexto, a aplicação da metabolômica baseada em ressonância magnética nuclear (RMN) emerge como uma ferramenta poderosa, capaz de identificar mudanças em amostras biológicas relacionadas a alterações morfológicas e bioquímicas da epilepsia (Canuto et al., 2017).

A identificação de metabólitos associados a fenótipos específicos pode fornecer insights sobre os mecanismos subjacentes à epilepsia, contribuindo para o desenvolvimento de abordagens terapêuticas mais eficazes (Canuto et al., 2017; Wang et al., 2023). Assim, a metabolômica representa não apenas uma promessa para melhorar a gestão clínica da epilepsia, mas também uma oportunidade de ampliar o entendimento científico sobre os processos envolvidos na epileptogênese.

Embora os dois temas – o ritmo circadiano e os metabólitos – tenham sido estudados separadamente, ambos apresentam potenciais implicações clínicas relevantes para a epilepsia. A investigação das flutuações nos ritmos biológicos e dos perfis metabólicos dos pacientes epilépticos pode contribuir para a identificação de novos alvos terapêuticos e para o desenvolvimento de métodos diagnósticos mais rápidos e eficazes. Diante disso, o presente estudo visa explorar essas duas abordagens de maneira distinta, mas complementar, utilizando modelos experimentais de epilepsia para fornecer uma visão mais abrangente sobre os mecanismos subjacentes à doença.

5 OBEJTIVOS

5.1 Objetivo Geral

5.1.1 Artigo 1

Explorar como a modulação ambiental, por meio da luz constante, influencia o processo epileptogênico.

5.1.2 Artigo 2

Identificar potenciais biomarcadores associados às fases da epileptogênese por meio da metabôlomica.

5.2 Objetivos Específicos

5.2.1 Artigo 1

- Avaliar o efeito na luz constante em animais naïves e nas crises agudas durante o SE;
- Avaliar o efeito da luz constante na expressão gênica de genes que fazem parte do processo epileptogênico;
- Avaliar o efeito da luz constante, quanto a neurodegeneração, em hipocampo de ratos submetidos ao SE;
- Avaliar o efeito da luz constante na epilepsia.

5.2.2 Artigo 2

- Analisar o metaboloma diferencial de águas fecais de ratos submetidos ao SE.

6 CAPÍTULO 1

6.1 Effects of Constant Light on Acute and Chronic Phases Post-Status Epilepticus

Abstract

Background: Epilepsy is a chronic neurological disorder characterized by recurrent seizures, with status epilepticus (SE) being a severe condition that can initiate epileptogenesis. Circadian rhythms regulate various physiological functions and have been implicated in modulating seizure susceptibility and severity. However, the precise role of circadian disruption in SE and chronic epilepsy remains poorly understood. This study investigates the effects of constant light exposure (LC), a model for circadian disruption, on the acute and chronic phases of epileptogenesis. **Methods:** Male Wistar rats were exposed to either a 12-hour light/dark (LD) cycle or constant light (LC) for two weeks. SE was induced using the lithium-pilocarpine model. The acute phase was assessed by analyzing seizure severity, latency to SE onset, and neurodegeneration using NeuN immunostaining 24 hours post-SE. Molecular markers, including Npy, Gad65, and Tnf-α, were analyzed to explore underlying mechanisms. The chronic phase was evaluated by monitoring spontaneous recurrent seizures (SRS), locomotor activity rhythms, and circadian patterns over several weeks. **Results:** LC exposure disrupted circadian rhythms, evidenced by an increased circadian period and reduced relative amplitude of locomotor activity. In the acute phase, LC attenuated SE severity, with fewer stage 3–5 seizures, prolonged latency to SE onset, and reduced neurodegeneration in the dentate gyrus. These effects were associated with decreased expression of Npy and Gad65. In the chronic phase, LC eliminated the circadian rhythmicity of SRS observed under LD conditions, but there were no significant differences in SRS frequency or severity between LC and LD groups. **Conclusions:** LC-induced circadian disruption exerts phase-specific effects on epilepsy. In the acute phase, LC reduces SE severity and neurodegeneration, suggesting a protective role. In the chronic phase, LC alters seizure timing without impacting overall seizure burden. These findings highlight the complex interplay between circadian rhythms and epilepsy, suggesting that circadian-based interventions should be tailored to specific phases of epileptogenesis.

Keywords: epilepsy, status epilepticus, circadian rhythms, constant light, neurodegeneration, spontaneous recurrent seizures.

Introduction

Epilepsy affects millions worldwide and is characterized by recurrent, unprovoked seizures that vary in frequency and severity. Status epilepticus (SE) represents a critical condition involving prolonged seizures, often initiating the process of epileptogenesis, where the brain undergoes changes leading to chronic epilepsy (1, 2, 3). Understanding factors influencing both the acute phase of SE and chronic epileptogenesis is essential for developing effective therapeutic interventions.

Circadian rhythms, endogenous ~24-hour cycles regulating physiological functions, significantly modulate neuronal excitability and seizure susceptibility (4,5). Clinical observations indicate that seizure occurrence and severity are influenced by the time of day (6,7,8). For instance, patients with temporal lobe epilepsy often experience seizures during sleep or upon waking, highlighting circadian regulation's role (9). Additionally, seizure thresholds vary throughout the day, being lower at certain times, suggesting intrinsic circadian modulation of neuronal excitability (10). Despite these insights, the mechanisms by which circadian rhythms affect SE and epileptogenesis remain poorly understood.

Environmental light is a primary synchronizer of circadian rhythms via its influence on the suprachiasmatic nucleus (SCN), the master circadian clock (11, 12, 13, 14).

Constant exposure to light disrupts the natural light-dark cycle, leading to desynchronization of circadian rhythms. This effect includes attenuation of *Per1* and *Per2* gene and protein expression in the SCN, suppression of *Bmal1*, *Per1*, *Per2*, *Cry1*, *Cry2*, and *Rev-Erb* expression in the liver, as well as the flattening of cortisol and melatonin rhythms and attenuation of body temperature oscillations. (15, 16, 17) Studies using constant light (LC) exposure have demonstrated altered circadian gene expression and increased seizure frequency in animal models, suggesting that light-induced circadian disruption may impact epileptic activity (18). However, the effects of LC on epilepsy are complex and may vary depending on factors such as timing, intensity, and duration (19). While constant light exposure is often associated with negative outcomes, including increased seizure frequency, it may also have beneficial effects under certain conditions (20). For example, LC exposure has been shown to modulate metabolic processes that reduce neuronal excitability, potentially decreasing seizure activity (21, 22). Despite these intriguing possibilities, there is a significant gap in studies exploring the dual effects of LC exposure on epilepsy across different phases of epileptogenesis.

This study aims to address this gap by investigating the impact of LC exposure on both the acute and chronic phases of epileptogenesis induced by SE. By examining seizure severity, latency, neurodegeneration, and patterns of locomotor activity under conditions of disrupted circadian rhythms, we seek to enhance understanding of the complex relationship between circadian rhythms and epilepsy. The findings may provide valuable insights into developing light-based therapeutic strategies that optimize the beneficial effects of circadian modulation while minimizing potential risks.

2 Materials and Methods

2.1 Animals

The experiment was conducted using male Wistar rats (250-300 g) obtained from the central animal facility of the Federal University of Alagoas. Initially, all animals were kept under controlled conditions of temperature (22 ± 2°C), humidity (50-60%), and a 12-hour light/dark cycle. All animal procedures were performed in accordance with a protocol approved by the Ethics Committee for Animal Research at the Federal University of Alagoas (permit numbers: 62-2014, 17-2017, 20/2018), and were

consistent with international guidelines for ethical animal use, such as those established by the Society for Neuroscience.

2.2 Experimental Groups

2.2.1 Light/Dark Cycle (LD) Group

Animals were maintained under a 12-hour light/12-hour dark cycle. The LD group consisted of non-manipulated animals (naïve) (n=10), the LD-PÓS-SE group included animals euthanized 24 hours after SE (n=10) (Figure 1), and the Epileptic–LD group comprised epileptic animals (n=21) (Figure 3).

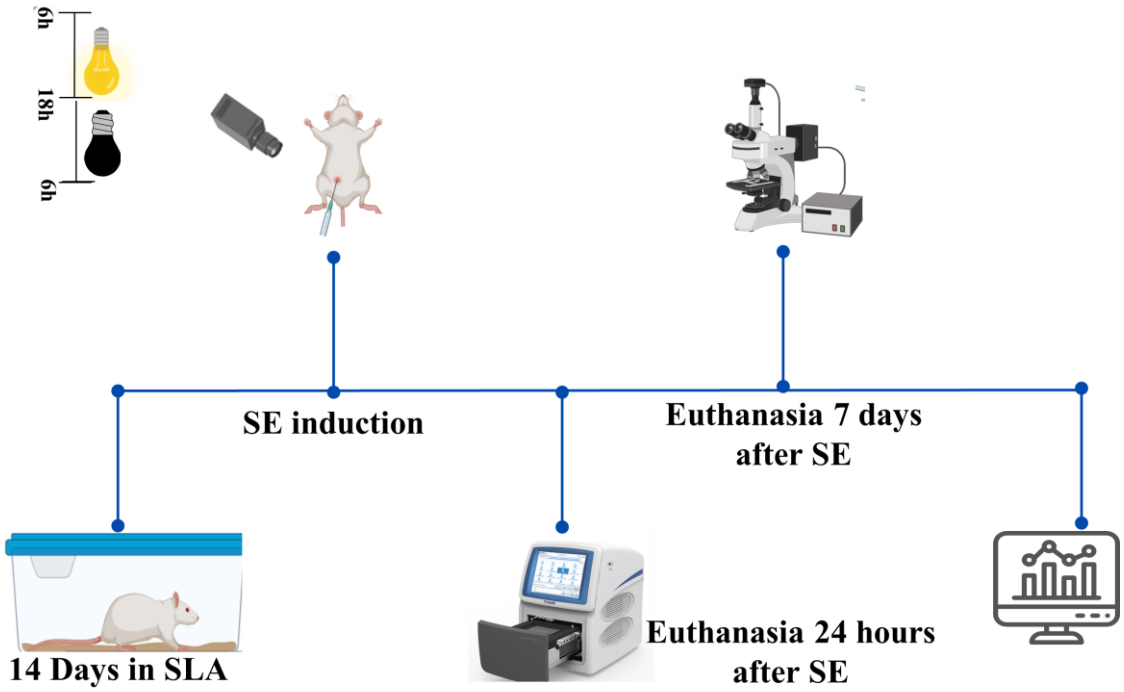


FIGURE1: Experimental design light/dark group Pós-SE

2.2.2 Constant Light (LC) Group

Animals were kept under constant light conditions. The LC group consisted of non-manipulated animals (n=10); the LC-PÓS-SE group included animals euthanized 24 hours after SE (n=10) (Figure2), and the Epileptic-LC, Epileptic-LC Rhythmic, and Epileptic-LC Arrhythmic groups comprised epileptic animals (n=21) (Figure 3).

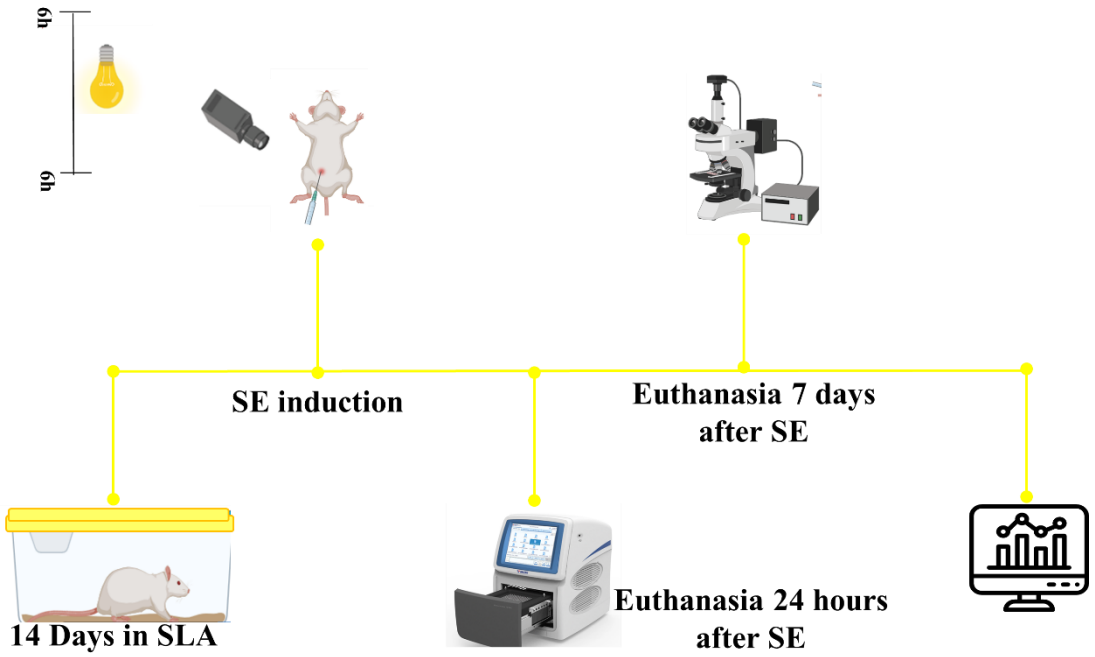


FIGURE 2: Experimental design constant light group Pós-SE

2.3 Naïve Experiment

Ten animals were housed individually and assigned to experimental groups. The LD group was maintained under a 12-hour light/12-hour dark cycle and underwent locomotor activity recording (LAR) for 14 days. The LC group was kept under constant light for 2 weeks and also underwent LAR for 14 days. After the activity rhythm recording, all animals were subjected to SE, with seizures assessed during SE and euthanasia performed 24 hours after SE.

2.4 SE Experiment

Ten animals were housed individually and assigned to experimental groups. The LD- Pós-SE group was maintained under a 12-hour light/12-hour dark cycle for 14 days and then subjected to SE, with euthanasia performed 24 hours and 7 days after SE. The same protocol was applied to the LC-pós-SE group, where animals were exposed to constant light.

2.5 Chronic Experiment

Animals (n=21) were subjected to SE. One month after SE, following the establishment of spontaneous recurrent seizures (SRS), animals were video-monitored during the following periods: 5 weeks after SE (for 9 days), 7 weeks after SE (for 14 days), and 9 weeks after SE (for 10 days). LAR was recorded between the 7th and 9th weeks after SE (for 10 days). Following the analysis of SRS during the SE cycle, all animals were kept under constant light. Upon initiating LC treatment, animals were video-monitored for 14 days and underwent LAR recording (for 10 days). Three weeks after the start of constant light, SRS were monitored, and LAR was assessed (for 10 days). Five weeks after LC treatment, LAR was again analyzed (for 10 days). Video monitoring of SRS and LAR recording were conducted 24 hours per day.

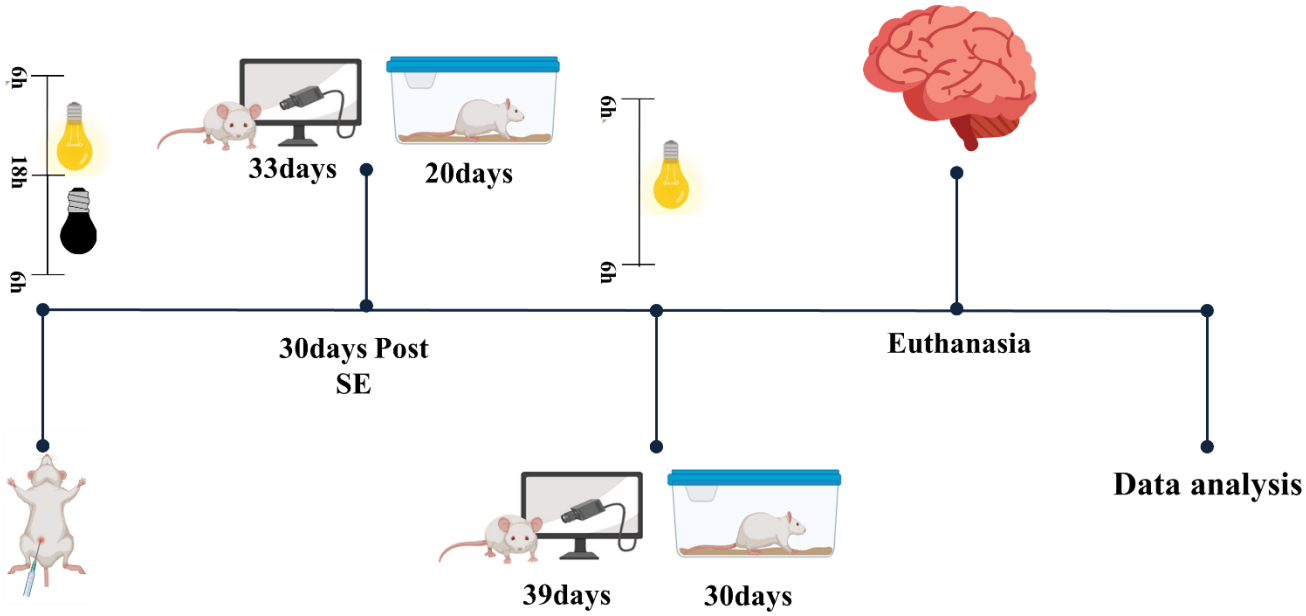


FIGURE 3: Experimental design light/dark epileptic group and constant light.

