UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS:
BIOQUÍMICA

Marcelo Salimen Rodolphi

O PARADOXO DO ABUSO DE ESTEROIDES ANABÓLICOS ANDROGÊNICOS: DA AGRESSIVIDADE À NEUROPROTEÇÃO NO TRAUMATISMO CRANIOENCEFÁLICO

Porto Alegre
2025

CIP - Catalogação na Publicação

RODOLPHI, MARCELO
O paradoxo do abuso de esteroides anabólicos androgênicos: da agressividade à neuroproteção / MARCELO RODOLPHI. -- 2025.
164 f.
Orientador: Luis Valmor Portela.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Instituto de Ciências Básicas da Saúde, Programa de Pós-Graduação em Ciências Biológicas: Bioquímica, Porto Alegre, BR-RS, 2025.

1. Esteroides Anabólicos Androgênicos. 2. Comportamento Agressivo. 3. Mitocôndria Sináptica. 4. Trauma Cranioencefálico. 5. Neurodegeneração. I. Portela, Luis Valmor, orient. II. Título.

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

Marcelo Salimen Rodolphi

O PARADOXO DO ABUSO DE ESTEROIDES ANABÓLICOS ANDROGÊNICOS: DA AGRESSIVIDADE À NEUROPROTEÇÃO NO TRAUMATISMO CRANIOENCEFÁLICO

Tese apresentada ao Programa de Pós- Graduação em Ciências Biológicas: Bioquímica do Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul como requisito parcial para a obtenção do título de doutor(a) em Bioquímica.

Orientador(a): Prof. Dr. Luis Valmor Cruz Portela

Porto Alegre

2025

AGRADECIMENTOS

A construção desta tese só foi possível graças ao esforço de diversas pessoas diretamente envolvidas com o laboratório de neurotrauma e biomarcadores que, ao longo desses anos, participaram do meu desenvolvimento como pesquisador e contribuíram fortemente para as produções desenvolvidas nesta tese. A todas as pessoas que fazem ou já fizeram parte deste grupo de pesquisa, fica a minha eterna gratidão.

Um agradecimento especial para os colegas e ex-colegas Nathan, Afonso e Mônia, possivelmente as pessoas que mais me apoiaram nessa jornada, pois sempre estiveram presentes nos momentos de muito trabalho e nos momentos de diversão. Vocês foram essenciais para a manutenção da minha sanidade mental.

Agradeço ao amigo Eduardo Zimmer, pelo empurrão para a ciência, pelas primeiras dicas desse mundo novo e principalmente pelo exemplo e amizade diária acima de tudo.

Agradeço aos ex-colegas e amigos Kamila Zenki e Eduardo Kalinine, pela grande ajuda no início da minha caminhada na ciência.

Um agradecimento especial à professora Lisiane Porciúncula e a todos os seus alunos, que dividem o laboratório 26 com nosso grupo, pela convivência diária, companheirismo e colaborações científicas ao longo desses anos.

Agradeço à professora Rachel Krolov e às suas alunas Alessandra e Ariadni pela colaboração e ajuda essenciais para a finalização de um paper desta tese.

Agradeço ao professor Luis Valmor Cruz Portela, pela oportunidade concedida, pela confiança depositada, pelos ensinamentos diários tanto na ciência quanto na vida, e por ser o meu mestre e exemplo na pesquisa científica. Obrigado por me "forçar" a dar aulas de bioquímica para os alunos da Nutrição da UFRGS – foi dentro de sala de aula que descobri uma vocação que nem eu sabia que tinha e tive a oportunidade de aprender com mais clareza a bioquímica. Quero agradecer também por todo o esforço que ele empregou para a produção desta tese e pela amizade verdadeira desenvolvida em todos esses anos de convivência. Não poderia deixar de agradecer à sua família, aqui representada pela Elsa, Juliana e Isabela, que abriram as portas de sua casa e sempre me receberam com carinho e amizade.

Agradeço profundamente aos componentes e amigos das secretarias do PPG Bioquímica e Educação, Cléia, Giordano e Douglas, pelo apoio e paciência em todos esses anos. Agradeço também aos servidores e funcionários do Departamento de Bioquímica, responsáveis pelo bom funcionamento do biotério e outras amenidades indispensáveis para a execução do trabalho aqui apresentado.

Agradeço à minha mãe, pelo apoio incondicional em todos os momentos da minha vida e aos meus familiares que estiveram comigo nesta jornada.

Agradeço ao CNPq, pela bolsa concedida.

SUMÁRIO

PARTE I 8
RESUMO 9
ABSTRACT 10
LISTA DE TABELAS 11
LISTA DE FIGURAS 12
LISTA DE ABREVIATURAS 13
1. INTRODUÇÃO 17
1.1 Esteroides anabólicos androgênicos 17
1.2 Histórico dos EAA 17
1.3 Estrutura e classe dos EAA 19
1.4 Mecanismo de ação dos EAA 23
1.5 Relação estrutura atividade dos EAA 26
1.6 Aplicações terapêuticas dos EAA 28
1.7 Efeitos Adversos e Toxicidade Sistêmica dos EAA 29
1.7.1 Efeitos Reprodutivos e Endócrinos 30
1.7.2 Efeitos Cardiovasculares 30
1.7.3 Efeitos Hepáticos 30
1.7.4 Efeitos Dermatológicos 31
1.7.5 Efeitos no Crescimento e Desenvolvimento 31
1.7.6. Sintomas Neuropsiquiátricos 31
1.8 Efeitos dos EAA no SNC e na Agressividade 31
1.8.1 Agressividade e disfunção corticolímbica 32
1.8.2 Modulação de sistemas neurotransmissores 32
1.8.3 Efeitos sobre cognição e morfologia cerebral 33
1.8.4 Efeitos afetivos e psiquiátricos 34
1.9 Mitocôndria: estrutura e função 35
1.10 Evolução do entendimento da função mitocondrial e a teoria quimiosmótica 37
1.11 A arquitetura funcional das cristas e o papel bioenergético do $\Delta\psi$ 41
1.12 Sinalização celular, ERO e manejo do Ca^{2+} mitocondrial 42
1.13 Integração funcional entre retículo endoplasmático e mitocôndria 44
1.14 Mitocôndrias sinápticas e extrasinápticas 46
1.15 TCE – Epidemiologia 47

1.16 TCE- Causas e Mecanismos Patofisiológicos.....49
1.17 Aspectos clínicos e fisiopatológicos do TCE 51
1.18 TCE – dano mitocondrial.....53
1.19 Traumatismo Cranioencefálico – Terapias 54
2 OBJETIVOS.....58
2.1 Objetivo Geral.....58
PARTE II.....59
Capítulo I: Synaptic and extrasynaptic mitochondria: features and impact of high anabolic androgen-steroid use.....61
Capítulo II: Glutamate transporter-1 links astrocytes with heightened aggressive behavior induced by steroid abuse in male CF1 mice 83
Capítulo III: Nandrolone abuse prior to head trauma mitigates endoplasmic reticulum stress, mitochondrial bioenergetic deficits, and markers of neurodegeneration......95
PARTE III 114
3 DISCUSSÃO.....115
4 CONCLUSÃO.....118
5 REFERÊNCIAS.....119
6 PRODUÇÃO CIENTÍFICA COMPLEMENTAR À TESE 151
ANEXO I: REPEATED HEAD INJURIES PROGRESSIVELY PROMOTE FUNCTIONAL AND METABOLIC IMPAIRMENT IN MICE.....152
ANEXO II: ORIENTAÇÃO DE TRABALHO DE CONCLUSÃO DE CURSO 161
ANEXO III: BANCA DE TRABALHO DE CONCLUSÃO DE CONCLUSÃO DE CURSO. 162
ANEXO IV: CO-ORIENTAÇÃO DE TRABALHO DE CONCLUSÃO DE CURSO...163
ANEXO V: PARTICIPAÇÃO EM COMISSÃO AVALIADORA DE TRABALHOS NO SALÃO DE INICIAÇÃO CIENTÍFICA DA UFRGS. 164

PARTE I

RESUMO

O abuso de esteroides anabólicos androgênicos (EAA) é um importante problema de saúde pública. Doses suprafisiológicas de testosterona (T) e decanoato de nandrolona (ND) são amplamente utilizadas para o aumento da massa muscular e do desempenho físico, e para moldar uma estética corporal "perfeita". No entanto, o abuso pode desencadear graves consequências físicas e neuropsiquiátricas, como problemas cardíacos, agressividade, ansiedade, depressão, surtos psicóticos e maior propensão ao uso de drogas, evidenciando suas ações tanto periféricas como centrais. Embora os EAA sejam reconhecidos por impulsionar o metabolismo periférico, do ponto de vista neurobiológico, seus impactos sobre a bioenergética mitocondrial, especialmente nas mitocôndrias dos terminais sinápticos, ainda são pouco compreendidos. **No Capítulo I,** revisamos os efeitos da ND sobre a função mitocondrial sináptica, destacando a redução da capacidade bioenergética, do potencial de membrana ($\Delta\Psi$), e aumento da produção de H_2O_2 e do desequilíbrio no fluxo de Ca^{2+}, sugerindo prejuízos no suporte energético da neurotransmissão. **No Capítulo II**, replicamos o fenótipo agressivo induzido por ND (15 mg/kg, 19 dias) em camundongos CF-1, evidenciado pelo aumento de ataques no teste do intruso-residente. Esse fenótipo foi consistentemente relacionado à hiperexcitabilidade glutamatérgica e à redução do transportador astroglial de glutamato GLT-1. A coadministração de ceftriaxona (200 mg/kg) e ND sustentou a expressão de GLT-1, diminuiu os parâmetros de agressividade e reverteu alterações bioenergéticas, confirmando assim, o papel do GLT-1 e das mitocôndrias dos terminais sinápticos na modulação do fenótipo agressivo. **No Capítulo III,** avaliamos a relação entre o abuso prévio de ND, agressividade e vulnerabilidade ao traumatismo cranioencefálico (TCE). Camundongos CF-1 submetidos ao impacto cortical controlado tiveram aumento dos níveis de APP, $A\beta_{42}$, $pTau^{Ser396}$ e de marcadores de estresse do retículo endoplasmático (GRP78, ATF6, CHOP), sugerindo que foi ativada a resposta à proteínas mal dobradas (UPR). Além disso, o TCE causou alterações significativas na bioenergética e marcadores de biogênese mitocondrial (PGC-1α, TOM20). Surpreendentemente, quando camundongos foram submetidos a doses suprafisiológicas de ND previamente ao TCE, foi observado uma redução dos marcadores moleculares da UPR e neurodegeneração, e uma atenuação dos prejuízos às funções das mitocôndrias sinápticas. O conjunto dos resultados dessa tese, indicam que o abuso de ND afeta componentes associados à atividade bioenergética mitocondrial no compartimento sináptico e exacerba a agressividade através de mecanismos de hiperativação glutamatérgica. Contudo, no contexto do TCE, o abuso de ND atenua mecanismos secundários que culminam em neurodegeneração precoce (acúmulo de proteínas neurotóxicas, redução da UPR e ROS, e do hipometabolismo). Este comportamento configura um paradoxo dos EAA: mudanças comportamentais e neurotoxicidade em condições fisiológicas, e neuroproteção sob estresse no contexto desencadeado pelo TCE severo. Esses resultados evidenciam a complexidade/potencialidades dos efeitos neurobiológicos dos EAA e destacam o papel dos astrócitos na modulação do comportamento agressivo induzido por ND. Essa tese desafia o conceito tradicional de neurotoxicidade associadas a doses suprafisiológicas, sugerindo efeitos neuroprotetores que dependem fortemente do contexto microambiental.

Palavras-chave: Esteroides anabólicos androgênicos, decanoato de nandrolona, comportamento agressivo, mitocôndria sináptica, glutamato, GLT-1, UPR, neurodegeneração, trauma cranioencefálico.

ABSTRACT

The abuse of anabolic-androgenic steroids (AAS) represents a major public health issue. Supraphysiological doses of testosterone (T) and nandrolone decanoate (ND) are widely used to increase muscle mass and physical performance, and to shape a "perfect" body aesthetic. However, such abuse can trigger severe physical and neuropsychiatric consequences, including cardiovascular problems, aggressive phenotypes, anxiety, depression, psychotic outbreaks, and increased propensity for drug abuse, demonstrating both peripheral and central actions. Although AAS are known to stimulate peripheral metabolism, from a neurobiological perspective, their effects on mitochondrial bioenergetics, particularly within synaptic terminal mitochondria, remain poorly understood. **In Chapter I**, we reviewed the effects of ND on synaptic mitochondrial function, highlighting reduced bioenergetic capacity, decreased membrane potential ($\Delta\Psi$), increased H_2O_2 production, and disruption of Ca^{2+} flux, suggesting impaired energetic support for neurotransmission. **In Chapter II**, we replicated the aggressive phenotype induced by ND (15 mg/kg, 19 days) in CF-1 mice, as evidenced by an increased number of attacks in the resident-intruder test. This phenotype was consistently associated with glutamatergic hyperexcitability and a reduction in the astroglial glutamate transporter GLT-1. Coadministration of ceftriaxone (200 mg/kg) with ND preserved GLT-1 expression, reduced aggressive behavior parameters, and reversed bioenergetic impairments thereby confirming the involvement of GLT-1 and synaptic terminal mitochondria in modulating the aggressive phenotype. **In Chapter III**, we investigated the relationship between prior ND abuse, aggressiveness, and vulnerability to traumatic brain injury (TBI). CF-1 mice subjected to controlled cortical impact showed increased levels of APP, $A\beta_{42}$, and $pTau^{Ser396}$, as well as markers of endoplasmic reticulum stress (GRP78, ATF6, CHOP), suggesting activation of the unfolded protein response (UPR). Moreover, TBI caused significant alterations in bioenergetics and in markers of mitochondrial biogenesis (PGC-1α, TOM20). Surprisingly, when mice were exposed to supraphysiological ND doses prior to TBI, there was a reduction in molecular markers of UPR activation and neurodegeneration, along with attenuation of impairments in synaptic mitochondrial function. Altogether, these findings indicate that ND abuse impairs mitochondrial bioenergetic function within synaptic compartments and exacerbates aggressive behavior through mechanisms involving glutamatergic hyperactivation. However, in the context of TBI, ND exposure appears to attenuate secondary injury mechanisms, as evidenced by reduced accumulation of neurotoxic proteins, decreased UPR activation, lower ROS production, and mitigation of post-traumatic hypometabolism. These findings illustrate a paradox of AAS roles: behavioral alterations and neurotoxicity under physiological conditions, yet neuroprotection under severe stress induced by TBI. Also, we underscore the complexity and potential duality of the neurobiological effects of AAS and highlight the role of astrocytes in modulating ND-induced aggressive behavior. This thesis challenges the traditional view that supraphysiological doses of AAS are solely neurotoxic, proposing instead that their effects are context-dependent and may confer neuroprotection under specific microenvironmental conditions.

Keywords: Anabolic-androgenic steroids, nandrolone decanoate, aggressive behavior, synaptic mitochondria, glutamate, GLT-1, UPR, neurodegeneration, traumatic brain injury.

LISTA DE TABELAS

Tabela 1. Escala de coma de Glasgow (ECG)

LISTA DE FIGURAS

Figura 1. Estrutura química da testosterona

Figura 2. Resumo das diferentes classes dos esteroides anabólicos androgênicos

Figura 3. Diferentes vias metabólicas da testosterona

Figura 4. Modificações químicas na molécula de testosterona

Figura 5. Organização estrutural da mitocôndria

Figura 6. Funcionamento do sistema de transporte de elétrons e organização dos supercomplexos mitocondriais

Figura 7. Vias ativadas pela resposta a proteínas mal enoveladas (UPR) frente ao estresse do retículo endoplasmático

Figura 8. Mitocôndrias sinápticas e extrasinápticas

Figura 9. Causas de trauma cranioencefálico (TCE)

Figura 10. Progressão do dano secundário associado ao trauma cranioencefálico (TCE)

LISTA DE ABREVIATURAS

5α-DHT – 5α-diidrotestosterona

19-NOR – 19-nortestosterona

Aβ42 – Proteína beta-amiloide 1-42

ADP – Adenosina difosfato

AMA – Agência mundial antidoping

APP – Proteína precursora amilóide

ATF6 – Fator ativador de transcrição 6

ATP – Adenosina trifosfato

BAK – Antagonista/assassino de Bcl-2

BAX – Proteína X associada a Bcl-2

BCE – Eficiência do acoplamento bioenergético

Bcl-2 – Linfoma de célula beta-2

BO – Boldenona

Ca^{2+} - Cálcio

CEF – Ceftriaxona

CHOP - Proteína homóloga C/EBP

CK – Ciclo de Krebs

CL – Clostebol

CO_2 - Dióxido de carbono

COI – Comitê olímpico internacional

CR – Concussões repetidas

FADH2 – Dinucleotídeo de flavina e adenina reduzido

NADH – Dinucleotídeo de nicotinamida e adenina reduzido

Δψ – Potencial de membrana

Δp – Força próton motriz

ΔpH – Diferença de concentração de íons hidrogênio

DHN – Diidronandrolona

DHT – Diidrotestosterona

E'_0 - Potencial redox

EAA – Esteroides anabólicos androgênicos

EB – Enobosarm

ECGl – Escala de coma de Glasgow

EI – Espaço intermembranas

EN – Enostebol

ERAD – Mecanismo de degradação de proteínas associadas ao retículo endoplasmático

ERO – Espécies reativas de oxigênio

ES – Estanozolol

ET – Ectidisteroide

ETC – Encefalopatia traumática crônica

FDA – US Food and Drug Administration

F – Fitoecdiesteroides

GADD153 – Gene 153 Induzível por parada de crescimento e dano ao DNA

GAPDH – Gliceraldeído-3-fosfato desidrogenase

GLT-1 – Transportador astrocitário de glutamato

GPER – Receptor de estrogênio acoplado à Proteína G

GRP78/BiP – Proteína 78 regulada por glicose

H^+ - Íon hidrogênio

H_2O – Água

H_2O_2 - Peróxido de hidrogênio

ICC – Impacto cortical controlado

IDH – Isocitrato desidrogenase

IKK – Cinase do inibidor de κB

$InsP_3R$ – Receptores de inositol 1,4,5-Trifosfato

IRE1α – Enzima que requer inositol 1

JNK - c-Jun N-Terminal cinase

LCR – Líquido céfalo-raquidiano

m-AAA – Proteases de ATP da matriz mitocondrial

MAM – Membranas do retículo endoplasmático associadas a mitocôndria

MCU – Uniporte de cálcio mitocondrial

ME – Metandienona

MET – Metandrostenelona

Mfn1 – Mitofusina 1

Mfn2 – Mitofusina 2

MME – Membrana mitocondrial externa

MMI – Membrana mitocondrial interna

MN – Metenolona

MT – Metribolona

MTT – Metiltestosterona

mV – Milivolts

Na^+ - Sódio

NCLX – Canal trocador Sódio/Cálcio (Lítio) mitocondrial

ND – Decanoato de nandrolona

NMDA – N-metil-D-aspartato

O_2 - Oxigênio

$O_2^{\bullet -}$ - Superóxido

OGDH – Complexo da α-oxoglutarato desidrogenase

OPA1 – Proteína de atrofia óptica 1

OT – Oximetolona

OX – Oxandrolona

PARL – Proteína semelhante a rombóide associada à presinilinas

PDH – Complexo piruvato desidrogenase

PERK – Proteína RNA-like endoplasmic reticulum cinase

PGC-1α – Coativador 1α do receptor gama ativado por proliferador de peroxissoma

PHB-SLP2 – Proteína semelhante a Proibitina-Estomatina 2

Pi – Fosfato inorgânico

POR – Porinas

PTP – Proteínas tirosina-fosfatases

QH_2 - Ubiquinol reduzido

RAAS – Sistema renina angiotensina aldosterona

RA – Receptores androgênicos

RE – Receptores estrogênicos

RG – Receptores glicocorticóides

ROMO1 – Modulador de espécies reativas 1

SARMs – Moduladores seletivos do receptor de androgênio

SERMs – Moduladores seletivos do receptor de estrogênio

SHBG – Globulina ligadora de hormônios sexuais

SNC – Sistema nervoso central

SUS – Sistema único de saúde

T – Testosterona

TAU – Proteína estabilizadora de microtúbulo

TCE – Trauma cranioencefálico

TI – Tribolona

TIM – Translocases de membrana interna

TOM – Translocases de membrana externa

TR – Trembolona

UCP – Proteína desacopladora

UPR – Resposta a proteínas mal enoveladas

VDAC – Canais iônicos dependentes de voltagem

1. INTRODUÇÃO

1.1 Esteroides anabólicos androgênicos

Os esteroides anabólicos androgênicos (EAA) são derivados sintéticos da testosterona (T) e da diidrotestosterona (DHT), classificados como hormônios com efeitos anabólicos (síntese proteica e crescimento tecidual) e androgênicos (desenvolvimento do trato reprodutivo e características sexuais masculinas secundárias). Seus efeitos anabólicos incluem aumento da massa muscular e força, hipertrofia de fibras musculares, redução da gordura corporal e modulação de funções do sistema nervoso central (Bond et al., 2022; Shahidi, 2001).

A ação dos EAA é mediada principalmente por receptores androgênicos (RA), amplamente distribuídos em tecidos de mamíferos, especialmente no músculo esquelético, onde promovem a biossíntese proteica e o aumento da área transversal das fibras musculares. Além dos mecanismos genômicos clássicos, evidências recentes demonstram que os EAA também modulam processos celulares por vias não genômicas, ampliando a compreensão sobre seus efeitos moleculares (Dubois et al., 2012; Pelton et al., 2023). A T, principal hormônio endógeno dessa classe, é considerada o protótipo para o desenvolvimento de análogos sintéticos.

Em homens eugonadais, cerca de 95% da T é produzida pelas células de Leydig nos testículos; pequenas quantidades são sintetizadas nos ovários e nas glândulas adrenais, que também secretam andrógenos precursores da T e da 5α-diidrotestosterona (5α-DHT) (Kochakian, 1993; Midzak et al., 2009). Embora possuam aplicações terapêuticas em condições como hipogonadismo, infecção por HIV, queimaduras extensas, osteoporose e anemia aplásica, o uso não terapêutico de EAA em doses suprafisiológicas, geralmente para fins estéticos ou de desempenho físico, representa um desvio de suas indicações médicas e está associado a efeitos adversos significativos (Anawalt, 2019; Bond et al., 2022; Moyle et al., 2004).

1.2 Histórico dos EAA

O interesse por substâncias com efeitos semelhantes aos dos EAA acompanha o desenvolvimento histórico da endocrinologia. Há mais de seis mil anos, observações empíricas sobre os efeitos da castração em animais já sugeriam a influência das gônadas no comportamento e na fisiologia. No Egito e em Roma antigas, partes de animais, como testículos, eram usadas em práticas medicinais e rituais, enquanto atletas gregos empregavam extratos de

plantas e tecidos animais para melhorar o desempenho físico (Dotson & Brown, 2007; Freeman et al., 2001).

Em 1849, o fisiologista Arnold Adolph Berthold demonstrou que a remoção dos testículos de galos reduzia suas características sexuais secundárias e comportamento agressivo, e que esses efeitos eram revertidos pela reimplantação do tecido testicular, mesmo sem reinervação, fornecendo as primeiras evidências da ação hormonal sistêmica (Dotson & Brown, 2007; Nieschlag & Nieschlag, 2019). Décadas depois, Édouard Brown-Séquard retomou essas investigações e, em 1889, relatou melhora subjetiva de vigor e força após autoinjeções de extratos testiculares, episódio que se tornaria um dos primeiros relatos de efeito placebo documentado (Cussons et al., 2002; Janssen, 2024).

No início do século XX, Oskar Zoth propôs o uso experimental de extratos testiculares para aprimorar o desempenho atlético (Dotson & Brown, 2007), e em 1905, Ernest Starling introduziu oficialmente o termo hormônio, consolidando a endocrinologia como campo científico (Henriksen, 2005). Nos anos seguintes, André Pezard (1911) demonstrou a relação dose-dependente entre extratos testiculares e características sexuais secundárias em galos, e Eugen Steinach explorou intervenções cirúrgicas e transplantes gonadais com o intuito de restaurar vitalidade e libido (Hoberman & Yesalis, 1995; Nieschlag & Nieschlag, 2014).

A síntese química da T representou um marco. Em 1935, Laqueur, na Organon (Holanda), isolou a T a partir de testículos bovinos, enquanto Butenandt e Ruzicka publicaram métodos de síntese a partir do colesterol, conquista que lhes rendeu o Prêmio Nobel de Química de 1939 (Dotson & Brown, 2007; Shahidi, 2001). A descoberta permitiu o rápido avanço na produção de derivados com propriedades anabólicas e androgênicas moduladas.

Na década de 1940, derivados como propionato de T e metiltestosterona (MTT) foram introduzidos clinicamente para tratar hipogonadismo e impotência (Kicman & Gower, 2003). Paralelamente, estudos em mulheres relataram aumento de libido e bem-estar com o uso tópico de T, embora os efeitos virilizantes limitassem sua aplicação terapêutica (Basaria et al., 2001).

Nos anos 1950, a utilização de T e seus derivados expandiu-se entre atletas e fisiculturistas, especialmente após a introdução da metandrostenolona (MET) em 1958 (Frati et al., 2015). Na mesma década, o decanoato de nandrolona (ND) foi desenvolvido, sendo o primeiro análogo sintético da T com dissociação significativa entre efeitos anabólicos e androgênicos, favorecendo os primeiros (Hershberger et al., 1953; Krüskemper & Morgner, 1968).

Com a popularização dos EAA nas décadas de 1960 e 1970, aumentaram os relatos de uso ilícito em competições esportivas, levando o Comitê Olímpico Internacional (COI) a proibir

oficialmente seu uso em 1975 (Mottram & George, 2000). O aprimoramento de métodos analíticos, como radioimunoensaios e espectrometria de massa, possibilitou o controle mais rigoroso do doping (Kicman & Brooks, 1988; Shackleton, 2009). O caso de Ben Johnson nos Jogos de Seul (1988) consolidou a necessidade de políticas antidoping globais, culminando na criação da Agência Mundial Antidoping (WADA) em 1999 (WADA, 2003).

Atualmente, o abuso de EAA extrapola o contexto esportivo e atinge populações não atletas, incluindo adolescentes e adultos jovens que buscam fins estéticos. Essa tendência transformou o uso não médico de EAA em um problema de saúde pública, agravado pela circulação de produtos falsificados e formulações caseiras (Dal Pizzol et al., 2006; DiMeo & Wood, 2006).

1.3 Estrutura e classe dos EAA

A estrutura básica dos EAA é formada pelo núcleo de ciclopentanoperidrofenantreno, constituído por quatro anéis fusionados, três anéis de ciclohexano e um de ciclopentano, uma característica comum a todos os esteroides. A T, precursor estrutural dos EAA, contém 19 átomos de carbono organizados nos anéis A, B, C e D, e apresenta um grupo hidroxila (-OH) ligado ao carbono 17 da cadeia lateral (Figura 1).

A T representa, portanto, a molécula-base para o desenvolvimento dos diferentes análogos sintéticos de EAA. Modificações químicas específicas em sua estrutura molecular permitem ajustar a relação entre efeitos anabólicos e androgênicos, com o objetivo de potencializar o crescimento muscular e reduzir os efeitos de masculinização, embora esses dois aspectos sejam, na prática, dificilmente dissociáveis.

As modificações estruturais mais comuns incluem a esterificação no carbono 17-β, que aumenta a lipossolubilidade e prolonga a meia-vida; Alquilação no carbono 17-α, que confere resistência ao metabolismo hepático e viabilidade oral; Remoção ou substituição de átomos no carbono 19, alterando seletividade androgênica; Aromatização parcial da molécula, convertendo-a em derivados estrogênicos; e Alterações nos anéis A e B por meio de ciclização ou introdução de duplas ligações, que modulam a afinidade pelos RA (Ding et al., 2021; Kicman, 2008; Melnik, 2009). Essas modificações químicas conferem aos EAA perfis farmacológicos distintos, determinando diferenças significativas em potência anabólica, biodisponibilidade, metabolismo e toxicidade.

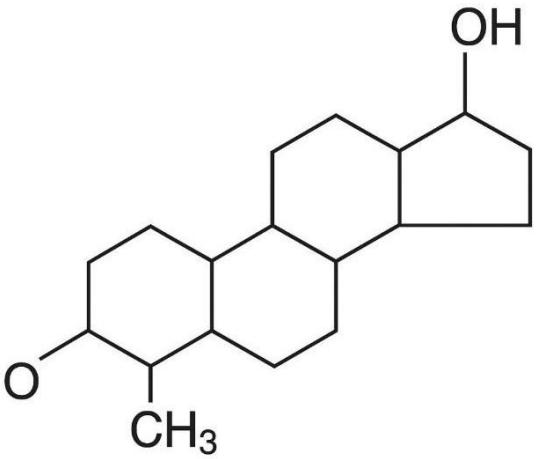


Figura 1. Estrutura química da Testosterona. Composta por um núcleo esteroidal formado por 4 anéis carbonados com a adição de uma hidroxila no carbono 17.

Com base nas modificações estruturais no anel esteroidal, os EAA podem ser classificados em três principais categorias, que se distinguem por suas propriedades químicas, farmacocinéticas e vias de administração. A primeira classe compreende os compostos injetáveis contendo ésteres de cadeia longa ligados ao grupo 17-β-hidroxila. Essa esterificação confere maior estabilidade e prolonga o tempo de ação dos fármacos, pois reduz sua taxa de degradação e promove liberação gradual do hormônio ativo na circulação. Entre os exemplos mais comuns estão o propionato e o cipionato de T (Forsdahl et al., 2015; Kicman, 2008; Shahidi, 2001).

Após a administração, os ésteres de T sofrem hidrólise enzimática, liberando a forma ativa do hormônio. A T pode então ser metabolizada por duas vias principais: (i) pela 5α-redutase, gerando a 5α-DHT, metabólito com maior afinidade pelos RA e, portanto, com atividade anabólica mais potente (Martini, 1982; Wilson, 1988); e (ii) pela aromatase, que converte a T em estrógenos, como o estradiol, com funções fisiológicas relevantes, mas que, em casos de uso abusivo de EAA, podem levar a efeitos colaterais, como ginecomastia e desequilíbrios hormonais (Cherrier et al., 2005; Finkelstein et al., 1981; Swislocki & Eisenberg, 2025). Assim, compreender essas vias metabólicas é essencial tanto para o uso clínico seguro quanto para a mitigação de efeitos adversos.

A segunda classe inclui os compostos injetáveis derivados da 19-nortestosterona (19-NOR), que apresentam diferenças estruturais em relação aos ésteres da T. Nessa classe, a adição de uma longa cadeia carbonada no carbono 17 reduz a afinidade pelo RA quando comparada à DHT, resultando em atividade androgênica atenuada. Além disso, a substituição de um hidrogênio por um grupo metila no carbono 19 aumenta a meia-vida dos compostos e favorece sua esterificação, prolongando a ação farmacológica (Shahidi, 2001). Um exemplo clássico é o

ND, cuja metabolização pela aromatase é menos eficiente que a da T, produzindo menores quantidades de metabólitos estrogênicos, mas mantendo elevado potencial anabólico (de Gooyer et al., 2003; Sirianni et al., 2012).

Os EAA das classes I e II exercem efeitos sobre o sistema nervoso central (SNC) tanto por meio da interação direta com os RA quanto pela ação de metabólitos aromatizados sobre RE no cérebro. Essas interações estão relacionadas a alterações de humor, comportamento e neuroplasticidade, podendo contribuir tanto para efeitos neuroprotetores quanto para reações adversas, como aumento da agressividade e instabilidade emocional, especialmente em contextos de uso abusivo (Grönbladh et al., 2016; Penatti et al., 2009; Wilson, 1988).

A terceira classe compreende os EAA de administração oral, caracterizados pela alquilação no carbono 17 (17-α-alquilados). Essa modificação impede a oxidação hepática e confere resistência ao metabolismo de primeira passagem, possibilitando a administração oral. Diferentemente das classes injetáveis, esses compostos não são convertidos em DHT nem em 17-β-estradiol, mas podem originar outros metabólitos biologicamente ativos com propriedades androgênicas ou estrogênicas (Basaria et al., 2001). Entre os principais representantes estão a MTT, oximetolona (OT), metandrostenolona (MET) e estanozolol (ES). Embora eficazes, o uso prolongado ou em altas doses desses agentes está associado a maior risco de hepatotoxicidade e complicações sistêmicas, decorrentes da resistência ao metabolismo hepático (Bond et al., 2016; Pavlatos et al., 2001).

Um resumo esquemático das três classes e principais características estruturais dos EAA é apresentado na Figura 2.

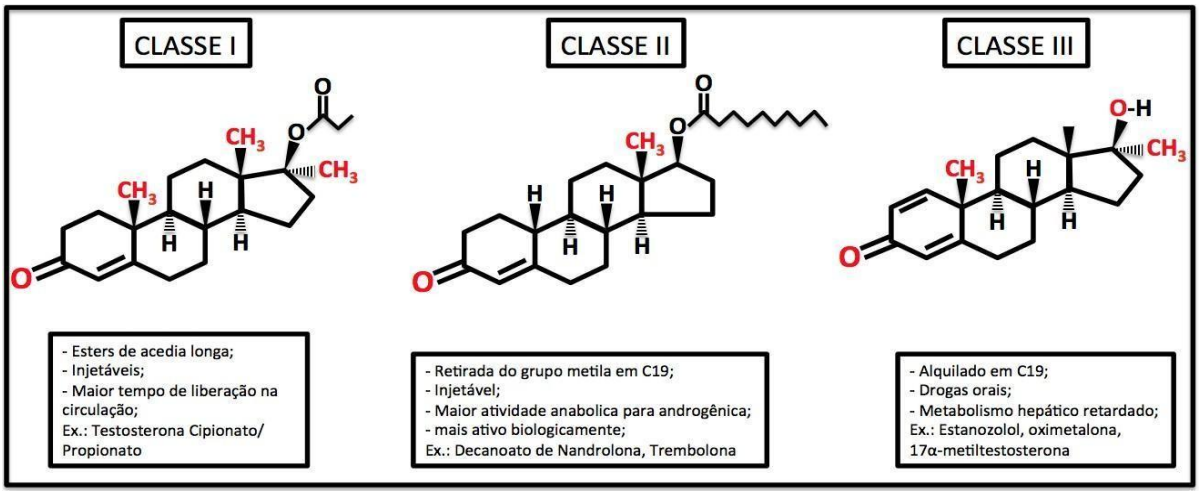


Figura 2. Estrutura química básica e principais características das três maiores classes de EAA. Adaptado de Oberlander & Henderson (2012).

Além das três classes clássicas de EAA, surgiu recentemente uma nova categoria de compostos esteroides denominada moduladores seletivos do receptor de androgênio (SARMs). Esses agentes são pequenas moléculas sintéticas que interagem seletivamente com os RA em diferentes tecidos, exercendo efeitos agonistas, parciais ou antagonistas, conforme o contexto molecular e celular. Uma analogia útil para compreender seu funcionamento é o tamoxifeno, um modulador seletivo do receptor de estrogênio (SERMs) amplamente utilizado no tratamento do câncer de mama. O tamoxifeno atua como antagonista nos receptores mamários, mas agonista no tecido ósseo e agonista parcial no endométrio, ilustrando o potencial terapêutico de moduladores seletivos capazes de exercer efeitos teciduais específicos com menor incidência de efeitos adversos sistêmicos (Solomon et al., 2019).

O RA é uma proteína multifuncional composta por domínios responsáveis pelo reconhecimento do ligante, dimerização e transativação gênica. Em seu estado inativo, encontra-se no citoplasma associado a proteínas de choque térmico (HSP90). Após a ligação de um SARM, o receptor é dissociado dessas proteínas e o complexo SARM–RA transloca-se para o núcleo, onde se liga a elementos de resposta androgênica nos genes-alvo, modulando a transcrição gênica. A magnitude e especificidade da resposta transcricional dependem da presença de co-reguladores proteicos em cada tecido, o que explica a seletividade funcional dos SARMs (Hikichi et al., 2015; Saeed et al., 2016; Schmidt et al., 2010).

Outra classe emergente de compostos esteroides é representada pelos fitoecdiesteroides (F), análogos naturais da ecdisona, o principal hormônio de muda e metamorfose dos insetos. Espécies vegetais como Rhaponticum carthamoides, Cyanotis vaga e Ajuga turkestanica constituem fontes ricas desses compostos, cujas estruturas e concentrações variam conforme o tecido e o estágio de desenvolvimento da planta (Lafont & Dinan, 2003). Embora a atividade hormonal dos ecdiesteroides (ET) seja comprovada apenas em artrópodes, estudos experimentais demonstram que eles podem exercer efeitos fisiológicos relevantes em mamíferos, incluindo ações anabólicas, hepatoprotetoras, imunomodulatórias, antioxidantes e hipoglicemiantes (Báthori et al., 2008; Mirzaev et al., 2000).

Do ponto de vista estrutural, os ET compartilham o esqueleto ciclopentanoperidrofenantreno, comum aos esteroides vertebrados, mas diferem pelo maior grau de oxigenação, número e posição de grupos hidroxila e presença de ligações duplas adicionais. Tais modificações impedem a ligação eficaz aos receptores esteroides nucleares clássicos (androgênico, estrogênico e glicocorticoide), sugerindo mecanismos de ação não genômicos (Gorelick-Feldman et al., 2008).

Evidências in vitro e in vivo indicam que os F podem atuar via receptores de membrana e cascatas de sinalização intracelular, especialmente pela ativação da via PI3K/Akt/mTOR, essencial à síntese proteica e hipertrofia muscular (Gorelick-Feldman et al., 2010; Parr et al., 2015). Além disso, há indícios de que esses compostos modulam a bioenergética mitocondrial e a resposta antioxidante, favorecendo um ambiente anabólico e protetor contra o estresse oxidativo (Tang et al., 2021).

O interesse farmacológico nos F decorre de sua baixa toxicidade, ausência de atividade androgênica e perfil de segurança superior aos EAA, o que os torna candidatos promissores como alternativas terapêuticas ou adjuvantes em condições associadas à perda de massa e função muscular, como sarcopenia, caquexia, osteopenia e síndromes catabólicas crônicas (Lafont & Dinan, 2003). Apesar disso, a evidência experimental sobre seus efeitos anabólicos em humanos ainda é inconclusiva. Faltam ensaios clínicos controlados e dados consistentes que confirmem sua eficácia. Portanto, os F representam um campo de pesquisa promissor, que requer investigações mais robustas sobre seus mecanismos de ação e potencial aplicação terapêutica (Báthori et al., 2008; Parr et al., 2015).

1.4 Mecanismo de ação dos EAA

A T exerce seus efeitos fisiológicos em múltiplos tecidos por meio de mecanismos complexos que envolvem tanto sua conversão metabólica quanto a interação com receptores nucleares e de membrana. Em muitos tecidos-alvo, a T é convertida localmente em DHT pela ação da enzima 5α-redutase, o que potencializa sua atividade biológica. A DHT apresenta uma afinidade aproximadamente três vezes maior pelos receptores RA em comparação à T, o que resulta em efeitos androgênicos mais intensos (Wilson, 1996).

Além disso, a T também pode ser metabolizada em estradiol pela ação da enzima aromatase, originando um metabólito hormonalmente ativo com funções distintas. O estradiol exerce seus efeitos principalmente por meio da ativação dos RE, localizados em tecidos como o SNC, tecido adiposo e tecido ósseo (Buchner, 1999; Busardò et al., 2015; Simpson et al., 2002).

As principais rotas metabólicas da T, incluindo sua conversão em DHT e estradiol, estão ilustradas na Figura 3.

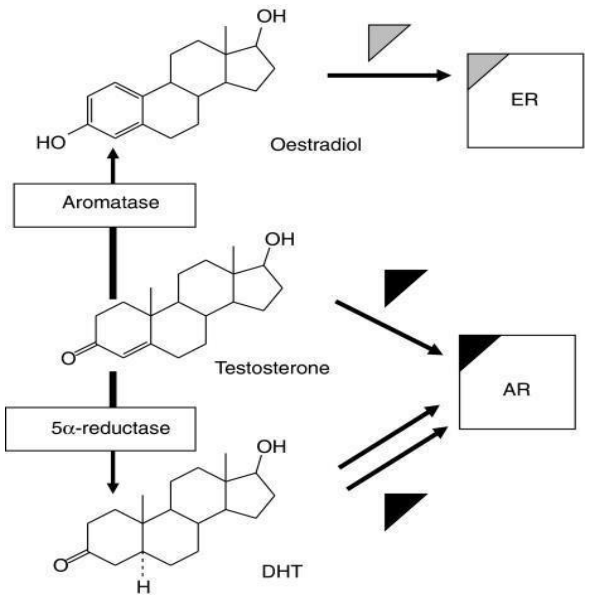


Figura 3. Vias metabólicas endógenas da T. Abreviações: DHT, Diidrotestosterona; ER, Receptores estrógenos; R, Receptores andrógenos. Figura adaptada de Kicman (2008).

A distribuição e a atividade das enzimas moduladoras do metabolismo androgênico variam conforme o tecido. A 5α-redutase apresenta maior expressão na próstata, pele e genitália externa, enquanto a aromatase é mais abundante no cérebro e no tecido adiposo (Labrie, 1991; Russell & Wilson, 1994). Evidências indicam que a atividade da 5α-redutase é praticamente ausente na musculatura esquelética, o que explica a baixa conversão local de T em DHT nesse tecido (Bartsch et al., 1980; Ferrando et al., 2002).

Devido ao seu caráter lipofílico, a T, os EAA e outros hormônios sexuais difundem-se livremente através das membranas plasmáticas. Na circulação, esses compostos podem estar na forma livre ou ligados a proteínas carreadoras, como a globulina ligadora de hormônios sexuais (SHBG). Apenas a fração livre é capaz de atravessar a membrana celular e exercer efeitos biológicos. Uma vez no interior da célula, a molécula se liga de maneira específica ao seu receptor, formando o complexo hormônio–receptor, cuja estabilidade e afinidade dependem das propriedades químicas de cada esteroide (Hammond, 2011; Kicman, 2008).

A ação dos esteroides pode ocorrer por duas vias principais: a clássica (genômica) e a rápida (não genômica). Na via clássica, o esteroide difunde-se até o citoplasma, onde se liga ao seu receptor específico, RA ou RE. O complexo hormônio–receptor sofre modificações conformacionais e dissocia-se de proteínas chaperonas, como a HSP90, translocando-se em seguida para o núcleo. Nesse compartimento, o complexo se liga a elementos de resposta hormonal no DNA, regulando positiva ou negativamente a transcrição gênica e,

consequentemente, modulando a síntese proteica (Falkenstein et al., 2000; Mangelsdorf et al., 1995; Wehling, 1997).

A via rápida ou não genômica é caracterizada por respostas celulares que ocorrem em segundos a minutos, independentes da transcrição gênica direta (Wehling, 1997). Esses efeitos podem resultar de alterações na fluidez da membrana plasmática e da ativação de cascatas intracelulares de sinalização, as quais se estabelecem por três mecanismos principais: (i) ligação do esteroide a receptores nucleares (RA ou RE) localizados na membrana, que, após modificações pós-traducionais como a palmitoilação, tornam-se capazes de acionar vias extracitoplasmáticas; (ii) interação com receptores de membrana não clássicos, como o receptor acoplado à proteína G para estrogênio (GPER), TRPM8 e ZIP9, que não pertencem à família dos receptores nucleares, mas mediam ações esteroidais específicas (Thomas et al., 2017; Wang et al., 2014); e (iii) efeitos físico-químicos diretos sobre a bicamada lipídica, alterando suas propriedades independentemente de qualquer receptor (Atkovska et al., 2018; Crowley et al., 2022; Solar & Velasquez, 2013). Essas respostas rápidas desempenham papéis fisiológicos relevantes, incluindo o controle do influxo de cálcio (Ca^{2+}) a regulação da secreção celular, a modulação da resposta imunológica e a sensibilização dos próprios receptores nucleares, atuando como um mecanismo de amplificação da via clássica (Wang et al., 2014; Wehling, 1997).

Quando administrados em doses suprafisiológicas, os EAA suprimem a biossíntese endógena de T por meio da inibição do eixo hipotálamo–hipófise–gonadal. Nessas condições, também podem desencadear efeitos adicionais independentes dos RA, incluindo o antagonismo aos receptores glicocorticoides (RG), a inibição da gliconeogênese e da degradação proteica (Kicman, 2008). Além disso, os EAA estimulam o eixo GH/IGF-1, promovendo efeitos anabólicos adicionais, como o aumento da síntese proteica, a ativação de células satélites e a hipertrofia muscular (Joubert & Tobin, 1989; Sinha-Hikim et al., 2003).

O uso de EAA em altas doses também está associado a uma série de efeitos adversos, como aumento do estresse oxidativo, disfunção mitocondrial e ativação de vias apoptóticas. Diversos estudos relatam elevação das espécies reativas de oxigênio (ERO), danos ao DNA e disfunção endotelial mesmo em indivíduos jovens e saudáveis (Arazi et al., 2017; Carteri et al., 2021; Kvorning et al., 2006). O uso crônico, por sua vez, pode ativar o sistema renina–angiotensina–aldosterona (RAAS), implicado no remodelamento vascular e miocárdico, o que contribui para o desenvolvimento de doenças cardiovasculares em usuários de EAA (D'Errico et al., 2011; Ma et al., 2010; Rocha et al., 2007).

1.5 Relação estrutura atividade dos EAA

Os EAA foram desenvolvidos com o objetivo de potencializar seletivamente os efeitos anabólicos, como o aumento da síntese proteica e o balanço nitrogenado positivo, sobre a musculatura esquelética, minimizando os efeitos androgênicos, como virilização e alterações prostáticas ou cutâneas. A relação estrutura–atividade desses compostos baseia-se na química do esqueleto androstano e nas interações com o domínio de ligação a ligantes do RA. Duas características estruturais são essenciais para um agonismo potente: o grupo 3-ceto conjugado a uma dupla ligação Δ4 no anel A e a hidroxila 17β, responsável pela ancoragem do ligante ao domínio de ligação por meio de pontes de hidrogênio (Fragkaki et al., 2009; Kicman & Gower, 2003; Singam et al., 2019; Xu et al., 2011).

Embora se busque dissociar completamente os efeitos anabólicos dos androgênicos, essa separação é impossível, pois ambos são mediados pelo mesmo tipo de RA (Mooradian et al., 1987). Uma explicação parcial para a predominância anabólica em determinados tecidos está na atividade diferencial da enzima 5α-redutase. Em tecidos androgênicos, como próstata e pele, a elevada atividade dessa enzima converte a T em DHT, um agonista mais potente do RA, amplificando os efeitos androgênicos. Em contraste, no músculo esquelético, onde a atividade da 5α-redutase é mínima, a T exerce predominantemente ação anabólica direta (Celotti & Negri Cesi, 1992).

Um segundo mecanismo de dissociação envolve o metabolismo dos derivados da 19-NOR. O ND, por exemplo, apresenta alta afinidade pelo RA, mas sua redução pela 5α-redutase origina a di-hidronandrolona (DHN), um agonista mais fraco. Assim, em tecidos androgênicos, a conversão de ND em DHN atenua a atividade androgênica, enquanto no músculo esquelético, onde a redução é limitada, a ND exerce plena atividade anabólica. Esse mecanismo confere à ND uma razão anabólica/androgênica superior à da T, embora ainda restrita pela ausência de isoformas seletivas do RA (Kicman & Gower, 2003; Tóth & Zakár, 1982).

Diversas modificações estruturais têm sido introduzidas no esqueleto da T para alterar sua potência, seletividade e farmacocinética: Remoção do grupo metil no carbono 19, característica da ND e da trembolona (TR), reduz o caráter androgênico e acentua o efeito anabólico (Busardò et al., 2015; Fragkaki et al., 2009; Kuuranne et al., 2008; Takeda et al., 2007); Adição de grupos metil ou etil no carbono 17α, como na oxandrolona (OX) e no estanozolol (ES), confere resistência ao metabolismo hepático e maior biodisponibilidade oral, embora aumente o risco de hepatotoxicidade (“Androgenic Steroids”, 2012; Petrovic et al., 2022); Inserção de ligações duplas entre os carbonos 1–2, observada na metandienona (ME) e

na boldenona (BO), reduz a conversão por 5α-redutase e aromatase, diminuindo a formação de DHT e estradiol (Fragkaki et al., 2009; Viljanto et al., 2019); Substituições por grupos hidroxila ou cloro, como no clostebol (CL) e no enestebol (EN), limitam a aromatização e a conversão por 5α-redutase, favorecendo o perfil anabólico (Fragkaki et al., 2009)., Ligações duplas adicionais entre os carbonos 9–10 e 11–12, presentes na TR e na metribolona (MT), aumentam significativamente a potência anabólica (Kuuranne et al., 2008; Takeda et al., 2007); Metilações específicas, como no carbono 7α (tibolona, TI) e no carbono 1 (metenolona, MN), conferem resistência à ação da 5α-redutase e maior estabilidade metabólica, além de melhorar a biodisponibilidade oral (Davis et al., 2003).; Substituição carbônica ou inserção de oxigênio (O_2) no anel A, como na OX (substituição do carbono 2 por um átomo de O_2), aumenta a estabilidade molecular e reduz a hepatotoxicidade (Petrovic et al., 2022); Esterificações nos carbonos 3 e 17β, comuns em formulações injetáveis como o ND, resultam em liberação lenta a partir de veículos oleosos, prolongando a meia-vida plasmática (Busardò et al., 2015; Fragkaki et al., 2009; Kicman, 2008; Kicman & Gower, 2003; Saartok et al., 1984; Shahidi, 2001; van der Vies, 1993).

A Figura 4 ilustra algumas das principais modificações químicas que podem ocorrer na molécula de testosterona e seus impactos sobre o perfil farmacodinâmico e farmacocinético dos EAA.

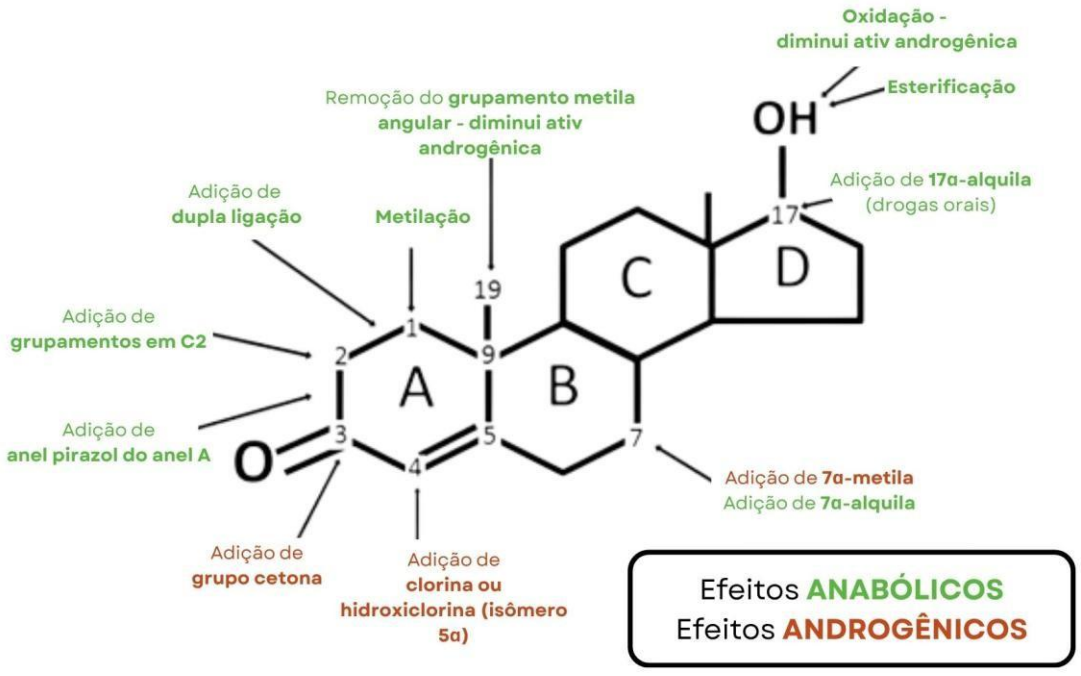


Figura 4. Resumo das modificações na molécula de T que alteram o potencial anabólico e androgênico. Adaptado de Oberlander & Henderson (2012) e Kicman (2008).

1.6 Aplicações terapêuticas dos EAA

O uso clínico dos EAA é determinado por suas características estruturais, seletividade tecidual, perfil farmacocinético e potencial de toxicidade sistêmica (Basaria et al., 2001; Kicman, 2008; Snyder, 1984). A principal indicação terapêutica da terapia androgênica é o hipogonadismo masculino, condição em que os EAA restauram os níveis séricos de T e normalizam funções fisiológicas dependentes do hormônio, com exceção da espermatogênese (Basaria et al., 2001). Além disso, análogos sintéticos como o ND e a OX têm sido empregados no tratamento de osteoporose, anemias refratárias e casos específicos de deficiência androgênica (Handelsman, 2000; Matsumoto & Bremner, 2004; Kicman, 2008). A OX, por exemplo, foi utilizada em meninas com síndrome de Turner e baixa estatura idiopática, embora atualmente seu uso tenha sido substituído pelo hormônio do crescimento (Arslan et al., 2010; Juul, 2001).

Evidências clínicas indicam que os EAA podem favorecer a recuperação em estados hipercatabólicos, como queimaduras graves, politraumatismos e períodos pós-operatórios. Nessas condições, compostos como OX e ND demonstraram reduzir a degradação proteica, melhorar o balanço nitrogenado e acelerar a reabilitação funcional (Bhasin et al., 2010, 2018; Demling & DeSanti, 1997, 2001). Em pacientes queimados, a OX mostrou-se particularmente eficaz em atenuar a resposta hipermetabólica, preservar massa magra e restaurar o peso corporal durante a recuperação (Demling & DeSanti, 1997; Wolf et al., 2006). Embora o impacto sobre desfechos clínicos ainda seja incerto, estudos multicêntricos sugerem que a OX pode aumentar a sobrevida em queimaduras extensas sem elevar a incidência de disfunção de múltiplos órgãos (Wolf et al., 2006).

Em contextos ortopédicos e esportivos, há relatos de benefício dos EAA na recuperação muscular e em procedimentos como reconstrução de ligamento cruzado anterior e artroplastias, embora os ensaios clínicos disponíveis sejam heterogêneos e limitados (Hartgens & Kuipers, 2004; Sinha-Hikim et al., 2002; Woodhouse et al., 2004).

Os EAA também têm sido explorados em síndromes metabólicas associadas à perda de massa muscular, como caquexia relacionada ao HIV, câncer e doenças crônicas debilitantes. Embora revisões sistemáticas indiquem que OX e ND possam atenuar a perda de massa magra, a evidência ainda é insuficiente para recomendar seu uso de rotina, principalmente devido ao risco de eventos adversos (Basaria et al., 2001; Bhasin et al., 2010; Grunfeld et al., 2006). Recentemente, surgiram alternativas como os SARMs, como o enobosarm (EB), que aumentam

a massa magra em ensaios de fase II, mas não demonstraram melhora funcional consistente em ensaios de fase III (Crawford, 2016; Dalton et al., 2011).

Outra aplicação relevante dos EAA é no tratamento de anemias, uma vez que indivíduos com hipogonadismo apresentam níveis reduzidos de hemoglobina em comparação aos eugonadais. A reposição de T ou de seus análogos eleva a hemoglobina por meio da estimulação da eritropoietina renal, promovendo eritropoiese compensatória (Bhasin et al., 2010; Coviello et al., 2008). Em contraste, em homens eugonadais, a administração de T causa apenas discreta elevação da hemoglobina, indicando um efeito dependente do ambiente hormonal. A magnitude dessa resposta varia conforme a formulação e via de administração, ésteres injetáveis de T produzem elevação mais robusta da hemoglobina em comparação às formas orais, devido à maior biodisponibilidade e estabilidade plasmática (Basaria et al., 2001; Cunningham et al., 1979; Snyder, 1984). Da mesma forma, compostos como a ND também elevam os níveis de hemoglobina, reforçando seu potencial terapêutico em anemias refratárias (Gaughan et al., 1997; Malgor & Fisher, 1970).

Em síntese, a eficácia dos EAA na preservação da massa corporal e recuperação metabólica em diversas enfermidades reforça seu potencial clínico. No entanto, o uso seguro e racional requer avaliação criteriosa da estrutura química, perfil farmacodinâmico e risco-benefício individual de cada composto, de modo a maximizar seus efeitos anabólicos e minimizar as complicações sistêmicas (Basaria et al., 2001; Kicman, 2008; Snyder, 1984).

1.7 Efeitos Adversos e Toxicidade Sistêmica dos EAA

O uso não terapêutico de EAA tem se expandido para além do contexto esportivo profissional, atingindo a população geral, especialmente entre indivíduos que buscam melhora estética e de desempenho físico. Estudos indicam que uma proporção significativa de atletas de elite faz uso de EAA, isoladamente ou em combinação, apesar dos riscos conhecidos (Hartgens & Kuipers, 2004). Esse padrão de uso, caracterizado por doses suprafisiológicas, ciclos prolongados e associação com outras substâncias, está relacionado a efeitos adversos multissistêmicos, potencialmente irreversíveis e, em alguns casos, fatais (Bahrke & Yesalis, 2004; Kicman & Gower, 2003; Maravelias et al., 2005; Pope et al., 2014). Os efeitos tóxicos variam conforme o tipo de derivado da T , a via de administração, a frequência e duração do uso, e fatores individuais como idade, sexo e predisposições genéticas (Maravelias et al., 2005).

1.7.1 Efeitos Reprodutivos e Endócrinos

O eixo hipotálamo–hipófise–gonadal é particularmente sensível à retroalimentação negativa induzida pelos EAA. O uso prolongado leva à supressão da secreção endógena de gonadotrofinas, resultando em hipogonadismo secundário, atrofia testicular, oligozoospermia ou azoospermia, e infertilidade potencialmente duradoura (Bhasin et al., 2018; Kanayama & Pope, 2018; Maravelias et al., 2005).

A ginecomastia masculina é comum, decorrente da aromatização de andrógenos em estrógenos. Em mulheres, os EAA promovem virilização, incluindo amenorreia, hirsutismo, engrossamento da voz, hipertrofia clitoriana e alopecia androgênica. A recuperação completa da função gonadal após cessação do uso é incerta, especialmente em usuários crônicos (Wagner, 1991).

1.7.2 Efeitos Cardiovasculares

Há forte evidência de associação entre o uso crônico de EAA e eventos cardiovasculares graves, como dislipidemia aterogênica (redução do HDL e aumento do LDL), hipertensão arterial, trombose, aterosclerose acelerada, hipertrofia ventricular e cardiomiopatia não isquêmica (Baggish et al., 2017; Torrisi et al., 2020; Fadah et al., 2023). Estudos histológicos e de imagem revelam fibrose miocárdica, disfunção sistólica e diastólica, e evidências de dano estrutural persistente mesmo após a suspensão do uso. Casos de infarto agudo do miocárdio em jovens e morte súbita têm sido descritos em múltiplas séries clínicas, reforçando o potencial cardiotóxico cumulativo dos EAA.

1.7.3 Efeitos Hepáticos

Alguns EAA orais, particularmente os 17α-alquilados como oximetolona (OT) e a MTT, estão associados a hepatotoxicidade significativa. As manifestações incluem elevação de transaminases, colestase, hipertensão portal, adenomas hepáticos e, em casos raros, carcinoma hepatocelular (Albano et al., 2021).

A toxicidade é dose e tempo-dependente, sendo agravada pelo uso concomitante de álcool e outras drogas hepatotóxicas. Esses efeitos resultam, em parte, da metabolização oxidativa intensa e do estresse oxidativo hepático induzido pelos metabólitos dos EAA.

1.7.4 Efeitos Dermatológicos

Entre os efeitos cutâneos mais comuns estão acne severa, alopecia androgênica em indivíduos predispostos e estrias associadas à rápida hipertrofia muscular (Albano et al., 2021; van Amsterdam et al., 2010). Esses sinais refletem a hiperatividade sebácea androgênio-dependente e a disfunção na renovação do colágeno dérmico.

1.7.5 Efeitos no Crescimento e Desenvolvimento

Em adolescentes e crianças, os EAA podem causar fechamento prematuro das epífises ósseas, levando à interrupção precoce do crescimento longitudinal e à redução da estatura final (Maravelias et al., 2005). Além disso, mesmo em doses clínicas, foram relatados casos de nefrotoxicidade, hepatotoxicidade e disfunção cardiovascular (Shahidi, 2001).

Em conjunto, os dados disponíveis demonstram que o uso não terapêutico e abusivo de EAA implica em risco substancial de disfunção multissistêmica, com possível dano irreversível a órgãos-alvo e efeitos psiquiátricos duradouros. Essa toxicidade reflete a complexa interação entre estrutura química dos compostos, metabolismo periférico, e susceptibilidade individual, o que reforça a necessidade de modelos experimentais translacionais para investigar seus mecanismos moleculares e neurobiológicos.

1.7.6. Sintomas Neuropsiquiátricos

O abuso de EAA está fortemente correlacionado a alterações comportamentais e neuropsiquiátricas, incluindo episódios maníacos, depressão, agressividade exacerbada, impulsividade, violência e sintomas psicóticos em indivíduos suscetíveis (Kanayama et al., 2009; Pope et al., 2014). A interrupção abrupta do uso pode desencadear síndromes de abstinência graves, com depressão profunda e ideação suicida. Evidências emergentes sugerem que esses efeitos decorrem da modulação dos sistemas serotoninérgico, dopaminérgico e GABAérgico, além de alterações neuroinflamatórias e mitocondriais em regiões límbicas e corticais.

1.8 Efeitos dos EAA no SNC e na Agressividade

Embora os efeitos periféricos dos EAA sobre tecidos musculoesqueléticos sejam amplamente documentados, evidencia-se cada vez mais o impacto profundo dessas substâncias

sobre o SNC. Devido à sua natureza lipofílica, os EAA atravessam facilmente a barreira hematoencefálica e modulam múltiplos processos neurobiológicos por mecanismos genômicos, mediados pelo RA nuclear, e não genômicos, envolvendo receptores de membrana e interações com RE após aromatização para estradiol (Foradori et al., 2008; Miller & Auchus, 2011).

No SNC, os EAA regulam a expressão de genes associados à plasticidade sináptica, mielinização, sobrevivência neuronal e neurogênese, além de modular sistemas neurotransmissores centrais como dopamina, serotonina, GABA e glutamato (Atwi et al., 2016; Hajszan et al., 2008). Em concentrações fisiológicas, podem exercer efeitos moduladores e até neuroprotetores; contudo, quando administrados em doses suprafisiológicas, típicas de regimes de abuso, induzem alterações comportamentais, cognitivas e neuroquímicas, com potenciais consequências neurotóxicas (Basile et al., 2013; Busardò et al., 2015).

1.8.1 Agressividade e disfunção corticolímbica

Entre os efeitos comportamentais mais relatados estão aumento da agressividade, irritabilidade e impulsividade (Pope et al., 2014). Essas manifestações estão relacionadas à modulação anômala de circuitos corticolímbicos, particularmente da amígdala, do hipotálamo e do córtex pré-frontal medial, regiões ricas em RA e altamente sensíveis à ação dos andrógenos (Hauger et al., 2019; Morrison et al., 2016; Westlye et al., 2017). Alterações estruturais e funcionais nessas áreas reduzem o controle inibitório e comprometem a regulação emocional, favorecendo respostas agressivas e impulsivas.

A agressividade, comportamento com função biológica adaptativa (defesa, competição, sobrevivência), torna-se patológica quando desregulada pela ação crônica de EAA, resultando em comportamentos de risco e episódios violentos, incluindo brigas, homicídios e violência doméstica (Choi & Pope, 1994; McGinnis, 2004; Pope & Katz, 1990). A intensidade desses efeitos depende de variáveis individuais e contextuais, como idade, sexo, predisposição genética, ambiente social, dose e tipo de EAA utilizado (Henderson et al., 2006).

1.8.2 Modulação de sistemas neurotransmissores

Os EAA alteram múltiplos sistemas neurotransmissores, promovendo um desequilíbrio neuroquímico que sustenta seus efeitos comportamentais.

