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VANESSA DA SILVA SOUZA

EFEITOS DO TRATAMENTO COM RESVERATROL SOBRE O DESENVOLVIMENTO POSTURAL, FUNÇÃO MOTORA E BALANÇO OXIDATIVO NO CÓRTEX SOMATOSSENSORIAL DE RATOS SUBMETIDOS A PARALISIA CEREBRAL

Recife
2023

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Dissertação apresentada ao Programa de Pós-Graduação em Neuropsiquiatria e Ciências do Comportamento da Universidade Federal de Pernambuco, como requisito parcial para obtenção do título de mestre(a) em Neurociências. Área de concentração: Neurociências.

Orientador (a): Profª. Drª. Ana Elisa Toscano Meneses da Silva Castro

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RESUMO

A Paralisia Cerebral (PC) é um grupo heterogêneo de doenças que resultam de um dano não progressivo ao cérebro em um estágio inicial de desenvolvimento. Os sintomas de PC incluem irregularidades nos movimentos e na postura. O resveratrol tem recebido atenção considerável recentemente por seus potenciais efeitos neuroprotetores em vários distúrbios neurodegenerativos. O objetivo do estudo foi investigar os efeitos do tratamento neonatal com resveratrol sobre o desenvolvimento postural, função motora e balanço oxidativo no cérebro de ratos submetidos a um modelo experimental de paralisia cerebral. Foram utilizados filhotes ratos machos Wistar que após o nascimento foram distribuídos nos grupos experimentais, com base na indução da paralisia cerebral e na administração do resveratrol: 1- Controle salina (CS, n=12), com filhotes que receberam solução salina; 2- Controle resveratrol (CR, n=11), com filhotes que receberam resveratrol; 3- PC salina (PCS, n=151, com filhotes submetidos à PC experimental e que receberam solução salina; 4- PC resveratrol (PCR, n=12), com filhotes submetidos à PC experimental e que receberam resveratrol. O modelo de PC se baseia na associação entre anóxia no período perinatal e restrição sensório-motora do 2º ao 28º dia de vida. O tratamento neonatal com o resveratrol foi realizado diariamente, sendo administrado via intraperitoneal, do 3º ao 21º dia pós-natal. Foram coletados dados de evolução ponderal, análise da marcha em catwalk, força muscular, análise postural e análise dos biomarcadores do estresse oxidativo. O tratamento neonatal com resveratrol nos animais submetidos a PC experimental atenuou déficits no crescimento somático, desenvolvimento postural e força muscular, diminuiu os níveis de MDA e carbonilas e a atividade enzimática da superóxido dismutase e catalase, além de aumentar os níveis de mRNA do TFAM e aumentar a atividade da citrato sintase. Nossos dados demonstram um efeito promissor do tratamento neonatal com resveratrol, melhorando os déficits posturais e musculares induzidos pela paralisia cerebral, associado a melhorias no balanço oxidativo e na biogênese mitocondrial no cérebro de ratos submetidos à PC.

Palavras-chave: paralisia cerebral; resveratrol; postura; função mitocondrial; estresse oxidativo.

ABSTRACT

Cerebral Palsy (CP) is a heterogeneous group of diseases that result from non-progressive damage to the brain at an early stage of development. CP symptoms include irregularities in movement and posture. Resveratrol has received considerable attention recently for its potential neuroprotective effects in various neurodegenerative disorders. The aim of the study was to investigate the effects of neonatal treatment with resveratrol on postural development, motor function, and oxidative balance in the brain of rats submitted to an experimental model of cerebral palsy. Male Wistar rat pups were used and, after birth, they were distributed into experimental groups, based on the induction of cerebral palsy and the administration of resveratrol: 1- Saline control (CS, n=12), with pups that received saline solution; 2- Control resveratrol (CR, n=11), with pups that received resveratrol; 3- CP saline (CPS, n=11), with pups submitted to experimental CP and who received saline solution; 4- PC resveratrol (CPR, n=12), with pups submitted to experimental CP and receiving resveratrol. The CP model is based on the association between perinatal anoxia and sensorimotor restriction from the 2nd to the 28th day of life. Neonatal treatment with resveratrol was performed daily, being administered intraperitoneally, from the 3rd to the 21st postnatal day. Data on weight gain, reflex ontogenesis, catwalk gait analysis, muscle strength, postural analysis, and analysis of oxidative stress biomarkers were collected. Neonatal treatment with resveratrol in animals submitted to experimental CP attenuated deficits in somatic growth, postural development, and muscle strength, decreased levels of MDA and carbonyls, and enzymatic activity of superoxide dismutase and catalase, in addition to increasing mRNA levels of TFAM and increase citrate synthase activity. Our data demonstrate a promising effect of neonatal treatment with resveratrol, improving postural and muscular deficits induced by cerebral palsy, associated with improvements in oxidative balance and mitochondrial biogenesis in the brain of rats submitted to CP.

Keywords: cerebral palsy; resveratrol; posture; mitochondrial function; oxidative stress.

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LISTA DE ABREVIATURAS E SIGLAS

CAT	Catalase
COX2	Ciclooxigenase-2
EROs	Espécies Reativas de Oxigênio
GPx	Glutationa Peroxidase
GSH	Glutationa
GSSG	Dissulfeto de Glutationa
H_2O_2	Peróxido de hidrogênio
HO1	Heme oxygenase
IL	Interleucinas
NADPH+	Dinocleotídeo Fosfato Oxidase
OH	Hidroxila
O_{2-}	Ânion superóxido
PC	Paralisia Cerebral
RES	Resveratrol
SDR	Síndrome do Desconforto Respiratório
SOD	Superóxido Dismutase
TNF-α	Fator de Necrose Tumoral-α
TRX	Tioredoxina

SUMÁRIO

1 INTRODUÇÃO

A Paralisia Cerebral (PC) é um grupo heterogêneo de doenças que resultam de um dano não progressivo ao cérebro em um estágio inicial de desenvolvimento. Os sintomas de PC incluem irregularidades nos movimentos e na postura. Os problemas coexistentes podem incluir distúrbios intelectuais, cognitivos e sensoriais, além de distúrbios comportamentais. De acordo com a definição, o dano não é progressivo, mas o estado geral pode mudar com o passar dos anos devido ao tratamento e à plasticidade do sistema nervoso em maturação (Mlodawski, 2019).

Pessoas com PC frequentemente apresentam baixo desempenho nas atividades de vida diária devido ao controle limitado de membros, tronco e cabeça (Velasco et al., 2017). Esses indivíduos apresentam tônus muscular e controle motor anormais, o que contribui para o prejuízo do controle postural e coordenação motora, comprometendo a saúde (McNish et al., 2019). O desenvolvimento postural adequado depende das ações do sistema sensório-motor, musculoesquelético e cognitivo, de modo que, qualquer mudança em um desses sistemas pode resultar em um desenvolvimento postural atípico.

A PC é o distúrbio de desenvolvimento motor mais comum em crianças, com incidência de 2- 3 por 1000 nascidos vivos no mundo (Stavsky et al., 2017). A incidência, prevalência e causas mais comuns de PC variam ao longo do tempo e variam de acordo com as localizações geográficas com base no desenvolvimento da assistência pediátrica pré-natal e pós-natal. A etiologia da PC é diversa e multifatorial. Geralmente é causada por lesão cerebral antes ou durante o nascimento. Este é o principal fator causal (Sewell, 2014). São descritos os seguintes fatores de risco como associados à PC em bebês a termo: síndrome do desconforto respiratório neonatal, aspiração de mecônio, asfixia no parto, convulsões neonatais, hipoglicemia e infecções neonatais (MCintyre, 2012). Dentre esses fatores, destaca-se os mecanismos que causam a privação de oxigênio e nutrientes para o sistema nervoso central, como a asfixia perinatal, onde o fluxo sanguíneo ou troca gasosa para o feto no período imediatamente anterior, durante ou após o parto, leva à hipóxia e isquemia (Hakobyana, 2019, Perlman, 2011), que estão associados às graves consequências neurofuncionais da PC (Rainaldi, 2016, Volpe, 2001, Low, 2004, Vannucci, 2004, Aridas, 2014).

Dessa forma, o dano cerebral secundário ao evento hipóxico-isquêmico no cérebro em desenvolvimento tem como mecanismo fisiopatológico o suprimento de glicose e oxigênio prejudicados (Northington, 2011). Isso resulta em lesão neuronal imediata e exaustão dos estoques de energia celular, que levam a uma cascata multifacetada de eventos bioquímicos (Northington, 2011). Ademais, após a hipóxia-isquemia, a reperfusão do cérebro marca o início da hiperoxigenação que leva à produção aumentada e adversa de radicais livres, inchaço celular e edema do tecido devido a desequilíbrios iônicos e, em última análise, apoptose por vários mecanismos. Nos dias, semanas e meses após um insulto isquêmico, pode haver a instalação da resposta neuroinflamatória (Koronowski, 2015). Sabe-se que tanto exposições agudas (Faiss, 2013) quanto exposições hipóxicas de longo prazo (Dosek, 2007) levam ao estresse oxidativo.

O estresse oxidativo, que é definido como um desequilíbrio entre os fatores oxidantes e antioxidantes, é considerado o principal contribuinte para a lesão cerebral isquêmica (Warner, 2004), porque é uma consequência importante da toxicidade mediada por neurotransmissores que segue a hipóxia-isquemia perinatal (Ferriero, 2001). A maturação do sistema de defesa antioxidante humano acelera no final do terceiro trimestre da gravidez e, como consequência, o recém-nascido humano tem uma deficiência relativa de superóxido dismutase, catalase e glutationa peroxidase no cérebro (Baba, 2008).

Neste contexto, modelos experimentais surgem como ferramentas de reprodução dos mecanismos fisiopatológicos da PC e suas desordens motoras semelhantes ao que ocorre em humanos. Dentre os modelos de PC, atualmente, destaca-se o modelo desenvolvido por Strata e colaboradores (2004) onde roedores são expostos a uma condição de anoxia perinatal associada a imobilização dos membros posteriores, a fim de reproduzir os déficits motores observados em crianças com paralisia cerebral. Esse tipo de modelo ocasionou alterações duradouras e semelhantes às ocorridas em crianças com PC, tais como aumento do tônus muscular (Strata, Coq, Byl, & Merzenich, 2004), redução da densidade de sarcômeros (Stigger et al, 2011), redução na atividade locomotora (Silva et al, 2016), modificações nas funções orais (Lacerda et al, 2017), além de prejuízos no desempenho motor (Marcuzzo et al., 2010).

Novak e colaboradores (2017) enfatizaram a importância do diagnóstico precoce para otimizar os resultados funcionais de longo prazo com base na modulação positiva de seu impacto na neuroplasticidade (Novak, 2017). Sabe-se que todos os organismos são capazes de produzir diferentes fenótipos em resposta a condições ambientais distintas (Moczek, 2011). Nesse contexto, podemos falar sobre plasticidade fenotípica que é definida como 'a capacidade de genótipos individuais de produzir diferentes fenótipos quando expostos a diferentes condições ambientais' (Pigliucci, 2006). Isso inclui a possibilidade de modificar as trajetórias de desenvolvimento em resposta a estímulos ambientais específicos e também a capacidade de um organismo individual de alterar seu estado ou atividade fenotípica (por exemplo, seu metabolismo) em resposta a variações nas condições ambientais (Garland, 2006). Estudos epidemiológicos e experimentais têm demonstrado que variações ambientais na vida precoce podem levar às mudanças fenotípicas com repercussões permanentes no metabolismo, estrutura e função dos sistemas fisiológicos (Hales et al., 1991; Toscano et al., 2008). Intervenções precoces são importantes, pois podem fornecer um padrão de atividade neuronal que pode levar a uma diferenciação normal dos neurônios motores e consequente desempenho neuromuscular ótimo na vida adulta, ressaltando que a restrição sensório-motora a qual é submetido o modelo animal piora o desenvolvimento e a performance (Stigger et al, 2011).

Apesar das conhecidas repercussões clínicas da PC, atualmente o manejo terapêutico da paralisia cerebral é principalmente de acompanhamento das sequelas e nenhum dos tratamentos atuais oferecidos tentam corrigir o problema principal de uma lesão cerebral. Entretanto, a alteração da plasticidade cerebral é um dos mecanismos de tratamentos de problemas neurológicos. Estudos com modelos experimentais e que se propõem a agregar novas perspectivas de tratamento para esta condição são muito relevantes. Intervenções sobre o sistema nervoso central foram sugeridas como as terapias destinadas a reduzir o estresse oxidativo, interrompendo a cascata lesional (Sandra E., 2014).

O resveratrol (RES) tem recebido atenção considerável recentemente por seus potenciais efeitos neuroprotetores em vários distúrbios neurodegenerativos (Wu, 2011). O RES é uma substância que se mostrou promissora no tratamento de uma ampla gama de patologias, incluindo as do sistema nervoso central (Baur and Sinclair,

2006). O resveratrol é um composto polifenólico presente em muitas espécies vegetais (Song, 2014) e está entre os polifenóis que estão sendo utilizados em modelos de neurodegeneração (doença de Alzheimer, Parkinson ou Huntington) e modelos de hipóxia-isquemia como estratégia neuroprotetora (Artega, 2014, Sandra E., 2014; Shulin Pan, 2016), além de se mostrar eficaz na redução de sequelas neuropatológicas e comportamentais em modelos de acidente vascular cerebral (Wang et al., 2002; West et al., 2007), lesão da medula espinhal (Ates et al., 2006; Kaplan et al., 2005; Kiziltepe et al., 2004; Yang e Piao, 2003) e epilepsia (Gupta et al., 2002a; 2001; 2002b; Wu et al., 2009). Várias linhas de evidência também demonstraram suas propriedades antiinflamatórias, antiagregantes e neuroprotetoras (Jing, 2013; Marques, 2009; Pandey, 2009). Um estudo recente usando um modelo de PC experimental (Pan, 2016) demonstrou que o resveratrol tem efeito neuroprotetor na lesão cerebral hipóxico-isquêmica neonatal e que o efeito está relacionado à inibição da inflamação e apoptose. Além disso, linhas de evidência demonstraram que a atividade antioxidante do resveratrol protegeu os tecidos insultados pelo estresse oxidativo.

Assim, o objetivo desse estudo é investigar os efeitos do tratamento neonatal com resveratrol sobre o desenvolvimento postural, função motora e balanço oxidativo no cerebelo de ratos submetidos a um modelo de paralisia cerebral.

2 REVISÃO DA LITERATURA

2.1 PARALISIA CEREBRAL

A Paralisia Cerebral é a deficiência física mais comum na infância (Novak et al., 2020). Essa doença é definida como um grupo de distúrbios que afetam o movimento, a postura e o equilíbrio de um indivíduo. Os achados clínicos, decorrentes de uma lesão no cérebro em desenvolvimento, são permanentes e não progressivos, mas podem mudar com o tempo (Vitrikas et al., 2020).

Rosenbaum e Bax em 2005 propuseram uma definição para a PC: “A paralisia cerebral descreve um grupo de distúrbios permanentes do desenvolvimento, do movimento e da postura, causando limitação da atividade, que são atribuídos a distúrbios não progressivos que ocorrem no desenvolvimento do cérebro fetal ou infantil. Seus distúrbios motores são frequentemente acompanhados por distúrbios de sensação, percepção, cognição, comunicação e comportamento, por epilepsia e por problemas musculoesqueléticos secundários.” (Wimalasundera, 2016). Essa abordagem holística se reflete na forma como a paralisia cerebral é classificada, por tipo, distribuição motora e por nível funcional. Os descritores funcionais permitiram pesquisas mais significativas sobre sua história natural e o benefício das intervenções (Wimalasundera, 2016).

O diagnóstico da paralisia cerebral é clínico, baseado na identificação das características definidoras (Vitrikas, 2020; O’Shea, 2008). A PC pode ser classificada com base na natureza do distúrbio do movimento: músculos rígidos (espasticidade), movimentos incontroláveis (discinesia), coordenação inadequada (ataxia), instabilidade postural, entre outros (Vitrikas et al., 2020).