2.6 SE Induction

SE was induced using the lithium-pilocarpine model. Animals received an intra-peritoneal injection of 127 mg/kg lithium chloride (LiCl), 16 hours before pilocarpine (PILO) administration at 30 mg/kg. To mitigate peripheral effects of pilocarpine, scopolamine butylbromide (1 mg/kg, i.p.) was injected 30 minutes before pilocarpine administration. Following pilocarpine injection, animals were video-monitored using a digital camera (DCR-SR68, Sony Brasil LTDA), with SE defined as continuous seizures lasting 5 minutes or more at level 2 or higher on the Racine scale (1972) (23) After 90 minutes, SE was terminated with intraperitoneal Diazepam (5 mg/kg), administered every 30 minutes until cessation of behavior-al seizures.

2.7 Spontaneous Locomotor Activity

Animals were individually housed in the Locomotor Activity System (LAS). The system recorded movements over a 10-day period (baseline). Infrared motion sensors detected any movement within the cage. Sensors were placed 15 cm above the cage lids and automatically recorded movement time every 5 minutes on a computer via the SAP system (Dr. Marconi Camara Rodrigues, Federal University of Rio Grande do Norte, Natal, Brazil, 2011).

2.8 Perfusion, Tissue Processing, and Immunohistochemistry

Animals from the LD-Naïve, LD-7days, LC, and LC-7 days groups (n = 6/group) were anesthetized with isoflurane and then perfused through the heart with 4% paraformaldehyde. Brains were carefully removed from the skull and post-fixed in 4% paraformaldehyde for approximately 24 hours at 4°C. Brain tissues were treated with 30% sucrose in PB until they sank to the bottom of the container. Brains were then sectioned coronally through the entire septotemporal axis of the hippocampus. Sections, 30 micrometers thick, were cut using a cryostat, collected, and stored in 24-well plates

containing phosphate buffer (PB). For immunohistochemistry, serial sections (every 20) of the entire hippocampus from each animal were selected. Immunohistochemistry was performed using NeuN, a specific marker for mature neurons (24). Sections were first treated with phosphate-buffered saline (PBS) containing 20% methanol and 3% hydrogen peroxide for 20 minutes and then washed three times with PBS. Sections were then treated for 30 minutes in PBS containing 0.1% Triton X-100 and an appropriate serum (10%) selected based on the species from which the secondary antibody was derived. Histological sections were incubated for 24 hours in primary antibody solution prepared in PBS. The primary antibody used was a monoclonal rat anti-NeuN (1:1000, Millipore). After incubation with the primary antibody, sections were washed three times in PBS, incubated with secondary antibody solution for 60 minutes, and washed three times in PBS. Sections were then treated with avidin-biotin complex (ABC, Vector) reagent for 60 minutes. The peroxidase re-action was performed using 3,3'-diaminobenzidine (DAB, Vector). Sections were mounted on gelatin-coated glass slides. After drying, slides were dehydrated, cleared, and coverslipped for stereological analysis.

2.9 Stereological Quantification

Samples were analyzed using stereology with an optical fractionator tool (25). The optical fractionator used was Stereo Investigator (Microbrightfield Inc., Williston, VT), attached to a Nikon E600 microscope and a video camera (Optronics Inc., Muskogee, OK). The number of NeuN+ immunoreactive neurons in the hilus of the dentate gyrus was quantified, estimated from 6 serial sections.

2.10 Statistical Analysis

Circadian rhythmicity analysis of Spontaneous Recurrent Seizures (SRS) and spontaneous locomotor activity (LAR) was performed using MetaCycle, an R package for evaluating periodicity in time series data, to analyze the temporal profile of SRS and LAR over 24 hours (26). MetaCycle results provided p-values, with a group considered rhythmic when $p < 0.05$. Circadian analysis was conducted using ImageJ and MetaCycle software. Comparisons between conditions were made using unpaired t-tests or Mann-Whitney tests for non-parametric data. Statistical significance levels were set at * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. For SE seizure analysis and SRS (latency, total seizures/SRS, and seizure severity), as well as rhythm variables, GraphPad Prism 8 (GraphPad Soft-ware, La Jolla, CA) was used. Data assuming normal distribution (tested by Kolmogorov-Smirnov test) were analyzed using one-way ANOVA followed by Bonferroni or Dunnett post-hoc tests for multiple comparisons. For comparisons between two groups, Student's t-test was used. For non-parametric samples, Kruskal-Wallis test was employed for multiple comparisons, while Mann-Whitney test was used for comparisons between two groups. Data are presented as mean ± standard error of the mean (± SEM) from at least four independent experiments. Significance levels were considered as $p < 0.05$, $p < 0.01$, and $p < 0.001$.

3 Resulted

3.1 Effect of constant light exposure on animals

Initially, we evaluated the effect of two weeks of constant light (LC) exposure on circadian rhythms based on locomotor activity. Actograms revealed a significant disruption of circadian rhythms in LC animals compared to those maintained under a light-dark (LD) cycle. Specifically, LC exposure resulted in a significant increase in circadian period ($p < 0.0001$) and a marked reduction in the relative amplitude of

locomotor activity ($p < 0.0001$) (Figures 4A and 4B). Subsequently, the study investigated whether these LC-induced alterations in circadian rhythms influenced the characteristics of status epilepticus (SE). During SE, LC animals exhibited a significantly longer latency to SE onset ($p < 0.05$), a reduced total number of seizures ($p < 0.01$), and fewer stage 3, 4, and 5 seizures ($p < 0.001$) compared to LD animals (Figures 4C and 4D). These findings indicate that two weeks of constant light exposure not only disrupt circadian rhythms but also significantly modulate SE severity, particularly reducing the frequency of more severe seizure stages.

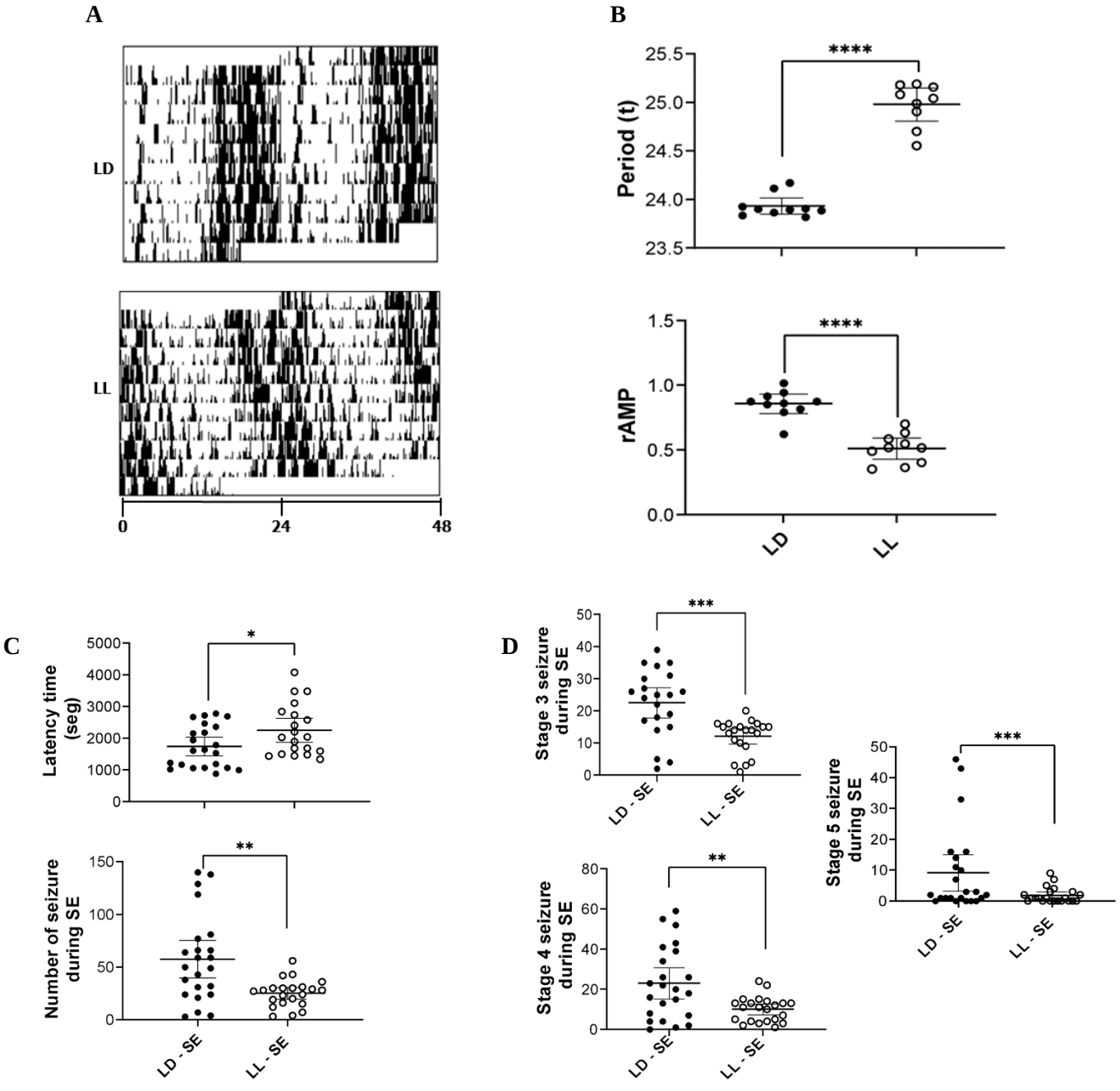


FIGURE 4| Effects of exposure to constant light on animals. **(A)** Representative attogram of double plotting of locomotor activity (SLA) in epileptic animals maintained in the light-dark cycle (LD) or constant light (LC). **(B)** Circadian rhythm variables. The period was longer than 24 hours in the group kept in constant light compared to the LD. Note that the animals in the LC group have a less robust rhythm compared to the LD group, represented by the smaller relative amplitude (rAMP) of the SLA. **(C-D)** For variables related to seizures during Status epilepticus (SE) there was an anticonvulsant effect in animals

exposed to LC compared to the LD group. Statistical testing for circadian analysis was performed using the MetaCycle software in the R package and the Meta2d function. Comparisons between conditions were made using the unpaired t-test or the Mann Whitney test, when data were nonparametric. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

3.2 Expression levels of key genes

To investigate the molecular mechanisms underlying the effects of constant light (LC) exposure and its interaction with status epilepticus (SE), the expression levels of key genes were analyzed, including Npy, Tnf-α, Gad65, Bdnf, Gfap, and Bmal (Figure 5). In the Light-Dark (LD) condition, SE significantly increased the expression levels of Npy, Tnf-α, Gad65, Bdnf, Gfap, and Bmal compared to LD controls (LD-CTR) (Figure 5A–F). Similarly, in the Constant-Light (LC) condition, SE also increased the expression levels of these genes compared to LC controls (LC-CTR). However, in the LC- PÓS-SE group, the expression levels of Npy and Gad65 were significantly lower compared to the LD- Pós-SE group ($p < 0.001$ and $p < 0.01$, respectively) (Figure 5A, 5C). These results highlight that while SE consistently upregulates key genes involved in neuronal activity, inflammation, and circadian regulation under both LD and LC conditions, LC exposure modulates this response, particularly by attenuating the expression of Npy and Gad65 post-SE. This suggests that constant light exposure alters the molecular landscape of the post-SE state, potentially influencing both excitatory and inhibitory pathways.

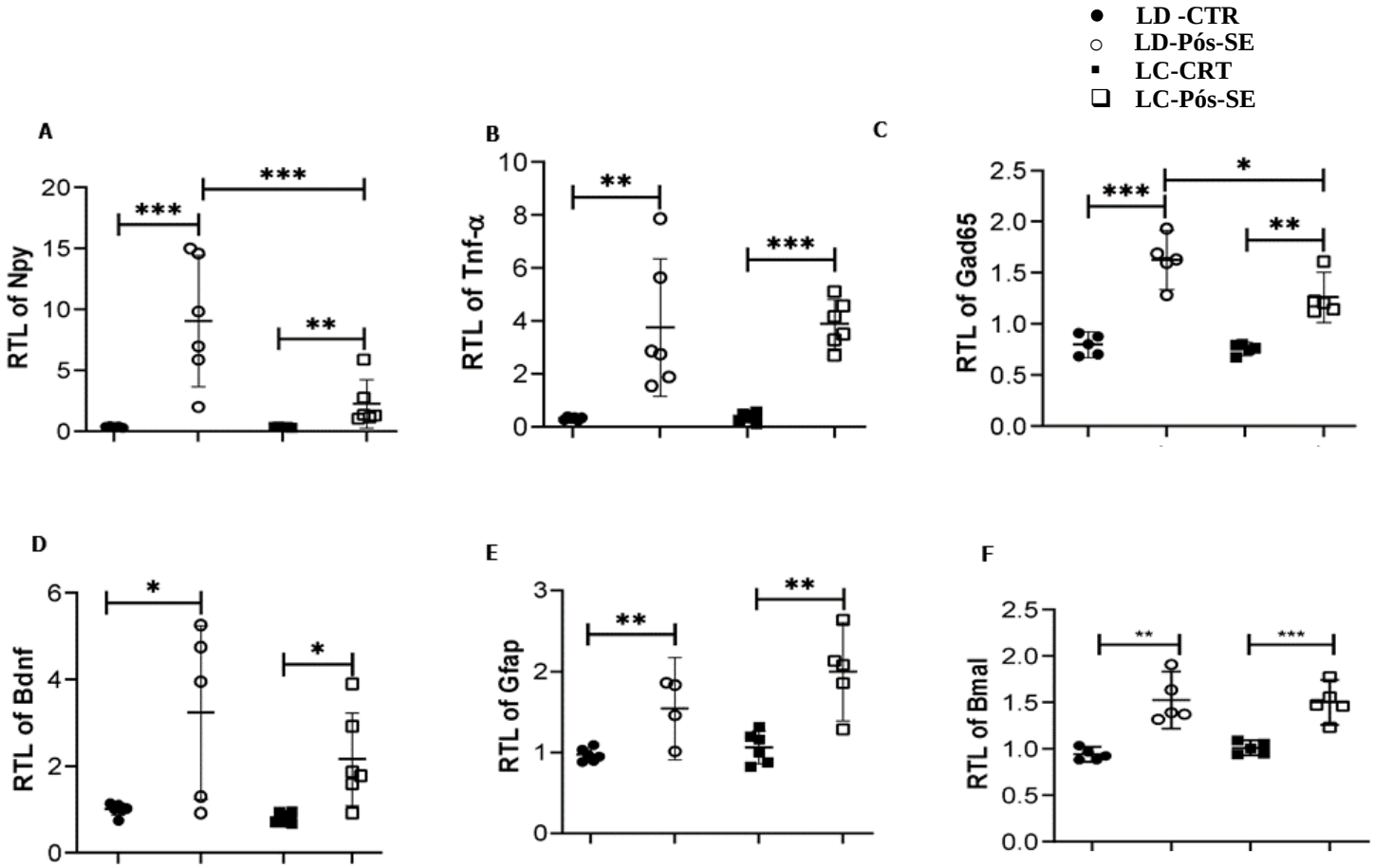
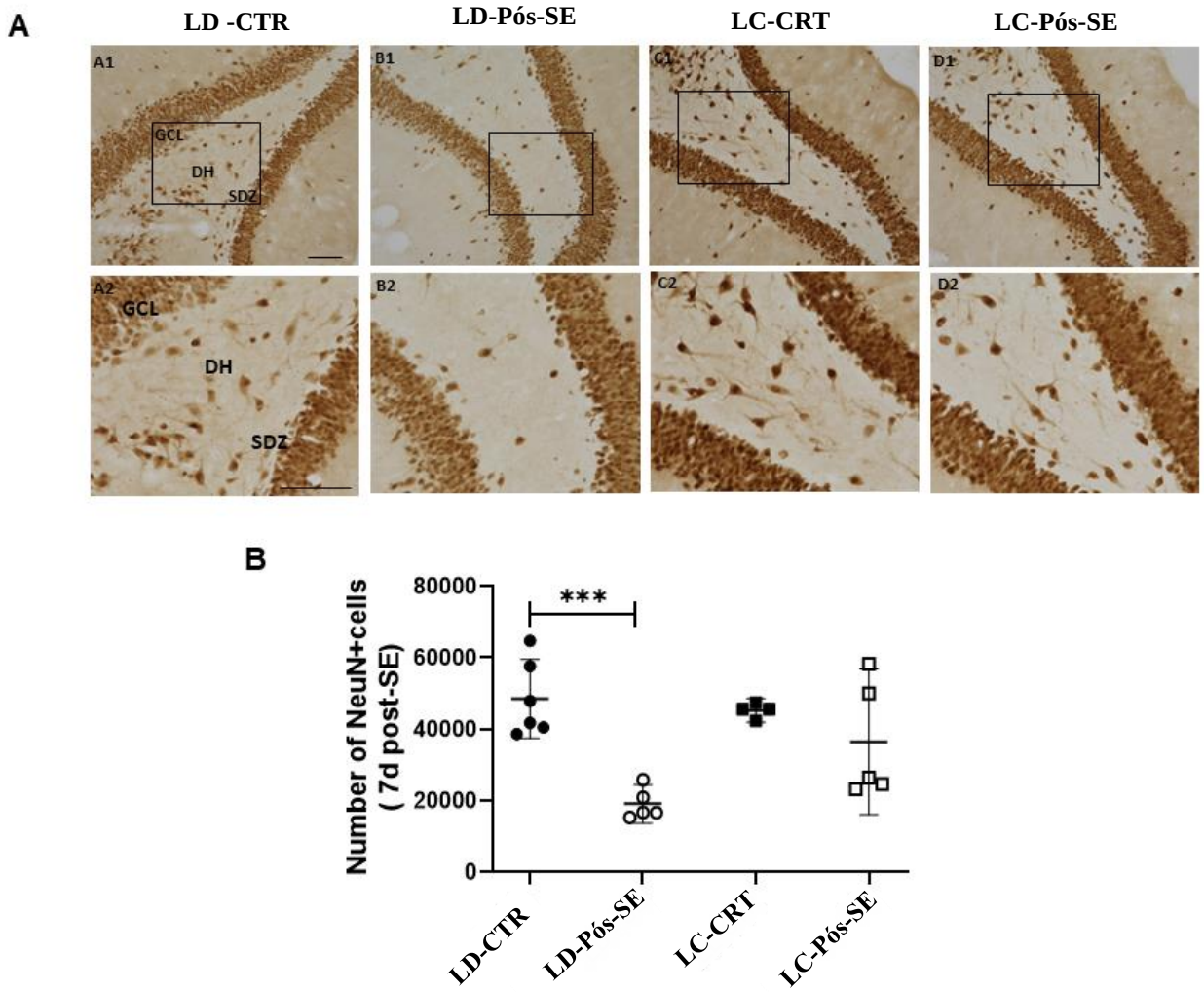


FIGURE 5 | Gene expression of Npy **(A)**, Tnf-α **(B)**, Gad65 **(C)**, Bdnf **(D)**, Gfap **(E)**, and Bmal (**F**) in the post-SE model. Values are displayed as Relative Transcript Levels (RTL). The mean RTL of Npy, Tnf-α,

Gad65, Bdnf, Gfap, and Bmal are increased in the Light-Dark post-*Status Epilepticus* (LD-Pós-SE) condition compared to Light-Dark Control (LD-CTR), and in Light-Light post-*Status Epilepticus* (LC-PÓS-SE) compared to Light-Light control (LC-CTR). However, the RTL of Npy and Gad65 decrease in the LC – SE compared to LD – SE. ALL data are represented as mean ± SEM with $n = 6$/group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, unpaired two-tailed *t*-test. Fonte: Marques, 2018.