- Sistema serotoninérgico: há redução na expressão e disponibilidade de receptores 5-HT_1A e 5-HT_2A em regiões como amígdala e córtex pré-frontal, diminuindo a capacidade inibitória e favorecendo impulsividade e agressividade (Ambar & Chiavegatto, 2009).
- Sistema dopaminérgico: a modulação da via mesolímbica de recompensa aumenta a liberação de dopamina no núcleo accumbens, reforçando padrões compulsivos de uso e contribuindo para disfunção afetiva (Grönbladh et al., 2016b; Naseri et al., 2018).
- Sistema GABAérgico e neuroesteroides: os EAA reduzem a síntese de alopregnanolona, um potente modulador positivo do receptor GABA A, comprometendo os mecanismos ansiolíticos e de regulação emocional, o que intensifica a irritabilidade e a agressividade (Pinna et al., 2008).
- Sistema glutamatérgico: destaca-se a redução da expressão do transportador astrocitário de glutamato (GLT-1), que prejudica a remoção de glutamato da fenda sináptica. O acúmulo extracelular do neurotransmissor leva à hiperexcitabilidade neuronal, influxo excessivo de Ca^{2+}, disfunção mitocondrial e excitotoxicidade, comprometendo a homeostase sináptica (Fischer et al., 2007; Kalinine et al., 2014; Le Grevès et al., 1997; Rodolphi et al., 2021).

Esses mecanismos apontam para uma interface crítica entre disfunção astroglial e circuitos de controle emocional, sugerindo que a regulação glial do glutamato constitui um alvo chave para mitigar os efeitos neurocomportamentais dos EAA.

1.8.3 Efeitos sobre cognição e morfologia cerebral

A exposição prolongada a EAA em altas doses está associada a prejuízos cognitivos e alterações morfofuncionais cerebrais. Estudos de neuroimagem em usuários crônicos demonstraram redução do volume do hipocampo, alterações na conectividade funcional do córtex pré-frontal e déficits de memória de trabalho, tomada de decisão e flexibilidade cognitiva (Kaufman et al., 2015; Westlye et al., 2017).

Essas alterações se correlacionam com prejuízos nas funções executivas, incluindo planejamento e controle inibitório, indicando que os EAA podem comprometer circuitos neurais essenciais à autorregulação e aprendizado adaptativo (Bjørnebekk et al., 2021).

Em nível celular, os EAA exercem efeitos duais: em doses fisiológicas, promovem mielinização e plasticidade sináptica (Foradori et al., 2008), enquanto em doses

suprafisiológicas induzem estresse oxidativo, neuroinflamação e apoptose neuronal, implicando um potencial efeito neurotóxico cumulativo (Pomara et al., 2015).

1.8.4 Efeitos afetivos e psiquiátricos

O uso crônico e a abstinência de EAA associam-se a sintomas depressivos, ansiedade, ideação suicida e transtornos de humor, especialmente durante a supressão pós-ciclo do eixo hipotálamo–hipófise–gonadal, quando há queda abrupta dos níveis endógenos de T (Karagun & Altug, 2024; Thiblin et al., 1999). Esses resultados reforçam o papel dos EAA na desregulação neuroendócrina e afetiva, sugerindo uma interseção entre disfunção mitocondrial, inflamação neural e desequilíbrio sináptico como base fisiopatológica dos efeitos psiquiátricos observados.

No Quadro 1 apresentamos um resumo dos efeitos adversos do abuso de EAA.

Órgão/Sistema	Efeitos adversos	Referências
Fígado	Icterícia; carcinoma hepático; peliose; hepatite; lesões benignas	Hartgens & Kuipers (2004)
Rins	Glomeruloesclerose; dano túbulo-intersticial; níveis elevados de ureia sérica e ácido úrico; hiperfosfatemia	Maravelias et al. (2005)
Sistema cardiovascular	Arteriosclerose; formação de trombos; vasoespasmo de artéria coronária; isquemia miocárdica; insuficiência cardíaca	Melchert & Welder (1995)
Sistema reprodutor	Diminuição da contagem e motilidade dos espermatozoides	Christou et al. (2017)
Hematologia	Aumento da contagem de hemácias, hemoglobina e hematócrito	Mingxing & Yanfei (2025)
Efeitos dermatológicos	Acne; alopecia	Walker & Adams (2009)
Efeitos musculoesqueléticos	Retardo do crescimento; deterioração da plasticidade muscular; destruição aguda do músculo esquelético	Mingxing & Yanfei (2025)
Efeitos comportamentais	Dependência/vício; hostilidade; agressividade; episódios psicóticos e maníacos; aumento da autoestima; paranoia	van Amsterdam et al. (2010)

Quadro 1. Efeitos adversos do abuso de EAA. Lista de efeitos colaterais em diferentes órgãos devido ao uso prolongado de EAA.

1.9 Mitocôndria: estrutura e função

A história das mitocôndrias antecede em muito o reconhecimento de seu papel central na bioenergética celular. Os primeiros registros dessas estruturas datam do século XIX. Em 1841, Friedrich Gustav Jacob Henle descreveu grânulos subcelulares utilizando microscopia óptica, referindo-se a eles com diversos termos, como plastocôndrias e microssomas (Müller & Henle, 1841). Posteriormente, Richard Altmann (1890) observou estruturas granulares em diferentes tipos celulares e destacou sua semelhança com bactérias, propondo que se tratava de componentes autônomos intracelulares, aos quais denominou bioblastos, definidos como a "menor unidade morfológica do material organizado". Altmann sugeriu ainda que esses bioblastos poderiam representar organismos vivos residentes no interior das células eucarióticas (Altmann, 1890).

O termo mitocôndria foi introduzido por Carl Benda, em 1898, derivado das palavras gregas mitos ("fio") e chondrion ("grão"), refletindo sua aparência filamentar e granular característica (Benda, 1898). A compreensão funcional das mitocôndrias surgiu posteriormente. Embora sua morfologia já fosse conhecida, sua importância fisiológica foi reconhecida à medida que se estabeleceu a relação entre essas organelas e os processos de respiração celular e produção de energia. A noção de que a respiração seria uma "combustão lenta de carbono e hidrogênio" havia sido proposta por Antoine Lavoisier, em 1790, antecipando o conceito bioquímico de oxidação celular. Com o avanço da bioquímica e da microscopia eletrônica no século XX, consolidou-se o entendimento das mitocôndrias como o principal centro de produção de adenosina trifosfato (ATP) e elementos centrais da evolução dos organismos eucarióticos (Ernster & Schatz, 1981; Pagliarini & Rutter, 2013).

As mitocôndrias estão presentes em praticamente todas as células eucarióticas e são caracterizadas por uma membrana dupla, uma externa lisa e uma interna altamente invaginada, formando as cristas mitocondriais. Essa compartimentalização cria gradientes iônicos e metabólicos essenciais para os processos bioenergéticos. Essas organelas são responsáveis pelo consumo de O_2 e pela fosforilação oxidativa, sendo reconhecidas como os principais centros de geração de energia celular. Além disso, participam de processos vitais como a síntese de metabólitos, a regulação da homeostase de Ca^{2+} intracelular e o controle de vias de morte celular programada (Khacho et al., 2019).

A mitocôndria é delimitada por duas membranas concêntricas, a membrana mitocondrial externa (MME) e a membrana mitocondrial interna (MMI), que definem três compartimentos: o espaço intermembranas (EI), a matriz mitocondrial (MM) e as próprias

membranas (Cogliati et al., 2016). A MME atua como interface entre a organela e o citoplasma, sendo relativamente permeável a pequenas moléculas e íons devido à presença de porinas (POR), canais iônicos dependentes de voltagem. Essa membrana também contém proteínas da família TOM (Translocases of the Outer Membrane), responsáveis pelo transporte de precursores proteicos sintetizados no citosol (Kühlbrandt, 2015).

Além disso, a MME abriga proteínas envolvidas na dinâmica mitocondrial, como as mitofusinas 1 e 2 (Mfn1 e Mfn2), que participam da fusão da membrana externa, e a proteína de fissão mitocondrial 1 (FIS1), mediadora do processo de fissão. Também estão presentes proteínas da família BCL-2, como a BAK (Bcl-2 antagonist/killer), essenciais na regulação da permeabilidade mitocondrial e na indução de apoptose (Cogliati et al., 2016).

A comunicação entre a MME e a MMI depende de interações proteicas específicas. A Gαq/11, localizada na MME, interage com a proteína de atrofia óptica 1 (OPA1) da MMI, enquanto a proteína SAM (Sorting and Assembly Machinery) conecta-se ao complexo MICOS (Mitochondrial Cristae Organizing System), fundamental para a formação e manutenção das cristas mitocondriais (Cogliati et al., 2016). A MME apresenta ainda regiões especializadas de contato com o retículo endoplasmático, denominadas MAMs (Mitochondria-Associated Membranes), que regulam a troca de lipídios e de Ca^{2+} entre as duas organelas (Hayashi et al., 2009). Além disso, junções intermitocondriais (JIMs) permitem a comunicação entre diferentes mitocôndrias, contribuindo para a coordenação funcional dentro da rede mitocondrial (Picard et al., 2015).

O EI é delimitado pela MMI e pelas cristas mitocondriais, que o separam da matriz (Cogliati et al., 2016). A MMI é altamente seletiva e contém proteínas TIM (Translocases of the Inner Membrane), responsáveis pela importação de proteínas para a matriz. Ela também abriga a OPA1 e cardiolipina, um fosfolipídio essencial para a montagem e estabilidade dos complexos do sistema de transporte de elétrons (STE) (Cogliati et al., 2016).

A OPA1 é regulada por proteínas da MME, como a Gαq/11, e por proteínas intrínsecas da MMI, incluindo PARL (Presenilin-Associated Rhomboid-Like protein), ROMO1 (Reactive Oxygen Modulator 1) e proteases da matriz (m-AAA). O funcionamento da OPA1 também depende do complexo PHB–SLP2 (Prohibitin–Stomatin-Like Protein 2), ancorado à cardiolipina (Cogliati et al., 2016). Essas interações são cruciais para a integridade estrutural das cristas e para a organização dos complexos do STE (Frey & Mannella, 2000).

As cristas mitocondriais não são meras invaginações da MMI, mas estruturas dinâmicas e altamente especializadas. Elas são separadas do EI por junções tubulares estreitas, cuja arquitetura é regulada principalmente pela OPA1 e pelo complexo MICOS. Essa organização

cria compartimentos funcionais que otimizam a difusão de moléculas e maximizam a eficiência bioenergética (Cogliati et al., 2013).

A Figura 5 (Devlin, 2011) ilustra a estrutura mitocondrial: a MME, a MMI e o EI, que contém enzimas importantes do metabolismo energético, como adenilato cinase e creatina cinase. O interior delimitado pela MMI corresponde à MM, onde se concentram as enzimas do ciclo de Krebs (CK) e da β-oxidação, essenciais para a produção de energia celular (Devlin, 2011).

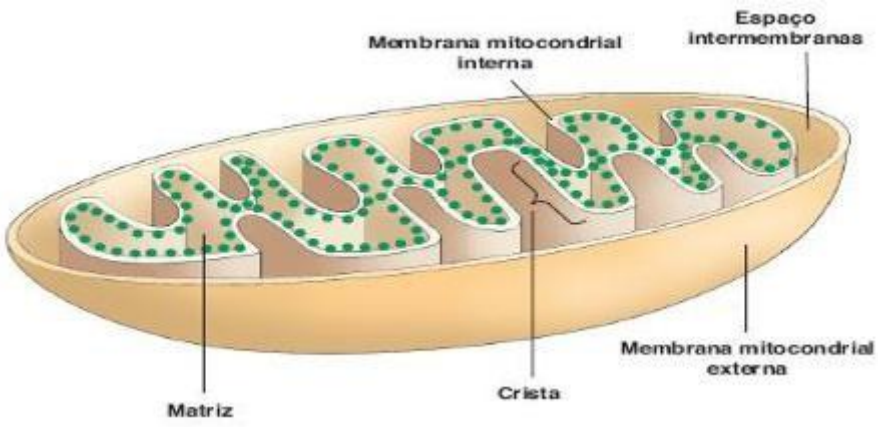


Figura 5. Organização estrutural da mitocôndria. Na figura estão indicadas a membrana mitocondrial externa, a membrana mitocondrial interna, o espaço intermembranas, as cristas mitocondriais e a matriz mitocondrial. Os pontos verdes representam a porção F_1 do complexo V-ATP sintase.

1.10 Evolução do entendimento da função mitocondrial e a teoria quimiosmótica

Durante grande parte do século XX, a compreensão sobre a função mitocondrial esteve centrada na ideia de que essas organelas atuavam primordialmente como “usinas de energia” celulares, responsáveis por converter a energia química proveniente da oxidação de substratos carbonados em ATP. Esse conceito consolidou-se à medida que os mecanismos de oxirredução e transporte eletrônico foram sendo elucidandos com maior precisão (Mitchell, 1961).

Um ponto de inflexão nesse campo ocorreu com a formulação da Teoria Quimiosmótica, proposta por Peter Dennis Mitchell em 1961. Em contraposição ao modelo químico direto de acoplamento vigente na época, Mitchell postulou que a energia liberada durante as reações de oxidação no STE seria convertida em um gradiente eletroquímico de prótons através da MMI. Esse gradiente, por sua vez, serviria como força motriz para a síntese de ATP (Mitchell, 1961).

Posteriormente, Mitchell expandiu esse modelo e formalizou o conceito de acoplamento quimiosmótico, no qual a energia livre gerada pela oxidação dos nutrientes é temporariamente armazenada como potencial de membrana ($\Delta\psi$). Dessa forma, os processos de transporte eletrônico, metabolismo e síntese de ATP foram unificados em um único sistema de transdução de energia (Mitchell, 1966). Por essa formulação e pela demonstração de seu papel central na bioenergética celular, Peter Mitchell foi agraciado com o Prêmio Nobel de Química em 1978. Essa descoberta representou uma verdadeira mudança de paradigma, permitindo compreender as mitocôndrias não apenas como geradoras de energia, mas também como centros de regulação da homeostase redox, da sinalização celular e da morte celular programada (Khacho et al., 2019).

De acordo com o modelo quimiosmótico, os complexos I, III e IV da CTE utilizam a energia liberada durante a transferência de elétrons provenientes dos equivalentes redutores NADH e $FADH_2$ para bombear prótons (H^+) da MM para o EI. Esse processo gera a chamada força próton-motriz ($\Delta\rho$), composta por dois componentes: uma diferença de potencial elétrico e um gradiente de concentração de prótons (ΔpH) através da MMI (Mitchell, 1961; Deshpande & Mohiuddin, 2025; Jonckheere et al., 2012).

A energia acumulada nesse gradiente é então utilizada pela FOF_1-ATP sintase (ou ATPase mitocondrial) para catalisar a síntese de ATP a partir de ADP e fosfato inorgânico (Pi). O fluxo de prótons através do domínio FO impulsiona a rotação mecânica do domínio F_1, localizado na matriz, promovendo as mudanças conformacionais necessárias para a formação da ligação fosfodiéster do ATP (Jonckheere et al., 2012b; Walker, 2013).

O processo culmina na redução do O_2, que atua como aceptor final de elétrons, resultando na formação de água (H_2O), um evento que envolve a transferência de quatro elétrons e quatro prótons para cada molécula de O_2 (Alabduladhem & Bordoni, 2025). Em paralelo, na MM, ocorrem reações de descarboxilação oxidativa mediadas pelo complexo da piruvato desidrogenase (PDH) e pelas desidrogenases do CK, como a isocitrato desidrogenase (IDH) e o complexo α-oxoglutarato desidrogenase (OGDH). Esses processos liberam CO_2 e produzem equivalentes redutores (NADH e $FADH_2$) que alimentam continuamente o STE (Alabduladhem & Bordoni, 2025).

Assim, o metabolismo mitocondrial representa uma forma controlada e altamente eficiente de combustão biológica, na qual moléculas orgânicas são oxidadas a CO_2 e H_2O. Diferentemente da combustão química direta descrita por Lavoisier, a energia liberada não é dissipada integralmente como calor, mas armazenada transitoriamente no gradiente de H^+,

permitindo a síntese contínua de ATP e a manutenção da homeostase energética celular (Cooper, 2000; Nolfi-Donegan et al., 2020).

Conceitualmente, a oxidação mitocondrial de substratos no CK, e adicionalmente no citoplasma, por meio das lançadeiras malato-aspartato e glicerol-3-fosfato, direciona os elétrons do NADH e $FADH_2$ para o STE, localizada na MMI (Nelson & Cox, 2023). Esses elétrons fluem sequencialmente através de complexos multiproteicos e transportadores móveis, culminando na redução do O_2 a H_2O. Concomitantemente, a energia liberada por essas reações redox é utilizada para bombear H^+, reforçando o gradiente eletroquímico que impulsiona a síntese de ATP (Ahmad et al., 2025; Nolfi-Donegan et al., 2020; Mitchell, 1961).

O STE é composto por cinco grandes complexos enzimáticos (Figura 6A):

- Complexo I (NADH:ubiquinona oxidorredutase), que catalisa a oxidação do NADH e transfere elétrons para a ubiquinona (coenzima Q);
- Complexo II (succinato desidrogenase), que integra o CK e transfere elétrons do succinato para a ubiquinona, sem bombeamento de H^+;
- Complexo III (ubiquinol:citocromo c oxidorredutase), que transfere elétrons do ubiquinol (QH_2) para o citocromo c;
- Complexo IV (citocromo c oxidase), que realiza a transferência final de elétrons do citocromo c reduzido para o O_2, produzindo H_2O;
- Complexo V (F_1F_0-ATP sintase), responsável pela fosforilação oxidativa, acoplando o refluxo de H^+ à síntese de ATP (Walker, 2013).

Evidências recentes demonstram que esses complexos não estão dispostos de maneira aleatória na MMI, mas organizados em supercomplexos respiratórios, ou respirossomos (Figura 6B), estruturas supramoleculares que otimizam o fluxo de elétrons, minimizam a produção de ERO e aumentam a eficiência bioenergética (Cogliati et al., 2016; Lenaz & Genova, 2010).

Esses supercomplexos podem apresentar composições diversas, como I–III–IV, II–III–IV e o arranjo clássico I–V, convergindo funcionalmente para os dímeros do Complexo V (F_1F_0-ATP sintase), localizados preferencialmente nas cristas mitocondriais, regiões em que o gradiente de H^+ é mais intenso (Cogliati et al., 2016).

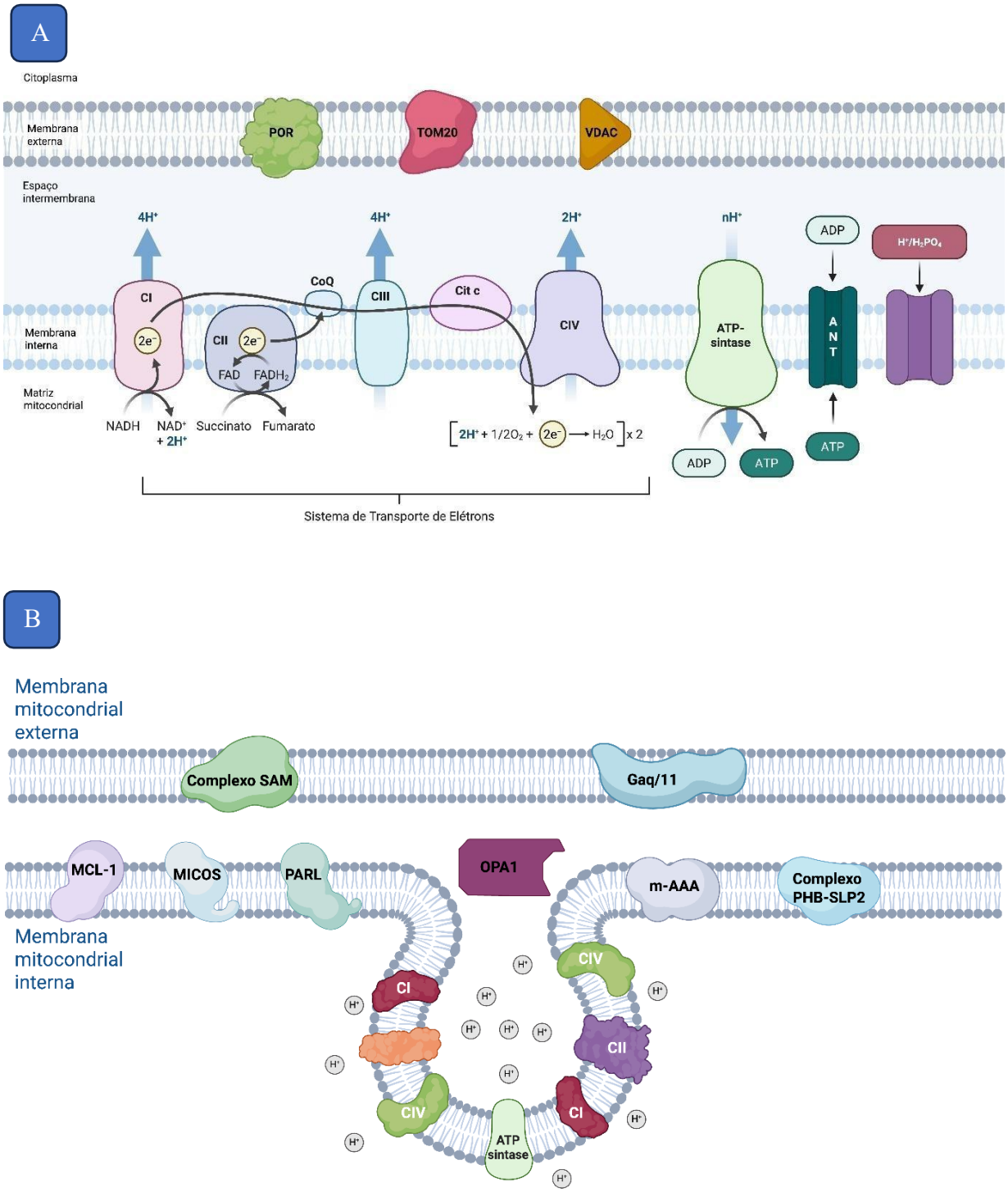


Figura 6. O Sistema de Transporte de Elétrons. (A) A membrana mitocondrial externa (OM) contém proteínas principais, incluindo porinas (POR), o canal aniônico dependente de voltagem (VDAC) e as translocases de proteínas da membrana externa (TOM). Na membrana mitocondrial interna (IM) localizam-se os componentes do STE (complexos I a V), o translocador de nucleotídeos de adenina (ANT) e o translocador de fosfato (PT). A matriz mitocondrial abriga os componentes restantes envolvidos na bioenergética, incluindo coenzimas em suas formas redox (NADH/NAD^+ e $FADH_2$/FAD^+), prótons (H^+), adenosina difosfato (ADP) e adenosina trifosfato (ATP). (B) Modelo proposto para a organização dos

complexos da MMI em supercomplexos, geralmente localizados nas cristas mitocondriais. Nesse modelo, os complexos de transporte de elétrons estão organizados de forma hierárquica para otimizar a função do complexo V (F_1F_0-ATP sintase)**.** Proteínas adicionais presentes tanto na MMI quanto na MME participam da montagem proteica**,** regulação da arquitetura das cristas mitocondriais**,** biogênese **e** manutenção da integridade estrutural da organela.

1.11 A arquitetura funcional das cristas e o papel bioenergético do Δψ

A organização tridimensional das cristas mitocondriais confere à organela uma arquitetura altamente especializada, capaz de maximizar a transdução de energia e otimizar a comunicação entre os módulos oxidativos e fosforilativos. Essa estrutura, sustentada por interações precisas entre proteínas da membrana interna e lipídios como a cardiolipina, é essencial para manter a eficiência metabólica e a homeostase bioenergética (Cogliati et al., 2013; Frey & Mannella, 2000).

A separação de H^+ através da MMI gera um potencial eletroquímico transmembrana, conhecido como força próton-motriz (Δρ). Esse potencial é composto por dois elementos interdependentes: um componente elétrico, representado pelo Δψ; e um componente químico, correspondente ao gradiente de pH (ΔpH) entre a MM e o EI. Ambos podem ser expressos em milivolts (mV) e refletem a capacidade da mitocôndria de armazenar energia potencial de maneira reversível (Mitchell, 1961).

A manutenção de Δρ depende do fluxo ordenado de elétrons ao longo do STE, mediado pelos complexos I, III e IV, e pelos transportadores móveis ubiquinona (CoQ) e citocromo c. Esse fluxo segue o gradiente termodinâmico definido pelo potencial redox (E′₀) dos pares envolvidos, culminando na redução do O_2, o aceptor final de elétrons. O par $NADH/NAD^+$ apresenta E′₀ de cerca de –320 mV, enquanto o par O_2/H_2O possui E′₀ de +820 mV, estabelecendo uma forte tendência para a transferência de elétrons do NADH para o O_2 (Nelson & Cox, 2023).

O potencial eletroquímico pode ser modulado pelo vazamento de H^+, que ocorre de duas formas: Induzível, mediado por proteínas desacopladoras (UCPs), as quais dissipam o gradiente de maneira controlada, regulando a termogênese e limitando a produção de ERO; não induzível, associada a condições patológicas, nas quais o gradiente é dissipado de forma desordenada, comprometendo a síntese de ATP e a integridade mitocondrial.

Além disso, o Complexo II (succinato desidrogenase) desempenha papel fundamental ao transferir elétrons do succinato para a ubiquinona, alimentando o Complexo III independentemente do Complexo I. Esse mecanismo, conhecido como fluxo convergente,

aumenta a flexibilidade bioenergética e confere resiliência ao sistema respiratório (Gnaiger, 2014).

Embora o Δρ seja classicamente reconhecido por impulsionar a síntese de ATP via F_1F_0-ATP sintase, sua função vai muito além da fosforilação oxidativa. Ele participa ativamente de diversos processos fisiológicos, como a regulação do metabolismo durante hipóxia ou isquemia. Nessas condições, a privação de O_2 leva à dissipação do gradiente de H^+ e à redução da produção de ATP mitocondrial, ativando uma resposta compensatória glicolítica baseada nas reservas de glicogênio. Parte do ATP resultante é consumida pela própria mitocôndria para manter o Δψ através da atividade reversa da F_1F_0-ATP sintase (Di Lisa & Bernardi, 1998).

A separação de cargas também gera uma MM eletricamente negativa, condição que favorece o influxo de cátions e moléculas catiônicas. Esse potencial é crucial para processos como: o transporte e importação de proteínas para a matriz (Geissler et al., 2000); a regulação da sinalização intracelular e da qualidade mitocondrial; a retenção do citocromo c no EI, impedindo sua liberação para o citosol e, portanto, suprimindo a ativação apoptótica até que estímulos de estresse mitocondrial sejam deflagrados (Gottlieb et al., 2003).

Assim, o Δρ emerge não apenas como um mecanismo de geração de ATP, mas como um eixo integrador da fisiologia mitocondrial, conectando a bioenergética, a sinalização celular e o controle da morte programada.

1.12 Sinalização celular, ERO e manejo do Ca^{2+} mitocondrial

As mitocôndrias desempenham papel duplo nas células: além de gerarem energia, funcionam como centros integradores de sinalização, capazes de traduzir seu estado bioenergético em respostas adaptativas celulares. Essa comunicação ocorre por meio da liberação de metabólitos e ERO, bem como através de microdomínios de contato interorganelar, como as membranas associadas à mitocôndria (MAM) formadas com o retículo endoplasmático (Chandel, 2015; Rowland & Voeltz, 2012; van Vliet et al., 2014). A MME também serve como plataforma de ancoragem para proteínas sinalizadoras citosólicas (Chandel, 2014; Q. Chen et al., 1997).

Entre as moléculas sinalizadoras derivadas da mitocôndria, o H_2O_2 se destaca por sua relativa estabilidade e capacidade de difusão através de membranas biológicas, atuando como mensageiro intracelular e parácrino. No meio int**ra**celular, o H_2O_2 é produzido principalmente por oxidases, como a família NADPH oxidase, e pela transferência parcial de elétrons no STE, resultando na formação de superóxido ($O_2^{\bullet -}$) e subsequente dismutação em H_2O_2 (Mailloux,

2015).Foram identificados cerca de 11 sítios mitocondriais de produção de $O_2^{\bullet -}/H_2O_2$, localizados em desidrogenases da matriz e da MMI e em oxidases associadas à MME. Esses sítios estão funcionalmente organizados em torno de dois pares redox principais: $NADH/NAD^+$ e QH_2/Q, cuja razão determina a taxa de geração de ERO. Assim, um aumento na razão $NADH/NAD^+$ ou QH_2/Q intensifica a produção de $O_2^{\bullet -}$ e H_2O_2. O grupo QH_2/Q, em particular, mostra sensibilidade à $\Delta\rho$ e ao $\Delta\Psi$ (Korshunov et al., 1997).

O H_2O_2 regula a função celular por meio da oxidação reversível de resíduos de cisteína em proteínas-alvo, formando ácido sulfênico (Cys-SOH), posteriormente reduzido por glutationa e outras vias redox (Finkel, 2012). Essa modificação pós-traducional constitui um mecanismo de sinalização redox reversível e altamente específico. Diversas proteínas-chave são moduladas por H_2O_2, incluindo: Fosfatases como PTP1B e PTEN (U et al., 2003; Kwon et al., 2004); Enzimas glicolíticas, como a GAPDH (Hildebrandt et al., 2015); GTPases reguladoras (Heo, 2011); Cinases como a IKK, cuja oxidação inibe a ativação do fator NF-κB (Korn et al., 2001). Essas vias redox modulam adaptação ao estresse, proliferação, diferenciação e migração celular, desempenhando papel essencial no desenvolvimento e na resposta ao dano tecidual (Zhang et al., 2016).

O Ca^{2+} é outro mensageiro intracelular fundamental, regulando processos de excitação, metabolismo e apoptose. Em condições basais, sua concentração citosólica é mantida em torno de 100 nM, graças a um delicado equilíbrio entre influxo, efluxo e armazenamento (Brini & Carafoli, 2011). Nas mitocôndrias, o uniporte de Ca^{2+} mitocondrial (MCU) é o principal canal responsável pela entrada de Ca^{2+} através da MMI, sensível a aumentos transitórios do Ca^{2+} citosólico. A ativação do MCU promove influxo de Ca^{2+}, que estimula a atividade de enzimas-chave do CK, PDH, IDH e α-cetoglutarato desidrogenase, e aumenta a eficiência respiratória pelos complexos III e V do STE (Glancy & Balaban, 2012; Tarasov et al., 2012).

O efluxo de Ca^{2+} da matriz é mediado pelo trocador sódio/cálcio (NCLX), que exporta um íon Ca^{2+} em troca de três íons Na^+, garantindo o equilíbrio entre entrada e saída do íon (Emrich et al., 2022; Jafri & Kumar, 2014; Majumder, 2025). Quando o influxo supera a capacidade de extrusão, ocorre sobrecarga mitocondrial de Ca^{2+}, evento altamente deletério. O excesso de Ca^{2+} leva à ruptura da integridade da membrana, aumento da geração de ERO e falência irreversível da fosforilação oxidativa. Tal desequilíbrio pode resultar de: liberação exacerbada de Ca^{2+} pelo retículo endolplasmático e influxo extracelular; redução da atividade do NCLX, ou; falha no tamponamento mitocondrial de Ca^{2+} (Lemasters et al., 2009; Luongo et al., 2017; Walkon et al., 2022).

O comprometimento da homeostase de Ca^{2+} constitui um marcador precoce de neurodegeneração, com relevância direta no traumatismo cranioencefálico (TCE). Nesse contexto, a tríade disfunção mitocondrial, sobrecarga de Ca^{2+}, estresse oxidativo atua sinergicamente, amplificando a morte celular e a perda de função neuronal (Calì et al., 2012; Mattson, 2007).

1.13 Integração funcional entre retículo endoplasmático e mitocôndria

A interface física entre o retículo endoplasmático e a mitocôndria é mediada por regiões especializadas conhecidas como MAMs. Essas zonas de contato são estruturas altamente dinâmicas que permitem o intercâmbio de sinais, lipídeos e íons Ca^{2+}, coordenando a função metabólica e a sobrevivência celular (Rowland & Voeltz, 2012; van Vliet et al., 2014).

O Ca^{2+} liberado do retículo endoplasmático, principalmente através dos receptores de inositol 1,4,5-trifosfato ($InsP_3R$), é rapidamente captado pela mitocôndria via MCU. Esse fluxo coordenado de Ca^{2+} entre as organelas modula a atividade de enzimas desidrogenases da MM, como PDH, IDH e α-cetoglutarato desidrogenase, e regula o fluxo de elétrons no STE (Glancy & Balaban, 2012; Tarasov et al., 2012). Esse acoplamento funcional retículo endoplasmático–mitocôndria permite ajustar a produção de ATP à demanda metabólica e integrar os eixos de bioenergética e sinalização celular (Tarasov, Griffiths et al., 2012).

Entretanto, em condições de estresse celular, como isquemia ou TCE, o aumento exacerbado do Ca^{2+} transferido do retículo endoplasmático para as mitocôndrias pode provocar sobrecarga de Ca^{2+}, ruptura da integridade da membrana mitocondrial, elevação da produção de ERO e ativação de vias apoptóticas (Calì et al., 2012; Mattson, 2007). Assim, o mesmo sistema que, em condições fisiológicas, sustenta a eficiência metabólica, torna-se um elo crítico de vulnerabilidade em estados patológicos.

A produção de ERO, em especial o H_2O_2, também participa ativamente dessa comunicação interorganelar. As ERO mitocondriais podem oxidar reversivelmente resíduos de cisteína em proteínas-alvo, modulando enzimas e fatores de transcrição envolvidos em adaptação ao estresse, proliferação e morte celular. Entre essas proteínas estão PTP1B, PTEN, GAPDH, GTPases e cinases como IKK, cuja inibição redox regula a ativação do NF-κB (Finkel, 2012; Zhang et al., 2016).

Por outro lado, o estresse do retículo endoplasmático (representado na figura 7), induzido pelo acúmulo de proteínas mal enoveladas, ativa a resposta a proteínas mal dobradas (UPR) e pode desencadear apoptose via CHOP/GADD153, JNK e caspase-12. Além das vias

dependentes de UPR, há mecanismos que envolvem efluxo de Ca^{2+} do retículo endoplasmático para as mitocôndrias, promovendo a liberação de fatores pró-apoptóticos, como o citocromo c (Oyadomari & Mori, 2004; Strasser et al., 2000).

Dessa forma, a comunicação retículo endoplasmático–mitocôndria constitui uma plataforma integradora de sinais metabólicos e de estresse, coordenando homeostase de Ca^{2+}, geração de ATP e decisões de vida ou morte celular. A disfunção dessa interface, observada em doenças neurodegenerativas e no TCE, desorganiza o equilíbrio entre bioenergética e sinalização apoptótica, favorecendo a degeneração neuronal progressiva (Marchi et al., 2018; Ureshino et al., 2019).

Perturbações nas MAMs podem amplificar a neurodegeneração pós-TCE ao intensificar a sobrecarga mitocondrial de Ca^{2+} e o estresse oxidativo, levando à falha energética e ativação de vias apoptóticas dependentes de CHOP e citocromo c. A restauração funcional das MAMs e do controle Ca^{2+}, ERO pode representar um alvo terapêutico estratégico para limitar o dano secundário e preservar a viabilidade neuronal.

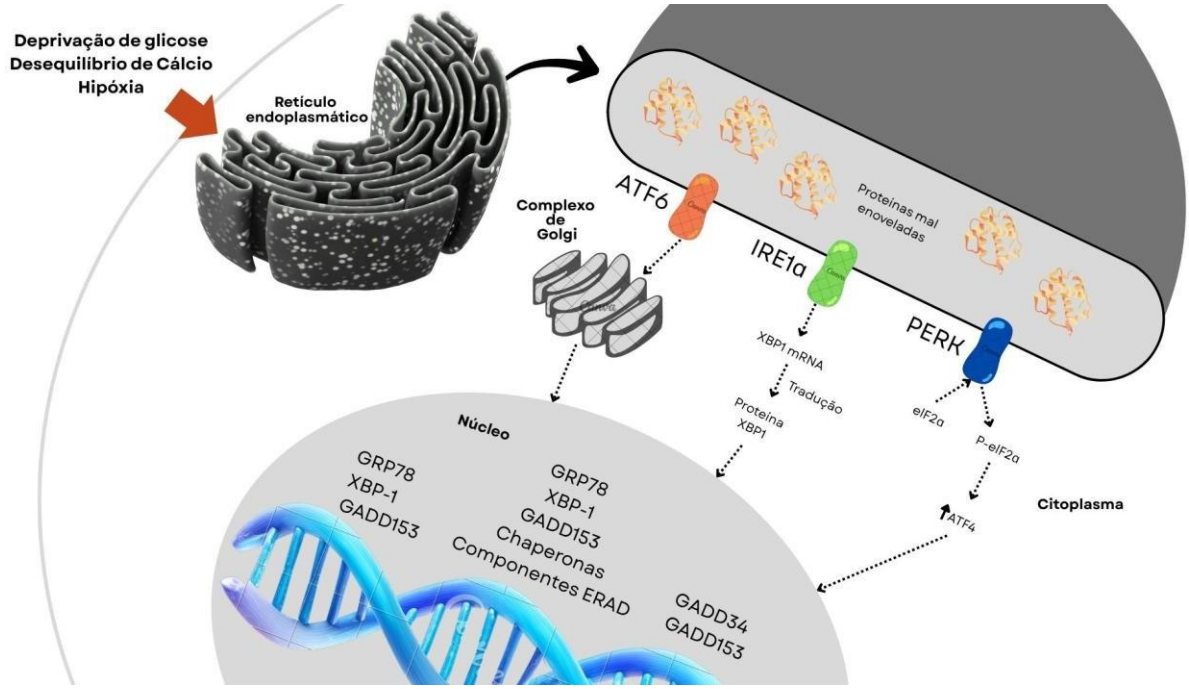


Figura 7. Estresse do retículo endoplásmatico. Vias associadas a resposta a proteínas mal enoveladas (UPR). Em condições de homeostasia, a chaperona GRP78 se liga às proteínas transmembrana ATF6, IRE1α e PERK, mantendo-as inativas. Sob estresse do retículo endoplasmático, o acúmulo de proteínas mal enoveladas promove a dissociação de GRP78, permitindo a oligomerização e ativação dessas proteínas sensoras. A ativação de IRE1α desencadeia sua atividade ribonuclease, gerando XBP1, um fator de transcrição que induz genes associados ao restabelecimento do enovelamento protéico e à degradação de proteínas

defeituosas (ERAD). A ativação de PERK leva à fosforilação de eIF2α, reduzindo globalmente a tradução de mRNAs, exceto de alguns, como ATF4, que induz genes relacionados à homeostase do retículo endoplasmático e à autofagia. A liberação de ATF6 permite seu transporte ao complexo de Golgi, onde sofre clivagem proteolítica e gera a forma ativa que regula a expressão de genes da UPR e ERAD. Quando o estresse do retículo endoplasmático persiste, as vias mediadas por ATF4**,** XBP1 **e** ATF6 convergem para a indução de GADD153 (CHOP)**,** que modula a expressão de genes como BIM e Bcl-2, culminando em apoptose celular. Figura adaptada de Hersey & Zhang (2008).

1.14 Mitocôndrias sinápticas e extrasinápticas

O tecido nervoso apresenta notável heterogeneidade mitocondrial, refletindo a diversidade funcional das populações celulares e a especialização regional das organelas. Em preparações cerebrais, distinguem-se duas populações principais: mitocôndrias sinápticas, localizadas em terminais axonais e espinhas dendríticas, e mitocôndrias extrasinápticas, derivadas da soma neuronal, astrócitos, oligodendrócitos e células endoteliais (Flippo & Strack, 2017; Kulbe et al., 2017; Stauch et al., 2014; Völgyi et al., 2015a).

Essas populações diferem marcadamente em capacidade bioenergética, cinética de Ca^{2+}, composição proteômica e resistência ao estresse oxidativo. As mitocôndrias sinápticas, derivadas de neurônios, exibem alto acoplamento entre consumo de O_2 e fosforilação oxidativa, permitindo responder rapidamente às flutuações de energia durante a neurotransmissão (Kulbe et al., 2017; Song et al., 2024). Já as mitocôndrias extrasinápticas apresentam perfil metabólico mais heterogêneo, refletindo a contribuição de células gliais que dependem mais da glicólise do que da fosforilação oxidativa (Bolaños, 2016; Mulica et al., 2021; Rose et al., 2020). Essa distinção confere às mitocôndrias sinápticas maior vulnerabilidade a falhas no suprimento energético e ao colapso do $\Delta\Psi$ sob sobrecarga de Ca^{2+}, enquanto as extrasinápticas possuem maior flexibilidade metabólica e capacidade antioxidante (L. Chen et al., 2023; Granath-Panelo & Kajimura, 2024).

O NCLX desempenha papel central nesse equilíbrio, controlando o efluxo de Ca^{2+} e o restabelecimento do $\Delta\Psi$ após sobrecarga (Fan et al., 2025; Ramos et al., 2024). Diferenças na regulação do NCLX entre mitocôndrias sinápticas e extrasinápticas podem explicar a vulnerabilidade seletiva de regiões cerebrais a insultos metabólicos e ao uso de EAA, que modulam o transporte de Ca^{2+} e favorecem o colapso mitocondrial (Hagenston et al., 2021; Palty et al., 2010; Stavsky et al., 2021).

Modelos experimentais mostram que compostos como o ND aumentam a produção de H_2O_2 e alteram o $\Delta\Psi$ em ambas as populações mitocondriais, promovendo neurodegeneração

apoptótica (Carteri et al., 2019; Turillazzi et al., 2016). Disfunções em mitocôndrias sinápticas são frequentemente eventos iniciais na perda de conectividade neuronal em modelos de isquemia, TCE e doenças neurodegenerativas (Brown et al., 2006; Song et al., 2024).

Sendo assim, estratégias que visem otimizar o acoplamento respiratório, potencializar a função do NCLX ou reforçar os sistemas antioxidantes emergem como abordagens promissoras para preservar a função sináptica e reduzir o dano neuronal (Fan et al., 2025; Ramos et al., 2024).

A distinção entre as populações mitocondriais, portanto, é essencial não apenas para compreender a fisiologia cerebral, mas também para o desenvolvimento de intervenções farmacológicas direcionadas à proteção mitocondrial em condições de excitotoxicidade e trauma.

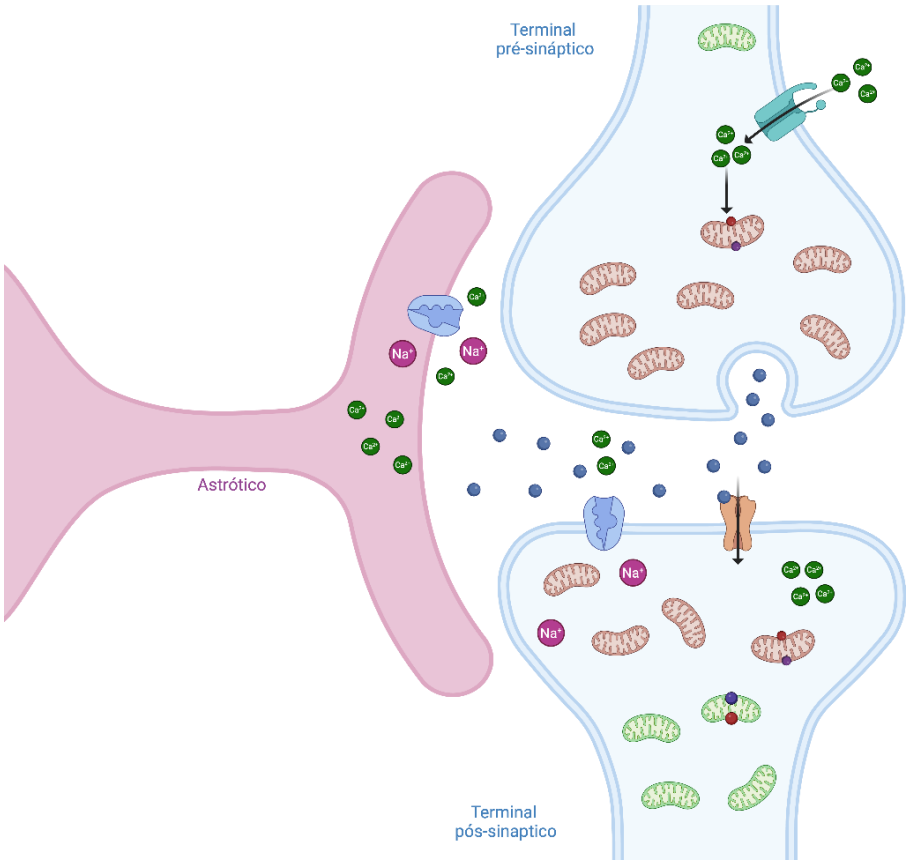


Figura 8. Sinapse Tripartite. Componentes dos terminais nervosos com Mitocôndrias sinápticas e extrasinápticas.

1.15 TCE – Epidemiologia

O TCE representa uma das principais causas de morbimortalidade em escala global, acometendo predominantemente indivíduos jovens e economicamente ativos, bem como

idosos, em decorrência de quedas (Dewan et al., 2019; GBD 2023 Disease and Injury and Risk Factor Collaborators, 2025; Maas et al., 2022). Estima-se que o TCE seja responsável por cerca de 69 milhões de novos casos anuais, configurando importante carga socioeconômica e impacto sobre os sistemas de saúde (Dewan et al., 2019).

Modelagens epidemiológicas globais indicam uma taxa padronizada de incidência de aproximadamente 259 casos por 100.000 habitantes, com tendência de estabilidade nas últimas décadas. Apesar disso, o número absoluto de casos tem aumentado em virtude do envelhecimento populacional (Dewan et al., 2019; Maas et al., 2022). Regiões de baixa e média renda apresentam elevação proporcional de casos moderados e graves, e os homens permanecem desproporcionalmente mais afetados que as mulheres (GBD 2023 Disease and Injury and Risk Factor Collaborators, 2025; Maas et al., 2022).

No Brasil, o TCE constitui um relevante problema de saúde pública, associado a elevadas taxas de internação, mortalidade e custos hospitalares. Entre 2018 e 2022, foram registradas 511.480 internações por TCE no Sistema Único de Saúde (SUS), com predomínio do sexo masculino (75,8%) e faixa etária de 20 a 39 anos (Costa et al., 2024). A média de permanência hospitalar variou entre 6 e 8 dias, com custo total superior a R$150 milhões no período (Costa et al., 2024).

Na população pediátrica, o impacto também é expressivo: entre 2012 e 2022 ocorreram 247.116 internações por traumatismo intracraniano em indivíduos de 0 a 19 anos, resultando em 8.936 óbitos e taxa média de mortalidade hospitalar de 3,61% (R. B. K. Carteri & Silva, 2021). A permanência média foi de 4,2 dias e o custo médio por internação estimado em R$1.124,00. Outro estudo nacional identificou média anual de 29.017 hospitalizações e cerca de 941 mortes hospitalares, com incidência estimada de 45,35 internações por 100.000 habitantes/ano (R. B. K. Carteri & Silva, 2021). Nessas faixas etárias, adolescentes de 15 a 19 anos representam o grupo de maior risco (Fonsêca et al., 2024).

Em todas as regiões do país, observa-se predomínio de vítimas do sexo masculino (70–80%), refletindo maior exposição a fatores de risco comportamentais, ocupacionais e de mobilidade urbana (Dias & Guerra, 2024). Adultos jovens (20–39 anos) são acometidos em maior número, enquanto idosos (>60 anos) exibem maior letalidade, atribuída à gravidade dos traumas e à presença de comorbidades (Dias & Guerra, 2024; Koerich et al., 2024). O impacto econômico é expressivo: o custo médio de internação varia entre R$1.000 e R$2.500, dependendo da gravidade e duração da hospitalização, resultando em custo anual estimado de R$156 milhões ao SUS (Costa et al., 2024). Além dos custos diretos, há ônus indiretos

significativos, relacionados à reabilitação prolongada, afastamento laboral e sequelas neurológicas persistentes (Maas et al., 2017; Roozenbeek et al., 2013).

Durante a pandemia de COVID-19, observou-se redução temporária nas taxas de hospitalização por TCE, provavelmente associada à diminuição da mobilidade urbana e do tráfego de veículos. Essa tendência, contudo, reverteu-se a partir de 2022, com o retorno das atividades e aumento progressivo dos casos (Campos et al., 2023; Tôrres et al., 2021).

Em síntese, o TCE permanece como causa expressiva de internações e mortalidade no Brasil, com grande variabilidade regional e predominância entre homens jovens. A subnotificação de casos leves e a falta de integração entre sistemas de vigilância dificultam estimativas precisas de incidência. Estratégias preventivas, como o uso de equipamentos de proteção individual, políticas de segurança no trânsito e programas de prevenção de quedas em idosos, continuam essenciais para reduzir a carga dessa condição (Campos et al., 2023; Dewan et al., 2019; Neurological Disorders: Public Health Challenges, s.d.).

1.16 TCE- Causas e Mecanismos Patofisiológicos

O TCE é definido como uma alteração da função cerebral decorrente da aplicação de forças biomecânicas externas que geram estresse mecânico sobre o crânio e o parênquima cerebral (Menon et al., 2010). Essas forças podem manifestar-se como impactos diretos, desacelerações ou acelerações lineares e rotacionais abruptas, além de ondas de choque produzidas por deslocamentos súbitos de ar, como em explosões, que se propagam no interior da caixa craniana (Gennarelli, 1982), como mostrado na figura 9. A lesão pode ou não envolver penetração na estrutura neural, variando desde fraturas ósseas simples até perfurações que atingem diretamente o tecido encefálico (Mt & Ak, 2017).

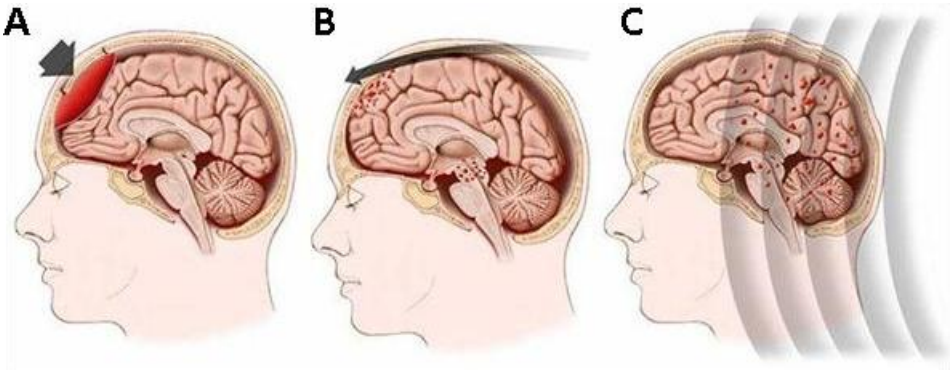


Figura 9. Causas do traumatismo cranioencefálico. (A) impacto direto; (B) aceleração, desaceleração do crânio; (C) ondas de choque.

Embora a etiologia do TCE seja multifatorial, os principais eventos desencadeantes incluem quedas, colisões veiculares (envolvendo automóveis ou motocicletas), práticas esportivas de contato, atos de violência e lesões penetrantes. Em escala global, o TCE é reconhecido como uma das principais causas de morbidade neurológica crônica e constitui um grave problema de saúde pública, em razão de sua elevada incidência, sequelas persistentes e expressivo impacto socioeconômico (Yan et al., 2025).

A classificação do TCE quanto à gravidade clínica baseia-se na Escala de Coma de Glasgow (ECGl), representada na Tabela 1, instrumento padronizado que avalia o nível de consciência por meio das respostas ocular, verbal e motora, refletindo a extensão do comprometimento neurológico (Se et al., 2024; Teasdale & Jennett, 1974). De modo geral, considera-se TCE leve aquele com pontuação entre 13 e 15, moderado entre 9 e 12, e grave entre 3 e 8 na ECGl (Hs & Rr, 2015; Meaney et al., 2014).

A gravidade da lesão está diretamente relacionada à magnitude das forças biomecânicas aplicadas ao encéfalo e à duração da perda de consciência, sendo determinante para o prognóstico e para a ocorrência de sequelas neurológicas e cognitivas posteriores.

Variáveis		Escore
Abertura Ocular	Espontânea À voz À dor Nenhuma	4 3 2 1
Resposta Verbal	Orientada Confusa Palavras inapropriadas Palavras incompreensíveis Nenhuma	5 4 3 2 1
Resposta Motora	Obedece a comandos Localiza a dor Movimentos de retirada Flexão normal Extensão anormal Nenhuma	6 5 4 3 2 1
Resposta Pupilar	Nenhuma Apenas uma reage ao estímulo luminoso Reação bilateral ao estímulo	2 1 0

Tabela 1. Escala de Coma de Glasgow. A coluna da esquerda representa os tipos de respostas avaliadas de cada paciente, a coluna central representa a qualidade das respostas atribuídas a cada tipo de resposta avaliada e a coluna da direta o score dado a cada qualidade de resposta atribuída.

1.17 Aspectos clínicos e fisiopatológicos do TCE

Em casos de lesões cerebrais leves (concussões), sintomas como perda de consciência, amnésia, cefaleia, tontura, hipersensibilidade a estímulos sensoriais, confusão mental e, ocasionalmente, convulsões são comuns. Embora a maioria desses sintomas apresenta remissão espontânea, há ampla documentação sobre a síndrome pós-concussional, caracterizada pela persistência de déficits cognitivos, fadiga e instabilidade emocional por semanas ou meses após o evento inicial (McIntosh et al., 2025).

Evidências crescentes apontam que episódios repetidos de concussão configuram um importante fator de risco para o desenvolvimento da encefalopatia traumática crônica (ETC), uma condição neurodegenerativa progressiva associada à degeneração axonal, hiperfosforilação e acúmulo de proteína tau, disfunção neurovascular e neuroinflamação persistente. Indivíduos com histórico de múltiplas concussões exibem maior propensão a déficits motores, cognitivos e transtornos neuropsiquiátricos tardios (Butler et al., 2025; Daneshvar et al., 2015; Khan & Talley, 2025; Munakomi & Puckett, 2025).

Os principais desfechos clínicos do TCE incluem mortalidade precoce, déficits cognitivos duradouros, alterações neuropsiquiátricas (como depressão e ansiedade pós-traumática), além de prejuízos motores e sensoriais que comprometem significativamente a qualidade de vida (Bryant et al., 2023; Ogonah et al., 2025). Esses efeitos têm sido associados à neuroinflamação crônica, disfunção mitocondrial progressiva e degeneração axonal persistente, sustentando a hipótese de que o TCE constitui uma condição de curso neuropatológico contínuo e multifatorial, e não apenas o resultado de um evento agudo isolado (Bramlett & Dietrich, 2015; Johnson et al., 2013; Maas et al., 2022).

A fisiopatologia do TCE envolve uma sequência complexa de eventos celulares e moleculares, classicamente divididos em dano primário e dano secundário (Figura 10) (Maas et al., 2022; Roozenbeek et al., 2013; Werner & Engelhard, 2007). O dano primário resulta da ação direta das forças biomecânicas, impacto, aceleração e desaceleração, que promovem deformação tecidual, ruptura de membranas celulares, lesão axonal difusa, e micro-hemorragias. Essas alterações comprometem a arquitetura neuronal e vascular, levando à liberação abrupta de conteúdo intracelular e iniciando as cascatas neurotóxicas subsequentes (Dash et al., 2010; Maas et al., 2022).

Já o dano secundário compreende uma série de eventos bioquímicos e moleculares que se estendem de minutos a anos após o insulto inicial, amplificando o processo lesional e contribuindo para a neurodegeneração progressiva (Johnson et al., 2013; Roozenbeek et al.,

2013). Nessa fase, ocorre liberação excessiva de glutamato e consequente excitotoxicidade, acompanhada de desequilíbrio da homeostase de Ca^{2+}, produção exacerbada de ERO, estresse oxidativo e disfunção mitocondrial. Paralelamente, a ativação sustentada da resposta neuroglial inflamatória perpetua o dano celular (Fesharaki-Zadeh, 2022; Hemphill et al., 2015; Olatona et al., 2025).

Essas cascatas culminam em perda de função neuronal, hipometabolismo cerebral, patologia sináptica e apoptose, comprometendo progressivamente a integridade e a conectividade das redes neurais, tanto nas regiões próximas ao foco lesional quanto em áreas cerebrais distantes, um fenômeno conhecido como diasquese (Jarrahi et al., 2020; Maas et al., 2022).

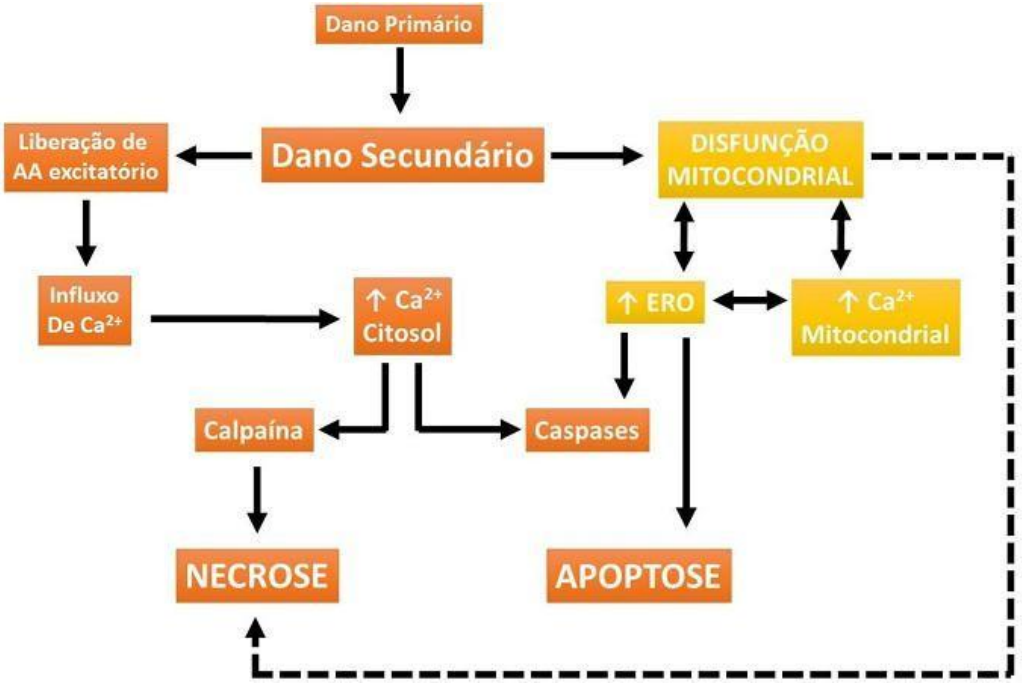


Figura 10. Progressão do dano secundário associado ao TCE. As forças biomecânicas decorrentes do trauma induzem deformações nos tecidos, caracterizando o dano primário. Em sequência, ocorre disfunção do fluxo sanguíneo cerebral e liberação exacerbada de aminoácidos excitatórios (principalmente glutamato), o que contribui para a excitotoxicidade neuronal. O aumento subsequente das concentrações intracelulares de Ca^{2+} ativa proteases dependentes de Ca^{2+} (como as calpainas), responsáveis pela degradação do citoesqueleto neuronal. Concomitantemente, a sobrecarga mitocondrial de Ca^{2+} compromete o metabolismo energético, intensifica a geração de ERO e desencadeia vias de sinalização associadas à morte celular apoptótica e necrótica. Figura adaptada de Cheng et al. (2012).

A severidade e a extensão dos processos secundários do TCE estão diretamente relacionadas à intensidade do impacto, à região cerebral acometida e à vulnerabilidade metabólica intrínseca dos neurônios. Nesse cenário, a mitocôndria emerge como um elemento

central na cascata fisiopatológica, dada sua participação essencial na manutenção da homeostase energética e redox, na regulação do Ca^{2+} intracelular e no controle das vias de apoptose intrínseca.

Após o trauma, a disfunção mitocondrial manifesta-se por redução da fosforilação oxidativa e da produção de ATP, colapso do ΔΨ, liberação de ERO e ativação de vias pró-apoptóticas. Esses eventos desencadeiam um ciclo autoalimentado de estresse oxidativo e falência bioenergética, que agrava a perda neuronal e compromete os mecanismos de reparo tecidual (Cheng et al., 2012; Modi et al., 2024; Olatona et al., 2025).

Dessa forma, a mitocôndria deixa de atuar apenas como fonte de energia celular e passa a representar um ponto crítico de vulnerabilidade e, simultaneamente, um alvo terapêutico promissor para estratégias voltadas à neuroproteção e restauração metabólica pós-TCE.

1.18 TCE – dano mitocondrial

O Ca^{2+} intracelular constitui um dos principais mediadores da fisiopatologia do dano secundário pós-TCE, estando diretamente implicado em múltiplos mecanismos de disfunção celular que envolvem o retículo endoplasmático rugoso, vias autofágicas e, de modo particularmente crítico, a atividade bioenergética mitocondrial (Mira et al., 2023; Olatona et al., 2025; Verma et al., 2022). Em condições fisiológicas, as mitocôndrias captam Ca^{2+} citosólico de forma controlada, modulando a atividade das desidrogenases do CK e ajustando a produção de ATP à demanda energética celular. Após o TCE, contudo, a ruptura da homeostase iônica leva a uma sobrecarga mitocondrial de Ca^{2+}, que desencadeia uma cascata de alterações metabólicas e estruturais incompatíveis com a manutenção da viabilidade neuronal (Mira et al., 2023; Verweij et al., 2000).

O equilíbrio do Ca^{2+} mitocondrial depende de um sistema dinâmico de transporte, no qual o NCLX exerce papel essencial na extrusão do cátion da matriz (Luongo et al., 2017; Palty et al., 2010). Quando esse equilíbrio é perdido, ocorre inchaço mitocondrial, formação de poros de transição na membrana interna (mPTP) e colapso do Δρ, comprometendo a fosforilação oxidativa e a síntese de ATP (R. B. Carteri, Kopczynski, Menegassi, et al., 2019; R. B. Carteri, Kopczynski, Rodolphi, et al., 2019).

A sobrecarga de íons e elétrons no STE favorece o vazamento de elétrons e a geração exacerbada de ERO, como o ânion $O_2^{\bullet -}$ e o H_2O_2, que intensificam o estresse oxidativo e amplificam o dano mitocondrial (Schieber & Chandel, 2014; Zorov et al., 2014). Esse processo estabelece um circuito de retroalimentação positiva, no qual o acúmulo de ERO perpetua a

perda da homeostase redox e energética, deteriorando progressivamente a integridade estrutural e funcional da organela (Fischer et al., 2016).

Como consequência, a eficiência do transporte eletrônico, a manutenção do potencial eletroquímico e a integração entre consumo de O_2 e fosforilação oxidativa tornam-se ineficazes, predispondo os neurônios à degeneração apoptótica (Daneshvar et al., 2015b; Johnson et al., 2017). A disfunção persistente leva ao desacoplamento do STE, ao aumento da produção de ERO e ao hipometabolismo tecidual, acompanhados pela ativação de vias pró-apoptóticas caracterizadas pela translocação de BAX para a membrana mitocondrial, redução de BCL-2 citosólico e liberação de citocromo c, culminando na ativação da caspase-3 clivada e na morte celular programada (Lorente et al., 2015; Qian et al., 2022; Singh et al., 2015).

Assim, a capacidade mitocondrial de detectar a demanda energética e modular a produção de ATP emerge como determinante crítico da sobrevivência celular pós-trauma. As mitocôndrias configuram-se, portanto, como nodos centrais de integração metabólica e de Ca^{2+}, definindo o equilíbrio entre recuperação e apoptose. Apesar dos avanços recentes, os aspectos neuroenergéticos e o papel do Ca^{2+} mitocondrial em concussões e traumas moderados ainda permanecem subexplorados (Pfanner et al., 2019), reforçando que a disfunção no manejo mitocondrial de Ca^{2+} constitui um pilar mecanístico essencial da fisiopatologia secundária do TCE.

1.19 Traumatismo Cranioencefálico – Terapias

O manejo clínico do TCE, incluindo as concussões repetidas (CR), continua sendo um dos maiores desafios da neurologia contemporânea. Apesar dos avanços na compreensão dos mecanismos celulares e moleculares do dano secundário, as abordagens terapêuticas permanecem predominantemente sintomáticas, fragmentadas e de eficácia limitada (Lawrence & Hutchison, 2017).

Os protocolos clínicos atuais concentram-se na avaliação da severidade e na monitorização de sintomas, com o repouso ainda sendo recomendado como principal intervenção inicial. Entretanto, sua eficácia e fundamentação fisiológica permanecem controversas, sobretudo em concussões leves e moderadas (Lawrence & Hutchison, 2017). O arsenal farmacológico disponível é escasso, e nenhum agente neuroprotetor demonstrou benefício clínico consistente nas últimas duas décadas, refletindo o insucesso de múltiplos ensaios clínicos que falharam em traduzir achados promissores de modelos experimentais.

O reposicionamento de fármacos, incluindo EAA e agentes metabólicos, tem sido explorado em modelos pré-clínicos, com resultados inicialmente encorajadores; contudo, a ausência de eficácia clínica robusta em humanos limita seu uso terapêutico (El-Hattab et al., 2017; Winkler et al., 2016). Intervenções dietéticas e metabólicas, como suplementação de ácidos graxos e controle glicêmico intensivo, também têm sido avaliadas, mas os resultados permanecem inconclusivos e heterogêneos entre estudos (El-Hattab et al., 2017).