A prevalência global de PC é estimada em 2 por 1000 nascidos vivos, variando de acordo com a localização geográfica com base no desenvolvimento do cuidado obstétrico e neonatal (GULATI; SONDHI, 2017). O baixo peso ao nascer (BPN) é frequentemente citado como fator de risco em vez da idade gestacional, pois é uma medida mais precisa; no entanto, o BPN comumente implica em nascimento prematuro (Wimalasundera, 2016). A prematuridade é o fator de risco mais importante para a paralisia cerebral (Graham, 2016; Moster, 2010). O risco aumenta de forma constante com o declínio da idade gestacional no nascimento: a probabilidade de bebês nascidos antes de 28 semanas de gestação de ter a PC é de aproximadamente

50 vezes mais do que os nascidos a termo (Graham et al., 2016)(Kuban, 2010). Uma revisão sistemática mostrou 10 fatores de risco significativamente associados à paralisia cerebral em crianças nascidas a termo: anormalidades placentárias, defeitos congênitos, baixo peso ao nascer, aspiração de mecônio, cesariana de emergência, asfixia ao nascer, convulsões neonatais, síndrome do desconforto respiratório (SDR), hipoglicemia e infecções neonatais (McIntyre, 2013). Atualmente, acredita-se que a asfixia perinatal seja responsável por 10 a 20% dos casos de PC (Colver, 2014).

A PC permanece como a maior causa de mortalidade neonatal e de danos permanentes no desenvolvimento neurológico infantil ocorrendo antes, durante e depois do nascimento, além de infecções maternais, que também ocasionam alto risco para Paralisia Cerebral (COQ et al.,2008).

Vários modelos animais (incluindo roedores, primatas, ovelhas e porcos) foram desenvolvidos para investigar os mecanismos das lesões cerebrais e os resultados funcionais dessas injúrias (Graham et al., 2016; Johnston et al., 2005). Modelos experimentais foram propostos a fim de reproduzir os danos neurofuncionais e esclarecer os mecanismos subjacentes à PC (LACERDA et al., 2017a), além de também permitir que os investigadores testem potenciais abordagens terapêuticas para esta patologia (Graham et al., 2016). Os estudos com animais reproduzem as alterações encontradas em humanos, colaboram para o desenvolvimento de uma adequada intervenção terapêutica nessa injuria e são fundamentais para elucidar mecanismos subjacentes e consequentemente para o desenvolvimento de estratégias de neuroproteção e reabilitação dos quadros adquiridos (Strata, 2004; Coq, 2016). No entanto, os modelos experimentais baseados em um único insulto, parecem não ser eficazes em reproduzir o fenótipo típico da criança com PC (Marcuzzo et al., 2010).

Diante disso, o modelo proposto por Strata e colaboladores (2004), que associa asfixia perinatal e restrição sensório-motora consegue mimetizar mais características da doença, além de ser simples, barato e facilmente reproduzível (Marcuzzo et al., 2010). Essa combinação de insultos reproduz diversas anormalidades na atividade locomotora, coordenação, equilíbrio, além de atrasos nos marcos do desenvolvimento motor (Coq et al., 2008; Strata et al., 2004).

2.2 PLASTICIDADE FENOTÍPICA E NEUROPLASTICIDADE

A plasticidade fenotípica pode ser definida como um fenômeno biológico no qual o organismo é capaz de reagir aos desafios impostos pelo ambiente modificando a sua forma, estado, movimento ou funcionamento (West-Eberhard, 1986). Refere-se também a todos os tipos de variação fenotípica induzida pelo ambiente e pode afetar aspectos morfológicos, fisiológicos e comportamentais do fenótipo de um organismo, além da sua história de vida (Sommer, 2020). O fenótipo de um indivíduo é a expressão última da interação do seu genótipo (conjunto de gens) e do ambiente (West-Eberhard, 1986), portanto, a plasticidade fenotípica possibilita a modificação das trajetórias de desenvolvimento em resposta a estímulos ambientais específicos e também a capacidade de um organismo individual de alterar seu estado ou atividade fenotípica (por exemplo, seu metabolismo) em resposta a variações nas condições ambientais (Fusco & Minelli, 2010; Garland & Kelly, 2006).

Recentemente, as pesquisas em neurociência começaram a caracterizar a capacidade adaptativa do sistema nervoso central (Kleim, 2008). Diferentes estudos mostram que a manipulação do ambiente no período que vai da concepção à infância pode estar associada a mudanças permanentes na fisiologia e/ou estrutura do organismo (Gluckman D. et al., 2006). Nessa fase do desenvolvimento, a estrutura cerebral é altamente receptiva (Shonkoff et al., 2009). Os neurônios, entre outras células cerebrais, possuem a importante capacidade de alterar sua estrutura e função em resposta a uma variedade de pressões internas e externas (Kleim, 2008) e a ausência de estímulos, ou a ocorrência de estímulos negativos, podem deixar marcas duradouras, não somente pela elevada vulnerabilidade dos indivíduos nesse período, mas também pelo efeito cumulativo desses fatores ao longo da vida (Shonkoff et al., 2009). Além disso, é importante reconhecer que o sistema nervoso continua a se remodelar e mudar não apenas no início da vida, mas durante todo o período de desenvolvimento e durante a idade adulta, em resposta a influências ambientais, bem como a eventos geneticamente programados (Rice & Barone, 2000).

Danos cerebrais no período perinatal resultam em muitas mudanças em neurônios e células cerebrais não neuronais que podem comprometer o desenvolvimento das funções motoras e cognitivas (KLEIM 2008 E OUTRA REFERÊNCIA). Por exemplo, ratos com paralisia cerebral apresentaram redução da massa muscular do masseter, o principal músculo da mastigação, resultando em

disfunções mastigatórios e redução do consumo alimentar (Lacerda et al., 2017). Roedores submetidos a estresse perinatal apresentaram anormalidades comportamentais, como aprendizagem e memoria prejudicadas, déficit de atenção e resposta ao estresse elevada (Kolb B & Gibb R, 2011; Weinstock, 2008). Em estudos com humanos, por exemplo, o estresse pré-natal é fator de risco no desenvolvimento de esquizofrenia, TDAH, depressão e dependência de drogas (Rice & Barone, 2000).

No sistema nervoso, há muitos processos plásticos de aprendizagem, alguns deles podendo ser mediados por substâncias. Pesquisas recentes têm chamado atenção para os efeitos benéficos dos polifenóis à saúde humana (Ammar et al., 2020). Seus benefícios incluem modulação da inflamação, redução do risco de doença cardiovascular e efeitos anticancerígenos, antioxidantes e neuroprotetores (Gim et al., 2021; Rossi et al., 2006; X. Wang et al., 2014). Além desses, os polifenóis também geram mudanças positivas no comportamento motor de pacientes com doenças neurológicas (Bonyadi et al., 2022; L. Gao et al., 2015). O resveratrol, um polifenol abundante e encontrado em inúmeras plantas, tem sido alvo de inumemos estudos, pois tem forte atividade neuroprotetora, anti-inflamatória e antioxidante (Cichon et al., 2020) e por melhorar a função motora de indivíduos com doenças que acometem o sistema nervoso (Kawamura et al., 2020; Shi et al., 2017).

Dessa forma, entende-se que a plasticidade fenotípica possibilita mudanças no desenvolvimento de um organismo em resposta à estímulos específicos. Estudos experimentais são fundamentais para a compreensão dos mecanismos associados à neuroplasticidade e suas repercussões nos outros sistemas.

2.3 DESENVOLVIMENTO POSTURAL

Controle postural é definido como o correto posicionamento das partes do corpo em relação ao mundo externo, tanto mantendo uma postura estável durante o repouso, quanto ajustando o corpo durante todas as fases do movimento (Lalonde & Strazielle, 2007). O controle postural é tradicionalmente considerado altamente imaturo em mamíferos altriciais recém-nascidos, amadurecendo lentamente nas primeiras semanas de vida (Lelard et al., 2006). Nessa idade, o desenvolvimento postural segue um gradiente rostro-caudal, onde a maturação dos membros anteriores precede a dos membros posteriores (Altman & Sudarshan, 1975; Brocard et al., 1999;

Lelard et al., 2006). Esse desenvolvimento depende da maturação de vários sistemas, como os sistemas musculoesquelético e sensório-motor (L. Vinay et al., 2005) e os sistemas periféricos: vestibular, exteroceptivo e proprioceptivo (Altman & Sudarshan, 1975).

Às 48 horas de vida, em repouso, o filhote consegue fazer alguns movimentos de rotação da cabeça e iniciar algumas sequências de engatinhar. Durante a primeira semana pós-natal, apenas a parte frontal do corpo apresenta algumas adaptações posturais, como elevação da cabeça (Clarac et al., 1998) e dos membros anteriores (Altman & Sudarshan, 1975). A partir do 5º dia pós-natal, os filhotes levantam a cabeça com controle (Clarac et al., 1998; Mendez-Gallardo et al., 2017), embora ainda permaneçam em decúbito ventral. Estudos sugerem que nessa fase o animal já apresenta um rastejamento induzido por odor, mesmo não tendo desenvolvido o controle postural, porém só quando os filhotes conseguem sustentar a cabeça e o tronco é que eles conseguem se locomover por mais tempo (Mendez-Gallardo et al., 2017). Durante a segunda semana, a região da pelve começa a suportar o peso do corpo e após 10 dias o filhote é capaz de ter uma postura quadrúpede elevada, com a barriga elevada do chão. Durante este período, parece que há uma aceleração repentina na maturação funcional dos membros posteriores (Clarac et al., 1998). No P12-13, o animal começa a caminhar e os olhos se abrem. Porém, os movimentos das pernas ainda são lentos, com pouco controle do tronco. No 14º dia pós-natal começam os movimentos verticais, o animal se senta nas patas traseiras e usa as patas dianteiras para fazer manipulações. Depois do P15, todos os comportamentos complexos são fluidos e muito mais eficientes (Clarac et al., 1998).

O desenvolvimento pós-natal da postura acontece junto com o desenvolvimento das aferências vestibulares e das vias descendentes da medula espinhal, necessárias para o controle postural (Clarac et al., 1998; Mendez-Gallardo et al., 2017). Os primeiros dez dias pós-natal do rato estão relacionados principalmente com o desenvolvimento do sistema vestibular, as vias descendentes e a regulação do reflexo postural. Este período é fundamental para definir adequadamente como as informações vestibulares são essenciais para a estruturação do comportamento e função motora (Clarac et al., 1998). Muitas projeções do cérebro para a medula espinhal que são necessários para o controle da postura e locomoção

não se desenvolvem totalmente até o final da segunda semana pós-natal (L. Vinay et al., 2005).

Como todos esses processos nos sistemas sensório-motor e musculoesquelético continuam a se desenvolver no rato infantil, a postura vai se tornando estável e controlada, o que permite que o filhote expresse comportamentos motores mais complexos, como por exemplo a locomoção (Mendez-Gallardo et al., 2017). Os ratos recém-nascidos adaptam sua postura durante as atividades regulares no ninho e durante as interações maternas (Eilam & Smotherman, 1998; Mendez-Gallardo et al., 2017). O desenvolvimento do controle postural e as adaptações posturais nesta idade ocorre enquanto os processos sensoriais, motores e neuromusculares amadurecem e se desenvolvem (Laurent Vinay et al., 2002).

Características específicas da postura podem se desenvolver no início do curso da doença neurológica, muitas vezes antes que outros sinais e sintomas sejam evidentes, e podem oferecer pistas importantes que levam ao diagnóstico do distúrbio subjacente ou auxiliar no diagnostico diferencial (Nonnekes et al., 2018). Poe exemplo, na doença de Parkinson, o desenvolvimento de instabilidade postural e quedas recorrentes no primeiro ano após o início da hipocinesia ou características de rigidez sugerem a presença de uma forma de parkinsonismo atípico, como paralisia supranuclear ou atrofia de múltiplos sistemas (Litvan et al., 1996; G. K. Wenning et al., 1994; Gregor K. Wenning et al., 2013).

Nas crianças com PC, problemas posturais desempenham um papel central na disfunção motora (Brogren Carlberg & Hadders-Algra, 2005). O desempenho das atividades cotidianas é notavelmente influenciado por tais déficits posturais; a extensão desses déficits, no entanto, varia com o grau da deficiência (Brogren Carlberg & Hadders-Algra, 2005). Embora crianças com PC tenham um estado musculoesquelético geralmente normal ao nascimento, as deformidades posturais podem surgir progressivamente com o desenvolvimento (SATO, 2020). A postura de crianças com PC tendem a apresentar um certo grau de deformidade, a chamada “deformidade postural” (Porter et al., 2007). Tais deformidades podem causar dificuldades nas atividades diárias de crianças com PC, como manter a posição sentada e/ou rolar no colchonete (Mercado et al., 2007; Rodby-Bousquet et al., 2013; SATO, 2020), levando a atraso e também déficits no desenvolvimento motor dessas

crianças. Além disso, a deformidade postural também pode afetar a caixa torácica, o que contribui para a diminuição da função respiratória (SATO, 2020).

2.4 ESTRESSE OXIDATIVO

O oxigênio é uma molécula essencial em todos os organismos aeróbicos. Ele está envolvido em diversas reações fisiológicas, como as produzidas na cadeia de transporte de elétrons, hidroxilação e oxigenação. Aproximadamente 1% do oxigênio usado nas células é transformado em radicais livres (Villafuerte et al., 2015). Os radicais livres são espécies químicas, altamente instáveis e extremamente reativas, que, por terem um número ímpar de elétrons na seu orbital externo, são ávidos por interagir com outras substâncias em busca de um elétron para atingir a estabilidade (FERREIRA E MATSUBARA, 1997). Em sistemas biológicos, a maioria destes radicais são espécies reativas de oxigênio (EROs), sendo as mais comuns o radical aniônico superóxido (O_2-), o radical hidroxila (OH) e o peróxido de hidrogênio (H_2O_2), e todos são subprodutos formados como parte do metabolismo aeróbico normal das mitocôndrias e dos peroxissomos (Sies, 1997; Villafuerte et al., 2015).

O estresse oxidativo é resultado do desequilíbrio entre a indução de espécies reativas de oxigênio e a capacidade das células em metabolizá-las (Beyfuss & Hood, 2018), danificando assim macromoléculas biológicas, incluindo proteínas, ácidos nucléicos e lipídios (Fila et al., 2021). O cérebro, assim como outros órgãos e tecidos, possui um sistema de defesa antioxidante para lidar com o aumento do estresse oxidativo. Este sistema inclui enzimas antioxidantes, proteínas de reparo de DNA e antioxidantes de baixo peso molecular (Fila et al., 2021).

Os antioxidantes são um grupo estrutural heterogêneo que compartilha a capacidade de eliminar os radicais livres e são a primeira defesa contra o dano potencial das EROs (Beckman & Ames, 1998; Villafuerte et al., 2015). Um dos antioxidantes endógenos mais significativos é o tripeptídeo glutationa (GSH), que é oxidado por diferentes radicais em dissulfeto de glutationa (GSSG) e confere proteção principalmente em tecidos altamente metabólicos. Em condições fisiológicas, os efeitos tóxicos das EROs podem ser prevenidos pela ação de enzimas como superóxido dismutase (SOD), glutationa peroxidase (GPx) e catalase (CAT) (Villafuerte et al., 2015). Essas são as enzimas antioxidantes mais estudadas e

responsáveis por transformar os radicais livres em formas químicas mais estáveis e, junto com a glutationa e a peroxidação lipídica, constituem as principais defesas antioxidantes dos animais (Melo et al., 2011). Além desses, há também antioxidantes que podem ser adquiridos através da administração, entre eles destacam-se o ácido ascórbico (vitamina C), α-tocoferol (vitamina E), minerais e derivados fenólicos, como os flavonóides (Bianchi & Antunes, 1999).