3.3 Effects of constant light exposure on neurodegeneration following status epilepticus

To evaluate the effects of constant light (LC) exposure on neurodegeneration following status epilepticus (SE), NeuN immunostaining was performed to quantify the number of surviving neurons in the dentate gyrus 7 days post-SE (Figure 6A). In the Light-Dark (LD) condition, SE significantly reduced the number of NeuN-positive cells compared to LD controls (LD-CTR; $p < 0.001$). However, in the Light-Light (LC) condition, the number of NeuN-positive cells in LC- PÓS-SE animals was comparable to LC controls (LC-CTR), indicating a reduction in SE-induced neurodegeneration under LC exposure (Figure 6B). Representative photomicrographs illustrate the marked neuronal loss in LD-Pós-SE animals compared to LD-CTR (Figures 6A1–B1), while LC-PÓS-SE animals displayed neuronal preservation similar to LC-CTR (Figures 6C1–D1). These results suggest that constant light exposure attenuates SE-induced neuronal loss in the dentate gyrus, highlighting a potential neuroprotective effect of LC.

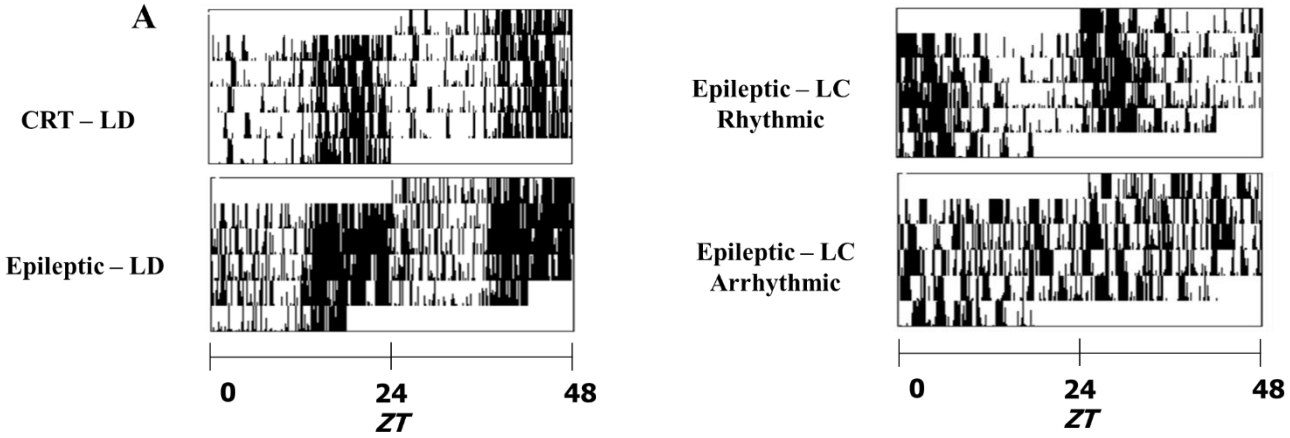


Fonte: Marques, 2018.

FIGURE 6 | Exposure to constant light reduced neurodegeneration in the dentate hilus of the hippocampus. **(A)** Images of immunostaining of NeuN+ cells dentate hilus of the hippocampus (A1,B1,C1,D1 - Scale bar =20 μm), (A2,B2,C2,D2, Scale bar =100 μm) in the Light-Dark Control (LD-CTR), Light-Dark post-*Status Epilepticus* (LD- Pós-SE) condition, Light-Light Control (LC-CTR), and in Light-Light post-*Status Epilepticus* (LC- Pós-SE). **(B)** Quantification of immunostaining data of NeuN+ at 7D (7 days) post-SE. The *y*-axis shows the number of NeuN positive cells. Data are shown as mean ± SEM. Statistical significance was determined by two-sided Welch's ANOVA. $^{***}p < 0.001$

3.4 Effects of constant light exposure on circadian rhythmicity and spontaneous recurrent seizures

To investigate the effects of constant light (LC) exposure on circadian rhythmicity and spontaneous recurrent seizures (SRS), locomotor activity and seizure patterns were analyzed in epileptic animals maintained under light-dark (LD) or constant light (LC) conditions (Figure 7). Representative actograms highlight that epileptic animals in LC conditions were categorized into rhythmic and arrhythmic phenotypes based on circadian rhythm robustness, determined by the MetaCy-cle statistical analysis (Figure 7A). Circadian rhythm analysis revealed that animals in the Epileptic - LC rhythmic and Epileptic - LC arrhythmic groups exhibited longer circadian periods compared to LD and Epileptic - LD animals ($p < 0.01$). Furthermore, the Epileptic - LC animals showed reduced rhythm robustness, as evidenced by a lower relative amplitude (rAMP) compared to LD and Epileptic - LD animals ($p < 0.01$). Among the Epileptic - LC groups, arrhythmic animals exhibited significantly reduced rAMP, higher intracycle variability, and lower interdaily stability compared to rhythmic animals, characterizing their arrhythmic phenotype (Figure 7B). In terms of SRS occurrence, no significant differences were observed between the Epileptic - LC and Epileptic - LD groups in the number of daily SRS or the frequency of sei-zures at stages 3, 4, and 5 (Figure 7C). However, the temporal distribution of SRS revealed a distinct pattern: while LD animals exhibited a circadian rhythm of SRS peaking during the light phase (acrophase: ZT 13.5; $p < 0.0001$), Epileptic - LC animals, including both rhythmic and arrhythmic groups, lost this circadian oscil-lation ($p = 0.60$) (Figure 7D). These findings indicate that constant light exposure disrupts circadian regulation of locomotor activity and SRS, with a subset of an-imals developing an arrhythmic phenotype characterized by fragmented rhythms and loss of seizure timing.



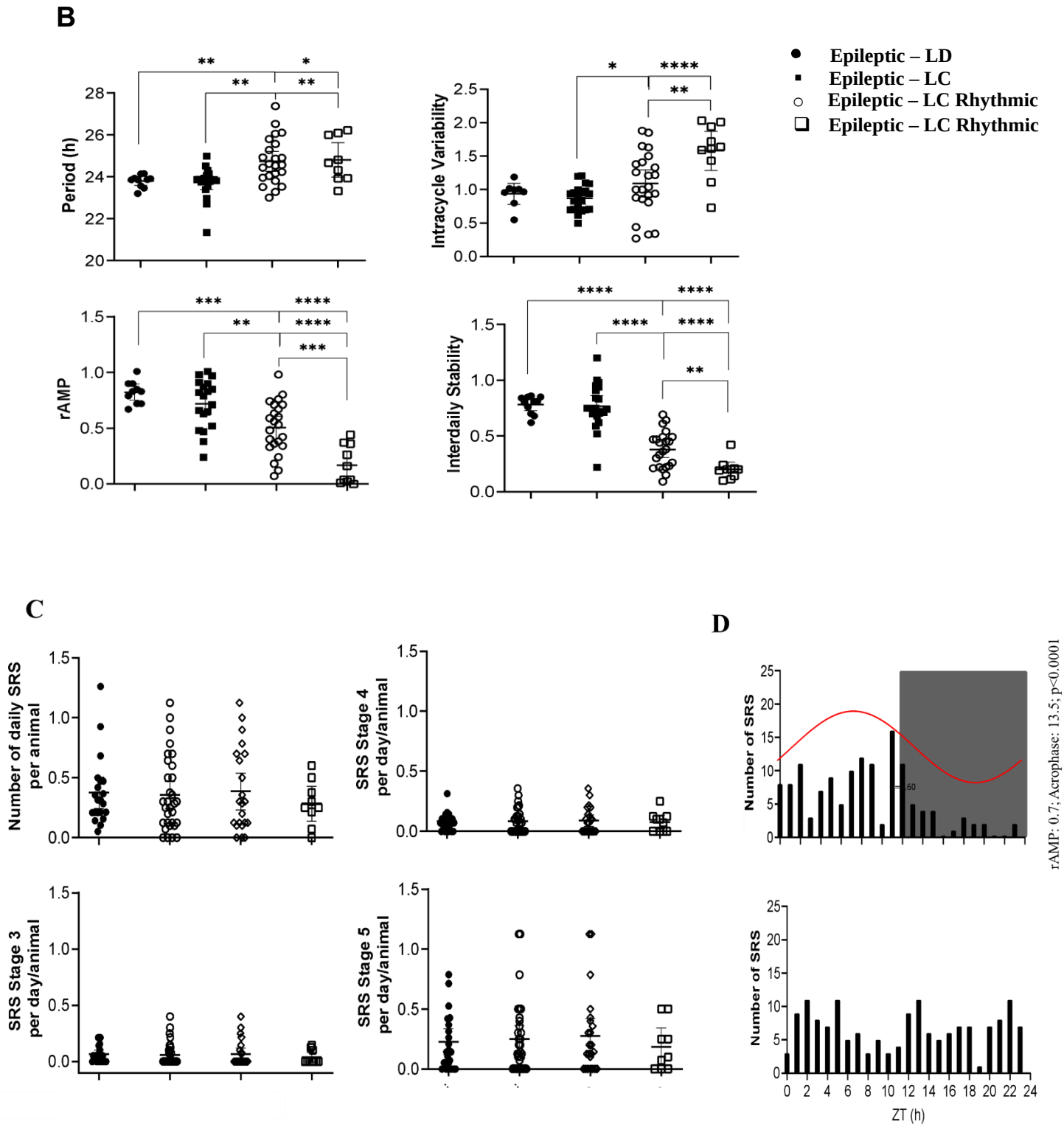


FIGURE 7 | Effects of constant light exposure in epilepsy**. (A)** Representative attogram of double plotting of locomotor activity (SLA) in epileptic animals maintained in the light-dark cycle (LD) or constant light (LC). Note that the Epileptic - LC group was divided into rhythmic LC and arrhythmic LC. **(B) Circadian rhythm variables.** The **period** was longer than 24 hours in epileptic groups kept in constant light (LC-Rhythmic and LC- Arrhythmic) compared to LD and Epileptic - LD rats. Note that the animals in the Epileptic - LC groups have a less robust rhythm compared to the LD and Epileptic - LD groups, represented by the lower **relative amplitude (**rAMP) of the SLA. As expected, the Epileptic - LC - Arrhythmic group presents less robustness of the circadian rhythm than the Epileptic - LC - Rhythmic group, which could characterize it as an arrhythmic group for SLA. The epileptic groups maintained in LC present a more fragmented rhythm, represented by the higher values of **intracycle variability**. The coupling strength of the rhythm to the environment between days, represented by the **Interdaily Stability**, was lower in the

Epileptic - LC groups compared to the LD and Epileptic - LD animals, being even more reduced in the Epileptic - LC - Arrhythmic group. **(C)**. There was no significant difference in variables related to spontaneous and recurrent seizures (SRS) between animals exposed to LC compared to the Epileptic - LD group. **(D)** 24-hour frequency of SRS in LD and LC. The SRS activity in the LD groups peaks during the day and decreases at night. However, the SRS of animals kept in the LC do not show circadian osciLCation The LC animals were divided into two groups, rhythmic and arrhythmic, according to the MetaCycle statistical test in the R package and the Meta2d function. The group was considered rhythmic when $p < 0.05$. The statistical test for circadian analysis was made by ImageJ and MetaCycle software. Comparisons between conditions were made using the Unpaired t test or the Mann Whitney test, when data were nonparametric * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

4 Discussion

Our study demonstrates that constant light (LC) exposure exerts phase specific effects on epilepsy, highlighting the complex interplay between circadian rhythms and epileptogenesis.

4.1 Disruption of Circadian Rhythms Modulates SE Severity

LC exposure disrupted circadian rhythms, as evidenced by alterations in locomotor activity patterns and increased circadian period. This desynchronization likely affects the SCN and downstream neural circuits regulating excitability (15,11,17,27). In the acute phase, LC exposure attenuated SE severity, with fewer severe seizures and increased latency to SE onset. This contrasts with studies suggesting that circadian disruption exacerbates seizure activity (18,28). However, our findings align with reports that LC can modulate metabolic processes (29), reducing neuronal excitability under certain conditions (21,30,31). The molecular data support this notion, showing that animals exposed to LC exhibit reduced expression of Npy (neuropeptide Y) and Gad65 (glutamate decarboxylase 65) compared to animals in the light-dark (LD) condition. Neuropeptide Y (NPY) is widely recognized for its anticonvulsant properties, primarily through Y2 receptor activation, which inhibits glutamate release at excitatory synapses (32,33). However, excessive NPY levels in hyperexcitable states may desensitize Y2 receptors, potentially exacerbating excitatory feedback over time (34,35,36,37,38,39). The observed reduction in Npy expression in LC-Pós-SE animals may prevent such desensitization, contributing to a more stable excitatory inhibitory balance. This adjustment could help explain the reduced seizure frequency and severity observed during SE. GAD65 is essential for synthesizing GABA, the brain's primary inhibitory neurotransmitter. While reduced Gad65 expression might initially seem detrimental, it could mitigate excessive inhibitory tone that can promote network in-stability and synchronization during SE. By lowering GABA synthesis, LC- Pós-SE animals may avoid pathological over-inhibition, which could otherwise enhance the propagation of severe seizures (40). Together, the reductions in Npy and Gad65 will likely increase the threshold for seizure initiation, prolonging the latency to SE onset and attenuating the severity of seizures. Additionally, LC-Pós-SE animals exhibited preservation of NeuN-positive neurons in the dentate gyrus, indicating neuroprotection. This effect may result from the modulation of excitatory and inhibitory balance, reducing excitotoxicity during SE. While pro-inflammatory markers such as Tnf-α and Gfap did not show consistent decreases, the neuroprotective effect suggests alternative mechanisms influenced by LC exposure.

LC Disrupts Circadian Regulation of SRS Without Affecting Seizure Burden

In the chronic phase, LC exposure disrupted the circadian rhythmicity of SRS observed in LD animals, which exhibited a clear circadian peak in seizure occurrence during the light phase (Figure 7D). This loss of circadian modulation is consistent with the observed desynchronization of locomotor activity rhythms, as LC exposure fragmented circadian patterns, resulting in reduced rhythm amplitude and stability. These findings align with studies demonstrating that circadian rhythms, mediated by the suprachiasmatic nucleus (SCN), regulate the temporal distribution of seizures by influencing cortical excitability and neurotransmitter release (4,5,41).

Contrary to the acute phase, where LC reduced seizure severity during SE, no significant differences were observed between Epileptic - LC and Epileptic - LD groups in the number or severity of SRS during the chronic phase (Figure 7C). This indicates that while LC profoundly affects circadian rhythms, it does not modulate the overall seizure burden once chronic epilepsy is established. The loss of circadian rhythmicity in SRS under LC conditions likely reflects a redistribution of seizures across the 24-hour cycle rather than a reduction or increase in total seizure activity. This observation suggests that the mechanisms driving chronic epileptogenesis and SRS are less influenced by circadian factors than the acute phase of SE. The emergence of rhythmic and arrhythmic phenotypes in LC-exposed animals highlights the heterogeneity of responses to circadian disruption. Rhythmic animals maintained a weak but detectable circadian rhythm in locomotor activity, whereas arrhythmic animals exhibited complete desynchronization. Despite these differences, no significant changes in SRS frequency or severity were observed between these phenotypes, suggesting that circadian desynchronization alone does not significantly impact the epileptic network's overall activity during the chronic phase. However, the loss of circadian modulation in arrhythmic animals may contribute to greater unpredictability in seizure timing, which could have implications for clinical management and quality of life. The disruption of circadian rhythms in SRS timing under LC conditions underscores the importance of considering circadian factors in epilepsy management. While light-based therapies are often used to stabilize circadian rhythms and improve seizure predictability (20,42), our findings suggest that circadian modulation may not significantly alter seizure frequency or severity in the chronic phase of epilepsy. Instead, interventions targeting other mechanisms, such as neuroinflammation, synaptic reorganization, or network excitability, may be more effective in reducing the overall epileptic burden.

Implications for Therapeutic Interventions

These findings suggest that circadian-based interventions may need to be tailored to specific phases of epilepsy. In the acute phase, strategies that modulate circadian rhythms could potentially reduce seizure severity and neurodegeneration. In contrast, during the chronic phase, interventions may focus on restoring circadian regulation to improve seizure predictability and patient quality of life.