Entre os alvos emergentes, a mitocôndria tem se destacado como componente central da fisiopatologia e um potencial foco terapêutico. Agentes capazes de modular a função mitocondrial, como indutores do coativador PGC-1α, um regulador mestre da biogênese mitocondrial, buscam restaurar a eficiência bioenergética e reduzir o estresse oxidativo (Hirano et al., 2018; Kehrein et al., 2013). No entanto, a transposição desses achados para a clínica enfrenta obstáculos consideráveis, devido às diferenças metabólicas e farmacocinéticas entre modelos animais e humanos, e à falta de marcadores sensíveis de eficácia mitocondrial in vivo.

Abordagens mais inovadoras, como o transplante celular e organelar, têm mostrado potencial em modelos experimentais de TCE e outras doenças neurodegenerativas. Essas estratégias visam restaurar o equilíbrio bioenergético e promover a regeneração tecidual, mas ainda carecem de validação clínica robusta e padronização metodológica (Ben-Shachar & Ene, 2018; Emani et al., 2017; Shin et al., 2017).

Atualmente, não há terapia aprovada pela FDA ou ANVISA que modifique de forma comprovada o curso da lesão cerebral pós-trauma. O tratamento permanece essencialmente de suporte, e o fracasso repetido de ensaios com neuroprotetores destaca a complexidade do dano secundário, que envolve múltiplos processos interdependentes, inflamatórios, excitotóxicos, oxidativos e metabólicos. Nesse cenário, estratégias combinatórias e multimodais podem oferecer maior potencial terapêutico, mas a ausência de ensaios clínicos multicêntricos de grande escala ainda limita sua implementação (Levin & Diaz-Arrastia, 2015).

Aspectos metabólicos também influenciam de forma decisiva o prognóstico. A hiperglicemia persistente, comum após o TCE, associa-se a piores desfechos clínicos e maior mortalidade. Estudos indicam que o controle glicêmico rigoroso, especialmente nas duas primeiras semanas pós-trauma, pode reduzir complicações infecciosas e pressão intracraniana, embora os limites ideais de glicemia ainda sejam debatidos (Bilotta et al., 2009; Meier et al., 2008).

A nutrição precoce e adequada é outro determinante prognóstico relevante. Iniciar a alimentação enteral dentro das primeiras 24 horas após o trauma reduz a mortalidade e melhora a resposta imunometabólica (Härtl et al., 2008; Perel et al., 2006). Contudo, mesmo com

estratégias otimizadas de suporte nutricional, a manutenção da massa muscular e do balanço nitrogenado ainda é limitada, refletindo a persistência do estado catabólico e da disfunção mitocondrial induzida pelo trauma (Jensen et al., 2010).

Assim, apesar dos avanços conceituais sobre os mecanismos bioenergéticos, inflamatórios e neuroendócrinos do TCE, as terapias disponíveis permanecem paliativas e ineficazes para prevenir a degeneração neuronal progressiva. O hipogonadismo traumático, por exemplo, reflete um dos desfechos endócrinos mais frequentes do TCE, resultante do comprometimento do eixo hipotálamo-hipófise-gonadal e da consequente redução dos níveis de T (Barton et al., 2016; Hohl et al., 2009; Tanriverdi et al., 2006). Evidências em modelos animais e humanos sugerem que essa deficiência androgênica pode contribuir para o hipometabolismo cerebral e a neurodegeneração pós-trauma, reforçando a necessidade de explorar novas terapias neuroendócrinas e mitocondriais como estratégias complementares à neuroproteção (R. B. Carteri, Kopczynski, Rodolphi et al., 2019).

Apesar das inúmeras tentativas de intervenção farmacológica, o TCE permanece sem terapias capazes de interromper de forma eficaz o dano secundário, em grande parte devido à natureza multifatorial da lesão e à ausência de alvos terapêuticos validados. O reconhecimento da mitocôndria como ponto de convergência entre disfunção bioenergética, acúmulo de Ca^{2+} e ativação apoptótica tem transformado a forma como se compreende a fisiopatologia do trauma cerebral. Nesse contexto, torna-se evidente que estratégias capazes de modular a homeostase mitocondrial e restaurar o acoplamento bioenergético representam uma fronteira promissora para o desenvolvimento de terapias efetivas.

Partindo do pressuposto de que o uso suprafisiológico de EAA, como o ND, interfere na homeostase sináptica e mitocondrial, esta tese propõe que a exposição prolongada à ND induz um estado de hiperexcitabilidade glutamatérgica mediado pela redução da expressão do transportador astroglial de glutamato GLT-1 e pela fosforilação das subunidades pGluN2BTyr1472 e pGluN2ATyr1246 do receptor NMDAr. Tal desbalanço excitotóxico comprometeria a função bioenergética das mitocôndrias sinápticas, reduzindo a atividade da succinato desidrogenase (SDH), o $\Delta\Psi$ e a eficiência da fosforilação oxidativa, ao mesmo tempo em que aumentaria a produção de H_2O_2 e a disfunção no manejo de Ca^{2+}. Além disso, postulamos que a modulação positiva do GLT-1 pela ceftriaxona (CEF) seja capaz de restaurar a homeostase glutamatérgica, atenuando tanto o comportamento agressivo quanto as disfunções mitocondriais induzidas pelo ND.

Por fim, hipotetiza-se que a exposição prévia à ND aumenta a vulnerabilidade do encéfalo a eventos traumáticos subsequentes, como o TCE, favorecendo a ativação de vias de

neurodegeneração associadas ao acúmulo de APP, $A\beta_{42}$ e tau fosforilada. Essa condição seria acompanhada por estresse do reticulo, prejuízos na biogênese mitocondrial, redução da eficiência de acoplamento bioenergético e déficits cognitivos, particularmente na memória espacial.

2 OBJETIVOS

2.1 Objetivo Geral

Elucidar mecanismos glutamatérgicos e mitocondriais subjacentes ao comportamento agressivo induzido por ND, bem como avaliar se a exposição prévia a este anabolizante aumenta a vulnerabilidade à neurodegeneração após o TCE.

2.2 Objetivos Específicos

a. Analisar criticamente as evidências disponíveis sobre como o abuso de EAA afeta a dinâmica e a eficiência bioenergética das mitocôndrias sinápticas e extrasinápticas;
b. Avaliar o impacto da administração de uma dose suprafisiológica de ND sobre o comportamento agressivo em camundongos;
c. Quantificar os níveis de glutamato no líquor e avaliar a expressão de GLT-1, pGluN2B^{Tyr1472} e pGluN2A^{Tyr1246} nos terminais sinápticos;
d. Investigar indicadores da atividade bioenergética mitocondrial em sinaptossomas, incluindo a atividade da SDH, o manejo de Ca^{2+}, o $\Delta\Psi$ e a produção de H_2O_2;
e. Analisar o efeito da modulação do GLT-1 pela CEF sobre o comportamento agressivo e a bioenergética mitocondrial;
f. Quantificar os níveis de proteína precursora amiloide (APP), peptídeo β-amiloide 42 ($A\beta_{42}$) e tau fosforilada nos terminais nervosos de animais previamente expostos à ND e submetidos ao TCE;
g. Avaliar nas mitocôndrias sinápticas desses animais a biossíntese de ATP acoplada ao consumo de O_2 e a capacidade de manejo de Ca^{2+};
h. Investigar o desempenho na memória espacial em camundongos previamente expostos à ND e submetidos ao TCE.

PARTE II

Esta seção apresentará toda a produção concluída e publicada durante o curso dessa tese de doutorado. As produções serão anexadas neste documento junto com uma breve descrição antes de cada capítulo.

O capítulo I aborda diversos aspectos do uso de EAA, desde efeitos desejados, efeitos adversos oriundos do abuso destas substâncias, dados epidemiológicos globais e, também, o impacto em mitocôndrias sinápticas e extrasinápticas. Para isso, foi feito um levantamento bibliográfico da literatura vigente gerando a publicação de um capítulo do livro "The handbook of Substance Misuse and Addictions: From Biology to Public health", intitulado: Synaptic and Extrasynaptic Mitochondria: Features and Impact of High Anabolic-Androgenic Steroid, publicado em 2022 pela editora Springer.

O capítulo II demonstra um possível mecanismo para o fenótipo agressivo em camundongos tratados com ND, envolvendo a cooperação de neurônios e astrócitos no manejo dos níveis extracelulares de glutamato. Os resultados dessa pesquisa geraram o artigo "Glutamate Transport-1 link Astrocytes with Heightened Aggressive Behavior Induced by Steroid Abuse in male CF1 Mice", publicado na revista Hormones and Behavior, 2021.

No capítulo III utilizamos o mesmo regime de tratamento do capítulo anterior, seguido de um TCE induzido por ICC e avaliamos como o abuso de EAA impactou na magnitude do dano secundário causado pelo TCE. Os resultados dessa pesquisa geraram o artigo "Nandrolone Abuse Prior to Head Trauma Mitigates Endoplasmic Reticulum Stress, Mitochondrial Bioenergetic Deficits and Markers of Neurodegeneration", publicado na revista Molecular Neurobiology, 2025.

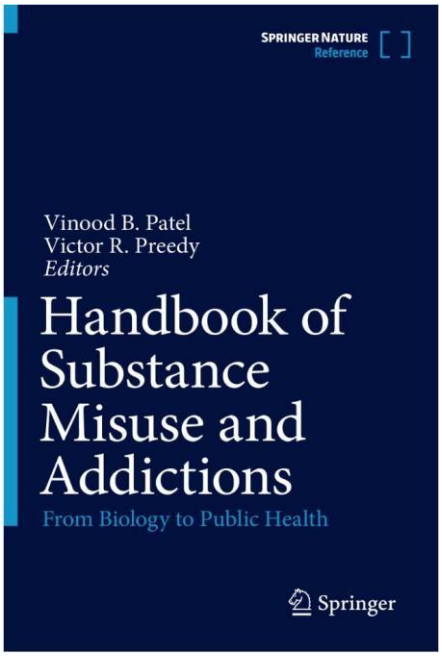
Capítulo I: Synaptic and extrasynaptic mitochondria: features and impact of high anabolic androgen-steroid use.

No capítulo I apresentamos o capítulo de livro produzido para a obra "*The handbook of Substance Misuse and Addictions: From Biology to Public health*"

O uso de substâncias como os EAA vem se tornando muito comum entre atletas de elite, atletas recreacionais e a população em geral. A promessa de ganho de performance, melhora em componentes estéticos e a facilidade de obtenção dessas substâncias contribuem para este uso, muitas vezes, indiscriminado e o aparecimento de uma série de efeitos adversos decorrentes do abuso destes compostos.

Neste capítulo de livro fizemos um levantamento bibliográfico abordando dados epidemiológicos dos EAA, os efeitos adversos em diversos tecidos do corpo, explorando, mais especificamente, o aumento da impulsividade e agressividade em usuários de EAA que, frequentemente, se envolvem em episódios de violência.

Abordamos também como os EAA poderiam afetar mitocôndrias cerebrais e, neste contexto, observamos duas populações diferentes de mitocôndrias: as sinápticas e as extrasinápticas. Existem algumas diferenças entre estas duas populações de mitocôndrias no que diz respeito ao manejo Ca^{2+}, produção de ATP e produção de ERO.



Synaptic and Extrasynaptic Mitochondria

Features and Impact of High Anabolic-Androgenic Steroids Use

Vitória Girelli de Oliveira, Jijo Stebin Justus,
Luis Valmor Cruz Portela, and Marcelo Salimen Rodolphi

Contents

Introduction 3
Anabolic-Androgenic Steroids: A Brief Epidemiological Tour D'horizon 3
Testosterone and Synthetic Derivatives 4
Side Effects of Anabolic-Androgenic Steroid Abuse 6
Impact of AAS on Anger and Aggression 8
Mitochondria: The "Powerhouse of the Cell" and More 8
Effects of AAS on Synaptic and Extrasynaptic Mitochondria 11
Applications to Other Areas of Public Health 13
Applications to Other Areas of Addiction 14
Mini-dictionary of Terms 14
Key Facts 15
Key Facts of Anabolic-Androgenic Steroids 15
Key Facts of Mitochondria 15
Key Facts of the Effects of AAS on Synaptic and Nonsynaptic Mitochondria 16
Summary Points 16
References 16

Abstract

The abuse of anabolic androgenic steroid (AAS) drugs has turned into a public health issue over the last years. Supraphysiological dosage of AAS is widespread among the elite sports community to increase skeletal muscle mass, oxidative metabolism, and physical performance. Also, recreational athletes have abused trying to improve aesthetic image or gain economic or social benefits. Unfortunately, AAS drug abuse may result in undesirable mental and physical consequences like aggressiveness, depression, and cardiovascular damage and is also an open gate for the consumption of other illicit drugs. Indeed, AAS have been

V. G. de Oliveira · J. S. Justus · L. V. C. Portela · M. S. Rodolphi (✉)
Laboratory of Neurotrauma and Biomarkers, Department of Biochemistry, ICBS, Universidade Federal do Rio Grande do Sul – UFRGS, Porto Alegre, Brazil
e-mail: luis.portela@ufrgs.br

© Springer Nature Switzerland AG 2022

V. B. Patel, V. R. Preedy(eds.), *Handbook of Substance Misuse and Addictions*,
https://doi.org/10.1007/978-3-030-67928-6_136-1

considered abuse substances, sharing addiction mechanisms with drugs like opioids. Though AAS misuse targets many neural systems and accessory components, particular effects on mitochondrial bioenergetic mechanisms associated with synapse have been neglected. This chapter discusses general effects and how common AAS such as testosterone and nandrolone decanoate abuse impact the bioenergetic responsiveness of the synaptic and extrasynaptic mitochondria.

Keywords

Aggressive phenotype · Anabolic androgenic steroids · Brain · Extrasynaptic mitochondria · Nandrolone decanoate · Neuropsychiatric disorders · Sports · Synaptic mitochondria · Synthetic steroids · Testosterone · Violence

Abbreviations

ΔΨm	Mitochondrial membrane potential
AAS	Anabolic androgenic steroids
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AR	Androgen receptor
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
CAT	Catalase
CK	Creatine kinase
CNS	Central nervous system
DHT	5-Alpha-dihydrotestosterone
DNA	Deoxyribonucleic acid
ER	Endoplasmic reticulum
ETC	Electron transport chain
ETS	Electron transfer system
FADH2	Flavin adenine dinucleotide
FDA	Food and Drug Administration
GABA	γ-Aminobutyric acid
GPx	Glutathione peroxidase
HAE	Hereditary angioedema
HIV/AIDS	Human immunodeficiency virus, acquired immunodeficiency syndrome
HPG	Hypothalamic-pituitary-gonadal
IBM	Inner boundary membrane
IM	Inner membrane
LDH	Lactic dehydrogenase
LH	Luteinizing hormone
MAM	Mitochondria-associated endoplasmic reticulum membrane
NADH	Nicotinamide adenine dinucleotide
NCLX	$Na^+/Ca^{2+}/Li^+$ exchanger
ND	Nandrolone decanoate

NFL	National Football League
NIDA	National Institute on Drug Abuse
OM	Outer membrane
OXPHOS	Oxidative phosphorylation
RBC	Red blood cell
ROS	Reactive oxygen species
SARM	Selective androgen receptor modulators
SHBG	Sex hormone-binding globulin
T	Testosterone
TBI	Traumatic brain injury
TCA	Tricarboxylic acid cycle
TOM	Translocase of outer membrane proteins
VDAC	Voltage-dependent anion channel

Introduction

Anabolic-androgenic steroids (AAS) are synthetic compounds derived from testosterone (T), a steroid hormone with androgenic and anabolic effects in several tissues, including the skeletal muscle and brain. These synthetic compounds were synthesized to maximize anabolic effects and minimize androgenic ones (Evans 2004; Yesalis and Bahrke 1995).

In the clinical setting, AAS are often prescribed to treat delayed puberty and muscle loss due to catabolic disorders (Shahidi 2001), to treat hypogonadism (Basaria et al. 2001), and to restore T levels in men with an associated medical condition. AAS are commercially found in formulations for oral, intramuscular, and transdermal administration, which facilitates application by patients in the clinical setting. However, there are many reports of AAS use unrelated with therapeutic approval, and in these cases, individuals tend to self-administer supraphysiological doses causing disturbances in various biological systems, including in the brain. This may be accompanied by neuropsychiatric symptoms like depression, anxiety, and exacerbated aggressiveness (Piacentino et al. 2014). Considering the worldwide reports of AAS abuse and their undesirable mental and physical side effects, there are increasing public health concerns about the non-prescribed consumption of these drugs.

Anabolic-Androgenic Steroids: A Brief Epidemiological Tour D'horizon

Brief statistics on AAS use around the world show a steady increase in the use of steroids as a drug that erroneously associates the perfect body shape and success in sport with a healthy lifestyle. According to the US Food and Drug Administration (FDA 2015), approximately 375,000 young men and 175,000 young women are abusers of AAS every year. Based on the reports of the Substance Abuse and Mental

Health Services Administration's National Household Survey on Drug Abuse, 0.5% of the adult population were confirmed to have used steroids, and about 1% had ever used steroids in the 18 to 34 age group (NIDA 2004).

In the USA, the AAS abuse by underage students belonging to 8th, 10th, and 12th graders were 2.0%, 1.7%, and 2.0%, respectively, which represent a steady increase relative to the previous data (NIDA 2020). In 2014, approximately 2% of students (1 in 50) belonging to the 12th grade used steroids, although these numbers were substantially lower than high school seniors. Earlier in 2007, a survey reported about 77% of college students from the USA who have taken steroids had also been under the addiction of at least one other drug (McCabe et al. 2007). In a survey of retired National Football League (NFL) players, 9.1% admitted the use of AAS during their careers (Horn et al. 2009). A published report based on self-reporting questionnaires by random school students has shown that more than 83,000 young Canadians (2.8% of the respondents) have used anabolic-androgenic steroids in the year before the survey. Among those taking such drugs, 29.4% injected them and around 29.2% shared needles while injecting AAS (Melia et al. 1996). A report among the Australian secondary school students suggested that 2.4%, aged 12–17 years, had ever used AAS. In this study, the AAS abuse was quite similar between boys and girls (1.7% vs 1.3%, respectively) albeit worldwide men are the highest AAS abusers (Dunn and White 2011).

In the UK, the overall number of adults using AAS in England and Wales in 2018 was around 411,000. This data suggested a solid increase, roughly twice the amount over the last 10-year period (Mullen et al. 2020). Further, an analysis of the lifetime nonmedical AAS use in the Nordic countries showed that Sweden had the highest frequency of utilization with 4.4%, followed by Norway with 2.4%, Finland with 0.8%, Iceland with 0.7%, and finally Denmark with 0.5% (Sagoe et al. 2017).

These epidemiological data highlight that teenagers, young adults, professional athletes, and even children of both sexes are being exposed to health risks of using AAS for different proposals than the therapeutic recommendations. Social aspects, the requirement to succeed and achieve better school scores, and the need to win at all costs in competitive sports to achieve personal benefits (i.e., scholarships, professional contracts, financial) are potential drivers of such harmful behavior.

Testosterone and Synthetic Derivatives

Testosterone, isolated and characterized in 1935 (David 1935), is responsible for developing male sex organs and secondary sex characteristics, spermatogenesis, nitrogen fixation, and increased muscle mass synthesis. It acts on reproductive tissues, such as the testes and ovaries, and nonreproductive tissues, such as the central nervous system (CNS), bone tissue, and skeletal muscle. In men, it is mainly synthetized and secreted by Leydig cells and in women by the ovaries. It is transported in the bloodstream predominantly conjugated to sex hormone-binding globulin (SHBG) or, to a lesser extent, with other plasma proteins (Dunn et al. 1981).

The decrease in T is accompanied by clinical manifestations like lack of sexual interest, excessive tiredness, hair loss, loss of muscle mass, and increase in body fat.

Like other androgens, T is synthesized from cholesterol ring (Miller and Auchus 2019). In mitochondria of Leydig cells, cholesterol is converted to pregnenolone under luteinizing hormone (LH) regulation. The following enzymatic reactions occur in the smooth endoplasmic reticulum (ER), in which pregnenolone is metabolized to androgens. Finally, androstenedione is synthesized and converted to T. In the male sex organs, T is converted to 5-alpha-dihydrotestosterone (DHT) by the enzyme 5-alpha-reductase. In other tissues, such as adipose tissue, T is converted to estradiol by the aromatase enzyme (Mooradian et al. 1987). Androgenic hormones bind to the androgen receptor (AR), which is translocated to the nucleus to regulate gene transcription. Alternatively, AR mediates rapid cellular events such as increased intracellular calcium concentration, release of neurotransmitters, and activation of intracellular signaling molecules (Foradori et al. 2008). In mature adipocytes, AR expression increases in a dose-dependent manner to testosterone levels (De Pergola et al. 1990). It causes lipolytic effects through the increase in the number of beta-adrenergic receptors in adipocytes, as well as an increase in the activity of adenylate cyclase and protein kinase A (Xu et al. 1991). On the other hand, an increase in nitrogen fixation, protein synthesis, and strength are recognized effects of AAS in the skeletal muscle, and for this reason it has been used as doping substances in elite sports (Duntas and Popovic 2013).

Recently, attention has been given to selective androgen receptor modulators (SARMs), a class of drugs that exerts agonist effects on AR and may target specific tissues depending on the molecular engineering (Solomon et al. 2019). The lack of undesirable androgenic effects of SARMs relative to AAS encompasses pharmacological advantages for the bodybuilding community, creating the potential for abuse among athletes (Grata et al. 2011).

Pharmacologically, the AAS are divided into three classes (Clark and Henderson 2003), according to the chemical alterations performed in the steroidal ring of T, as shown in Fig. 1:

- Esters of the 17-beta-hydroxyl group, such as T propionate and cypionate, whose esterification slows the systemic release, prolonging the action time and delaying the degradation of the compound.
- Derivatives of T esters, such as nandrolone decanoate (ND), whose demethylation increases the half-life and which has reduced androgenic activity when compared to DHT.
- Alkylated compounds, such as oxymetholone and stanozolol, have decreased hepatic metabolism and are not converted to DHT or estradiol.

Importantly, these chemical alterations modulate the metabolism of these testosterone analogs by the 5-alpha reductase and aromatase pathways, as well as their affinity to AR. This allows the designing of AAS with a wide range of effects that may drive an androgenic enhancement of secondary sexual characteristics or even target enhancement of anabolic metabolism and tissue growth, particularly in

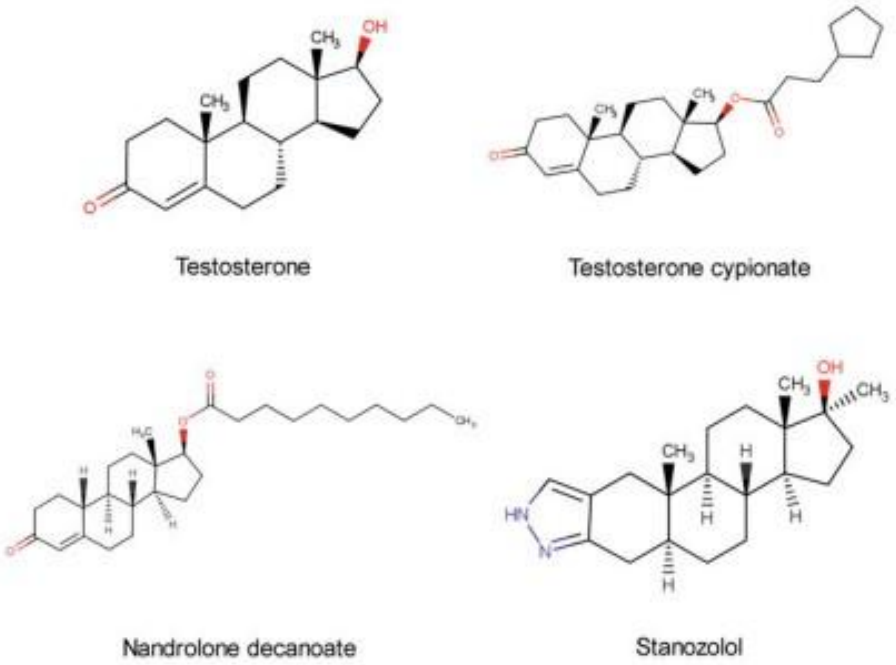


Fig. 1 The hormone testosterone and its synthetic derivatives. AAS are substances that are derived from the hormone testosterone. They are divided into three classes, each one with different anabolic/androgenic ratios

skeletal muscle. These anabolic effects are associated with improved physical performance in sports, which has favored the misuse of these drugs in elite athletes but also by nonathletes' population for aesthetic purposes. Regardless of the goal pursued by the AAS abusers, they underestimate the side effects of these drugs.

Side Effects of Anabolic-Androgenic Steroid Abuse

Among the side effects caused by the administration of AAS are testicular atrophy, gynecomastia, acne, liver toxicity, and cardiovascular complications. As shown in Table 1, the prolonged use of AAS is associated with cardiovascular damage, myocardial dysfunction, coronary atherosclerosis, and an increased risk of heart attack (Baggish et al. 2010, 2017). High doses of AAS can lead to increased levels of liver enzymes, increased chance of developing hepatocellular carcinoma, hepatocellular necrosis, hepatitis, and liver hemorrhage (Gorayski et al. 2008; Maravelias et al. 2005; Patil et al. 2007). Particularly in women AAS abuse leads to the development of male characteristics such as deep voice, hirsutism, acne, clitoris hypertrophy, and fluid retention.

Table 1 Adverse effects of AAS abuse. A list of side effects in different organs due to prolonged use of anabolic-androgenic steroids

	Adverse effects	References
Liver	Jaundice; hepatic carcinoma; peliosis; hepatitis; benign lesions	Samuel et al. 2019
Kidney	Glomerulosclerosis; tubulointerstitial damage; elevated levels of serum urea and serum uric acid; hyperphosphatemia	Samuel et al. 2019
Cardiovascular system	Arteriosclerosis; thrombus formation; coronary artery vasospasm; myocardial ischemia; heart failure	Samuel et al. 2019
Reproductive system	Decreased sperm count and motility	Kanayama et al. 2020
Hematology	Increased RBC count, hemoglobin, and hematocrit	Kanayama et al. 2020
Dermatological effects	Acne; alopecia	Samuel et al. 2019
Musculoskeletal effects	Growth retardation; deterioration in muscular plasticity; acute skeletal muscle destruction	Samuel et al. 2019
Behavioral effects	Dependence/addiction; hostility; aggression; psychosis and maniac episodes; increase in self-esteem; paranoia	Samuel et al. 2019

Remarkably, the side effects in the brain are associated with changes in neuroendocrine and neurotransmitter signaling with consequences affecting whole body homeostasis. It has been estimated that 30% of AAS abusers are addicted (Kanayama et al. 2010) and display neuropsychological characteristics that resemble substance dependence, such as drug self-administration and withdrawal syndrome. Exogenous AAS administration promotes a negative feedback on hypothalamic-pituitary-gonadal (HPG) axis and changes in the dopaminergic and noradrenergic system, reducing endogenous androgen hormone production (Hochberg et al. 2003). Following removal of exogenous AAS, an immediate lack of endogenous androgen hormones leads to a consequent hypogonadism that could drive the symptoms observed with AAS withdrawal, including muscle pain, insomnia, headache, fatigue, and irritability (Brower et al. 1990; Kashkin and Kleber 1989; Malone et al. 1995).

Moreover, AAS abuse is associated with increased dopamine levels (Hannan et al. 1991), vasopressin receptor binding (DeLeon et al. 2002), and glutamatergic tonus (Kalinine et al. 2014b) and also linked with GABAergic and serotoninergic signals. The changes promoted by AAS abuse in neurotransmission contribute to the additional neuropsychological side effects, like depression, altered mood, anxiety, and heightened aggressive phenotype. Modulations in components of these neurotransmitter systems have proved to be effective for attenuating behavioral changes induced by AAS, including aggressive behavior.

Impact of AAS on Anger and Aggression

Mood swings, hostility, and violent impulsive and aggressive behavior are among the behavioral hallmarks of AAS abuse (Galligani et al. 1996; Pope et al. 2014; Thiblin et al. 2008). Such behavior has been replicated in rodents using AAS concentrations corresponding to those administered in humans, allowing to build up a mechanistic basis for the aggressive behavior. In hamsters, AAS administration in adolescence increases aggressive behavior, which is attenuated following pharmacological treatments to increase serotonergic activity (Grimes and Melloni 2002; Melloni et al. 1997). As for GABAergic systems, AAS administration modifies GABA neurotransmission in areas of the brain related to fear and anxiety (Clark et al. 2006). Further, the use of a specific AAS, with an increased anabolic effect compared to T, ND, was associated with significant increases in brain extracellular glutamate levels and an exacerbated aggression (Kalinine et al. 2014). This highlighted the potential role of the glutamatergic system in this specific phenotype following AAS abuse. Interestingly, this effect of AAS on neurotransmission and behavior seems to not be limited to neuronal modulation, as increasing the activity of the astrocytic glutamate receptor transporter, GLT-1, decreased brain extracellular glutamate levels and the aggressive phenotype (Rodolphi et al. 2021). This conceptual finding highlights that beyond neurons, other key components of synaptic transmission, such as the astrocytes, also mediate the heightened aggression under influence of AAS abuse.

Mitochondria: The "Powerhouse of the Cell" and More

Mitochondria are eukaryotic multifunctional cell organelles that perform crucial functions that ultimately keep the integrity of cells (Khacho et al. 2019; Pfanner et al. 2019). The structure of these organelles consists of two membranes with different structures and functions. The outer membrane (OM) separates the organelle from the cytosol, while the inner membrane (IM) encompasses the mitochondrial matrix. These two membranes are separated by an intermembrane space, as shown in Fig. 2 (Cogliati et al. 2016). The OM is more permeable, allowing the passage of ions and small, uncharged molecules through pore-forming membrane proteins (porins), such as the voltage-dependent anion channel (VDAC) and the translocase of outer membrane proteins (TOM) for any larger molecule, such as proteins, as demonstrated in Fig. 3 (Cogliati et al. 2016). Moreover, the OM establishes intimate physical connections with various intracellular membranes, such as ER through mitochondria-associated ER membrane (MAM) that creates a unique environment for shared interorganellar functions, such as calcium (Ca^{2+}) homeostasis, influencing mitochondrial dynamics (Nunnari and Suomalainen 2012). The intermembrane space is a gap between OM and IM where all matrix proteins imported into the mitochondrion from the cytoplasm must pass through (Kühlbrandt 2015). The IM is a tight diffusion barrier to all ions and molecules and can be subdivided into the inner boundary membrane (IBM) and the cristae, as displayed in Fig. 2 (Cogliati et al.

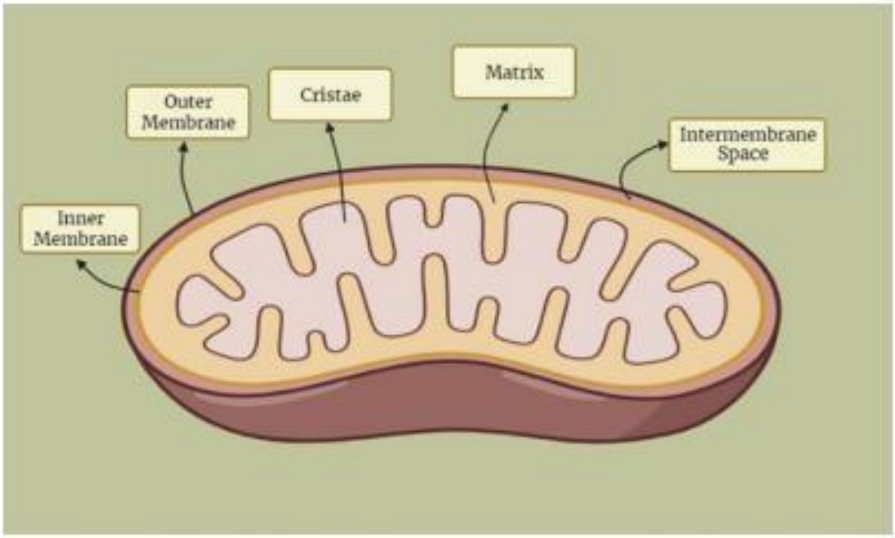


Fig. 2 Mitochondrial membrane structure. The OM separates mitochondria from the cytoplasm. It surrounds the IM, which separates the inner-membrane space from the matrix. The IM is differentiated into the IBM and the cristae

2016; Kühlbrandt 2015). The mitochondrial cristae are invaginations of the IM, which contains the oxidative phosphorylation system, comprising the respiratory complexes I to IV and the F1Fo-ATP synthase (Fig. 3). Systematic analysis of mitochondria proteome has comprehensively covered the mitochondrial proteins and the large variety of functions performed by mitochondria (Pfanner et al. 2019). They are mostly known as providers of energy in the form of ATP, but current knowledge expands this strict assumption. Indeed, mitochondria functions range from redox and apoptotic signaling to regulating nuclear gene expression and epigenetics (Chandel 2014; Khacho and Slack 2017, 2018). However, the mitochondrial role as energy provider remains fundamental for maintaining cell function, particularly in the brain where the rate of energy demand and utilization by gram of tissue surpass all other organs.

The mitochondrial oxidation of metabolic substrates in the tricarboxylic acid cycle (TCA) directs the flow of electrons from reduced nicotinamide adenine dinucleotide (NADH) and reduced flavin adenine dinucleotide ($FADH_2$) through the respiratory complexes until the final electron acceptor, oxygen. This flux generates an electrochemical gradient used to drive ATP synthesis by the F1Fo-ATP synthase, the import of precursor proteins, and the transfer of some metabolites across the IM. Also, the electron transfer to complexes generates a mitochondrial membrane potential ($\Delta\Psi m$), which represents a considerable driving force for both Ca^{2+} influx into the matrix, and reactive oxygen species (ROS) production. The mitochondrial Ca^{2+} buffering plays an essential role in signaling apoptosis. An exacerbated Ca^{2+} influx into mitochondria results in the opening of the permeability

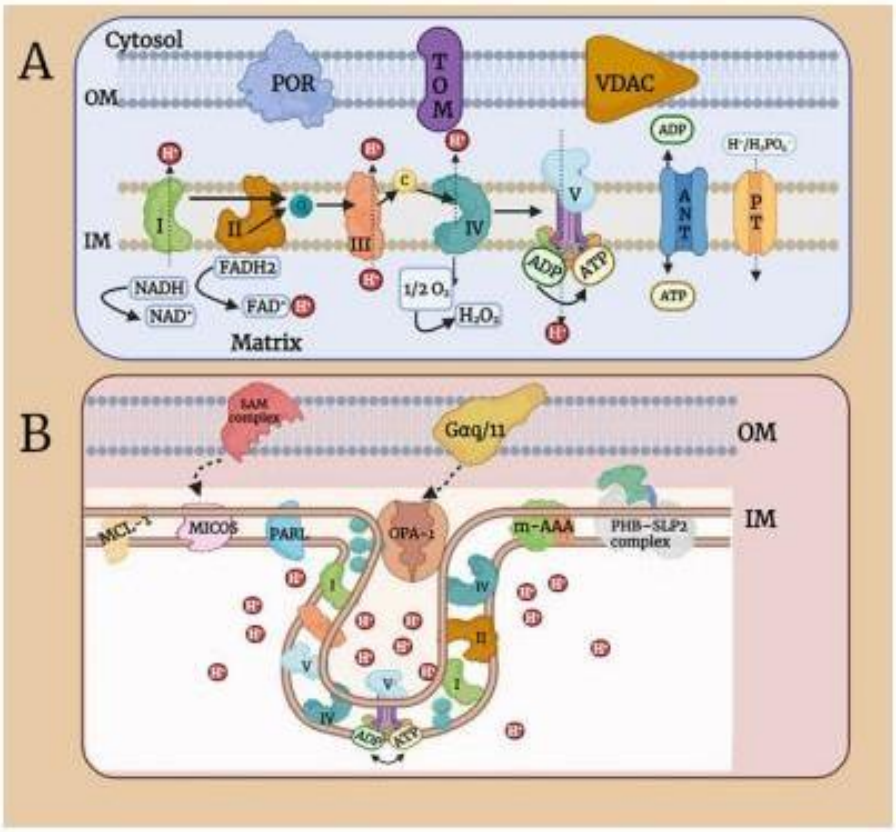


Fig. 3 The electron transport system (ETS). (**a**) Major outer mitochondrial membrane (OM) proteins including porins (POR), voltage-dependent anion channel (VDAC), and protein translocases of outer membrane (TOM). Inner mitochondrial membrane (IM) proteins including the components of the ETS (complexes I to V), adenine nucleotide translocase (ANT), and phosphate translocase (PT). The mitochondrial matrix has the remaining components involved in bioenergetics including coenzymes in redox forms ($NADH/NAD^+$ and $FADH_2/FAD^+$), protons (H^+), adenosine diphosphate (ADP), and adenosine triphosphate (ATP). (**b**) Proposed model for the arrangements of inner mitochondrial membrane (IM) proteins in supercomplexes usually located in mitochondrial cristae. In this model, the electron transport complexes are organized in a hierarchy manner to favor the function of complex V (F1-Fo ATP synthase). Additional proteins present in inner (IM) and OM are involved in the assemble of proteins, regulation of mitochondrial cristae architecture, biogenesis, and structural integrity

transition pore, followed by morphology changes and release of cytochrome c, which may serve as a proapoptotic stimulus (Calì et al. 2012; Mattson 2007). Moreover, the excess of Ca^{2+} can lead to activation of calpains, mainly calpain-2, which induces the fragmentation of structural proteins of the neuronal cytoskeleton along with hyperphosphorylation of TAU, classical molecular biomarker of neurodegeneration (Ji et al. 2012). In general, mitochondria are dynamic organelles that are submitted to continuous remodeling (Labbé et al. 2014; Westermann 2010)

in response to physiological and pathological stimuli (Nunnari and Suomalainen 2012). This mitochondrial plasticity is mandatory for the flux of information in the nerve terminals, which suffer unpredictable oscillations due to variations in ionic concentrations, plasma membrane potential, receptor activations, and metabolic demands.

Effects of AAS on Synaptic and Extrasynaptic Mitochondria

It is noteworthy that most studies with AAS focus on organ toxicity due to supra-physiological doses. However, there are recognized clinical therapeutic indications for these drugs. Further, AAS encompasses pharmacological properties that deserve to be explored in conditions where the anabolic support becomes deficient and exposes cells to the risk of death or decreased function. It is known that AAS mediates benefits for highly oxidative tissues like the heart and skeletal muscle, which implicates mitochondria bioenergetic as a target of anabolic-induced effects. The increased bioenergetic capacity is necessary to increase protein synthesis, for example, which is an ATP-demanding process and is often impaired in several pathologies. In general, impaired energy metabolism is a common finding in brain pathologies, including traumatic brain injury (TBI), Parkinson's disease, and Alzheimer's disease, and there are no pharmacological therapies directed to restore brain bioenergetics, thus hampering the progression of neurological diseases. Beyond pathologies, decreased brain energy metabolism is a hallmark of the aging process, and, similarly, senescent brain cells may benefit from pharmacological strategies that avoid the decay in neuroenergetic function (Kang et al. 2014). Hence, AAS supraphysiological doses generate a stigma against the therapeutic potential of these drugs. Indeed, AAS has characteristics that represent the duality between "Dr. Jekyll and Mr. Hyde." Regardless of the character, AAS through AR signaling or secondary effects trigger increased mitochondrial bioenergetics in skeletal muscle, whereas a direct metabolic benefit to neural cells is still controversial.

Targeting mitochondria pharmacologically has been complex, since mitochondria are heterogeneous (Kulbe et al. 2017). In the brain tissue preparations of nerve terminals, at least two distinct populations of mitochondria can be found (Fig. 4): synaptic mitochondria derived from the axon, dendritic, and soma and also an extrasynaptic population containing mitochondria, derived from glial and endothelial cell types (Flippo and Strack 2017; Kulbe et al. 2017; Li et al. 2004).

Studies have shown that these two populations of mitochondria displayed distinct properties, including differences in Ca^{2+} buffering capacity and protein expression and different responses to injury and pharmacotherapy (Brown et al. 2006; Gilmer et al. 2010; Kulbe et al. 2017; Naga et al. 2007; Patel et al. 2009; Stauch et al. 2014; Yarana et al. 2012). It is well established in the literature that supraphysiological doses of AAS can disrupt the glutamatergic synapse culminating in over-activation of the N-methyl-d-aspartate (NMDA) receptor calcium channel (Kalinine et al. 2014; Rodolphi et al. 2021), leading to elevated mitochondrial Ca^{2+} influx

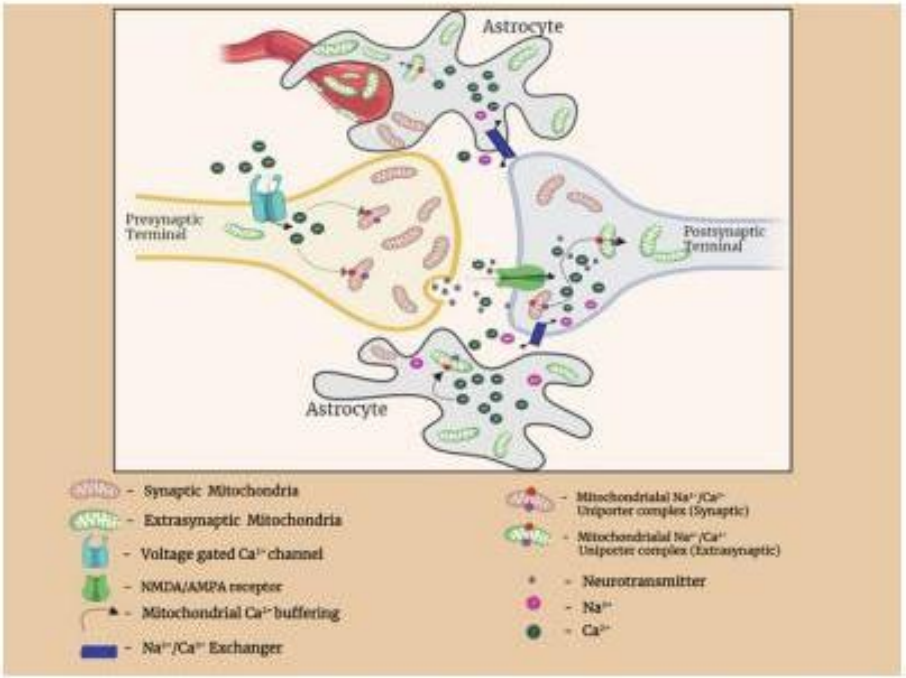


Fig. 4 Synaptic and extrasynaptic mitochondria. Synapses are highly active zones in which mitochondria bioenergetic efficiency paves the way for the flux of information

(Lin and Beal 2006; Nicholls 2010). This may alter the capability of handling Ca^{2+} by both synaptic (Rodolphi et al. 2021) and extrasynaptic mitochondria (Carteri et al. 2019a). Remarkably, in extrasynaptic mitochondria, this effect is related to the type and potency of the AAS. For instance, the molecular modification that originates ND provided higher anabolic potency than T. Based on this, ND mediates an increase in Ca^{2+} influx to extrasynaptic mitochondria but stimulates the Ca^{2+} efflux only in synaptic preparations (Carteri et al. 2019a; Rodolphi et al. 2021). This latter effect suggests a regulation on NCLX, the main responsible for balancing Ca^{2+}efflux in exchange with Na^{+} influx. However, it is not known the exact regulatory site that suffers such regulation.

Moreover, the Ca^{2+} influx brings to matrix positive charges that may alter the electrochemical potential of the mitochondrial membrane ($\Delta\Psi_m$) and trigger metabolic uncoupling, meaning oxygen consumption is not associated with ATP synthesis. A well-known consequence of a deficient formation and dissipation of $\Delta\Psi_m$ is an exacerbated production of ROS like hydrogen peroxide (H_2O_2). Nandrolone but not T was shown to alter both synaptic and extrasynaptic $\Delta\Psi_m$, increasing H_2O_2 production. Such rupture in the synchronicity of mitochondrial bioenergetic components caused by nandrolone may act as a driver for oxidative damage and apoptotic neurodegeneration (Carteri et al. 2019a; Rodolphi et al. 2021).

Due to their assumed differences in localization (Stauch et al. 2014; Yarana et al. 2012), mitochondria are subject to different stimuli that could affect their functional properties in distinct manners (Carteri et al. 2019a). It is understood that synaptic mitochondria are more sensitive to inhibition of the electron transport chain (ETC) proteins. This may be related to the higher energy demand requirement for synaptic transmission and regulation (Kulbe et al. 2017). Additionally, neurons possess a higher dependency of synaptic Ca^{2+} signaling, making mitochondria located at this site more susceptible to Ca^{2+} overloads resulting from the synaptic firings (Brown et al. 2006; Davey et al. 1997; Nicholls et al. 2007; Yarana et al. 2012). Another critical feature is regarding the specific cellular contributions to the extrasynaptic mitochondrial populations isolated from cortical samples. In this case, astroglia likely represents a large proportion of the population (Kulbe et al. 2017). Astrocytes differ from neurons regarding energy sources: while neurons utilize ATP generated by oxidative phosphorylation, astrocytes primarily utilize glycolysis for ATP production (Almeida et al. 2001; Bolaños 2016). Interestingly, astrocytes also have greater antioxidant capacity when compared to neurons (Raps et al. 1989; Sagara et al. 1993); therefore, they are more equipped to handle elevated levels of ROS (Kulbe et al. 2017). Remarkably, the AAS easily crosses the brain-blood barrier and reaches all brain areas, thereby influencing specific functional outcomes. However, the brain consumes large amounts of energy, which is required to maintain the electrochemical gradients, ionic balance, protein synthesis, trafficking of synaptic vesicles, and recycling neurotransmitters. Therefore, the mitochondrial bioenergetic machinery needs to respond immediately to the neurons' demands; otherwise, the flux of information through the synapse can be obliterated. In brain pathologies, such communication is likely impaired due to secondary mechanism affecting synaptic and extrasynaptic bioenergetics. Hence, preclinical findings reveal that AAS may have potential as mitochondrial bioenergetic enhancers with therapeutic applicability, albeit the resulting clinical outcomes associated with this approach are limited by the undesirable consequences of abuse in the brain.

Applications to Other Areas of Public Health

The clinical studies on AAS as neuroprotective agents are scarce, but there are positive reports. Oxandrolone (17β-hydroxy-17α-methyl-2-oxa-5α-androstane-3-one) has shown clinical benefits in neuromuscular diseases such as Duchenne muscular dystrophy (Fenichel et al. 2001), chronic catabolic disorders such as the treatment of HIV/AIDS-associated wasting (Berger et al. 1996), and amyotrophic lateral sclerosis (Rosenfeld et al. 2009). In long-term corticosteroid use, oxandrolone is known to hamper the protein catabolism and relieve bone pain associated with osteoporosis (Orr and Fiatarone Singh 2004). Furthermore, it does not display significant hepatic side effects such as cholestasis, peliosis hepatis, hepatic adenomas, and hepatocellular carcinomas (Rosenfeld et al. 2009). Other AAS showing therapeutic benefits have been mentioned in Table 2, based on the clinical research data worldwide.

Table 2 Anabolic-androgenic steroids and its therapeutic potential in several diseases. The clinical research data showing positive outcomes associated with anabolic-androgenic steroids and as potential drugs for different medical conditions. All these experiments were carried out with a controlled dosage under the supervision of medical practices, some of them with human trials while others on animal models

AAS	Treatment	References
Fluoxymesterone	Hypogonadism.	Buckle 1959
Oxymetholone	Advanced osteomyelitis; HIV/AIDS wasting syndrome	Skarberg 1973; Hengge et al. 1996
Mesterolone	Male infertility	Gerris et al. 1991; Liu et al. 2003; Madhukar and Rajender 2009
Stanozolol	Hereditary angioedema	Sloane et al. 2007; Cicardi et al. 1991
Testosterone cypionate	Testosterone replacement therapy for prostate cancer	Agarwal and Oefelein 2005
Mibolerone	Breast cancer	Rizza et al. 2014

Applications to Other Areas of Addiction

Opioids are pain-relieving drugs that act on nerve cells in the body and brain. They are usually safe when taken for a short period and are prescribed by a doctor. They are also known to produce euphoria, opening the possibility of misuse. For this reason, these drugs can lead to dependence, overdose incidents, and deaths. The brain abnormalities caused by abuse of AAS and other drugs such as heroin and oxycodone are underlying causes of opioid dependence since they lead to the use of opioids to relieve the withdrawal symptoms (Kosten and George 2002). The opioid overdose symptoms showed after testosterone ICV self-administration by male hamsters were reversed with naloxone, an opioid receptor antagonist (Peters and Wood 2005). According to Arvary and Pope (2000), 9.3% of patients admitted for treatment of dependence on heroin or other opioids had used anabolic steroids before, of which 67% had used it to counteract the withdrawal symptoms, suggesting that AAS may serve as a gateway to opioid dependence (Arvary and Pope 2000). It may happen due to the enhanced activity of central endogenous opioids led by AAS, which decreases after the cessation of use, leading to an acute hyperadrenergic syndrome (Kashkin and Kleber 1989).

Mini-dictionary of Terms

- **Cycling**: Taking a steroid for a period combined with frequent breaks to prevent tolerance buildup and return to homeostasis.
- **Electron transfer system**: Sequences of electron transporters embedded in the inner mitochondrial membrane that convert the available redox energy from oxidation of NADH and FADH2 into proton-motive force.

- **Homeostasis**: Homeostasis is the state of optimal condition required for the functioning of organisms. These include internal, physical, and chemical conditions such as body temperature and fluid balance.
- **Hypothalamic-pituitary-gonadal axis**: Has a crucial role in the regulation and development of the reproductive and immune systems.
- **Oxidative phosphorylation**: Oxidative phosphorylation (OXPHOS) is the process through which ATP is generated as a result of the transfer of electrons from NADH or $FADH_2$ to O_2 by a series of electron carriers. It is otherwise known as electron transport-linked phosphorylation or terminal oxidation.
- **Reward system**: Group of neural structures responsible for the positive validation of emotions and associative learning.
- **Stacking**: Mixed use of multiple steroids at the same period.

Key Facts

Key Facts of Anabolic-Androgenic Steroids

- Anabolic-androgenic steroids are synthetic substances derived from testosterone.
- The AAS are class III-controlled substances since 1991 in the USA.
- AAS users usually cycle and stack steroids to prevent side effects and tolerance buildup.
- AAS abuse affects dopaminergic, glutamatergic, serotonergic, and GABAergic systems.
- AAS modifies GABA neurotransmitter transmission and improves mental health.
- Prolonged use of AAS can cause damage to the cardiovascular, hepatic, kidney, and reproductive system.
- AAS use if frequently associated with an aromatase inhibitor to prevent androgenic side effects.
- Since steroids can be injected directly into the muscles, the use of unsterile equipment can lead to infections and life-threatening diseases like hepatitis or HIV.

Key Facts of Mitochondria

- Mitochondrial cristae are invaginations of the IM which contains the oxidative phosphorylation (OXPHOS) system.
- It generates energy by metabolic pathways such as the TCA, amino acid, lipid, and nucleotide metabolism pathways.
- Ca^{2+} buffering plays an important role in signaling for metabolic pathway regulation and apoptosis.

Key Facts of the Effects of AAS on Synaptic and Nonsynaptic Mitochondria

- Supraphysiological doses of AAS can cause a disruption in the glutamatergic synapses.
- Alterations in NMDAr activity and abnormal Ca^{2+} buffering cause mitochondrial dysfunction.
- These dysfunctions include increase in H_2O_2 production and reduction of mitochondrial dehydrogenase activity.
- Synaptic mitochondria are more sensitive to inhibition of ETC protein complex activity.
- Neurons utilize oxidative phosphorylation for ATP generation, while astrocytes primarily utilize glycolysis.

Summary Points

- The testosterone is an androgenic hormone responsible for developing male sex organs and secondary sex characteristics, and anabolic androgenic steroids (AAS) are synthetic substances derived from the testosterone, commonly used by individuals to enhance performance, esthetics, and self-esteem.
- The prolonged use of AAS is associated with cardiovascular, hepatic, kidney, and reproductive system damage and behavioral changes including serious addiction within various ages.
- The abuse of AAS can disrupt the glutamatergic synapse, resulting in overactivation of the N-methyl-D-aspartate receptor (NMDAr) ion channel, contributing to the elevation of mitochondrial Ca^{2+}.
- The AAS addictions are skyrocketing as each year passes, although the true extent globally remains unknown.
- According to the major public health surveys conducted by FDA, NIDA, NSP, and various rehabilitation research groups across the world, the statistics on the use of AAS shows a shocking amount of steadily increasing steroid abuse culture.
- Although addiction and abuse are serious growing concerns, the AAS, as therapeutic agents, also provide various breakthroughs in the scientific community for the research and development associated with many diseases and disorders.

References

Almeida A, Almeida J, Bolaños JP, Moncada S (2001) Different responses of astrocytes and neurons to nitric oxide: the role of glycolytically generated ATP in astrocyte protection. Proc Natl Acad Sci U S A 98:15294–15299. https://doi.org/10.1073/pnas.261560998

Arvary D, Pope HG (2000) Anabolic–androgenic steroids as a gateway to opioid dependence. N Engl J Med 342:1532–1532. https://doi.org/10.1056/nejm200005183422018

Baggish AL, Weiner RB, Kanayama G, Hudson JI, Picard MH, Hutter AM, Pope HG (2010) Long-term anabolic-androgenic steroid use is associated with left ventricular dysfunction. Circ Heart Fail 3:472–476. https://doi.org/10.1161/CIRCHEARTFAILURE.109.931063

Baggish AL, Weiner RB, Kanayama G, Hudson JI, Lu MT, Hoffmann U, Pope HG (2017) Cardiovascular toxicity of illicit anabolic-androgenic steroid use. Circulation 135:1991–2002. https://doi.org/10.1161/CIRCULATIONAHA.116.026945

Basaria S, Wahlstrom JT, Dobs AS (2001) Anabolic-androgenic steroid therapy in the treatment of chronic diseases. J Clin Endocrinol Metab 86:5108–5117. https://doi.org/10.1210/jcem.86.11.7983

Berger JR, Pall L, Hall CD, Simpson DM, Berry PS, Dudley R (1996) Oxandrolone in AIDS-wasting myopathy. AIDS 10:1657–1662. https://doi.org/10.1097/00002030-199612000-00010

Bolaños JP (2016) Bioenergetics and redox adaptations of astrocytes to neuronal activity. J Neurochem 139(Suppl):115–125. https://doi.org/10.1111/jnc.13486

Brower KJ, Eliopulos GA, Blow FC, Catlin DH, Beresford TP (1990) Evidence for physical and psychological dependence on anabolic androgenic steroids in eight weight lifters. Am J Psychiatry 147:510–512. https://doi.org/10.1176/ajp.147.4.510

Brown MR, Sullivan PG, Geddes JW (2006) Synaptic mitochondria are more susceptible to Ca2+overload than nonsynaptic mitochondria. J Biol Chem 281:11658–11668. https://doi.org/10.1074/jbc.M510303200

Cali T, Ottolini D, Brini M (2012) Mitochondrial Ca(2+) and neurodegeneration. Cell Calcium 52:73–85. https://doi.org/10.1016/j.ceca.2012.04.015

Carteri RB, Kopczynski A, Menegassi LN, Salimen Rodolphi M, Strogulski NR, Portela LV (2019a) Anabolic-androgen steroids effects on bioenergetics responsiveness of synaptic and extrasynaptic mitochondria. Toxicol Lett 307:72–80. https://doi.org/10.1016/j.toxlet.2019.03.004

Chandel NS (2014) Mitochondria as signaling organelles. BMC Biol 12:34. https://doi.org/10.1186/1741-7007-12-34

Clark AS, Henderson LP (2003) Behavioral and physiological responses to anabolic-androgenic steroids. Neurosci Biobehav Rev 27:413–436. https://doi.org/10.1016/S0149-7634(03)00064-2

Clark AS, Costine BA, Jones BL, Kelton-Rehkopf MC, Meerts SH, Nutbrown-Greene LL, Penatti CAA, Porter DM, Yang P, Henderson LP (2006) Sex- and age-specific effects of anabolic androgenic steroids on reproductive behaviors and on GABAergic transmission in neuroendocrine control regions. Brain Res 1126:122–138. https://doi.org/10.1016/j.brainres.2006.08.081

Cogliati S, Enriquez JA, Scorrano L (2016) Mitochondrial cristae: where beauty meets functionality. Trends Biochem Sci 41:261–273. https://doi.org/10.1016/j.tibs.2016.01.001

Davey GP, Canevari L, Clark JB (1997) Threshold effects in synaptosomal and nonsynaptic mitochondria from hippocampal CA1 and paramedian neocortex brain regions. J Neurochem 69:2564–2570. https://doi.org/10.1046/j.1471-4159.1997.69062564.x

David K (1935) Über des Testosteron, des Kfistallisierte Mäinnliche Hormon aus Steerentestes. Acta Brev Neerl. Physiol Pharmacol Microbiol 5:85–86

De Pergola G, Holmang A, Svedberg J, Giorgino R, Bjomtorp P (1990) Testosterone treatment of ovariectomized rats: effects on lipolysis regulation in adipocytes. Acta Endocrinol 123:61–66. https://doi.org/10.1530/acta.0.1230061

DeLeon KR, Grimes JM, Melloni RH (2002) Repeated anabolic-androgenic steroid treatment during adolescence increases vasopressin V1A receptor binding in Syrian hamsters: correlation with offensive aggression. Horm Behav 42:182–191. https://doi.org/10.1006/hbeh.2002.1802

Dunn M, White V (2011) The epidemiology of anabolic-androgenic steroid use among Australian secondary school students. J Sci Med Sport 14:10–14. https://doi.org/10.1016/j.jsams.2010.05.004

Dunn JF, Nisula BC, Rodbard D (1981) Transport of steroid hormones: binding of 21 endogenous steroids to both testosterone-binding globulin and corticosteroid-binding globulin in human plasma. J Clin Endocrinol Metab 53:58–68. https://doi.org/10.1210/jcem-53-1-58

Duntas LH, Popovic V (2013) Hormones as doping in sports. Endocrine. https://doi.org/10.1007/s12020-012-9794-9

Evans NA (2004) Current concepts in anabolic-androgenic steroids. Am J Sports Med. https://doi.org/10.1177/0363546503262202

Fenichel GM, Griggs RC, Kissel J, Kramer TI, Mendell JR, Moxley RT, Pestronk A, Sheng K, Florence J, King WM, Pandya S, Robison VD, Wang H (2001) A randomized efficacy and safety trial of oxandrolone in the treatment of Duchenne dystrophy. Neurology 56:1075–1079. https://doi.org/10.1212/WNL.56.8.1075

Flippo KH, Strack S (2017) An emerging role for mitochondrial dynamics in schizophrenia. Schizophr Res. https://doi.org/10.1016/j.schres.2017.05.003

Food and Drug Administration. Testosterone Information (2015). https://www.fda.gov/drugs/drugsafety/postmarketdrugsafetyinformationforpatientsandproviders/ucm161874.htm. Accessed 26 May 2021

Foradori CD, Weiser MJ, Handa RJ (2008) Non-genomic actions of androgens. Front Neuroendocrinol. https://doi.org/10.1016/j.yfrne.2007.10.005

Galligani N, Renck A, Hansen S (1996) Personality profile of men using anabolic androgenic steroids. Horm Behav 30:170–175. https://doi.org/10.1006/HBEH.1996.0021

Gilmer LK, Ansari MA, Roberts KN, Scheff SW (2010) Age-related mitochondrial changes after traumatic brain injury. J Neurotrauma 27:939–950. https://doi.org/10.1089/neu.2009.1181

Gorayski P, Thompson CH, Subhash HS, Thomas AC (2008) Hepatocellular carcinoma associated with recreational anabolic steroid use. Br J Sports Med 42:74–75. https://doi.org/10.1136/bjsm.2007.03932

Grata E, Perrenoud L, Saugy M, Baume N (2011) SARM-S4 and metabolites detection in sports drug testing: a case report. Forensic Sci Int 213:104–108. https://doi.org/10.1016/j.forsciint.2011.07.014

Grimes JM, Melloni RH (2002) Serotonin modulates offensive attack in adolescent anabolic steroid-treated hamsters. Pharmacol Biochem Behav 73:713–721. https://doi.org/10.1016/S0091-3057(02)00880-8

Hannan CJ, Friedl KE, Zold A, Kettler TM, Plymate SR (1991) Psychological and serum homovanillic acid changes in men administered androgenic steroids. Psychoneuroendocrinology 16:335–343. https://doi.org/10.1016/0306-4530(91)90019-P

Hochberg Z, Pacak K, Chrousos GP (2003) Endocrine withdrawal syndromes. Endocr Rev 24:523–538. https://doi.org/10.1210/ER.2001-0014

Horn S, Gregory P, Guskiewicz KM (2009) Self-reported anabolic-androgenic steroids use and musculoskeletal injuries: findings from the center for the study of retired athletes health survey of retired NFL players. Am J Phys Med Rehabil 88:192–200. https://doi.org/10.1097/PHM.0b013e318198b622

Ji J, Tyurina YY, Tang M, Feng W, Stolz DB, Clark RSB, Meaney DF, Kochanek PM, Kagan VE, Bayır H (2012) Mitochondrial injury after mechanical stretch of cortical neurons in vitro: biomarkers of apoptosis and selective peroxidation of anionic phospholipids. J Neurotrauma 29:776–788. https://doi.org/10.1089/neu.2010.1602

Kalinine E, Zimmer ER, Zenki KC, Kalinine I, Kazlauckas V, Haas CB, Hansel G, Zimmer AR, Souza DO, Muller AP, Portela LV (2014) Nandrolone-induced aggressive behavior is associated with alterations in extracellular glutamate homeostasis in mice. Horm Behav 66:383–392. https://doi.org/10.1016/j.yhbeh.2014.06.005

Kanayama G, Hudson JI, Pope HG (2010) Illicit anabolic-androgenic steroid use. Horm Behav 58:111–121. https://doi.org/10.1016/j.yhbeh.2009.09.006

Kang L, Li S, Xing Z, Li J, Su Y, Fan P, Wang L, Cui H (2014) Dihydrotestosterone treatment delays the conversion from mild cognitive impairment to Alzheimer's disease in SAMP8 mice. Horm Behav 65:505–515. https://doi.org/10.1016/j.yhbeh.2014.03.017

Kashkin KB, Kleber HD (1989) Hooked on hormones?: an anabolic steroid addiction hypothesis. JAMA J Am Med Assoc 262:3166–3170. https://doi.org/10.1001/jama.1989.03430220089036

Khacho M, Slack RS (2017) Mitochondrial activity in the regulation of stem cell self-renewal and differentiation. Curr Opin Cell Biol 49:1–8. https://doi.org/10.1016/j.ceb.2017.11.003

Khacho M, Slack RS (2018) Mitochondrial and reactive oxygen species signaling coordinate stem cell fate decisions and life long maintenance. Antioxid Redox Signal 28:1090–1101. https://doi.org/10.1089/ars.2017.7228

Khacho M, Harris R, Slack RS (2019) Mitochondria as central regulators of neural stem cell fate and cognitive function. Nat Rev Neurosci. https://doi.org/10.1038/s41583-018-0091-3

Kosten TR, George TP (2002) The neurobiology of opioid dependence: implications for treatment. Sci Pract Perspect. https://doi.org/10.1151/spp021113

Kühlbrandt W (2015) Structure and function of mitochondrial membrane protein complexes. BMC Biol 13:89. https://doi.org/10.1186/s12915-015-0201-x

Kulbe JR, Hill RL, Singh IN, Wang JA, Hall ED (2017) Synaptic mitochondria sustain more damage than non-synaptic mitochondria after traumatic brain injury and are protected by cyclosporine a. J Neurotrauma 34:1291–1301. https://doi.org/10.1089/neu.2016.4628

Labbé K, Murley A, Nunnari J (2014) Determinants and functions of mitochondrial behavior. Annu Rev Cell Dev Biol 30:357–391. https://doi.org/10.1146/annurev-cellbio-101011-155756

Li Z, Okamoto K-I, Hayashi Y, Sheng M (2004) The importance of dendritic mitochondria in the morphogenesis and plasticity of spines and synapses. Cell 119:873–887. https://doi.org/10.1016/j.cell.2004.11.003

Lin TM, Beal MF (2006) Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature 443:787–795

Malone DA, Dimeff RJ, Lombardo JA, Sample RHB (1995) Psychiatric effects and psychoactive substance use in anabolic-androgenic steroid users. Clin J Sport Med 5:25–31. https://doi.org/10.1097/00042752-199501000-00005

Maravelias C, Dona A, Stefanidou M, Spiliopoulou C (2005) Adverse effects of anabolic steroids in athletes: a constant threat. Toxicol Lett. https://doi.org/10.1016/j.toxlet.2005.06.005

Mattson MP (2007) Calcium and neurodegeneration. Aging Cell 6:337–350. https://doi.org/10.1111/j.1474-9726.2007.00275.x

McCabe SE, Brower KJ, West BT, Nelson TF, Wechsler H (2007) Trends in non-medical use of anabolic steroids by U.S. college students: results from four national surveys. Drug Alcohol Depend 90:243–251. https://doi.org/10.1016/J.DRUGALCDEP.2007.04.004

Melia P, Pipe A, Greenberg L (1996) The use of anabolic-androgenic steroids by Canadian students. Clin J Sport Med 6:9–14. https://doi.org/10.1097/00042752-199601000-00004

Melloni RH, Connor DF, Xuan Hang PT, Harrison RJ, Ferris CF (1997) Anabolic-androgenic steroid exposure during adolescence and aggressive behavior in golden hamsters. Physiol Behav 61:359–364. https://doi.org/10.1016/S0031-9384(96)00373-3

Miller WL, Auchus RJ (2019) The "backdoor pathway" of androgen synthesis in human male sexual development. PLoS Biol 17. https://doi.org/10.1371/journal.pbio.3000198

Mooradian AD, Morley JE, Korenman SG (1987) Biological actions of androgens. Endocr Rev 8:1–28. https://doi.org/10.1210/edrv-8-1-1

Mullen C, Whalley BJ, Schifano F, Baker JS (2020) Anabolic androgenic steroid abuse in the United Kingdom: an update. Br J Pharmacol 177:2180–2198. https://doi.org/10.1111/BPH.14995

Naga KK, Sullivan PG, Geddes JW (2007) High cyclophilin D content of synaptic mitochondria results in increased vulnerability to permeability transition. J Neurosci 27:7469–7475. https://doi.org/10.1523/JNEUROSCI.0646-07.2007

Nicholls DG (2010) Stochastic aspects of transmitter release and bioenergetic dysfunction in isolated nerve terminals. Biochem Soc Trans 38:457–459. https://doi.org/10.1042/BST0380457

Nicholls DG, Johnson-Cadwell L, Vesce S, Jekabsons M, Yadava N (2007) Bioenergetics of mitochondria in cultured neurons and their role in glutamate excitotoxicity. J Neurosci Res 85:3206–3212. https://doi.org/10.1002/jnr.21290

NIDA (2004) Steroid Abuse in Today's Society by Drug enforcement administration office of diversion control Washington, D.C 20537 Available at: https://books.google.com.br/books?

id=14GyX4r8nwoC&pg=PA12&lpg=PA12&dq=Steroid+Abuse+in+Today%27s+Society+Dr.+James+Tolliver&source=bl&ots=H9H0crfNy9&sig=ACfU3U0edv1pACbeJ02XZIwH9IS-Be9B4vw&hl=en&sa=X&ved=2ahUKEwiW1O-GlPP2AhUDH7kGHXifBsQQ6AF6BAg-SEAM#v=onepage&q=Steroid%20Abuse%20in%20Today's%20Society%20Dr.%20James%20Tolliver&f=false

NIDA (2020) Lifetime prevalence of use of steroids for grades 8, 10, and 12 from 2006 to 2021 Available at: https://www.statista.com/statistics/696572/us-lifetime-prevalence-of-steroid-use-in-grades-8-10-12-since-2006/

Nunnari J, Suomalainen A (2012) Mitochondria: in sickness and in health. Cell 148:1145–1159. https://doi.org/10.1016/j.cell.2012.02.035

Orr R, Fiatarone Singh M (2004) The anabolic androgenic steroid oxandrolone in the treatment of wasting and catabolic disorders: review of efficacy and safety. Drugs. https://doi.org/10.2165/00003495-200464070-00004

Patel SP, Sullivan PG, Pandya JD, Rabchevsky AG (2009) Differential effects of the mitochondrial uncoupling agent, 2,4-dinitrophenol, or the nitroxide antioxidant, Tempol, on synaptic or nonsynaptic mitochondria after spinal cord injury. J Neurosci Res 87:130–140. https://doi.org/10.1002/jnr.21814

Patil JJ, O'Donohoe B, Loyden CF, Shanahan D (2007) Near-fatal spontaneous hepatic rupture associated with anabolic androgenic steroid use: a case report. Br J Sports Med 41:462–463. https://doi.org/10.1136/bjsm.2006.031617

Peters KD, Wood RI (2005) Androgen dependence in hamsters: overdose, tolerance, and potential opioidergic mechanisms. Neuroscience 130:971–981. https://doi.org/10.1016/J.NEUROSCIENCE.2004.09.063

Pfanner N, Warscheid B, Wiedemann N (2019) Mitochondrial proteins: from biogenesis to functional networks. Nat Rev Mol Cell Biol 20:267–284. https://doi.org/10.1038/s41580-018-0092-0

Piacentino D, Kotzalidis G, Casale A, Aromatario M, Pomara C, Girardi P, Sani G (2014) Anabolic-androgenic steroid use and psychopathology in athletes. A systematic review. Curr Neuropharmacol 13:101–121. https://doi.org/10.2174/1570159x13666141210222725

Pope HG, Wood RI, Rogol A, Nyberg F, Bowers L, Bhasin S (2014) Adverse health consequences of performance-enhancing drugs: an endocrine society scientific statement. Endocr Rev. https://doi.org/10.1210/er.2013-1058

Raps SP, Lai JC, Hertz L, Cooper AJ (1989) Glutathione is present in high concentrations in cultured astrocytes but not in cultured neurons. Brain Res 493:398–401. https://doi.org/10.1016/0006-8993(89)91178-5

Rodolphi MS, Kopczynski A, Carteri RB, Sartor M, Fontella FU, Feldmann M, Hansel G, Strogulski NR, Portela LV (2021) Glutamate transporter-1 link astrocytes with heightened aggressive behavior induced by steroid abuse in male CF1 mice. Horm Behav 127:104872. https://doi.org/10.1016/J.YHBEH.2020.104872

Rosenfeld J, King RM, Smith JE (2009) Oxandrolone in ALS: preliminary analysis. Amyotroph Lateral Scler Other Motor Neuron Disord 1:21–26. https://doi.org/10.1080/14660820050515674

Sagara JI, Miura K, Bannai S (1993) Maintenance of neuronal glutathione by glial cells. J Neurochem 61:1672–1676. https://doi.org/10.1111/j.1471-4159.1993.tb09802.x

Sagoe D, Torsheim T, Molde H, Andreassen CS, Pallesen S (2017) Anabolic-androgenic steroid use in the Nordic countries: a meta-analysis and meta-regression analysis. Nordic Stud Alcohol Drugs 32:7–20. https://doi.org/10.1515/NSAD-2015-0002

Shahidi NT (2001) A review of the chemistry, biological action, and clinical applications of anabolic-androgenic steroids. Clin Ther 23:1355–1390. https://doi.org/10.1016/S0149-2918(01)80114-4

Solomon ZJ, Mirabal JR, Mazur DJ, Kohn TP, Lipshultz LI, Pastuszak AW (2019) Selective androgen receptor modulators: current knowledge and clinical applications. Sex Med Rev. https://doi.org/10.1016/j.sxmr.2018.09.006

Stauch KL, Purnell PR, Fox HS (2014) Quantitative proteomics of synaptic and nonsynaptic mitochondria: insights for synaptic mitochondrial vulnerability. J Proteome Res 13: 2620–2636. https://doi.org/10.1021/pr500295n

Thiblin I, Kristiansson M, Rajs J (2008) Anabolic androgenic steroids and behavioural patterns among violent offenders. J Forensic Psychiatry 8:299–310. https://doi.org/10.1080/09585189708412012

Westermann B (2010) Mitochondrial fusion and fission in cell life and death. Nat Rev Mol Cell Biol 11:872–884. https://doi.org/10.1038/nrm3013

Xu X, de Pergola G, Björntorp P (1991) Testosterone increases lipolysis and the number of β-adrenoceptors in male rat adipocytes. Endocrinology 128:379–382. https://doi.org/10.1210/endo-128-1-379

Yarana C, Sanit J, Chattipakorn N, Chattipakorn S (2012) Synaptic and nonsynaptic mitochondria demonstrate a different degree of calcium-induced mitochondrial dysfunction. Life Sci 90: 808–814. https://doi.org/10.1016/j.lfs.2012.04.004

Yesalis CE, Bahrke MS (1995) Anabolic-androgenic steroids: current issues. Sport Med. https://doi.org/10.2165/00007256-199519050-00003

Capítulo II: Glutamate transporter-1 links astrocytes with heightened aggressive behavior induced by steroid abuse in male CF1 mice.