O sistema nervoso central está sempre sob risco de dano oxidativo devido a: (i) alto consumo de oxigênio; (ii) riqueza em ácidos graxos mais facilmente peroxidáveis (fosfolipídios); e (iii) níveis relativamente baixos de antioxidantes protetores (Aycicek & Iscan, 2006). Os principais causadores de danos ao sistema nervoso central são as Espécies Reativas de Oxigênio (Barbosa et al., 2010). Quando as EROs são produzidas em grandes quantidades, elas colocam uma grande demanda nos sistemas de desintoxicação e levam à interrupção de numerosas vias metabólicas, causando mais de 200 doenças humanas (Beyfuss & Hood, 2018; Hybertson et al., 2011).

O estresse oxidativo desempenha um papel fundamental na patogênese de distúrbios neurológicos, causando uma série de eventos fisiopatológicos (Fatemi et al., 2009). Um estudo realizado por Manzanero e colaboradores (2013) mostrou que a geração de radicais livres e consequente estresse oxidativo está relacionado com o surgimento de lesão neuronal, causando neurodegeneração e disfunção cognitiva. Esses efeitos têm sido observados na patogênese de diferentes doenças que afetam o cérebro, como distúrbios mitocondriais, isquemia cerebral, epilepsia e paralisia cerebral (Aycicek & Iscan, 2006). Em crianças com Paralisia Cerebral, deficiência na ingestão de vitaminas e alimentos, fatores ambientais e crises epilépticas podem causar o estresse oxidativo (Aycicek & Iscan, 2006). Neste sentido, entender o papel do estresse oxidativo na PC é importante porque a terapia com agentes que aumentam a capacidade antioxidante pode ser uma opção de tratamento útil para bloquear a alteração destrutiva de lipídios, proteínas e ácidos nucleicos por radicais livres derivados de oxigênio, a fim de prevenir insulto neurológico (Aycicek & Iscan, 2006).

2.5 EFEITOS DO RESVERATROL NAS DOENÇAS NEUROLÓGICAS

Os polifenóis são micronutrientes presentes em uma grande variedade de alimentos, que ganharam interesse nos últimos 30 anos devido às suas propriedades antioxidantes e seu papel emergente na prevenção de diversas doenças ligadas ao estresse oxidativo, como câncer, doenças cardiovasculares e neurodegenerativas (Pacifici et al., 2021). Entre os polifenóis, o resveratrol vem ganhando bastante atenção devido ao seu potencial terapêutico (Griñán-Ferré et al., 2021).

O resveratrol é um ingrediente vegetal polifenóico natural que pode ser encontrado no vinho tinto, uvas, frutas vermelhas e nozes (Kairisalo et al., 2011). É usado em uma ampla variedade de campos biológicos por conta de suas propriedades antioxidantes, anti-inflamatórias e anticâncer (Gim et al., 2021; Rossi et al., 2006; X. Wang et al., 2014). Ele tem uma função anticancerígena pois inibe o fator indutor de hipóxia e o fator de crescimento endotelial vascular (Diaz-Gerevini et al., 2016). O resveratrol também reduz a produção de espécies de radicais livres e aumenta os antioxidantes mitocondriais, protegendo as células contra os danos do estresse oxidativo (Kairisalo et al., 2011).

Takaoka isolosou pela primeira vez o resveratrol das raízes do heléboro branco (*Veratrum grandiflorum O. Loes*), em 1939 (Takaoka). Após esse relato, artigos esporádicos apareceram na literatura, dos quais a maioria era de natureza descritiva (Pezzuto, 2019). Foi somente em 1992 que o resveratrol atraiu mais interesse da comunidade cientifica: um estudo observou que os altos níveis de resveratrol encontrado no vinho tinto promoviam um efeito cardioprotetor (Baur & Sinclair, 2006). Desde então, vários estudos têm demonstrado que o resveratrol pode prevenir ou retardar a progressão de uma ampla variedade de doenças (Griñán-Ferré et al., 2021), incluindo lesões isquêmicas, doenças neurodegenerativas, câncer e doenças cardiovasculares. Recentemente, Dumont e colaboradores demonstraram que a suplementação materna permitiu diminuir a incidência e os volumes de lesões cerebrais detectadas por ressonância magnética, a extensão da perda celular e, mais importante, preservar as habilidades motoras e cognitivas de filhotes submetidos a hipoxia-isquemia (Dumont et al., 2021).

Os efeitos antioxidantes do resveratrol são objeto de interesse de uma grande quantidade de estudos. Dentre as diversas pesquisas nesta área, destacamos as pesquisas de Gao e Wang, que relataram que o resveratrol protege contra vários tipos

de danos cerebrais (Z. B. Gao et al., 2006; Q. Wang et al., 2004) por conta de suas propriedades antioxidantes e anti-inflamatórias (Gim et al., 2021). Há também evidências fortes sobre a capacidade do Resveratrol de proteger contra danos cerebrais isquêmicos (Shah et al., 2014). Além disso, ele também pode ser um composto útil no tratamento de doenças neurodegenerativas como a doença de Huntington, Parkinson e Alzheimer (Kairisalo et al., 2011).

A atividade antioxidante do resveratrol consiste em sua capacidade de eliminar radicais livres e metais do organismo, como íons de cobre, alumínio e zinco. Essa ação antioxidante acontece porque o resveratrol reduz a formação de EROs ao inibir genes que codificam proteínas pró-oxidantes, como Nicotinamida Adenina Dinocleotídeo Fosfato Oxidase (NADPH+) e Mieloperoxidase, induzindo a expressão de genes que codificam várias enzimas antioxidantes, como SOD, CAT, tioredoxina (TRX) e GPx (Carrizzo et al., 2013; Griñán-Ferré et al., 2021; Liu et al., 2017b). Além disso, o RES atua melhorando as respostas gliais, oxidativas e inflamatórias, aumentando a expressão de Heme oxygenase-1 (HO1) e o conteúdo extracelular de GPx em células C6 induzidas por H_2O_2 (Chen et al., 2005).

Diversos estudos mostram que a neuroinflamação é um contribuidor importante para as doenças neurológicas (Brambilla, 2019). As propriedades anti-inflamatórias do resveratrol estão principalmente associadas à ativação de SIRT1 (Ohtsu et al., 2017) e à seguinte regulação negativa do fator pró-inflamatório NF-κβ (Yi et al., 2011). Suas respostas anti-inflamatórias incluem a ativação da micróglia, astrócitos, linfócitos e macrófagos que desencadeiam numerosos mediadores pró-inflamatórios e neurotransmissores. A ativação da micróglia é considerada a marca registrada da neuroinflamação cerebral, pois libera citocinas altamente pró-inflamatórias, EROs e NO, o que leva a oxidação de proteínas, peroxidação lipídica, fragmentação de DNA, inflamação neuronal e morte celular (Bellaver et al., 2014; Zhang et al., 2010). A literatura mostra que o resveratrol medeia a regulação negativa de vários marcadores pró-inflamatórios, como Fator de Necrose Tumoral-α (TNF-α), Ciclooxigenase-2 (COX2), iNOS e interleucinas (IL) (Huang et al., 2012; Lu et al., 2010; Yao et al., 2015). Assim, as atividades do RES contra a neuroinflamação parecem ter como alvo a micróglia ativada e resultar na regulação negativa de fatores pró-inflamatórios (Bastianetto et al., 2015; Tomé-Carneiro et al., 2013).

Sendo assim, o resveratrol mostra-se como uma substância promissora no tratamento de doenças neurológicas, apoiando-se principalmente nos seus efeitos antioxidantes, anti-inflamatórios e neuroprotetores (Griñán-Ferré et al., 2021).

3 HIPÓTESE

A administração neonatal de resveratrol diminui os danos na postura, na marcha e na força e reduz o estresse oxidativo cerebral em animais submetidos a paralisia cerebral.

4 OBJETIVOS

4.1 OBJETIVO GERAL

Investigar os efeitos do tratamento neonatal com resveratrol sobre o desenvolvimento postural, função motora e balanço oxidativo e função no cérebro de ratos submetidos a um modelo de paralisia cerebral.

4.2 OBJETIVOS ESPECÍFICOS

Avaliar em ratos submetidos ou não a paralisia cerebral:

a) a evolução ponderal até o 28° dia de vida;
b) a análise da marcha em catwalk;
c) a força muscular;
d) a análise postural;
e) a análise dos biomarcadores do estresse oxidativo e da função mitocondrial.

5 METODOLOGIA

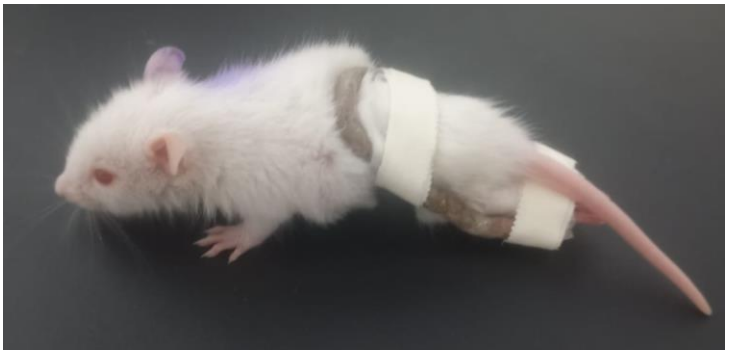
5.1 ANIMAIS E CONDIÇÕES DE BIOTÉRIO

Este é um estudo experimental com animais em que foram utilizados filhotes ratos machos *Rattus Norvegicus Albinus* da linhagem *Wistar* provenientes do biotério de criação do Departamento de Nutrição da UFPE. Eles foram mantidos no biotério de manutenção do Departamento de Nutrição da UFPE com temperatura de 22 ± 2 °C, ciclo claro-escuro invertido de 12/12 horas (Ciclo claro - 20:00 as 8:00h; Ciclo escuro - 08:00 às 20:00 h), alojados em gaiolas de polipropileno (46cmx34cmx20cm) forrada com maravalha estéril, com acesso livre a água e dieta. Todos os procedimentos foram realizados em conformidade com as diretrizes do Conselho Nacional de Controle de Experimentação Animal (CONCEA) e com as normas internacionais do National Institute of Health Guide for Care and Use of Laboratory Animals (8ª ed). O projeto foi aprovado pelo Comitê de Ética em Uso Animal (CEUA) da Universidade Federal de Pernambuco (número do protocolo CEUA: 0032/2021) (Anexo A).

5.2 MODELO EXPERIMENTAL DE PARALISIA CEREBRAL

O modelo experimental de PC utilizado foi o mesmo descrito por Strata et al. (2004), Coq et al. (2008), Silva et al. (2016) e Lacerda et al. (2017), o qual associa anóxia perinatal a restrição sensório-motora das patas posteriores. Os filhotes dos grupos PC foram submetidos a dois episódios de anóxia pós-natal, no dia do nascimento e no primeiro dia de vida (P0 e P1). Os filhotes foram colocados dentro de uma câmara de acrílico parcialmente imersa em água a 37 graus celsius e expostos a nitrogênio (100%) a 9L/min durante 12 minutos e, em seguida, recuperados em ar e temperatura ambiente e devolvidos às suas respectivas mães. Do 2° ao 28° dia de vida (P2 ao P28) foi feita a restrição sensório-motora das patas posteriores durante 16 horas por dia, sendo permitida nas 8 horas restantes, a livre movimentação do animal. Para a restrição sensório-motora foi utilizada uma órtese feita com molde de epóxi, deixando as patas posteriores estendidas, sem que a eliminação de urina e fezes e os cuidados maternos fossem prejudicados (Strata et al., 2004).

Figura 1. Rato utilizando órtese durante a restrição sensório-motora.



Fonte: acervo do autor (2022)

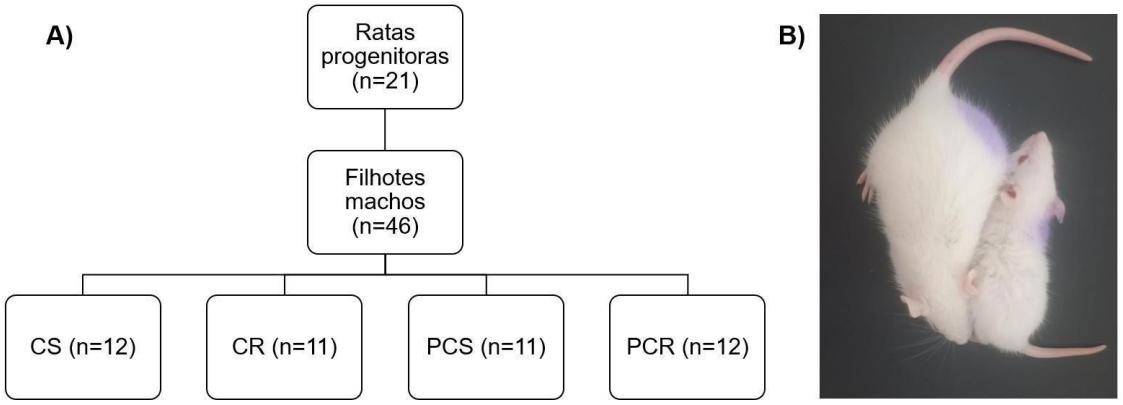
5.3 GRUPOS EXPERIMENTAIS

Os filhotes foram obtidos através de 21 ratas nulíparas da espécie *Rattus Norvegicus Albinus* da linhagem *Wistar* provenientes do biotério de criação do Departamento de Nutrição da UFPE que foram acasaladas com machos reprodutores na proporção de duas fêmeas para um macho (não consanguíneos), com idade entre 90 e 120 dias e peso entre 220 e 260g. Após a confirmação da gravidez através de esfregaço vaginal quando observado a presença de espermatozoide na secreção vaginal, as gestantes foram distribuídas em gaiolas individuais e acompanhadas durante todo período gestacional.

No dia do nascimento foi realizada uma randomização dos filhotes machos, com um peso corporal ideal (6 a 8 gramas) e eles foram distribuídos em quatro grupos, baseados na manipulação farmacológica e na indução à paralisia cerebral: 1- Controle salina (CS, n=12), constituído por filhotes que receberam solução salina do 3º ao 21º dia de vida; 2- Controle resveratrol (CR, n=11), constituído por filhotes que receberam resveratrol do 3º ao 21º dia de vida; 3- PC Salina (PCS, n=11), constituído por filhotes submetidos à modelo experimental de PC e que receberam solução salina do 3º ao 21º dia de vida; 4- PC resveratrol (PCR, n=12), constituído por filhotes submetidos à modelo experimental de PC e que receberam resveratrol do 3º ao 21º dia de vida.

Cada ninhada foi composta por 8 filhotes que permaneceram com as mães até o 25º dia pós-natal quando eles foram desmamados e os machos colocados em gaiolas individuais até a eutanásia por decapitação no 29 dia pós-natal.

Figura 2. Representação da formação dos grupos experimentais. A) Fluxograma com a divisão dos grupos, onde CS = controle salina; CR = controle resveratrol; PCS = paralisia cerebral e salina; PCR = paralisia cerebral e resveratrol. B) Animais com 26 dias de vida. O animal da esquerda representa o grupo controle e o da direita representa o grupo submetido à paralisia cerebral



Fonte: acervo do autor (2022)

5.4 ADMINISTRAÇÃO DO RESVERATROL

Após o nascimento dos animais, os filhotes machos foram alocados de forma aleatória conforme os grupos experimentais no período neonatal e a administração da droga foi feita do 3º ao 21º dia de vida pós-natal, por via intraperitoneal (Girbovan & Plamondon, 2015). Os animais foram distribuídos em: 1- tratados com resveratrol (dose diária, 10mg/kg) e 2-salina (0,9% NaCl), o volume de injeção foi de 0,1ml/100g peso do rato). Os ratos foram pesados diariamente e o volume de injeção foi ajustado para corresponder ao peso corporal do animal.