5 Conclusion

Constant light-induced circadian disruption has distinct effects on epilepsy across different phases. LC exposure reduces SE severity and neurodegeneration in the acute phase, potentially through modulation of inhibitory neurotransmission. In the chronic phase, LC disrupts the circadian timing of seizures without affecting overall seizure burden. These findings highlight the importance of considering circadian rhythms in epilepsy management and suggest that interventions should be phase-specific.

6 References

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7 CAPITULO 2

7.1 Metabolomic Analysis of Fecal Water in an Animal Model of Temporal Lobe Epilepsy

Abstract

Epilepsy is a neurological disorder characterized by spontaneous and recurrent seizures. Temporal lobe epilepsy (TLE) is the most common form in adults and is related to alterations in the limbic system, commonly triggered by epileptogenic events, such as *status epilepticus* (SE). The diagnosis of epilepsy presents a clinical challenge due to high rates of incorrect diagnoses. The study of organic metabolites has proven relevant for understanding homeostasis and pathogenesis, as their concentrations vary in response to cellular dysfunction, making them potential biomarkers. Therefore, the present study aimed to evaluate the differential metabolome of fecal waters in rats subjected to the TLE protocol. To investigate the presence of metabolites in feces, 52 *Wistar* rats were divided into five experimental groups (Pós-SE, NS, SRS, NSRS, Naive) and induced into the SE protocol using the Lithium-Pilocarpine method. Fecal samples were collected, prepared and analyzed by Nuclear Magnetic Resonance spectroscopy. The results showed that the epilepsy-resistant groups (NS and NSRS) exhibited distinct metabolic profiles compared to the naïve animals and those that underwent recurrent seizures. An increase in glutamate and glutamine levels was observed in the SRS group, a reduction in butyrate levels in the 24h group, and increases in valine, isoleucine and leucine levels in the NS and NSRS groups. These metabolic signatures highlight the complexity of TLE and open new perspectives for the diagnosis and treatment of the condition, although further research is needed for effective clinical application.

Keywords: epilepsy, metabolome, feces, epileptogenesis, nuclear magnetic resonance spectroscopy.

1 Introduction

Temporal lobe epilepsy (TLE) is a neurological disorder that impacts an estimated 50 million people globally. This condition is characterized by spontaneous, recurrent limbic seizures (SRS), which occur due to hypersynchronous neuronal activity. The origin of epilepsy is related to progressive structural and physiological changes that begin when susceptible individuals are exposed to specific environmental events, such as status epilepticus (SE). This process, known as epileptogenesis, is responsible for transforming a normal brain into an epileptic brain, making it susceptible to the triggering of epileptic seizures (1).

Epilepsy presents a complex classification and various clinical manifestations, making diagnosis particularly challenging. Currently, the diagnosis of epilepsy primarily relies on symptoms and electroencephalography (EEG) (2). However, in the absence of ictal EEG and video monitoring, it becomes challenging to differentiate epilepsy from other paroxysmal disorders (3-5).

The clinical management of epilepsy primarily relies on the use of antiepileptic drugs (AEDs), such as valproic acid, benzodiazepines, carbamazepine, phenytoin, and phenobarbital, commonly referred to as first-line AEDs (6). However, the literature indicates that although initial treatment with AEDs is effective in reducing the short-term risk of seizure recurrence, approximately 30% of patients still experience uncontrolled

seizures, often accompanied by side effects (7), a condition known as drug-resistant epilepsy (DRE) (8).

The metabolome represents the profile of metabolites present in cells, tissues and biofluids, and is routinely used as a tool for biomarker discovery. Metabolites are substrates and products of metabolism responsible for essential cellular functions, such as energy production and storage, signal transduction, and apoptosis (9). Metabolites can influence local and systemic cellular responses, providing insights into the mechanisms underlying disease processes and progression (10).

In the context of temporal lobe epilepsy (TLE), studies have demonstrated a significant correlation between seizure severity and fluctuations in the levels of metabolites such as acetate, alanine, creatine, glutamine, glycerophosphocholine, taurine, and valine in the hippocampi of drug-resistant patients (11). Although the metabolic profile associated with epileptogenesis is still poorly understood, its investigation in animal models has the potential to provide rapid and accessible diagnostic tools, in addition to enabling the identification of new therapeutic targets for anticonvulsants (AEDs) (12).

The choice of fecal samples in this study reflects their relevance as an analytical matrix, given their ability to represent systemic metabolic processes and complex interactions of the gut-brain axis. This axis plays a central role in the regulation of neurological and metabolic functions, and is increasingly associated with conditions such as epilepsy. Alterations in the gut microbiota and fecal metabolites have been implicated in the development and progression of neurological disorders, highlighting the importance of exploring these relationships (13,14).

Furthermore, stool analysis is a non-invasive and practical approach, particularly in animal models, allowing longitudinal monitoring of metabolic alterations without compromising the well-being of the individuals studied. This highlights its value as a tool in translational studies and in the search for metabolic markers.

By investigating metabolic alterations in fecal samples, this work seeks to deepen the understanding of the pathological mechanisms underlying TLE, with a view to facilitating faster and more accurate diagnoses and, simultaneously, identifying new targets for the development of innovative treatments. This approach has the potential to significantly impact the clinical management of resistant epilepsy, contributing to a substantial improvement in the quality of life of patients.

2 Materials and Methods

2.1 Experimental Animals

For this study, 52 male *Wistar* rats (*Rattus norvegicus*), approximately 60 days old and weighing between 250-300g, were obtained from the Multidisciplinary Center for Biological Research in Laboratory Animal Science (CEMIB - UNICAMP). The animals were housed in the Animal Facility of the Cellular and Molecular Biology Laboratory, with a temperature of 22°C, on a 12-hour light/dark cycle (06:00/18:00), and were provided with free access to food and water. The study was approved by the Animal Ethics Committee of UFAL under protocol number 06/2021.

2.2 Status Epilepticus Induction

The induction of Status Epilepticus (SE) followed the Lithium-Pilocarpine chemical model. Initially, the animals were given an intraperitoneal (i.p.) injection of 127 mg/kg of lithium chloride (LiCl) to enhance the effect of pilocarpine. 15 hours and 30 minutes after LiCl administration, 1 mg/kg of scopolamine butylbromide was administered i.p. to limit the peripheral effects of pilocarpine (PILO). Thirty minutes after the scopolamine

injection, 30 mg/kg of pilocarpine was administered i.p. Immediately after pilocarpine administration, the animals' behavior was closely monitored (video monitoring was also performed using a digital video camera DCR-SR68, SonyBrasil LTDA), with animals showing 5 minutes of continuous level 2 or higher seizures on the Racine scale (15) considered to have entered SE. After 90 minutes, SE was terminated by administering 5 mg/kg of Diazepam i.p., with additional doses given every 30 minutes until the seizures were fully controlled.

2.3 Analysis of Spontaneous and Recurrent Seizures

On the first day after SE, the animals were individually housed in acrylic cages and monitored 24 hours a day by a digital video camera (Intelbras, iMX-397H) for a period of 86 days, to detect the latency of the first limbic seizures (SRS), as well as their severity, frequency, and duration. The characterization of the chronic phase was based on the occurrence of at least two seizures, according to the limbic seizure classification established by Racine (15). Animals that exhibited at least three seizures of level 3 or higher were considered epileptic (SRS group), while those that did not meet this criterion were assigned to the NSRS group.

2.4 Experimental Groups

Five experimental groups were defined (Figure1), as follows:
Naïve group (n=10): animals that were not subjected to any procedure and were kept in the Sectorial Animal Facility of the LBCM until euthanasia.
Pós-SE group (n=10): animals subjected to SE and euthanized 24 hours after induction.
NS group (n=11): animals that were subjected to induction at least 3 times but did not enter SE, considered resistant to the method.
NSRS group (n=10): animals subjected to SE that did not present spontaneous recurrent seizures (SRS) in the chronic phase.
SRS group (n=11): animals subjected to SE that exhibited spontaneous recurrent seizures (SRS) in the chronic phase.

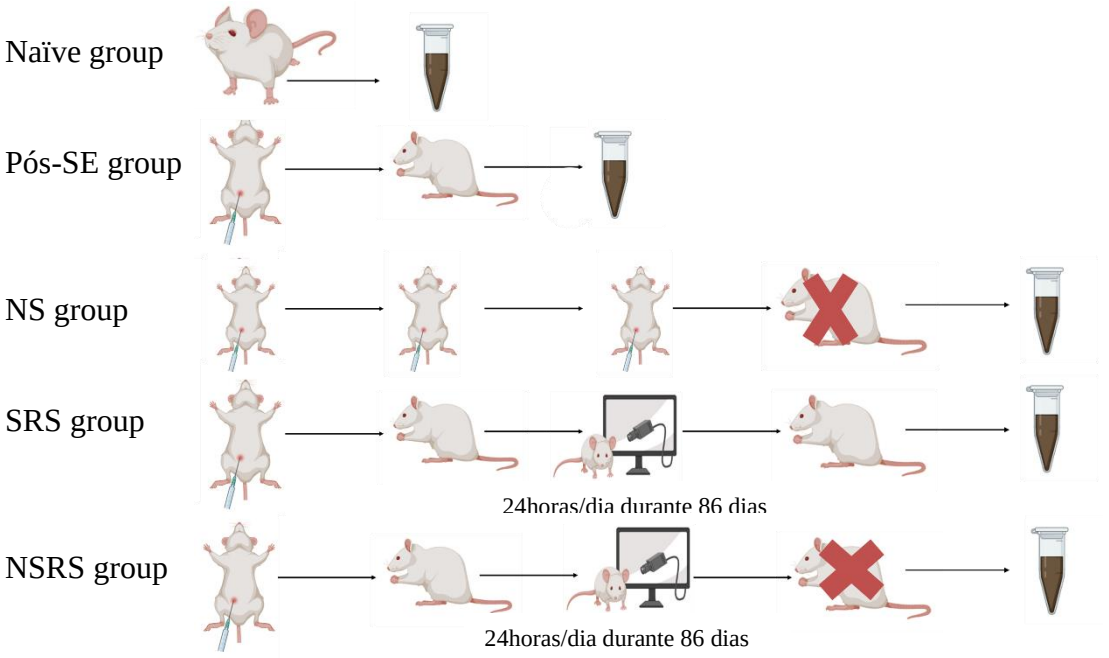


FIGURE 1: Experimental design for establishing experimental groups.

2.5 Fecal Sample Collection

One hour before euthanasia, the animals were placed in sanitized boxes (chloro diluted 50% 50%, 70% ethanol) for fecal sample collection in cryogenic tubes (2 mL), which were immediately frozen in liquid nitrogen for later storage at -80°C. For animals that did not defecate (due to SE-related weakness), feces were collected *post-mortem* from the intestines of the rodents.

2.6 Fecal Waters Extraction

Fecal waters were extracted by adding 1200 µL of phosphate buffer (pH = 7.0 ~ 7.4) to 50 mg of fecal material in an Eppendorf tube, followed by vortex mixing (4x20s) until homogenization. A spatula was used to mash and assist in the homogenization process when needed. Finally, the suspension was centrifuged for 15 minutes at 14,000 rpm and 4°C, and 700 µL of the supernatant was transferred to a new Eppendorf tube, then stored in a freezer at -80°C until metabolomic analysis.

2.7 Nuclear Magnetic Resonance (NMR) Analysis

Fecal metabolites were identified using Nuclear Magnetic Resonance (NMR) spectroscopy. After thawing the fecal water samples at room temperature, 350 µL were pipetted into an NMR tube (5 mm) with 350 µL of phosphate buffer (containing sodium trimethylsilyl propionate-d4 (TSP 0.1 mM and 100% D2O). NMR spectra were acquired using a Bruker 600 MHz Avance III spectrometer (Bruker BioSpin, Germany), and were recorded using the *noesypr1d* pulse sequence. The metabolite signals were identified in the one-dimensional spectra with the aid of The Human Metabolome Database Version 4.0 and the ChenomX NMR Suite (Chenomx Inc., Edmonton, Canada).

For spectral processing, the statistical software R, version 3.6.0 (http://cran.rproject.org/) with the R package mrbin (version 1.5.0) was used. The 1H NMR dataset was divided into consecutive bins of 0.03 ppm fixed width over the region from 0.5 to 9.5 ppm. The spectral region between 4.7 and 5.1 ppm (containing the residual water signal) was excluded. The spectral dataset was analyzed using the online platform MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/) (16). ALL container intensities were normalized by median to minimize the effect of different concentrations. Unsupervised principal component analysis (PCA) was used as an initial exploratory analysis to gain a preliminary overview of the data and facilitate an overall view of the groups in principal components (PC1 vs. PC2) in score plots, revealing trends and potential outliers. In addition, the methodology employed involved the use of PLS-DA and OPLS-DA, supervised statistical methods recognized for highlighting intergroup disparities while simultaneously reducing intragroup variations. The confidence interval was set at 95%, and 2000 permutations were defined for model evaluation. Additionally, permutation plots and VIP scores were used. Differences in metabolite concentrations between groups were assessed through Analysis of Variance (ANOVA).

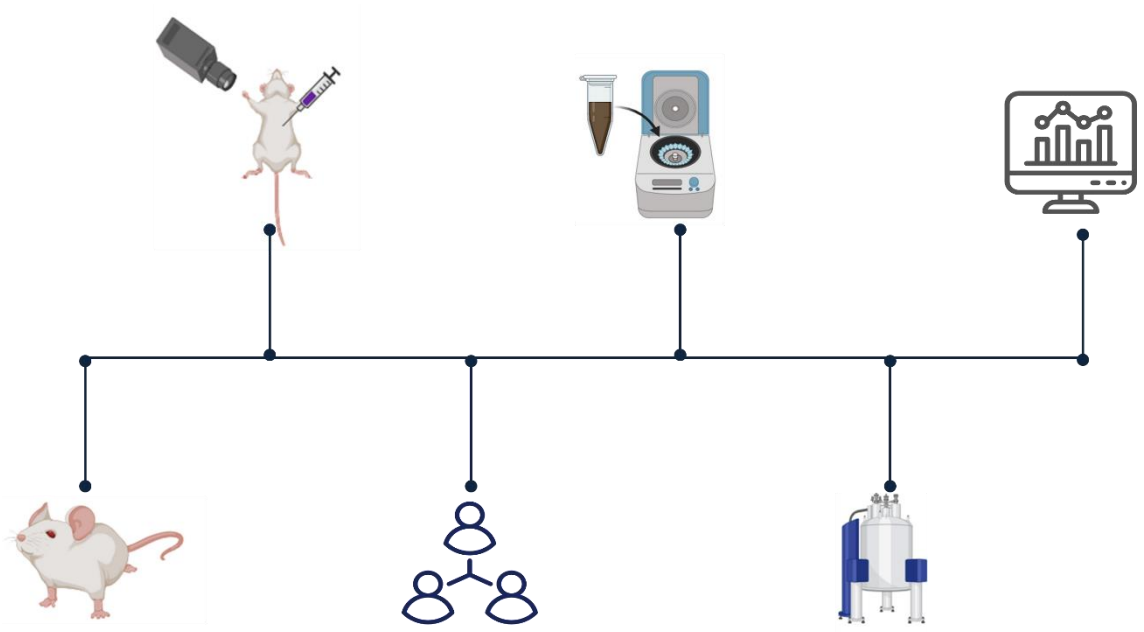


FIGURE 2: Experimental design.

3 Resulted

3.1 Heat map com clustering

For better visualization of the metabolic patterns of the samples, a heat map with clustering was used (Figure 3), providing a clearer visualization of the patterns and similarities inherent to the data. A similar metabolic pattern was identified between the 24h, naïve and SRS groups, as well as between the NS and NSRS groups. Glucose and ethanol stand out as characteristic metabolites of the naïve, 24h and SRS groups, while leucine, valine, propionate, nicotinic acid, isoleucine and pyridoxine are predominant metabolites common to the NS and NSRS groups. On the other hand, hypoxanthine, fumarate and phenylalanine are distinctive markers of the Pós-SE group, while glutamine and glutamate are prominent in the SRS group, and lysine and butyrate are predominant in the naïve group.