No capítulo II apresentamos o artigo publicado na revista *"Hormones and Behavior"*

Esse estudo teve como objetivo principal investigar se o aumento da expressão da GLT-1 pela CEF seria capaz de modular os níveis de glutamato e de atividade do receptor NMDA via subunidade pNR2B^{ser1232} e reduzir o comportamento agressivo induzido pelo ND. Para isso, realizamos um tratamento de 19 dias com uma dose diária de ND e, para testar nossa hipótese, administramos CEF nos últimos 5 dias dessa janela de 19 dias. Os resultados demonstraram que a co-administração da CEF diminuiu a agressividade induzida pelo ND. Mecanisticamente estes efeitos foram associados ao aumento da expressão de GLT-1, e diminuição dos níveis de glutamato e de fosforilação de pNR2B^{ser1232}. Desta maneira, estes resultados sugerem que a modulação de um alvo astrocitário (GLT-1) promove uma diminuição da hiperexcitabilidade glutamatérgica via receptor NMDA e o comportamento agressivo.

Hormones and Behavior 127 (2021) 104872

Contents lists available at ScienceDirect

Hormones and Behavior

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

Glutamate transporter-1 link astrocytes with heightened aggressive behavior induced by steroid abuse in male CF1 mice

Marcelo S. Rodolphi[a], Afonso Kopczynski[a], Randhall B. Carteri[a,b], Monia Sartor[a], Fernanda U. Fontella[c], Marceli Feldmann[a], Gisele Hansel[a,d,e], Nathan R. Strogulski[a], Luis V. Portela[a,*]

[a] Laboratório de Neurotrauma e Biomarcadores, Departamento de Bioquímica, Programa de Pós-Graduação Bioquímica, Universidade Federal do Rio Grande do Sul-UFRGS, Ramiro Barcelos 2600, anexo, Porto Alegre, RS 90035-003, Brazil
[b] Centro Universitário Metodista – Instituto Porto Alegre (IPA), Coronel Joaquim Pedro Salgado 80, Porto Alegre, RS 90420-060, Brazil
[c] Departamento de Bioquímica, Programa de Pós-Graduação Bioquímica, Universidade Federal do Rio Grande do Sul-UFRGS, Ramiro Barcelos 2600, anexo, Porto Alegre, RS 90035-003, Brazil
[d] Robert A. Groff Professor of Teaching and Research in Neurosurgery Department: Neurosurgery, University of Pennsylvania, 105 Hayden Hall 3320 Smith Walk, Philadelphia, PA 19104-6316, USA
[e] Laboratório de Neuroinflamação e Neuroimunologia, Instituto do Cérebro do Rio Grande do Sul, Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Porto Alegre, RS, Brazil

ARTICLE INFO

Keywords:
GLT-1 transporter
GluN2B
NMDA
Synaptic mitochondria
Calcium
Na^+/K^+-ATPase
Aggression

ABSTRACT

The astrocytic glutamate transporter GLT-1 performs glutamate uptake thereby mediating NMDAr responses in neurons. Ceftriaxone (CEF) upregulates astrocytic GLT-1 expression/activity, which could counteract excessive glutamate levels and aggressive behavior induced by anabolic synthetic steroids such as nandrolone decanoate (ND). Here, adult male CF-1 mice were allocated to oil (VEH), ND, CEF, and ND/CEF groups. Mice were subcutaneously (s.c.) injected with ND (15 mg/kg) or VEH for 19 days, and received intraperitoneal (i.p.) injections of CEF (200 mg/kg) or saline for 5 days. The ND/CEF group received ND for 19 days plus coadministration of CEF in the last 5 days. On the 19th day, the aggressive phenotypes were evaluated through the resident-intruder test. After 24 h, cerebrospinal fluid was collected to measure glutamate levels, and the pre-frontal cortex was used to assess GLT-1, pGluN2B^{Tyr1472}, and pGluN2A^{Tyr1246} by Western blot. Synaptosomes from the left brain hemisphere was used to evaluate mitochondrial function including complex II-succinate dehydrogenase (SDH), Ca^{2+} handling, membrane potential ($\Delta\Psi_m$), and H_2O_2 production. ND decreased the latency for the first attack and increased the number of attacks by the resident mice against the intruder, mechanistically associated with an increase in glutamate levels and pGluN2B^{Tyr1472} but not pGluN2A^{Tyr1246}, and GLT-1 downregulation. The abnormalities in mitochondrial Ca^{2+} influx, SDH, $\Delta\Psi_m$, and H_2O_2 implies in deficient energy support to the synaptic machinery. The ND/CEF group displayed a decreased aggressive behavior, normalization of glutamate and pGluN2B^{Tyr1472}levels, and mitochondrial function at synaptic terminals. In conclusion, the pharmacological modulation of GLT-1 highlights its relevance as an astrocytic target against highly impulsive and aggressive phenotypes.

1. Introduction

There are worldwide health concerns regarding the widespread misuse of anabolic-androgen steroids (AAS) among young male and female subjects aiming to enhance sports performance and body image. For instance, supraphysiological doses of AAS may cause undesirable neuropsychiatric manifestations including psychosis, depression, anxiety, and exacerbated aggressive behavior (Clark and Henderson, 2003; Yesalis, 2001).

Reports indicate an increasing association between young male individuals engaged in violent crimes and the abuse of these substances, though socioeconomic status and polydrug abuse may favor the effects of AAS on violent behavior (Beaver et al., 2008; Lundholm et al., 2015). Studies in rodents mimicking AAS abuse in humans have also replicated

* Corresponding author at: Laboratory of Neurotrauma and Biomarkers, Department of Biochemistry, ICBS, UFRGS, 2600 Ramiro Barcelos street, 90035-003 Porto Alegre, RS, Brazil.
E-mail address: roskaportela@gmail.com (L.V. Portela).

https://doi.org/10.1016/j.yhbeh.2020.104872
Received 31 March 2020; Received in revised form 2 October 2020; Accepted 3 October 2020
Available online 22 October 2020
0018-506X/ © 2020 Elsevier Inc. All rights reserved.

the increased aggressive impulse and dominant phenotype (Breuer et al., 2001; Carrillo et al., 2011; Le Grevès et al., 1997) suggesting that supraphysiological doses trigger aggressive behavior *per se*. Although both experimental and epidemiological studies have reinforced such a causal role, there are still gaps to fill regarding the brain mechanisms underlying this behavior.

Attention has been given to the involvement of glutamatergic ionotropic receptors in heightened aggressive behavior induced by AAS, alcohol, or other drugs (Newman et al., 2018a, 2018b; Rossbach et al., 2007). Glutamate is the main excitatory neurotransmitter in the mammalian brain and is engaged in a variety of neurophysiological roles; however, its excessive levels in the synaptic terminals may trigger neuronal hyperexcitability mainly through the *N*-methyl-D-aspartate receptor (NMDAr) – a calcium (Ca^{2+}) ion channel (Danbolt et al., 2016; Dingledine et al., 1999). The magnitude of the rise in intracellular Ca^{2+} concentrations mediated by glutamate levels may affect crucial synaptic mechanisms such as mitochondrial function, and the activity downstream signaling proteins leading to hyperphosphorylation of NMDAr subunits (Carteri et al., 2019a; Gnegy, 2000). Remarkably, mechanistic studies have linked AAS abuse with changes in the protein content and phosphorylation levels of NMDAr subunits in brain regions involved in emotional behavior like the hypothalamus, hippocampus, and prefrontal cortex (Rossbach et al., 2007). Further, the pharmacological administration of NMDAr channel blockers has reinforced its mechanistic involvement in the development of aggressive phenotypes (Kotermanski et al., 2013; Newman et al., 2018c).

Although exacerbated aggression has been linked to abnormalities in the NMDAr function, complementary mechanisms based on neuron-to-astrocyte interactions cannot be ruled out (Fischer et al., 2007). Existing transporters in astrocytes, particularly the more abundant Na^+-dependent glutamate-transporter-1 (GLT-1), is responsible for more than 90% of glutamate removal from the synaptic cleft in the cortex and hippocampus thereby contributing significantly to the homeostasis of glutamatergic signaling (Danbolt, 2001; Danbolt et al., 2016). The GLT-1 should work in a synchronized manner with other synaptic components including Na^+/K^+-ATPase, Ca^{2+}-ATPase, NMDAr, and mitochondria to sustain the ionic homeostasis, energy support, and prevent glutamatergic hyperactivation (Allen, 2014; Bordone et al., 2019; Danbolt et al., 2016).

We previously used a mouse model of nandrolone (ND)-induced aggressive behavior and found a decreased GLT-1 expression/activity in the prefrontal cortex coupled with increased glutamate levels in the cerebrospinal fluid (CSF). We significantly attenuated exacerbated aggression using ketamine and MK-801 – two well-known NMDAr channel blockers (Kalinine et al., 2014). In several experimental models that imply an excessive increase of glutamate levels in the synaptic space, the administration of the β-lactam antibiotic ceftriaxone (CEF) counteracts the behavioral and molecular effects associated with NMDAr hyperactivation (Mineur et al., 2007; Rothstein et al., 2005). Particularly, Rothstein et al. (2005) demonstrated that intraperitoneal administration of CEF (200 mg/kg) for 7 days in male rats increased the expression and activity of GLT-1 in the brain, which remained elevated for at least 7 days after the last administration. This seminal work highlighted the potentialities of CEF as a modulator of GLT-1 in neurological conditions that evolve with glutamatergic hyperactivation and provided a solid background for further studies exploring alternative time regimens like five days treatment in both rats and mice (Krzyzanowska et al., 2016; Miller et al., 2008; Sari et al., 2010). However, these recognized neuropharmacological properties were not tested against exacerbated aggressive phenotypes.

Here, we pharmacologically interrogate the role of GLT-1 in astrocyte-neuron-mediated aggressive behavior induced by AAS. We show that ruptures in the glutamatergic-synaptic mechanisms are based on GLT-1, glutamate levels, phosphorylation of GluN2B, and synaptic mitochondrial metabolic dysfunctions that lead to an escalation of aggressive behavior.

2. Methods

2.1. Mice

Two-month old CF1 male mice ($n = 120$) weighing 40–48 g were provided by the Centro de Reprodução e Experimentação de Animais de Laboratório, (CREAL, UFRGS, Porto Alegre, Brazil). Mice were housed in standard plexiglass cages (48 × 26 cm) and had free access to food and water. The home-cages were maintained in a room with controlled temperature (22 °C) under a 12 h light/12 h dark cycle (lights on at 7 a.m.). To avoid social isolation, we maintained four mice per cage (Muller et al., 2011). All experiments were conducted according to the NIH Guide for Care and Use of Laboratory Animals, the Brazilian Society for Neuroscience and Behavior (SBNeC), and the official governmental guidelines in compliance with the Federation of Brazilian Societies for Experimental Biology. The experiments were approved by the Ethical Committee of the Federal University of Rio Grande do Sul, Brazil (CEUA#29844).

2.2. Experimental design and drug administration

The ND dose and time-regimen were chosen based on previously published works by our group and other groups who reported changes in the glutamatergic parameters associated with exacerbated aggressive behavior induced by administration of ND (15 mg/kg) for 14 to 19 days (Kalinine et al., 2014; Le Grevès et al., 1997; Rossbach et al., 2007). The dose of CEF 200 mg/kg was well-established by Rothstein et al., 2005 to increase GLT-1 expression and biochemical function in the brain of rats. Other works performed in rats and mice also reported increments in GLT-1 expression/activity induced by CEF 200 mg/kg after 5 days administration (Krzyzanowska et al., 2016; Miller et al., 2008; Sari et al., 2010). To effectively prevent the effects of ND on glutamatergic synaptic machinery and aggressive behavior, we decided to overlap CEF and ND injections in the final 5 days of ND treatment; in which time-window the establishment of molecular alterations that lead to heightened aggression induced by ND is already observed. This was reported by Kalinine et al. (2014) and in the data composing the M.Sc. dissertation work of Kalinine (Kalinine and Portela, 2011).

Mice home-cages were randomized into one of the five groups: i) (ND) injected subcutaneously (s.c.) with Deca-Durabolin®, Organon (15 mg/kg/day in a volume of 1 ml/kg, dissolved in corn oil) for 19 days; simultaneous intraperitoneal (i.p.) injection of saline solution for 19 days ($n = 15$); ii) (CEF) injected s.c. with corn oil for 19 days and simultaneous i.p. saline solution for 14 days; saline was then exchanged for 5 days of i.p. administration of ceftriaxone (ceftriaxone sodium, Eurofarma, SP, Brasil) 200 mg/kg/day in a total of 3 ml/kg volume (n = 15) (Cui et al., 2014; Hu et al., 2015; Tyson et al., 2018); iii) (ND/CEF), injected s.c. with ND for 19 days and simultaneous i.p. saline solution for 14 days; saline was then exchanged for 5 days i.p. CEF ($n = 15$); iv) (VEH) injected s.c. with corn oil for 19 days and simultaneous i.p. saline for 19 days ($n = 15$); and v) intruder mice allocated to this group were not injected with drugs, oil, or saline solution, or suffered prior manipulation until they were submitted to the intruder test ($n = 60$). Mice from the intruder group were housed separately from other experimental groups. Each mice received two daily injections in the morning shift (between 10:00 and 11:00 am). The experimental timeline and procedures are shown in Fig. 1.

2.3. Open field test (OF)

On the 18th day of treatment, mice were submitted to an open field test to evaluate spontaneous locomotion and exploratory activity. The apparatus was made of a black-painted box measuring 50 cm × 50 cm surrounded by 50 cm high walls. The experiments were conducted in a sound-attenuated room under low-intensity light (12 lx). Each mouse ($n = 6$ per group) was placed in the right corner of the arena and

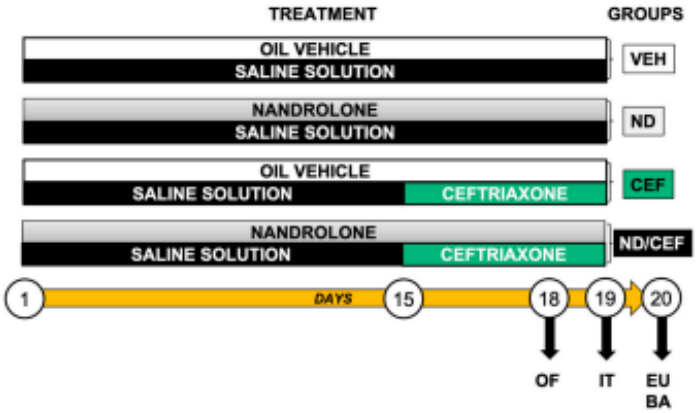


Fig. 1. Experimental design. Schematic design of pharmacologic treatments and regimens for each experimental group. Abbreviations: VEH, vehicle; ND, nandrolone decanoate; CEF, ceftriaxone; IT, resident-intruder test; OF, open field test; EU, euthanasia; BA, biochemical assessments.

locomotor activity was recorded with a video camera placed above the arena according to protocol already described (Kazlauckas et al., 2005). The analysis was performed using a computer-operated tracking system (Any-maze, Stoelting, Woods Dale, IL). The apparatus was cleaned with 70% alcohol between each test. The total distance traveled, distance traveled minute by minute, maximum speed, mean speed, total mobile time, total immobile time, and time spent in peripheral and central zones were recorded and used as indicators of locomotor activity and exploratory behavior.

2.4. Intruder/resident test (IT)

The intruder test was performed on the 19th day of the experimental protocol ($n = 12$ per group). Two days prior to testing the home cage, sawdust was not changed to preserve the territorial demarcation clues of the resident mice, which is important for the sense of territoriality. The test was performed during the light phase. Three resident mice were removed from the box and placed in identical clean cages while one remained alone in the home cage for 2 min before beginning the test. Next, an intruder male was inserted into the territory of the resident male and the behavior of the mice was recorded for 10 min if possible. The mouse was then removed from the home cage and another mouse was subsequently introduced and tested. Afterward, two blind researchers analyzed the dominant, subordinate, and neutral postures and the latency to the first aggressive attack and number of attacks of the resident mouse toward an intruder. The posture of the resident in relation to the intruder mouse was stratified as follows: i) Dominant when the resident mouse attacked the intruder; ii) Submissive when he was attacked by the intruder; and iii) Neutral when there was no aggressive interaction.

In rare cases, intruder mice presented an offensive profile and attacked the residents first. In such cases, the test was discontinued, and both resident and intruder mice were excluded from the experiment. The test was immediately discontinued when the aggressive behavior inflicted visible injuries and bleeding—both mice were excluded from all analyses in this case (Kalinine et al., 2014; Newman et al., 2018a). After exclusions both due to injuries and intruder-first attack, the total number of mice was 96 with an $n = 12$ per group including 48 intruder mice. For a more detailed protocol see Kalinine et al. (Kalinine et al., 2014).

2.5. Sample collection

Twenty-four hours after the intruder test, mice were anesthetized in an induction chamber for cerebrospinal fluid (CSF) collection directly from the cisterna-magna (Muller et al., 2012). Mice were subsequently euthanized by decapitation, and the right prefrontal cortex was dissected on ice and bathed with cold homogenization buffer (320 mM sucrose, 10 mM TRIS, 0.1 EDTA, pH 7.4). The left hemisphere was dissected and processed according to the techniques described below ($n = 12$ per group).

2.6. HPLC procedure

The concentrations of glutamate and aspartate in the cell-free CSF were determined by high-performance liquid chromatography (HPLC) (Rowley et al., 1995; Stefani et al., 2017). Samples were derivatized with 100.5 µl of o-phthalaldehyde (OPA) (5.4 mg OPA in 1 ml silicon borate 0.2 M, pH 9.5) plus 25.5 µl of mercaptoethanol 4% for 3 min, and separation was carried out with a reverse- phase column (Supelcosil LC-18, 250 mm × 4,6 mm × 5 µm, Supelco) in a Shimadzu instruments liquid chromatograph (50 µl loop valve injection, 40 µl injection volume). The mobile phase flowed at a rate of 1.4 ml/min, and the column temperature was 25 °C. The buffer composition was A: 0.04 mol/l sodium dihydrogen phosphate monohydrate buffer, pH 5.5 containing 80% of methanol; and B: 0.01 mol/l dihydrogen phosphate monohydrate buffer, pH 5.5 containing 20% of methanol. The gradient profile was modified according to the content of buffer B in the mobile phase: 100% in 0.10 min, 90% in 15 min, 48% in 10 min, and 100% in 60 min. The excitation and emission were 360 nm and 455 nm and studied in a Shimadzu fluorescence detector. Samples (5 µl) were used, and the concentration was expressed in µM. The amino acids were identified by their retention time and were quantitatively determined using their peak chromatographic area. A standard amino acid mixture was used for calibration (Joseph and Davies, 1983); the total was $n = 8$ per group.

2.7. Western blotting

Shortly after euthanasia, the pre-frontal cortex was dissected and homogenized in a buffer solution (EDTA 2 mM, protease and phosphatase inhibitor cocktails I and II (Sigma, St. Louis, USA) 0.001%, Tris HCl 50 mM, (pH 7.4), glycerol 10% and Triton X-100 1%) and centrifuged (15,000 *g* for 10 min). The supernatant was collected and diluted in an appropriate sample buffer. Protein samples (20 µg) from each sample ($n = 5$–6 group) were separated by electrophoresis on a 10% polyacrylamide gel and electrotransferred to nitrocellulose membranes (Amersham, GE Healthcare, Little Chalfont, UK) as previously

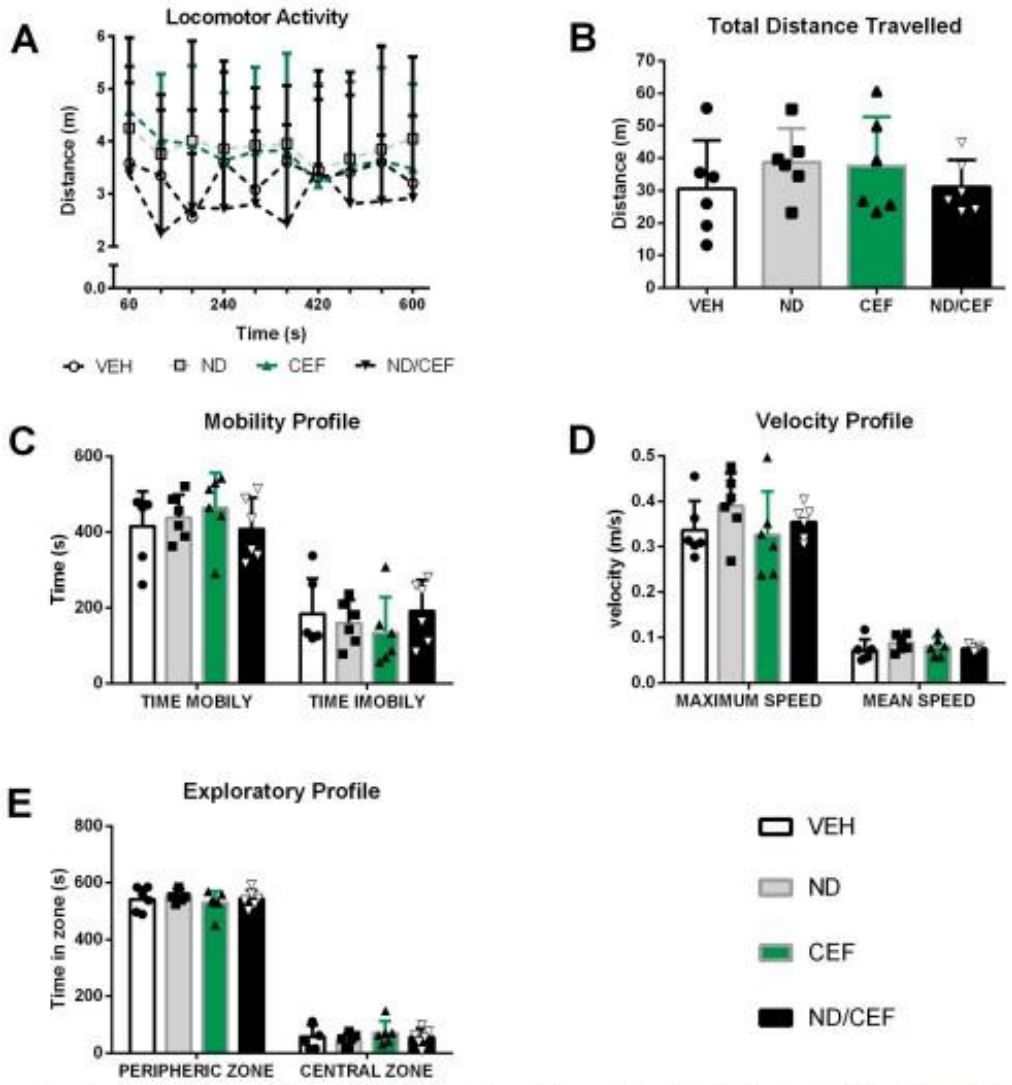


Fig. 2. Locomotor and exploratory behavior on the open field test. (A) Locomotor activity per minute, (B) total distance traveled, (C) mobility profile, (D) velocity profile, and (E) exploratory profile. Data are shown as group Mean ± S.D. There were no differences between groups (n = 6 per group).

reported (Muller et al., 2011). Blocking procedures were performed according to the manufacturer's instructions. Primary antibodies against anti-EAAT2 (ABCAM, Cambridge, UK, 1:1000), GluNR2B (Cell Signaling Technology, Denver, USA, 1:1000), pGluNR2B(Tyr1472) (Tocris, Bristol, UK, 1:1000), GluNR2A (Cell Signaling Technology, Denver, USA, 1:1000), pGluNR2A(Tyr1246) (Cell Signaling Technology, Denver, USA, 1:1000) were incubated overnight followed by 2 h incubation with horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG (1:5000) (GE, Little Chalfont, UK) secondary antibodies. Membranes were incubated with Amersham ECL Western Blotting Detection Reagent (GE Healthcare Life Sciences, Little Chalfont, UK) for 5 min and the immunodetection used chemiluminescence with an Image Quant LAS 4000 system (GE, Little Chalfont, UK). The protein expression was quantified using Image J® software (Rockville, USA). The optical density values were expressed as % of control. All procedures were conducted according to specific antibody manufacturer's instruction.

2.8. Synaptosomes preparation

The left cerebral hemisphere was rapidly dissected from the cerebellum and olfactory bulb and homogenized in 500 µl of homogenization with 10 strokes of a 7 ml glass Douce tissue homogenizer. The cerebral homogenates were centrifuged, and the pellet was resuspended in a 15% Percoll® (GE Healthcare®, Little Chalfont, UK) solution and submitted to a discontinuous Percoll® gradients centrifugation at 30,700g × 5 min resulting in 15% and 23% Percoll® bands, respectively, enriched in synaptosomes (Carteri et al., 2019b; Sims and Anderson, 2008). Synaptosomes were then resuspended in sucrose and Tris buffer (320 mM sucrose, 10 mM Tris, pH 7.4) and immediately

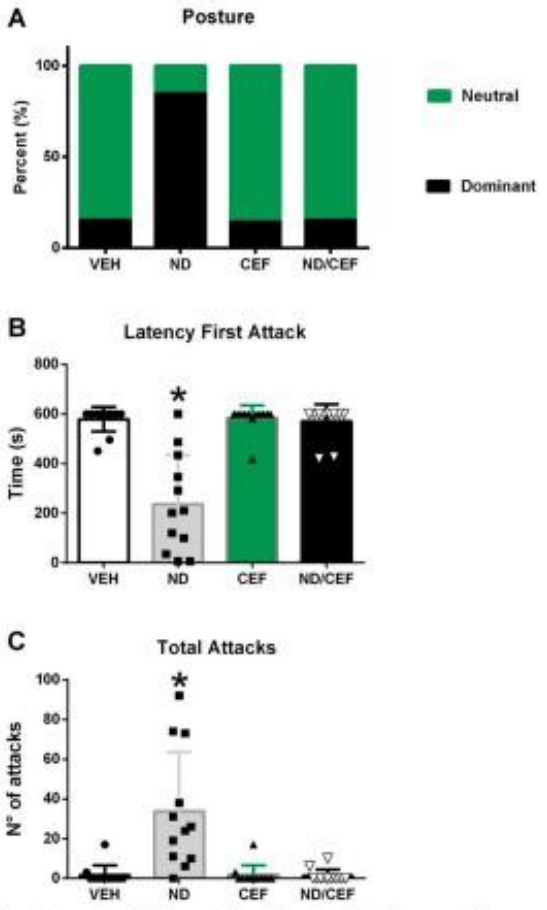


Fig. 3. Aggressive behavior profiling in the Intruder-Resident test. (A) posture of resident mice against the intruder, VEH (15.38% dominant, 84.62% neutral), ND (85% dominant; 15% neutral), CEF (14.29% dominant; 85.71% neutral), ND/CEF (15.38% dominant; 84.62% neutral), (B) Latency to first attack, (C) Total number of attacks inflicted by the resident against the intruder. Data are shown as group Mean ± S.D. *Indicates significant statistical differences from other groups ($p < 0.05$). Abbreviations: VEH, vehicle; ND, nandrolone decanoate; CEF, ceftriaxone; ND/CEF, nandrolone plus ceftriaxone ($n = 12$ per group).

used to assess Na^+/K^+-ATPase and Ca^{2+}-ATPase activity including a panel of mitochondrial parameters like Ca^{2+} handling, general dehydrogenases activity, mitochondrial oxygen consumption, membrane potential ($\Delta\Psi_m$) induced by succinate (substrate of succinate dehydrogenase also known as mitochondrial Complex II), and H_2O_2 production ($n = 7$–9 per group). Aliquots of synaptosomes were used to quantify the total protein levels.

2.9. Synaptosomal Na^+/K^+-ATPase and Ca^{2+}-ATPase activities

The activity of these enzymes was measured in synaptosomes through a colorimetric method that uses malachite green dye. For a Na^+/K^+-ATPase assay, the synaptosomes were incubated with reaction buffer (5 mM $MgCl_2$, 80 mM NaCl, 20 mM KCl, and 40 mM Tris-HCl (pH 7.4)). After 10 min of pre-incubation at 37 °C, 3 mM of ATP was added to the medium followed by an additional 20 min incubation. The same procedure was made with the addition of 1 mM ouabain. The Na^+/K^+-ATPase activity was calculated by the difference between the assays with and without ouabain (Wyse et al., 2000). The color formation by the release of Pi was monitored at 630 nm (Spectra Max M5, Molecular Devices), and the enzymatic activity was expressed as nmol Pi (hydrolyzed from ATP) released/min/mg of protein.

The Ca^{2+}-ATPase activity was measured using a reaction medium buffer (50 mM, KCl 25 mM, Tris HCl [pH 7.4], 4.0 μg of oligomycin, and 0.1 mM ouabain). Synaptosomes were pre-incubated with or without 0.155 mM of $CaCl_2$ for 10 min. ATP at a final concentration of 3 mM was added to the medium and then reaction proceeded for 20 min at 37 °C. It was then stopped with molybdate (Michaelis et al., 1983). The Ca^{2+}-ATPase activity was calculated as the difference in ATP consumption in the presence and absence of Ca^{2+}. The intensity of color formation due to Pi reaction with green malachite dye was monitored at 630 nm (Spectra Max M5, Molecular Devices), and the activity was expressed as nmol Pi released/min/mg of protein ($n = 7$–9 per group).

2.10. Mitochondrial panel

The mitochondria within the synaptosomes provide ATP to the cytoplasm and plasmatic membrane. This preserves the metabolism and maintains the functioning of receptors, ionic channels, and the machinery for exocytosis and reuptake of neurotransmitters (Nicholls, 2003). To assess mitochondrial calcium buffering capacity in synaptosomes – namely calcium influx and efflux (n = 7–9 per group) – we evaluated absorbance spectrophotometrically at 540 nm (Spectra Max M5, Molecular Devices). Synaptosomes (50 ul) were added to standard swelling incubation medium (100 mM KCl, 50 mM sucrose, 10 mM HEPES, and 5 mM KH_2PO_4); basal absorbance was monitored for 3 min. Mitochondrial substrates were added to support ATP production (3.5 mM pyruvate, 4.5 mM malate, 4.5 mM glutamate, 1.2 mM succinate, and 100 uM ADP), and the mitochondrial absorbance during coupled oxidative phosphorylation (PMGSA) was monitored for 5 min. Subsequently, 20 mM Ca^{2+} was added to induce large-amplitude swelling driven by the influx into the mitochondrial matrix (Ca^{2+}) and was monitored for 10 min. The mitochondrial Ca^{2+} efflux was monitored for 10 min after the addition of 30 mM Na^+ to stimulate the mitochondrial sodium-calcium exchanger (NCLX). For a mitochondrial enriched suspension, the swelling and shrinkage lead a decrease and increase in absorbance, respectively (Carteri et al., 2019a; Nunez-Figueredo et al., 2014).

The assessments of mitochondrial dehydrogenase activity were performed with a colorimetric method [3(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (MTT) (Chen et al., 2008). Synaptosomes were pre-incubated by adding 0.5 mg/ml of MTT followed by incubation at 37 °C for 45 min ($n = 7$–9 per group). The formazan product generated during the incubation was solubilized in dimethyl sulfoxide and measured at 560 and 630 nm (Spectra Max M5 (Molecular Devices, USA)). The results were expressed as arbitrary units and as a percentage of control.

Oxygen consumption per tissue mass was measured using the high-resolution Oxygraph-2k system and recorded in real-time using DatLab software (Oroboros, Innsbruck, Austria). The results were normalized to the protein content. The experiments were performed at 37 °C in a 2-ml chamber with a modified substrate titration protocol (Gnaiger, 2014) that consisted of the addition of succinate (Suc, 10 mM) in saturating ADP concentrations. Non-mitochondrial respiration (ROX) was measured after the addition of cyanide (KCN, 5 mM). ROX was extracted from all of the above-mentioned states and tissue-mass specific oxygen fluxes were compared across different substrates and coupling states. Based on the acquired data, we calculated succinate dehydrogenase (SDH) oxygen consumption (Gnaiger, 2014; Muller et al., 2013).

The ΔΨm was measured using the fluorescence signal of the cationic dye safranin-O (n = 7–9 per group). The synaptosomes were added to the incubation medium supplemented with succinate in saturating

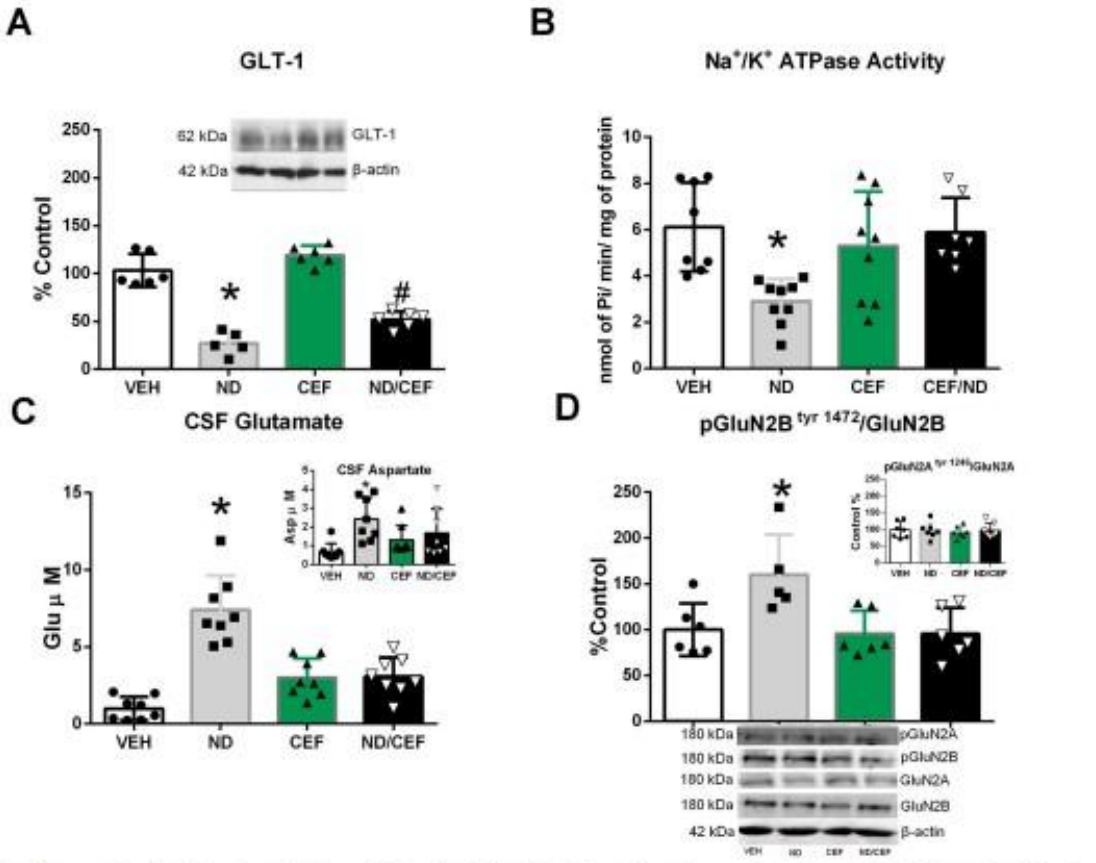


Fig. 4. Brain glutamatergic related biomarkers. (A) Western blotting bands for GLT-1 in the prefrontal cortex of mice (n = 5–6). ND downregulated the immunocontent of the astrocytic glutamate transporter GLT-1 whereas ND/CEF sustained GLT-1 at control level; (B) ND impaired Na^+/K^+ ATPase activity in synaptosomes (n = 7–9) (C) ND increased CSF glutamate and aspartate (inset) levels and ND/CEF avoided such changes (n = 8). (D) Western blotting for phosphorylated NMDA receptor subunits GluN2B and GluN2A (inset) in the prefrontal cortex (n = 5–7). * Indicates significant statistical differences from other groups. Data are shown as Mean ± S.D. Abbreviations: VEH, vehicle; ND, nandrolone decanoate; CEF, ceftriaxone; ND/CEF, nandrolone plus ceftriaxone.

concentrations of ADP and 10 μM safranin-O. The fluorescence was detected with an excitation wavelength of 495 nm and an emission wavelength of 586 nm (Spectra Max M5, Molecular Devices). A decrease in fluorescence represents a high ΔΨm. The results were normalized to the protein content. Data are reported as arbitrary fluorescence units (AFUs) (Muller et al., 2013).

The mitochondrial production of hydrogen peroxide (H_2O_2) in cortex synaptosomes was assessed using the Amplex Red® oxidation method (n = 7–9 per group). The level of H_2O_2 was measured using the respiration buffer supplemented with succinate and ADP plus 10 μM Amplex Red ® and 2 units/ml horseradish peroxidase – an indicator of H_2O_2 formation. The fluorescence was monitored at excitation (563 nm) and emission (587 nm) wavelengths with a Spectra Max M5 microplate reader (Molecular Devices, USA) (Portela et al., 2017).

2.11. Protein quantification

The BCA Pierce™ Protein Assay Kit (Thermo Fischer Scientific, USA, ref. # 23225) was used to quantify the protein content of all samples. The procedures were performed according to the manufacturer's instructions.

2.12. Statistical analysis

Results were calculated and expressed as the mean ± S.E.M. Normality was assessed through the Shapiro-Wilk normality test. To normally distributed data, we used a two-way analysis of variance (ANOVA) followed by a post-hoc Tukey test to analyze the differences between groups; furthermore, repeated measures of two-way ANOVA for minute-by-minute locomotor activity and mitochondrial calcium swelling assessments was performed. Kruskal-Wallis test was used for non-parametric cortical GLT-1, pGluN2B/GluN2B, and pGluN2A/GluN2A immunocontent ratios. Partial eta-squared effect size was assessed using the R package "*effectsize*" (Makowski et al., 2019). The pairwise correlation between variables was assessed through Pearson's correlation coefficient. All procedures were performed using GraphPad Prism 6.0. The differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Ceftriaxone attenuated aggressive behavior induced by nandrolone

There were no significant differences in the distance traveled

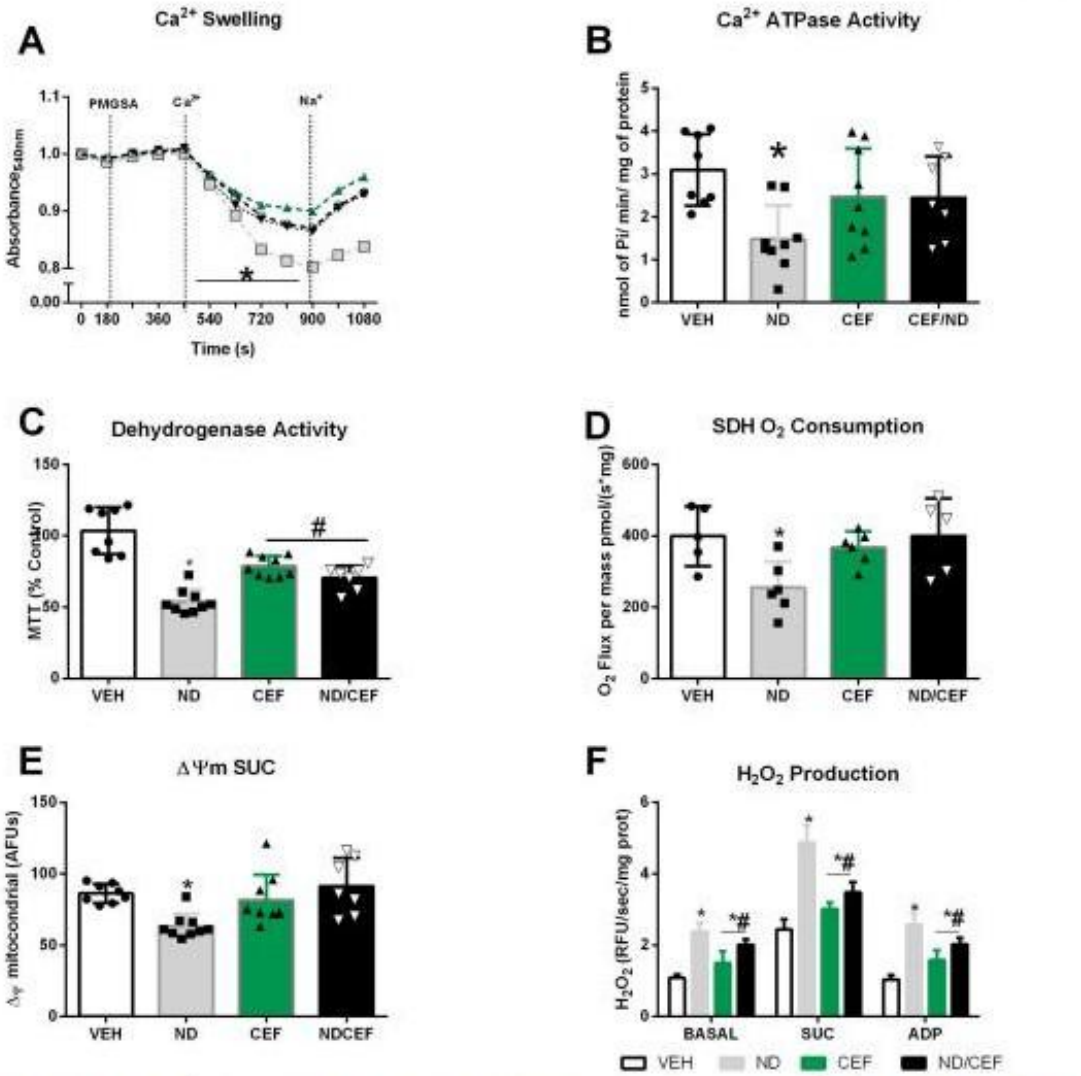


Fig. 5. Mitochondrial panel and Ca^{2+} ATPase in synaptosomes. (A) Energized mitochondria were challenged regarding Ca^{2+} influx (swelling) and Na^{+} efflux (shrinkage) measured through changes in absorbance (540 nm). ND increases the mitochondrial swelling (decrease in absorbance) and ND/CEF decreases mitochondrial swelling induced by calcium influx (increase in absorbance) (n = 7–9); (B) ND impairs Ca^{2+} ATPase activity, which is reversed by ceftriaxone (ND/CEF) (n = 7–9); (C) ND impairs total dehydrogenases activity and ND/CEF attenuates this change (n = 7–9); (D) Oxygen consumption linked to Complex II-succinate dehydrogenase (SDH) (n = 5–6); (E) ND causes a disturbance in the membrane potential dynamics after SDH stimulation, while ND/CEF display potential dynamics similar to VEH levels (n = 7–9); (F) Ceftriaxone attenuates the increased H_2O_2 production caused by ND treatment (7–9). * indicates significant statistical differences from other groups and # indicates difference only from VEH. Data are shown as Mean ± S.D. Abbreviations: VEH, vehicle; ND, nandrolone decanoate; CEF, ceftriaxone; ND/CEF, nandrolone plus ceftriaxone.

minute-by-minute (Fig. 2A) and in total distance traveled (Fig. 2B). Additional analysis acquired from the open field test showed no significant differences in the mobility (Fig. 2C) and velocity profiles (Fig. 2D). The time exploring the peripheral and central zone was not different between groups (Fig. 2E). Overall, mice showed preserved locomotor and exploratory capabilities in the open field test.

In the intruder test, the ND-treated mice were mostly dominant (73%) while in the other groups, the neutral posture – defined when the resident mice did not engage in hostile behavior toward the intruder during 10 min of evaluation (Fig. 3A) – was predominant. The aggressive behavior was quantified by the latency for the first attack and the total number of attacks. The ND group displayed decreased latency for the first attack ([F (3, 44) = 28.55, p = 0.0001, Partial η^2 = 0.6606], Fig. 3B) and an increased number of attacks in comparison to other groups ([F (3, 44) = 12.86, p = 0.0001, Partial η^2 = 0.4671], Fig. 3C). VEH, CEF, and NAND/CEF displayed similar aggressive phenotypes.

Table 1
Summary of statistical values obtained for behavioral and neurochemical outcomes. F-value, degrees of freedom (DF), the *p*-value for the interaction between factors, and the Effect size (partial eta squared) are presented.

Dependent variable	F-value	DF	P-value	Partial η^2
Latency to attack	28.55	3,44	< 0.0001	0.6606
Number of attacks	12.86	3,44	< 0.0001	0.4671
GLT-1	66.03	3,19	< 0.0001	0.9124
Na^+/K^+-ATPase	5.916	3,29	< 0.0001	0.3797
CSF glutamate	26.88	3,28	< 0.0001	0.7422
$pGluN2B^{Y1472}$/GluN2B	5.149	3,19	0.0089	0.4484
Ca^{2+} swelling	3.033	3,29	0.0451	0.2388
NCLX	4.215	3,29	0.0137	0.3036
Ca^{2+}-ATPase	4.308	3,29	0.0125	0.3082
DH activity	31.24	3,29	< 0.0001	0.7637
CII OCR	4.409	3,18	0.0172	0.4235
CII $\Delta\Psi_m$	6.241	3,29	0.00211	0.3922
Basal H_2O_2	49.24	3,29	< 0.0001	0.8359
Leak H_2O_2	85.21	3,29	< 0.0001	0.8981
CII H_2O_2	59.29	3,29	< 0.0001	0.8598

Abbreviations: Effect size (Partial Eta-squared, Partial η^2); GLT-1, Glutamate transporter 1; CSF, cerebrospinal fluid; pGluN2B, phosphorylated NMDA subunit 2B, CII OCR, Oxygen consumption rate linked to mitochondrial complex II; CII $\Delta\Psi_m$, Mitochondrial membrane potential linked to complex II; NCLX, mitochondrial sodium-calcium exchanger; DH, total dehydrogenase activity; CII H_2O_2, hydrogen peroxide linked to mitochondrial complex II.

3.2. Ceftriaxone mediated interconnected mechanisms linked to glutamatergic homeostasis

ND administration decreased the immunocontent of the Na^+-dependent transporter GLT-1 in the prefrontal cortex ([F (3, 19) = 66.03; $p < 0.0001$ for ND *vs* all groups, Partial $\eta^2 = 0.9124$]) Fig. 4A). However, the GLT-1 protein level was significantly higher in the ND/CEF relative to ND group ([F (3, 19) = 66.03, $p = 0.0186$] Fig. 4A). No significant difference was observed between CEF and VEH groups (Fig. 4a). The activity of the enzyme Na^+/K^+- ATPase – which is crucial for pumping Na^+ ions from the cytosol of astrocytes and neurons to extracellular space – was decreased by ND. The administration of CEF in ND-treated mice prevented this decrease in enzymatic activity ([F (3, 29) = 5.916, $p = 0.0118$, Partial $\eta^2 = 0.3797$] ND/CEF *vs* ND, Fig. 4B).

ND increased the CSF glutamate level relative to other groups ([F (3, 28) = 26.88, $p < 0.0001$, Partial $\eta^2 = 0.7422$] ND *vs* all the groups, Fig. 4C). Glutamate levels were not different between ND/CEF, VEH, and CEF groups (Fig. 4C, $n = 6$–7 per group). Aspartate, a co-agonist of NMDAr, potentializes glutamate binding to the receptor and intracellular signals mediated by Ca^{2+}. The levels of aspartate displayed a similar profile of glutamate as indicated in the inset (Fig. 4C). The activity of the NMDAr can be estimated by the ratio between the phosphorylated status of specific subunits and total protein content. Here, we showed that ND increased the $pGluN2B^{Tyr1472}$/GluN2B ratio relative to other groups ([F (3, 19) = 5.149, $p = 0.009$] $p = 0.0263$; $p = 0.0165$; $p = 0.0161$, ND *vs* VEH, ND *vs* CEF and ND *vs* ND/CEF respectively, Partial $\eta^2 = 0.4488$, Fig. 4D). No differences between CEF, ND/CEF, and VEH groups were observed (Fig. 4D, $n = 5$–6 per group). Also, there was no significant difference between groups in the $pGluN2A^{Tyr1246}$/GluN2A ratio (Fig. 4D, inset).

3.3. Ceftriaxone attenuated disturbances in the function of synaptic mitochondria and Ca^{2+}-ATPase

Increased cytosolic Ca^{2+} influx through NMDAr should be promptly counterbalanced by mitochondrial buffering and plasma membrane Ca^{2+}-ATPase activity in order to maintain the homeostasis of Ca^{2+}-dependent processes (Giacomello et al., 2013). Although Ca^{2+} positively regulates the activity of some mitochondrial dehydrogenases and the bioenergetics flux, an excessive load may impair the net charge and oxidative metabolism (Bordone et al., 2019; Luongo et al., 2017; Zsurka and Kunz, 2015). No significant differences in the mitochondrial Ca^{2+}

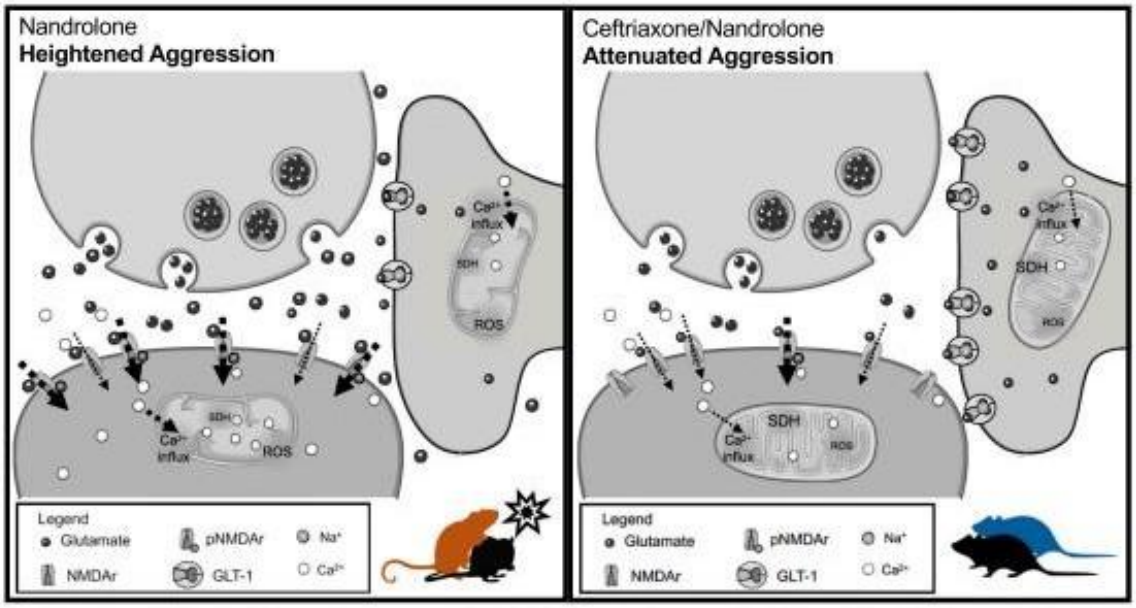


Fig. 6. Integrative model. The figure illustrates the components of the tripartite glutamatergic synapse involved in nandrolone-induced aggressive behavior (ND), and the pharmacological anti-aggressive effects of ceftriaxone (ND/CEF). Abbreviations: GLT-1, Glutamate transporter-1; pNMDAr, Phosphorylated *N*-methyl-D-aspartate; SDH, Succinate Dehydrogenase; ROS, Reactive Oxygen Species. The schematic art pieces used in this figure were adapted from material provided by Servier Medical art (https://smart.servier.com). Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License.

influx between groups were found at baseline state and after the addition of metabolic substrates (pyruvate, malate, glutamate, succinate, and ADP; PMGSA) to energize synaptic mitochondria (Fig. 5A). The addition of Ca^{2+} leads to an increased mitochondrial influx (decrease absorbance) in the ND group relative to other groups ([F (3, 29) =3.033, p = 0.0021], p = 0.0001, p = 0.0104, ND *vs* VEH, ND *vs* CEF and ND *vs* ND/CEF respectively, Partial η^2 = 0.2388, Fig. 5A). The subsequent addition of Na^+ is expected to increase the absorbance mirroring mitochondrial Ca^{2+} extrusion from the matrix. There was no difference in the capacity of exchanging Na^+ to Ca^{2+} (Fig. 5A). Looking at the Ca^{2+} variation in synaptic mitochondria, we saw that the CEF/ND group maintained an equilibrium in the influx-efflux similar to VEH and CEF (Fig. 5A). The activity of Ca^{2+}-ATPase in the synaptic terminals was significantly decreased in ND compared to other groups ([F (3, 29) =4.308, p = 0.0125], ND *vs* VEH p = 0.0072, Partial η^2 = 0.3082, Fig. 5B). Comparisons between VEH, CEF, and CEF/ND groups showed no significant differences (Fig. 5B).

Mitochondrial dehydrogenase activity was decreased in the synaptic terminals of the ND group *versus* VEH, CEF, and ND/CEF groups ([F (3, 29) =31.24, p = 0.0001]; p = 0.0001; p = 0.0002, p = 0.0210, ND *vs* VEH, ND *vs* CEF and ND *vs* ND/CEF respectively, Partial η^2 = 0.7637, Fig. 5C). CEF and ND/CEF groups showed decreased activity relative to VEH ([F (3, 29) =31.24, p = 0.0001]; p = 0.002, p = 0.0001, VEH *vs* CEF and VEH *vs* ND/CEF respectively) but attenuated relative to ND (Fig. 5C).

The O_2 consumption stimulated by the activity of SDH was decreased by ND administration *versus* other groups ([F (3, 18) =4.409 p = 0.0172], p = 0.0095, p = 0.0398, p = 0.0012, ND *vs* VEH, ND *vs* CEF and ND *vs* ND/CEF, respectively, Partial η^2 = 0.4235, Fig. 5D). Also, the mitochondrial membrane potential formation stimulated by succinate increased in the ND group relative to other groups ([F (3, 29) =6.241 p = 0.0021]; p = 0.0113; p = 0.0498; p = 0.0026; ND *vs* VEH, ND *vs* CEF and ND *vs* ND/CEF respectively, Partial η^2 = 0.3922, Fig. 5E). ND/CEF maintained the O_2 consumption profile and membrane potential similar to the VEH group.

The baseline levels of mitochondrial H_2O_2 were increased in ND, CEF, and ND/CEF groups compared to the VEH group ([F (3, 29) = 49.24 p = 0.0001; ND *vs* all other groups, Partial η^2 = 0.8359, Fig. 5F). After the addition of succinate and ADP, the H_2O_2 level was higher in the ND than in the other groups ([F (3, 29) =59.28, p = 0.0001]; p = 0.0001 ND *vs* all other groups, Partial η^2 = 0.8598). CEF and ND/CEF groups showed H_2O_2 levels higher than VEH ([F (3, 87) =189.8, p = 0.0001]; p = 0.0002, p = 0.0001, VEH *vs* CEF and VEH *vs* ND/CEF respectively) but significantly lower than ND indicating that CEF attenuated the stimulatory effects of ND on H_2O_2 formation (Fig. 5F). Table 1 summarizes the observed statistical values obtained for F values, df values, interaction p-values, and effect size (Partial η^2).

We developed an integrative synaptic mechanism involved in the aggressive behavior mediated by ND and by ND/CEF (see Fig. 6). In this model, the differences in the neurochemical and molecular parameters in relation to aggressive phenotypes are visually demonstrated.

4. Discussion

In this study, injections of supraphysiological doses of ND induced an aggressive phenotype in mice as orchestrated by GLT-1 downregulation, which was accompanied by synaptic abnormalities including increased CSF glutamate levels, NMDAr hyperactivity, and mitochondria dysfunction. The sustained GLT-1 expression due to coadministration of ND and CEF prevented GLT-1 downregulation, and NMDAr mechanisms that boosted aggressive behavior.

The expression of aggressive behavior requires that mice recognize their environmental clues in the resident home cage and have preserved locomotor capacity to engage in physical conflicts and even to display defensive strategies (Blanchard et al., 2003; Koolhaas et al., 2013). The chosen time and dose regimens used here did not alter locomotor and exploratory behaviors before submitting mice to the intruder test.