5.5 PROCEDIMENTOS EXPERIMENTAIS

5.5.1 Análise da evolução ponderal

O peso dos animais foi registrado diariamente desde o dia do nascimento até o P29. Para isso, foi utilizado uma balança Marte, modelos S-100, capacidade de 1kg e sensibilidade de 0,01g.

5.5.2 Análise da maturação das características físicas

Esta análise foi feita diariamente onde para cada animal registrou-se o tempo, em dias, desde o nascimento até a maturação da característica física avaliada (SMART; DOBBING, 1971). As seguintes características foram avaliadas:

- Abertura do pavilhão auditivo (APA): Os dois pavilhões, primitivamente dobrados ao nascimento sobre o orifício auricular, desfazeram a dobra, ficando livres e palpáveis pelo pesquisador.
- Abertura do conduto auditivo (ACA): Os dois orifícios auriculares, primitivamente fechados, abriram-se, tornando-se visíveis pelo pesquisador.
- Erupção dos incisivos inferiores (EII): A erupção dos incisivos inferiores foi observada quando ocorreu o rompimento da gengiva com exposição incisal.
- Erupção dos incisivos superiores (EIS): A erupção dos incisivos superiores foi observada quando ocorreu uma exposição incisal juntamente com o rompimento da gengiva.
- Abertura dos olhos (AO): Observadas quando os dois olhos estavam abertos e com a presença de movimento palpebral.

5.5.3 Avaliações murinométricas

Os animais foram avaliados nos dias 1, 3, 6, 9, 12, 15, 18 e 21 após o nascimento, com auxílio de um paquímetro digital (JOMARCA®), quanto às seguintes medidas (SILVA, DA et al., 2005):

- Comprimento da cauda (CC): O animal foi contido gentilmente com uma das mãos do pesquisador. Logo após, a cauda foi estirada horizontalmente na borda de uma mesa lisa e plana. Com uma caneta, foram feitas marcas na mesa, coincidentes com a extremidade e a base da cauda. Mediu-se então, a distância entre os pontos obtidos em milímetros (mm).
- Eixo látero-lateral do crânio (ELLC): Considerou-se como referência a linha imaginária perpendicular ao eixo longitudinal do crânio, dividindo os pavilhões auriculares ao meio. O animal foi contido com uma das mãos, tendo a cabeça dele entre os dedos indicadores e polegar, medindo a distância em milímetros.
- Eixo ântero-posterior do crânio (EAPC): A referência considerada para a medida do eixo ântero-posterior do crânio foi a linha média que vai da

extremidade do focinho até o ponto de intersecção com outra linha perpendicular imaginária. Essa última passa tangencialmente às extremidades posteriores dos pavilhões auriculares. O animal foi contido com uma das mãos, mantendo a cabeça deste entre os dedos indicadores e polegar, medindo a distância em milímetros.

- Eixo longitudinal (EL): O animal teve as regiões do dorso-anterior, dorso-posterior do corpo comprimidas e a cauda do animal de encontro a uma superfície plana (mesa). Em seguida, com uma caneta, foram feitas marcas na mesa, coincidentes com o focinho e a base da cauda do animal. Mediu-se então a distância em mm entre os pontos obtidos.

5.5.4 Análise da marcha em Catwalk

A análise da marcha foi realizada através do Catwalk (Noldus), conforme descrito por Herold et al. (2016), no P28. As alterações nos componentes do ciclo da marcha são determinadas e quantificados os déficits na locomoção. O CatWalk System consiste em uma passarela fechada (placa de vidro) que é iluminada por luz fluorescente. O sistema é equipado com uma câmera colorida de alta velocidade conectada a um computador com o software de detecção adequado (CatwalkXT9.1), que é capaz de detectar vários parâmetros estáticos e dinâmicos durante a locomoção espontânea do rato. Dessa forma, o animal é posicionado em um corredor de um metro de diâmetro e filmado individualmente, enquanto atravessa o Catwalk para mensuração de parâmetros estáticos e dinâmicos da locomoção espontânea.

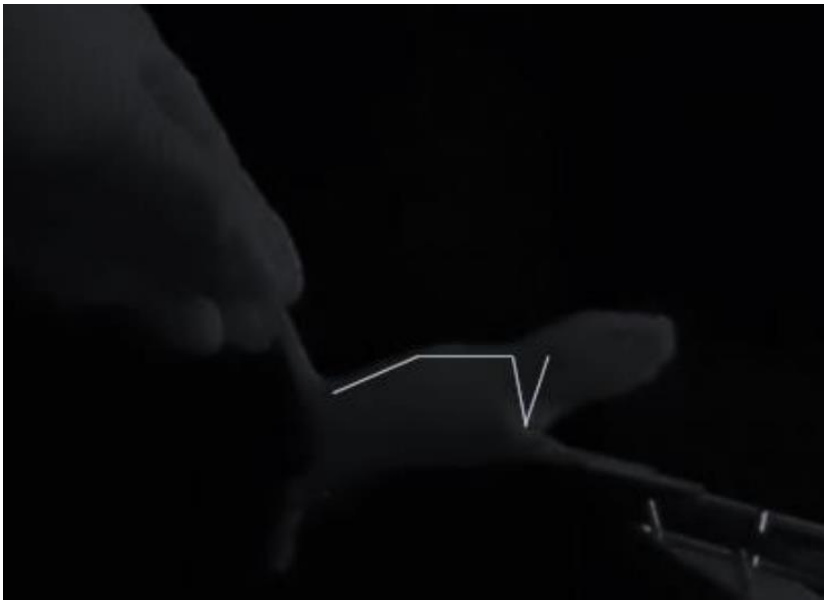
5.5.5 Avaliação da força muscular

A análise de força muscular foi feita através do teste de força da preensão dos membros anteriores nos dias 22 e 28 de vida pós-natal. Cada animal agarrou a barra de apoio, exercendo uma força de tração sobre ela em posição horizontal enquanto suspensos pela cauda. O captor de força permite quantificar o pico de força de cada animal (g). Este teste foi realizado no equipamento Animal Grip Strength System (SD Instruments) com capacidade de 200Kgf, resolução 0,1Kgf e acurácia de ± 0,2% (Takeshita et al., 2017).

5.5.6 Análise Postural

A postura foi analisada através de vídeo referente a avaliação de força muscular pelo teste de suspensão, nas idades de 14, 21 e 28 dias de vida pós-natal. Foram capturadas duas imagens do vídeo correspondente, uma imagem após os 5 segundos iniciais de filmagem, quando o animal estiver parado, e outra imagem antes dos 5 segundos finais, da mesma forma. Determinamos assim dois momentos de análise para se obter uma média de cada animal nas idades estudadas. Os parâmetros avaliados foram: alinhamento da cabeça e do tronco na postura horizontal (Figura 3). Para avaliação do alinhamento de cabeça será delimitado um ângulo utilizando como pontos de referência o trago, a transição occipto-cervical e o ponto médio na transição entre a cabeça e o ventre do animal. Para avaliação do alinhamento do tronco outro ângulo será delimitado, sendo os pontos de referência a transição entre o dorso e cauda, o ponto médio no dorso do animal e a transição occipto-cervical (adaptado de Lelard et al. 2006).

Figura 3. Representação da análise postural evidenciando os ângulos correspondentes ao alinhamento da cabeça e do tronco.



Fonte: acervo do autor (2019).

5.5.7 Extração, preparação de tecidos e quantificação de proteínas

Aos 29 dias de vida pós-natal, os animais foram eutanasiados por decapitação. O córtex somatossensorial foi removido e mantido a -80 °C até o preparo adequado. O córtex somatossensorial direito foi preparado para ensaios de RT-PCR e o córtex somatossensorial esquerdo foi preparado para análise bioquímica. O córtex

somatossensorial esquerdo foi homogeneizado em tampão de extração frio (base Tris 100 mM, pH 7,5; EDTA 10 mM; contendo um coquetel de inibidores de protease). Após a homogeneização, as amostras foram centrifugadas a 4°C a 1180 x g por 5 minutos, e os sobrenadantes foram utilizados para quantificação de proteínas de acordo com o método de Bradford (BRADFORD 1976).

5.5.8 Medição da atividade da citrato sintase

A atividade enzimática da citrato sintase foi determinada conforme descrito por da Silva et al. (2015). Resumidamente, foi feito uma reação contendo (em mM) 100 Tris-HCl (pH 8,2), 1 MgCl2, 1 EDTA, 0,2 5,5-ditio-bis (ácido 2-nitrobenzóico) (DTNB) (ε = 13,6 µmol - ml- 1 cm-1), 3 acetil-CoA, 5 oxaloacetato e 0,3 mg / ml de homogeneizado. A atividade CS foi medida avaliando a taxa de alteração na absorbância a 412 nm durante 3 min (intervalos de leitura de 30 s) e os resultados foram expressos como U/mg de proteína (Patel, 1976).

5.5.9 Biomarcadores de estresse oxidativo:

- Avaliação da produção de malondialdeído (MDA)

0,2 mg/ml de amostra de proteína foi usado para medir o ácido tiobarbitúrico (TBA). Neste ensaio, o malondialdeído ou substâncias semelhantes a MDA produzem um pigmento rosa com absorção máxima em 535 nm. A reação foi realizada com ácido tricloroacético (TCA) 30% e Tris-HCl (3mM), seguida de centrifugação a 2500 x g por 10 min. Em seguida, o sobrenadante foi transferido para um tubo, misturado com o mesmo volume de TBA 0,8% (v/v) e fervido por 30 min. A absorbância da fase orgânica foi lida a 535 nm em um espectrofotômetro, e os resultados foram expressos em mmol por mg de proteína (Buege e Aust 1978).

- Avaliação da oxidação de proteínas

O conteúdo de carbonila é o marcador primário de dano oxidativo à proteína, medido conforme publicado anteriormente (Reznick e Packer 1994). Resumidamente, 30% de TCA foi adicionado à amostra (0,2 mg/ml de proteína) em gelo, misturado e centrifugado por 15 min a 1180 x g. O pellet foi suspenso em 10 mM de 2,4-dinitrofenilhidrazina (DNPH) e imediatamente incubado em uma sala escura por uma hora com agitação a cada 15 minutos. Em seguida, as amostras foram centrifugadas,

lavadas três vezes com tampão etil/acetato e o pellet suspenso em cloridrato de guanidina 6 M, seguido de incubação por 5 minutos em banho-maria a 30°C. A absorbância foi lida até 370 nm, e os resultados foram expressos em mmol por mg de proteína.

5.5.10 Defesa Antioxidante Enzimática:

- Medição da atividade da superóxido dismutase (SOD)

A atividade total da enzima superóxido dismutase (t-SOD) foi determinada de acordo com o método descrito anteriormente (Misra & Fridovich, 1972). Os sobrenadantes do córtex (0,2 mg/ml) foram incubados com 880 µl de carbonato de sódio (0,05%, pH 10,2, 0,1 mM EDTA) a 25 °C e a reação foi iniciada por 30 mM de epinefrina (em 0,05% de ácido acético). A cinética da inibição da auto-oxidação da adrenalina foi monitorada por 180 segundos a 480 nm, e o resultado foi expresso em U/mg de proteína.

- Medição da atividade da catalase (CAT)

A atividade de CAT foi medida de acordo com o método descrito por Aebi (Aebi 1984). O ensaio consistiu em tampões fosfato 50 mM (pH 7,0), H2O2 0,300 mM e amostra de córtex 0,3 mg/mL. A taxa constante da enzima foi determinada medindo a mudança de absorbância a 240 nm por 4 minutos a 25□C. A atividade de CAT foi expressa como U/mg de proteína.

- Medição da atividade da glutationa-S-transferase (GST)

A atividade de GST foi medida como descrito anteriormente (Habig et al., 1974). Duzentos microgramas do sobrenadante do córtex foram adicionados a tampão fosfato 0,1 M (pH 6,5) contendo um mM-EDTA a 25°C. O ensaio foi iniciado com 1 mM de 1-cloro-2,4-dinitrobenzeno mais 1 mM-GSH. A formação de 2,4-dinitrofenil-S-glutationa foi monitorada a 340 nm de absorbância, e a atividade enzimática foi definida como a quantidade de proteína necessária para catalisar a formação de 1µmol de 2,4-dinitrofenil S-glutationa. Os resultados foram expressos em U/mg de proteína.

5.5.11 Defesa não enzimática

- Estado REDOX

A relação Glutationa reduzida/Glutationa oxidada (GSH/GSSG) foi avaliada conforme descrito anteriormente por (Hissin e Hilf 1976). As amostras foram incubadas em tampão fosfato 0,1M contendo 5mM-EDTA (pH 8,0) e com 1µg/ml de o-ftaldialdeído (OPT) em temperatura ambiente (RT) por 15 min e avaliadas por fluorescência com comprimentos de onda de 350nm e 420nm. Os níveis de GSSG foram avaliados incubando as mesmas amostras com N-etilmaleimida 40mM por 30 min em temperatura ambiente com a adição de um tampão NaOH 100mM. Os mesmos passos do ensaio de GSH foram seguidos para determinar os níveis de GSSG. A razão de GSH/GSSH determinou o estado REDOX.

- Quantificação de grupos tiol totais

A amostra foi misturada em uma solução contendo tampão Tris-EDTA (pH 7,4), com 10 mM de 5,5'-ditio-bis (ácido 2-nitrobenzóico) (DTNB) e incubada à temperatura ambiente por 30 min. A absorbância foi medida a 412nm e os resultados são expressos em mmol/mg de proteína (Aksenov & Markesbery, 2001).

5.5.12 Expressão do mRNA via RT-PCR

O RNA total foi obtido pelo método de extração com isotiocianato de guanidina usando o reagente Trizol. Inicialmente os tecidos foram lisados usando Triol, depois de 5 minutos de incubação, à temperatura ambiente, foi acrescentado clorofórmio e foram centrifugados a 1200g por 15 minutos. A fase aquosa foi transferida para outro tubo, adicionados de isopropanol gelado e depois foi incubado por 10 minutos à temperatura ambiente e nova centrifugação a 12000 g por 10 minutos. O RNA formado foi então lavado com etanol a 75% e centrifugado a 7000 g por 5 minutos. O sedimento do RNA (pellet) foi então ressuspenso em água livre de RNase e armazenado. A quantificação do RNA foi realizada em duplicata, diluindo as amostras 1:50 em água isenta de RNase. A absorbância da amostra foi determinada por espectrofotometria nos comprimentos de onda de 260nm (correspondente ao pico de absorção de RNA) e 280nm (correspondente ao pico de absorção de proteínas). Para a análise da pureza de RNA, o valor da absorbância obtido a 260nm foi dividido pelo obtido a 280nm e a

amostra que apresentou a razão de 260/280 igual ou superior a 1.8, foi utilizada (indicativo de alto grau de pureza) (Lagranha et al, 2007).

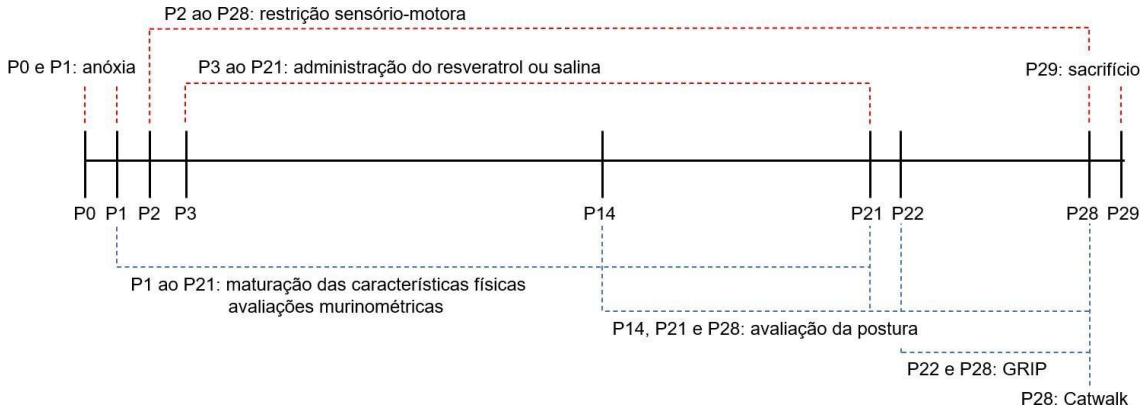
As reações foram realizadas para cada grupo de “primers” e todos os parâmetros foram avaliados utilizando concentrações constantes de RNA e seguindo as normas do fabricante do Kit SuperScriptIII® SYBR® GREEN One-Step qRT-PCR (Invitrogen, USA). Usando também a expressão do gene B2M como o gene normalizador para cada amostra e a quantificação da expressão será de acordo com o cálculo de 2-CT (LIVAK e SCHMITTGEN 2001; PFAFFL, 2001).