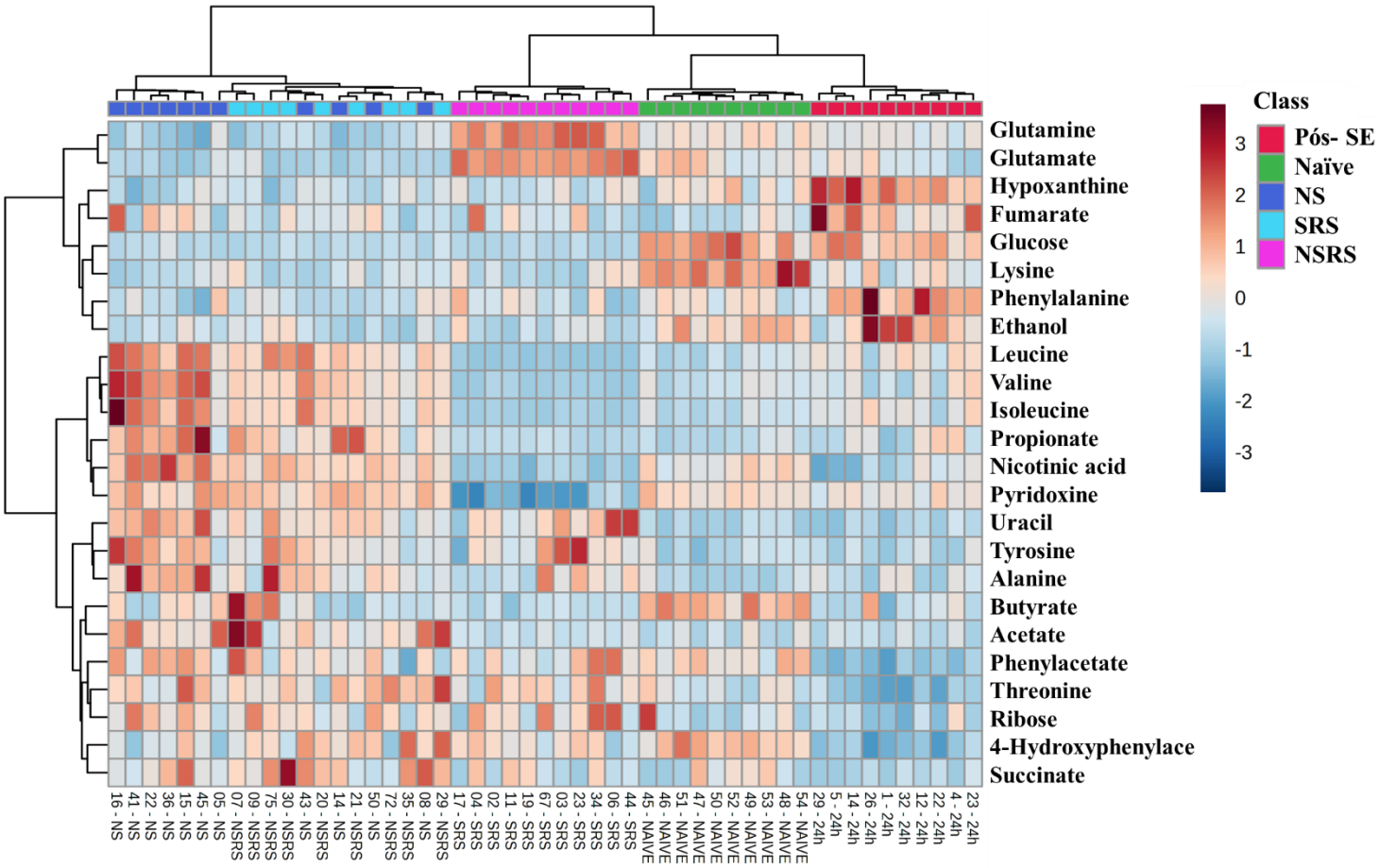


FIGURE 3 -Heat map with clustering of fecal water samples from the experimental groups.

3.2 NMR spectrum

The NMR spectrum, along with the respective metabolite signals, is presented in figure 4. A total of 24 metabolites were identified in the fecal water samples, consistently present across all experimental groups. These metabolites include: 4-hydroxyphenylacetic acid, phenylacetate, nicotinic acid, alanine, acetate, butyrate, ethanol, phenylalanine, fumarate, glucose, glutamate, glutamine, hypoxanthine, isoleucine, leucine, lysine, pyridoxine, propionate, ribose, succinate, tyrosine, threonine, uracil, and valine.

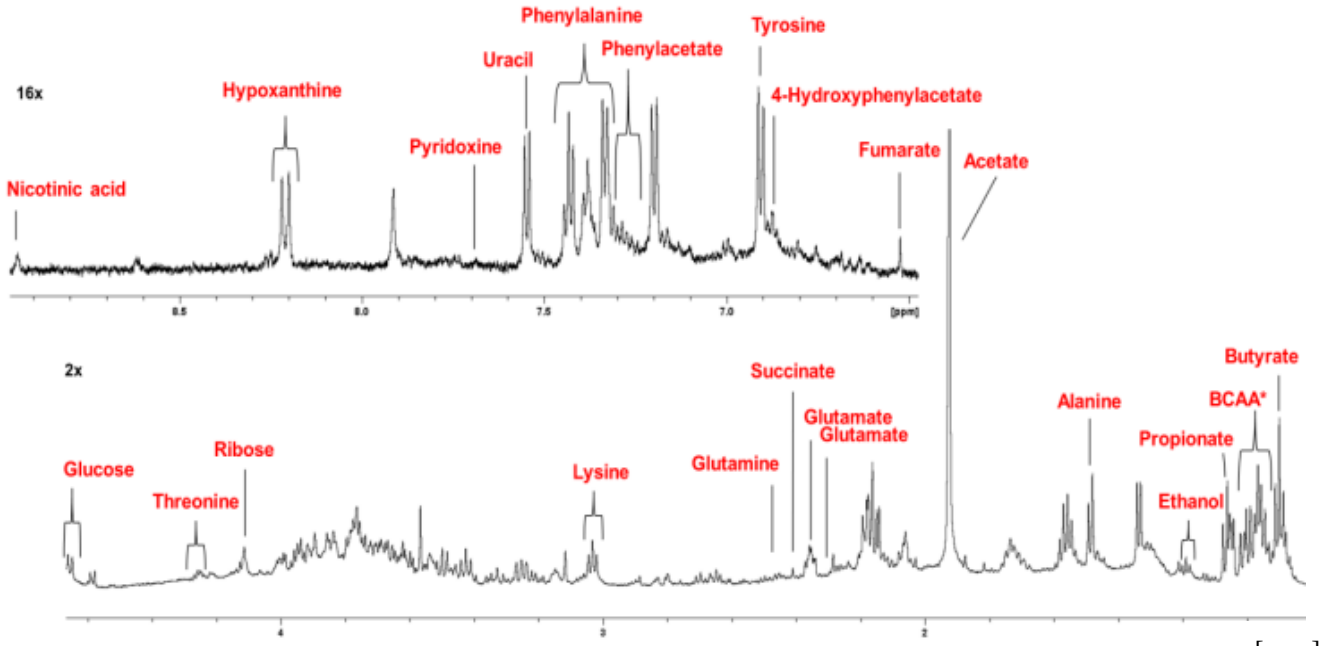


FIGURE 4 – 1H NMR spectrum of fecal water samples**.**

3.2 Metabolomic Analyses

Principal Component Analysis (PCA), as shown in the 2D plot (Figure 5A), revealed a clear trend of separation between the five groups, while samples within the same group exhibited similarities, with a total variance of 93.1%, with PC1 accounting for 84.2% and PC2 for 8.9%. The separation is more clearly observed in the 3D plot (Figure 5B). Partial Least Squares Discriminant Analysis (PLS-DA) further confirmed the separation of the groups, with a total variance of 65% (Figure 6).

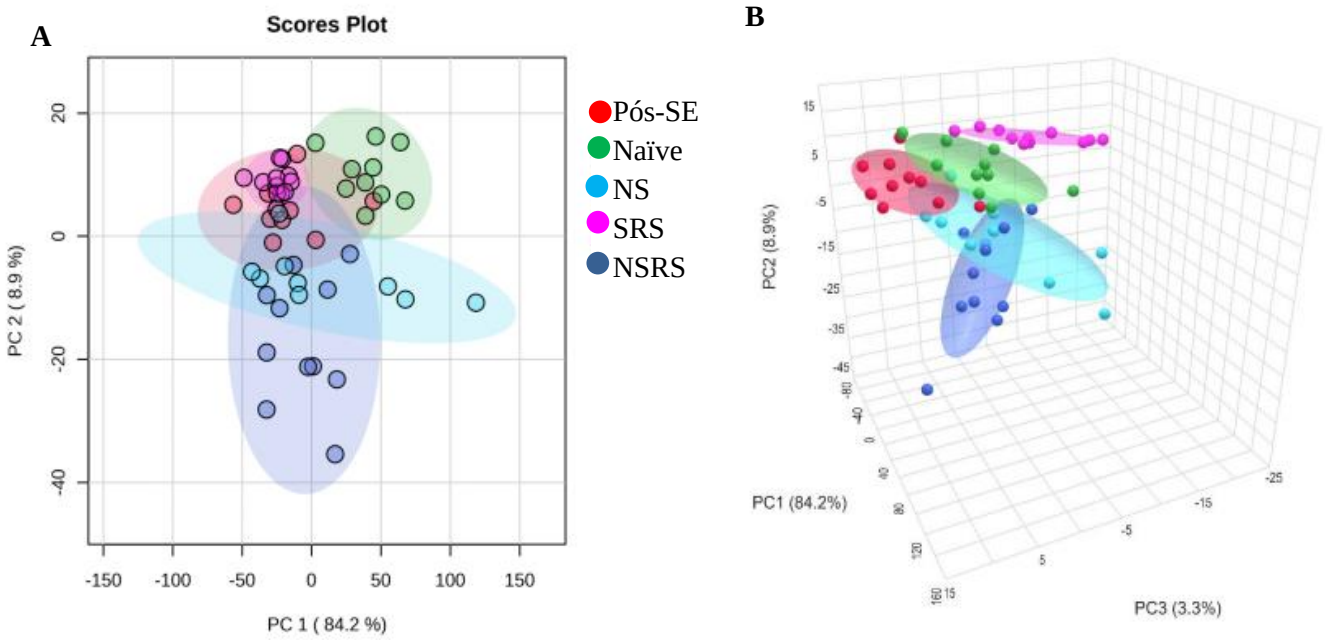


FIGURE 5 – Principal Component Analysis (PCA) of fecal water samples. **(A)** 2D PCA score plot. **(B)** 3D plot.

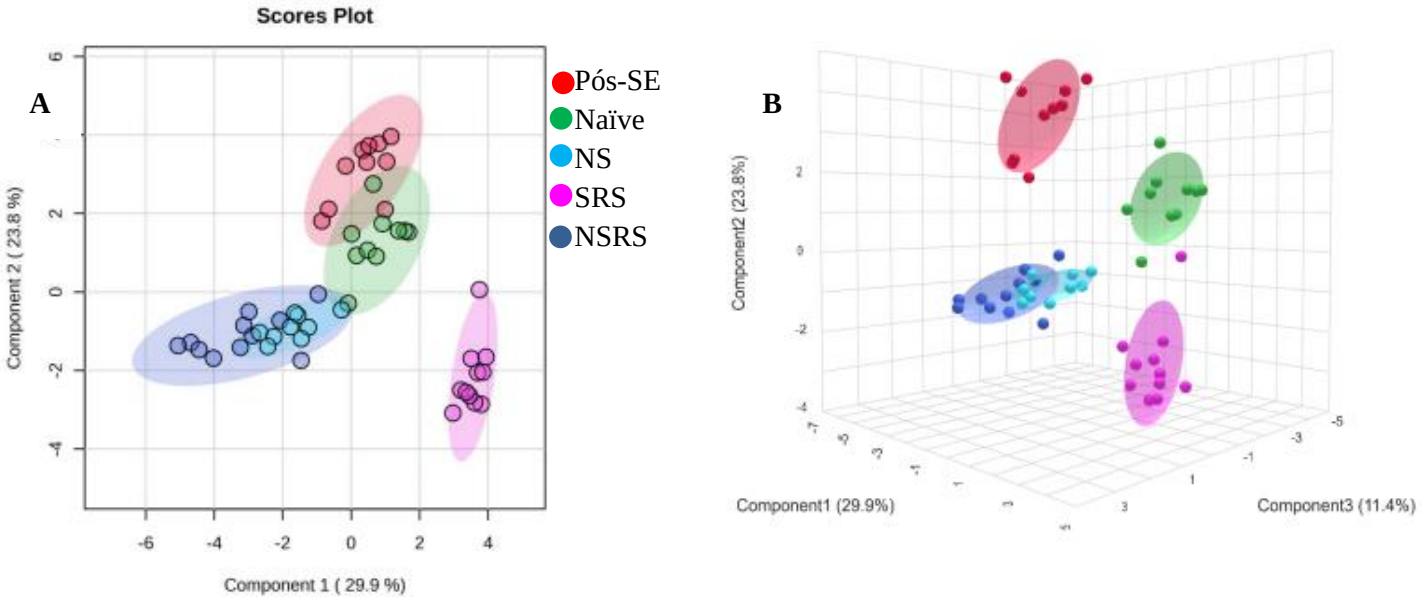
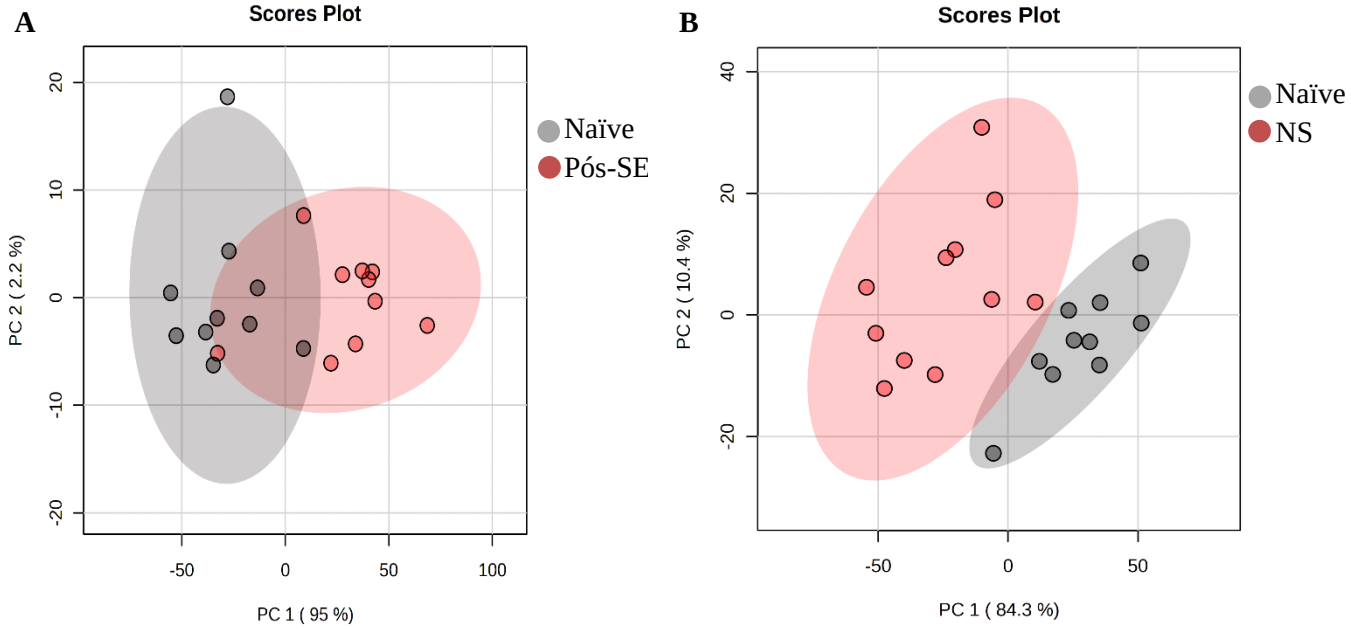


FIGURE 6 – Discriminant analysis with multivariate calibration by Partial Least Squares Discriminant Analysis (PLS-DA). **(A)** 2D PLS-DA score plot. **(B)** 3D plot.

PCA analysis (Figure 7), conducted to compare the naïve group with the respective experimental groups (Pós-SE, NS, SRS, and NSRS), showed the following variance: 97.2% (PC1: 95% and PC2: 2.2%) for naïve vs 24h; 94.7% (PC1: 84.3% and PC2: 10.4%) for naïve vs NS; 97.4% (PC1: 93.7% and PC2: 3.7%) for naïve vs SRS; and 96% (PC1: 90.7% and PC2: 5.7%) for naïve vs NSRS.