The behavioral composite provided by the intruder test like the postural dominance, decreased latency for the first attack, and increased the number of attacks of the resident mice against the intruder, highlighted the connection between synthetic steroid signaling and escalated aggression (Clark and Henderson, 2003; McGinnis, 2004). Our study replicated the ND-induced aggressive behavior model reported by Long et al. (1996) and Lindqvist et al. (2002) both performed in rats. The heightened aggression occurs in a variety of neuropsychiatry disorders and would benefit from the identification of common mechanistic effectors for address anti-aggressive therapies. Particularly, the tripartite glutamatergic synapse offers a variety of compartmentalized effectors in neurons and astrocytes (Bordone et al., 2019; Danbolt et al., 2016). Based on this, we and others have previously suggested that increased brain glutamate level increases NMDAr activity leading to heightened aggression (Kalinine et al., 2014; Newman et al., 2018c). There is a higher predominance of NMDAr subunits GluN2A and GluN2B in the adult brain areas involved in aggressive behavior like the hippocampus and cortex (Monyer et al., 1994). The glutamate binding to GluN2A and B subunits generate "high conductance" channel openings and Ca^{2+} permeability leading to amplification of synaptic mechanisms like vesicle release, protein expression, and ROS signaling. These processes are highly dependent on energy supply; however, an abnormal Ca^{2+} influx through NMDAr ultimately impairs mitochondrial function and these synaptic properties (Dingledine et al., 1999). Also, secondary neurobiochemical mechanisms leading to the phosphorylation of NMDAr subunits sites regulate receptor activity beyond the expression level. Here, we found that ND-induced aggression caused pGluN2B^{Tyr1472} upregulation but not pGluN2A^{Tyr1246} in the prefrontal cortex suggesting that aggressive behavior may be dependent on specific NMDA subunit phosphorylation though this issue requires further investigation. Frequently, the cytosolic protein kinase Fyn phosphorylates pGluN2B^{Tyr1472} in the postsynaptic terminal. Such pGluN2B^{Tyr1472}–Fyn interaction has been connected with NMDAr hyperactivity and may account for our behavioral findings (Knox et al., 2014; Li et al., 2017; Tsavou and Curtis, 2019). Additionally, the accessory enzymes, Na^+/K^+-ATPase, and Ca^{2+}-ATPase are critical for maintaining the membrane action-potential, astrocytic glutamate-uptake, and ionic homeostasis in the synaptic terminals. A rupture in these processes mediated by glutamate excess may impact NMDAr activity and mitochondrial function -(Attwell and Gibb, 2005) as corroborated here by the increase in pGluN2B^{Tyr1472}, altered mitochondrial Ca^{2+} influx, complex II activity (SDH), membrane potential, and H_2O_2 production observed in ND treated mice.

Although the brain tissue is immersed in large amounts of endogenously produced glutamate, only a lower amount is physiologically detected in the extracellular fluid. This is mandatory because of the paradoxical function of glutamate that mimics the struggle between good and evil present in the character of "Dr. Jekyll and Mr. Hyde" (Zhou and Danbolt, 2014). Recently, the molecular machinery of astrocytes has gained visibility as mediators of anxiety and hyperactivity disorders (Nagai et al., 2019), whose patients often manifest aggressive episodes (Krishnan, 1999). Indeed, we previously showed that the GLT-1 protein level and activity are downregulated in highly aggressive mice implying that it could be a candidate target for anti-aggressive therapies (Kalinine et al., 2014) (Rothstein et al., 2005).

In this study, the coadministration of CEF in ND-treated mice attenuated the aggressive behavior and neurochemical abnormalities mechanistically shared by neurons and astrocytes in the glutamatergic tripartite synapse. Remarkably, the sustained GLT-1 level/activity in ND/CEF was accompanied by a decreased extracellular glutamate levels (and aspartate) and pGluN2B^{tyr1472} as well as by normalization of synaptic mitochondria function. Interestingly, the glutamate uptake by GLT-1 is dependent on a concomitant Na^+ influx whereas the Na^+ efflux is dependent on astrocytic Na^+/K^+ ATPase. We found that increased GLT-1 levels in ND/CEF paralleled with normalization of the

enzymes Na^+/K^+ ATPase and Ca^{2+} ATPase activities in the synaptic terminals. Based on the functional interactions between glutamatergic effectors in the synaptic terminals, we proposed an illustrated model (see Fig. 6) containing the interplay between neurobiological variables found in ND (highly aggressive) and ND/CEF (attenuated aggressive) groups. This model highlights the role of glutamate signaling in aggression and the GLT-1 as a pharmacological target for anti-aggressive therapy.

Our work has some limitations. First, we used a single dose of both ND and CEF within a short time-window. This strategy has limitations regarding drug efficacy and safety. Second, we do not demonstrate the relation between drug concentration in blood and brain and the duration of the biological response mediated by GLT-1. In this context, a curve dose-response may help to determine these parameters for optimizing the anti-aggressive effects of GLT-1. Despite these limitations, our work highlights the mechanistic relevance of astrocytes and paves the way to further explore the GLT-1 in models that evolve with heightened aggression.

In conclusion, GLT-1 downregulation by ND arises as an astrocytic molecular effector mediating aggressive phenotypes and synaptic abnormalities in glutamate levels, pGluN2BTyr, and mitochondrial function. Preventing GLT-1 downregulation with the CEF administration avoids these neurochemical alterations and attenuates the aggressive behavior induced by ND.

Declaration of competing interest

The authors have nothing to disclose.

Acknowledgements

This research was funded by resources from Brazilian Agencies FAPERGS #1010267, FAPERGS/PPSUS#17/2551-0001, FAPERGS/PRONEX#16/2551- 0000499-4, CAPES/PNPD#1663, Program Science without Borders CNPQ #4011645/2012-6, and CNPq INNT #5465346/2014-6.

References

Allen, N.J., 2014. Astrocyte regulation of synaptic behavior. Annu. Rev. Cell Dev. Biol. 30, 439–463. https://doi.org/10.1146/annurev-cellbio-100913-013053.

Attwell, D., Gibb, A., 2005. Neuroenergetics and the kinetic design of excitatory synapses. Nat. Rev. Neurosci. 6, 841–849. https://doi.org/10.1038/nrn1784.

Beaver, K.M., Vaughn, M.G., Delisi, M., Wright, J.P., 2008. Anabolic-androgenic steroid use and involvement in violent behavior in a nationally representative sample of young adult males in the United States. Am. J. Public Health 98, 2185–2187. https://doi.org/10.2105/AJPH.2008.137018.

Blanchard, R.J., Wall, P.M., Blanchard, D.C., 2003. Problems in the study of rodent aggression. Horm. Behav. 44, 161–170. https://doi.org/10.1016/s0018-506x(03)00127-2.

Bordone, M.P., Salman, M.M., Titus, H.E., Amini, E., Andersen, J.V., Chakraborti, B., Diuba, A.V., Dubouskaya, T.G., Ehrke, E., Espindola de Freitas, A., Braga de Freitas, G., Gonçalves, R.A., Gupta, D., Gupta, R., Ha, S.R., Hemming, I.A., Jaggar, M., Jakobsen, E., Kumari, P., Lakkappa, N., Marsh, A.P.L., Mitlöhner, J., Ogawa, Y., Kumar, P.R., Ribeiro, F.C., Salamian, A., Saleem, S., Sharma, S., Silva, J.M., Singh, S., Sulakhiya, K., Tefera, T.W., Vafadari, B., Yadav, A., Yamazaki, R., Seidenbecher, C.I., 2019. The energetic brain - a review from students to students. J. Neurochem. 151, 139–165. https://doi.org/10.1111/jnc.14829.

Breuer, M.E., McGinnis, M.Y., Lumia, A.R., Possidente, B.P., 2001. Aggression in male rats receiving anabolic androgenic steroids: effects of social and environmental provocation. Horm. Behav. 40, 409–418. https://doi.org/10.1006/hbeh.2001.1706.

Carrillo, M., Ricci, L.A., Melloni, R.H., 2011. Glutamate and the aggression neural circuit in adolescent anabolic steroid-treated Syrian hamsters (Mesocricetus auratus). Behav. Neurosci. 125, 753–763. https://doi.org/10.1037/a0025127.

Carteri, Randhall B., Kopczynski, A., Menegassi, L.N., Salimen Rodolphi, M., Strogulski, N.R., Portela, L.V., 2019a. Anabolic-androgen steroids effects on bioenergetics responsiveness of synaptic and extrasynaptic mitochondria. Toxicol. Lett. 307, 72–80. https://doi.org/10.1016/j.toxlet.2019.03.004.

Carteri, Randhall B., Kopczynski, A., Rodolphi, M.S., Strogulski, N.R., Sartor, M., Feldmann, M., De Bastiani, M.A., Duval Wannmacher, C.M., De Franceschi, I.D., Hansel, G., Smith, D.H., Portela, L.V., 2019b. Testosterone administration after traumatic brain injury reduces mitochondrial dysfunction and neurodegeneration. J. Neurotrauma. https://doi.org/10.1089/neu.2018.6266.

Chen, C.-M., Lin, J.-K., Liu, S.-H., Lin-Shiau, S.-Y., 2008. Novel regimen through combination of memantine and tea polyphenol for neuroprotection against brain excitotoxicity. J. Neurosci. Res. 86, 2696–2704. https://doi.org/10.1002/jnr.21706.

Clark, A.S., Henderson, L.P., 2003. Behavioral and physiological responses to anabolic-androgenic steroids. Neurosci. Biobehav. Rev. 27, 413–436.

Cui, C., Cui, Y., Gao, J., Sun, L., Wang, Y., Wang, K., Li, R., Tian, Y., Song, S., Cui, J., 2014. Neuroprotective effect of ceftriaxone in a rat model of traumatic brain injury. Neurol. Sci. Off. J. Ital. Neurol. Soc. Ital. Soc. Clin. Neurophysiol. 35, 695–700. https://doi.org/10.1007/s10072-013-1585-4.

Danbolt, N.C., 2001. Glutamate uptake. Prog. Neurobiol. 65, 1–105.

Danbolt, N.C., Furness, D.N., Zhou, Y., 2016. Neuronal vs glial glutamate uptake: resolving the conundrum. Neurochem. Int. 98, 29–45. https://doi.org/10.1016/j.neuint.2016.05.009.

Dingledine, R., Borges, K., Bowie, D., Traynelis, S.F., 1999. The glutamate receptor ion channels. Pharmacol. Rev. 51, 7–61.

Fischer, S.G., Ricci, L.A., Melloni, R.H.J., 2007. Repeated anabolic/androgenic steroid exposure during adolescence alters phosphate-activated glutaminase and glutamate receptor 1 (GluR1) subunit immunoreactivity in Hamster brain: correlation with offensive aggression. Behav. Brain Res. 180, 77–85. https://doi.org/10.1016/j.bbr.2007.02.025.

Giacomello, M., De Mario, A., Scarlatti, C., Primerano, S., Carafoli, E., 2013. Plasma membrane calcium ATPases and related disorders. Int. J. Biochem. Cell Biol. 45, 753–762. https://doi.org/10.1016/j.biocel.2012.09.016.

Gnaiger, E., 2014. Mitochondrial Pathways and Respiratory Control an Introduction to OXPHOS Analysis the Blue Book Mitochondrial Pathways and Respiratory Control an Introduction to OXPHOS Analysis, 4th ed. OROBOROS MiPNet Publications.

Gnegy, M.E., 2000. Ca2+/calmodulin signaling in NMDA-induced synaptic plasticity. Crit. Rev. Neurobiol. 14, 91–129.

Hu, Y.-Y., Xu, J., Zhang, M., Wang, D., Li, L., Li, W.-B., 2015. Ceftriaxone modulates uptake activity of glial glutamate transporter-1 against global brain ischemia in rats. J. Neurochem. 132, 194–205. https://doi.org/10.1111/jnc.12958.

Joseph, M.H., Davies, P., 1983. Electrochemical activity of o-phthalaldehyde-mercaptoethanol derivatives of amino acids. Application to high-performance liquid chromatographic determination of amino acids in plasma and other biological materials. J. Chromatogr. 277, 125–136.

Kalinine, E., Portela, L.V., 2011. Efeitos comportamentais, neuroquímicos e metabólicos do tratamento com decanoato de nandrolona em camundongos. UFRGS.

Kalinine, E., Zimmer, E.R., Zenki, K.C., Kalinine, I., Kazlauckas, V., Haas, C.B., Hansel, G., Zimmer, A.R., Souza, D.O., Muller, A.P., Portela, L.V., 2014. Nandrolone-induced aggressive behavior is associated with alterations in extracellular glutamate homeostasis in mice. Horm. Behav. 66, 383–392. https://doi.org/10.1016/j.yhbeh.2014.06.005.

Kazlauckas, V., Schuh, J., Dall'Igna, O.P., Pereira, G.S., Bonan, C.D., Lara, D.R., 2005. Behavioral and cognitive profile of mice with high and low exploratory phenotypes. Behav. Brain Res. 162, 272–278. https://doi.org/10.1016/j.bbr.2005.03.021.

Knox, R., Brennan-Minnella, A.M., Lu, F., Yang, D., Nakazawa, T., Yamamoto, T., Swanson, R.A., Ferriero, D.M., Jiang, X., 2014. NR2B phosphorylation at tyrosine 1472 contributes to brain injury in a rodent model of neonatal hypoxia-ischemia. Stroke 45, 3040–3047. https://doi.org/10.1161/STROKEAHA.114.006170.

Koolhaas, J.M., Coppens, C.M., de Boer, S.F., Buwalda, B., Meerlo, P., Timmermans, P.J.A., 2013. The resident-intruder paradigm: a standardized test for aggression, violence and social stress. J. Vis. Exp. e4367. https://doi.org/10.3791/4367.

Kotermanski, S.E., Johnson, J.W., Thiels, E., 2013. Comparison of behavioral effects of the NMDA receptor channel blockers memantine and ketamine in rats. Pharmacol. Biochem. Behav. 109, 67–76. https://doi.org/10.1016/j.pbb.2013.05.005.

Krishnan, K.R., 1999. Brain imaging correlates. J. Clin. Psychiatry 60 (Suppl. 15), 50–54.

Krzyzanowska, W., Pomierny, B., Budziszewska, B., Filip, M., Pera, J., 2016. N-Acetylcysteine and ceftriaxone as preconditioning strategies in focal brain ischemia: influence on glutamate transporters expression. Neurotox. Res. https://doi.org/10.1007/s12640-016-9602-z.

Le Grevès, P., Huang, W., Johansson, P., Thörnwall, M., Zhou, Q., Nyberg, F., 1997. Effects of an anabolic-androgenic steroid on the regulation of the NMDA receptor NR1, NR2A and NR2B subunit mRNAs in brain regions of the male rat. Neurosci. Lett. 226, 61–64. https://doi.org/10.1016/s0304-3940(97)00244-9.

Li, S., Cai, J., Feng, Z.-B., Jin, Z.-R., Liu, B.-H., Zhao, H.-Y., Jing, H.-B., Wei, T.-J., Yang, G.-N., Liu, L.-Y., Cui, Y.-J., Xing, G.-G., 2017. BDNF contributes to spinal long-term potentiation and mechanical hypersensitivity via Fyn-mediated phosphorylation of NMDA receptor GluN2B subunit at tyrosine 1472 in rats following spinal nerve ligation. Neurochem. Res. 42, 2712–2729. https://doi.org/10.1007/s11064-017-2274-0.

Lindqvist, A.-S., Johansson-Stenman, P., Nyberg, F., Fahlke, C., 2002. Anabolic androgenic steroid affects competitive behaviour, behavioural response to ethanol and brain serotonin levels. Behav. Brain Res. 133, 21–29.

Long, S.F., Wilson, M.C., Sufka, K.J., Davis, W.M., 1996. The effects of cocaine and nandrolone co-administration on aggression in male rats. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 20, 839–856.

Lundholm, L., Frisell, T., Lichtenstein, P., Langstrom, N., 2015. Anabolic androgenic steroids and violent offending: confounding by polysubstance abuse among 10,365 general population men. Addiction 110, 100–108. https://doi.org/10.1111/add.12715.

Lutsenko, T.S., Lambert, J.P., Gross, P., Nwokedi, M., Lombardi, A.A., Shanmughapriya, S., Carpenter, A.C., Kolmetzky, D., Gao, E., van Berlo, J.H., Tsai, E.J., Molkentin, J.D., Chen, X., Madesh, M., Houser, S.R., Elrod, J.W., 2017. The mitochondrial Na+/Ca2+ exchanger is essential for Ca2+ homeostasis and viability. Nature 545, 93–97. https://doi.org/10.1038/nature22082.

Makowski, D., Ben-Shachar, M.S., Lüdecke, D., 2019. Compute and Interpret Indices of

Effect Size. CRAN.
McGinnis, M.Y., 2004. Anabolic androgenic steroids and aggression: studies using animal models. Ann. N. Y. Acad. Sci. 1036, 399–415. https://doi.org/10.1196/annals.1330.024.
Michaelis, E.K., Michaelis, M.L., Chang, H.H., Kitos, T.E., 1983. High affinity Ca2+ -stimulated Mg2 + -dependent ATPase in rat brain synaptosomes, synaptic membranes, and microsomes. J. Biol. Chem. 258, 6101–6108.
Miller, B.R., Dorner, J.L., Shou, M., Sari, Y., Barton, S.J., Sengelaub, D.R., Kennedy, R.T., Rebec, G.V., 2008. Up-regulation of GLT1 expression increases glutamate uptake and attenuates the Huntington's disease phenotype in the R6/2 mouse. Neuroscience. https://doi.org/10.1016/j.neuroscience.2008.02.004.
Mineur, Y.S., Picciotto, M.R., Sanacora, G., 2007. Antidepressant-like effects of ceftriaxone in male C57BL/6J mice. Biol. Psychiatry 61, 250–252. https://doi.org/10.1016/j.biopsych.2006.04.037.
Monyer, H., Burnashev, N., Laurie, D.J., Sakmann, B., Seeburg, P.H., 1994. Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. Neuron 12, 529–540. https://doi.org/10.1016/0896-6273(94)90210-0.
Muller, A.P., Gnoatto, J., Moreira, J.D., Zimmer, E.R., Haas, C.B., Lulhier, F., Perry, M.L.S., Souza, D.O., Torres-Aleman, I., Portela, L.V., 2011. Exercise increases insulin signaling in the hippocampus: physiological effects and pharmacological impact of intracerebroventricular insulin administration in mice. Hippocampus 21, 1082–1092. https://doi.org/10.1002/hipo.20822.
Muller, A.P., Fernandez, A.M., Haas, C., Zimmer, E., Portela, L.V., Torres-Aleman, I., 2012. Reduced brain insulin-like growth factor I function during aging. Mol. Cell. Neurosci. 49, 9–12. https://doi.org/10.1016/j.mcn.2011.07.008.
Muller, A.P., Haas, C.B., Camacho-Pereira, J., Brochier, A.W., Gnoatto, J., Zimmer, E.R., de Souza, D.O., Galina, A., Portela, L.V., 2013. Insulin prevents mitochondrial generation of H(2)O(2) in rat brain. Exp. Neurol. 247, 66–72. https://doi.org/10.1016/j.expneurol.2013.03.007.
Nagai, J., Rajbhandari, A.K., Gangwani, M.R., Hachisuka, A., Coppola, G., Masmanidis, S.C., Fanselow, M.S., Khakh, B.S., 2019. Hyperactivity with disrupted attention by activation of an astrocyte synaptogenic cue. Cell 177, 1280–1292.e20. https://doi.org/10.1016/j.cell.2019.03.019.
Newman, E.L., Albrechet-Souza, L., Andrew, P.M., Auld, J.G., Burk, K.C., Hwa, L.S., Zhang, E.Y., DeBold, J.F., Miczek, K.A., 2018a. Persistent escalation of alcohol consumption by mice exposed to brief episodes of social defeat stress: suppression by CRF-R1 antagonism. Psychopharmacology 235, 1807–1820. https://doi.org/10.1007/s00213-018-4905-9.
Newman, E.L., Leonard, M.Z., Arena, D.T., de Almeida, R.M.M., Miczek, K.A., 2018b. Social defeat stress and escalation of cocaine and alcohol consumption: focus on CRF. Neurobiol. Stress 9, 151–165. https://doi.org/10.1016/j.ynstr.2018.09.007.
Newman, E.L., Terranova, M., Wang, T.L., Hewage, N., Bicakci, M.B., Moss, S.J., DeBold, J.F., Miczek, K.A., 2018c. A role for prefrontal cortical NMDA receptors in murine alcohol-heightened aggression. Neuropsychopharmacology 43, 1224–1234. https://doi.org/10.1038/npp.2017.253.
Nicholls, D.G., 2003. Bioenergetics and transmitter release in the isolated nerve terminal. Neurochem. Res. 28, 1433–1441.
Nunez-Figueredo, Y., Pardo-Andreu, G.L., Ramirez-Sanchez, J., Delgado-Hernandez, R., Ochoa-Rodriguez, E., Verdecia-Reyes, Y., Naal, Z., Muller, A.P., Portela, L.V., Souza, D.O., 2014. Antioxidant effects of JM-20 on rat brain mitochondria and synaptosomes: mitoprotection against Ca(2)(+)-induced mitochondrial impairment. Brain Res. Bull. 109, 68–76. https://doi.org/10.1016/j.brainresbull.2014.10.001.
Portela, L.V., Brochier, A.W., Haas, C.B., de Carvalho, A.K., Gnoato, J.A., Zimmer, E.R., Kalinine, E., Pellerin, L., Muller, A.P., 2017. Hyperpalatable diet and physical exercise modulate the expression of the glial monocarboxylate transporters MCT1 and 4. Mol. Neurobiol. 54, 5807–5814. https://doi.org/10.1007/s12035-016-0119-5.
Rossbach, U.L.W., Steensland, P., Nyberg, F., Le Greves, P., 2007. Nandrolone-induced hippocampal phosphorylation of NMDA receptor subunits and ERKs. Biochem. Biophys. Res. Commun. 357, 1028–1033. https://doi.org/10.1016/j.bbrc.2007.04.037.
Rothstein, J.D., Patel, S., Regan, M.R., Haenggeli, C., Huang, Y.H., Bergles, D.E., Jin, L., Dykes Hoberg, M., Vidensky, S., Chung, D.S., Toan, S.V., Bruijn, L.I., Su, Z.-Z., Gupta, P., Fisher, P.B., 2005. Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. Nature 433, 73–77. https://doi.org/10.1038/nature03180.
Rowley, H.L., Martin, K.F., Marsden, C.A., 1995. Determination of in vivo amino acid neurotransmitters by high-performance liquid chromatography with o-phthalaldehyde-sulphite derivatisation. J. Neurosci. Methods 57, 93–99.
Sari, Y., Prieto, A.L., Barton, S.J., Miller, B.R., Rebec, G.V., 2010. Ceftriaxone-induced up-regulation of cortical and striatal GLT1 in the R6/2 model of Huntington's disease. J. Biomed. Sci. https://doi.org/10.1186/1423-0127-17-62.
Sims, N.R., Anderson, M.F., 2008. Isolation of mitochondria from rat brain using Percoll density gradient centrifugation. Nat. Protoc. 3, 1228–1239. https://doi.org/10.1038/nprot.2008.105.
Stefani, M.A., Modkovski, R., Hansel, G., Zimmer, E.R., Kopczynski, A., Muller, A.P., Strogulski, N.R., Rodolphi, M.S., Carteri, R.K., Schmidt, A.P., Oses, J.P., Smith, D.H., Portela, L.V., 2017. Elevated glutamate and lactate predict brain death after severe head trauma. Ann. Clin. Transl. Neurol. https://doi.org/10.1002/acn3.416.
Tsarvou, A., Curtis, D., 2019. In-silico investigation of coding variants potentially affecting the functioning of the glutamatergic N-methyl-D-aspartate receptor in schizophrenia. Psychiatr. Genet. 29, 44–50. https://doi.org/10.1097/YPG.0000000000000216.
Tyson, G.H., Bodeis-Jones, S., Caidi, H., Cook, K., Dessai, U., Haro, J., McCullough, A.E., Meng, J., Morales, C.A., Lawrence, J.P., Tillman, G.E., Winslow, A., Miller, R.A., 2018. Proposed epidemiological cutoff values for ceftriaxone, cefepime, and colistin in salmonella. Foodborne Pathog. Dis. 15, 701–704. https://doi.org/10.1089/fpd.2018.2490.
Wyse, A.T., Streck, E.L., Barros, S.V., Brusque, A.M., Zugno, A.I., Wajner, M., 2000. Methylmalonate administration decreases Na + ,K + -ATPase activity in cerebral cortex of rats. Neuroreport 11, 2331–2334. https://doi.org/10.1097/00001756-200007140-00052.
Yesalis, C.E., 2001. Use of steroids for self-enhancement: an epidemiologic/societal perspective. Adv. Pharmacol. 11, 157–160.
Zhou, Y., Danbolt, N.C., 2014. Glutamate as a neurotransmitter in the healthy brain. J. Neural Transm. 121, 799–817. https://doi.org/10.1007/s00702-014-1180-8.
Zsurka, G., Kunz, W.S., 2015. Mitochondrial dysfunction and seizures: the neuronal energy crisis. Lancet Neurol. 14, 956–966. https://doi.org/10.1016/S1474-4422(15)00148-9.

Capítulo III: Nandrolone abuse prior to head trauma mitigates endoplasmic reticulum stress, mitochondrial bioenergetic deficits, and markers of neurodegeneration.

Neste capítulo III apresentamos o artigo publicado na revista *Molecular Neurobiology.*

Os usuários de EAA frequentemente se envolvem em episódios de violência de diversas magnitudes, devido ao aumento de impulsividade e agressividade associados ao abuso dessas substâncias. Este aumento de agressividade e envolvimento em comportamento violento também aumenta significativamente a chance de um TCE. Um dos principais mecanismos secundários das TCE é o aumento da excitotoxicidade glutamatérgica, também observada em regimes de abuso de EAA, como demonstrado no capítulo 2. Dessa maneira, essa combinação poderia tornar as células neurais mais vulneráveis e agravar mecanismos neurodegenerativos associados ao TCE.

Considerando que o abuso de drogas aumenta o risco de ocorrência de TCE e que há desfechos patofisiológicos semelhantes e compartilhados entre o uso de EAA e o TCE, submetemos camundongos previamente tratados com ND por 19 dias, conforme o mesmo regime descrito no capítulo anterior, a um modelo experimental de TCE, com o objetivo de investigar o impacto da exposição prévia aos EAA sobre a progressão do dano secundário pós-traumático.

O TCE reduziu o efluxo mitocondrial de Ca^{2+}, perturbou a formação e dissipação do potencial de membrana mitocondrial, aumentou a produção de H_2O_2, diminuiu a biogênese mitocondrial (PGC-1*a* e TOM20) e a biossíntese de ATP acoplada ao consumo de O_2. De forma inesperada, o abuso de ND antes do TCE atenuou as elevações de APP, $A\beta_{42}$ e $pTau^{Ser396}$, acompanhadas por uma diminuição nos níveis de biomarcadores de estresse do retículo GRP78, ATF6 e CHOP, além de uma normalização parcial dos parâmetros bioenergéticos mitocondriais. Uma análise de componentes principais revelou uma assinatura hierárquica chave, composta por efluxo mitocondrial de Ca^{2+}, CHOP, GRP78, TOM20, H_2O_2 e eficiência bioenergética, configurando uma variável única (PC1) capaz de explicar tanto os déficits de memória causados pelo TCE quanto a preservação da capacidade cognitiva induzida pelo abuso prévio de ND.

Molecular Neurobiology (2025) 62:6951–6967
https://doi.org/10.1007/s12035-024-04488-8

RESEARCH

Nandrolone Abuse Prior to Head Trauma Mitigates Endoplasmic Reticulum Stress, Mitochondrial Bioenergetic Deficits, and Markers of Neurodegeneration

Marcelo S. Rodolphi[1] · Nathan R. Strogulski[1,2] · Afonso Kopczynski[1] · Monia Sartor[1] · Gabriela Soares[1] · Vitoria G. de Oliveira[1] · Lucia Vinade[3] · Chariston Dal-Belo[3,4] · Juliana V. Portela[1] · Cesar A. Geller[5] · Marco A. De Bastiani[6] · Jijo S. Justus[1] · Luiz Osorio C. Portela[5] · Douglas H. Smith[7] · Luis V. Portela[1]

Received: 7 March 2024 / Accepted: 9 September 2024 / Published online: 23 September 2024
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2024

Abstract

The abuse of synthetic steroids, such as nandrolone decanoate (ND), is often associated with violent behavior, increasing the risk of traumatic brain injury (TBI). After a TBI, proteins like APP, β-amyloid peptide-42 (Aβ42), and phosphorylated tau (pTau) accumulate and trigger endoplasmic reticulum (ER) stress associated with an unfolded protein response (UPR). The involvement of mitochondrial bioenergetics in this context remains unexplored. We interrogate whether the abuse of ND before TBI alters the responses of ER stress and mitochondrial bioenergetics in connection with neurodegeneration and memory processing in mice. Male CF1 adult mice were administered ND (15 mg/kg) or vehicle (VEH) s.c. for 19 days, coinciding with the peak day of aggressive behavior, and then underwent cortical controlled impact (CCI) or sham surgery. Spatial memory was assessed through the Morris water maze task (MWM) post-TBI. In synaptosome preparations, i) we challenged mitochondrial complexes (I, II, and V) in a respirometry assay, employing metabolic substrates, an uncoupler, and inhibitors; and ii) assessed molecular biomarkers through Western blot. TBI significantly increased APP, Aβ42, and $pTau^{Ser396}$ levels, along with ER-stress proteins, GRP78, ATF6, and CHOP, implying it primed apoptotic signaling. Concurrently, TBI reduced mitochondrial Ca^{2+} efflux in exchange with Na^+, disturbed the formation/dissipation of membrane potential, increased H_2O_2 production, decreased biogenesis (PGC-1α and TOM20), and ATP biosynthesis coupled with oxygen consumption. Unexpectedly, ND abuse before TBI attenuated the elevations in APP, Aβ42, and $pTau^{Ser396}$, accompanied by a decrease in GRP78, ATF6, and CHOP levels, and partial normalization of mitochondrial-related endpoints. A principal component analysis revealed a key hierarchical signature featuring mitochondrial Ca^{2+} efflux, CHOP, GRP78, TOM20, H_2O_2, and bioenergetic efficiency as a unique variable (PC1) able to explain the memory deficits caused by TBI, as well as the preservation of memory fitness induced by prior ND abuse.

Keywords Nandrolone · Abuse · Traumatic brain injury

✉ Luis V. Portela
roskaportela@gmail.com

[1] Laboratory of Neurotrauma and Biomarkers, Departamento de Bioquímica, Universidade Federal Do Rio Grande Do Sul, UFRGS, Anexo, Rua Ramiro Barcelos 2600, Porto Alegre, RS 90035-003, Brazil

[2] School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Leinster, Ireland

[3] Laboratory of Neurobiology and Toxinology (LANETOX), Universidade Federal Do Pampa (UNIPAMPA), São Gabriel, RS, Brazil

[4] Departamento Multidisciplinar - Escola Paulista de Política, Economia E Negócios (EPPEN), Universidade Federal de São Paulo (UNIFESP), Osasco, SP, Brazil

[5] Laboratory of Performance in Simulated Environment (LAPAS), Centro de Educação Física, Universidade Federal de Santa Maria - UFSM, Santa Maria, RS, Brazil

[6] Zimmer Neuroimaging Lab, Departamento de Bioquímica, ICBS, Universidade Federal Do Rio Grande Do Sul, Porto Alegre, RS, Brazil

[7] Center for Brain Injury and Repair, Department of Neurosurgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Content courtesy of Springer Nature, terms of use apply. Rights reserved.

Introduction

Traumatic brain injury (TBI) is an important cause of disability associated with large-scale economic burden. This mechanical injury induces structural and biochemical damage to the brain that can induce neurological dysfunction for hours to years after the injury [1–3]. Importantly, while the global incidence of TBI is on the rise, certain populations, such as military personnel in conflict zones, individuals engaged in contact sports, and substance abusers, face a higher risk of head injuries [4, 5].

Notably, anabolic–androgenic steroids (AAS) are among the most abused substances by young adult populations worldwide, mostly for aesthetics and athletic performance [5, 6]. While therapeutic doses of AAS, including nandrolone decanoate (ND), can exert beneficial anabolic effects for specific disorders, supraphysiologic doses have been linked to organ damage and the development of neuropsychiatric disorders, including psychosis, depression, anxiety, and heightened aggressive behavior [7–10]. In particular, AAS abuse is associated with increased engagement in acts of violence and physical fights, as perpetrator or victim, among young adult males in the USA [11]. Although drug abuse and violence account for a significant number of TBI victims admitted in the emergency care [12], the specific contribution of AAS to these cases is unknown and likely underreported. Furthermore, preclinical studies evaluating aggressive behavior have demonstrated that supraphysiologic doses of AAS increase social dominance, aggressive impulses, and the number of violent attacks inflicted by a resident mouse against an intruder mouse (resident-intruder paradigm) [7, 13]. This suggests that the administration of AAS alone in rodents may lead to exacerbated violent behavior, replicating the profile of a subject at an increased risk of suffering TBI.

Important overlaps in the neurochemical effects of both AAS abuse and TBI may provide substrates for a synergistic effect that could influence the extent and type of brain pathology. Indeed, alone, AAS abuse or TBI can replicate brain abnormalities such as elevated glutamate levels, impaired astrocytic glutamate transporters, oxidative stress, accumulation of amyloid precursor protein (APP) and its derived β-amyloid peptides (Aβ), and increased tau phosphorylation. Remarkably, both Aβ and hyperphosphorylated tau levels increase soon after initiating supraphysiologic dose of AAS, and after a head trauma [14, 15]. Accordingly, these observations suggest that AAS abuse could exacerbate progressive pathophysiological changes after TBI, including neurotoxicity and protein misfolding and aggregation [14–17].

Regardless of the etiology, the presence of protein aggregates triggers endoplasmic reticulum (ER) stress, initiating an adaptive unfolded protein response (UPR) mechanism to prevent proteotoxicity and maintain cellular homeostasis. The UPR involves the dissociation of the chaperone protein glucose-regulated protein 78 (GRP78 or BiP) from ER transmembrane proteins, leading to the activation of transcription factors such as ATF6, IRE1α, and PERK (PKR-like ER kinase) [18]. Downstream responses orchestrated by both PERK and ATF6 culminate in increased levels of CHOP, a critical proapoptotic protein. Persistent ER stress can contribute to the progression of neurodegenerative conditions associated with proteotoxicity, particularly in Alzheimer's disease (AD) and TBI [19, 20]. Nevertheless, the mechanisms of ER stress are closely linked with mitochondrial dysfunction, although it remains unclear which factor precedes the other. Further, it is uncertain whether the multitarget effects of AAS modulate this interplay. In this context, impairments in mitochondrial Ca^{2+} regulation, the production of reactive oxygen species (ROS), a decline in biogenesis, and reduced protein import capacity through translocase of the outer membrane 20 (TOM20) collectively conduct cells to a vicious loop characterized by neurobioenergetic deficits, persistent ER stress, and neurodegeneration [19, 20]. Accordingly, it is now accepted that TBI is a complex disorder in which multiple interacting mechanisms operate to influence neurological outcomes [21].

In this study, we investigate whether the abuse of ND before TBI alters the responses of ER stress and mitochondrial bioenergetics in connection with neurodegeneration and memory processing in mice.

Material and Methods

Animals and Treatment Groups

Two-month-old CF1 male mice ($n = 32$) were utilized for the study. The mice were obtained from the Centro de Reprodução e Experimentação de Animais de Laboratório (CREAL, UFRGS, Porto Alegre, Brazil). They were housed in Plexiglass cages (38 cm × 32 cm × 16 cm) with stainless-steel coverage and had ad libitum access to water and food [22–25]. To prevent social isolation, animals were maintained in $n = 4$ mice/per cage [24]. The animal facility was maintained at a temperature of 22 °C with a 12-h light/12-h dark cycle (lights on at 7 a.m.).

The mice were randomly assigned to the following treatment groups: vehicle/sham received oil vehicle and underwent sham surgery (VEH/SHAM); nandrolone/sham received nandrolone decanoate and underwent sham surgery (ND/SHAM); vehicle/CCI received oil vehicle and underwent surgery for controlled cortical impact (VEH/CCI); and nandrolone/CCI received nandrolone and underwent surgery

Content courtesy of Springer Nature, terms of use apply. Rights reserved.

for controlled cortical impact (ND/CCI). Animals received one daily subcutaneous injection of either oil vehicle (VEH) or nandrolone decanoate 50 mg (ND; Deca-Durabolin®160, Organon; 15 mg/kg/day in a volume of 1 ml/kg, dissolved in corn oil) for 19 days (see experimental timeline, Fig. 1). Previous studies of our laboratory have shown that this time and dosage regimen induces heightened aggressive behavior in mice challenged in the intruder test [7, 13]. All experiments were conducted in accordance with the guidelines provided by the NIH Guide for Care and Use of Laboratory Animals, the National Research Council's Guide for the Care and Use of Laboratory Animals, and the Brazilian Society for Neuroscience and Behavior (SBNeC). Additionally, the experimental procedures followed the governmental guidelines in compliance with the Federation of Brazilian Societies for Experimental Biology. The Ethical Committee of the Federal University of Rio Grande do Sul, Brazil, approved all procedures (#33764).

Controlled Cortical Impact

On the 19th day of ND or VEH administration, traumatic brain injury (TBI) was induced using a controlled cortical impact (CCI) procedure (see timeline, Fig. 1). Anesthesia was maintained using a mixture of 2.5% isoflurane, in nitrogen (N_2), and oxygen (O_2). The mice were placed on a heated stereotaxic bed (Kopf Instruments, Tujunga, CA, USA) maintained at a temperature of 37 ± 1 °C. The level of anesthesia was monitored throughout the surgery by testing the withdrawal reflex of the feet. A 4-mm-diameter craniotomy was performed on the central part of the left parietal bone without damaging the dura mater. During the time course of surgical procedure, an eye lubricant was used for avoiding damage to corneal membranes. The CCI injury was inflicted using the benchmark stereotaxic impactor (myNeuroLab®, Leica, St. Louis, MO, USA), which utilized a 3.0-mm-diameter piston with an impact velocity of 5.7 m/s. The impact duration was 100 ms, resulting in a 1.5 mm depth of penetration on the exposed surface of the dura mater. This protocol was adapted from Smith and colleagues [26]. After the injury, a concave lamella bonded with dental cement was placed to isolate the craniotomy area, and the scalp was sutured. The entire surgery lasted approximately 7 min, and lidocaine was locally applied to alleviate pain and discomfort. Following the procedure, the mice were placed in a heated chamber to maintain a core temperature of 37 °C and were monitored for up to 4 h post-injury, during which they exhibited a recovery in spontaneous locomotor activity. Subsequently, the mice were returned to their home cages, and daily inspections were carried out by the researchers and animal care staff. These inspections were conducted to check for signs of distress, discomfort, and pain, which included assessing for bleeding and inflammation at the surgical site, piloerection, a lack of spontaneous activity, changes in body temperature, decreased water or food pellet consumption, and a hunched posture. Sham surgery mice were subjected to anesthesia and craniotomy to expose the dura mater but were not subjected to the CCI protocol. If mice displayed at least one of these phenotypes, they were removed from the study and euthanized humanely,

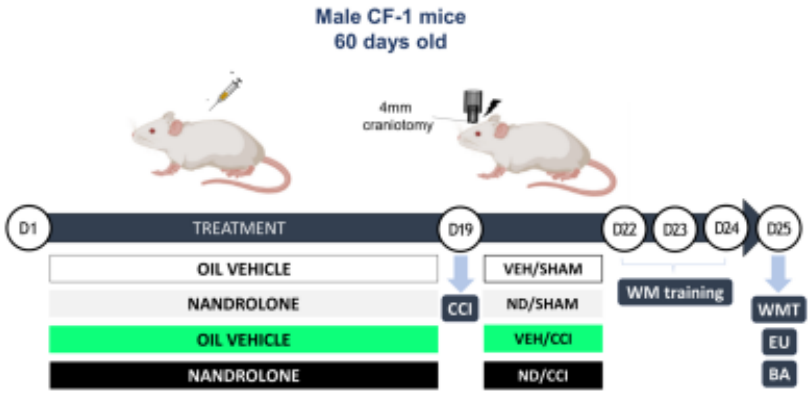


Fig. 1 Experimental timeline. Sixty-day-old CF1 albino mice were randomly allocated to 25-day experimental protocol. Mice received for 19 days nandrolone decanoate (ND) or oil vehicle. Afterward, mice were randomly allocated into the groups that underwent either a sham operation or a CCI to induce TBI resulting in VEH/SHAM, ND/SHAM, VEH/CCI, and ND/CCI ($n = 5$–6 mice/group). On day 21 mice were submitted to open field test, and from days 22 to 25 to Morris water maze training (WM training) and test (WMT). After the Morris water maze task mice were euthanized, and the brain was dissected for biochemical analysis. Abbreviations: D, day; VEH, vehicle; ND, nandrolone decanoate; CCI, controlled cortical impact; WM, Morris water maze; WMT, Morris water maze test; EU, euthanasia; BA, biochemical assays

Content courtesy of Springer Nature, terms of use apply. Rights reserved.

following the guidelines outlined by Andersen et al. [27] and the National Research Council [28]. To minimize variations, all surgical procedures were performed by the same expert experimenter.

Open Field Test

After a 48-h recovery period from the surgical procedure, the mice were assessed for spontaneous locomotion and exploratory activity using the open field (OF) test. The OF apparatus consisted of a black box measuring 50 cm × 50 cm, surrounded by 50-cm-high walls, and placed in a sound-attenuated room under low-intensity light conditions (12 lx). Each mouse was placed in the right corner of the arena, and its locomotor activity was recorded for 10 min using a video camera [7]. The analysis of locomotor activity and exploratory behavior was performed using a computer-operated tracking system (Any-maze, Stoelting, Woods Dale, IL). The apparatus was cleaned with 70% alcohol between each test. Various parameters such as total distance traveled, maximum speed, mean speed, total mobile time, total immobile time, and time spent in peripheral and central zones were recorded and utilized as indicators of locomotor activity and exploratory behavior.

Morris Water Maze Task

To assess spatial learning and memory, we used a modified version of the protocol established by Smith et al. (1995). Twenty-four hours after the open field test, the mice underwent daily training in a four-trial Morris water maze (MWM) task to locate a hidden platform (experimental days 22 to 24). Visual clues were provided on the wall for guidance. The latency to find the submerged platform was recorded as an indicator of learning during the training phase. On day 25, a 60-s probe test was conducted without the submerged platform to evaluate memory retention. The time spent in the target quadrant, where the platform was previously located, was measured as an indicator of memory retention. Any-maze software (Stoelting, Woods Dale, IL) was used for data analysis [24].

Synaptosomes Preparation

The whole brain was rapidly dissected from the cerebellum and olfactory bulb and homogenized in 500 µl of homogenization buffer with 10 strokes of a 7-ml glass Douce tissue homogenizer. The cerebral homogenates were centrifuged, and the pellet was resuspended in a 15% Percoll® (GE Healthcare®, Little Chalfont, UK) solution and submitted to a discontinuous Percoll® gradient centrifugation at 30,700 g × 5 min resulting in 15% and 23% Percoll® bands, respectively, enriched in synaptosomes [7, 29]. Subsequently, synaptosomes were resuspended in sucrose and Tris buffer (320 mM sucrose, 10 mM Tris, pH 7.4) and immediately used to assess mitochondrial parameters and immune quantification of target proteins by Western blotting. Aliquots of synaptosomes were used to quantify the total protein levels.

Western Blot Analysis

Levels of proteins associated with neurodegeneration such as APP, Ab42, pTau^{Ser396}, ER-stress (GRP78, ATF6, CHOP), mitochondrial biogenesis (PGC1-α), and the mitochondrial import precursor proteins (TOM20) were evaluated using Western blotting ($n = 5$–6 per group). Synaptosomes of whole-brain mice (20 µg of protein) were subjected to electrophoresis using a 10 to 14% polyacrylamide gel and transferred onto PVDF membranes (Amersham, GE Healthcare, Little Chalfont, UK) [23]. Primary antibodies against APP (Cell Signaling, USA ref. 2452; 1:1000), Aβ-42 (Cell Signaling, USA, ref. 7672; 1:1000), anti-tau-phospho-SER396 (Abcam, UK, ref. ab109390; 1:1000), anti-tau antibody (Abcam, UK, ref. ab32057; 1:1000), anti-GRP-78 BIP antibody (Abcam, UK, ref. ab21685; 1:1000), anti-ATF-6 antibody (Abcam, UK, ref. ab11909; 1:1000), CHOP (Cell Signaling, USA, ref. 2895; 1:1000), PGC1-α (Cell Signaling, USA, ref. 2178; 1:1000), and anti-TOM20 (Cell Signaling, USA, ref. 42,406; 1:1000). All procedures were conducted according to specific antibody manufacturer's instruction. Primary antibodies were incubated with the membranes overnight. Afterward, the membranes were incubated with horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG secondary antibodies (1:5000) (GE, Little Chalfont, UK) for 2 h. Chemiluminescence detection was performed using the Amersham ECL Western Blotting Detection Reagent (GE Healthcare Life Sciences, Little Chalfont, UK) for 5 min, and the immunodetection signals were visualized using the Image Quant LAS 4000 system (GE, Little Chalfont, UK). Protein expression levels were quantified using Image J® software (Rockville, USA), and the optical density values were expressed as a percentage of the control [7].

Mitochondrial Panel

Mitochondrial Calcium Management

To assess the calcium influx and efflux in mitochondria from synaptosomes of whole-brain mice ($n = 6$ per group), we used spectrophotometric readings at an absorbance of 540 nm (Spectra Max M5, Molecular Devices). Synaptosomes (50 µl) prepared were added to a standard swelling incubation medium containing 100 mM KCl, 50 mM sucrose, 10 mM HEPES, and 5 mM KH_2PO_4, and the basal absorbance was monitored for 3 min. Mitochondrial

Content courtesy of Springer Nature, terms of use apply. Rights reserved.

metabolic substrates were then added to support ATP production (3.5 mM pyruvate, 4.5 mM malate, 4.5 mM glutamate, 1.2 mM succinate, and 100 uM ADP), and the mitochondrial absorbance during coupled oxidative phosphorylation (PMGSA) was monitored for 5 min. Subsequently, 20 mM Ca^{2+} was introduced to induce large-amplitude swelling driven by the influx into the mitochondrial matrix (Ca^{2+}), and the absorbance was monitored for 10 min. Following this, the mitochondrial Ca^{2+} efflux was monitored for 10 min after the addition of 30 mM Na^{+} to stimulate the mitochondrial sodium-calcium exchanger 1 (NCLX). In a mitochondrial-enriched suspension, swelling caused a decrease in absorbance, while shrinkage led to an increase in absorbance [7].

Mitochondrial Membrane Potential (Δψm) and Hydrogen Peroxide (H_2O_2) Production

The mitochondrial membrane potential (Δψm) was evaluated by monitoring the fluorescence signal (excitation wavelength of 495 nm and emission wavelength of 586 nm) incubated in a respiration buffer supplemented with 10 µM safranin-O (SpectraMax M5, Molecular Devices). An increase in fluorescence units indicated a decreased Δψm, while a decrease in fluorescence units indicated an increased Δψm. The baseline Δψm was determined during incubation without substrates. Substrates for complexes I, II (PMGSA), and V (ADP) were used to modulate the dynamic responses of Δψm, and uncoupling with FCCP (1 µM) was also employed. The fluorescence units of each coupling state were used to calculate the relative percentage change in Δψm. The data were expressed as arbitrary fluorescence units (AFUs) and normalized to protein content [30].

Hydrogen peroxide (H_2O_2) production by the mitochondria of synaptosomes was assessed in different metabolic states using a respiration buffer supplemented with 10 µM Amplex Red and 2 units/ml horseradish peroxidase. Baseline H_2O_2 levels were measured without the presence of exogenous substrates in the incubation medium. The coupling states L (PMGS), P, and Lomy were induced to assess H_2O_2 production using the same substrates and inhibitors as previously mentioned in the respirometry assay. Fluorescence was monitored at excitation (563 nm) and emission (587 nm) wavelengths using a Spectramax M5 microplate reader (Molecular Devices, USA) [7, 30].

Real-Time Respirometry

Oxygen consumption rates (OCR) were measured at 37 °C using a high-resolution Oxygraph-2 k system and recorded in real time with DatLab software (Oroboros, Innsbruck, Austria) in a respiration buffer (100 mM KCl, 75 mM mannitol, 25 mM sucrose, 5 mM phosphate, 0.05 mM EDTA, and 10 mM Tris–HCl, pH 7.4). After a 5-min period to establish routine OCR, a multi-substrate titration protocol was initiated using pyruvate, malate, glutamate, and succinate (PMGS; 10, 10, 20, and 10 mM, respectively) to stimulate the formation of an electrochemical gradient. Adenosine diphosphate (ADP, 2.5 mM) was then titrated to obtain maximal oxidative phosphorylation (OxPHOS) capacity (P). Subsequently, oligomycin was titrated to obtain leak OCR, and the maximal electron transport capacity (ETS) was measured following titration of the proton ionophore carbonyl cyanide 4-trifluoromethoxy-phenylhydrazone (FCCP, 1 µM), a mitochondrial oxidative phosphorylation uncoupler. Non-mitochondrial respiration (ROX) was calculated after inhibiting complexes I, III, and IV with rotenone, antimycin-A, and cyanide (ROT, AA, and KCN, 2, 2.5, and 5 mM, respectively) [31]. Several parameters were calculated based on the OCR values, including biochemical coupling efficiency (P – Lomy)/P, electron transport system coupling efficiency (E – P)/E, and proton leak ratio, Lomy/E [31, 32].

Protein Quantification

The BCA Pierce™ Protein Assay Kit (Thermo Fisher Scientific, USA, ref # 23,225) was utilized to measure the protein content in synaptosome samples. The protocols were executed in accordance with the manufacturer's instructions.

Systems Biology Approach

We performed pairwise Pearson correlations for each continuous variable studied for each experimental group. We then estimated the association between paired variables using the Pearson's product moment correlation coefficient (rho) testing a null hypothesis of rho being zero. Results were corrected for multiple comparisons. Statistically significant correlations were plotted into a correlation matrix using GraphPad Prism 9, and results were confirmed using the *Psych* package in R statistical environment by applying the corr.test function.

Statistical Analysis

The results were calculated and expressed as the mean ± S.E.M. Normality was assessed through the Shapiro–Wilk normality test. To analyze the differences between groups regarding CCI and the drug treatment, we used a two-way analysis of variance (ANOVA) followed by a post-hoc Tukey's multiple comparison test. Further, a two-way repeated measures ANOVA was used to assess statistical significance relative to differences in learning and test phase latency in the MWM. Kruskal–Wallis test was used for comparison of non-parametric data. All procedures were

Content courtesy of Springer Nature, terms of use apply. Rights reserved.

performed using GraphPad Prism 7.0 software. The differences were considered statistically significant at $p < 0.05$.

Results

Effect of Nandrolone and/or TBI on Accumulation of Proteins

The level of amyloid precursor protein (APP), Aβ42 peptide, and phosphorylated tau at Ser396 ($pTau^{Ser396}$) in the brain was evaluated through Western blotting. Mice from the VEH/CCI group showed 58.68% increment in amyloid precursor protein (APP), and 118% in Aβ42 in relation to VEH/SHAM (Fig. 2A and 2B). Levels of APP in VEH/CCI mice were significantly elevated compared to ND/SHAM ($p = 0.043$). No significant difference in APP levels between VEH/CCI and VEH/SHAM was observed ($p = 0.0505$). APP levels in ND/CCI versus VEH/SHAM were not statistically different, as well as there was no difference between ND/CCI versus VEH/CCI ($p = 0.054$). Aβ42 protein fragment was significantly elevated in VEH/CCI compared to VEH/SHAM (Fig. 2B, $p = 0.044$). ND/CCI displayed a decrease of 111% in Aβ42 in relation to VEH/CCI ($p = 0.061$). There was a 64% increase in $pTau^{Ser396}$ in VEH/CCI and 39% ND/CCI relative to VEH/SHAM (Fig. 2C). VEH/CCI displayed significant increments in $pTau^{Ser396}$ relative to VEH/SHAM and ND/SHAM (Fig. 2C, $p = 0.037$ and $p = 0.039$, respectively). There was a non-significant 24.7% reduction in $pTau^{Ser396}$ in the ND/CCI group relative to VEH/CCI ($p = 0.639$). ND significantly decreased markers of neurodegeneration associated with CCI.

Effect of Nandrolone and/or TBI on Endoplasmic Reticulum (ER) Stress Biomarkers

The proteins GRP78, ATF6, and CHOP, which are considered biomarkers of the ER stress response to accumulation

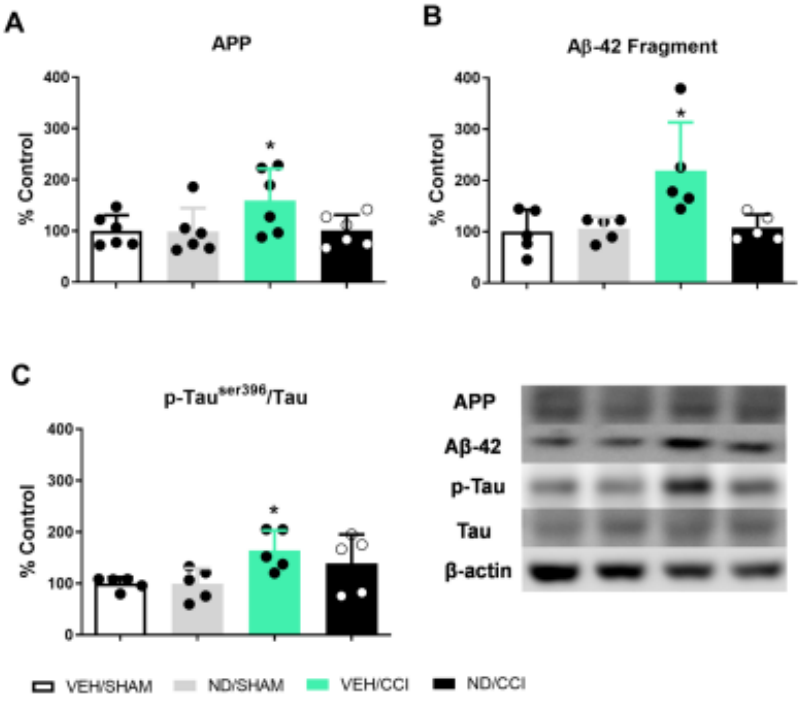


Fig. 2 Brain accumulated proteins. The immune content of proteins whose accumulation in the brain is associated with neurodegenerative mechanisms was estimated using Western blot analysis with synaptosomes preparation from whole-brain mice. A traumatic brain injury (CCI) led to a significant increase in the levels of **A** protein precursor amyloid (APP), **B** amyloid-β peptide 42 (Aβ42), and **C** phosphorylated tau at serine 396 ($p\text{-}Tau^{ser}396$) and total tau protein (tau) ratio. Statistical differences were noticed in the following comparisons: **A** VEH/CCI vs ND/SHAM; **B** VEH/CCI vs VEH/SHAM; **C** VEH/CCI vs VEH/SHAM; and VEH/CCI vs ND/SHAM. Nandrolone prior to TBI sustained APP and Aβ42 at the level of VEH/SHAM ($n = 5$–6 mice per group). * indicates statistical significance at $p < 0.05$ for all comparisons. Data is presented as mean ± S.D. Abbreviations: VEH, vehicle; ND, nandrolone decanoate; CCI, controlled cortical impact

Content courtesy of Springer Nature, terms of use apply. Rights reserved.

of protein, were assessed using Western blotting. The level of GRP78 increased significantly in VEH/CCI relative to VEH/SHAM ($p=0.002$), ND/SHAM ($p=0.002$), and ND/CCI ($p=0.011$). GRP78 in the ND/CCI group displayed an increment of 45.6% in relation to VEH/SHAM, and a reduction of 175% in relation to VEH/CCI (Fig. 3A). ATF6 content in the VEH/CCI group increased significantly compared to VEH/SHAM ($p=0.022$), ND/SHAM ($p=0.034$), and ND/CCI ($p=0.048$) (Fig. 3B). CHOP, a dynamic regulator of ER stress, was significantly increased in the brain of VEH/CCI mice relative to VEH/SHAM ($p=0.0001$) and ND/SHAM ($p=0.0001$), and ND/CCI (0.0004) (Fig. 3C). ND/CCI displayed a 207% attenuation in CHOP level relative to VEH/CCI. ND significantly reduced markers of ER stress in the brains of mice submitted to CCI.

Influence of Nandrolone and/or TBI on Mitochondrial Plasticity

Mitochondrial plasticity in response to microenvironmental stress is an important component for neural cell survival. Here, protein levels of molecular biomarkers of mitochondrial biogenesis (PGC1-α) and mitochondrial protein import (TOM20) were evaluated using Western blotting. There was a significant decrease in PGC1-α in VEH/CCI compared to VEH/SHAM ($p=0.031$), ND/SHAM ($p=0.025$), and ND/CCI ($p=0.027$) (Fig. 4A). ND/CCI preserved the brain PGC1-α at levels similar to VEH/SHAM. The abundance of TOM20 in VEH/CCI decreased significantly relative to VEH/SHAM ($p=0.003$), ND/SHAM ($p=0.002$), and ND/CCI ($p=0.002$) (Fig. 4B). The ND/CCI group displayed TOM20 levels similar to VEH/SHAM. ND significantly maintained markers of mitochondrial dynamics relative to VEH/CCI.

Nandrolone and/or TBI Mediates Mitochondrial Bioenergetics Responses

It is acknowledged that mitochondrial Ca^{2+} influx/efflux plays a crucial role in the homeostatic regulation on membrane potential, oxidative stress, and bioenergetic capacity. These variables are functionally interconnected with ER stress responses. Mitochondria were challenged with metabolic substrates (PMGSA), and following the addition of Ca^{2+}, the influx into energized mitochondria was significantly increased in ND/SHAM, VEH/CCI, and ND/CCI compared to VEH/SHAM ($p=0.005$, $p=0.023$, and $p=0.006$, respectively) (Fig. 5A). There was no significant difference between ND/SHAM, VEH/CCI, and ND/CCI groups ($p>0.05$). The Ca^{2+} efflux from mitochondria stimulated by Na^{+} was significantly impaired in VEH/CCI compared to other groups (Fig. 5A, $p<0.05$).

The net charge relevant for the formation and dissipation of mitochondrial membrane potential may be influenced by the influx/efflux of Ca^{2+} ions. The variation in mitochondrial membrane potential ($\Delta\psi m$) was significantly impaired

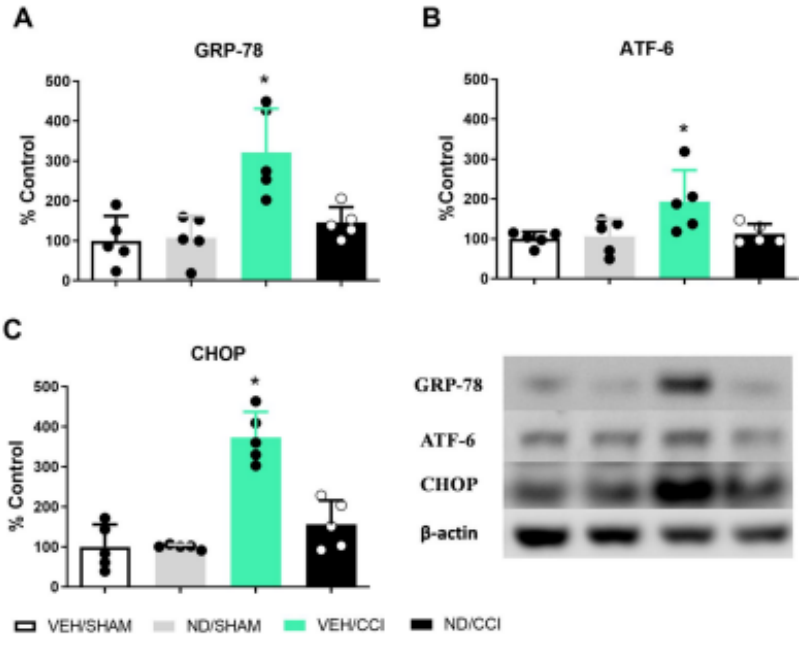


Fig. 3 Endoplasmic reticulum (ER) stress. The immune content of proteins that serve as biomarkers of ER stress was estimated using Western blot analysis with synaptosomes preparation from whole-brain mice. A trauma to the brain (CCI) led to a significant upregulation in the abundance of **A** 78-kDa glucose-regulated protein (GRP-78), **B** activating transcription factor 6 (ATF-6), and **C** C/EBP homologous protein (CHOP). Nandrolone prior to TBI sustained GRP-78, ATF-6, and CHOP at the level of VEH/SHAM ($n=5$ mice per group). * indicates statistical significance at $p<0.05$ for all comparisons. Data are shown as mean ± S.D. Abbreviations: VEH, vehicle; ND, nandrolone decanoate; CCI, controlled cortical impact

Content courtesy of Springer Nature, terms of use apply. Rights reserved.

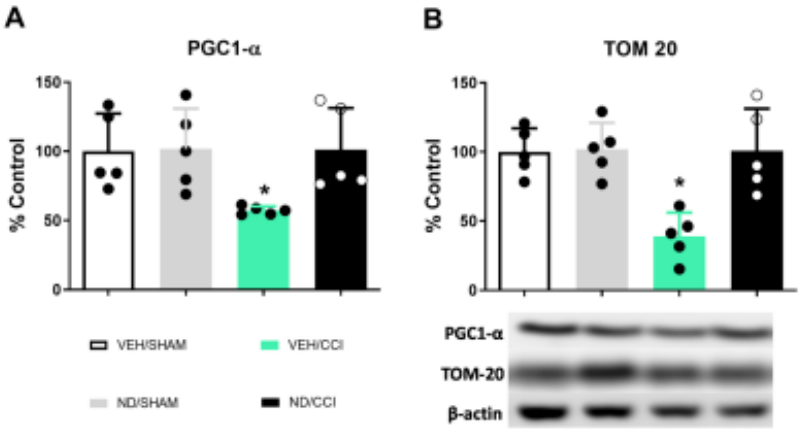


Fig. 4 Mitochondrial plasticity biomarkers. The immune content of proteins related to mitochondrial biogenesis and capability to import cytosolic proteins to the matrix was assessed using Western blot analysis with synaptosomes preparation from whole-brain mice. A traumatic brain injury causes downregulation in the abundance of **A** peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1-α) and **B** translocase of outer membrane 20 (TOM 20). Nandrolone prior to CCI sustained PGC1-α and TOM20 at the level of VEH/SHAM ($n=5$ mice per group). * indicates statistical significance at $p<0.05$ for all comparisons. Data are shown as mean ± S.D. Abbreviations: VEH, vehicle; ND, nandrolone decanoate; CCI, controlled cortical impact

in VEH/CCI compared to VEH/SHAM, ND/SHAM, and ND/CCI in the following respiratory states: i) stimulated by metabolic substrates (PMGS) to complexes I and II (CI + CII); ii) stimulated by PMGSA to complexes I, II, and V (CI + CII) + (P); iii) after inhibition of complex V with oligomycin (Lomy); and iv) in the presence of the uncoupler FCCP ($p<0.05$ for all comparisons). In the Lomy and FCCP states, ND/CCI sustained Δψm at levels of VEH/SHAM (see Supplementary Fig. 1A).

Mitochondrial H_2O_2 production was significantly increased in the VEH/CCI group compared to VEH/SHAM ($p=0.006$) and ND/CCI ($p=0.010$) in basal conditions (presence of endogenous substrates). Also, H_2O_2 production by VEH/CCI and ND/CCI groups were significantly increased in relation to VEH/SHAM and ND/SHAM, in the following respiratory states: i) stimulated by metabolic substrates (PMGS) to complexes I and II (CI + CII); and ii) stimulated by PMGSA to complexes I, II, and V (CI + CII) + (P) (both comparisons, $p<0.05$). After the inhibition of complex V with oligomycin (Lomy), the H_2O_2 levels in VEH/CCI remained elevated compared to VEH/SHAM ($p=0.0001$), whereas ND/CCI decreased significantly in relation to VEH/CCI ($p=0.001$). In the presence of the uncoupler FCCP, VEH/CCI group displayed elevated H_2O_2 levels in relation to VEH/SHAM ($p=0.0016$) (Supplementary Fig. 1B).

Considering the close functional association between mitochondrial membrane potential with mitochondrial H_2O_2 production, we generated an integrative figure combining the aforementioned data. This proposed integrative figure juxtaposes both mitochondrial variables and shows how changes in membrane potential influence H_2O_2 production in the context of CCI and ND pretreatment. This combined figure became possible because mitochondria from synaptosomes were challenged in microplate assays using the same protocol of metabolic substrates (PMGS) and (PMGSA), inhibitor (oligomycin), and uncoupler (FCCP) (Fig. 5 B).