Tabela 1. Tabela dos primers utilizados para as análises de RT-PCR

Gene	Forward Primer	Reverse Primer
β2M	TGACCGTGATCTTTCTGGTG	ACTTGAATTTGGGGAGTTTTCTG
PGC-1alfa	AAC AGC AAA AGC CAC AAA GA	AAG TTG TTG GTT TGG CTT GA
TFAM	TCT CAT GAT GAA AAG CAG GCA	GAG ATC ACT TCG CCC AAC TT
Complexo II (Sdhb)	TTT ACC GAT GGG ACC CGG AC	CGT GTT GCC TCC GTT GAT GT
Complexo V (Atp5f1a)	TCC CTG AAC TTG GAA CCC GA	GGC ATT TCC CAG GGC ATC AA

Fonte: acervo do autor (2022)

Figura 4. Cronograma de experimentos e testes desde o dia do nascimento até o dia de sacrifício do animal.



Fonte: acervo do autor (2022).

6 ANÁLISE ESTATÍSTICA

A normalidade das variáveis investigadas foi estabelecida pelo teste de Kolmogorov-Smirnov. Quando constatada distribuição normal, foram realizados os testes paramétricos adequados como o ANOVA two-way para comparação dos grupos nas análises realizadas em apenas uma idade do animal, ou o ANOVA two-way Medidas repetidas para comparação dos grupos nas análises realizadas em mais de uma idade. Quando não foi observada distribuição normal, foram adotados testes estatísticos não paramétricos como o teste de Kruskal-Wallis e Friedman. Os resultados foram expressos como média ± erro padrão, sendo o nível de significância utilizado de 5%. Foi utilizado o software GraphPadPrism® versão 9 para análises dos dados e construção dos gráficos.

7 RESULTADOS

7.1 ARTIGO ORIGINAL

Os resultados e discussão deste estudo foram apresentados em forma de artigo original.

Artigo Original: NEONATAL TREATMENT WITH RESVERATROL DECREASES POSTURAL AND STRENGTH IMPAIRMENTS AND IMPROVES MITOCHONDRIAL FUNCTION IN THE SOMATOSENSORY CORTEX RATS SUBMITTED TO CEREBRAL PALSY

Artigo original elaborado a partir dos resultados do presente estudo, seguindo as normas da revista a qual foi publicado: Neurochemistry International Journal (Qualis A2 na área 21 da CAPES, fator de impacto 4,297) (APÊNDICE A)

7.2 REVISÃO SISTEMÁTICA

Publicação do artigo intitulado “Locomotion is impacted differently according to the perinatal brain injury model: Meta-analysis of preclinical studies with implications for cerebral palsy” (APÊNDICE B)

8 CONSIDERAÇÕES FINAIS

A paralisia cerebral promoveu prejuízos nos animais com relação ao ganho de peso, as funções motoras e o balanço oxidativo. Assim, observamos a redução do peso corporal e do crescimento corporal a partir do 8° e 9° dia de vida, respectivamente, alteração na marcha aos 28 dias, redução da força muscular, alteração no alinhamento da cabeça a partir do 14° dia de vida, além de aumento nos níveis de carbonilas, diminuição da expressão de TFAM e da atividade da citrato sintase.

O tratamento neonatal com o resveratrol nos animais submetidos à paralisia cerebral melhorou o desenvolvimento postural e a força muscular, reduziu os níveis de MDA e carbonilas (principais marcadores do estresse oxidativo) e promoveu um aumento da expressão do gene TFAM e da atividade da enzima citrato sintase, sugerindo que o polifenol aumentou a função mitocondrial nas células.

Nos animais do grupo controle, a administração do resveratrol diminuiu a concentração de MDA e a atividade das enzimas SOD e CAT, além de aumentar as expressões dos genes dos complexos II e V e a atividade da citrato sintase. Estes dados sugerem uma possível atuação preventiva do resveratrol contra insultos futuros.

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APÊNDICE A — NEONATAL TREATMENT WITH RESVERATROL DECREASES POSTURAL AND STRENGTH IMPAIRMENTS AND IMPROVES MITOCHONDRIAL FUNCTION IN THE SOMATOSENSORY CORTEX RATS SUBMITTED TO CEREBRAL PALSY

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ABSTRACT

Cerebral palsy is a neurodevelopmental disease characterized by postural, motor, and cognitive disorders, being one of the main causes of physical and intellectual disability in childhood. To minimize functional impairments, the use of resveratrol as a therapeutic strategy is highlighted due to its neuroprotective and antioxidant effects in different regions of the brain. Thus, this study aimed to investigate the effects of neonatal treatment with resveratrol on postural development, motor function, oxidative balance, and mitochondrial biogenesis in the brain of rats submitted to a cerebral palsy model. Neonatal treatment with resveratrol attenuated deficits in somatic growth, postural development, and muscle strength in rats submitted to cerebral palsy. Related to oxidative balance, resveratrol in cerebral palsy decreased the levels of MDA and carbonyls. Related to mitochondrial biogenesis, was observed in animals with cerebral palsy treated with resveratrol, an increase in mRNA levels of TFAM, in association with the increase of citrate synthase activity. The data demonstrated a promising effect of neonatal resveratrol treatment, improving postural and muscle deficits induced by cerebral palsy. These findings were associated with improvements in oxidative balance and mitochondrial biogenesis in the brain of rats submitted to cerebral palsy.

1. Introduction

Cerebral palsy (CP) is a heterogeneous group of diseases that result from non-progressive damage to the brain at an early stage of development. CP symptoms include irregular movement and irregularities in posture (Hadders-algra, 2014). According to the definition, the damage is not progressive, but the general condition can change over the years due to treatment and the plasticity of the maturing nervous system (Mlodawski et al., 2019). People with CP often perform poorly in activities of daily living due to limited limb, trunk, and head control (Velasco et al., 2017), have abnormal muscle tone and motor control, which contributes to the loss of postural control and motor coordination, compromising functionality and, consequently, quality of life (McNish et al., 2019).

CP is the most common motor development disorder in children (McIntyre et al., 2012), with an incidence of 3,4 per 1000 live births in regions from low- and middle-income countries and 1,5 per 1000 live

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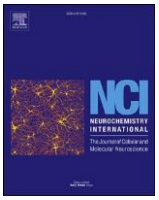

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Cerebral palsy (CP) is a heterogeneous group of diseases that result from non-progressive damage to the brain at an early stage of development. CP symptoms include irregular movement and irregularities in posture (Hadders-algra, 2014). According to the definition, the damage is not progressive, but the general condition can change over the years due to treatment and the plasticity of the maturing nervous system (Mlodawski et al., 2019). People with CP often perform poorly in activities of daily living due to limited limb, trunk, and head control (Velasco et al., 2017), have abnormal muscle tone and motor control, which contributes to the loss of postural control and motor coordination, compromising functionality and, consequently, quality of life (McNish et al., 2019).

CP is the most common motor development disorder in children (McIntyre et al., 2012), with an incidence of 3,4 per 1000 live births in regions from low- and middle-income countries and 1,5 per 1000 live

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Abbreviations

CP	Cerebral Palsy
ROS	Reactive Oxygen Species
RES	Resveratrol
TBA	Thiobarbituric Acid
MDA	Malondialdehyde
TCA	Trichloroacetic Acid
DNPH	Dinitrophenylhydrazine
SOD	Superoxide dismutase
CAT	Catalase
GST	Glutathione-S-Transferase
GSH/GSSG	Glutathione/Oxidized Glutathione ratio
OPT	o-phthaldialdehyde
DTNB	5,5′-dithio-bis (2-nitrobenzoic acid)
β2M	β-2 microglobulin
RT-PCR	Real-time polymerase chain reaction
LPS	liposaccharide
OS	oxidative stress
PGC-1α	Peroxisome proliferator-activated receptor-gamma coactivator-1α
TFAM	transcription factor A

births in regions from high-income countries (McIntyre et al., 2022). The etiology of CP is diverse and multifactorial, and the risk factors are found in the periods before and around the time of conception, and up to 2 years of age (McIntyre et al., 2022; Pakula et al., 2009). The following risk factors are described as associated with CP: genetic variants, congenital anomalies, neonatal respiratory distress syndrome, meconium aspiration, birth asphyxia, kernicterus, hypoxic-ischemia, and cerebrovascular insults during pregnancy and in infancy, and accidental and non-accidental brain injury (McIntyre et al., 2012, 2022). Among these factors, the mechanisms that cause oxygen and nutrient deprivation to the central nervous system stand out, such as perinatal asphyxia (Hakobyan et al., 2019; Perlman and Shah, 2011), which are associated with the severe neurofunctional consequences of CP (Low and Roland, 2004; Rainaldi and Perlman, 2016; Vannucci et al., 2004; Volpe, 2001).

Oxidative stress (OS) is considered a major contributor to ischemic brain injury (Warner et al., 2004) because it is an important consequence of neurotransmitter-mediated toxicity in combination with hyperoxygenation after hypoxia-ischemia (Ferriero, 2001). The generation of ROS and consequent oxidative stress and mitochondrial dysfunction is related to the onset of neuronal injury, leading to neurodegeneration (Manzanero et al., 2013; Sun et al., 2022). The effects of OS are observed in the pathogenesis of different diseases that affect the brain, such as mitochondrial disorders, cerebral ischemia, epilepsy, and cerebral palsy (Aycicek and Iscan, 2006). Understanding the role of OS in cerebral palsy is important as therapy with agents that increase the body's antioxidant capacity may be a treatment option for preventing the neurological insult (Aycicek and Iscan, 2006).

In this context, experimental models emerge as tools that mimic the pathophysiological mechanisms of CP and its motor disorders similar to those that occur in humans (Lacerda et al., 2017; Pereira et al., 2021a; Pereira et al., 2021b). In this sense, the model that associates perinatal anoxia and sensory-motor restriction of the lower limb reproduces the motor deficits found in children with cerebral palsy, such as reduced locomotor activity (Marcuzzo et al., 2010; Pereira et al., 2021a), reduced sarcomere density (Stigger et al., 2011), changes in oral functions (Lacerda et al., 2017), in addition to impairments in motor performance (Marcuzzo et al., 2010).

Through experimental models, early therapeutic perspectives may emerge for the treatment of CP based on phenotypic plasticity. Phenotypic plasticity is defined as 'the ability of individual genotypes to produce different phenotypes when exposed to different environmental conditions (Pigliucci et al., 2006). This includes the ability of neural synapses and brain pathways to be modified by altered thoughts and emotions, as well as environmental, behavioral, and neural stimuli, thus enabling neural plasticity (Kourosh-Arami et al., 2021). Studies with polyphenols have shown that the consumption of these natural compounds can improve neural plasticity (Bensalem et al., 2018) and resveratrol (RES), a polyphenolic compound present in many plant species (Peng et al., 2022; Song et al., 2014), improves neuroplasticity through a variety of mechanisms (Peng et al., 2022). It is important to highlight the importance of interventions due to their ability to modulate neuronal activity, generating adaptive responses that can cause normal differentiation of motor neurons and consequent ideal neuromuscular performance in adulthood (Stigger et al., 2011).

Several researches have indicated that the use of neuroprotective drugs can improve the prognostic perspectives for patients with cerebral palsy (Sun et al., 2021) and interventions on the central nervous system, mainly in early life, have been suggested as therapies aimed at reducing oxidative stress by interrupting the lesion cascade (Juul and Ferriero, 2014; Visco et al., 2022). Resveratrol has received considerable attention recently for its strong brain protection (Peng et al., 2022). RES is a substance that has shown effective treatment of a wide range of pathologies, including neurodevelopmental disorders and neurocognitive disorders (Baur and Sinclair, 2006). Resveratrol is among the polyphenols that are being used in acute central nervous system injury (Peng et al., 2022) and hypoxia-ischemia models as a neuroprotective strategy and (Juul and Ferriero, 2014; Ortega et al., 2014; Pan et al., 2016). Several lines of evidence have also demonstrated its antioxidant, anti-inflammatory and anti-aggregating properties (Jing et al., 2013; Marques et al., 2009; Pandey and Rizvi, 2009). In addition, a study on early intervention with RES in hypoxia-ischemia brain damage was reported (Karalis et al., 2011), however, the outcomes evaluated in this study have not yet been explored in the literature. Thus, this study aims to investigate the effects of neonatal treatment with resveratrol on postural development, motor function, and oxidative balance in the brain of rats submitted to a cerebral palsy model through neonatal anoxia and sensorimotor restriction.

2. Methodology

2.1. Animals

This study was performed by the guidelines of the National Council for the Control of Animal Experiments (CONCEA) and with the international standards of the National Institute of Health Guide for Care and Use of Laboratory Animals (8th ed) and has been approved by the Ethics Committee for Animal Use (CEUA) of the Federal University of Pernambuco (process number CEUA: 0032/2021). Twenty-one female *Wistar* rats were mated with breeding males in the proportion of two females to one male in a 12h dark-light-cycle (dark 8 a.m.-8 p.m.) at 22 ± 2 °C with free access to water and diet. On the day of birth, male pups were randomly assigned with an ideal body weight (6–8g) and they were divided into four groups, based on resveratrol manipulation and cerebral palsy induction: 1- saline control (CS, n = 12), consisting of pups that received a saline solution from the 3rd to the 21st day of life; 2- resveratrol control (CR, n = 11), consisting of pups that received resveratrol from the 3rd to the 21st day of life; 3- CP salina (CPS, n = 11), constituted by pups submitted to the experimental model of CP and that received a saline solution from the 3rd to the 21st day of life; 4- CP resveratrol (CPR, n = 12), constituted by pups submitted to the experimental model of CP and that received resveratrol from the 3rd to the 21st day of life. Each litter consisted of 8 pups that remained with their mothers until the 25th postnatal day (P25) when they were weaned, and the males were placed in individual cages until euthanasia by decapitation at P29.

2.2. Experimental model of cerebral palsy

The experimental CP model used was the same as described by Strata et al. (2004), Coq et al. (2008), Lacerda et al. (2017), and Pereira (2021a) which associates perinatal anoxia with sensorimotor restriction of the hind paws. The pups in the CP groups underwent two episodes of postnatal anoxia, on the day of birth and the first day of life (P0 and P1). Pups were placed inside an acrylic chamber partially immersed in water at 37 °C and exposed to nitrogen (100%) at 9L/min for 12 min, then recovered in air and at room temperature and returned to their respective mothers. From P2 to P28 sensorimotor restriction of the hind paws was performed for 16 h a day, with the animal being allowed free movement for the remaining 8 h. For the sensorimotor restriction, an orthosis made with an epoxy mold was used, leaving the hind legs extended, without affecting the elimination of urine and feces and maternal care (Strata et al., 2004).

2.3. Administration of resveratrol

After the birth of the animals, the male offspring were randomly allocated according to the experimental groups in the neonatal period, and the drug was administered intraperitoneally from P3 to P21 (Girbovan and Plamondon, 2015). The animals were divided into 1- treated with resveratrol (daily dose, 10 mg/kg) and 2-saline (0.9% NaCl), injection volume was 0.1 ml/100g rat weight). Rats were weighed daily, and the injection volume was adjusted to match the animal's body weight.