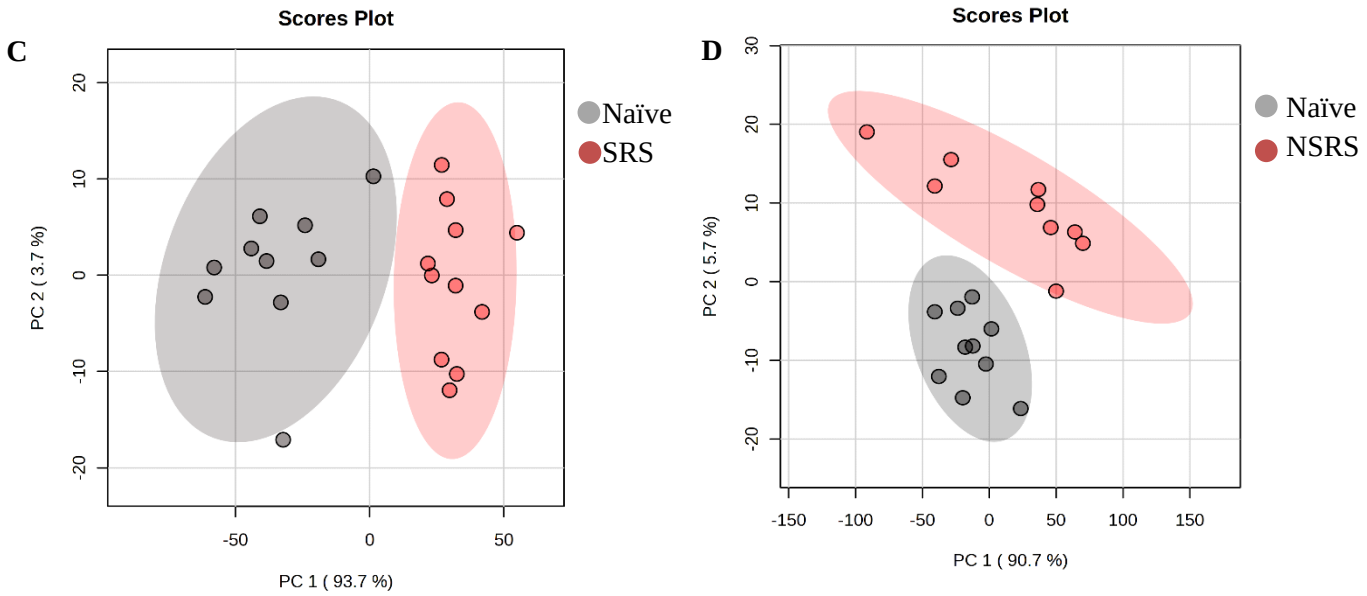
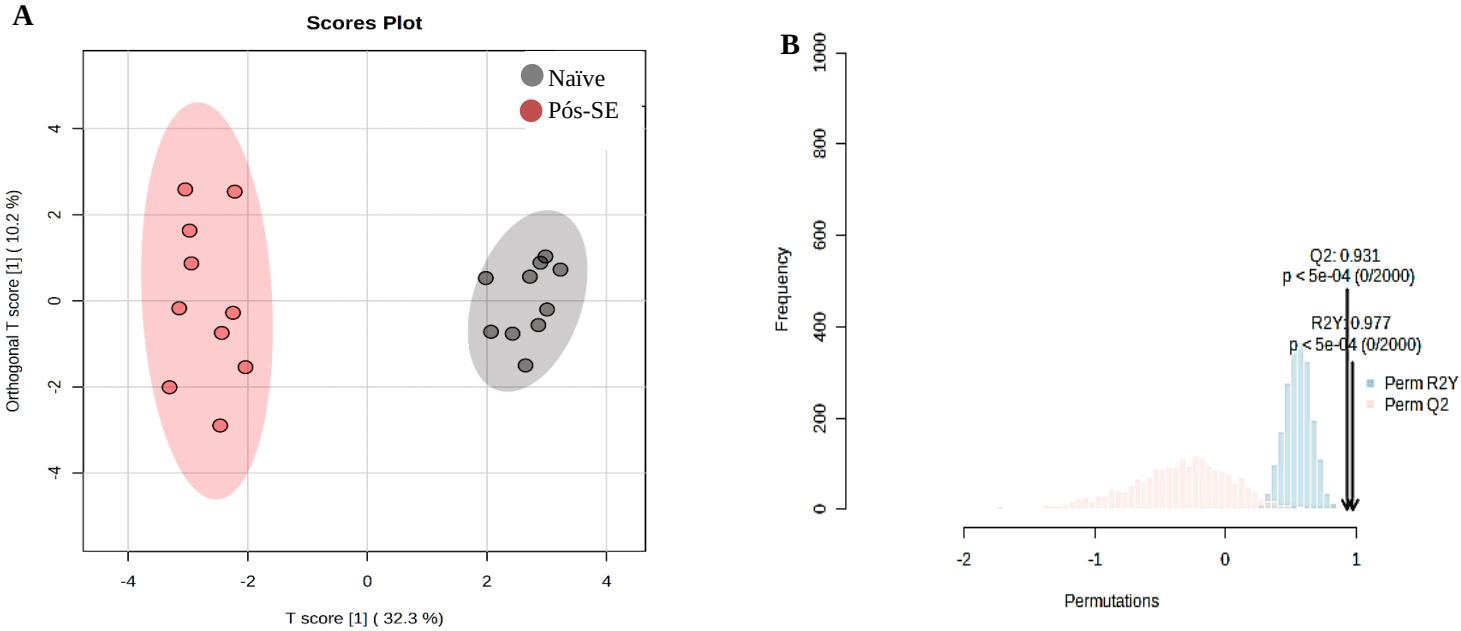


FIGURE 7 – Principal Component Analysis (PCA) showing **(A)** naïve vs 24h, **(B)** naïve vs NS, **(C)** naïve vs SRS and **(D)** naïve vs NSRS.

To confirm and enhance the separation of the samples, an Orthogonal Partial Least Squares Discriminant Analysis (OPLS) was performed, where the samples were compared to each other (Figures 8A, 9A, 10A, and 11A). To validate the performance of the OPLS model, a permutation plot was generated (Figures 8B, 9B, 10B, and 11B). This plot shows the coefficient of determination (R^2) and the predictive ability of the model (Q^2). For satisfactory performance, these values should be greater than 0.5. In this study, the following values were obtained: naïve vs Pós-SE ($R^2 = 0.977$ and $Q^2 = 0.931$); naïve vs NS ($R^2 = 0.972$ and $Q^2 = 0.923$); naïve vs SRS ($R^2 = 0.979$ and $Q^2 = 0.945$) and naïve vs NSRS ($R^2 = 0.98$ and $Q^2 = 0.913$).

Figures 8C, 9C, 10C, and 11C show the Variable Importance in Projection (VIP) score plots, along with the corresponding heatmap, where red and blue indicate the metabolite levels presented. This plot highlights which metabolites contribute most significantly to the differences between the groups.



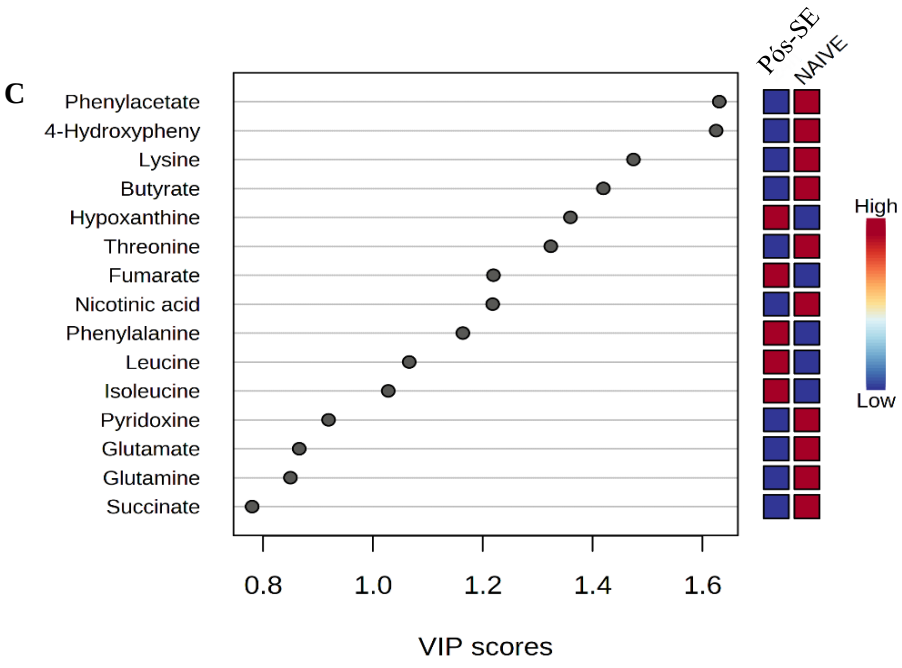
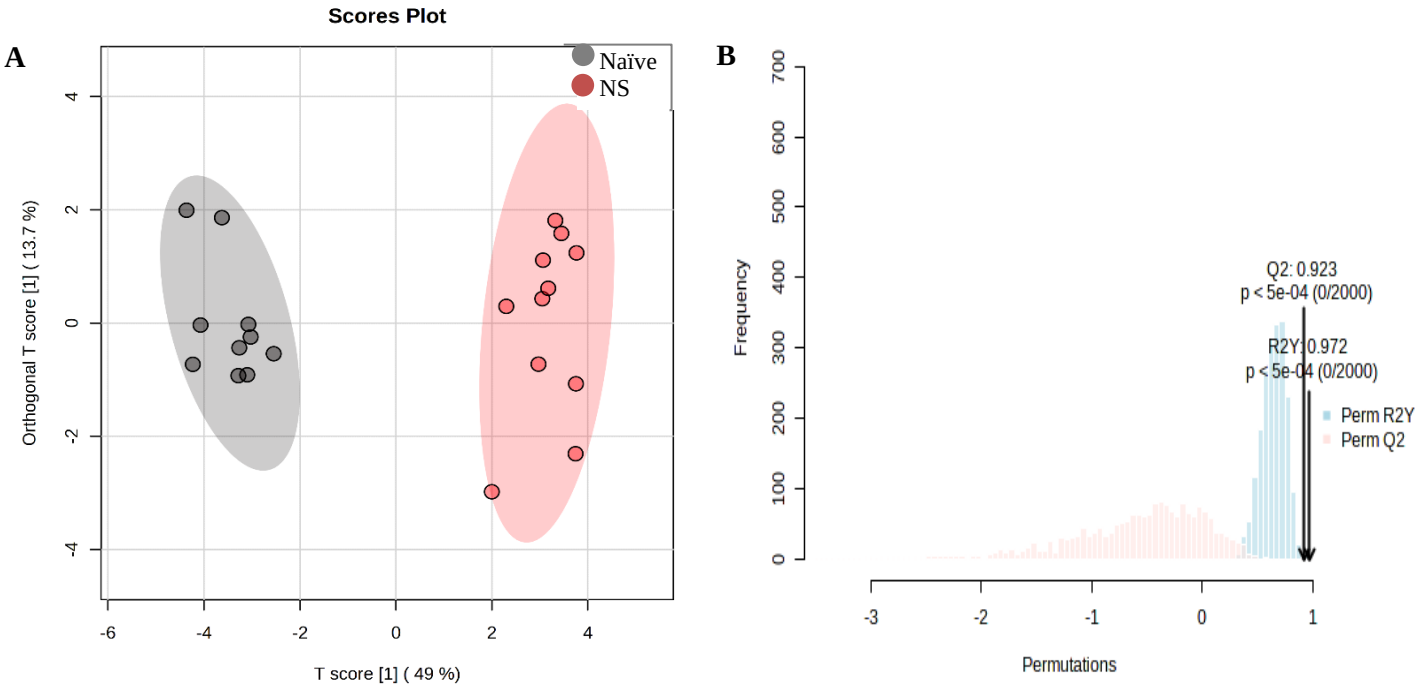


FIGURE 8 – (A) Orthogonal Partial Least Squares Discriminant Analysis (OPLS) of the metabolic profile of the animals. (B) Permutation plot and (C) VIP score plot, with the corresponding heatmap where red and blue indicate metabolite levels for naïve vs Pós-SE group.



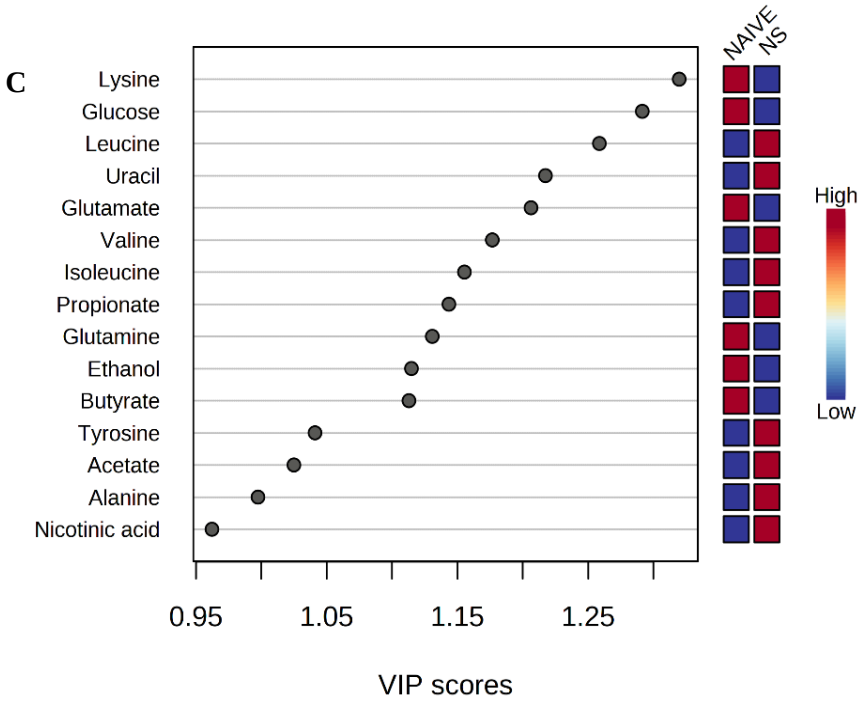
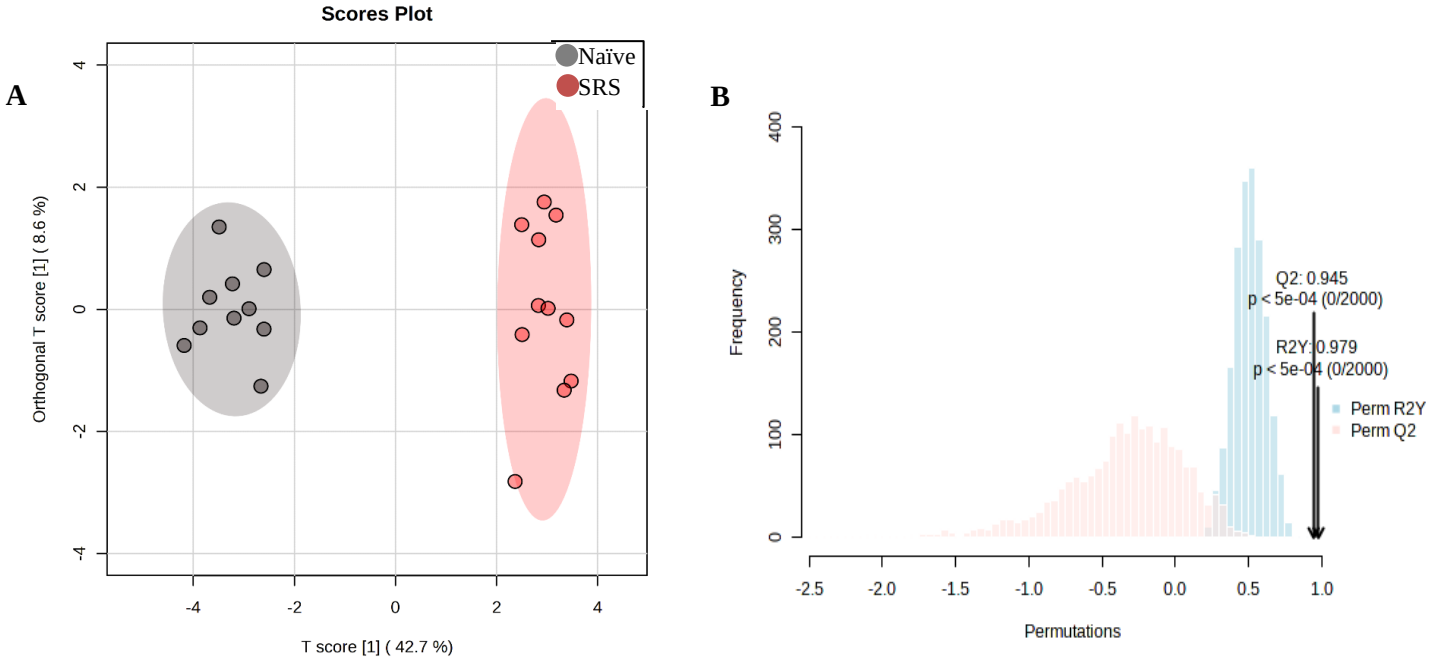


FIGURE 9 – **(A)** Orthogonal Partial Least Squares Discriminant Analysis (OPLS) of the metabolic profile of the animals. **(B)** Permutation plot and **(C)** VIP score plot, with the corresponding heatmap where red and blue indicate metabolite levels for naïve vs NS group.



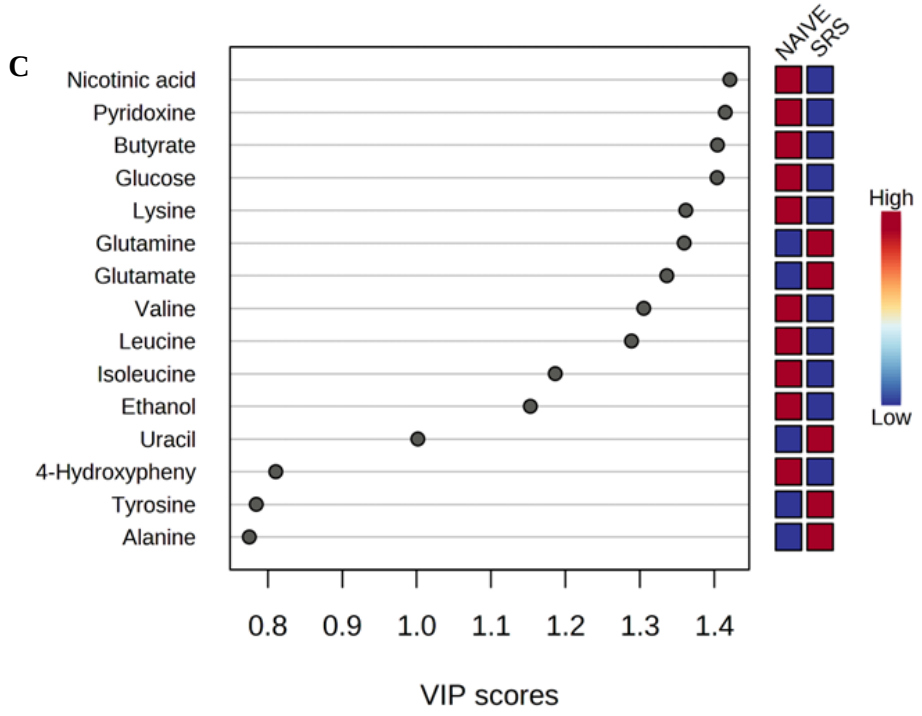
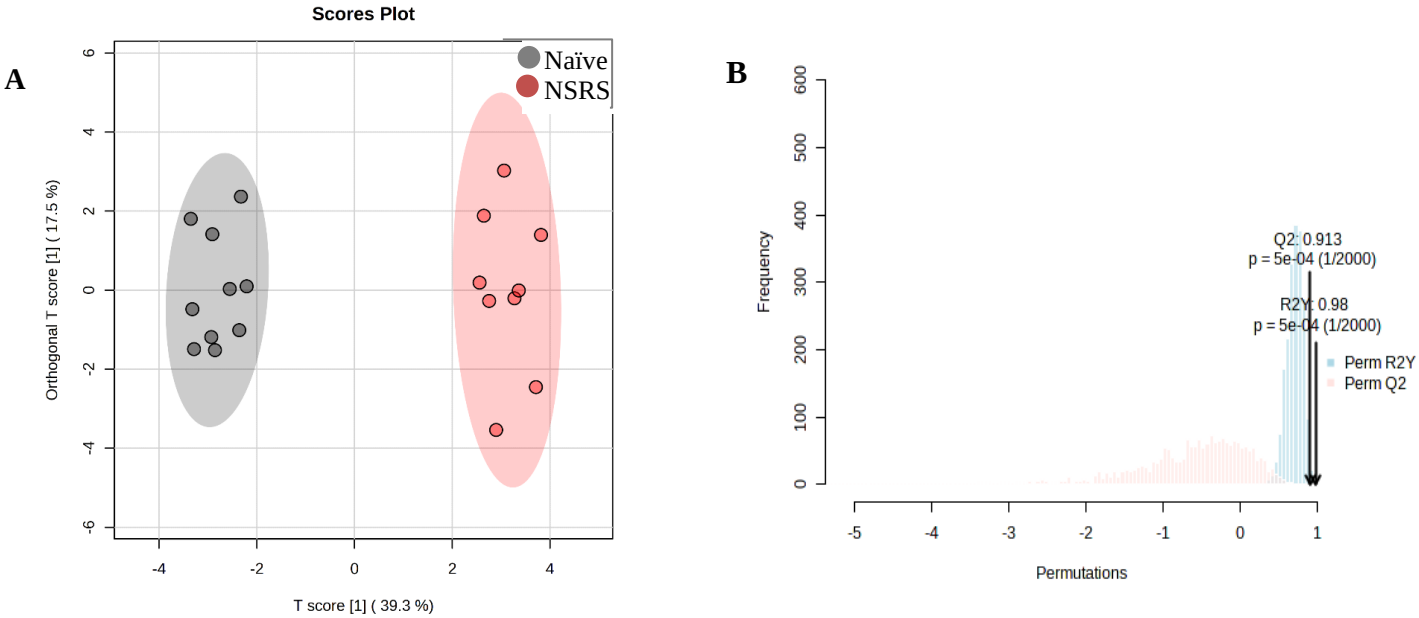


FIGURE 10 – **(A)** Orthogonal Partial Least Squares Discriminant Analysis (OPLS) of the metabolic profile of the animals**. (B)** Permutation plot and **(C)** VIP score plot, with the corresponding heatmap where red and blue indicate metabolite levels for naïve vs SRS group.