The real-time mitochondrial oxygen consumption rate (OCR) at different respiratory states is shown in Fig. 5 C. In basal conditions, ND/SHAM displayed increased OCR relative to VEH/SHAM and ND/CCI ($p=0.009$ and $p=0.040$, respectively). Using substrates PMGSA for complexes I, II, and V (CI + CII (P)), VEH/CCI displayed decreased OCR relative to VEH/SHAM and ND/SHAM ($p=0.008$ for both comparisons). In the Lomy state, there was no significant difference between groups. In the presence of FCCP, VEH/CCI showed decreased OCR relative to other groups (for all comparisons $p<0.05$). Also, OCR from the ND/CCI group was different from both SHAM groups ($p<0.05$) (Fig. 5C).

Specific mitochondrial signatures were derived from raw OCR data, such as proton leak ratio (L/E) and electron transfer system (ETS). We found an increase in proton leak in VEH/CCI relative to VEH/SHAM, ND/SHAM, and ND/CCI ($p<0.0001$ for all group comparisons) (Fig. 5D). ND/CCI displayed significant elevated proton leak compared to VEH/SHAM ($p=0.012$) (Fig. 5D). ETS efficiency was decreased in VEH/CCI compared to VEH/SHAM ($p=0.0002$), ND/SHAM ($p=0.0001$), and ND/CCI ($p=0.0059$) (Fig. 5E).

Moreover, the biochemical coupling efficiency (BCE) stems from the metabolic capacity of mitochondria in

Content courtesy of Springer Nature, terms of use apply. Rights reserved.

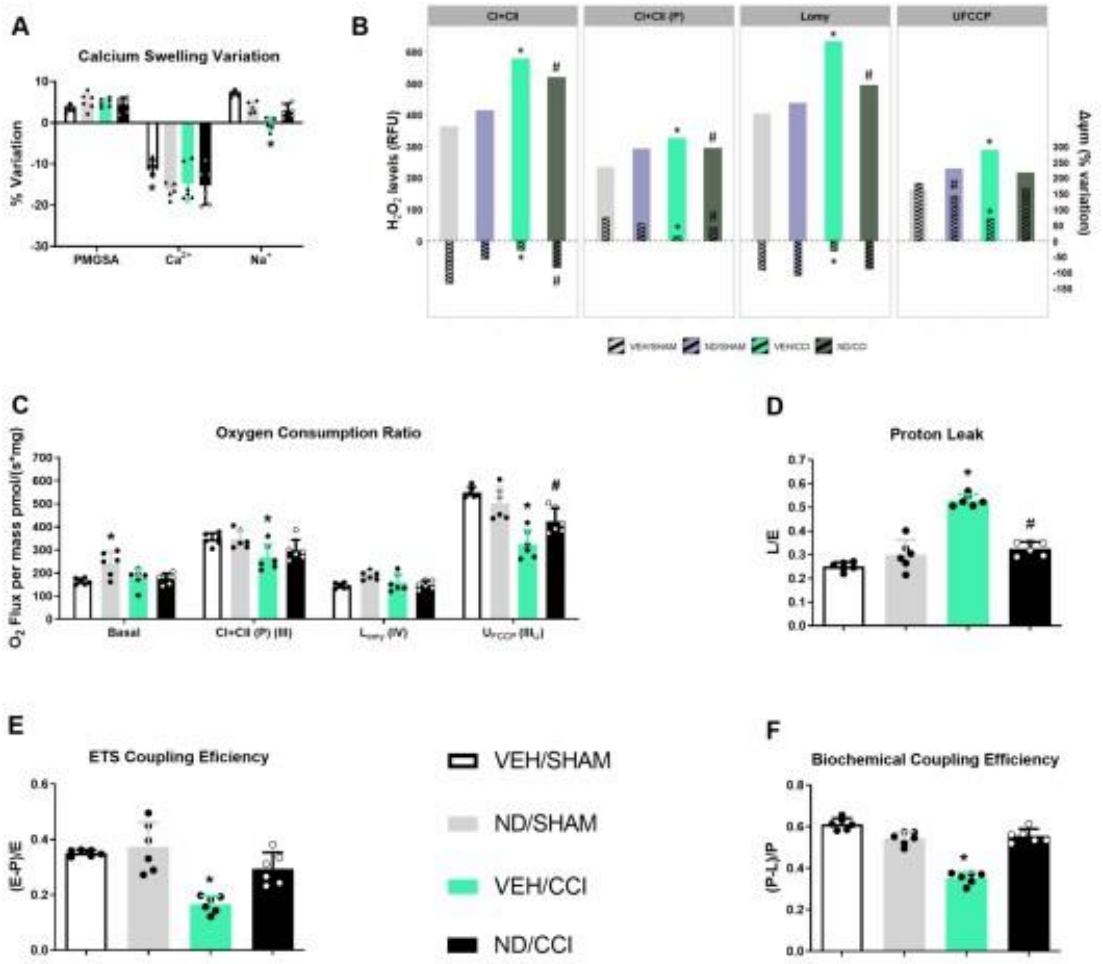


Fig. 5 Panel of mitochondrial-related bioenergetic endpoints. Mitochondrial function was assessed in synaptosomes from whole-brain mice. **A** Mitochondria energized with metabolic substrates for the complexes I, II, and V (pyruvate, malate, glutamate, succinate, and ADP; PMGSA) were challenged regarding Ca^{2+} influx (swelling after Ca^{2+} addition) and Ca^{2+} efflux (shrinkage after Na^{+} addition) capacity. VEH/SHAM showed significantly less swelling relative to other groups. Calcium efflux was impaired in VEH/CCI relative to other groups, and ND prior to CCI partially sustained the capacity of mitochondria to extrude calcium (*$p<0.05$ for all comparisons). **B** Formation and dissipation of mitochondrial membrane potential (Δψm) in relation to mitochondrial H_2O_2 production. VEH/CCI group showed a lower Δψm concurrent with increased H_2O_2 production when compared to VEH/SHAM after the addition of i) substrates to complexes I and II (CI + CII); ii) complex V (CI + CII (P)); iii) an inhibitor of complex V (Lomy); and iv) the uncoupler UFCCP (*$p<0.05$). ND/CCI group displayed different values of Δψm and H_2O_2 (CI + CII and CI + CII(P)) relative to VEH/SHAM (#$p<0.05$). Relative to VEH/CCI, ND/CCI alleviated the disruption in Δψm, leading to a reduced H_2O_2 production particularly in Lomy (# $p<0.05$). In the UFCCP state, ND/CCI sustained the H_2O_2 production at the level of VEH/SHAM. The bars below the dashed lines represent Δψm formation and bars above dashed lines represent Δψm dissipation. The columns above dashed lines represent H_2O_2 production. **C** Oxygen consumption rate in real-time respirometry after challenging synaptosomes with metabolic substrates to CI (PMG), CII (S), CV (ADP), an inhibitor of CV (oligomycin), and an uncoupler (UFCCP). In the basal state, ND/SHAM showed significant increase in oxygen consumption relative to VEH/SHAM and VEH/CCI. Further, VEH/CCI displayed decreased respiratory capacity in CI + CII(P) and uncoupling (UFCCP) states relative to VEH/SHAM (*$p<0.05$). In UFCCP state, ND/CCI was different from VEH/SHAM (#$p<0.05$). Parameters **D** proton leak, **E** electron transfer efficiency, and **F** biochemical coupling efficiency were significantly impaired in VEH/CCI relative to VEH/SHAM, ND/SHAM, and ND/CCI (*$p<0.05$). Prior nandrolone administration attenuated the impairment in mitochondrial bioenergetics caused by CCI ($n=5$–6 per group). Data are shown as mean ± S.D. Abbreviations: VEH, vehicle; ND, nandrolone decanoate; CCI, controlled cortical impact

Content courtesy of Springer Nature, terms of use apply. Rights reserved.

coupling membrane potential, effective electron transfer, and oxygen consumption coupled with ATP biosynthesis by the FoF1-ATP synthase (complex V). We found a significant decrease of BCE in VEH/CCI relative to VEH/SHAM, ND/SHAM, and ND/CCI ($p = 0.0001$ for all group comparisons) (Fig. 5F). This setting of results highlights that ND administration prior to CCI significantly maintained the integrity of the components involved in mitochondrial bioenergetic function following injury.

System Biology Approach

A correlogram was generated using pairwise Pearson correlations to illustrate relevant associations between ER stress proteins and mitochondrial endpoints. The correlogram highlights both positive and negative associations (− 1 to 1) between the variables, which mathematically indicates their functional interconnectedness and mutual influence. For instance, mitochondrial BCE presents negative correlation (− 1) with ER stress proteins GRP78 and CHOP, whereas mitochondrial H_2O_2 production correlates positively (1) with GRP78 and CHOP (Fig. 6A).

Furthermore, a principal component analysis (PCA) was conducted using key factors for mitochondrial metabolism and ER stress. The variables comprising the PC1 explained 87% of the variances (Fig. 6B). The contribution of each variable to PC1 is shown in Fig. 6C, where variables beyond the red line are considered significant. When considering all these variables, the PC1 values can effectively illustrate the distinctions among all groups when contrasted with the VEH/CCI group (Fig. 6D). The six significant variables for PC1 provide compelling evidence of the crucial role played

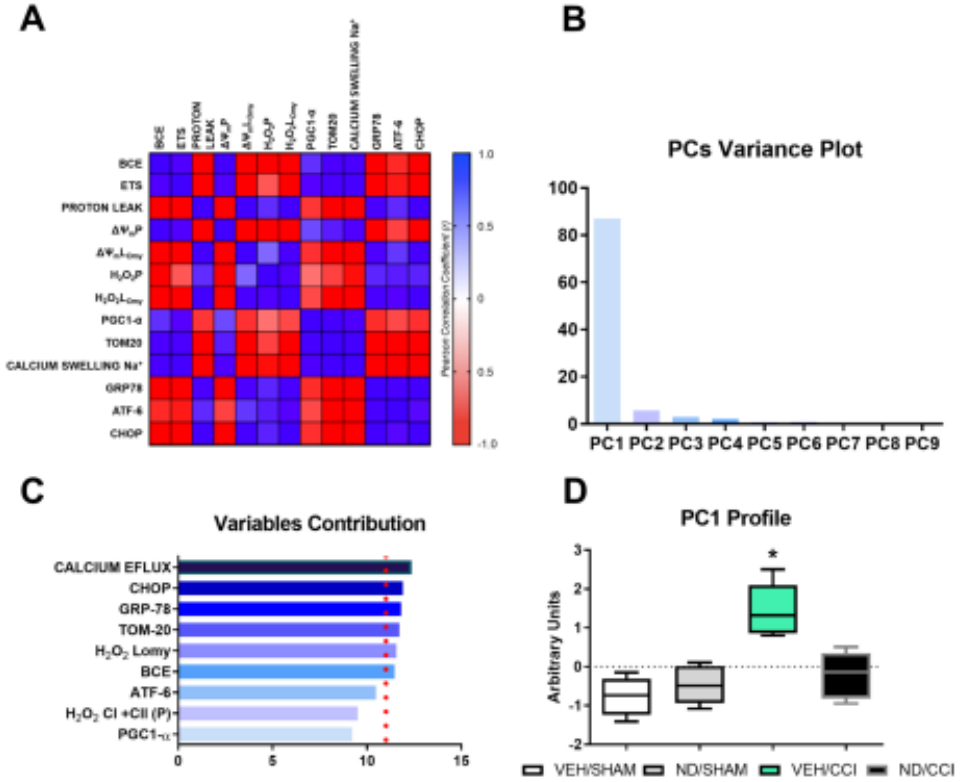


Fig. 6 System biology approach of molecular and functional endpoints. **A** Correlogram of ER stress biomarkers and mitochondrial variables. The bivariate correlation analysis confirms a significant association between ER stress and mitochondrial endpoints, suggesting that they may exert varying degrees of positive and/or negative functional influence on each other within the range of − 1 to + 1. **B** Principal component analysis (PCs). The molecular and biochemical variables were grouped in 9 PCAs being that PC1 seemed to explain 87% of the variances found in the results. **C** Variable contribution to PC1. The variables that contribute to the PC1 were plotted according to the higher to lower magnitude contribution. Six variables that cross beyond the dashed red line presented a statistical significant contribution to the PC1. **D** Group profiling of PC1. Considering the six significant variables, the PC1 values can effectively provide a discrimination among all groups ($^*p < 0.05$)

Content courtesy of Springer Nature, terms of use apply. Rights reserved.

by ER stress markers and mitochondrial metabolism in understanding the differences between the VEH/CCI group and the other groups, including VEH/SHAM ($p < 0.0001$), ND/SHAM ($p = 0.004$), and ND/CCI ($p = 0.0015$).

Spatial Memory Modulation by ND and/or TBI

Before the MWM task, mice performed an open field test to evaluate their locomotor and exploratory profile. There were no significant differences between groups in total distance traveled, average speed, mobile and immobile time, and exploratory zone profile (periphery and center) (Supplementary Fig. 1).

In the training phase of MWM (day 1), both TBI groups (VEH/CCI and ND/CCI) spent significant time searching for a hidden submersed platform when compared to VEH/SHAM and ND/SHAM groups ($p < 0.05$) (Fig. 7A). At day 3 of training, all groups presented a significant decrease in the latency to find the hidden platform when compared with day 1 ($p < 0.05$), implying that all groups learned the task and may advance to the test phase (day 4) (Fig. 7A).

In the test phase of the MWM, we found that VEH/CCI spent significantly less time in the target quadrant compared to other groups (for all comparisons, $p < 0.0001$; Fig. 7B). ND/CCI and VEH/SHAM spent similar time in the target zone. There was no significant difference in the average speed between groups (Fig. 7C). Further, there was significant correlation between the time spent in the target quadrant and PC1 ($r = -0.944$, $p = 0.0001$) (Fig. 7D). In Fig. 7E, we presented a representative tracking plot that illustrates the path taken by a single mouse of each group during the test phase. ND taken prior to CCI reduced spatial memory deficits in the MWM paradigm.

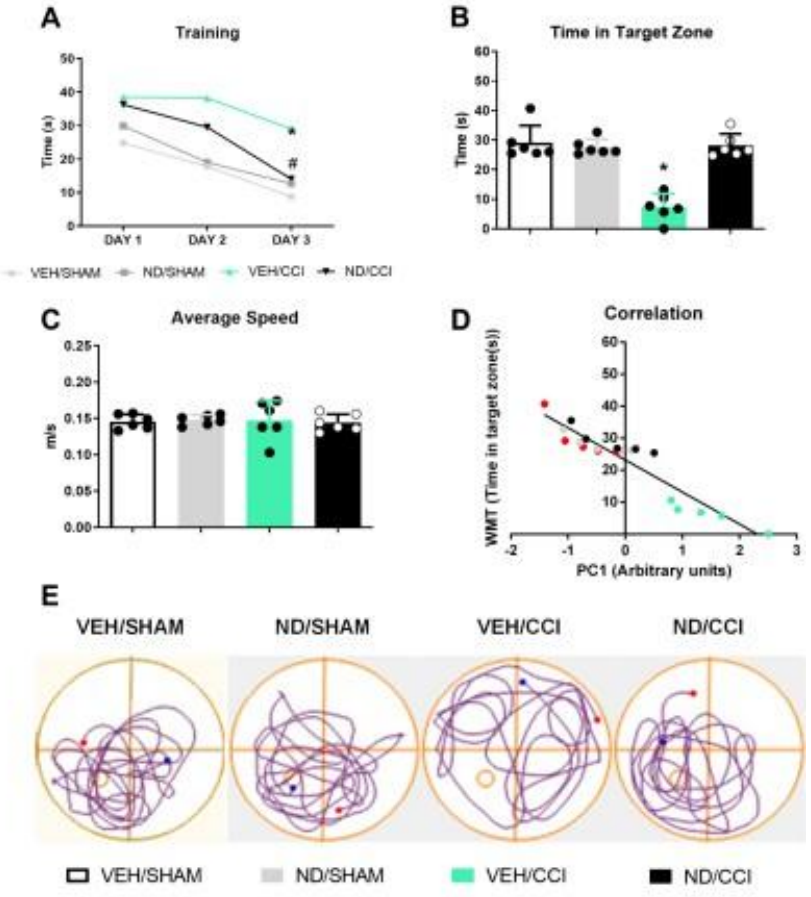


Fig. 7 Spatial memory task. Spatial memory was assessed using the Morris water maze. **A** During the 3-day learning phase, mice from all groups successfully learned to locate a submerged hidden platform (*$p < 0.05$). On the third day of training, mice in the VEH/CCI group spent more time searching for the platform compared to the other groups (#$p < 0.05$). **B** In the test phase, mice from VEH/CCI group spent less time in the quadrant where the hidden platform was previously located (*$p < 0.05$). **C** Average speed did not differ between groups. **D** Correlation between PC1 and time in quadrant ($r = -0.944$, $p = 0.0001$). **E** Representative image one animal from each group during the test trial. Abbreviations: VEH, vehicle; ND, nandrolone decanoate; CCI, controlled cortical impact

Content courtesy of Springer Nature, terms of use apply. Rights reserved.

Discussion

In this study, we aimed to investigate the overlap between the responses of ER stress and mitochondrial bioenergetics to both TBI and prior AAS abuse. Surprisingly, despite anticipating an increase in protein aggregation and mitochondrial dysfunction, we found a downregulation of aggregation-prone proteins, as well as decreased ER stress markers and mitochondrial dysfunction. These observations were found to correlate with a reduction in spatial memory deficits after TBI, suggesting a paradoxical effect of AAS abuse prior to injury.

Previous studies have shown that the administration of ND (15 mg/kg/day) to mice for 19 days, as opposed to 5 or 11 days, resulted in heightened impulsive and aggressive behavior compared to the control group [13]. The underlying mechanism for this behavior was associated with reduced astrocytic glutamate uptake and elevated brain glutamate levels, inducing *N*-methyl-D-aspartate receptor (NMDAr) hyperexcitability [7], which are also hallmarks of TBI pathogenesis. Importantly, locomotor activity, aversive and spatial memory, and anxiety-like behavior remained unaffected, suggesting that 19 days of ND abuse preferentially exacerbated aggressive behavior [7, 33]. This predisposes individuals abusing AAS to an increased risk of experiencing head impacts, which is particularly prevalent in young adults engaged in violent behavior or participating in contact sports [4, 11, 34–37]. Given this, our study was tailored to induce highly impulsive and aggressive phenotypes in mice on the day they underwent TBI. Tabor and colleagues showed that prior methandienone, another AAS, administration to sham-TBI surgery rats elicited no significant aggressive behavior in three-trial dominance tube test [38]. Also, methandienone alone affects short-term working memory and causes depressive-like behavior, with the latter being associated with prefrontal cortex atrophy and amygdala enlargement. The authors did not report any alterations in locomotor behavior due to methandienone or repeated head trauma in rats, which aligns with our results using a single TBI in mice [38].

A significant gap exists in the mechanistic understanding and therapeutic management of TBI, which relates to whether and how preexisting genetic and lifestyle factors influence the pathophysiological mechanisms and neurological outcomes [39–42]. In particular, the abuse of drugs including AAS has gained increasing interest as a factor interacting with TBI. Indeed, evidence highlights that the widespread hyperphosphorylation of tau protein, the accumulation of Aβ peptide derived from alternative APP processing, impaired mitochondrial Ca^{2+} control combined with the dysregulation of redox status, and progressive axonal pathology serve as substrates for the propagation of neurodegeneration following TBI [3, 17, 43]. Similarly, supraphysiological doses of AAS mediate the abundance of some of these neurotoxic substrates rendering the brain more vulnerable to TBI mechanisms, likely increasing the risk of dementia [14, 40]. In this study, we confirmed that the biomechanical forces applied to the brain through the CCI model resulted in an increase in APP, Aβ42, and $pTau^{Ser396}$, building a molecular context that favors proteotoxic aggregation and neural cell damage via ER stress coupled with mitochondrial dysfunction [44, 45]. Indeed, ER stress along with abnormal protein accumulation has been reported following TBI [44]. Interestingly, the downregulation of ER stress proteins induced by a polyunsaturated fatty acid was associated with decreasing APP and pTau accumulation. The authors also found a decreased aggregation of ubiquitinated proteins in the TBI group, suggesting an increasing proteasomal degradation/recycling of neurotoxic proteins contributes to the proteostasis [46]. Our study demonstrated that ND abuse alone does not increase APP, Aβ42, and pTau in the brains of mice. Further, we showed that ND prevents the abnormal accumulation of these proteins caused by TBI, likely alleviating triggers of ER stress, mitochondrial dysfunction, and neurodegeneration.

In the context of neurodegenerative disorders, the physical and functional misconnection of ER with mitochondria contact sites has been implicated in neuronal and astrocytic death following TBI [44–48]. Chen et al. (2022) reported that ER length adjacent to mitochondria peaked at 6 h, coinciding with disorganized cristae, increased swelling, expression of ER-mitochondria tethering proteins (PACS2 and Mfn2), and reduced mitochondrial number lasting for up to 24 h after CCI [45]. The levels of ER stress biomarkers GRP78, PERK, eIF2α, ATF4 also peaked at 6 h following TBI, favoring the activation of pro-apoptotic signaling via CHOP for more than 72 h. Another study showed an upregulation of ATF4 levels for 21 days after a CCI model, implying prolonged ER stress-induced apoptotic responses to TBI. Also, the levels of GRP78, the master regulator of the UPR, did not change from up to 21 days after CCI, suggesting that ER Ca^{2+} depletion and protein aggregates may serve as alternative upstream starters of UPR, regardless of the chaperone GRP78 [46].

Considering that ER stress has three main branches (PERK, IRE-1, ATF6), all activated by the main upstream effector GRP78, our findings suggest an integral response of UPR branches. In our study, we found that GRP78, ATF6, and CHOP were upregulated on day 6 after CCI. This biomarker panel is based on ATF6 as a transcriptional protein activated by proteolysis in the Golgi, from which it migrates to the nucleus to promote the biosynthesis of chaperones like GRP78, and CHOP activation.

Content courtesy of Springer Nature, terms of use apply. Rights reserved.

Also, CHOP upregulation is representative of apoptotic cell death in response to the upstream IRE-1, and PERK activation, implying we cannot rule out the participation of these two branches [49]. Remarkably, we found that supraphysiological administration of ND prior to TBI in mice mitigated the upregulation of GRP78, ATF6, and CHOP and kept these apoptotic effectors under physiological control for up to 6 days after injury [50].

Importantly, protein accumulation and heightened ER stress affect many aspects of mitochondrial function [45, 51]. Here, the mitochondrial biomarkers PGC-1α and TOM20 in the mice brain were downregulated, reflecting a scenario of decreasing adaptive mechanisms such as metabolic plasticity and biogenesis in response to TBI-induced proteotoxic stress. These findings are corroborated by earlier evidence indicating reduced mitochondrial number, irregular cristae, fragmented appearance, increased ROS production, and mitochondrial apoptotic markers subsequent to TBI-induced ER stress [45]. Moreover, triggers of ER stress such as thapsigargin and tunicamycin deplete Ca^{2+} from ER leading to accumulation in the mitochondrial matrix. While physiological Ca^{2+} influx into the mitochondrial matrix improves dehydrogenase activity and metabolic substrate utilization, excessive load may lead to dysfunctional outcomes [52].

Our study revealed that TBI-induced ER stress increases Ca^{2+} influx and diminishes the NCLX-dependent mitochondrial Ca^{2+} efflux in exchange with Na^{+}, thus reflecting increased mitochondrial swelling previously documented by others through transmission electron microscopy [45, 53]. In addition, an elevation in mitochondrial Ca^{2+} levels disrupts electrochemical potential, increases H_2O_2 production, compromises electron transfer between complexes, and decreases ATP biosynthesis. While ROS production and a deficient membrane potential are commonly considered biomarkers of mitochondrial dysfunction due to ER stress, our study expands this assumption by highlighting the involvement of multiple components of the mitochondrial bioenergetic machinery. We also show metabolic signatures of individual mitochondrial complexes (CI + CII + CV) based on addition of substrates to drive oxygen consumption for ATP biosynthesis (OxPhos). Particularly, the mitochondrial-fitness-derived respiratory parameter, BCE, provides a comprehensive assessment of whether electron transfer through the complexes, the formation and dissipation of membrane potential, and reduction of ½ O_2 to H_2O are synchronously working toward ATP synthesis or not [45, 54, 55]. The reduction of this multi-parameter endpoint of mitochondrial function after CCI suggests a loss of mitochondrial fitness after injury, which is attenuated by prior ND administration. Altogether, these results indicate that prior ND administration preserved neural bioenergetic function, including components of both ER and mitochondria.

Remarkably, ND administration prior to CCI mitigated the downregulation of PGC-1α and TOM20, along with partially preventing the decrease in mitochondrial Ca^{2+} efflux. In addition, all components of the bioenergetic machinery are efficiently engaged in ATP biosynthesis, thereby overcoming the energy downregulation reported previously [56, 57]. We already showed that testosterone supplementation after a CCI improves NCLX-driven Ca^{2+} efflux, improved mitochondrial respiration, and decreased Ca^{2+}-dependent pro-apoptotic cleavage of caspase-3, suggesting that androgenic steroids can modulate Ca^{2+} efflux to improve cell survival, and possibly attenuate neurodegeneration after TBI [16]. Overall, this indicates a potential role of androgenic steroids as neuroprotective compounds for acute brain injury. While selective "laser-guided" neuroprotective strategies have been shown limited effectiveness, the use of multipotential drugs that modulate multiple secondary injury pathways is emerging as the next strategy approach. Here, we found that ND possesses neuroprotective characteristics that match this proposed mechanistic concept [21].

Adult TBI patients may present reduced central and systemic endogenous testosterone levels due to persisting hypopituitarism culminating in hypogonadotropic hypogonadism. This can be followed by both short-term and long-term vascular, metabolic, and neuropsychiatric problems [58–60]. Moreover, a previous report proposes that ER stress acts as a mediator of anabolic resistance in skeletal muscle, which may be a factor promoting body weight loss and muscle atrophy after TBI [61–63]. Our study reveals a paradoxical effect in relation to synthetic anabolic steroids abuse. Indeed, ND possessing over 10 times the anabolic potency of testosterone surprisingly acts as a neuroprotective barrier instead of amplifying the secondary changes induced by TBI. Testosterone levels have already been shown to modulate ER stress in other contexts. Castrated rats, which present reduced endogenous levels of testosterone, present a synergic effect to high fat diet on ER-stress related markers such as liver PERK and IRE1α expression, leading to downstream activation of CHOP, JNK, and NF-κB. Testosterone replacement to castrated rats fed the same diet resulted in normalization in the ER stress expression profile signaling and amelioration of inflammation and apoptosis [64]. Given the substantial ATP requirements for the recovery of neural cells post-TBI and the recognized impact of androgenic steroid hormones on anabolic processes, it seems that the supraphysiological dose of ND employed in this study sustained elevated steroid levels during the critical period, aligning with the onset of hypogonadism.

In fact, decreased testosterone levels and impaired androgen receptor signaling after TBI can alter various downstream mechanisms, thereby justifying the benefits observed with prior ND administration. However, the mechanistic support for our data is relatively contradictory, and the

Content courtesy of Springer Nature, terms of use apply. Rights reserved.

majority stems from preclinical studies unrelated to TBI. To date, testosterone supplementation in castrated rats has been shown to repress ubiquitin ligases (atrogin-1/MAFbx/FBXO32 and Murf-1/Trim63) and skeletal muscle protein breakdown, implying that testosterone levels can downregulate the ubiquitin–proteasome system to mediate proteostasis [65]. Further, steroid hormone signaling using 1,25(OH)$_2$D3 in human prostate cancer cells decreased endoplasmic reticulum-associated degradation (ERAD) and poly-ubiquitination, and suppressed PERK signaling. This androgenic signaling did not change BiP/GRP78 level, but caused an upregulation of IRE1α and XBP-1 proteins reflecting a specific activation of UPR response [66]. Erzurumlu et al. (2023) showed that progesterone positively regulates mRNA and protein levels of ERAD components in prostate cancer cells and upregulates the IREα and PERK branches of UPR signaling. These effects were exerted in parallel with androgen receptors [67]. Also, testosterone-induced CHOP expression and apoptosis was blocked with an androgen receptor antagonist flutamide in granulosa cells [68]. On opposite, in a model of doxorubicin-induced testicular damage, testosterone levels decreased by 85%, leading to significant increases in markers of oxidative stress (MDA and GSH), apoptosis (caspase-3 and P53), and autophagic markers (PAKT, mTOR, and LC3) in the testicles. These changes were followed by neurodegeneration in the brain cortex and hippocampus, which correlated with memory deficits. Remarkably, restoring testosterone levels attenuated the oxidative stress markers, apoptosis, autophagy in the testicles, and decreased neurodegeneration along with improvements in memory processing [69]. Although not evaluated by the referred study, apoptosis and oxidative stress damage have mechanistic connections with mitochondrial dysfunction, and post-CCI testosterone therapy attenuated these alterations [32]. Considering that autophagy promotes the removal of accumulating proteins and dysfunctional mitochondria [70, 71], and that the restoration of testosterone levels increased autophagic markers (PAKT, mTOR, and LC3), here we proposed that ND may induce the clearance of APP, Aβ42, and pTauSer396 proteins alleviating ER stress, and elicited the renewal of mitochondria in the brain [69]. Indeed, beyond increasing mitochondrial biogenesis through the upregulation of PGC1α, testosterone supports the integrity of internal membrane cristae, which foster the electron transport complexes and FoF1-ATP-synthase associated with bioenergetic function [72]. Of notice, castrated rodents displayed reduced expression of PGC1α in the skeletal muscle and testosterone reversed this profile [73]. Mitochondrial biogenesis requires nuclear-encoded pre-proteins, which are imported by translocase of the outer mitochondrial membrane (TOMs), and then assembled into subunits of mitochondrial complexes. Prominent evidence suggests that estradiol and estrogen receptors in mitochondria mediate TOM expression [74]. Like testosterone esters in the brain, ND can be aromatized to 17β-estradiol and other estrogens, implying it could contribute to TOM20 expression and biogenesis [75]. Overall, the alterations resulting from testosterone deficiency, as observed in hypopituitary hypogonadism associated with TBI, underscore the multidimensional mechanistic effects of androgenic signaling and provide therapeutic perspectives dependent on the target tissue.

While our correlogram approach provides suggestions of multiple functional associations between effectors participating in the crosstalk between ER stress and mitochondria, it does not specifically indicate the major contributing effector. We mathematically estimate a hierarchization of these effectors through PCA analysis. Data from PC variance indicates mitochondrial Ca^{2+} efflux as the variable that contributes with higher magnitude for the PC1. This suggests that Ca^{2+} efflux is the key hierarchic effector of ER-stress and mitochondrial dysfunction mediated by ND and TBI. Although in our model, Ca^{2+} efflux displays a higher grade of contribution. it is accessorized by CHOP, GRP78, TOM20, H_2O_2 Lomy, and BCE. These six variables provide a clear discrimination of VEH/CCI from other groups, leading to a comprehensive understanding of the dysfunctional interconnections raised by this study.

Moreover, silent neurodegeneration and neuropsychiatric deficits are concurrent in TBI patients and how lifestyle factors influence these outcomes still demand elucidation. Here we display that ND prior to CCI avoided impairments in spatial memory acquisition and retention. Noteworthy, a bivariate correlation analysis displayed an inverse correlation between time in the target quadrant and PC1 value, implying that beyond discriminating study groups, PC1 variables also explained unfavorable neurological outcomes.

In summary, our findings indicate that the abuse of ND prior to TBI mitigated ER stress and several dimensions of mitochondrial dysfunction. A principal component analysis revealed a key hierarchical signature featuring mitochondrial Ca^{2+} efflux, CHOP, GRP78, TOM20, H_2O_2, and bioenergetic efficiency (BCE) as a unique variable (PC1) able to explain the memory deficits caused by TBI, as well as the preservation of memory fitness induced by prior ND abuse. The paradoxical nature of these findings warrants further investigation into the mechanisms of ND abuse in improving TBI outcome.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12035-024-04488-8.

Acknowledgements The authors would like to thank the Center for Brain Injury and Repair, University of Pennsylvania (CBIR-UPENN) for the technical and scientific support.

Author Contribution MSR, LVP, LOCP, DHS, built the study concept, writing the original draft. NRS, LVP and DHS, acquired grants. MSR,

Content courtesy of Springer Nature, terms of use apply. Rights reserved.

AK, MS, GS, VGO, animal care, dissected the brain, and performed synaptosomal preparations, run behavior and WB experiments. LV, CDB, JVP, and JSJ performed the surgery and head trauma, and mitochondrial assays, MSR,CAG, NRS, MAD, analyzed the data and created the figures, performed systems biology approach. All authors reviewed and edited the final manuscript.

Funding This work was supported by the Brazilian Agencies/Programs FAPERGS #1010267, FAPERGS/PPSUS#17/2551–0001, FAPERGS/PRONEX#16/2551–0000499-4, CNPq Program Science without Borders #4011645/2012–6, CNPq INNT #5465346/2014–6, Programa de Internacionalização de Ciência FAPERGS/CAPES #19/25510000717–5, and Irish Research Council grant GOIPD/2022/792 (NRS). This research was made possible with support from National Institutes of Health grants R01NS092398 (DHS), R01NS038104 (DHS), R01NS094003 (DHS), R01EB021293 (DHS), U54NS115322 (WS and DHS), as well as support from Paul G. Allen Family Foundation (DHS).

Data Availability No datasets were generated or analysed during the current study.

Declarations

Conflict of Interest The authors declare no competing interests.

References

1. Pearn ML, Niesman IR, Egawa J et al (2017) Pathophysiology associated with traumatic brain injury: current treatments and potential novel therapeutics. Cell Mol Neurobiol 37:571–585. https://doi.org/10.1007/S10571-016-0400-1
2. Johnson VE, Stewart W, Arena JD, Smith DH (2017) Traumatic brain injury as a trigger of neurodegeneration. Adv Neurobiol 15:383–400. https://doi.org/10.1007/978-3-319-57193-5_15
3. Johnson VE, Stewart W, Smith DH (2013) Axonal pathology in traumatic brain injury. Exp Neurol 246:35–43. https://doi.org/10.1016/J.EXPNEUROL.2012.01.013
4. Maas AIR, Menon DK, Manley GT et al (2022) Traumatic brain injury: progress and challenges in prevention, clinical care, and research. Lancet Neurol 21:1004–1060. https://doi.org/10.1016/S1474-4422(22)00309-X
5. Sagoe D, Molde H, Andreassen CS et al (2014) The global epidemiology of anabolic-androgenic steroid use: a meta-analysis and meta-regression analysis. Ann Epidemiol 24:383–398. https://doi.org/10.1016/J.ANNEPIDEM.2014.01.009
6. Hartgens F, Kuipers H (2004) Effects of androgenic-anabolic steroids in athletes. Sports Med 34:513–554. https://doi.org/10.2165/00007256-200434080-00003
7. Rodolphi MS, Kopczynski A, Carteri RB, et al (2021) Glutamate transporter-1 link astrocytes with heightened aggressive behavior induced by steroid abuse in male CF1 mice. Horm Behav 127:. https://doi.org/10.1016/J.YHBEH.2020.104872
8. Clark AS, Henderson LP (2003) Behavioral and physiological responses to anabolic-androgenic steroids. Neurosci Biobehav Rev 27:413–436. https://doi.org/10.1016/S0149-7634(03)00064-2
9. Pomara C, Neri M, Bello S et al (2015) Neurotoxicity by synthetic androgen steroids: oxidative stress, apoptosis, and neuropathology: a review. Curr Neuropharmacol 13:132–145. https://doi.org/10.2174/1570159X13666141210221434
10. Piacentino D, Kotzalidis G, Casale A et al (2015) Anabolic-androgenic steroid use and psychopathology in athletes. A Systematic Rev Curr Neuropharmacol 13:101–121. https://doi.org/10.2174/1570159X13666141210222725
11. Beaver KM, Vaughn MG, DeLisi M, Wright JP (2008) Anabolic-androgenic steroid use and involvement in violent behavior in a nationally representative sample of young adult males in the United States. Am J Public Health 98:2185. https://doi.org/10.2105/AJPH.2008.137018
12. Taylor LA, Kreutzer JS, Demm SR, Meade MA (2003) Traumatic brain injury and substance abuse: a review and analysis of the literature. Neuropsychol Rehabil 13:165–188. https://doi.org/10.1080/09602010244000336
13. Kalinine E, Zimmer ER, Zenki KC et al (2014) Nandrolone-induced aggressive behavior is associated with alterations in extracellular glutamate homeostasis in mice. Horm Behav 66:383–392. https://doi.org/10.1016/j.yhbeh.2014.06.005
14. Kaufman MJ, Kanayama G, Hudson JI, Pope HG (2019) Supraphysiologic-dose anabolic-androgenic steroid use: a risk factor for dementia? Neurosci Biobehav Rev 100:180–207. https://doi.org/10.1016/J.NEUBIOREV.2019.02.014
15. Smith DH, Stewart W (2016) Tackling concussion, beyond Hollywood. Lancet Neurol 15:662–663. https://doi.org/10.1016/S1474-4422(16)30037-0
16. Carteri RB, Kopczynski A, Rodolphi MS et al (2019) Testosterone administration after traumatic brain injury reduces mitochondrial dysfunction and neurodegeneration. J Neurotrauma 36:2246–2259. https://doi.org/10.1089/NEU.2018.6266
17. Zetterberg H, Smith DH, Blennow K (2013) Biomarkers of mild traumatic brain injury in cerebrospinal fluid and blood. Nat Rev Neurol 9:201–210. https://doi.org/10.1038/NRNEUROL.2013.9
18. Dis N, Hoozemans JJM, Van Haastert ES, et al (2012) Activation of the unfolded protein response is an early event in Alzheimer's and Parkinson's disease. https://doi.org/10.1159/000334536
19. Ferreiro E, Baldeiras I, Ferreira IL et al (2012) Mitochondrial- and endoplasmic reticulum-associated oxidative stress in alzheimers disease: from pathogenesis to biomarkers. Int J Cell Biol. https://doi.org/10.1155/2012/735206
20. Roussel BD, Kruppa AJ, Miranda E, et al (2013) Endoplasmic reticulum dysfunction in neurological disease. Lancet Neurol 12:. https://doi.org/10.1016/S1474-4422(12)70238-7i
21. Loane DJ, Faden AI (2010) Neuroprotection for traumatic brain injury: translational challenges and emerging therapeutic strategies. Trends Pharmacol Sci 31:596–604. https://doi.org/10.1016/j.tips.2010.09.005
22. de Carvalho AK, da Silva S, Serafini E et al (2017) Prior exercise training prevent hyperglycemia in STZ mice by increasing hepatic glycogen and mitochondrial function on skeletal muscle. J Cell Biochem 118:678–685. https://doi.org/10.1002/JCB.25658
23. Dietrich MO, Mantese CE, Porciuncula LO et al (2005) Exercise affects glutamate receptors in postsynaptic densities from cortical mice brain. Brain Res 1065:20–25. https://doi.org/10.1016/J.BRAINRES.2005.09.038
24. Muller AP, Gnoatto J, Moreira JD et al (2011) Exercise increases insulin signaling in the hippocampus: physiological effects and pharmacological impact of intracerebroventricular insulin administration in mice. Hippocampus 21:1082–1092. https://doi.org/10.1002/HIPO.20822
25. Stefani MA, Modkovski R, Hansel G et al (2017) Elevated glutamate and lactate predict brain death after severe head trauma. Ann Clin Transl Neurol 4:392–402. https://doi.org/10.1002/ACN3.416
26. Smith DH, Soares HD, Pierce JS et al (1995) A model of parasagittal controlled cortical impact in the mouse: cognitive and histopathologic effects. J Neurotrauma 12:169–178. https://doi.org/10.1089/NEU.1995.12.169
27. Andersen ML, Lopes de Souza R, Sebben A, et al (2016) Normativas do CONCEA para Produção, Manutenção ou Utilização de Animais em Atividades de Ensino ou Pesquisa Científica.

Content courtesy of Springer Nature, terms of use apply. Rights reserved.

Ministério d a Ciência, Tecnologia, Inovações e Comunicações (MCTIC) do Brazil. 3a Edição, Brasília, 26 de setembro de 2016
28. Council NR (2007) Recognition and alleviation of distress in laboratory animals. recognition and alleviation of distress in laboratory animals. https://doi.org/10.17226/11931
29. Sims NR, Anderson MF (2008) Isolation of mitochondria from rat brain using Percoll density gradient centrifugation. Nat Protoc 3:1228–1239. https://doi.org/10.1038/NPROT.2008.105
30. Portela LV, Brochier AW, Haas CB et al (2017) Hyperpalatable Diet and Physical Exercise Modulate the Expression of the glial monocarboxylate transporters MCT1 and 4. Mol Neurobiol 54:5807–5814. https://doi.org/10.1007/S12035-016-0119-5
31. Gnaiger E (2014) Mitochondrial pathways and respiratory control. An introduction to OXPHOS analysis. Bioenergetics Commun 2014:2–2
32. Carteri RB, Kopczynski A, Menegassi LN et al (2019) Anabolic-androgen steroids effects on bioenergetics responsiveness of synaptic and extrasynaptic mitochondria. Toxicol Lett 307:72–80. https://doi.org/10.1016/j.toxlet.2019.03.004
33. Kalinine E (2011) Efeitos comportamentais, neuroquímicos e metabólicos do tratamento com decanoato de nandrolona em camundongos. Dissertation in Biochemistry. (LUME repository link: http://hdl.handle.net/10183/31788). Federal University of Rio Grande do Sul, UFRGS. Porto Alegre, RS, Brazil
34. Iverson GL, Büttner F, Caccese JB (2021) Age of first exposure to contact and collision sports and later in life brain health: a narrative review. Front Neurol 12:727089. https://doi.org/10.3389/FNEUR.2021.727089/BIBTEX
35. Ameen-Ali KE, Bretzin A, Lee EB et al (2022) Detection of astrocytic tau pathology facilitates recognition of chronic traumatic encephalopathy neuropathologic change. Acta Neuropathol Commun 10:1–11. https://doi.org/10.1186/S40478-022-01353-4/TABLES/4
36. Maroon JC, Winkelman R, Bost J, et al (2015) Chronic traumatic encephalopathy in contact sports: a systematic review of all reported pathological cases. PLoS One 10:. https://doi.org/10.1371/JOURNAL.PONE.0117338
37. Iverson GL, Terry DP (2021) High school football and risk for depression and suicidality in adulthood: findings from a national longitudinal study. Front Neurol 12:812604. https://doi.org/10.3389/FNEUR.2021.812604
38. Tabor J, Wright DK, Christensen J et al (2020) Examining the effects of anabolic–androgenic steroids on repetitive mild traumatic brain injury (RmTBI) outcomes in adolescent rats. Brain Sci 10(5):258. https://doi.org/10.3390/BRAINSCI10050258
39. Smith DH, Kochanek PM, Rosi S et al (2021) Roadmap for advancing pre-clinical science in traumatic brain injury. J Neurotrauma 38:3204–3221. https://doi.org/10.1089/NEU.2021.0094
40. Namjoshi DR, Cheng WH, Carr M, et al (2016) Chronic exposure to androgenic-anabolic steroids exacerbates axonal injury and microgliosis in the CHIMERA mouse model of repetitive concussion. PLoS One 11:. https://doi.org/10.1371/JOURNAL.PONE.0146540
41. Kopczynski A, Carteri RB, Rodolphi MS et al (2023) Lower and higher volumes of physical exercise build up brain reserves against memory deficits triggered by a head injury in mice. Exp Neurol 363:114352. https://doi.org/10.1016/J.EXPNEUROL.2023.114352
42. Mills JD, Bailes JE, Turner RC et al (2012) Anabolic steroids and head injury. Neurosurgery 70:205–209. https://doi.org/10.1227/NEU.0B013E3182250918
43. Smith DH, Johnson VE, Stewart W (2013) Chronic neuropathologies of single and repetitive TBI: substrates of dementia? Nat Rev Neurol 9:211. https://doi.org/10.1038/NRNEUROL.2013.29
44. Larner SF, Hayes RL, Wang KKW (2006) Unfolded protein response after neurotrauma. J Neurotrauma 23:807–829. https://doi.org/10.1089/NEU.2006.23.807
45. Chen X, Mi L, Gu G et al (2022) Dysfunctional endoplasmic reticulum-mitochondrion coupling is associated with endoplasmic reticulum stress-induced apoptosis and neurological deficits in a rodent model of severe head injury. J Neurotrauma 39:560–576. https://doi.org/10.1089/NEU.2021.0347
46. Begum G, Yan HQ, Li L et al (2014) Docosahexaenoic acid reduces ER stress and abnormal protein accumulation and improves neuronal function following traumatic brain injury. J Neurosci 34:3743–3755. https://doi.org/10.1523/JNEUROSCI.2872-13.2014
47. Kim I, Xu W, Reed JC (2008) Cell death and endoplasmic reticulum stress: disease relevance and therapeutic opportunities. Nature Rev Drug Discovery 7(12):1013–30. https://doi.org/10.1038/nrd2755
48. Bravo R, Gutierrez T, Paredes F et al (2012) Endoplasmic reticulum: ER stress regulates mitochondrial bioenergetics. Int J Biochem Cell Biol 44:16–20. https://doi.org/10.1016/J.BIOCEL.2011.10.012
49. Walter P, Ron D (2011) The unfolded protein response: from stress pathway to homeostatic regulation. Science 334:1081–1086. https://doi.org/10.1126/SCIENCE.1209038
50. Yang H, Niemeijer M, van de Water B, Beltman JB (2020) ATF6 is a critical determinant of CHOP dynamics during the unfolded protein response. iScience 23:. https://doi.org/10.1016/J.ISCI.2020.100860
51. Costa RO, Ferreiro E, Martins I et al (2012) Amyloid β-induced ER stress is enhanced under mitochondrial dysfunction conditions. Neurobiol Aging 33:824.e5-824.e16. https://doi.org/10.1016/J.NEUROBIOLAGING.2011.04.011
52. Delmotte P, Sieck GC (2019) Endoplasmic reticulum stress and mitochondrial function in airway smooth muscle. Front Cell Dev Biol 7:374. https://doi.org/10.3389/FCELL.2019.00374
53. Logsdon AF, Turner RC, Lucke-Wold BP, et al (2014) Altering endoplasmic reticulum stress in a model of blast-induced traumatic brain injury controls cellular fate and ameliorates neuropsychiatric symptoms. Front Cell Neurosci 8:. https://doi.org/10.3389/FNCEL.2014.00421
54. Nedel WL, Strogulski NR, Kopczynski A et al (2021) Association between hyperlactatemia, perfusional parameters and lymphocyte mitochondrial dysfunction in septic shock patients. Shock. https://doi.org/10.1097/SHK.0000000000001868
55. Qureshi S, Lee S, Steidl W et al (2023) Endoplasmic reticulum stress disrupts mitochondrial bioenergetics, dynamics and causes corneal endothelial cell apoptosis. Invest Ophthalmol Vis Sci 64:18. https://doi.org/10.1167/IOVS.64.14.18
56. Lyons DN, Vekaria H, Macheda T et al (2018) A mild traumatic brain injury in mice produces lasting deficits in brain metabolism. J Neurotrauma 35:2435–2447. https://doi.org/10.1089/NEU.2018.5663
57. Singh IN, Sullivan PG, Deng Y et al (2006) Time course of post-traumatic mitochondrial oxidative damage and dysfunction in a mouse model of focal traumatic brain injury: implications for neuroprotective therapy. J Cereb Blood Flow Metab 26:1407–1418. https://doi.org/10.1038/SJ.JCBFM.9600297
58. Gray S, Bilski T, Dieudonne B, Saeed S (2019) Hypopituitarism after traumatic brain injury. Cureus 11:. https://doi.org/10.7759/CUREUS.4163
59. Bavisetty S, Bavisetty S, McArthur DL et al (2008) Chronic hypopituitarism after traumatic brain injury: risk assessment and relationship to outcome. Neurosurgery 62:1080–1093. https://doi.org/10.1227/01.NEU.0000325870.60129.6A
60. Lopez-Rodriguez AB, Acaz-Fonseca E, Spezzano R et al (2016) Profiling neuroactive steroid levels after traumatic brain injury

Content courtesy of Springer Nature, terms of use apply. Rights reserved.

in male mice. Endocrinology 157:3983–3993. https://doi.org/10.1210/EN.2016-1316
61. Deldicque L, Bertrand L, Patton A, et al (2011) ER stress induces anabolic resistance in muscle cells through PKB-induced blockade of mTORC1. PLoS One 6:. https://doi.org/10.1371/JOURNAL.PONE.0020993
62. Shahidi B, Shah SB, Esparza M et al (2018) Skeletal muscle atrophy and degeneration in a mouse model of traumatic brain injury. J Neurotrauma 35:398–401. https://doi.org/10.1089/NEU.2017.5172
63. Charrueau C, Belabed L, Besson V et al (2009) Metabolic response and nutritional support in traumatic brain injury: evidence for resistance to renutrition. J Neurotrauma 26:1911–1920. https://doi.org/10.1089/NEU.2008.0737
64. Jia Y, Yee JK, Wang C et al (2018) Testosterone protects high-fat/low-carbohydrate diet-induced nonalcoholic fatty liver disease in castrated male rats mainly via modulating endoplasmic reticulum stress. Am J Physiol Endocrinol Metab 314:E366–E376. https://doi.org/10.1152/AJPENDO.00124.2017
65. Pires-Oliveira M, Maragno ALGC, Parreiras-e-Silva LT et al (2010) Testosterone represses ubiquitin ligases atrogin-1 and Murf-1 expression in an androgen-sensitive rat skeletal muscle in vivo. J Appl Physiol 108:266–273. https://doi.org/10.1152/japplphysiol.00490.2009
66. Erzurumlu Y, Aydogdu E, Dogan HK et al (2023) 1,25(OH)2 D3 induced vitamin D receptor signaling negatively regulates endoplasmic reticulum-associated degradation (ERAD) and androgen receptor signaling in human prostate cancer cells. Cell Signal 103:110577. https://doi.org/10.1016/j.cellsig.2022.110577
67. Erzurumlu Y, Dogan HK, Catakli D (2023) Progesterone regulates the endoplasmic reticulum-associated degradation and unfolded protein response axis by mimicking the androgenic stimulation in prostate cancer cells. Mol Biol Rep 50:1253–1265. https://doi.org/10.1007/s11033-022-08065-x
68. Azhary JMK, Harada M, Takahashi N et al (2019) Endoplasmic reticulum stress activated by androgen enhances apoptosis of granulosa cells via induction of death receptor 5 in PCOS. Endocrinology 160:119–132. https://doi.org/10.1210/en.2018-00675
69. Alafifi SA, Wahdan SA, Elhemiely AA et al (2023) Modulatory effect of liraglutide on doxorubicin-induced testicular toxicity and behavioral abnormalities in rats: role of testicular-brain axis. Naunyn Schmiedebergs Arch Pharmacol 396:2987–3005. https://doi.org/10.1007/s00210-023-02504-7
70. Aman Y, Schmauck-Medina T, Hansen M et al (2021) Autophagy in healthy aging and disease. Nat Aging 1:634–650. https://doi.org/10.1038/s43587-021-00098-4
71. Cai Z, Zhou Y, Liu Z, et al (2015) Autophagy dysfunction upregulates beta-amyloid peptides via enhancing the activity of γ-secretase complex. Neuropsychiatr Dis Treat 2091. https://doi.org/10.2147/NDT.S84755
72. Yin L, Luo M, Wang R, et al (2021) Mitochondria in sex hormone-induced disorder of energy metabolism in males and females. Front Endocrinol (Lausanne) 12:. https://doi.org/10.3389/fendo.2021.749451
73. Rossetti ML, Esser KA, Lee C et al (2019) Disruptions to the limb muscle core molecular clock coincide with changes in mitochondrial quality control following androgen depletion. Am J Physiology-Endocrinology Metabolism 317:E631–E645. https://doi.org/10.1152/ajpendo.00177.2019
74. Chen J-Q, Cammarata PR, Baines CP, Yager JD (2009) Regulation of mitochondrial respiratory chain biogenesis by estrogens/estrogen receptors and physiological, pathological and pharmacological implications. Biochimica et Biophysica Acta (BBA) - Mol Cell Res 1793:1540–1570. https://doi.org/10.1016/j.bbamcr.2009.06.001
75. Penatti CAA, Porter DM, Henderson LP (2009) Chronic exposure to anabolic androgenic steroids alters neuronal function in the mammalian forebrain via androgen receptor- and estrogen receptor-mediated mechanisms. J Neurosci 29:12484–12496. https://doi.org/10.1523/JNEUROSCI.3108-09.2009

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.

Content courtesy of Springer Nature, terms of use apply. Rights reserved.

Terms and Conditions

Springer Nature journal content, brought to you courtesy of Springer Nature Customer Service Center GmbH ("Springer Nature").
Springer Nature supports a reasonable amount of sharing of research papers by authors, subscribers and authorised users ("Users"), for small-scale personal, non-commercial use provided that all copyright, trade and service marks and other proprietary notices are maintained. By accessing, sharing, receiving or otherwise using the Springer Nature journal content you agree to these terms of use ("Terms"). For these purposes, Springer Nature considers academic use (by researchers and students) to be non-commercial.
These Terms are supplementary and will apply in addition to any applicable website terms and conditions, a relevant site licence or a personal subscription. These Terms will prevail over any conflict or ambiguity with regards to the relevant terms, a site licence or a personal subscription (to the extent of the conflict or ambiguity only). For Creative Commons-licensed articles, the terms of the Creative Commons license used will apply.
We collect and use personal data to provide access to the Springer Nature journal content. We may also use these personal data internally within ResearchGate and Springer Nature and as agreed share it, in an anonymised way, for purposes of tracking, analysis and reporting. We will not otherwise disclose your personal data outside the ResearchGate or the Springer Nature group of companies unless we have your permission as detailed in the Privacy Policy.
While Users may use the Springer Nature journal content for small scale, personal non-commercial use, it is important to note that Users may not:

1. use such content for the purpose of providing other users with access on a regular or large scale basis or as a means to circumvent access control;
2. use such content where to do so would be considered a criminal or statutory offence in any jurisdiction, or gives rise to civil liability, or is otherwise unlawful;
3. falsely or misleadingly imply or suggest endorsement, approval , sponsorship, or association unless explicitly agreed to by Springer Nature in writing;
4. use bots or other automated methods to access the content or redirect messages
5. override any security feature or exclusionary protocol; or
6. share the content in order to create substitute for Springer Nature products or services or a systematic database of Springer Nature journal content.

In line with the restriction against commercial use, Springer Nature does not permit the creation of a product or service that creates revenue, royalties, rent or income from our content or its inclusion as part of a paid for service or for other commercial gain. Springer Nature journal content cannot be used for inter-library loans and librarians may not upload Springer Nature journal content on a large scale into their, or any other, institutional repository.
These terms of use are reviewed regularly and may be amended at any time. Springer Nature is not obligated to publish any information or content on this website and may remove it or features or functionality at our sole discretion, at any time with or without notice. Springer Nature may revoke this licence to you at any time and remove access to any copies of the Springer Nature journal content which have been saved.
To the fullest extent permitted by law, Springer Nature makes no warranties, representations or guarantees to Users, either express or implied with respect to the Springer nature journal content and all parties disclaim and waive any implied warranties or warranties imposed by law, including merchantability or fitness for any particular purpose.
Please note that these rights do not automatically extend to content, data or other material published by Springer Nature that may be licensed from third parties.
If you would like to use or distribute our Springer Nature journal content to a wider audience or on a regular basis or in any other manner not expressly permitted by these Terms, please contact Springer Nature at

onlineservice@springernature.com

PARTE III

3 DISCUSSÃO

Nesta tese, investigamos os efeitos do uso abusivo de EAA sobre o SNC de camundongos, em dois contextos experimentais complementares. No primeiro, estabelecemos um regime de administração diária de ND (15 mg/kg), por 19 dias, um protocolo reconhecidamente associado ao surgimento de comportamento agressivo em roedores (Elfverson et al., 2011; Kalinine et al., 2014; Le Grevès et al., 1997; Rossbach et al., 2007). Buscamos reproduzir esse fenótipo e propor um mecanismo baseado na disfunção da cooperação neurônio–astrócito, associando a excitotoxicidade glutamatérgica à bioenergética mitocondrial sináptica. Após o tratamento, os animais foram submetidos a testes comportamentais e análises bioquímicas, incluindo avaliações detalhadas do perfil bioenergético de mitocôndrias sinápticas.

No segundo contexto, empregamos o mesmo regime de tratamento com ND, seguido da indução de TCE por ICC, 48 horas após o término da exposição. A escolha deste modelo baseou-se na observação de que o abuso de EAA, frequentemente associado a comportamento impulsivo e violento (Chegeni et al., 2021; Hauger et al., 2021; Lundholm et al., 2015), aumenta a probabilidade de envolvimento em situações de risco e, portanto, a incidência de TCE (Farrer et al., 2012; Mosti & Coccaro, 2018). Assim, investigamos se o uso prévio de ND influencia a vulnerabilidade e o curso do dano secundário pós-traumático, com ênfase na bioenergética mitocondrial, neurodegeneração e desempenho cognitivo. Por fim, uma revisão de literatura publicada como capítulo de livro abordou os efeitos do abuso de EAA sobre a função mitocondrial cerebral, destacando as diferenças entre mitocôndrias sinápticas e extrasinápticas.

No Capítulo 1, introduzimos o conceito de heterogeneidade mitocondrial dependente da localização espacial na sinapse tripartite e sua relevância para a neurotransmissão. Evidências de proteômica e bioenergética demonstram que mitocôndrias sinápticas, predominantemente neuronais, apresentam alto acoplamento respiratório e respondem rapidamente à demanda energética durante a neurotransmissão, mas são mais suscetíveis à despolarização e falhas oxidativas. Em contraste, mitocôndrias extrasinápticas, derivadas majoritariamente de células gliais, exibem metabolismo mais flexível, maior resistência a flutuações energéticas e sistemas antioxidantes mais robustos (Garone et al., 2024; Graham et al., 2017b; Stauch et al., 2014). Essa heterogeneidade funcional torna o tecido cerebral particularmente vulnerável a insultos que alteram o manejo de Ca^{2+} e o equilíbrio redox, aspectos centrais na excitotoxicidade e disfunção mitocondrial (Cabral-Costa & Kowaltowski, 2023; Datta & Jaiswal, 2021). O desenho experimental adotado nos capítulos subsequentes concentra-se em preparações de

sinaptossomas, que refletem predominantemente eventos dos compartimentos pré e pós-sinápticos.

No Capítulo 2, demonstramos que o tratamento prolongado com ND (15 mg/kg/dia) induziu comportamento agressivo e impulsividade em camundongos machos, sem alterar a atividade locomotora global, indicando disfunção de circuitos cortico-límbicos específicos responsáveis pela regulação emocional e social. Paralelamente, observamos redução expressiva do GLT-1 no córtex pré-frontal e aumento dos níveis de glutamato e aspartato no líquor, indicando falha na depuração glutamatérgica e consequente hiperativação de receptores NMDA. Esse mecanismo foi corroborado pelo aumento da fosforilação da subunidade GluN2B (pGluN2B^{Tyr1472}), um marcador de excitotoxicidade.

Nas mitocôndrias sinápticas, a ND induziu alterações bioenergéticas compatíveis com sobrecarga de Ca^{2+}: inchaço mitocondrial, redução da Ca^{2+}-ATPase, prejuízo na atividade de desidrogenases e desacoplamento respiratório. O consumo de O_2 acoplado ao complexo II (succinato desidrogenase) foi reduzido, acompanhado por aumento da produção de H_2O_2 e perda do $\Delta\Psi$, sugerindo colapso energético e estresse oxidativo sináptico.

A coadministração de CEF, um indutor farmacológico de GLT-1, restaurou os níveis do transportador, normalizou a homeostase glutamatérgica e preveniu completamente o comportamento agressivo induzido pela ND. Além disso, a CEF preservou a integridade mitocondrial e reduziu a geração de ERO, reforçando a importância da regulação astroglial na manutenção da função sináptica e da estabilidade bioenergética. Esses achados revelam um eixo funcional GLT-1 → excitação glutamatérgica → disfunção mitocondrial → agressividade, que conecta disfunções metabólicas a alterações comportamentais induzidas por EAA.

No Capítulo 3, avaliamos o impacto da exposição prévia à ND sobre a fisiopatologia pós-TCE. O TCE, uma das principais causas de morbimortalidade global, desencadeia cascatas de dano secundário envolvendo excitotoxicidade, inflamação, disfunção mitocondrial e estresse do retículo endoplasmático. Surpreendentemente, a exposição prévia à ND atenuou a resposta degenerativa pós-TCE: os animais tratados apresentaram menores níveis de APP, $A\beta_{42}$ e tau fosforilada em comparação aos controles lesionados, além de reduzida ativação de marcadores de estresse do retículo (GRP78, ATF6, CHOP).

Nas análises mitocondriais, a ND preservou o efluxo de Ca^{2+} mediado por NCLX, reduziu a produção de H_2O_2 e manteve o $\Delta\Psi$ e a eficiência de acoplamento bioenergético (BCE). Também observamos manutenção parcial da biogênese mitocondrial, com níveis preservados de PGC-1α e TOM20. No teste do labirinto aquático de Morris, os animais

ND/TCE exibiram desempenho cognitivo semelhante aos do controle, enquanto o grupo veículo/TCE apresentou déficit acentuado de memória espacial.

Esses resultados sugerem que a exposição prévia à ND induziu um estado de pré-condicionamento mitocondrial, promovendo adaptação bioenergética e resistência ao estresse oxidativo subsequente ao trauma. A ativação de vias regulatórias, como CREB e PGC-1α, pode ter contribuído para o aumento da plasticidade mitocondrial e da eficiência respiratória. Tal fenômeno paradoxal, em que um agente neurotóxico em condições basais confere neuroproteção sob estresse, ressalta conceitualmente que estímulos potencialmente deletérios poderiam desencadear respostas adaptativas citoprotetoras (Calabrese, 2008).

Assim, o ND apresenta uma dualidade funcional no SNC: em condições fisiológicas, promove disfunção glial, excitotoxicidade e estresse oxidativo; porém, frente ao insulto traumático, pode desencadear respostas compensatórias que limitam a neurodegeneração e preservam a função mitocondrial. Essa ambivalência é comparável à metáfora de “Dr. Jekyll e Mr. Hyde”, representando a coexistência de efeitos neurotóxicos e neuroprotetores dependentes do contexto fisiológico.

A elucidação dos mecanismos que determinam essa bifurcação funcional, incluindo a dosagem, o tempo de exposição e a interação com receptores androgênio e estrogênio, é essencial para compreender o papel da sinalização esteroidal na plasticidade neuronal e na bioenergética cerebral. Embora os EAA apresentem risco elevado de dependência, disfunções cardiovasculares e transtornos de humor, os resultados desta tese sugerem que o sistema mitocondrial pode ser um ponto de convergência entre seus efeitos deletérios e protetores, abrindo perspectivas para abordagens terapêuticas seletivas baseadas em moduladores da biogênese mitocondrial ou da homeostase de Ca^{2+}.

4 CONCLUSÃO

Os resultados desta tese demonstram que o abuso de EAA induz disfunções glutamatérgicas e mitocondriais que explicam, em parte, o comportamento agressivo e impulsivo. Ainda, revelamos que o GLT-1, é um componente astrocitário relevante para o comportamento agressivo. Por outro lado, nossos resultados evidenciam que a exposição prévia à ND pode conferir resistência mitocondrial ao dano e atenuar os mecanismos neurodegenerativos associados a um TCE.

Em condições fisiológicas, a ND promove excitotoxicidade, sobrecarga mitocondrial de Ca^{2+}, estresse oxidativo e colapso bioenergético. No entanto, frente ao TCE, desencadeia adaptações citoprotetoras associadas à preservação da biogênese mitocondrial e da homeostase do retículo endoplasmático. Assim, os EAA modulam a vulnerabilidade cerebral de forma bifásica e dependente do contexto, destacando a mitocôndria e o retículo endoplasmático como elementos centrais dessa dualidade. Assim, os EAA exibem um comportamento antagônico, análogo ao paradoxo de "Dr. Jekyll and Mr. Hyde". Desvendar essas características é fundamental para delimitar a fronteira entre os efeitos neurotóxicos e neuroprotetores dos EAA.

5 REFERÊNCIAS

AHMAD, M.; WOLBERG, A.; KAHWAJI, C. I. Biochemistry, Electron Transport Chain. In: StatPearls. **StatPearls Publishing**, 2025. Disponível em: http://www.ncbi.nlm.nih.gov/books/NBK526105/.

ALABDULADHEM, T. O.; BORDONI, B. Physiology, Krebs Cycle. In: StatPearls. **StatPearls Publishing**, 2025. Disponível em: http://www.ncbi.nlm.nih.gov/books/NBK556032/.