2.4. Weight evolution, somatic growth, and maturation of physical features

The weight of the animals was recorded daily from the day of birth to P29. For this, a Marte scale, models S-100, capacity of 1 kg and sensitivity of 0.01g was used.

The somatic growth and physical features were evaluated daily between 7:00 and 9:00h until maturation. For the somatic growth, the animals were evaluated with a digital caliper (JOMARCA®), for the following measurements, in centimeters (H. J. da Silva et al., 2005): lateral skull axis (distance between the ear holes), anteroposterior skull axis (distance between snout and head-neck articulation), tail length (distance from tail tip to tail base) and longitudinal axis (distance between snout and tail base).

The age at the maturation of a particular physical feature was defined as the day when it was first observed (Smart and Dobbing, 1971). The following features were evaluated: auricle opening, auditory conduit opening, eyes opening, upper incisors eruption, and lower incisors eruption.

2.5. Gait analysis on catwalk

Gait analysis was performed using the Catwalk (Noldus), as described by Herold et al. (2016), at P28. Changes in the components of the gait cycle are determined and deficits in locomotion are quantified. The CatWalk System consists of an enclosed walkway (glass plate) that is illuminated by fluorescent light. The system is equipped with a high-speed color camera connected to a computer with the appropriate detection software (CatwalkXT9.1), which can detect various static and dynamic parameters during the rat's spontaneous locomotion (Fig. 1). In this way, the animal is positioned in a 1-m-diameter corridor and individually filmed as it crosses the Catwalk to measure static and dynamic parameters of spontaneous locomotion.

2.6. Forelimb grip strength and postural analysis

Muscle strength analysis was performed using the forelimb grip strength test at P22 and P28. Each animal grabbed the support bar,

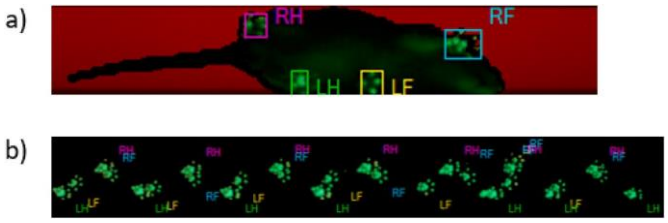

Fig. 1. Evaluation of static and dynamic gait parameters during animal's locomotion by the Catwalk's system. A – Rat walking through the platform. B - Representative images of paw prints during active locomotion of the animals on the platform. LF, left front paw; LH, left hind paw; RF, right front paw; RH, right hind paw.

exerting a traction force on it in a vertical position while suspended by the tail. The force sensor quantifies the peak force of each animal (g). This test was performed on the Animal Grip Strength System (SD Instruments) equipment with a capacity of 200Kgf, resolution 0.1Kgf, and accuracy of ± 0.2% (Takeshita et al., 2017).

For the postural analysis, the animals were submitted to the adapted forelimb grip test and evaluated in a horizontal position, at P14, P21 and P28 for the analysis of postural alignment. The performance of the animals during the test was recorded using video. After that, two images of the corresponding video were captured, an image after the initial 5 s of filming, when the animal was still, and another image before the final 5 s, in the same way. We thus determined two moments of analysis to obtain an average of each animal at the ages studied. The parameters evaluated were head and trunk alignment in the horizontal posture. To evaluate the head alignment, an angle will be delimited using the tragus, the occipito-cervical transition, and the midpoint in the transition between the head and the belly of the animal as reference points. To assess trunk alignment, another angle will be defined, the reference points being the transition between the back and tail, the midpoint on the animal's back, and the occipito-cervical transition (adapted from Lelard et al., 2006).

2.7. Extraction, tissue preparation, and protein quantification

At P29, the animals were euthanized by decapitation. The somatosensory cortex (here from now just mention as sample) was removed and kept at —80 °C and prepared for RT-PCR assays and biochemical analysis. The sample was homogenized in cold extraction buffer (100 mM Tris base, pH 7.5; 10 mM EDTA; containing a cocktail of protease inhibitors). After homogenization, samples were centrifuged at 4 °C at 1180×*g* for 5 min, and supernatants were used for protein quantification according to the Bradford method (BRADFORD, 1976).

2.8. Measurement of citrate synthase activity

Citrate synthase is the first enzyme in the Krebs cycle, its activity capacity is widely used as a marker of cellular and mitochondrial function. Citrate synthase activity was evaluated as previously described (Patel, 1976). Briefly, the reaction was carried out in a mixture containing Tris-HCl (pH = 8.2), magnesium chloride (MgCl), ethylenediamine-tetra-acetic acid (EDTA), 0.2 of 5.5 dithiobis (2-nitrobenzoic acid) (E = 13.6 μmol/(mL.cm), 3 acetyl CoA, 5 oxaloacetate and 0.1 mg/mL of sample. The activity was evaluated by measuring the change in the absorbance rate at 412 nm for 3 min at a temperature of 25 °C, using the EVEN UV-VIS spectrophotometer. The levels are expressed as U/mg of protein.

2.9. Oxidative stress biomarkers

- Evaluation of malondialdehyde production

0.2 mg/ml of protein sample was used to measure the thiobarbituric acid (TBA). In this assay, malondialdehyde (MDA) or MDA-like substances produce a pink pigment with maximum absorption at 535 nm. The reaction was carried out using 30% trichloroacetic acid (TCA) and Tris-HCl (3 mM), followed by centrifugation at 2500×*g* for 10 min. Then, the supernatant was transferred to a tube, mixed with the same volume of 0.8% TBA (v/v), and boiled for 30 min. The absorbance of the organic phase was read at 535 nm in a spectrophotometer, and the results were expressed as mmol per mg of protein (Buege and Aust, 1978).

3. Evaluation of protein oxidation

The carbonyl content is the primary marker for oxidative damage to protein, measured as previously published (Reznick and Packer, 1994). Briefly, 30% TCA was added to the sample (0.2 mg/ml of protein) on ice, mixed, and centrifuged for 15 min at 1180×*g*. The pellet was suspended in 10 mM 2,4-dinitrophenylhydrazine (DNPH) and immediately incubated in a dark room for 1 h with shaking every 15 min. Then, samples were centrifuged, washed three times with ethyl/acetate buffer, and the pellet suspended in 6 M guanidine hydrochloride, followed by incubation for 5 min in a water bath at 30 °C. Absorbance was read up to 370 nm, and the results were expressed as mmol per mg of protein.

3.1. Enzymatic antioxidant defense

3.1.1. - Measurement of superoxide dismutase (SOD) activity

The total superoxide dismutase enzyme activity (t-SOD) was determined according to the previously described method (Misra and Fridovich, 1972). Sample (0.2 mg/ml) were incubated with 880 μl sodium carbonate (0.05%, pH 10.2, 0.1 mM EDTA) at 25 °C, and the reaction started by 30 mM epinephrine (in 0.05% acetic acid). The kinetics of the inhibition of adrenalin auto-oxidation was monitored for 180 s at 480 nm, and the result was expressed as U/mg protein.

3.1.2. Measurement of catalase (CAT) activity

CAT activity was measured according to the method described by Aebi (Aebi, 1984). The assay consisted of 50 mM-phosphate buffers (pH 7.0), 0.300 mM H2O2 and 0.3 mg/mL of sample. The constant rate of the enzyme was determined by measuring the absorbance change at 240 nm for 4 min at 25 °C. CAT activity was expressed as U/mg protein.

- Measurement of Glutathione-S-Transferase (GST) activity

GST activity was measured as described previously (Habig et al., 1974). Two hundred micrograms of the sample were added to 0.1 M-phosphate buffer (pH 6.5) containing one mM-EDTA at 25 °C. The assay was initiated with 1 mM of 1-chloro-2.4-dinitrobenzene plus 1 mM-GSH. The formation of 2,4-dinitrophenyl-S-glutathione was monitored at 340 nm of absorbance, and the enzymatic activity was defined as the amount of protein required to catalyze the formation of 1 μmol 2, 4-dinitrophenyl S-glutathione. The results were expressed as U/mg protein.

3.2. Non-enzymatic defense

- REDOX State

Reduced Glutathione/Oxidized Glutathione ratio (GSH/GSSG) was evaluated as previously described by (Hissin and Hilf, 1976). The samples were incubated in a 0.1M phosphate buffer containing 5 mM-EDTA (pH 8.0) and with 1 μg/ml o-phthaldialdehyde (OPT) at room temperature (RT) for 15 min and evaluated by fluorescence with wavelengths of 350 nm and 420 nm. GSSG levels were assessed by incubating the same samples with 40 mM N-ethylmaleimide for 30 min in RT with the addition of a 100 mM NaOH buffer. The same steps of the GSH assay were followed to determine the GSSG levels. The ratio of GSH/GSSH determined the REDOX state.

- Quantification of total thiol groups

The quantification of sulfhydryls was based on the reduction of 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB). The sample was mixed in a solution containing Tris-EDTA buffer (pH 7.4), with 10 mM of DTNB and incubated at room temperature for 30 min. The absorbance was measured at 412 nm and results are expressed as mmol/mg protein.

3.3. mRNA evaluation

Total RNA was extracted from cortex tissue using TRIzol reagent and the guanidine isothiocyanate method according to the manufacturer's instructions (Invitrogen, USA). RNA quantification was performed in a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA), and purity was assessed using the ratio of 260/280 nm. Real-time polymerase chain reaction (RT-PCR) experiments were performed using the SuperScript® III Platinum® SYBR® Green One-Step qRT-PCR Kit (Invitrogen, USA). All genes used in the experiment were processed in duplicate, and the cycle threshold (Ct) value of each targeted gene was normalized to the β-2 microglobulin (β2M), and data expressed as $2^{-\Delta\Delta Ct}$ (Silva et al., 2023) (see Table 1).

3.4. Statistical analysis

For data analysis and graph construction we used the statistical software GraphPadPrism® version 9 and the results were expressed as mean ± standard error. A significance level of 95% was assigned. The Kolmogorov-Smirnov normality test was performed. As the normal distribution, for intergroup comparison, the ANOVA two-way parametric test was used with experimental cerebral palsy and pharmacological manipulation, followed by the Tukey post-test. For the variables in which the analysis was performed at several ages, the ANOVA two-way repeated measures followed by the Tukey post-test was used.

4. Results

4.1. Weight evolution, somatic growth and maturation of physical features

Fig. 2 presents the weight evolution of pups. The animals in the CPS group had lower body weight compared to the control saline group, from P8 to the last day of the experiment (P8: CPS: 12.45g ± 2.57g/CS: 18.79g ± 1.52g, $p < 0.01$; P14: CPS: 19.72g ± 4.27g/CS: 31.93g ± 2.65g, $p < 0.001$; P17: CPS: 22.45g ± 4.4g/CS: 37.42g ± 3.67g, $p < 0.001$; P21: CPS: 26.62g ± 4.93g/CS: 47.34g ± 4.68g, $p < 0.001$; P29: CPS: 47.87g ± 12.35g/CS: 78.35g ± 4.78g; $p < 0.001$) (Fig. 1). The treatment with resveratrol in CP attenuated the damage on weight gain only at P21 (P21: CPR: 32.69g ± 4.2g/CPS: 26.62g ± 4.93g, $p < 0.05$).

Somatic growth was also affected by experimental CP, evidenced by

Table 1
Primers sequence used in this study.

Gene Name	Forward Primer Sequence	Reverse Primer Sequence
β2M	TGACCGTGATCTTTCTGGTG	ACTTGAATTTGGGGAGTTTTCTG
PGC-1α	AAC AGC AAA AGC CAC AAA GA	AAG TTG TTG GTT TGG CTT GA
TFAM	TCT CAT GAT GAA AAG CAG GCA	GAG ATC ACT TCG CCC AAC TT
Complex II Subunit (Sdhb)	TTT ACC GAT GGG ACC CGG AC	CGT GTT GCC TCC GTT GAT GT
Complex V Subunit (Atp5f1a)	TCC CTG AAC TTG GAA CCC GA	GGC ATT TCC CAG GGC ATC AA

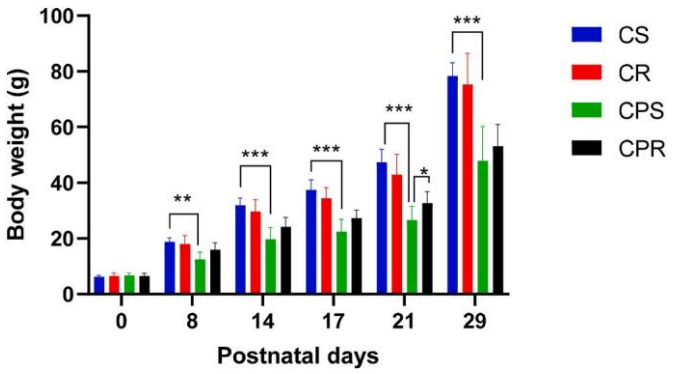

Fig. 2. Weight evolution at P0, P8, P14, P17, P21, and P29 according to the experimental groups: CS: Control Saline (n = 12); CR: Resveratrol Control (n = 11); CPS: CP + Saline (n = 11); CPR: CP + Resveratrol (n = 12). Data were expressed as mean ± standard error, $p < 0.05$ (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$).

smaller murinometric measurements in the CPS group compared to the CS group. Thus, in the measurements of the skull axes, the smallest lateral skull axis was observed in the CPS group at P12 (CPS: 1.55 cm ± 0.12 cm/CS: 1.79 cm ± 0.15 cm; $p < 0.001$), P15 (CPS: 1.67 cm ± 0.16 cm/CS: 1.82 cm ± 0.09 cm, $p < 0.05$), P21 (CPS: 1.69 cm ± 0.16 cm/CS: 1.90 cm ± 0.05 cm; $p < 0.01$) and P29 (CPS: 1.83 cm ± 0.11 cm/CS: 2.15 cm ± 0.15 cm, $p < 0.001$), as was observed smaller anteroposterior skull axis of the animals submitted to the CP model compared to the control animals at P3 (CPS: 2.23 cm ± 0.16 cm/CS: 1.93 cm ± 0.26 cm; $p < 0.05$), P12 (CPS: 2.85 cm ± 0.27 cm/CS: 3.20 cm ± 0.17 cm, $p < 0.05$), P15 (CPS: 3.09 cm ± 0.24 cm/CS: 3.39 cm ± 0.13 cm, $p < 0.05$), P18 (CPS: 3.24 cm ± 0.3 cm/CS: 3.59 cm ± 0.17 cm; $p < 0.05$), P21 (CPS: 3.32 cm ± 0.23 cm/CS: 3.81 cm ± 0.15 cm; $p < 0.05$) and P29 (CPS: 4.1 cm ± 0.32 cm; $p < 0.05$) (Fig. 3A and B).

Similarly, tail length was also impaired by experimental CP from the 9th postnatal day onwards (P9: CPS: 2.84 cm ± 0.51 cm/CS: 3.5 cm ± 0.36 cm, $p < 0.05$; P12: CPS: 3.31 cm ± 0.47 cm/CS: 4 cm ± 0.28 cm, $p < 0.05$; P15: CPS: 3.66 cm ± 0.64 cm/CS: 4.41 cm ± 0.35 cm, $p < 0.05$; P18: CPS: 4.19 cm ± 0.96 cm/CS: 4.99 cm ± 0.36 cm, $p < 0.05$, P21: CPS: 4.71 cm ± 0.7 cm/CS: 6.07 cm ± 0.47 cm, $p < 0.05$; and P29: CPS: 5.46 cm ± 1.23 cm/CS: 8.21 cm ± 0.91 cm, $p < 0.05$), as well as the longitudinal axis from the 12th postnatal day (P:12 CPS: 7.92 cm ± 0.95 cm/CS: 9.01 cm ± 0.48 cm, $p < 0.05$; P15: CPS: 8.73 cm ± 0.96 cm/CS: 10.51 cm ± 0.98 cm, $p < 0.001$; P18: CPS: 9.49 cm ± 1.12 cm/CS: 11.63 cm ± 0.59 cm, $p < 0.001$; P21: CPS: 10.02 cm ± 1.22 cm/CS: 12.45 cm ± 0.71 cm, $p < 0.001$; and P29: CPS: 12.32 cm ± 1.66 cm/CS: 13.55 cm ± 1.26 cm, $p < 0.01$) when compared to the control group (Fig. 3C and D).