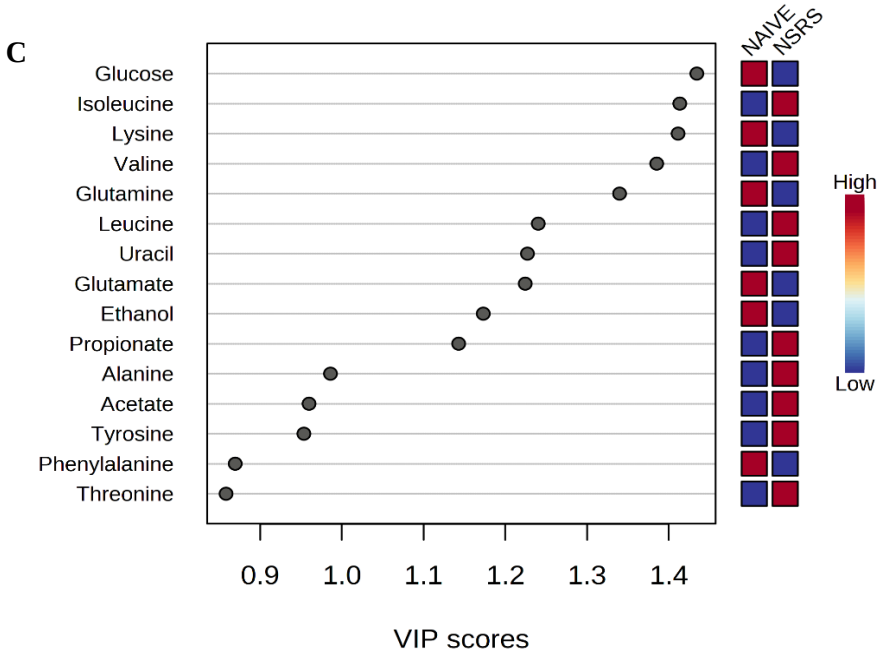
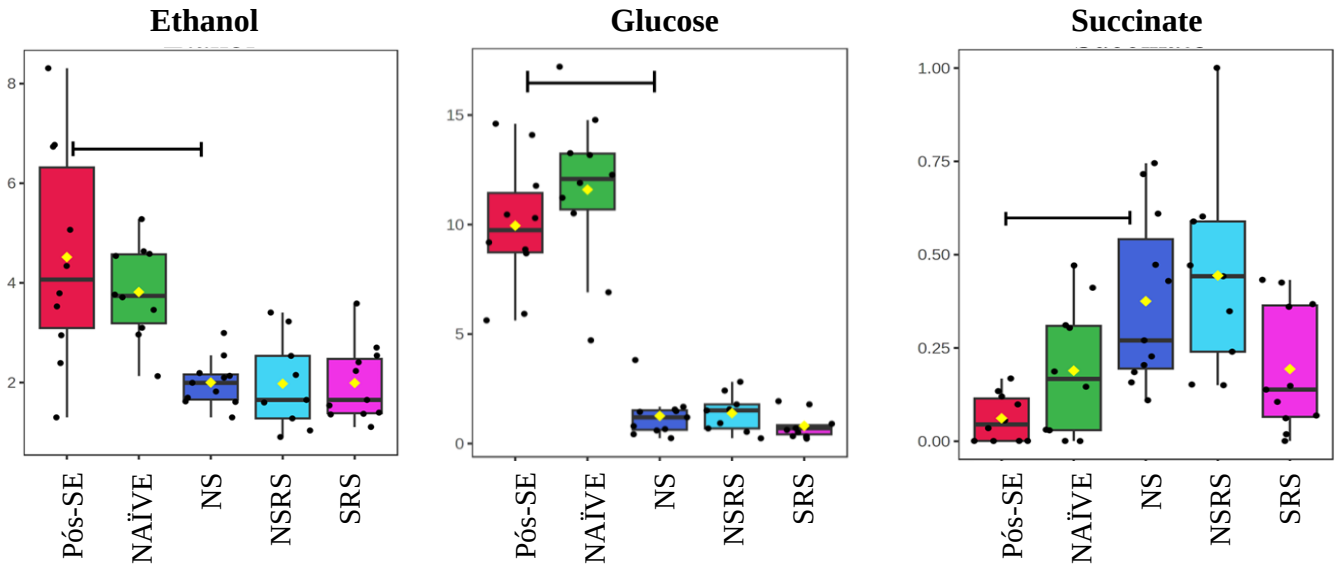
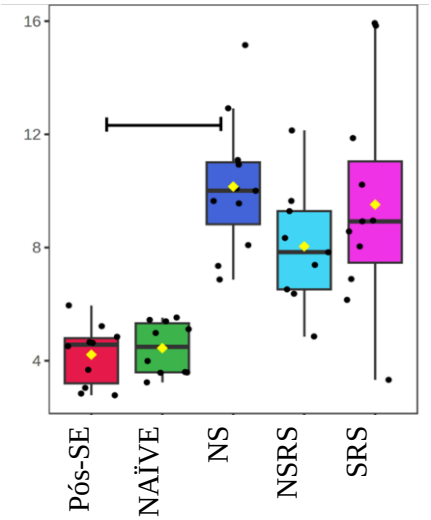


FIGURE 11 – **(A)** Orthogonal Partial Least Squares Discriminant Analysis (OPLS) of the metabolic profile of the animals. **(B)** Permutation plot and **(C)** VIP score plot, with the corresponding heatmap where red and blue indicate metabolite levels for naïve vs NSRS group

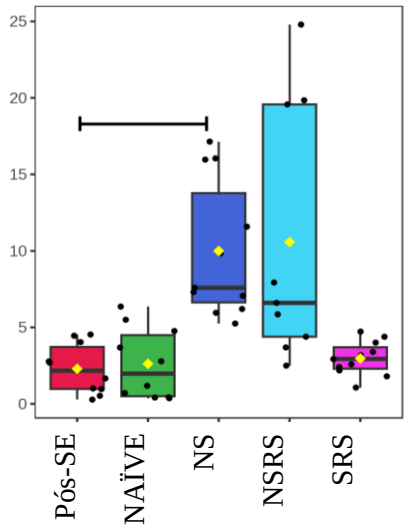
The analysis of variance (ANOVA), presented in Figure 12, allowed for the correlation of metabolite increases and decreases between the groups, with statistical significance at $p < 0.05$.