ALBANO, G. D. et al. Adverse Effects of Anabolic-Androgenic Steroids: A Literature Review. **Healthcare**, v. 9, n. 1, p. 97, 2021. DOI: https://doi.org/10.3390/healthcare9010097.

AL-DORZI, H. M.; ARABI, Y. M. Nutrition support for critically ill patients. **JPEN Journal of Parenteral and Enteral Nutrition**, v. 45, supl. 2, p. 47–59, 2021. DOI: https://doi.org/10.1002/jpen.2228.

ALTAŞ, M. et al. Effects of ceftriaxone on ischemia/reperfusion injury in rat brain. **Journal of Clinical Neuroscience**, v. 20, n. 3, p. 457–461, 2013. DOI: https://doi.org/10.1016/j.jocn.2012.05.030.

AMBAR, G.; CHIAVEGATTO, S. Anabolic-androgenic steroid treatment induces behavioral disinhibition... **Genes, Brain and Behavior**, v. 8, n. 2, p. 161–173, 2009. DOI: https://doi.org/10.1111/j.1601-183X.2008.00458.x.

ANAWALT, B. D. Diagnosis and Management of Anabolic Androgenic Steroid Use. **The Journal of Clinical Endocrinology and Metabolism**, v. 104, n. 7, p. 2490–2500, 2019. DOI: https://doi.org/10.1210/jc.2018-01882.

ANDROGENIC STEROIDS. In: LiverTox. National Institute of Diabetes and Digestive and Kidney Diseases, 2012. Disponível em: http://www.ncbi.nlm.nih.gov/books/NBK548931/.

ANGELOVA, P. R.; ABRAMOV, A. Y. Interplay of mitochondrial calcium signalling and reactive oxygen species. **Biochemical Society Transactions**, v. 52, n. 4, p. 1939–1946, 2024. DOI: https://doi.org/10.1042/BST20240261.

ARAZI, H.; MOHAMMADJAFARI, H.; ASADI, A. Use of anabolic androgenic steroids produces greater oxidative stress. **Toxicology Reports**, v. 4, p. 282–286, 2017. DOI: https://doi.org/10.1016/j.toxrep.2017.05.005.

ARSLAN, N.; ERDUR, B.; AYDIN, A. Hormones and cytokines in childhood obesity. **Indian Pediatrics**, v. 47, n. 10, p. 829–839, 2010. DOI: https://doi.org/10.1007/s13312-010-0142-y.

ATKOVSKA, K. et al. Rationalizing Steroid Interactions with Lipid Membranes. **ACS Central Science**, v. 4, n. 9, p. 1155–1165, 2018. DOI: https://doi.org/10.1021/acscentsci.8b00332.

ATWI, S. et al. Androgen Modulation of Hippocampal Structure and Function. **The Neuroscientist**, v. 22, n. 1, p. 46–60, 2016. DOI: https://doi.org/10.1177/1073858414558065.

BAGGISH, A. L. et al. Cardiovascular Toxicity of Illicit Anabolic-Androgenic Steroid Use. **Circulation**, v. 135, n. 21, p. 1991–2002, 2017. DOI: https://doi.org/10.1161/CIRCULATIONAHA.116.026945.

BAHRKE, M. S.; YESALIS, C. E. Abuse of anabolic androgenic steroids in sport. **Current Opinion in Pharmacology**, v. 4, n. 6, p. 614–620, 2004. DOI: https://doi.org/10.1016/j.coph.2004.05.006.

BARTON, D. J. et al. Persistent Hypogonadotropic Hypogonadism After TBI. **Journal of Head Trauma Rehabilitation**, v. 31, n. 4, p. 277–287, 2016. DOI: https://doi.org/10.1097/HTR.0000000000000188.

BARTSCH, W.; KRIEG, M.; VOIGT, K. D. Quantification of endogenous testosterone… **Journal of Steroid Biochemistry**, v. 13, n. 3, p. 259–264, 1980. DOI: https://doi.org/10.1016/0022-4731(80)90003-5.

BASARIA, S.; WAHLSTROM, J. T.; DOBS, A. S. Anabolic-androgenic steroid therapy. **JCEM**, v. 86, n. 11, p. 5108–5117, 2001. DOI: https://doi.org/10.1210/jcem.86.11.7983.

BASILE, J. R. et al. Supraphysiological doses of anabolic steroids exert toxic effects. **Frontiers in Cellular Neuroscience**, v. 7, p. 69, 2013. DOI: https://doi.org/10.3389/fncel.2013.00069.

BÁTHORI, M. et al. Phytoecdysteroids and anabolic-androgenic steroids. **Current Medicinal Chemistry**, v. 15, n. 1, p. 75–91, 2008. DOI: https://doi.org/10.2174/092986708783330674.

BAXTER, P. S.; HARDINGHAM, G. E. Adaptive regulation of antioxidant defences. **Free Radical Biology & Medicine**, v. 100, p. 147–152, 2016. DOI: https://doi.org/10.1016/j.freeradbiomed.2016.06.027.

BEN-SHACHAR, D.; ENE, H. M. Mitochondrial Targeted Therapies. **Biological Psychiatry**, v. 83, n. 9, p. 770–779, 2018. DOI: https://doi.org/10.1016/j.biopsych.2017.08.007.

BHASIN, S. et al. Testosterone Therapy in Men With Hypogonadism. **JCEM**, v. 103, n. 5, p. 1715–1744, 2018. DOI: https://doi.org/10.1210/jc.2018-00229.

BHASIN, S. et al. Testosterone therapy in men with androgen deficiency. **JCEM**, v. 95, n. 6, p. 2536–2559, 2010. DOI: https://doi.org/10.1210/jc.2009-2354.

BILOTTA, F. et al. Glycemia management in neurocritical care. **Journal of Neurosurgical Anesthesiology**, v. 21, n. 1, p. 2–9, 2009. DOI: https://doi.org/10.1097/ANA.0b013e31818f8a5c.

BJØRNEBEKK, A. et al. Long-term AAS Use Is Associated With Deviant Brain Aging. **Biological Psychiatry: Cognitive Neuroscience and Neuroimaging**, v. 6, n. 5, p. 579–589, 2021. DOI: https://doi.org/10.1016/j.bpsc.2021.01.001.

BOLAÑOS, J. P. Bioenergetics and redox adaptations of astrocytes. **Journal of Neurochemistry**, v. 139, supl. 2, p. 115–125, 2016. DOI: https://doi.org/10.1111/jnc.13486.

BOND, P.; LLEWELLYN, W.; VAN MOL, P. AAS-induced hepatotoxicity. **Medical Hypotheses**, v. 93, p. 150–153, 2016. DOI: https://doi.org/10.1016/j.mehy.2016.06.004.

BOND, P.; SMIT, D. L.; DE RONDE, W. AAS: mechanisms and risks. **Frontiers in Endocrinology**, v. 13, 1059473, 2022. DOI: https://doi.org/10.3389/fendo.2022.1059473.

BRAMLETT, H. M.; DIETRICH, W. D. Long-term consequences of TBI. **Journal of Neurotrauma**, v. 32, n. 23, p. 1834–1848, 2015. DOI: https://doi.org/10.1089/neu.2014.3352.

BRINI, M.; CARAFOLI, E. PMCA and NCX in cell calcium regulation. **Cold Spring Harbor Perspectives in Biology**, v. 3, n. 2, a004168, 2011. DOI: https://doi.org/10.1101/cshperspect.a004168.

BROWN, M. R.; SULLIVAN, P. G.; GEDDES, J. W. Synaptic mitochondria susceptibility to Ca2+. **The Journal of Biological Chemistry**, v. 281, n. 17, p. 11658–11668, 2006. DOI: https://doi.org/10.1074/jbc.M510303200.

BRYANT, A. M. et al. Cognitive functioning 6 months after TBI. **JAMA Network Open**, v. 6, n. 12, e2349118, 2023. DOI: https://doi.org/10.1001/jamanetworkopen.2023.49118.

BUCHNER, J. Hsp90 & Co. **Trends in Biochemical Sciences**, v. 24, n. 4, p. 136–141, 1999. DOI: https://doi.org/10.1016/s0968-0004(99)01373-0.

BUSARDÒ, F. P. et al. Impact of nandrolone decanoate on CNS. **Current Neuropharmacology**, v. 13, n. 1, p. 122–131, 2015. DOI: https://doi.org/10.2174/1570159X13666141210225822.

BUTLER, M. L. M. D. et al. Repeated head trauma causes neuron loss. **Nature**, p. 1–10, 2025. DOI: https://doi.org/10.1038/s41586-025-09534-6.

CABRAL-COSTA, J. V.; KOWALTOWSKI, A. J. Mitochondrial Ca2+ handling as a cell signaling hub: Lessons from astrocyte function. **Essays in Biochemistry**, v. 67, n. 1, p. 63–75, 2023. DOI: https://doi.org/10.1042/EBC20220094.

CALFEE, R.; FADALE, P. Popular ergogenic drugs and supplements in young athletes. **Pediatrics**, v. 117, n. 3, p. e577–589, 2006. DOI: https://doi.org/10.1542/peds.2005-1429.

CALÌ, T.; OTTOLINI, D.; BRINI, M. Mitochondrial Ca(2+) and neurodegeneration. **Cell Calcium**, v. 52, n. 1, p. 73–85, 2012. DOI: https://doi.org/10.1016/j.ceca.2012.04.015.

CAMPOS, F. M.; SUASSUNA, T. M.; VIETTA, G. G. Tendência temporal de internação por traumatismo cranioencefálico no Brasil entre 2008-2020. **Repositório Ânima**, 2023. Disponível em: https://repositorio.animaeducacao.com.br/handle/ANIMA/34135.

CARTERI, R. B. K.; SILVA, R. A. da. Incidência hospitalar de traumatismo craniencefálico no Brasil: Uma análise dos últimos 10 anos. **Revista Brasileira de Terapia Intensiva**, v. 33, p. 282–289, 2021. DOI: https://doi.org/10.5935/0103-507X.20210036.

CARTERI, R. B. et al. Anabolic-androgen steroids effects on bioenergetics responsiveness of synaptic and extrasynaptic mitochondria. **Toxicology Letters**, v. 307, p. 72–80, 2019a. DOI: https://doi.org/10.1016/j.toxlet.2019.03.004.

CARTERI, R. B. et al. Anabolic-androgen steroids effects on bioenergetics responsiveness of synaptic and extrasynaptic mitochondria. **Toxicology Letters**, v. 307, p. 72–80, 2019b. DOI: https://doi.org/10.1016/j.toxlet.2019.03.004.

CARTERI, R. B. et al. Testosterone Administration after Traumatic Brain Injury Reduces Mitochondrial Dysfunction and Neurodegeneration. **Journal of Neurotrauma**, v. 36, n. 14, p. 2246–2259, 2019. DOI: https://doi.org/10.1089/neu.2018.6266.

CARTERI, R. B. et al. Anabolic-androgenic steroids impair mitochondrial function and redox status in the heart and liver of mice. **Steroids**, v. 172, p. 108861, 2021. DOI: https://doi.org/10.1016/j.steroids.2021.108861.

CELOTTI, F.; NEGRI CESI, P. Anabolic steroids: A review of their effects on the muscles, of their possible mechanisms of action and of their use in athletics. **The Journal of Steroid Biochemistry and Molecular Biology**, v. 43, n. 5, p. 469–477, 1992. DOI: https://doi.org/10.1016/0960-0760(92)90085-w.

CHANDEL, N. S. Mitochondria as signaling organelles. **BMC Biology**, v. 12, p. 34, 2014. DOI: https://doi.org/10.1186/1741-7007-12-34.

CHANDEL, N. S. Evolution of Mitochondria as Signaling Organelles. **Cell Metabolism**, v. 22, n. 2, p. 204–206, 2015. DOI: https://doi.org/10.1016/j.cmet.2015.05.013.

CHEGENI, R. et al. Anabolic-androgenic steroid administration increases self-reported aggression in healthy males: A systematic review and meta-analysis of experimental studies. **Psychopharmacology**, v. 238, n. 7, p. 1911–1922, 2021. DOI: https://doi.org/10.1007/s00213-021-05818-7.

CHEN, L. et al. Mitochondrial heterogeneity in diseases. **Signal Transduction and Targeted Therapy**, v. 8, n. 1, p. 311, 2023. DOI: https://doi.org/10.1038/s41392-023-01546-w.

CHEN, Q.; LIN, R. Y.; RUBIN, C. S. Organelle-specific targeting of protein kinase AII (PKAII)... **The Journal of Biological Chemistry**, v. 272, n. 24, p. 15247–15257, 1997. DOI: https://doi.org/10.1074/jbc.272.24.15247.

CHEN, Y. et al. The role of astrocytes in oxidative stress of central nervous system: A mixed blessing. **Cell Proliferation**, v. 53, n. 3, e12781, 2020. DOI: https://doi.org/10.1111/cpr.12781.

CHENG, G.; KONG, R.; ZHANG, L.; ZHANG, J. Mitochondria in traumatic brain injury and mitochondrial-targeted multipotential therapeutic strategies. **British Journal of Pharmacology**, v. 167, n. 4, p. 699–719, 2012. DOI: https://doi.org/10.1111/j.1476-5381.2012.02025.x.

CHERRIER, M. M. et al. The role of aromatization in testosterone supplementation: Effects on cognition in older men. **Neurology**, v. 64, n. 2, p. 290–296, 2005. DOI: https://doi.org/10.1212/01.WNL.0000149639.25136.CA.

CHOI, P. Y.; POPE, H. G. Violence toward women and illicit androgenic-anabolic steroid use. **Annals of Clinical Psychiatry**, v. 6, n. 1, p. 21–25, 1994a. DOI: https://doi.org/10.3109/10401239409148835.

CHOI, P. Y.; POPE, H. G. Violence toward women and illicit androgenic-anabolic steroid use. **Annals of Clinical Psychiatry**, v. 6, n. 1, p. 21–25, 1994b. DOI: https://doi.org/10.3109/10401239409148835.

CINI, N. et al. Ceftriaxone has a neuroprotective effect... **Strahlentherapie und Onkologie**, v. 201, n. 9, p. 903–919, 2025. DOI: https://doi.org/10.1007/s00066-025-02405-z.

COGLIATI, S.; ENRIQUEZ, J. A.; SCORRANO, L. Mitochondrial Cristae: Where Beauty Meets Functionality. **Trends in Biochemical Sciences**, v. 41, n. 3, p. 261–273, 2016. DOI: https://doi.org/10.1016/j.tibs.2016.01.001.

COGLIATI, S. et al. Mitochondrial cristae shape determines respiratory chain supercomplexes assembly... **Cell**, v. 155, n. 1, p. 160–171, 2013. DOI: https://doi.org/10.1016/j.cell.2013.08.032.

COOPER, G. M. The Mechanism of Oxidative Phosphorylation. Em: **The Cell: A Molecular Approach**. 2. ed. Sinauer Associates, 2000. Disponível em: https://www.ncbi.nlm.nih.gov/books/NBK9885/.

COSTA, D. G. A. da et al. Análise epidemiológico da vítima de traumatismo intracraniano... **Brazilian Journal of Implantology and Health Sciences**, v. 6, n. 1, p. 81–90, 2024. DOI: https://doi.org/10.36557/2674-8169.2024v6n1p81-90.

COVIELLO, A. D. et al. Effects of graded doses of testosterone on erythropoiesis... **The Journal of Clinical Endocrinology and Metabolism**, v. 93, n. 3, p. 914–919, 2008. DOI: https://doi.org/10.1210/jc.2007-1692.

CRAWFORD, J. Clinical results in cachexia therapeutics. **Current Opinion in Clinical Nutrition and Metabolic Care**, v. 19, n. 3, p. 199–204, 2016. DOI: https://doi.org/10.1097/MCO.0000000000000274.

CROWLEY, J.; WITHANA, M.; DEPLAZES, E. The interaction of steroids with phospholipid bilayers and membranes. **Biophysical Reviews**, v. 14, n. 1, p. 163–179, 2022. DOI: https://doi.org/10.1007/s12551-021-00918-2.

CUNNINGHAM, G. R.; SILVERMAN, V. E.; THORNBY, J.; KOHLER, P. O. The potential for an androgen male contraceptive. **The Journal of Clinical Endocrinology and Metabolism**, v. 49, n. 4, p. 520–526, 1979. DOI: 10.1210/jcem-49-4-520.

CUSSONS, A. J.; BHAGAT, C. I.; FLETCHER, S. J.; WALSH, J. P. Brown-Séquard revisited: A lesson from history on the placebo effect of androgen treatment. **The Medical Journal of Australia**, v. 177, n. 11–12, p. 678–679, 2002. DOI: https://doi.org/10.5694/j.1326-5377.2002.tb05014.x.

DAL PIZZOL, T. da S.; BRANCO, M. M. N.; CARVALHO, R. M. A. de; PASQUALOTTI, A.; MACIEL, E. N.; MIGOTT, A. M. B. Non-medical use of psychoactive medicines among elementary and high school students in Southern Brazil. **Cadernos de Saúde Pública**, v. 22, n. 1, p. 109–115, 2006. DOI: https://doi.org/10.1590/s0102-311x2006000100012.

DALTON, J. T. et al. The selective androgen receptor modulator GTx-024 (enobosarm) improves lean body mass and physical function in healthy elderly men and postmenopausal women: results of a double-blind, placebo-controlled phase II trial. **Journal of Cachexia, Sarcopenia and Muscle**, v. 2, n. 3, p. 153–161, 2011. DOI: https://doi.org/10.1007/s13539-011-0034-6.

DANESHVAR, D. H. et al. Post-traumatic neurodegeneration and chronic traumatic encephalopathy. **Molecular and Cellular Neurosciences**, v. 66, Pt B, p. 81–90, 2015a. DOI: https://doi.org/10.1016/j.mcn.2015.03.007.

DASH, P. K.; ZHAO, J.; HERGENROEDER, G.; MOORE, A. N. Biomarkers for the diagnosis, prognosis, and evaluation of treatment efficacy for traumatic brain injury. **Neurotherapeutics**, v. 7, n. 1, p. 100–114, 2010. DOI: https://doi.org/10.1016/j.nurt.2009.10.019.

DATTA, S.; JAISWAL, M. Mitochondrial calcium at the synapse. **Mitochondrion**, v. 59, p. 135–153, 2021. DOI: https://doi.org/10.1016/j.mito.2021.04.006.

DAVIS, S. R. et al. Tibolone was associated with an increase in 7 alpha-methyl-ethinylestradiol. **Menopause**, v. 10, n. 1, p. 105–106, 2003. DOI: https://doi.org/10.1097/00042192-200310010-00018.

DE GOOYER, M. E. et al. Tibolone is not converted by human aromatase to 7alpha-methyl-17alpha-ethynylestradiol (7alpha-MEE): analyses with sensitive bioassays for estrogens and androgens and with LC-MSMS. **Steroids**, v. 68, n. 3, p. 235–243, 2003. DOI: https://doi.org/10.1016/s0039-128x(02)00184-8.

DEMLING, R. H.; DESANTI, L. Oxandrolone, an anabolic steroid, significantly increases the rate of weight gain in the recovery phase after major burns. **The Journal of Trauma**, v. 43, n. 1, p. 47–51, 1997. DOI: https://doi.org/10.1097/00005373-199707000-00012.

DEMLING, R. H.; DESANTI, L. The rate of restoration of body weight after burn injury, using the anabolic agent oxandrolone, is not age dependent. **Burns**, v. 27, n. 1, p. 46–51, 2001. DOI: https://doi.org/10.1016/s0305-4179(00)00064-4.

D'ERRICO, S. et al. Cardiac β1-adrenoceptor expression in two stress-induced cardiomyopathy-related deaths. **Forensic Science International**, v. 207, n. 1–3, p. e8–e11, 2011. DOI: https://doi.org/10.1016/j.forsciint.2010.11.007.

DESHPANDE, O. A.; MOHIUDDIN, S. S. Biochemistry, Oxidative Phosphorylation. In: **StatPearls**. Treasure Island: StatPearls Publishing, 2025.

DEWAN, M. C. et al. Estimating the global incidence of traumatic brain injury. **Journal of Neurosurgery**, v. 130, n. 4, p. 1080–1097, 2019. DOI: https://doi.org/10.3171/2017.10.JNS17352.

DI LISA, F.; BERNARDI, P. Mitochondrial function as a determinant of recovery or death in cell response to injury. **Molecular and Cellular Biochemistry**, v. 184, n. 1–2, p. 379–391, 1998.

DIAS, M. S.; GUERRA, H. S. Perfil epidemiológico dos pacientes internados por traumatismo cranioencefálico na região Centro-Oeste do Brasil. **Revista Científica da Escola Estadual de Saúde Pública de Goiás "Cândido Santiago"**, v. 10, p. 1–6, 2024. DOI: https://doi.org/10.22491/2447-3405.2024.756.

DIMEO, A. N.; WOOD, R. I. Self-administration of estrogen and dihydrotestosterone in male hamsters. **Hormones and Behavior**, v. 49, n. 4, p. 519–526, 2006. DOI: https://doi.org/10.1016/j.yhbeh.2005.11.003.

DING, J. B. et al. Anabolic-Androgenic Steroid Misuse: Mechanisms, Patterns of Misuse, User Typology, and Adverse Effects. **Journal of Sports Medicine**, 2021. DOI: https://doi.org/10.1155/2021/7497346.

DOTSON, J. L.; BROWN, R. T. The history of the development of anabolic-androgenic steroids. **Pediatric Clinics of North America**, v. 54, n. 4, p. 761–769, 2007. DOI: https://doi.org/10.1016/j.pcl.2007.04.003.

DU TOIT, E. F.; ROSSOUW, E.; VAN ROOYEN, J.; LOCHNER, A. Proposed mechanisms for the anabolic steroid-induced increase in myocardial susceptibility to ischaemia/reperfusion injury. **Cardiovascular Journal of South Africa**, v. 16, n. 1, p. 21–28, 2005.

DUBOIS, V. et al. Androgens and skeletal muscle: cellular and molecular action mechanisms underlying the anabolic actions. **Cellular and Molecular Life Sciences**, v. 69, n. 10, p. 1651–1667, 2012. DOI: https://doi.org/10.1007/s00018-011-0883-3.

DUCHEN, M. R. Mitochondria, calcium-dependent neuronal death and neurodegenerative disease. **Pflugers Archiv – European Journal of Physiology**, v. 464, n. 1, p. 111–121, 2012. DOI: https://doi.org/10.1007/s00424-012-1112-0.

ELFVERSON, M.; JOHANSSON, T.; ZHOU, Q.; LE GREVÈS, P.; NYBERG, F. Chronic administration of the anabolic androgenic steroid nandrolone alters neurosteroid action at the sigma-1 receptor but not at the sigma-2 or NMDA receptors. **Neuropharmacology**, v. 61, n. 7, p. 1172–1181, 2011. DOI: https://doi.org/10.1016/j.neuropharm.2011.01.005.

EL-HATTAB, A. W.; ZARANTE, A. M.; ALMANNAI, M.; SCAGLIA, F. Therapies for mitochondrial diseases and current clinical trials. **Molecular Genetics and Metabolism**, v. 122, n. 3, p. 1–9, 2017. DOI: https://doi.org/10.1016/j.ymgme.2017.09.009.

ELLGAARD, L.; HELENIUS, A. Quality control in the endoplasmic reticulum. **Nature Reviews Molecular Cell Biology**, v. 4, n. 3, p. 181–191, 2003. DOI: https://doi.org/10.1038/nrm1052.

EMANI, S. M. et al. Autologous mitochondrial transplantation for dysfunction after ischemia-reperfusion injury. **The Journal of Thoracic and Cardiovascular Surgery**, v. 154, n. 1, p. 286–289, 2017. DOI: https://doi.org/10.1016/j.jtcvs.2017.02.018.

EMRICH, S. M. et al. The mitochondrial sodium/calcium exchanger NCLX (Slc8b1) in B lymphocytes. **Cell Calcium**, v. 108, p. 102667, 2022. DOI: https://doi.org/10.1016/j.ceca.2022.102667.

ERNSTER, L.; SCHATZ, G. Mitochondria: a historical review. **The Journal of Cell Biology**, v. 91, n. 3 Pt 2, p. 227s–255s, 1981. DOI: https://doi.org/10.1083/jcb.91.3.227s.

FADAH, K. et al. Anabolic androgenic steroids and cardiomyopathy: an update. **Frontiers in Cardiovascular Medicine**, v. 10, p. 1214374, 2023. DOI: https://doi.org/10.3389/fcvm.2023.1214374.

FALKENSTEIN, E. et al. Multiple actions of steroid hormones—A focus on rapid, nongenomic effects. **Pharmacological Reviews**, v. 52, n. 4, p. 513–556, 2000.

FAN, M. et al. Structure and mechanism of the mitochondrial calcium transporter NCLX. **Nature**, v. 646, n. 8087, p. 1272–1280, 2025. DOI: https://doi.org/10.1038/s41586-025-09491-0.

FARRER, T. J.; FROST, R. B.; HEDGES, D. W. Prevalence of traumatic brain injury in intimate partner violence offenders compared to the general population: a meta-analysis. **Trauma, Violence, & Abuse**, v. 13, n. 2, p. 77–82, 2012. DOI: https://doi.org/10.1177/1524838012440338.

FERRANDO, A. A. et al. Testosterone administration to older men improves muscle function: molecular and physiological mechanisms. **American Journal of Physiology – Endocrinology and Metabolism**, v. 282, n. 3, p. E601–E607, 2002. DOI: https://doi.org/10.1152/ajpendo.00362.2001.

FESHARAKI-ZADEH, A. Oxidative stress in traumatic brain injury. **International Journal of Molecular Sciences**, v. 23, n. 21, p. 13000, 2022. DOI: 10.3390/ijms232113000.

FILIPOVIĆ, D.; TURCK, C. W. Proteomic signatures of hippocampal nonsynaptic and synaptosome-enriched mitochondria in rats resilient to chronic social isolation. **Biomolecules**, v. 15, n. 10, p. 1358, 2025. DOI: https://doi.org/10.3390/biom15101358.

FINKEL, T. From sulfenylation to sulfhydration: what a thiolate needs to tolerate. **Science Signaling**, v. 5, n. 215, p. pe10, 2012. DOI: https://doi.org/10.1126/scisignal.2002943.

FINKELSTEIN, M. et al. Comparative studies of the aromatization of testosterone and epitestosterone by human placental aromatase. **Endocrinology**, v. 108, n. 3, p. 943–947, 1981. DOI: https://doi.org/10.1210/endo-108-3-943.

FISCHER, S. G.; RICCI, L. A.; MELLONI, R. H. Repeated anabolic/androgenic steroid exposure during adolescence alters phosphate-activated glutaminase and glutamate receptor 1 (GluR1) subunit immunoreactivity in hamster brain: correlation with offensive aggression. **Behavioural Brain Research**, v. 180, n. 1, p. 77–85, 2007. DOI: https://doi.org/10.1016/j.bbr.2007.02.025.

FISCHER, T. D. et al. Altered mitochondrial dynamics and TBI pathophysiology. **Frontiers in Systems Neuroscience**, v. 10, p. 29, 2016. DOI: https://doi.org/10.3389/fnsys.2016.00029.

FLIPPO, K. H.; STRACK, S. Mitochondrial dynamics in neuronal injury, development and plasticity. **Journal of Cell Science**, v. 130, n. 4, p. 671–681, 2017. DOI: https://doi.org/10.1242/jcs.171017.

FONSÊCA, M. M. da et al. Hospitalizações por traumatismo cranioencefálico em crianças e adolescentes no estado da Bahia, 2008 a 2017. **Revista de Ciências Médicas e Biológicas**, v. 23, n. 1, p. 121–125, 2024. DOI: https://doi.org/10.9771/cmbio.v23i1.48571.

FORADORI, C. D.; WEISER, M. J.; HANDA, R. J. Non-genomic actions of androgens. **Frontiers in Neuroendocrinology**, v. 29, n. 2, p. 169–181, 2008. DOI: https://doi.org/10.1016/j.yfrne.2007.10.005.

FORSDAHL, G. et al. Detection of testosterone esters in blood. **Drug Testing and Analysis**, v. 7, n. 11–12, p. 983–989, 2015. DOI: https://doi.org/10.1002/dta.1914.

FRAGKAKI, A. G. et al. Structural characteristics of anabolic androgenic steroids contributing to binding to the androgen receptor and to their anabolic and androgenic activities: applied modifications in the steroidal structure. **Steroids**, v. 74, n. 2, p. 172–197, 2009. DOI: https://doi.org/10.1016/j.steroids.2008.10.016.

FRANKENFIELD, D.; SMITH, J. S.; COONEY, R. N. Validation of 2 approaches to predicting resting metabolic rate in critically ill patients. **JPEN – Journal of Parenteral and Enteral Nutrition**, v. 28, n. 4, p. 259–264, 2004. DOI: https://doi.org/10.1177/0148607104028004259.

FRATI, P. et al. Anabolic androgenic steroid (AAS) related deaths: autoptic, histopathological and toxicological findings. **Current Neuropharmacology**, v. 13, n. 1, p. 146–159, 2015. DOI: https://doi.org/10.2174/1570159X13666141210225414.

FREEMAN, E. R.; BLOOM, D. A.; McGUIRE, E. J. A brief history of testosterone. **The Journal of Urology**, v. 165, n. 2, p. 371–373, 2001. DOI: https://doi.org/10.1097/00005392-200102000-00004.

FREY, T. G.; MANNELLA, C. A. The internal structure of mitochondria. **Trends in Biochemical Sciences**, v. 25, n. 7, p. 319–324, 2000. DOI: https://doi.org/10.1016/s0968-0004(00)01609-1.

GARONE, C.; DE GIORGIO, F.; CARLI, S. Mitochondrial metabolism in neural stem cells and implications for neurodevelopmental and neurodegenerative diseases. **Journal of Translational Medicine**, v. 22, n. 1, p. 238, 2024. DOI: https://doi.org/10.1186/s12967-024-05041-w.

GAUGHAN, W. J.; LISS, K. A.; DUNN, S. R.; MANGOLD, A. M.; BUHSMER, J. P.; MICHAEL, B.; BURKE, J. F. A 6-month study of low-dose recombinant human erythropoietin alone and in combination with androgens for the treatment of anemia in chronic hemodialysis patients. **American Journal of Kidney Diseases**, v. 30, n. 4, p. 495–500, 1997. DOI: https://doi.org/10.1016/s0272-6386(97)90307-3.

GBD 2023 DISEASE AND INJURY AND RISK FACTOR COLLABORATORS. Burden of 375 diseases and injuries, risk-attributable burden of 88 risk factors, and healthy life expectancy in 204 countries and territories, including 660 subnational locations, 1990–2023: A systematic analysis for the Global Burden of Disease Study 2023. **Lancet**, v. 06, n. 10513, p. 1873–1922, 2025. DOI: https://doi.org/10.1016/S0140-6736(25)01637-X.

GEISSLER, A.; KRIMMER, T.; BÖMER, U.; GUIARD, B.; RASSOW, J.; PFANNER, N. Membrane potential-driven protein import into mitochondria. The sorting sequence of cytochrome b(2) modulates the deltapsi-dependence of translocation of the matrix-targeting sequence. **Molecular Biology of the Cell**, v. 11, n. 11, p. 3977–3991, 2000. DOI: https://doi.org/10.1091/mbc.11.11.3977.

GENNARELLI, T. A. Diffuse axonal injury and traumatic coma in the primate. **Annals of Neurology**, 1982. DOI: 10.1002/ana.410120611.

GLANCY, B.; BALABAN, R. S. Role of mitochondrial Ca2+ in the regulation of cellular energetics. **Biochemistry**, v. 51, n. 14, p. 2959–2973, 2012. DOI: https://doi.org/10.1021/bi2018909.

GORELICK-FELDMAN, J.; COHICK, W.; RASKIN, I. Ecdysteroids elicit a rapid Ca2+ flux leading to Akt activation and increased protein synthesis in skeletal muscle cells. **Steroids**, v. 75, n. 10, p. 632–637, 2010. DOI: https://doi.org/10.1016/j.steroids.2010.03.008.

GORELICK-FELDMAN, J. et al. Phytoecdysteroids increase protein synthesis in skeletal muscle cells. **Journal of Agricultural and Food Chemistry**, v. 56, n. 10, p. 3532–3537, 2008. DOI: https://doi.org/10.1021/jf073059z.

GOTTLIEB, E.; ARMOUR, S. M.; HARRIS, M. H.; THOMPSON, C. B. Mitochondrial membrane potential regulates matrix configuration and cytochrome c release during apoptosis. **Cell Death and Differentiation**, v. 10, n. 6, p. 709–717, 2003. DOI: https://doi.org/10.1038/sj.cdd.4401231.

GRAHAM, L. C. et al. Proteomic profiling of neuronal mitochondria reveals modulators of synaptic architecture. **Molecular Neurodegeneration**, v. 12, n. 1, p. 77, 2017a. DOI: 10.1186/s13024-017-0221-9.

GRANATH-PANELO, M.; KAJIMURA, S. Mitochondrial heterogeneity and adaptations to cellular needs. **Nature Cell Biology**, v. 26, n. 5, p. 674–686, 2024. DOI: 10.1038/s41556-024-01410-1.

GRÖNBLADH, A.; NYLANDER, E.; HALLBERG, M. The neurobiology and addiction potential of anabolic androgenic steroids and the effects of growth hormone. **Brain Research Bulletin**, v. 126, n. Pt 1, p. 127–137, 2016a. DOI: 10.1016/j.brainresbull.2016.05.003.

GRÖNBLADH, A.; NYLANDER, E.; HALLBERG, M. The neurobiology and addiction potential of anabolic androgenic steroids and the effects of growth hormone. **Brain Research Bulletin**, v. 126, n. Pt 1, p. 127–137, 2016b. DOI: 10.1016/j.brainresbull.2016.05.003.

GRUNFELD, C. et al. Oxandrolone in the treatment of HIV-associated weight loss in men: A randomized, double-blind, placebo-controlled study. **Journal of Acquired Immune Deficiency Syndromes**, v. 41, n. 3, p. 304–314, 2006. DOI: 10.1097/01.qai.0000197546.56131.40.

HAAS, I. G. BiP (GRP78), an essential hsp70 resident protein in the endoplasmic reticulum. **Experientia**, v. 50, n. 11–12, p. 1012–1020, 1994. DOI: 10.1007/BF01923455.

HAGENSTON, A. M. et al. Disrupted expression of mitochondrial NCLX sensitizes neuroglial networks to excitotoxic stimuli and renders synaptic activity toxic. **The Journal of Biological Chemistry**, v. 298, n. 2, p. 101508, 2021. DOI: 10.1016/j.jbc.2021.101508.

HAJSZAN, T.; MACLUSKY, N. J.; LERANTH, C. Role of androgens and the androgen receptor in remodeling of spine synapses in limbic brain areas. **Hormones and Behavior**, v. 53, n. 5, p. 638–646, 2008. DOI: 10.1016/j.yhbeh.2007.12.007.

HAMMOND, G. L. Diverse roles for sex hormone-binding globulin in reproduction. **Biology of Reproduction**, v. 85, n. 3, p. 431–441, 2011. https://doi.org/10.1095/biolreprod.111.092593.

HANDELSMAN, D. J. Androgen physiology, pharmacology, use and misuse. In: FEINGOLD, K. R. et al. (Org.). **Endotext**. MDText.com, Inc., 2000. http://www.ncbi.nlm.nih.gov/books/NBK279000/.

HARTGENS, F.; KUIPERS, H. Effects of androgenic-anabolic steroids in athletes. **Sports Medicine**, v. 34, n. 8, p. 513–554, 2004a. https://doi.org/10.2165/00007256-200434080-00003

HARTGENS, F.; KUIPERS, H. Effects of androgenic-anabolic steroids in athletes. **Sports Medicine**, v. 34, n. 8, p. 513–554, 2004b.

HÄRTL, R.; GERBER, L. M.; NI, Q.; GHAJAR, J. Effect of early nutrition on deaths due to severe traumatic brain injury. **Journal of Neurosurgery**, v. 109, n. 1, p. 50–56, 2008. https://doi.org/10.3171/JNS/2008/109/7/0050.

HATTON, C. K.; CATLIN, D. H. Detection of androgenic anabolic steroids in urine. **Clinics in Laboratory Medicine**, v. 7, n. 3, p. 655–668, 1987.

HAUGER, L. E.; HAVNES, I. A.; JØRSTAD, M. L.; BJØRNEBEKK, A. Anabolic androgenic steroids, antisocial personality traits, aggression and violence. **Drug and Alcohol Dependence**, v. 221, p. 108604, 2021. https://doi.org/10.1016/j.drugalcdep.2021.108604

HAUGER, L. E.; WESTLYE, L. T.; FJELL, A. M.; WALHOVD, K. B.; BJØRNEBEKK, A. Structural brain characteristics of anabolic-androgenic steroid dependence in men. **Addiction**, v. 114, n. 8, p. 1405–1415, 2019. https://doi.org/10.1111/add.14629

HAYASHI, T.; RIZZUTO, R.; HAJ NOCZKY, G.; SU, T.-P. MAM: More than just a housekeeper. **Trends in Cell Biology**, v. 19, n. 2, p. 81–88, 2009. https://doi.org/10.1016/j.tcb.2008.12.002

HEMPHILL, M. A.; DAUTH, S.; YU, C. J.; DABIRI, B. E.; PARKER, K. K. Traumatic brain injury and the neuronal microenvironment: A potential role for neuropathological mechanotransduction. **Neuron**, v. 85, n. 6, p. 1177–1192, 2015. https://doi.org/10.1016/j.neuron.2015.02.041.

HENDERSON, L. P.; PENATTI, C. A. A.; JONES, B. L.; YANG, P.; CLARK, A. S. Anabolic androgenic steroids and forebrain GABAergic transmission. **Neuroscience**, v. 138, n. 3, p. 793–799, 2006. https://doi.org/10.1016/j.neuroscience.2005.08.039.

HENRIKSEN, J. H. Ernest Henry Starling (1866–1927): The scientist and the man. **Journal of Medical Biography**, v. 13, n. 1, p. 22–30, 2005. https://doi.org/10.1177/096777200501300107.

HEO, J. Redox control of GTPases: From molecular mechanisms to functional significance in health and disease. **Antioxidants & Redox Signaling**, v. 14, n. 4, p. 689–724, 2011. https://doi.org/10.1089/ars.2009.2984.

HEPATIC EFFECTS OF 17 ALPHA-ALKYLATED ANABOLI-ANDROGENIC STEROIDS. **HIV Hotline**, v. 8, n. 5–6, p. 2–5, 1998.

HERSEY, P.; ZHANG, X. D. Adaptation to ER stress as a driver of malignancy and resistance to therapy in human melanoma. **Pigment Cell & Melanoma Research**, v. 21, n. 3, p. 358–367, 2008. https://doi.org/10.1111/j.1755-148X.2008.00467.x

HERSHBERGER, L. G.; SHIPLEY, E. G.; MEYER, R. K. Myotrophic activity of 19-nortestosterone and other steroids determined by modified levator ani muscle method. **Proceedings of the Society for Experimental Biology and Medicine**, v. 83, n. 1, p. 175–180, 1953. https://doi.org/10.3181/00379727-83-20301.

HIKICHI, Y.; YAMAOKA, M.; KUSAKA, M.; HARA, T. Selective androgen receptor modulator activity of a steroidal antiandrogen TSAA-291 and its cofactor recruitment profile. **European Journal of Pharmacology**, v. 765, p. 322–331, 2015. https://doi.org/10.1016/j.ejphar.2015.08.052.

HILDEBRANDT, T.; KNUESTING, J.; BERNDT, C.; MORGAN, B.; SCHEIBE, R. Cytosolic thiol switches regulating basic cellular functions: GAPDH as an information hub? **Biological Chemistry**, v. 396, n. 5, p. 523–537, 2015. https://doi.org/10.1515/hsz-2014-0295.

HILL, R. L.; KULBE, J. R.; SINGH, I. N.; WANG, J. A.; HALL, E. D. Synaptic mitochondria are more susceptible to traumatic brain injury-induced oxidative damage and respiratory dysfunction than non-synaptic mitochondria. **Neuroscience**, v. 386, p. 265–283, 2018. https://doi.org/10.1016/j.neuroscience.2018.06.028.

HIRANO, M.; EMMANUELE, V.; QUINZII, C. M. Emerging therapies for mitochondrial diseases. **Essays in Biochemistry**, v. 62, n. 3, p. 467–481, 2018. https://doi.org/10.1038/scientificamerican0295-76.

HOBERMAN, J. M.; YESALIS, C. E. The history of synthetic testosterone. **Scientific American**, v. 272, n. 2, p. 76–81, 1995.

HOHL, A.; MAZZUCO, T. L.; CORAL, M. H. C.; SCHWARZBOLD, M.; WALZ, R. Hypogonadism after traumatic brain injury. **Arquivos Brasileiros de Endocrinologia e Metabologia**, v. 53, n. 8, p. 908–914, 2009. https://doi.org/10.1590/s0004-27302009000800003.

HS, L.; RR, D.-A. Diagnosis, prognosis, and clinical management of mild traumatic brain injury. **The Lancet Neurology**, v. 14, n. 5, 2015. https://doi.org/10.1016/S1474-4422(15)00002-2.

JAFRI, M. S.; KUMAR, R. Modeling mitochondrial function and its role in disease. **Progress in Molecular Biology and Translational Science**, v. 123, p. 103–125, 2014. DOI: 10.1016/B978-0-12-397897-4.00001-2.

JANSSEN, D. F. Endocrinology's early historians (1889-1914) and the forgotten prehistory of testosterone. **Sexual Medicine Reviews**, v. 12, n. 2, p. 192–198, 2024. DOI: 10.1093/sxmrev/qeae001.

JARRAHI, A. et al. Revisiting Traumatic Brain Injury: From Molecular Mechanisms to Therapeutic Interventions. **Biomedicines**, v. 8, n. 10, p. 389, 2020. DOI: 10.3390/biomedicines8100389.

JENSEN, G. L. et al. Adult starvation and disease-related malnutrition: A proposal for etiology-based diagnosis in the clinical practice setting from the International Consensus Guideline Committee. **JPEN. Journal of Parenteral and Enteral Nutrition**, v. 34, n. 2, p. 156–159, 2010. DOI: 10.1177/0148607110361910.

JOHNSON, V. E.; STEWART, W.; ARENA, J. D.; SMITH, D. H. Traumatic Brain Injury as a Trigger of Neurodegeneration. **Advances in Neurobiology**, v. 15, p. 383–400, 2017. DOI: 10.1007/978-3-319-57193-5_15.

JOHNSON, V. E.; STEWART, W.; SMITH, D. H. Axonal pathology in traumatic brain injury. **Experimental Neurology**, v. 246, p. 35–43, 2013. DOI: 10.1016/j.expneurol.2012.01.013.

JONCKHEERE, A. I.; SMEITINK, J. A. M.; RODENBURG, R. J. T. Mitochondrial ATP synthase: Architecture, function and pathology. **Journal of Inherited Metabolic Disease**, v. 35, n. 2, p. 211–225, 2012a. DOI: 10.1007/s10545-011-9382-9.

JONCKHEERE, A. I.; SMEITINK, J. A. M.; RODENBURG, R. J. T. Mitochondrial ATP synthase: Architecture, function and pathology. **Journal of Inherited Metabolic Disease**, v. 35, n. 2, p. 211–225, 2012b. DOI: 10.1007/s10545-011-9382-9.

JOUBERT, Y.; TOBIN, C. Satellite cell proliferation and increase in the number of myonuclei induced by testosterone in the levator ani muscle of the adult female rat. **Developmental Biology**, v. 131, n. 2, p. 550–557, 1989. DOI: 10.1016/s0012-1606(89)80025-9.

JUUL, A. The effects of oestrogens on linear bone growth. **Human Reproduction Update**, v. 7, n. 3, p. 303–313, 2001. DOI: 10.1093/humupd/7.3.303.

KALININE, E. et al. Nandrolone-induced aggressive behavior is associated with alterations in extracellular glutamate homeostasis in mice. **Hormones and Behavior**, v. 66, n. 2, p. 383–392, 2014. DOI: 10.1016/j.yhbeh.2014.06.005.

KANAYAMA, G.; BROWER, K. J.; WOOD, R. I.; HUDSON, J. I.; POPE, H. G. Anabolic-androgenic steroid dependence: An emerging disorder. **Addiction**, v. 104, n. 12, p. 1966–1978, 2009. DOI: 10.1111/j.1360-0443.2009.02734.x.

KANAYAMA, G.; POPE, H. G. History and epidemiology of anabolic androgens in athletes and non-athletes. **Molecular and Cellular Endocrinology**, v. 464, p. 4–13, 2018. DOI: 10.1016/j.mce.2017.02.039.

KARAGUN, B.; ALTUG, S. Anabolic-androgenic steroids are linked to depression and anxiety in male bodybuilders. **Annals of Medicine**, v. 56, n. 1, p. 2337717, 2024. DOI: 10.1080/07853890.2024.2337717.

KAUFMAN, M. J. et al. Brain and cognition abnormalities in long-term anabolic-androgenic steroid users. **Drug and Alcohol Dependence**, v. 152, p. 47–56, 2015. DOI: 10.1016/j.drugalcdep.2015.04.023.

KEHREIN, K.; BONNEFOY, N.; OTT, M. Mitochondrial protein synthesis: Efficiency and accuracy. **Antioxidants & Redox Signaling**, v. 19, n. 16, p. 1928–1939, 2013. DOI: 10.1089/ars.2012.4896.

KHACHO, M.; HARRIS, R.; SLACK, R. S. Mitochondria as central regulators of neural stem cell fate and cognitive function. **Nature Reviews Neuroscience**, v. 20, n. 1, p. 34–48, 2019. DOI: 10.1038/s41583-018-0091-3.

KHAN, S.; TALLEY, L. Beyond the Hit: The Hidden Costs of Repetitive Head Trauma. **Neuroscience Insights**, v. 20, p. 26331055251316315, 2025. DOI: 10.1177/26331055251316315.

KICMAN, A. T. Pharmacology of anabolic steroids. **British Journal of Pharmacology**, v. 154, n. 3, p. 502–521, 2008. DOI: 10.1038/bjp.2008.165.

KICMAN, A. T.; BROOKS, R. V. A radioimmunoassay for the metabolites of the anabolic steroid nandrolone. **Journal of Pharmaceutical and Biomedical Analysis**, v. 6, n. 5, p. 473–483, 1988. DOI: 10.1016/0731-7085(88)80014-1.

KICMAN, A. T.; GOWER, D. B. Anabolic steroids in sport: Biochemical, clinical and analytical perspectives. **Annals of Clinical Biochemistry**, v. 40, n. 4, p. 321–356, 2003. DOI: 10.1258/000456303766476977.

KOCHAKIAN, C. D. History, chemistry and pharmacodynamics of anabolic-androgenic steroids. **Wiener Medizinische Wochenschrift**, v. 143, n. 14–15, p. 359–363, 1993.

KOERICH, A. H.; VOLINO, G. C.; VICARI, G. B. Perfil epidemiológico do trauma cranioencefálico na região Sul do Brasil: Uma análise comparativa entre estados (2018–2023). **Revista Multidisciplinar em Saúde**, v. 5, n. 2, p. 1–8, 2024. DOI: 10.51161/integrar/rems/4303.

KORN, S. H. et al. Cytokine-induced activation of nuclear factor-kappa B is inhibited by hydrogen peroxide. **The Journal of Biological Chemistry**, v. 276, n. 38, p. 35693–35700, 2001. DOI: 10.1074/jbc.M104321200.

KORSHUNOV, S. S.; SKULACHEV, V. P.; STARKOV, A. A. High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria. **FEBS Letters**, v. 416, n. 1, p. 15–18, 1997. DOI: 10.1016/s0014-5793(97)01159-9.

KRÜSKEMPER, H. L.; MORGNER, K. D. Studies on the clinical pharmacology of methyltestosterone, mesterolone and 4-chlortestosterone. **Internationale Zeitschrift für Klinische Pharmakologie, Therapie und Toxikologie**, v. 1, n. 5, p. 455–460, 1968.

KÜHLBRANDT, W. Structure and function of mitochondrial membrane protein complexes. **BMC Biology**, v. 13, p. 89, 2015. DOI: 10.1186/s12915-015-0201-x.

KULBE, J. R. et al. Synaptic mitochondria sustain more damage than non-synaptic mitochondria after traumatic brain injury. **Journal of Neurotrauma**, v. 34, n. 7, p. 1291–1301, 2017. DOI: 10.1089/neu.2016.4628.

KUURANNE, T. et al. Screening of in vitro synthesised metabolites of 4,9,11-trien-3-one steroids by liquid chromatography mass spectrometry. **European Journal of Mass Spectrometry**, v. 14, n. 3, p. 181–189, 2008. DOI: 10.1255/ejms.923.

KVORNING, T.; ANDERSEN, M.; BRIXEN, K.; MADSEN, K. Suppression of endogenous testosterone production attenuates the response to strength training. **American Journal of Physiology – Endocrinology and Metabolism**, v. 291, n. 6, p. E1325–E1332, 2006. DOI: 10.1152/ajpendo.00143.2006.

LABRIE, F. Intracrinology. **Molecular and Cellular Endocrinology**, [s.l.], v. 78, n. 3, p. C113–C118, 1991. DOI: 10.1016/0303-7207(91)90116-a.

LAFONT, R.; DINAN, L. Practical uses for ecdysteroids in mammals including humans: An update. **Journal of Insect Science**, [s.l.], v. 3, n. 1, p. 7–7, 2003. DOI: 10.1093/jis/3.1.7.

LAWRENCE, D. W.; HUTCHISON, M. Concerns with novel concussion protocol. **British Journal of Sports Medicine**, [s.l.], v. 51, n. 7, p. 620–621, 2017. DOI: 10.1136/bjsports-2016-097126.

LE GREVÈS, P.; HUANG, W.; JOHANSSON, P.; THÖRNWALL, M. et al. Effects of an anabolic-androgenic steroid on the regulation of the NMDA receptor NR1, NR2A and NR2B subunit mRNAs in brain regions of the male rat. **Neuroscience Letters**, [s.l.], v. 226, n. 1, p. 61–64, 1997. DOI: 10.1016/s0304-3940(97)00244-9.

LEE, S. C.; ZASLER, N. D.; KREUTZER, J. S. Male pituitary-gonadal dysfunction following severe traumatic brain injury. **Brain Injury**, [s.l.], v. 8, n. 6, p. 571–577, 1994. DOI: 10.3109/02699059409151009.

LEMASTERS, J. J.; THERUVATH, T. P.; ZHONG, Z.; NIEMINEN, Anna-Liisa. Mitochondrial calcium and the permeability transition in cell death. **Biochimica et Biophysica Acta**, [s.l.], v. 1787, n. 11, p. 1395–1401, 2009. DOI: 10.1016/j.bbabio.2009.06.009.

LENAZ, G.; GENOVA, M. L. Structure and organization of mitochondrial respiratory complexes: A new understanding of an old subject. **Antioxidants & Redox Signaling**, [s.l.], v. 12, n. 8, p. 961–1008, 2010. DOI: 10.1089/ars.2009.2704.

LEVIN, H. S.; DIAZ-ARRASTIA, R. R. Diagnosis, prognosis, and clinical management of mild traumatic brain injury. **The Lancet Neurology**, [s.l.], v. 14, n. 5, p. 506–517, 2015. DOI: 10.1016/S1474-4422(15)00002-2.

LIM, Siu-Wei; SU, Huang-Chi; NYAM, Tze-Tee Elaine; CHIO, Chih-Cheng et al. Ceftriaxone therapy attenuates brain trauma in rats by affecting glutamate transporters and neuroinflammation and not by its antibacterial effects. **BMC Neuroscience**, [s.l.], v. 22, n. 1, p. 54–54, 2021. DOI: 10.1186/s12868-021-00659-8.

LORENTE, L.; MARTÍN, M. M.; ARGUESO, M.; RAMOS, L. et al. Serum caspase-3 levels and mortality are associated in patients with severe traumatic brain injury. **BMC Neurology**, [s.l.], v. 15, p. 228–228, 2015. DOI: 10.1186/s12883-015-0485-z.

LOUGHTON, S. J.; RUHLING, R. O. Human strength and endurance responses to anabolic steroid and training. **The Journal of Sports Medicine and Physical Fitness**, [s.l.], v. 17, n. 3, p. 285–296, 1977.

LUNDHOLM, L.; FRISELL, T.; LICHTENSTEIN, P.; LÅNGSTRÖM, N. Anabolic androgenic steroids and violent offending: Confounding by polysubstance abuse among 10,365 general population men. **Addiction**, [s.l.], v. 110, n. 1, p. 100–108, 2015. DOI: 10.1111/add.12715.

LUONGO, T. S.; LAMBERT, J. P.; GROSS, P.; NWOKEDI, M.; LOMBARDI, A. A. et al. The mitochondrial Na+/Ca2+ exchanger is essential for Ca2+ homeostasis and viability. **Nature**, [s.l.], v. 545, n. 7652, p. 93–97, 2017. DOI: 10.1038/nature22082.

MA, T. K. W.; KAM, K. K. H.; YAN, B. P.; LAM, Y.-Y. Renin-angiotensin-aldosterone system blockade for cardiovascular diseases: Current status. **British Journal of Pharmacology**, v. 160, n. 6, p. 1273–1292, 2010. DOI: 10.1111/j.1476-5381.2010.00750.x.

MA, Y.; HENDERSHOT, L. M. ER chaperone functions during normal and stress conditions. **Journal of Chemical Neuroanatomy**, v. 28, n. 1–2, p. 51–65, 2004. DOI: 10.1016/j.jchemneu.2003.08.007.

MAAS, A. I. R. et al. Traumatic brain injury: Integrated approaches to improve prevention, clinical care, and research. **The Lancet. Neurology**, v. 16, n. 12, p. 987–1048, 2017. DOI: 10.1016/S1474-4422(17)30371-X.

MAAS, A. I. R. et al. Traumatic brain injury: Progress and challenges in prevention, clinical care, and research. **The Lancet Neurology**, v. 21, n. 11, p. 1004–1060, 2022. DOI: 10.1016/S1474-4422(22)00309-X.

MAILLOUX, R. J. Teaching the fundamentals of electron transfer reactions in mitochondria and the production and detection of reactive oxygen species. **Redox Biology**, v. 4, p. 381–398, 2015. DOI: 10.1016/j.redox.2015.02.001.

MAJUMDER, D. Calcium dynamics unplugged: NCLX in disease and therapeutic frontiers. **Molecular Biology Reports**, v. 53, n. 1, p. 12, 2025. DOI: 10.1007/s11033-025-11188-6.

MALGOR, L. A.; FISHER, J. W. Effects of testosterone on erythropoietin production in isolated perfused kidneys. **The American Journal of Physiology**, v. 218, n. 6, p. 1732–1736, 1970. DOI: 10.1152/ajplegacy.1970.218.6.1732.

MALHOTRA, J. D.; KAUFMAN, R. J. Endoplasmic reticulum stress and oxidative stress: A vicious cycle or a double-edged sword? **Antioxidants & Redox Signaling**, v. 9, n. 12, p. 2277–2293, 2007. DOI: 10.1089/ars.2007.1782.

MAMMUCARI, C. et al. Mitochondrial calcium uptake in organ physiology: From molecular mechanism to animal models. **Pflugers Archiv: European Journal of Physiology**, v. 470, n. 8, p. 1165–1179, 2018. DOI: 10.1007/s00424-018-2123-2.

MANGELSDORF, D. J. et al. The nuclear receptor superfamily: The second decade. **Cell**, v. 83, n. 6, p. 835–839, 1995. DOI: 10.1016/0092-8674(95)90199-x.

MARAVELIAS, C.; DONA, A.; STEFANIDOU, M.; SPILIOPOULOU, C. Adverse effects of anabolic steroids in athletes: A constant threat. **Toxicology Letters**, v. 158, n. 3, p. 167–175, 2005. DOI: 10.1016/j.toxlet.2005.06.005.

MARCHI, S. et al. Mitochondrial and endoplasmic reticulum calcium homeostasis and cell death. **Cell Calcium**, v. 69, p. 62–72, 2018. DOI: 10.1016/j.ceca.2017.05.003.

MARTINI, L. The 5alpha-reduction of testosterone in the neuroendocrine structures: Biochemical and physiological implications. **Endocrine Reviews**, v. 3, n. 1, p. 1–25, 1982. DOI: 10.1210/edrv-3-1-1.

MATSUMOTO, A. M.; BRENNER, W. J. Serum testosterone assays—Accuracy matters. **The Journal of Clinical Endocrinology and Metabolism**, v. 89, n. 2, p. 520–524, 2004. DOI: 10.1210/jc.2003-032175.

MATTSON, M. P. Calcium and neurodegeneration. **Aging Cell**, v. 6, n. 3, p. 337–350, 2007. DOI: 10.1111/j.1474-9726.2007.00275.x.

MAZZONI, I.; BARROSO, O.; RABIN, O. The list of prohibited substances and methods in sport: Structure and review process by the world anti-doping agency. **Journal of Analytical Toxicology**, v. 35, n. 9, p. 608–612, 2011. DOI: 10.1093/anatox/35.9.608.

MCEVOY, C. T.; CRAN, G. W.; COOKE, S. R.; YOUNG, I. S. Resting energy expenditure in non-ventilated, non-sedated patients recovering from serious traumatic brain injury. **Clinical Nutrition**, v. 28, n. 5, p. 526–532, 2009. DOI: 10.1016/j.clnu.2009.04.009.

MCGINNIS, M. Y. Anabolic androgenic steroids and aggression: Studies using animal models. **Annals of the New York Academy of Sciences**, v. 1036, p. 399–415, 2004. DOI: 10.1196/annals.1330.024.

MCINTOSH, S. J.; VERGEER, M. H.; GALARNEAU, J.-M.; ELIASON, P. H.; DEBERT, C. T. Factors associated with persisting symptoms after concussion in adults with mild TBI: A systematic review and meta-analysis. **JAMA Network Open**, v. 8, n. 6, e2516619, 2025. DOI: 10.1001/jamanetworkopen.2025.16619.

MEANEY, D. F.; MORRISON, B.; DALE BASS, C. The mechanics of traumatic brain injury: A review of what we know and what we need to know for reducing its societal burden. **Journal of Biomechanical Engineering**, v. 136, n. 2, 021008, 2014. DOI: 10.1115/1.4026364.

MEIER, R. et al. Differential temporal profile of lowered blood glucose levels in patients with severe traumatic brain injury. **Critical Care**, v. 12, n. 4, R98, 2008. DOI: 10.1186/cc6974.

MELNIK, B. C. Androgen abuse in the community. **Current Opinion in Endocrinology, Diabetes, and Obesity**, v. 16, n. 3, p. 218–223, 2009. DOI: 10.1097/MED.0b013e32832afdfe.

MENON, D. K. et al. Position statement: Definition of traumatic brain injury. **Archives of Physical Medicine and Rehabilitation**, v. 91, n. 11, p. 1637–1640, 2010. DOI: 10.1016/j.apmr.2010.05.017.

MEUSSER, B.; HIRSCH, C.; JAROSCH, E.; SOMMER, T. ERAD: The long road to destruction. **Nature Cell Biology**, v. 7, n. 8, p. 766–772, 2005. DOI: 10.1038/ncb0805-766.

MIDZAK, A. S.; CHEN, H.; PAPADOPOULOS, V.; ZIRKIN, B. R. Leydig cell aging and the mechanisms of reduced testosterone synthesis. **Molecular and Cellular Endocrinology**, v. 299, n. 1, p. 23–31, 2009. DOI: 10.1016/j.mce.2008.07.016.

MILLER, W. L.; AUCHUS, R. J. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. **Endocrine Reviews**, v. 32, n. 1, p. 81–151, 2011. DOI: 10.1210/er.2010-0013.

MIRA, R. G.; QUINTANILLA, R. A.; CERPA, W. Mild traumatic brain injury induces mitochondrial calcium overload and triggers the upregulation of NCLX in the hippocampus. **Antioxidants**, v. 12, n. 2, 403, 2023. DOI: 10.3390/antiox12020403.

MIRZAEV, I. R.; SYROV, V. N.; KHRUSHEV, S. A.; ISKANDEROVA, S. D. [Effect of ecdystene on parameters of the sexual function under experimental and clinical conditions]. **Eksperimental'naia i Klinicheskaia Farmakologiia**, v. 63, n. 4, p. 35–37, 2000.

MISHRA, J. et al. Differential Ca^{2+} handling by isolated synaptic and non-synaptic mitochondria: Roles of Ca^{2+} buffering and efflux. **Frontiers in Synaptic Neuroscience**, v. 17, 1562065, 2025. DOI: 10.3389/fnsyn.2025.1562065.

MODI, H. R. et al. Mitochondria-targeted antioxidant therapeutics for traumatic brain injury. **Antioxidants**, v. 13, n. 3, 303, 2024. DOI: 10.3390/antiox13030303.

MOORADIAN, A. D.; MORLEY, J. E.; KORENMAN, S. G. Biological actions of androgens. **Endocrine Reviews**, v. 8, n. 1, p. 1–28, 1987. DOI: 10.1210/edrv-8-1-1.

MORRISON, T. R.; SIKES, R. W.; MELLONI, R. H. Anabolic steroids alter the physiological activity of aggression circuits in the lateral anterior hypothalamus. **Neuroscience**, v. 315, p. 1–17, 2016. DOI: 10.1016/j.neuroscience.2015.12.001.

MOSTI, C.; COCCARO, E. F. Mild traumatic brain injury and aggression, impulsivity, and history of other- and self-directed aggression. **The Journal of Neuropsychiatry and Clinical Neurosciences**, v. 30, n. 3, p. 220–227, 2018. DOI: 10.1176/appi.neuropsych.17070141.

MOTTRAM, D. R.; GEORGE, A. J. Anabolic steroids. **Bailliere's Best Practice & Research. Clinical Endocrinology & Metabolism**, v. 14, n. 1, p. 55–69, 2000. DOI: 10.1053/beem.2000.0053.

MOYLE, G. J. et al. Efficacy of selected treatments of HIV wasting: A systematic review and meta-analysis. **Journal of Acquired Immune Deficiency Syndromes**, v. 37, suppl. 5, p. S262–S276, 2004. DOI: 10.1097/01.qai.0000144381.09350.5b.

MT, V.; AK, S. A review of penetrating brain trauma: Epidemiology, pathophysiology, imaging assessment, complications, and treatment. **Emergency Radiology**, v. 24, n. 3, 2017. DOI: 10.1007/s10140-016-1477-z.

MULICA, P.; GRÜNEWALD, A.; PEREIRA, S. L. Astrocyte-neuron metabolic crosstalk in neurodegeneration: A mitochondrial perspective. **Frontiers in Endocrinology**, v. 12, 668517, 2021. DOI: 10.3389/fendo.2021.668517.

MUNAKOMI, S.; PUCKETT, Y. Chronic traumatic encephalopathy. In: **StatPearls**. Treasure Island, FL: StatPearls Publishing, 2025. Disponível em: http://www.ncbi.nlm.nih.gov/books/NBK541013/.

NASERI, M.; ALI MOZAYENI, M.; SAFI, Y.; HEIDARNIA, M.; AKBARZADEH BAGHBAN, A.; NOROUZI, N. Root canal morphology of maxillary second molars according to age and gender in a selected Iranian population: A cone-beam computed tomography evaluation. **Iranian Endodontic Journal**, v. 13, n. 3, p. 373–380, 2018. DOI: 10.22037/iej.v13i3.19278.

NEUROLOGICAL DISORDERS: PUBLIC HEALTH CHALLENGES. (s.d.). Recuperado em 3 nov. 2025, de https://www.who.int/publications/i/item/9789241563369.

NIESCHLAG, E.; NIESCHLAG, S. Testosterone deficiency: A historical perspective. **Asian Journal of Andrology**, v. 16, n. 2, p. 161–168, 2014. DOI: 10.4103/1008-682X.122358.

NIESCHLAG, E.; NIESCHLAG, S. Endocrine history: The history of discovery, synthesis and development of testosterone for clinical use. **European Journal of Endocrinology**, v. 180, n. 6, p. R201–R212, 2019. DOI: 10.1530/EJE-19-0071.

NOLFI-DONEGAN, D.; BRAGANZA, A.; SHIVA, S. Mitochondrial electron transport chain: Oxidative phosphorylation, oxidant production, and methods of measurement. **Redox Biology**, v. 37, p. 101674, 2020. DOI: 10.1016/j.redox.2020.101674.

OBERLANDER, J. G.; HENDERSON, L. P. The Sturm und Drang of anabolic steroid use: Angst, anxiety, and aggression. **Trends in Neurosciences**, v. 35, n. 6, p. 382–392, 2012. DOI: 10.1016/j.tins.2012.03.001.

OGONAH, M. G. T.; BOTCHWAY, S.; YU, R.; SCHOFIELD, P. W.; FAZEL, S. An umbrella review of health outcomes following traumatic brain injury. **Nature Mental Health**, v. 3, n. 1, p. 83–91, 2025. DOI: 10.1038/s44220-024-00356-5.