Treatment with resveratrol in animals with CP attenuated the damage in somatic growth. Therefore, the animals in the CP group treated with resveratrol had greater measurements regarding tail length at P29 (CPR: 6.37 cm ± 1.15 cm/CPS: 5.46 cm ± 1.23 cm, $p < 0.05$) and the longitudinal axis at P6 (CPR: 7.49 cm ± 1.05 cm/CPS: 6.27 cm ± 0.42 cm, $p < 0.01$), P9 (CPR: 8.54 cm ± 1.39 cm/CPS: 7.19 cm ± 0.66 cm, $p < 0.01$) and P12 (CPR: 9.16 cm ± 1.32 cm/CPS: 7.93 cm ± 0.95 cm, $p < 0.01$) compared to the CPS group. Additionally, in control animals submitted to resveratrol treatment, it was also possible to observe an increase in the longitudinal axis of the body at P6 (CR: 7.75 cm ± 1.1 cm/CS: 6.38 cm ± 0.38 cm, $p < 0.01$) and P9 (CR: 8.95 cm ± 1.08 cm/CS: 7.66 cm ± 0.46 cm, $p < 0.01$) compared to the CS group (Fig. 3C and D).

Not only the somatic growth was affected by experimental CP, but the maturation of physical features was also impaired. There were a delay in the post-natal day of auricle opening (CPS: 3.41 ± 1.08/CS: 2.0 ± 0.0, $p = 0.0124$), auditory conduit opening (CPS: 14.33 ± 1.87/CS: 12.41 ± 0.51, $p = 0.0003$) and eyes opening (CPS: 14.66 ± 1.23/CS: 12.58 ± 0.51, $p < 0.0001$), in the animals submitted to the CP model compared to the control group. CP model did not cause changes in upper or lower incisor eruption. However, the treatment with resveratrol in the experimental CP did not influence the maturation of the evaluated somatic characteristics (Fig. 4).

4.2. Gait analysis on catwalk

Motor behavior after resveratrol treatment was evaluated. For this, the average speed of the animal during the Catwalk test was used as a kinetic parameter. In this analysis, the CPS group had a lower average speed when compared to the CS group (CS: 21.06 cm/s ± 2.13 cm/s/

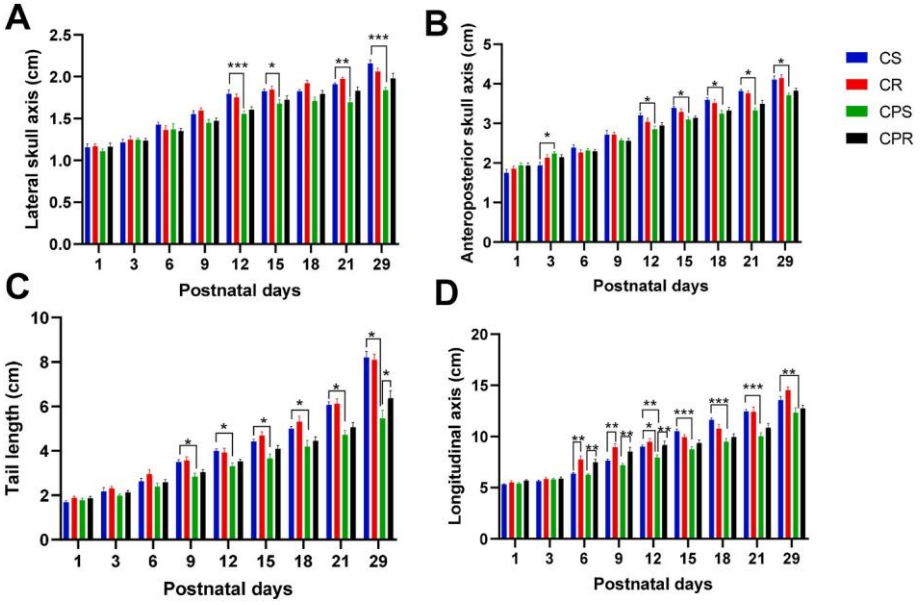

Fig. 3. Somatic growth at P1, P3, P6, P9, P12, P15, P18, P21 and P29 of postnatal life according to the experimental groups: CS: Control Saline (n = 12); CR: Resveratrol Control (n = 10); CPS: CP + Saline (n = 11); CPR: CP + Resveratrol (n = 12). Data were expressed as mean ± standard error, $p < 0.05$ (* = $p < 0,05$; ** = $p < 0,01$; *** = $p < 0.001$).

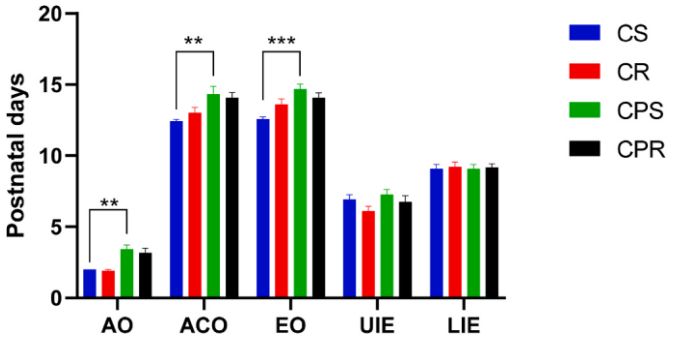

Fig. 4. Physical features between the P1 and P21 according to the experimental groups: CS: Control Saline (n = 12); CR: Resveratrol Control (n = 10); CPS: CP + Saline (n = 11); CPR: CP + Resveratrol (n = 12). AO: auricle opening; ACO: auditory conduit opening; EO: eyes opening UIE: upper incisors eruption; LIR: lower incisors eruption. Data were expressed as mean ± standard error, $p < 0.05$ (** = p < 0,01; *** = p < 0.001).

CPS: 18.35 cm/s ± 3.39 cm/s; $p < 0.05$) (Fig. 5).

In addition to the average velocity, the evaluation of spatial and temporal parameters showed important changes. Regarding the time of the swing phase, the CPS group had a long time when compared to the CS group (CS: 0.26s ± 0.04s/CPS: 0.32s ± 0.07s; $p < 0.05$) (Fig. 6B). In the analysis of swing velocity, the CPS group had a lower mean velocity compared to the saline control group (CS: 71.74 cm/s ± 3.58 cm/s/CPS: 55.61 cm/s ± 5.52 cm/s; $p < 0.05$) (Fig. 6C). The analysis of the maximum contact area showed that the CPS group showed a reduction in this area when compared to the CS group (CS: 0.78 cm^2 ± 0.04 cm^2/CPS: 0.52 cm^2 ± 0.05 cm^2; $p < 0.05$) (Fig. 6D). About the print length, the animals in the CPS group had a reduction in this length compared to the CS group (CS: 1.73 cm ± 0.04 cm/CS: 1.27 cm ± 0.06 cm; $p < 0.0001$) (Fig. 6E). The print width analysis showed that the CPS group had a reduced print width when compared to the saline control group (CS: 1.43 cm ± 0.04 cm/CPS: 1.18 cm ± 0.06 cm; $p < 0.0001$), but it showed no statistical difference between the groups CPS and CPR (Fig. 6F).

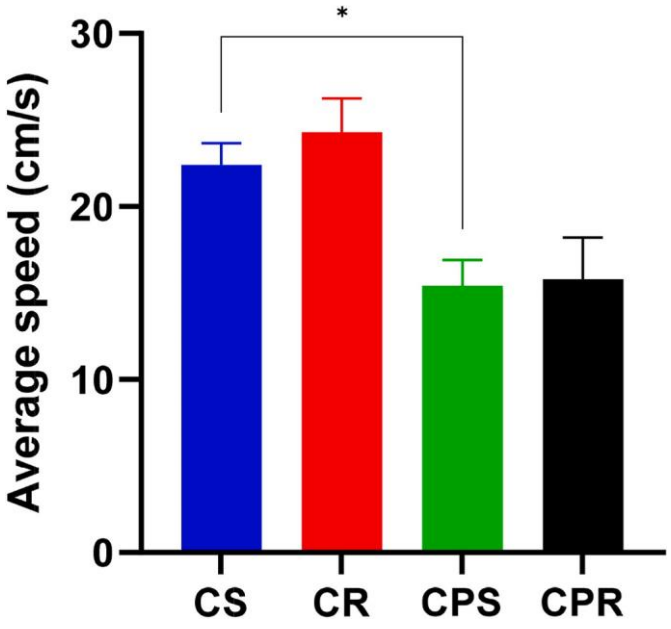

Fig. 5. Average speed during active locomotion on the platform. Experimental groups: CS: Control Saline (n = 12); CR: Resveratrol Control (n = 10); CPS: CP + Saline (n = 10); CPR: CP + Resveratrol (n = 10). Data were expressed as mean ± standard error, $p < 0.05$ (* = p < 0,05).

4.3. Forelimb grip strength and postural analysis

In the muscle strength analysis with the grip test, at P22 and P28, the CS group showed greater strength compared to the CPS group (P22: CS: 160.97g ± 6.22g/CPS: 113.58g ± 10.85g; $p < 0.01$; P28: CS: 235.05g ± 6.51g/CPS: 187.05g ± 8.5g; $p < 0.01$) (Fig. 6A and B). At the same ages, CP animals treated with RES showed greater strength compared to the CPS group (P22: CPR: 175.6g ± 9.17g/CPS: 113.58g ± 10.85g; $p < 0.001$; P28: CPR: 225, 19g ± 11.72g/CPS: 187.05g ± 8.5g; $p < 0.05$) (Fig. 7A and B). No significant difference was seen between the control groups ($p > 0.05$).

For the postural analysis, at P28 the animals in the CPS group presented greater trunk angulation when compared to the CPR group (CPR: 159.33° ± 1.44°/CPS: 167.38° ± 2.87°; $p < 0.05$). Likewise, the CS group presented a greater angulation compared to the CR group (CR: 153.98° ± 2.51°/163.95° ± 0.75°; $p < 0.05$) (Fig. 8A). The analysis of the head alignment showed that, from P14 to P28, the CPS animals presented greater angulation compared to the CS group (P14: CS: 45.34° ± 2.2°/CPS: 54.01° ± 2.28°; P21: CS: 48.4° ± 1.55°/57.65° ± 1.71°; P28: CS: 46.5° ± 1.46°/CPS: 62.06° ± 1.93°; $p < 0.05$) (Fig. 8B). Also, at P28, the CP animals submitted to the treatment with resveratrol presented smaller head angulation when compared to the pups of the CPS group (P28: CPR: 46.87° ± 2.09°/CPS: 62.06° ± 1.93°; $p < 0.05$) (Fig. 8B).

4.4. Oxidative state in the somatosensory cortex

After our previous analyzes, with the intent to understand how resveratrol treatment could improve the mechanical properties, we evaluate the oxidative stress biomarkers. Our data showed that treatment with resveratrol decreased MDA and Carbonyl markers in the animals with CP compared to the CPS group (MDA: CPR: 4.66 ± 1.27/CPS: 10.98 ± 1.30 μmol/mg prot; $p < 0.001$; Carbonyls: CPR: 13.81 ± 2.85/CPS: 29.14 ± 5.08 μmol/mg prot; $p < 0.01$) (Fig. 9A and B). Also, the control animals treated with resveratrol showed a decrease in MDA compared to the CS group (CR: 5.47 ± 1.04/CS: 12.12 ± 1.61 μmol/mg prot; $p < 0.001$) (Fig. 9A), while the CPS group showed an increase in Carbonyls compared to the CS group (CPS: 29.14 ± 5.08/CS: 13.55 ± 2.52 μmol/mg prot; $p < 0.01$) (Fig. 9B).

In addition, we performed several assays to investigate antioxidant capacity. Superoxide dismutase (SOD) and Catalase (CAT) activity in control animals treated with resveratrol were decreased when compared to the CS group (SOD: CR: 45.44 ± 4.34/CS: 72.81 ± 6.19 U/mg prot; $p < 0.05$; CAT: CR: 223.1 ± 45.78/CS: 405.3 ± 47.02 U/mg prot; $p < 0.05$) (Fig. 9C and D). No significant difference was seen in the others assays ($p > 0.05$) (Fig. 9E, F,9G, 9H and 9I).

4.5. Mitochondrial biogenesis and citrate synthase activity in the somatosensory cortex

After the observations in oxidative stress biomarkers, we further analyze some parameters linked with oxidative status. First, we measure the mitochondrial biogenesis mRNA levels. Our data showed a decrease in PGC-1α in the group of animals with cerebral palsy treated with resveratrol compared to the untreated (CPR: 0.6 ± 0.1/CPS: 1.4 ± 0.15; $p < 0.01$) (Fig. 10A) associated with an increase in TFAM levels (CPS: 0.3 ± 0.1/CPR: 0.9 ± 0.15; $p < 0.05$). In addition, we observed in CPS decreased levels of TFAM compared to CS (CPS: 0.3 ± 0.1/CS: 1 0.0 ± 0.1; $p < 0.05$) (Fig. 10B).

When we evaluated the mitochondrial complexes II and V, we observed in CR group an increase in both complexes' subunits compared to the CS group (Complex II: CR: 1.7 ± 0.2/CS: 1.0 ± 0.2; $p < 0.05$; Complex V: CR: 5.3 ± 0.25/CS: 1.0 ± 0.08; $p < 0.0001$). The CPS group showed an increase in V complex when compared with the CPR and CS groups (CPS: 2.8 ± 0.17/CPR: 0.51 ± 0.09/CS: 1.0 ± 0.08; $p < 0.0001$) (Fig. 10C and D).

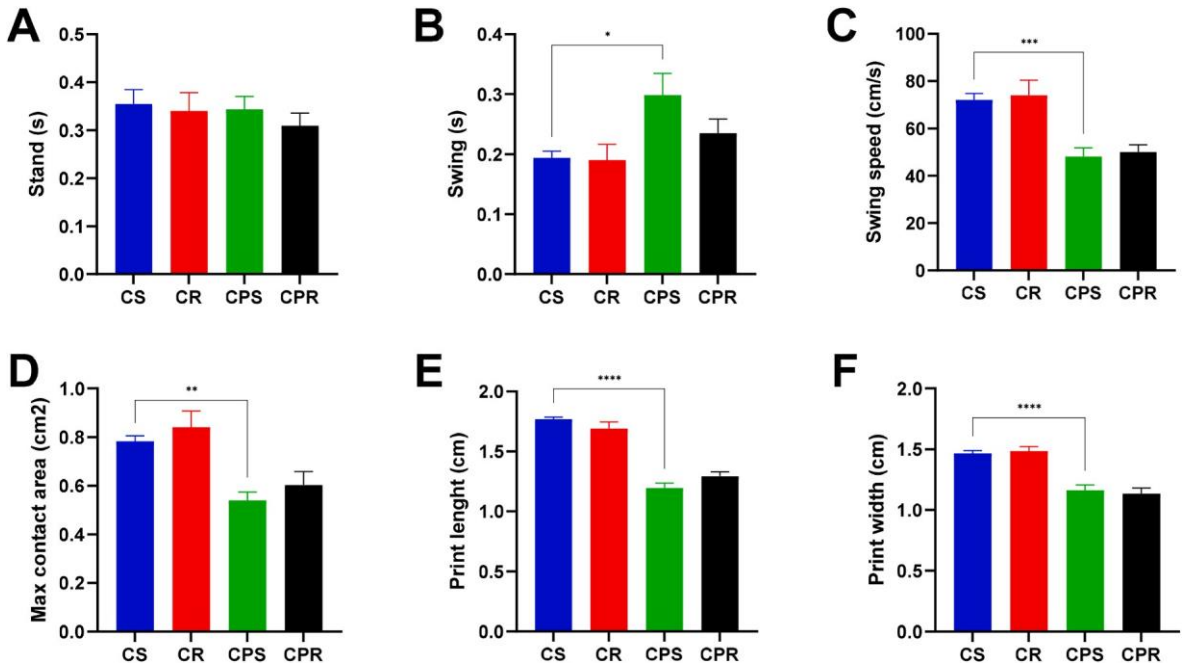

Fig. 6. Evaluation of temporal and spatial parameters of gait at P28. A – Paw support time during locomotion. B – Time during the swing phase on the paws. C – Velocity of the paws during the swing phase. D – Measurement of the area of the paws in contact with the platform during locomotion; E — paw print length during locomotion; F – paw print width during locomotion. Experimental groups: CS: Control Saline (n = 12); CR: Control Resveratrol (n = 10); CPS: CP + Saline (n = 10); CPR: CP + Resveratrol (n = 10). Data were expressed as mean ± standard error, $p < 0.05$ (* = $p < 0,05$; ** = $p < 0,01$; *** = $p < 0.001$; **** = $p < 0,0001$).