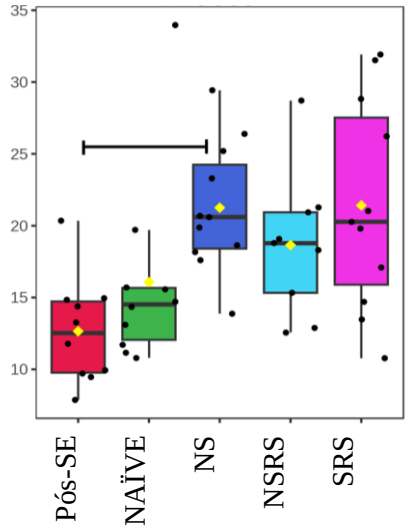
Uracil



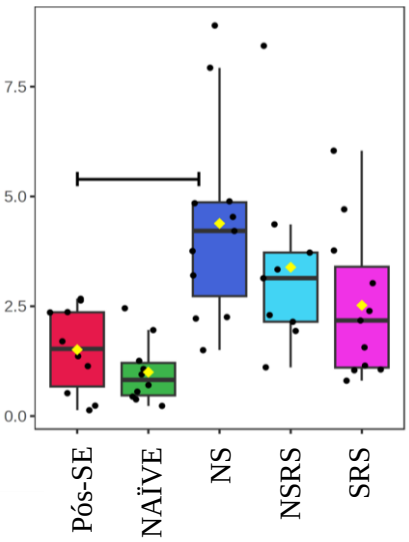
Acetate



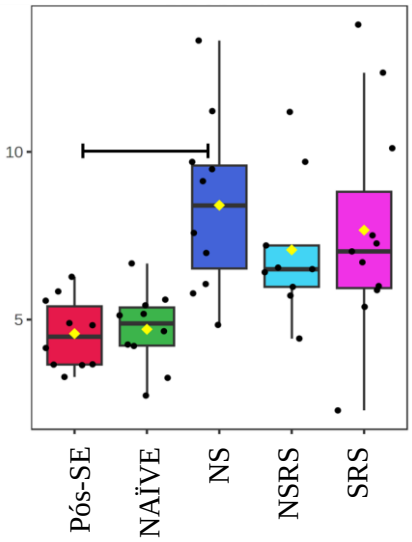
Ribose



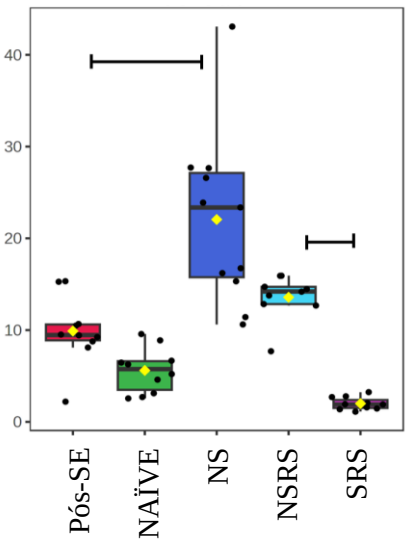
Alanine



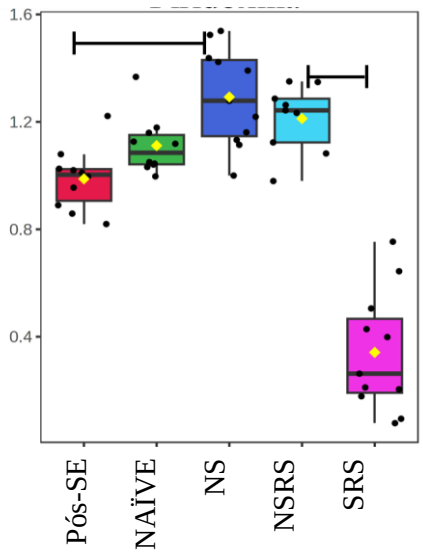
Tyrosine



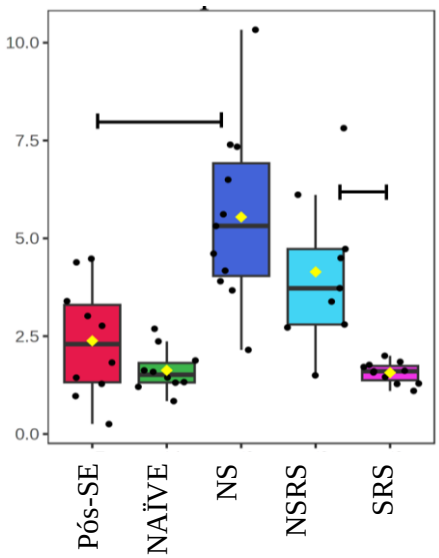
Isoleucine



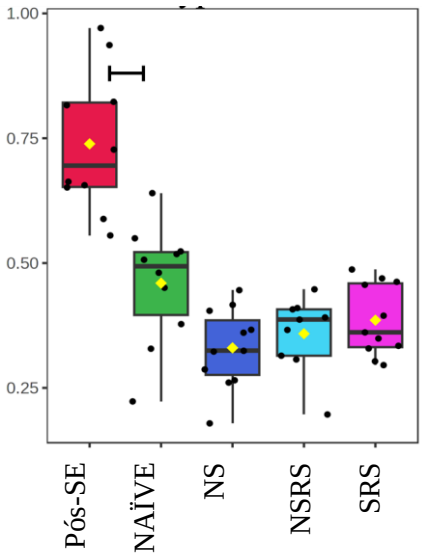
Pyridoxine



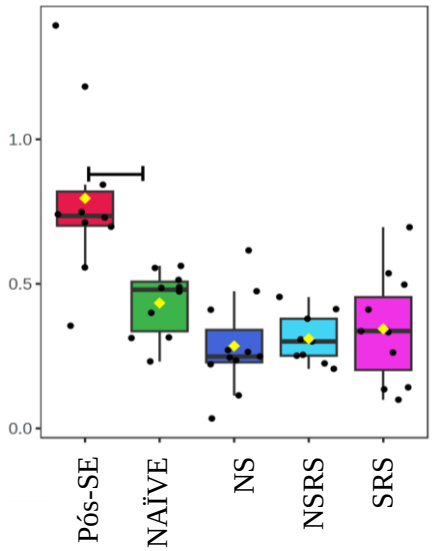
Propionate



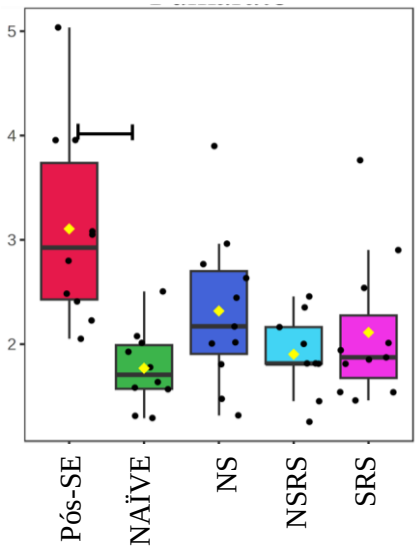
Hypoxanthine



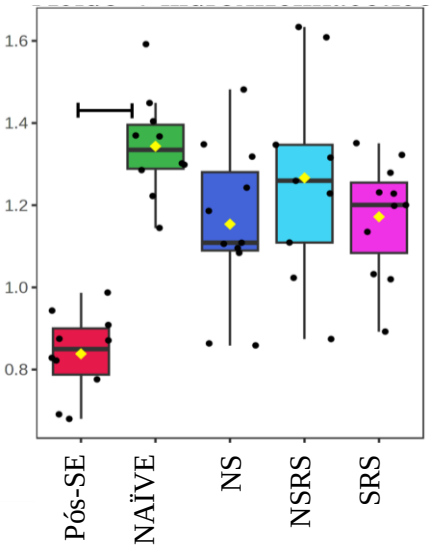
Phenylalanine



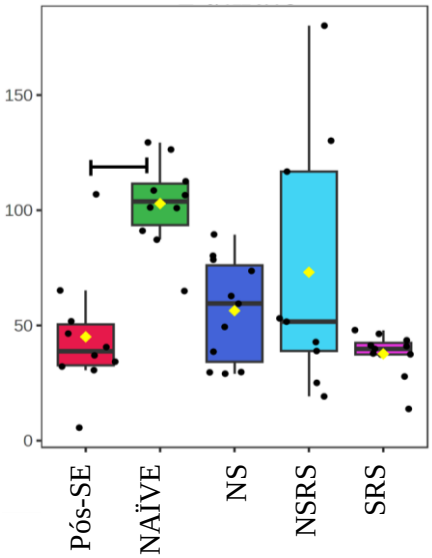
Fumarate



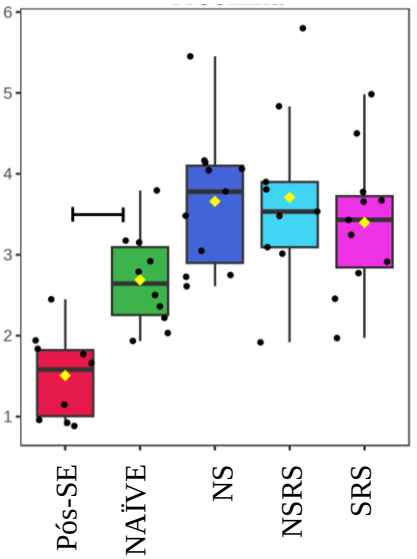
4-hydroxyphenylacetic acid



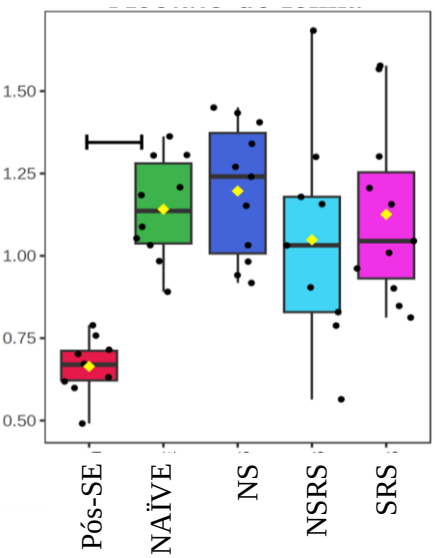
Butyrate



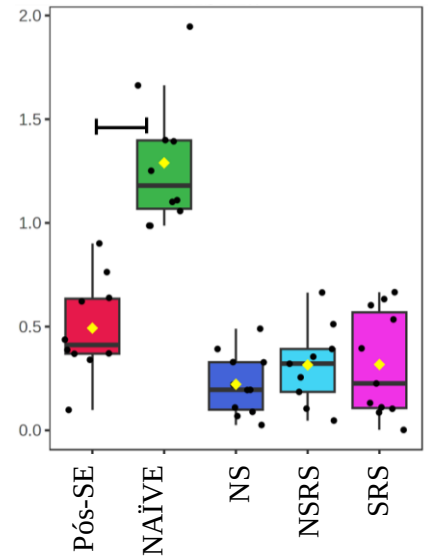
Threonine



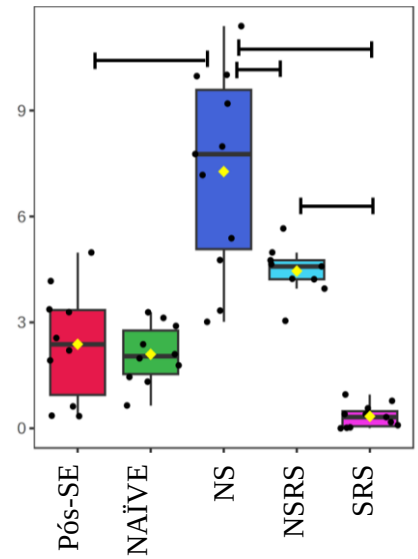
Phenyl acetate



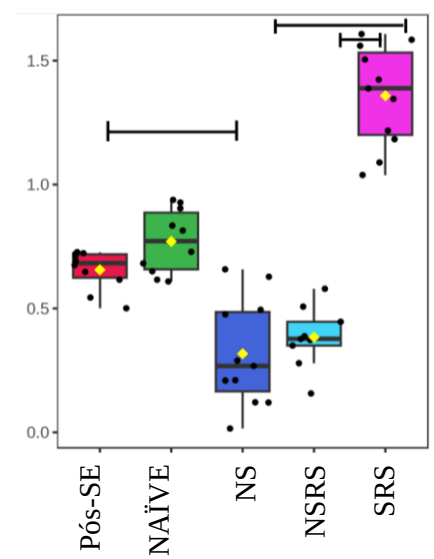
Lysine



Valine



Glutamine



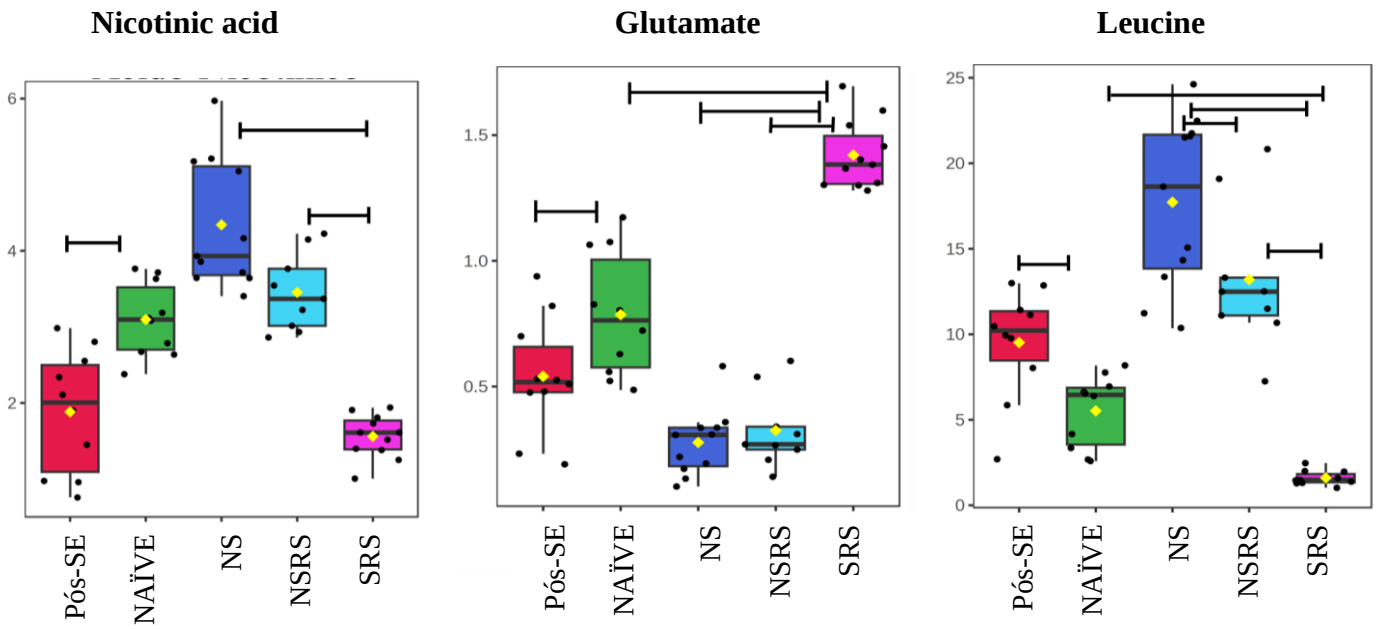


FIGURE 12 - Concentration of metabolites identified in the experimental groups from fecal water samples. Statistical analysis was performed using ANOVA, followed by Fisher's multiple comparison test ($p < 0.05$)

4 Discussion

Our study confirmed the distinction between the Pós-SE, naïve and SRS experimental groups, highlighting that these groups are differentiated from each other. On the other hand, the NS and NSRS groups exhibited very similar metabolite patterns. The analysis reveals that, following induction, there are two distinct profiles of animals: resistance and susceptibility to seizures.

The PCA and PLS-DA analyses confirm that the mechanisms of resistance involve an adaptive response distinct from that observed in both naïve animals and those that experienced seizures, suggesting the presence of specific biological processes that warrant further investigation. The OPLS analysis further emphasized the differences between the samples, enhancing their distinction.

Phenylacetate, 4-hydroxyphenyl, lysine and butyrate are the metabolites with the greatest contribution in the comparison of naïve vs Pós-SE; lysine, glucose, leucine and uracil in the comparison of naïve vs NS; nicotinic acid, pyridoxine, butyrate, glucose and lysine in naïve vs SRS; and, finally, glucose, lysine, glutamate, isoleucine and valine in the comparison of naïve vs NSRS. In this study, we observed a metabolic profile of branched-chain amino acids (BCAAs), such as isoleucine, leucine and valine, in the NS and NSRS groups. BCAAs play important roles in physiology and are widely used by athletes to promote muscle growth and recovery (17). They account for 35% of the essential amino acids in muscles (18) and stimulate growth by activating the mTOR signaling pathway (19). Furthermore, BCAAs, obtained through the diet and present in the bloodstream, can influence the regulation of glutamate levels in the brain, which is essential for efficient neurotransmission and the prevention of excitotoxicity (20). Studies have demonstrated that the administration of BCAAs increased the seizure susceptibility threshold, both when administered individually or in mixtures, in a picrotoxin model (21,22). Additionally, BCAA administration, either alone or in combination with a ketogenic diet, has been shown to reduce or even stop the frequency of seizures, as well

as increase the seizure threshold in children with refractory epilepsy (23). Therefore, the increased levels of BCAAs in the NS and NSRS groups may indicate a potential therapeutic effect, reflecting an increase in the threshold of seizure susceptibility.

Alanine, valine and leucine are reduced in the Pós-SE group compared to the resistant group. Reduced levels of glycine, valine, isoleucine, leucine, lysine, alanine and serine have been observed in patients with ischemic stroke (24).

A study demonstrated that the combined application of ribose and adenine promoted a higher accumulation of extracellular adenosine during electrographic seizures in hippocampal slices, resulting in a reduction in the intensity and frequency of epileptiform activity (25). These observations suggest that ribose and adenine may be valuable in severely injured brains, which supports our findings showing a higher concentration of ribose in the NS group compared to the Pós-SE group.

We observed elevated levels of phenylalanine in the Pós-SE group. Phenylalanine, essential for the synthesis of neurotransmitters important for cognitive and emotional functions, exhibits metabolic defects in conditions such as phenylketonuria, which can lead to neurological damage (26). In phenylketonuria, reduced activity of phenylalanine hydroxylase or deficiency of the cofactor tetrahydrobiopterin results in the accumulation of phenylalanine in the blood and an increase in its levels in the brain. This reduces the levels of large neutral amino acids (LNAAs), such as methionine and tyrosine, in the CNS (27,28), causing severe toxic effects that may lead to irreversible intellectual disability. The early symptoms include microcephaly, intellectual disability and epilepsy (29).

Hypoxanthine levels were also elevated in the Pós-SE group. It is important to highlight the role of purinergic signaling in epilepsy. Hypoxanthine, derived from adenosine metabolism, is associated with the development of epilepsy, with adenosine acting as an anticonvulsant modulator (30,31). ATP and adenosine increase in response to cellular damage or seizures, which also raises the levels of their metabolites, such as hypoxanthine, following neurological injuries (32-35). A study observed elevations in purine concentrations in the blood following experimental seizures and during epilepsy in humans. Blood purine levels correlated with the severity of seizures and brain damage in mice (36). These biomarkers are being studied to assist in the diagnosis and monitoring of seizures.

In our study, an increase in the levels of nicotinic acid (niacin or vitamin B3) and pyridoxine (vitamin B6) was observed in the resistant groups (NS and NSRS). B vitamins are essential in macronutrient metabolism, gene regulation, neurotransmitter synthesis and as antioxidants (37). Niacin, traditionally used in the treatment of pellagra and dyslipidemias, plays crucial roles in metabolism by activating the Hcar2 receptor, which regulates immune responses, phagocytosis of myelin debris and beta-amyloid, as well as promoting intracellular cholesterol efflux. Recent studies suggest that niacin may have therapeutic potential in neurological diseases, such as Alzheimer's, Parkinson's and multiple sclerosis, due to its ability to protect against oxidative damage and inflammation in the central nervous system (38). In clinical practice, particularly in the Far East, pyridoxine and pyridoxal phosphate have been used as anticonvulsant medications in infantile spasms and childhood generalized and focal epilepsy (39). Therefore, the increased levels of nicotinic acid and pyridoxine in the NS and NSRS groups may reflect neuroprotective mechanisms and anticonvulsant effects.

Interestingly, the NS group showed a higher concentration of succinate compared to the Pós-SE group. Research indicates that succinate accumulation stimulates the production of mitochondrial reactive oxygen species, being elevated in SE in animals subjected to the kainic acid induction protocol (40).

A study analyzing the serum metabolome of patients with epilepsy identified increased levels of alanine, butyric acid and glutamate, along with decreased levels of glucose and lactic acid (41). The same results were observed in the feces of rats subjected

to the experimental protocol of TLE, in comparison to their controls (42). In our study, we found that glucose concentration in the Pós-SE group was elevated compared to the NS group. Although the relationship between glucose levels and seizures in epileptic patients is not yet fully understood, studies suggest that both hypoglycemia and hyperglycemia may act as triggers for seizures (43). In patients with type 1 diabetes mellitus, seizures can be provoked by episodes of hypoglycemia and hyperglycemia (44-46). Rats with hyperglycemia experienced more intense seizures, leading to more severe damage in the hippocampus after SE. This damage resulted in a higher mortality rate and, in the surviving animals, impaired learning and cognition (47). Both hypoglycemia and hyperglycemia may play crucial roles in SE generation, although the intrinsic pathophysiological mechanisms explaining this association remain unclear (48).

In this study, we observed a decrease in butyrate levels after the SE event. Butyrate is a short-chain fatty acid (SCFA) produced in the colon. It has a beneficial effect on brain health by inhibiting histone deacetylases, activating GPCR receptors, releasing serotonin and generating ATP (49). Additionally, we observed a higher concentration of propionate in the NS group compared to the Pós-SE group. Similar results were observed for acetate, another short-chain fatty acid, in the NS group relative to the Pós-SE group. Both propionate and acetate are end products of carbohydrate and dietary amino acid fermentation by enterobacteria, playing important roles as signaling molecules (50). Propionic acid, in particular, has been associated with autism and is found at elevated levels in the feces of patients with this condition (51). The reduction in SCFA levels, such as propionate and acetate, may indicate a significant metabolic alteration during epileptogenesis.

Glutamine and glutamate are two metabolites that are characteristic of the SRS group, both with high concentrations in this group. Glutamate levels are disturbed in patients with epilepsy, with this alteration being more common in serum (12). Serum glutamate levels were found to be elevated in the serum of patients with epilepsy compared to healthy individuals (42, 52). However, no alterations in glutamate levels were observed in epilepsy patients compared to healthy controls in two studies using cerebrospinal fluid (CSF) samples and brain tissue (53, 54). In animal models, glutamate and glutamine levels have been both increased (55-56) and decreased (57, 58). This variation may result from different seizure-inducing mechanisms in each model, as well as from variations in sampling time points and the analytical platforms used (16).

Glutamate, the main excitatory neurotransmitter in the central nervous system, plays a crucial role in the initiation of epileptic seizures and in the induction of neuronal death (59). NMDA receptors for glutamate can trigger paroxysmal depolarizations and epileptic discharges during epileptogenesis (60) and the glutamatergic system can reduce the efficacy of GABA receptors, impairing the inhibition of epileptic seizures (61). Glutamine is essential for the biosynthesis of the neurotransmitters glutamate and GABA, playing a key role in the regulation of both excitatory and inhibitory synaptic transmission (62).

Recent studies have shown that the metabolism of alanine, aspartate and glutamate is altered in epilepsy models, both in humans and in various animal models. These alterations highlight the relevance of this metabolic pathway in the pathogenesis of epilepsy and suggest multiple potential therapeutic targets. Additionally, other metabolic pathways, such as those involving glycine, serine and threonine, as well as glycerophospholipid metabolism, have also been identified as altered in these models, underscoring the complexity of the biochemical changes involved in epilepsy (16). Finally, to better visualize the patterns and similarities inherent in the data, a heatmap with clustering was used, allowing for a more accessible and intuitive identification and interpretation of intergroup differences. A similar metabolic pattern was observed between the samples of the Pós-SE, naïve, and SRS groups, and between the samples of

the NS and NSRS groups. Glucose and ethanol are two metabolites characteristic of the naïve, Pós-SE, and SRS groups, while leucine, valine, propionate, nicotinic acid, isoleucine, and pyridoxine are predominant and common metabolites in the NS and NSRS groups. Glutamine and glutamate are notable metabolites in the SRS group, whereas hypoxanthine, fumarate, and phenylalanine are distinctive metabolites of the 24-hour group, and lysine and butyrate are predominant in the naïve group.

5 Conclusion

In this study, we investigated fecal metabolomics in a lithium-pilocarpine induced temporal lobe epilepsy experimental model and identified significant changes in 24 metabolites across the experimental groups. The metabolic analyses of fecal waters revealed a clear separation between the experimental groups, suggesting that the metabolic variations reflect different phases of epilepsy and potential biomarkers for disease progression and resistance. These metabolic signatures highlight the complexity of temporal lobe epilepsy and offer new perspectives to improve its understanding and treatment. However, further studies using different types of samples are needed to clarify the underlying mechanisms and validate these findings for clinical application.

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6 CONCLUSÃO

Nossos estudos destacam aspectos distintos, porém complementares, da epilepsia do lobo temporal e seus mecanismos subjacentes.

O primeiro artigo mostra que a interrupção circadiana causada pela exposição à luz constante afeta de maneira distinta cada fase da epilepsia. Observou-se alteração no ritmo circadiano, redução da gravidade das crises e da neurodegeneração na fase aguda, além de uma mudança no momento circadiano das crises na fase crônica, sem impacto na frequência total.

O segundo artigo identificou alterações significativas em 24 metabólitos, evidenciando uma clara separação entre os grupos experimentais, com exceção dos grupos resistentes, que apresentaram perfis metabólicos bastante semelhantes entre si, sugerem que as variações metabólicas estão associadas às diferentes fases da epilepsia, indicando potenciais possiveis biomarcadores para a progressão e resistência da doença. Essas assinaturas metabólicas destacam a complexidade da epilepsia do lobo temporal e abrem novas perspectivas para aprofundar seu entendimento e aprimorar estratégias terapêuticas. Entretanto, são necessários estudos em diferentes tipos de amostras para validar os dados.

Em conjunto, os achados ressaltam a relevância de abordagens multifatoriais no estudo da epilepsia, considerando tanto os ritmos circadianos quanto os perfis metabólicos para entender melhor os processos biológicos envolvidos. Ambos os estudos sugerem novas perspectivas para intervenções direcionadas e personalizadas, embora enfatizem a necessidade de mais estudos para validar e traduzir essas descobertas para a prática clínica.

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