OLATONA, O. A.; STERBEN, S. P.; KANSAKAR, S. B. S.; SYMES, A. J.; LIAUDANSKAYA, V. Mitochondria: The hidden engines of traumatic brain injury-driven neurodegeneration. **Frontiers in Cellular Neuroscience**, v. 19, 2025. DOI: 10.3389/fncel.2025.1570596.

OLEKSAK, P.; NEPOVIMOVA, E.; VALKO, M.; ALWASEL, S.; ALOMAR, S.; KUCA, K. Comprehensive analysis of prohibited substances and methods in sports: Unveiling trends, pharmacokinetics, and WADA evolution. **Environmental Toxicology and Pharmacology**, v. 108, p. 104447, 2024. DOI: 10.1016/j.etap.2024.104447.

OYADOMARI, S.; MORI, M. Roles of CHOP/GADD153 in endoplasmic reticulum stress. **Cell Death and Differentiation**, v. 11, n. 4, p. 381–389, 2004. DOI: 10.1038/sj.cdd.4401373.

PAGLIARINI, D. J.; RUTTER, J. Hallmarks of a new era in mitochondrial biochemistry. **Genes & Development**, v. 27, n. 24, p. 2615–2627, 2013. DOI: 10.1101/gad.229724.113.

PALTY, R.; SILVERMAN, W. F.; HERSHFINKEL, M.; CAPORALE, T. et al. NCLX is an essential component of mitochondrial Na+/Ca2+ exchange. **Proceedings of the National Academy of Sciences of the United States of America**, v. 107, n. 1, p. 436–441, 2010. DOI: 10.1073/pnas.0908099107

PARR, M. K.; BOTRÈ, F.; NAß, A.; HENGEVOSS, J.; DIEL, P.; WOLBER, G. Ecdysteroids: A novel class of anabolic agents? **Biology of Sport**, v. 32, n. 2, p. 169–173, 2015. DOI: 10.5604/20831862.1144420.

PAVLATOS, A. M.; FULTZ, O.; MONBERG, M. J.; VOOTKUR, A.; PHARMD. Review of oxymetholone: A 17alpha-alkylated anabolic-androgenic steroid. **Clinical Therapeutics**, v. 23, n. 6, p. 789–801; discussion 771, 2001. DOI: 10.1016/s0149-2918(01)80070-9.

PELTON, L. M.; MARIS, S. A.; LOSEKE, J. The effects of anabolic-androgenic steroids on gene expression in skeletal muscle: A systematic review. **International Journal of Exercise Science**, v. 16, n. 3, p. 53–82, 2023. DOI: 10.70252/QFNY6413.

NASERI, M.; ALI MOZAYENI, M.; SAFI, Y.; HEIDARNIA, M.; AKBARZADEH BAGHBAN, A.; NOROUZI, N. Root canal morphology of maxillary second molars according to age and gender in a selected Iranian population: A cone-beam computed tomography evaluation. **Iranian Endodontic Journal**, v. 13, n. 3, p. 373–380, 2018. DOI: 10.22037/iej.v13i3.19278.

NEUROLOGICAL DISORDERS: PUBLIC HEALTH CHALLENGES. (s.d.). Recuperado em 3 nov. 2025, de https://www.who.int/publications/i/item/9789241563369.

NIESCHLAG, E.; NIESCHLAG, S. Testosterone deficiency: A historical perspective. **Asian Journal of Andrology**, v. 16, n. 2, p. 161–168, 2014. DOI: 10.4103/1008-682X.122358.

NIESCHLAG, E.; NIESCHLAG, S. Endocrine history: The history of discovery, synthesis and development of testosterone for clinical use. **European Journal of Endocrinology**, v. 180, n. 6, p. R201–R212, 2019. DOI: 10.1530/EJE-19-0071.

NOLFI-DONEGAN, D.; BRAGANZA, A.; SHIVA, S. Mitochondrial electron transport chain: Oxidative phosphorylation, oxidant production, and methods of measurement. **Redox Biology**, v. 37, p. 101674, 2020. DOI: 10.1016/j.redox.2020.101674.

OBERLANDER, J. G.; HENDERSON, L. P. The Sturm und Drang of anabolic steroid use: Angst, anxiety, and aggression. **Trends in Neurosciences**, v. 35, n. 6, p. 382–392, 2012. DOI: 10.1016/j.tins.2012.03.001.

OGONAH, M. G. T.; BOTCHWAY, S.; YU, R.; SCHOFIELD, P. W.; FAZEL, S. An umbrella review of health outcomes following traumatic brain injury. **Nature Mental Health**, v. 3, n. 1, p. 83–91, 2025. DOI: 10.1038/s44220-024-00356-5.

OLATONA, O. A.; STERBEN, S. P.; KANSAKAR, S. B. S.; SYMES, A. J.; LIAUDANSKAYA, V. Mitochondria: The hidden engines of traumatic brain injury-driven neurodegeneration. **Frontiers in Cellular Neuroscience**, v. 19, 2025. DOI: 10.3389/fncel.2025.1570596.

OLEKSAK, P.; NEPOVIMOVA, E.; VALKO, M.; ALWASEL, S.; ALOMAR, S.; KUCA, K. Comprehensive analysis of prohibited substances and methods in sports: Unveiling trends, pharmacokinetics, and WADA evolution. **Environmental Toxicology and Pharmacology**, v. 108, p. 104447, 2024. DOI: 10.1016/j.etap.2024.104447.

OYADOMARI, S.; MORI, M. Roles of CHOP/GADD153 in endoplasmic reticulum stress. **Cell Death and Differentiation**, v. 11, n. 4, p. 381–389, 2004. DOI: 10.1038/sj.cdd.4401373.

PAGLIARINI, D. J.; RUTTER, J. Hallmarks of a new era in mitochondrial biochemistry. **Genes & Development**, v. 27, n. 24, p. 2615–2627, 2013. DOI: 10.1101/gad.229724.113.

PALTY, R.; SILVERMAN, W. F.; HERSHFINKEL, M.; CAPORALE, T. et al. NCLX is an essential component of mitochondrial Na+/Ca2+ exchange. **Proceedings of the National Academy of Sciences of the United States of America**, v. 107, n. 1, p. 436–441, 2010. DOI: 10.1073/pnas.0908099107.

PARR, M. K.; BOTRÈ, F.; NAß, A.; HENGEVOSS, J.; DIEL, P.; WOLBER, G. Ecdysteroids: A novel class of anabolic agents? **Biology of Sport**, v. 32, n. 2, p. 169–173, 2015. DOI: 10.5604/20831862.1144420.

PAVLATOS, A. M.; FULTZ, O.; MONBERG, M. J.; VOOTKUR, A.; PHARMD. Review of oxymetholone: A 17alpha-alkylated anabolic-androgenic steroid. **Clinical Therapeutics**, v. 23, n. 6, p. 789–801; discussion 771, 2001. DOI: 10.1016/s0149-2918(01)80070-9.

PELTON, L. M.; MARIS, S. A.; LOSEKE, J. The effects of anabolic-androgenic steroids on gene expression in skeletal muscle: A systematic review. **International Journal of Exercise Science**, v. 16, n. 3, p. 53–82, 2023. DOI: 10.70252/QFNY6413.

PENATTI, C. A. A.; PORTER, D. M.; HENDERSON, L. P. Chronic exposure to anabolic androgenic steroids alters neuronal function in the mammalian forebrain via androgen receptor- and estrogen receptor-mediated mechanisms. **The Journal of Neuroscience: The Official Journal of the Society for Neuroscience**, v. 29, n. 40, p. 12484–12496, 2009. DOI: 10.1523/JNEUROSCI.3108-09.2009.

PEREL, P.; YANAGAWA, T.; BUNN, F.; ROBERTS, I.; WENTZ, R.; PIERRO, A. Nutritional support for head-injured patients. **The Cochrane Database of Systematic Reviews**, v. 2006, n. 4, CD001530, 2006. DOI: 10.1002/14651858.CD001530.pub2.

PETROVIC, A.; VUKADIN, S.; SIKORA, R.; BOJANIC, K.; SMOLIC, R.; PLAVEC, D.; WU, G. Y.; SMOLIC, M. Anabolic androgenic steroid-induced liver injury: An update. **World Journal of Gastroenterology**, v. 28, n. 26, p. 3071–3080, 2022. DOI: 10.3748/wjg.v28.i26.3071.

PFANNER, N.; WARSCHEID, B.; WIEDEMANN, N. Mitochondrial proteins: From biogenesis to functional networks. **Nature Reviews Molecular Cell Biology**, v. 20, n. 5, p. 267–284, 2019. DOI: 10.1038/s41580-018-0092-0.

PICARD, M.; McMANUS, M. J.; CSORDÁS, G.; VÁRNAI, P.; DORN, G. W.; WILLIAMS, D.; HAJNÓCZKY, G.; WALLACE, D. C. Trans-mitochondrial coordination of cristae at regulated membrane junctions. **Nature Communications**, v. 6, p. 6259, 2015. DOI: 10.1038/ncomms7259.

PINNA, G.; AGIS-BALBOA, R. C.; PIBIRI, F.; NELSON, M.; GUIDOTTI, A.; COSTA, E. Neurosteroid biosynthesis regulates sexually dimorphic fear and aggressive behavior in mice. **Neurochemical Research**, v. 33, n. 10, p. 1990–2007, 2008. DOI: 10.1007/s11064-008-9718-5.

POMARA, C.; NERI, M.; BELLO, S.; FIORE, C.; RIEZZO, I.; TURILLAZZI, E. Neurotoxicity by synthetic androgen steroids: Oxidative stress, apoptosis, and neuropathology: A review. **Current Neuropharmacology**, v. 13, n. 1, p. 132–145, 2015. DOI: 10.2174/1570159X13666141210221434.

POPE, H. G.; KATZ, D. L. Homicide and near-homicide by anabolic steroid users. **The Journal of Clinical Psychiatry**, v. 51, n. 1, p. 28–31, 1990.

POPE, H. G.; WOOD, R. I.; ROGOL, A.; NYBERG, F.; BOWERS, L.; BHASIN, S. Adverse health consequences of performance-enhancing drugs: An Endocrine Society scientific statement. **Endocrine Reviews**, v. 35, n. 3, p. 341–375, 2014. DOI: 10.1210/er.2013-1058.

QIAN, S.; WEI, Z.; YANG, W.; HUANG, J.; YANG, Y.; WANG, J. The role of BCL-2 family proteins in regulating apoptosis and cancer therapy. **Frontiers in Oncology**, v. 12, 2022. DOI: 10.3389/fonc.2022.985363.

RAMOS, V. M.; SERNA, J. D. C.; VILAS-BOAS, E. A.; CABRAL-COSTA, J. V.; CUNHA, F. M.; KATAURA, T.; KOROLCHUK, V. I.; KOWALTOWSKI, A. J. Mitochondrial sodium/calcium exchanger (NCLX) regulates basal and starvation-induced autophagy through calcium signaling. **FASEB Journal**, v. 38, n. 3, e23454, 2024. DOI: 10.1096/fj.202301368RR.

ROCHA, F. L.; CARMO, E. C.; ROQUE, F. R.; HASHIMOTO, N. Y.; ROSSONI, L. V.; FRIMM, C.; ANÉAS, I.; NEGRÃO, C. E.; KRIEGER, J. E.; OLIVEIRA, E. M. Anabolic steroids induce cardiac renin-angiotensin system and impair the beneficial effects of aerobic training in rats. **American Journal of Physiology. Heart and Circulatory Physiology**, v. 293, n. 6, p. H3575–H3583, 2007. DOI: 10.1152/ajpheart.01251.2006.

RODOLPHI, M. S.; KOPCZYNSKI, A.; CARTERI, R. B.; SARTOR, M.; FONTELLA, F. U.; FELDMANN, M.; HANSEL, G.; STROGULSKI, N. R.; PORTELA, L. V. Glutamate transporter-1 link astrocytes with heightened aggressive behavior induced by steroid abuse in male CF1 mice. **Hormones and Behavior**, v. 127, p. 104872, 2021. DOI: 10.1016/j.yhbeh.2020.104872.

ROOZENBEEK, B.; MAAS, A. I. R.; MENON, D. K. Changing patterns in the epidemiology of traumatic brain injury. **Nature Reviews Neurology**, v. 9, n. 4, p. 231–236, 2013. DOI: 10.1038/nrneurol.2013.22.

ROSE, J.; BRIAN, C.; PAPPA, A.; PANAYIOTIDIS, M. I.; FRANCO, R. Mitochondrial metabolism in astrocytes regulates brain bioenergetics, neurotransmission and redox balance. **Frontiers in Neuroscience**, v. 14, 536682, 2020. DOI: 10.3389/fnins.2020.536682.

ROSSBACH, U. L. W.; STEENSLAND, P.; NYBERG, F.; LE GREVÈS, P. Nandrolone-induced hippocampal phosphorylation of NMDA receptor subunits and ERKs. **Biochemical and Biophysical Research Communications**, v. 357, n. 4, p. 1028–1033, 2007. DOI: 10.1016/j.bbrc.2007.04.037.

ROWLAND, A. A.; VOELTZ, G. K. Endoplasmic reticulum-mitochondria contacts: Function of the junction. **Nature Reviews Molecular Cell Biology**, v. 13, n. 10, p. 607–625, 2012. DOI: 10.1038/nrm3440.

RUSSELL, D. W.; WILSON, J. D. Steroid 5 alpha-reductase: Two genes/two enzymes. **Annual Review of Biochemistry**, v. 63, p. 25–61, 1994. DOI: 10.1146/annurev.bi.63.070194.000325.

SAARTOK, T.; DAHLBERG, E.; GUSTAFSSON, J. A. Relative binding affinity of anabolic-androgenic steroids: Comparison of the binding to the androgen receptors in skeletal muscle and in prostate, as well as to sex hormone-binding globulin. **Endocrinology**, v. 114, n. 6, p. 2100–2106, 1984. DOI: 10.1210/endo-114-6-2100.

SAEED, A.; VAUGHT, G. M.; GAVARDINAS, K.; MATTHEWS, D.; GREEN, J. E.; et al. 2-Chloro-4-[[(1R,2R)-2-hydroxy-2-methyl-cyclopentyl]amino]-3-methyl-benzonitrile: A transdermal selective androgen receptor modulator (SARM) for muscle atrophy. **Journal of Medicinal Chemistry**, v. 59, n. 2, p. 750–755, 2016. DOI: 10.1021/acs.jmedchem.5b01168.

SCHIEBER, M.; CHANDEL, N. S. ROS function in redox signaling and oxidative stress. **Current Biology**, v. 24, n. 10, p. R453–R462, 2014. DOI: 10.1016/j.cub.2014.03.034.

SCHMIDT, A. et al. Discovery of the selective androgen receptor modulator MK-0773 using a rational development strategy based on differential transcriptional requirements for androgenic anabolism versus reproductive physiology. **The Journal of Biological Chemistry**, v. 285, n. 22, p. 17054–17064, 2010. DOI: 10.1074/jbc.M109.099002.

SE, M.; C, L.; M, W.; KS, T.; TA, S. Traumatic brain injuries: Manifestations, evaluation, management, and outcomes. **The Primary Care Companion for CNS Disorders**, v. 26, n. 3, 2024. DOI: 10.4088/PCC.23f03667.

SHACKLETON, C. Steroid analysis and doping control 1960–1980: Scientific developments and personal anecdotes. **Steroids**, v. 74, n. 3, p. 288–295, 2009. DOI: 10.1016/j.steroids.2008.10.002.

SHAHIDI, N. T. A review of the chemistry, biological action, and clinical applications of anabolic-androgenic steroids. **Clinical Therapeutics**, v. 23, n. 9, p. 1355–1390, 2001. DOI: 10.1016/s0149-2918(01)80114-4.

SHIN, B.; COWAN, D. B.; EMANI, S. M.; DEL NIDO, P. J.; McCULLY, J. D. Mitochondrial transplantation in myocardial ischemia and reperfusion injury. **Advances in Experimental Medicine and Biology**, v. 982, p. 595–619, 2017. DOI: 10.1007/978-3-319-55330-6_31.

SIMPSON, E. R. et al. Aromatase — a brief overview. **Annual Review of Physiology**, v. 64, p. 93–127, 2002. DOI: 10.1146/annurev.physiol.64.081601.142703.

SINGAM, E. R. A.; TACHACHARTVANICH, P.; MERRILL, M. A. L.; SMITH, M. T.; DURKIN, K. A. Structural dynamics of agonist and antagonist binding to the androgen receptor. **The Journal of Physical Chemistry B**, v. 123, n. 36, p. 7657–7666, 2019. DOI: 10.1021/acs.jpcb.9b05654.

SINGH, L. et al. Expression of pro-apoptotic Bax and anti-apoptotic Bcl-2 proteins in human retinoblastoma. **Clinical & Experimental Ophthalmology**, v. 43, n. 3, p. 259–267, 2015. DOI: 10.1111/ceo.12397.

SINHA-HIKIM, I. et al. Testosterone-induced increase in muscle size in healthy young men is associated with muscle fiber hypertrophy. **American Journal of Physiology – Endocrinology and Metabolism**, v. 283, n. 1, p. E154–E164, 2002. DOI: 10.1152/ajpendo.00502.2001.

SINHA-HIKIM, I.; ROTH, S. M.; LEE, M. I.; BHASIN, S. Testosterone-induced muscle hypertrophy is associated with an increase in satellite cell number in healthy, young men. **American Journal of Physiology – Endocrinology and Metabolism**, v. 285, n. 1, p. E197–E205, 2003. DOI: 10.1152/ajpendo.00370.2002.

SIRIANNI, R. et al. Nandrolone and stanozolol upregulate aromatase expression and further increase IGF-I-dependent effects on MCF-7 breast cancer cell proliferation. **Molecular and Cellular Endocrinology**, v. 363, n. 1–2, p. 100–110, 2012. DOI: 10.1016/j.mce.2012.08.002.

SJÖQVIST, F.; GARLE, M.; RANE, A. Use of doping agents, particularly anabolic steroids, in sports and society. **The Lancet**, v. 371, n. 9627, p. 1872–1882, 2008. DOI: 10.1016/S0140-6736(08)60801-6.

SNYDER, P. J. Clinical use of androgens. **Annual Review of Medicine**, v. 35, p. 207–217, 1984. DOI: 10.1146/annurev.me.35.020184.001231.

SOLAR, P.; VELASQUEZ, L. Consequences of nongenomic actions of estradiol on pathogenic genital tract response. **Journal of Molecular Signaling**, v. 8, n. 1, p. 1, 2013. DOI: 10.1186/1750-2187-8-1.

SOLOMON, Z. J. et al. Selective androgen receptor modulators: Current knowledge and clinical applications. **Sexual Medicine Reviews**, v. 7, n. 1, p. 84–94, 2019. DOI: 10.1016/j.sxmr.2018.09.006.

SONG, N.; MEI, S.; WANG, X.; HU, G.; LU, M. Focusing on mitochondria in the brain: From biology to therapeutics. **Translational Neurodegeneration**, v. 13, n. 1, p. 23, 2024. DOI: 10.1186/s40035-024-00409-w.

STAUCH, K. L.; PURNELL, P. R.; FOX, H. S. Quantitative proteomics of synaptic and nonsynaptic mitochondria: Insights for synaptic mitochondrial vulnerability. **Journal of Proteome Research**, v. 13, n. 5, p. 2620–2636, 2014. DOI: 10.1021/pr500295n.

STAVSKY, A. et al. Aberrant activity of mitochondrial NCLX is linked to impaired synaptic transmission and is associated with mental retardation. **Communications Biology**, v. 4, n. 1, p. 666, 2021. DOI: 10.1038/s42003-021-02114-0.

STRASSER, A.; O'CONNOR, L.; DIXIT, V. M. Apoptosis signaling. **Annual Review of Biochemistry**, v. 69, p. 217–245, 2000. DOI: 10.1146/annurev.biochem.69.1.217.

SWISLOCKI, A. L. M.; EISENBERG, M. L. A review on testosterone: estradiol ratio — does it matter, how do you measure it, and can you optimize it? **The World Journal of Men's Health**, v. 43, n. 3, p. 453–464, 2025. DOI: 10.5534/wjmh.240029.

TAKEDA, A.-N. et al. The synthetic androgen methyltrienolone (r1881) acts as a potent antagonist of the mineralocorticoid receptor. **Molecular Pharmacology**, v. 71, n. 2, p. 473–482, 2007. DOI: 10.1124/mol.106.031112.

TANG, Y. et al. Regulation of osteoblast autophagy based on PI3K/AKT/mTOR signaling pathway: study on the effect of β-ecdysterone on fracture healing. **Journal of Orthopaedic Surgery and Research**, v. 16, n. 1, p. 719, 2021. DOI: 10.1186/s13018-021-02862-z.

TANRIVERDI, F. et al. High risk of hypopituitarism after traumatic brain injury: a prospective investigation of anterior pituitary function in the acute phase and 12 months after trauma. **The Journal of Clinical Endocrinology & Metabolism**, v. 91, n. 6, p. 2105–2111, 2006. DOI: 10.1210/jc.2005-2476.

TARASOV, A. I.; GRIFFITHS, E. J.; RUTTER, G. A. Regulation of ATP production by mitochondrial Ca2+. **Cell Calcium**, v. 52, n. 1, p. 28–35, 2012. DOI: 10.1016/j.ceca.2012.03.003.

TARASOV, A. I. et al. The mitochondrial Ca2+ uniporter MCU is essential for glucose-induced ATP increases in pancreatic β-cells. **PloS One**, v. 7, n. 7, e39722, 2012. DOI: 10.1371/journal.pone.0039722.

TEASDALE, G.; JENNETT, B. Assessment of coma and impaired consciousness: a practical scale. **The Lancet**, v. 2, n. 7872, p. 81–84, 1974. DOI: 10.1016/s0140-6736(74)91639-0.

THEVIS, M.; SCHÄNZER, W. Mass spectrometry in sports drug testing: structure characterization and analytical assays. **Mass Spectrometry Reviews**, v. 26, n. 1, p. 79–107, 2007. DOI: 10.1002/mas.20107.

THIBLIN, I.; RUNESON, B.; RAJS, J. Anabolic androgenic steroids and suicide. **Annals of Clinical Psychiatry**, v. 11, n. 4, p. 223–231, 1999. DOI: 10.1023/a:1022313529794.

THOMAS, P.; PANG, Y.; DONG, J. Membrane androgen receptor characteristics of human ZIP9 (SLC39A) zinc transporter in prostate cancer cells: androgen-specific activation and involvement of an inhibitory G protein in zinc and MAP kinase signaling. **Molecular and Cellular Endocrinology**, v. 447, p. 23–34, 2017. DOI: 10.1016/j.mce.2017.02.025.

TÔRRES, S. G.; BALDO, J. H. L.; PROPÉRCIO, A. A. Perfil epidemiológico do trauma cranioencefálico na região norte do Brasil entre 2010 e 2020. **Facit Business and Technology Journal**, v. 2, n. 31, 2021. Disponível em: https://revistas.faculdadefacit.edu.br/index.php/JNT/article/view/1327.

TORRISI, M. et al. Sudden cardiac death in anabolic-androgenic steroid users: a literature review. **Medicina**, v. 56, n. 11, p. 587, 2020. DOI: 10.3390/medicina56110587.

TÓTH, M.; ZAKÁR, T. Relative binding affinities of testosterone, 19-nortestosterone and their 5 alpha-reduced derivatives to the androgen receptor and to other androgen-binding proteins: a suggested role of 5 alpha-reductive steroid metabolism in the dissociation of “myotropic” and “androgenic” activities of 19-nortestosterone. **Journal of Steroid Biochemistry**, v. 17, n. 6, p. 653–660, 1982. DOI: 10.1016/0022-4731(82)90567-2.

TURILLAZZI, E. et al. Lipid peroxidation and apoptotic response in rat brain areas induced by long-term administration of nandrolone: the mutual crosstalk between ROS and NF-kB. **Journal of Cellular and Molecular Medicine**, v. 20, n. 4, p. 601–612, 2016a. DOI: 10.1111/jcmm.12748.

TURILLAZZI, E. et al. Lipid peroxidation and apoptotic response in rat brain areas induced by long-term administration of nandrolone: the mutual crosstalk between ROS and NF-kB. **Journal of Cellular and Molecular Medicine**, v. 20, n. 4, p. 601–612, 2016b. DOI: 10.1111/jcmm.12748.

UNSWORTH, D. J.; MATHIAS, J. L. Traumatic brain injury and alcohol/substance abuse: a Bayesian meta-analysis comparing the outcomes of people with and without a history of abuse. **Journal of Clinical and Experimental Neuropsychology**, v. 39, n. 6, p. 547–562, 2017. DOI: 10.1080/13803395.2016.1248812.

URESHINO, R. P. et al. The interplay between Ca2+ signaling pathways and neurodegeneration. **International Journal of Molecular Sciences**, v. 20, n. 23, p. 6004, 2019. DOI: 10.3390/ijms20236004.

VAN AMSTERDAM, J.; OPPÉRHUIZEN, A.; HARTGENS, F. Adverse health effects of anabolic-androgenic steroids. **Regulatory Toxicology and Pharmacology**, v. 57, n. 1, p. 117–123, 2010. DOI: 10.1016/j.yrtph.2010.02.001.

VAN DER VIES, J. Pharmacokinetics of anabolic steroids. **Wiener Medizinische Wochenschrift**, v. 143, n. 14–15, p. 366–368, 1993.

VAN VLIET, A. R.; VERFAILLIE, T.; AGOSTINIS, P. New functions of mitochondria associated membranes in cellular signaling. **Biochimica et Biophysica Acta**, v. 1843, n. 10, p. 2253–2262, 2014. DOI: 10.1016/j.bbamcr.2014.03.009.

VERMA, M.; LIZAMA, B. N.; CHU, C. T. Excitotoxicity, calcium and mitochondria: a triad in synaptic neurodegeneration. **Translational Neurodegeneration**, v. 11, n. 1, p. 3, 2022. DOI: 10.1186/s40035-021-00278-7.

VERWEIJ, B. H. et al. Impaired cerebral mitochondrial function after traumatic brain injury in humans. **Journal of Neurosurgery**, v. 93, n. 5, p. 815–820, 2000. DOI: 10.3171/jns.2000.93.5.0815.

VILJANTO, M. J. et al. Elucidation of the biosynthetic pathways of boldenone in the equine testis. **Steroids**, v. 146, p. 79–91, 2019. DOI: 10.1016/j.steroids.2019.03.011.

VÖLGYI, K. et al. Synaptic mitochondria: a brain mitochondria cluster with a specific proteome. **Journal of Proteomics**, v. 120, p. 142–157, 2015a. DOI: 10.1016/j.jprot.2015.03.005.

VÖLGYI, K. et al. Synaptic mitochondria: a brain mitochondria cluster with a specific proteome. **Journal of Proteomics**, v. 120, p. 142–157, 2015b. DOI: 10.1016/j.jprot.2015.03.005.

VOS, M.; LAUWERS, E.; VERSTREKEN, P. Synaptic mitochondria in synaptic transmission and organization of vesicle pools in health and disease. **Frontiers in Synaptic Neuroscience**, v. 2, 2010. DOI: 10.3389/fnsyn.2010.00139.

WAGNER, J. C. Enhancement of athletic performance with drugs: an overview. **Sports Medicine**, v. 12, n. 4, p. 250–265, 1991. DOI: 10.2165/00007256-199112040-00004.

WALKER, J. E. The ATP synthase: the understood, the uncertain and the unknown. **Biochemical Society Transactions**, v. 41, n. 1, p. 1–16, 2013. DOI: 10.1042/BST20110773.

WALKON, L. L.; STRUBBE-RIVERA, J. O.; BAZIL, J. N. Calcium overload and mitochondrial metabolism. **Biomolecules**, v. 12, n. 12, p. 1891, 2022. DOI: 10.3390/biom12121891.

WANG, C.; LIU, Y.; CAO, J.-M. G protein-coupled receptors: extranuclear mediators for the non-genomic actions of steroids. **International Journal of Molecular Sciences**, v. 15, n. 9, p. 15412–15425, 2014. DOI: 10.3390/ijms150915412.

WEHLING, M. Specific, nongenomic actions of steroid hormones. **Annual Review of Physiology**, v. 59, p. 365–393, 1997. DOI: 10.1146/annurev.physiol.59.1.365.

WERNER, C.; ENGELHARD, K. Pathophysiology of traumatic brain injury. **British Journal of Anaesthesia**, v. 99, n. 1, p. 4–9, 2007. DOI: 10.1093/bja/aem131.

WEST, S. L. Substance use among persons with traumatic brain injury: a review. **NeuroRehabilitation**, v. 29, n. 1, p. 1–8, 2011. DOI: 10.3233/NRE-2011-0671.

WESTLYE, L. T. et al. Brain connectivity aberrations in anabolic-androgenic steroid users. **NeuroImage: Clinical**, v. 13, p. 62–69, 2017. DOI: 10.1016/j.nicl.2016.11.014.

WILSON, J. D. Androgen abuse by athletes. **Endocrine Reviews**, v. 9, n. 2, p. 181–199, 1988. DOI: 10.1210/edrv-9-2-181.

WILSON, J. D. Role of dihydrotestosterone in androgen action. **The Prostate. Supplement**, v. 6, p. 88–92, 1996.

WINKLER, E. A.; MINTER, D.; YUE, J. K.; MANLEY, G. T. Cerebral edema in traumatic brain injury: pathophysiology and prospective therapeutic targets. **Neurosurgery Clinics of North America**, v. 27, n. 4, p. 473–488, 2016. DOI: 10.1016/j.nec.2016.05.008.

WOLF, S. E. et al. Effects of oxandrolone on outcome measures in the severely burned: a multicenter prospective randomized double-blind trial. **Journal of Burn Care & Research**, v. 27, n. 2, p. 131–139; discussion 140–141, 2006. DOI: 10.1097/01.BCR.0000202620.55751.4F.

WOODHOUSE, L. J. et al. Dose-dependent effects of testosterone on regional adipose tissue distribution in healthy young men. **The Journal of Clinical Endocrinology and Metabolism**, v. 89, n. 2, p. 718–726, 2004. DOI: 10.1210/jc.2003-031492.

XU, X. et al. Dynamic communication between androgen and coactivator: mutually induced conformational perturbations in androgen receptor ligand-binding domain. **Proteins**, v. 79, n. 4, p. 1154–1171, 2011. DOI: 10.1002/prot.22951.

YAN, J.; WANG, C.; SUN, B. Global, regional, and national burdens of traumatic brain injury from 1990 to 2021. **Frontiers in Public Health**, v. 13, p. 1556147, 2025. DOI: 10.3389/fpubh.2025.1556147.

ZHANG, J. et al. ROS and ROS-mediated cellular signaling. **Oxidative Medicine and Cellular Longevity**, 2016, p. 4350965, 2016. DOI: 10.1155/2016/4350965.

ZOROV, D. B.; JUHASZOVA, M.; SOLLOTT, S. J. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. **Physiological Reviews**, v. 94, n. 3, p. 909–950, 2014. DOI: 10.1152/physrev.00026.2013.

6 PRODUÇÃO CIENTÍFICA COMPLEMENTAR À TESE

Adicionalmente à produção central desta tese foi produzido um artigo científico que está em preparação, que apresenta um modelo de TCEs repetidos com uma ampla caracterização comportamental e correlações entre a bioenergética mitocôndrial e o metabolismo cerebral de glicose (Anexo I); orientação de trabalho de conclusão de curso, composta de uma revisão sistemática intitulada "depressão maior e seus estudos e modelos animais: uma revisão da literatura" do aluno Pedro Henrique da Rosa Corrêa do curso de Biomedicina da UFRGS (Anexo II); participação em banca de trabalho de conclusão de curso intitulado "Dietoterapia como estratégia adjuvante ao tratamento de sintomas de depressão: uma revisão sistemática" apresentado pela aluna Bruna Valdameri do curso de Nutrição da UFRGS (Anexo III); co-orientação de trabalho de conclusão de curso, composta de uma revisão sistemática intitulada "Suplementação de beta-alanina no treinamento de força: uma revisão narrativa da aluna Maria Eduarda Pinko Santa Helena do curso de Nutrição da UFRGS (Anexo IV); participação no salão de iniciação cientícifa da UFRGS como membro da banca avaliadora nas sessões do curso de medicina e de educação física (anexo V).

ANEXO I: REPEATED HEAD INJURIES PROGRESSIVELY PROMOTE FUNCTIONAL AND METABOLIC IMPAIRMENT IN MICE

Rodolphi, MS.1; Strogulski, NR.2; Portela, LV1.

1. Neurotrauma and Biomarkers Laboratory, Departamento de Bioquímica, Universidade Federal do Rio Grande do Sul, Brazil.
2. Neuroinflammation and Neurotrauma Research, School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland.

Results:

1. *Repeated head injury leads to progressive loss in fine, and not gross motor function.*

We observed a progressive loss in fine motor function that grows along with the number of hits. This is indicated by a significant reduction on the latency to fall in a rotarod apparatus comparing sham, 1, and 3 repeated head injuries. Latency to fall in mice submitted to 5 repeated head injuries was reduced compared to sham and 1 head injury, yet no significance is observed when comparing 5 and 3 repeated head injuries (Figure 2A). Further, no differences in gross locomotor function across all the RHI spectrum was observed, when assessing the total distance travelled in the open field maze, as well as the mean speed in the Morris water maze apparatus (Figures 2 B,C).

2. *Behavioral burden changes with increased exposure to injury*

We found that only mice submitted to 5 RHI displayed anxiety-related thigmotactic behaviour in the open field compared to Sham (Figure 3A). This dose-dependent effect of RHI over anxiety-associated endophenotypes is also observed in the elevated plus maze paradigm, where both 3 and 5 RHI lead to increase in the time spent in the closed arms of the apparatus (Figure 3B). Further, data from the light dark box paradigm suggests an overall decrease in time spent in the light zone across all RHI groups compared to sham (Figure 3C). Anhedonic behaviour was also observed across all the spectrum of RHI, indicated by an increase in immobile time in the forced swim test of all 1, 3 and 5 hit groups compared to Sham (Figure 3D).

Cognitive performance was progressively impaired by the number of head injuries. Mice display decreased working memory (indicated by the % of spontaneous alternations in the Y-maze), which is already apparent at 1 hit, yet only is significantly reduced at 3 and 5 hits (Figure 3E). Spatial memory is also impaired in a dose-dependent fashion by RHI. Training phase of the Morris water maze suggests an impairment in memory acquisition in mice submitted to 3 and 5 hits compared to 1 hit and Sham (Figure 3F). Further, data from the test phase of the Morris water maze suggests that there is a progressive increase in the time required to reach the platform zone, which parallels the number of hits (Figure 3G). Further, injury led to a reduction in the time spent in the target zone of the apparatus, regardless of the number of hits (Figure 3H).

3. Head injuries lead to additive impairment to brain metabolism.

Mitochondrial metabolism is impaired in injured mice, and a trend towards a progressive loss of mitochondrial basal respiration is observed across 1, 3 and 5 hits (Figure 4A). In a similar fashion, regional brain glucose uptake is also decreased following hits. Interestingly, the variation between uptake at baseline and after RHI is higher in 1 and 5 hits compared to 3 hits (Figure 4B and C). Further, a progressive loss of inter-regional correlations in glucose uptake is observed, which follows the number of hits. We found that the number of metabolic correlations lost after injury increases across the continuum of number of injuries (Figure 4 D). Despite glucose hypometabolism not displaying any particular pattern within the injured brain across injuries (Figure 4B and C), metabolic connectivity loss involves regions in common. Particularly, Amygdala and inferior colliculi display loss in connectivity already from 1 hit on, and at 5 hits are major nodes of interregional connectivity loss (Figure 4D).

Figura 1

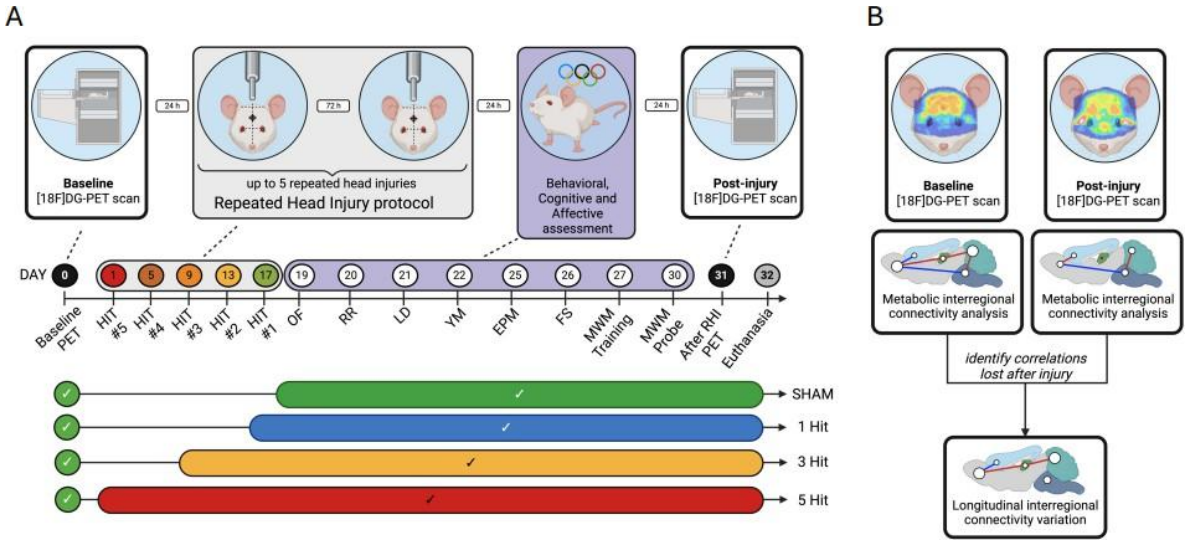


Figura 2

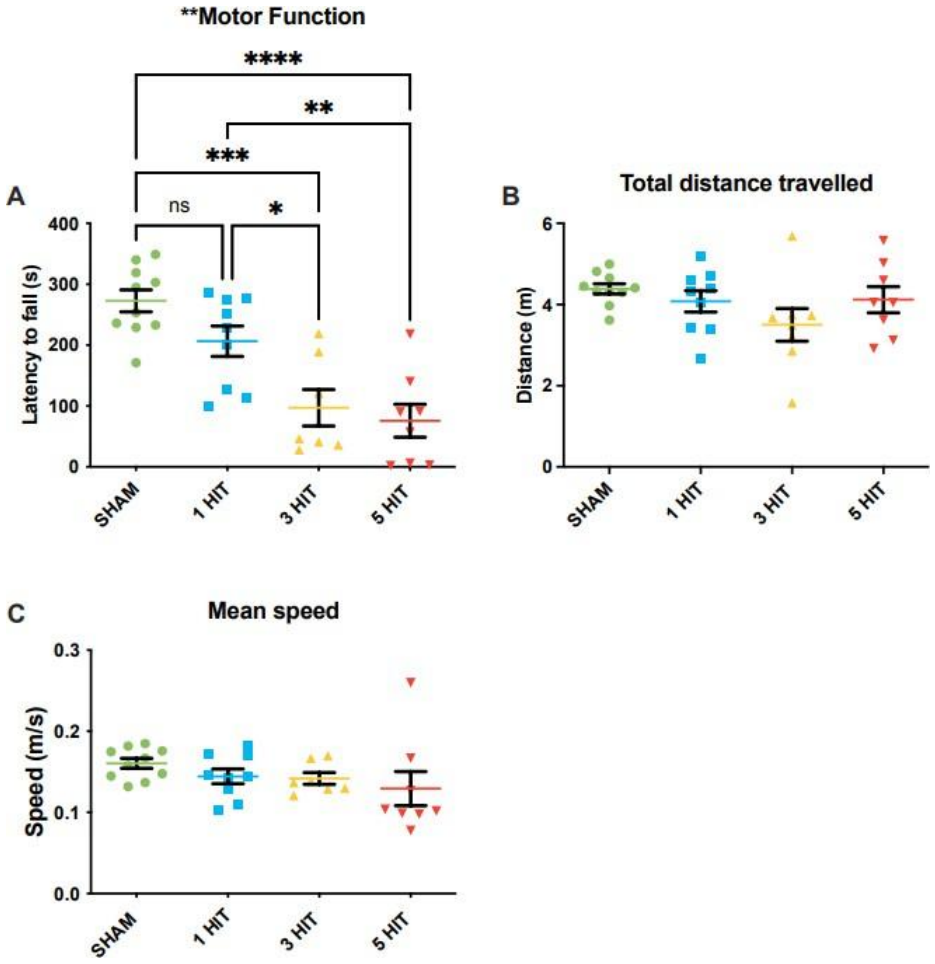


Figura 3

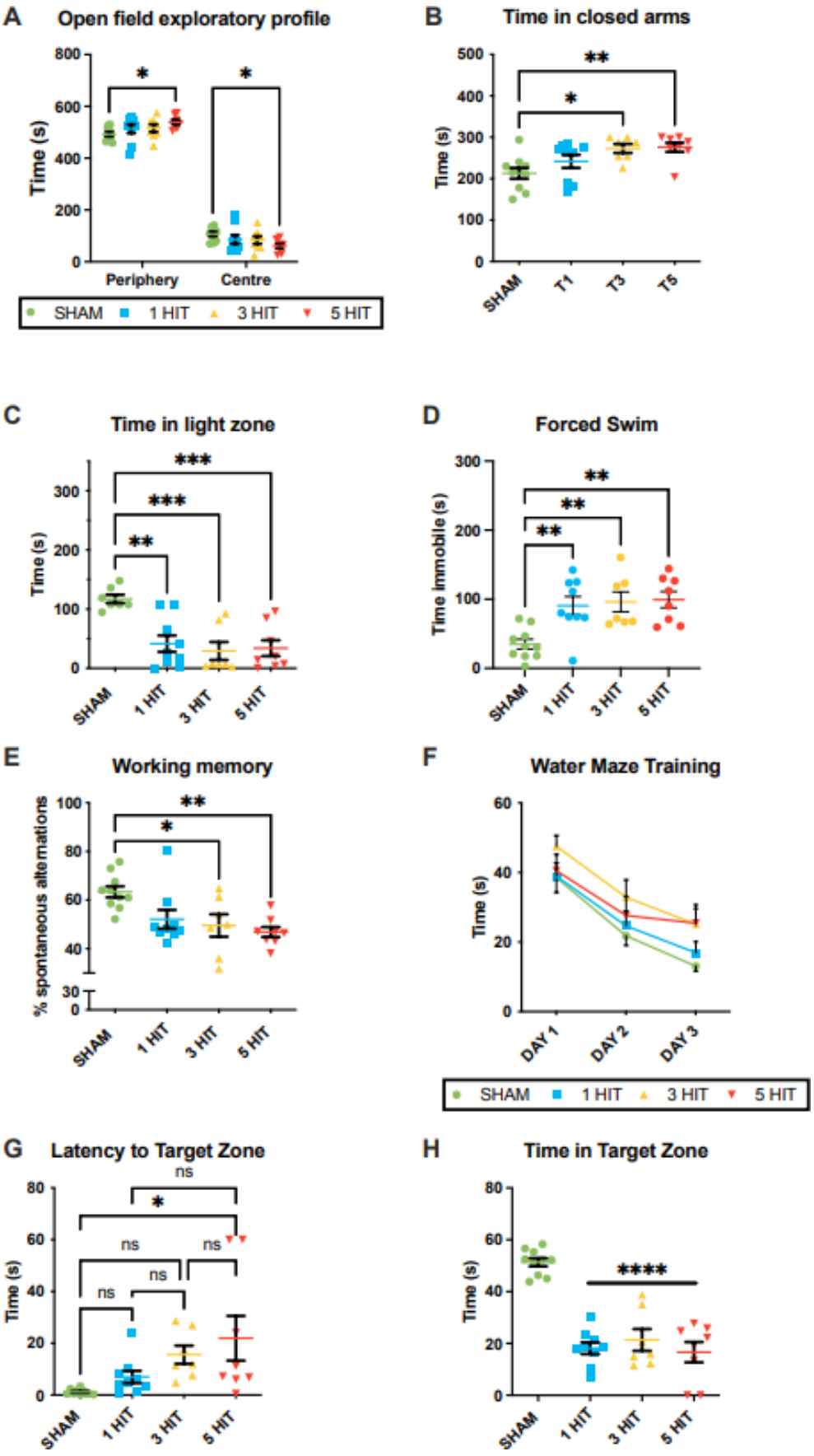

A
Open field exploratory profile
Time (s)
Periphery
Centre
SHAM
1 HIT
3 HIT
5 HIT
B
Time in closed arms
Time (s)
SHAM
T1
T3
T5
C
Time in light zone
Time (s)
D
Forced Swim
Time immobile (s)
E
Working memory
% spontaneous alternations
F
Water Maze Training
Time (s)
DAY 1
DAY 2
DAY 3
G
Latency to Target Zone
Time (s)
ns
H
Time in Target Zone
Time (s)

Figura 4

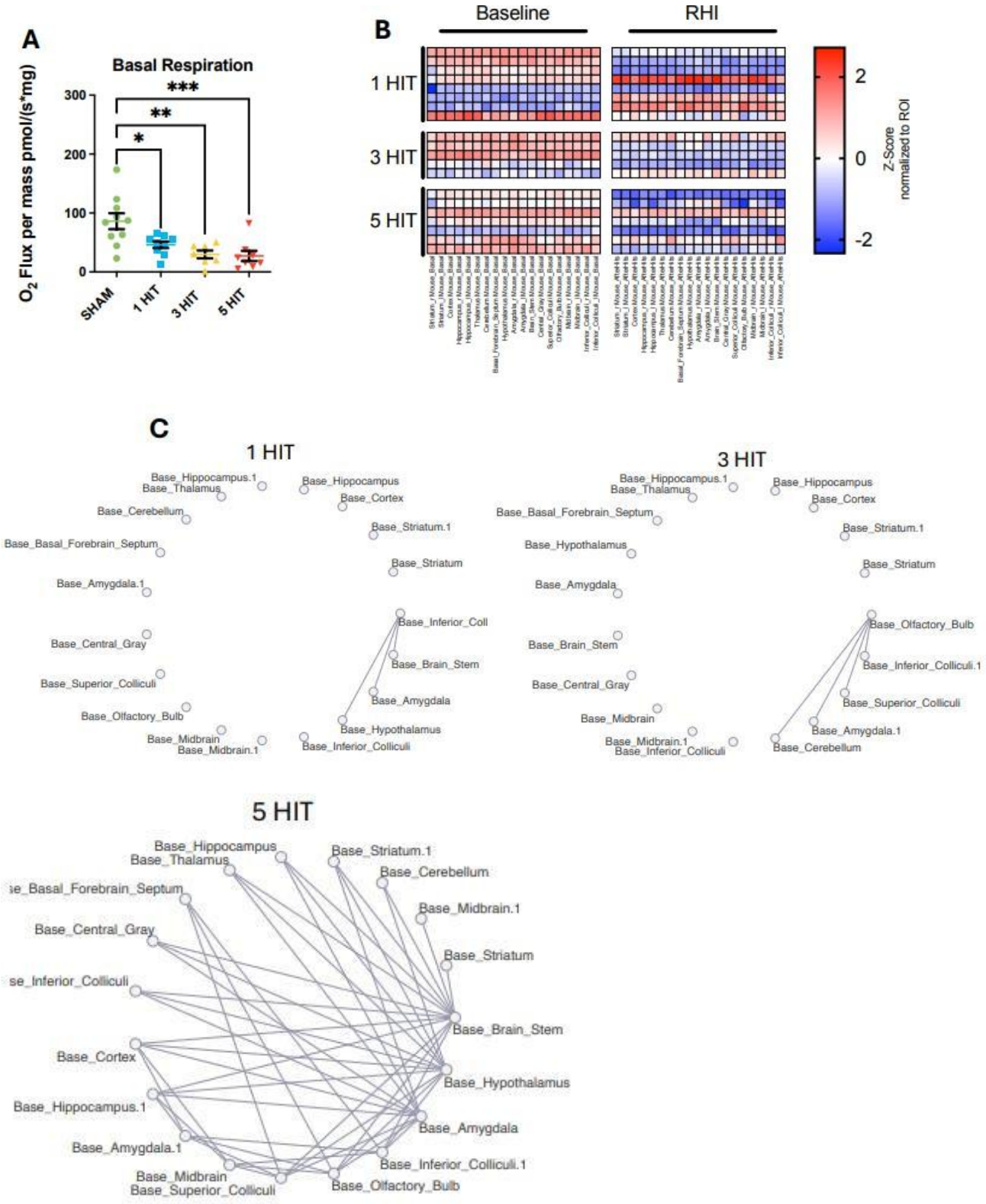

A
Basal Respiration
O_2 Flux per mass pmol/(s*mg)
300
200
100
0
***
**
*
SHAM
1 HIT
3 HIT
5 HIT
B
Baseline
RHI
1 HIT
3 HIT
5 HIT
2
0
-2
Z-Score
normalized to ROI
C
1 HIT
Base_Hippocampus.1
Base_Thalamus
Base_Hippocampus
Base_Cortex
Base_Cerebellum
Base_Striatum.1
Base_Basal_Forebrain_Septum
Base_Striatum
Base_Amygdala.1
Base_Inferior_Coll
Base_Central_Gray
Base_Brain_Stem
Base_Superior_Colliculi
Base_Amygdala
Base_Olfactory_Bulb
Base_Hypothalamus
Base_Midbrain
Base_Midbrain.1
Base_Inferior_Colliculi
3 HIT
Base_Hippocampus.1
Base_Thalamus
Base_Hippocampus
Base_Cortex
Base_Basal_Forebrain_Septum
Base_Striatum.1
Base_Hypothalamus
Base_Striatum
Base_Amygdala
Base_Olfactory_Bulb
Base_Brain_Stem
Base_Inferior_Colliculi.1
Base_Central_Gray
Base_Superior_Colliculi
Base_Midbrain
Base_Amygdala.1
Base_Midbrain.1
Base_Inferior_Colliculi
Base_Cerebellum
5 HIT
Base_Hippocampus
Base_Thalamus
Base_Striatum.1
Base_Cerebellum
ıe_Basal_Forebrain_Septum
Base_Midbrain.1
Base_Central_Gray
Base_Striatum
se_Inferior_Colliculi
Base_Brain_Stem
Base_Cortex
Base_Hypothalamus
Base_Hippocampus.1
Base_Amygdala
Base_Amygdala.1
Base_Inferior_Colliculi.1
Base_Midbrain
Base_Superior_Colliculi
Base_Olfactory_Bulb

Figura 4 B

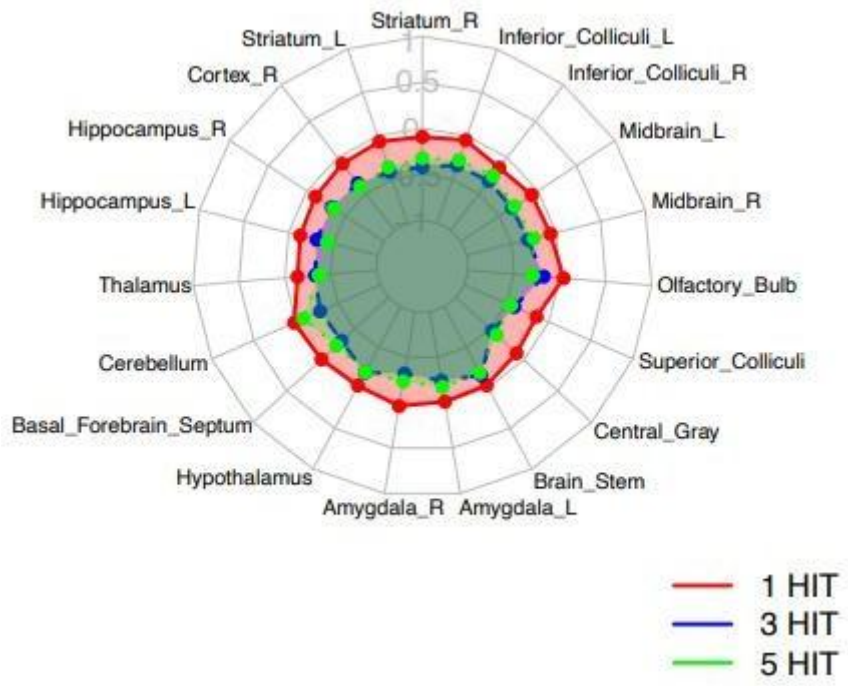
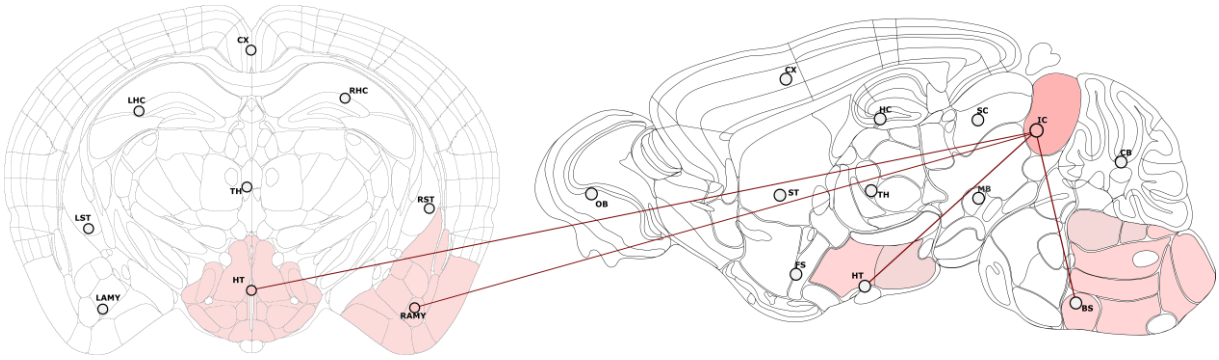
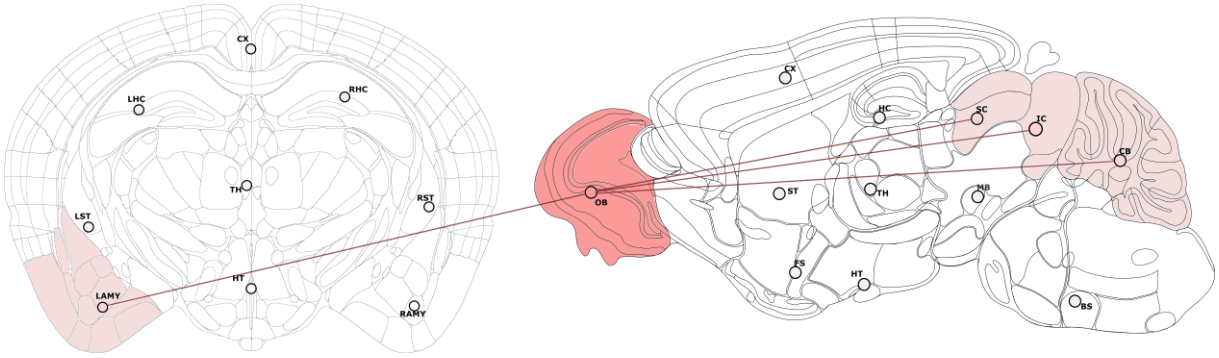


Figura 4 C D E

1 HIT



3 HIT



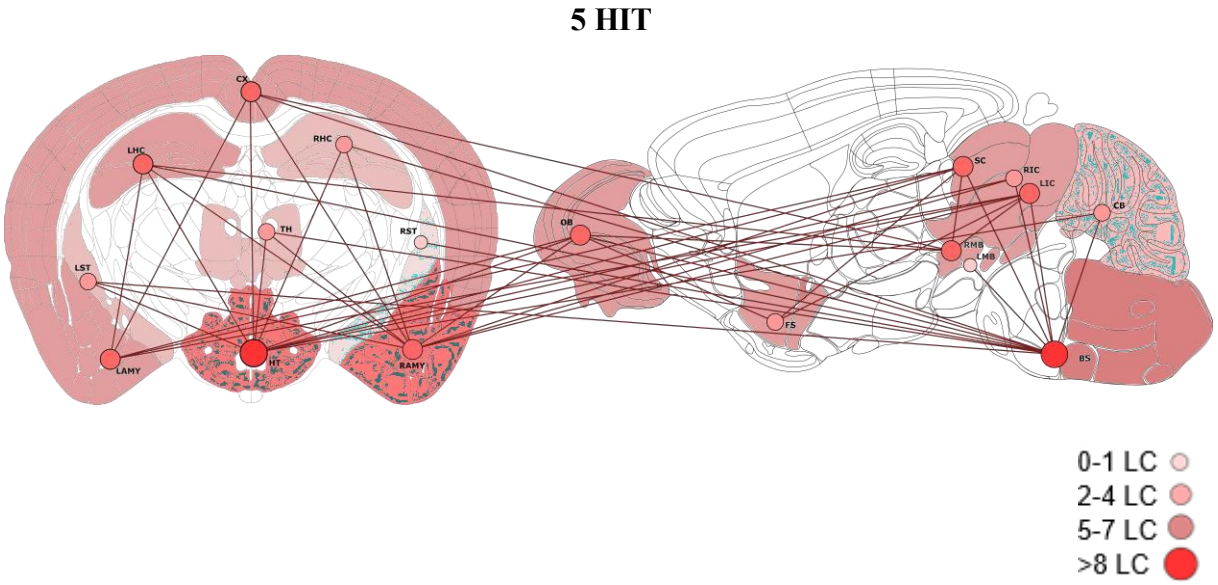
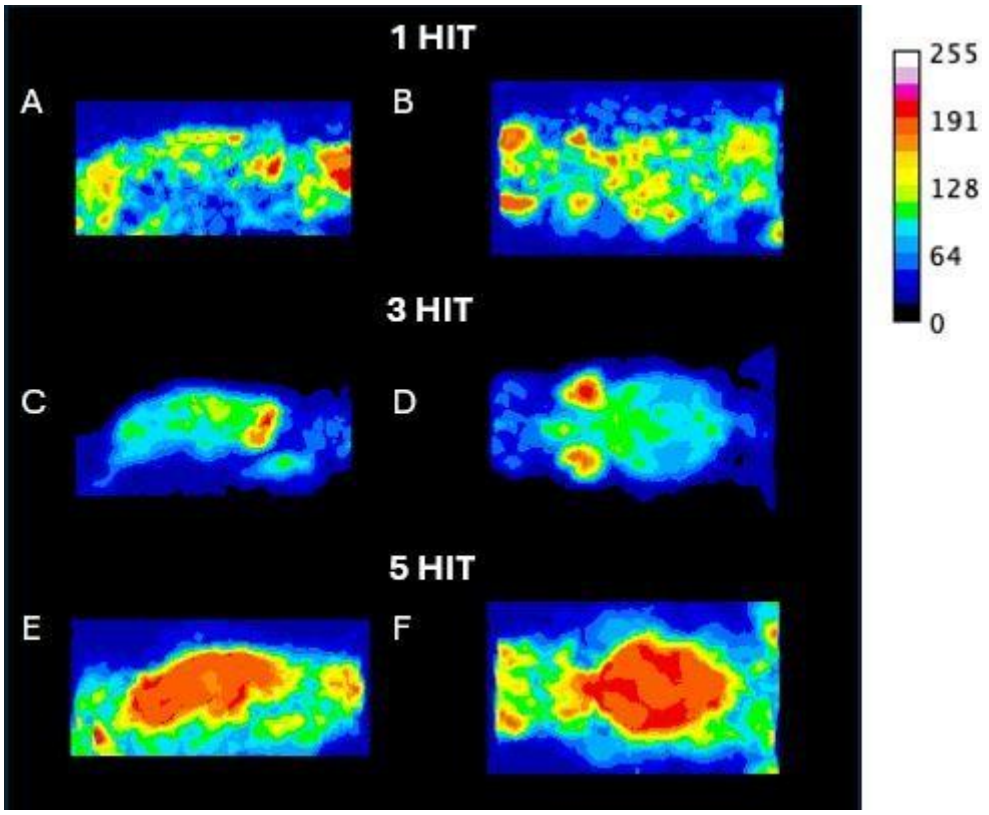


Figura 4 F



Discussion:

Rats exposed to 1 and 3 repeated head injuries, 48 hours apart display increased lose-shift tendencies, and risk-related behavior in a probabilistic discount task, which is exacerbated in females, and is associated with increased dopaminergic tonus in the orbitofrontal cortex [1].

Increases in lose-shift behaviours were exacerbated in hese behavioral effects are more prominent on the first two-weeks after injury, and resolved after three-weeks after injury. Further Further, suggesting that behavioral effects of repeated injuries are both sex- and time dependent.

Rats exposed to 3 repeated head rotation injuries, 48 hours apart display increases in fractional anisotropy in the cerebellum, hippocampus, somatosensory cortex 30 days post-injury. This is accompanied by a trend towards a reduction in the connectivity in the entorhinal cortex [2]. Albeit involving different rodent models, our findings corroborate this, suggesting a loss of metabolic connectivity in the cerebellum in mice submitted to 3 head injuries, along with a striking contribution of cerebellum, and sensorimotor processing regions (inferior and superior colliculi, olfactory bulb) to brain connectivity loss after 5 hits (Figure 4 D).

Rats exposed to 3 repeated injuries, 72 hours apart display increased immobility time in the forced swim test, poorer motor function on the beam walk test, and a reduction on the time spent in the open arms of the elevated plus maze [3]. Our findings replicate this, and suggest that motor and anxiety-related outcomes respond in a dose-dependent manner to injury, whilst anhaedonic behaviors fully manifest as early as after the first hit.

1. Knapp, C.P., et al., *Sex-dependent perturbations in risky choice behavior and prefrontal tyrosine hydroxylase levels induced by repetitive mild traumatic brain injury.* Behav Brain Res, 2024. **476**: p. 115244.
2. Everson, C.A., et al., *Subclinical brain manifestations of repeated mild traumatic brain injury are changed by chronic exposure to sleep loss, caffeine, and sleep aids.* Exp Neurol, 2024. **381**: p. 114928.
3. Yamakawa, G.R., et al., *Behavioral and pathophysiological outcomes associated with caffeine consumption and repetitive mild traumatic brain injury (RmTBI) in adolescent rats.* PLoS One, 2017. **12**(11): p. e0187218.

ANEXO II: ORIENTAÇÃO DE TRABALHO DE CONCLUSÃO DE CURSO

Depressão maior e seus estudos e modelos animais: uma revisão da literatura

Aluno: Pedro Henrique da Rosa Corrêa

Curso: Biomedicina UFRGS

Porto Alegre, 2020

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE MEDICINA
Graduação em Nutrição

CERTIFICADO

Atestamos para os devidos fins que **Marcelo Salimen Rodolphi**, no dia 11 de abril de 2023, participou como banca do Trabalho de Conclusão de Curso de Bruna Valdameri, intitulado "DIETOTERAPIA COMO ESTRATÉGIA ADJUVANTE AO TRATAMENTO DOS SINTOMAS DE DEPRESSÃO: UMA REVISÃO SISTEMÁTICA", da graduação de Nutrição da Universidade Federal do Rio Grande do Sul.

Porto Alegre, 11 de abril de 2023.

Ramiro Barcelos, 2400 - 4° andar - CEP 90035-003 - Porto Alegre - RS
tel. (51)3308-5122 fax.: (51)3308-5232 e-mail: nutricao@ufrgs.br

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE MEDICINA
Graduação em Nutrição

CERTIFICADO

Atestamos para os devidos fins que **Marcello Salimen Rodolphi** foi coorientador do Trabalho de Conclusão de Curso de Maria Eduarda Pinko Santa Helena, intitulado "SUPLEMENTAÇÃO DE BETA-ALANINA NO TREINAMENTO DE FORÇA: UMA REVISÃO NARRATIVA", da graduação de Nutrição da Universidade Federal do Rio Grande do Sul.

Porto Alegre, 25 de fevereiro de 2024.

Vivian Cristine Luft

Ramiro Barcelos, 2400 - 4° andar - CEP 90035-003 - Porto Alegre - RS
tel. (51)3308-5122 fax.: (51)3308-5232 e-mail: nutricao@ufrgs.br



CERTIFICADO

Certificamos que

MARCELO SALIMEN RODOLPHI

participou na qualidade de membro de Comissão Julgadora das sessões:

Sessão PSIQUIATRIA II

Sessão EDUCAÇÃO FÍSICA, FISIOTERAPIA E DANÇA- MOVIMENTO HUMANO, CULTURA E EDUCAÇÃO
no evento Salão UFRGS 2018: SIC - XXX SALÃO DE INICIAÇÃO CIENTÍFICA DA UFRGS, realizado no período de 15/10/2018 a 19/10/2018.

Documento gerado sob autenticação BTK.668.670.KQI

UFRGS
PROPESQ

15 A 19 DE OUTUBRO DE 2018
CAMPUS DO VALE . UFRGS