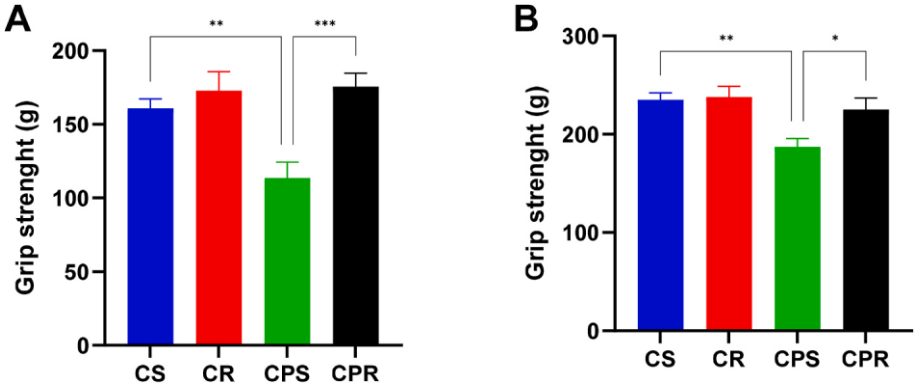

Fig. 7. Grip strength test at P22 (A) and P28 (B) according to the experimental groups: CS: Saline Control (n = 12); CR: Resveratrol Control (n = 10); CPS: CP + Saline (n = 11); CPR: CP + Resveratrol (n = 10). Data were expressed as mean ± standard error, $p < 0.05$ (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$).

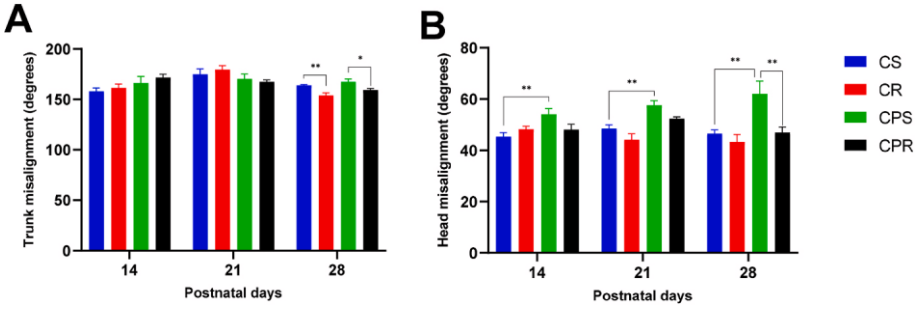

Fig. 8. Analysis of posture based on trunk (A) and head alignment (B) at P14, P21 and P28 according to the experimental groups: CS: Control Saline (n = 12); CR: Resveratrol Control (n = 10); CPS: CP + Saline (n = 10); CPR: CP + Resveratrol (n = 12). Data were expressed as mean ± standard error, $p < 0.05$ (* = $p < 0.05$; ** = $p < 0.01$).

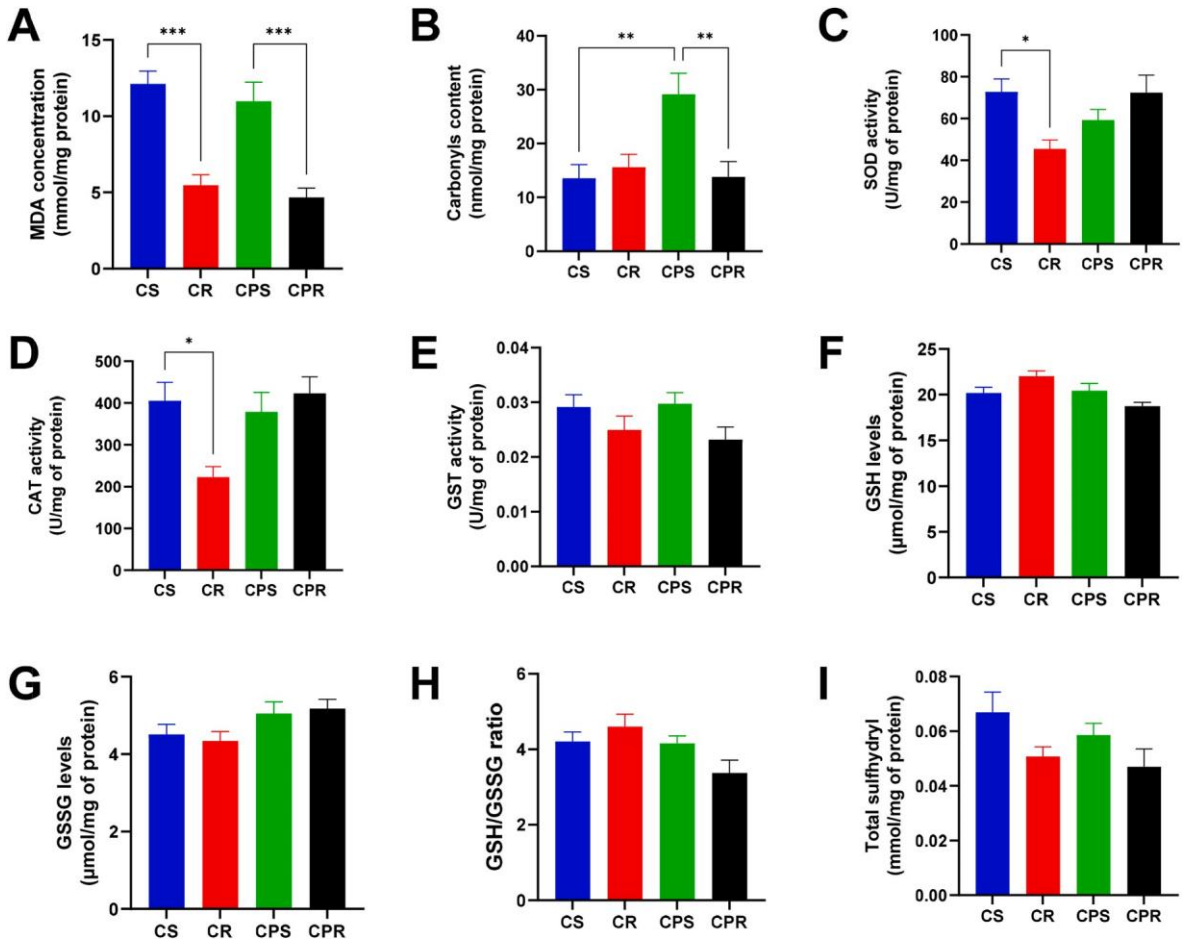

Fig. 9. Analysis of oxidative stress in the somatosensory cortex at P29 according to the experimental groups: CS: Control Saline (n = 8); CR: Resveratrol Control (n = 7); CPS: CP + Saline (n = 7); CPR: CP + Resveratrol (n = 8). Data were expressed as mean ± standard error, $p < 0.05$ (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$).

After our data related to the mRNA of important genes related to mitochondrial biogenesis, we further analyze the citrate synthase activity, since is the first enzyme in Krebs cycle a crucial enzyme for mitochondrial function. Our data showed an increase in the activity of this enzyme in the animals with CP treated with resveratrol compared to the CPS group (CPR: 21.83 ± 1.14/CPS: 13.43 ± 1.36; $p < 0.05$). Similarly, the CPS group showed a decrease in citrate synthase when compared to the CS group (CPS: 13.43 ± 1.36/CS: 25.0 ± 1.63; $p < 0.01$). Our data also showed that the resveratrol-treated control group had an increase in enzyme compared to the saline-treated control group (CR: 39 ± 3.66/CS: 25.0 ± 1.63; $p < 0.001$) (Fig. 11).

5. Discussion

The findings of this study demonstrated that neonatal treatment with resveratrol was able to improve muscle strength and posture in the animals, in addition to acting as a modulator of brain function, reducing markers of oxidative stress and increasing gene expression related to mitochondrial biogenesis in the somatosensory cortex of rats submitted to cerebral palsy. Furthermore, we observed an increase in the activity of the enzyme citrate synthase in the somatosensory cortex of the CPR group, which demonstrates an increase in mitochondrial function.

We analyzed the body weight of the experimental groups over the 28 days and significant differences were found between the groups from P14, in which the animals submitted to experimental CP showed lower weight gain compared to the control animals. Previous studies with this model of CP observed impairments in the musculoskeletal system, verifying a reduction in muscle fibers in the soleus, EDL muscles, and tibial bone mass associated with impairments in locomotion (Pereira et al., 2021a; Visco et al., 2023). Thus, it is understood that delays in locomotion can impair the animals' eating behavior, which would explain the low weight found in our study. In addition, findings point to a reduction in masseter muscle fibers (Lacerda et al., 2017), which is one of the main muscles involved in mastication, suggesting that there are chewing impairments present in this CP model. In addition, the association of sensorimotor restriction with anoxia exposes the animals to situations of early stress that can delay the weight development of the animals (Pereira et al., 2021a; Strata et al., 2004). However, treatment with resveratrol showed significant changes in the animal's body weight only in the P21. We suggest that more studies be done to better understand how RES influences on weight gain in cerebral palsy.

In addition to body weight analysis, we also evaluated the development of physical features and somatic growth. We observed alterations in the development of the physical features of the animals in the CP group, being possible to notice a delay in the auricle opening, auditory conduit opening and eyes opening in relation to the control animals. In a study carried out by Toso et al. (2005) a delay in eyes opening of animals submitted to a CP model was also observed. However, these authors used a different model of CP through the application of liposaccharides (LPS) during pregnancy, suggesting that brain injuries during pregnancy and after birth can harm the development of animals. Furthermore, the PC model delayed the animals' somatic growth, evidenced by the evaluations of the head axes, longitudinal axis, and tail length. Neonatal exposure to resveratrol was able to attenuate the delay in the growth of the longitudinal axis, from the P6 to the P12, and tail length, in the P29. Visco et al. (2023), using the same CP model, found a reduced body

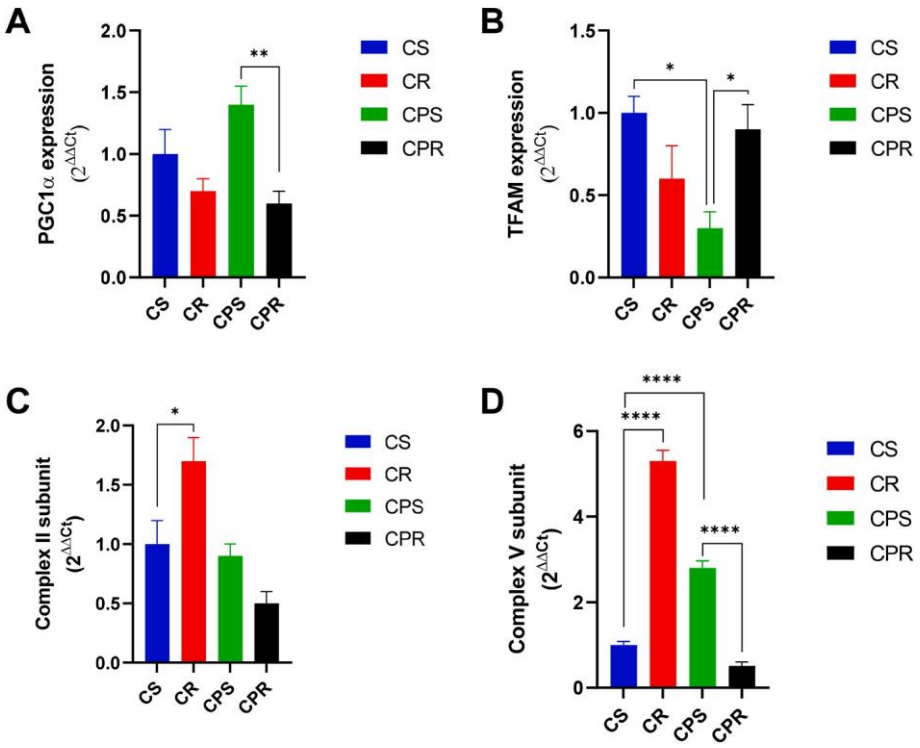

Fig. 10. Analysis of mitochondrial biogenesis genes in the somatosensory cortex at P29 according to the experimental groups: CS: Control Saline (n = 8); CR: Resveratrol Control (n = 7); CPS: CP + Saline (n = 7); CPR: CP + Resveratrol.Data were expressed as mean ± standard error, $p < 0.05$ (* = $p < 0.05$; ** = $p < 0.01$; **** = $p < 0.0001$).

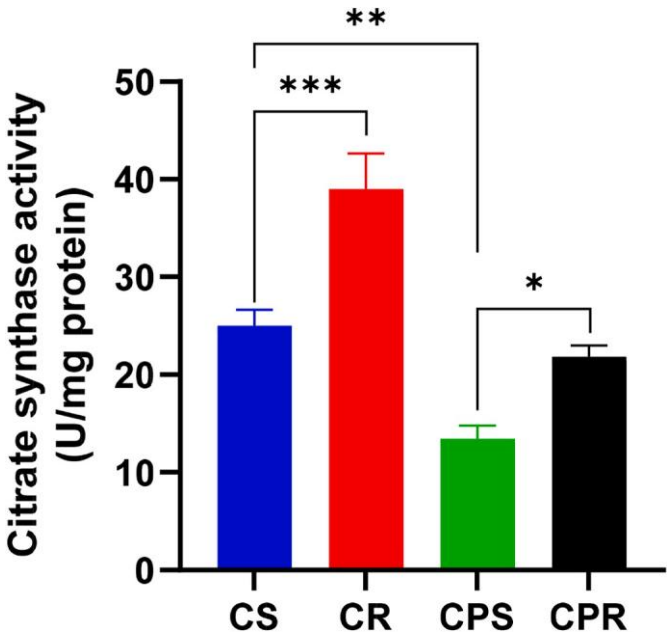

Fig. 11. Analysis of citrate synthase activity in the somatosensory cortex at P29 according to the experimental groups: CS: Control Saline (n = 8); CR: Resveratrol Control (n = 7); CPS: CP + Saline (n = 7); CPR: CP + Resveratrol (n = 8). Data were expressed as mean ± standard error, $p < 0.05$ (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$).

growth in animals with CP, which was attenuated with a neonatal treatment with kaempferol, another polyphenol. These results show that polyphenols have an important positive action on development. Thus, it is understood that cerebral palsy delays body growth and development, in addition to delaying the motor development of these children. To date, we are not aware of other studies evaluating the effects of resveratrol on these outcomes in models of cerebral palsy. We suggest that future studies be carried out to obtain more information about the effectiveness of RES in improving the somatic growth and physical features of animals with CP.

In the Catwalk analysis, we demonstrated that the induction of CP altered the gait pattern of the animals. Pups submitted to CP model showed lower average speed during walking and lower speed during the swing phase, resulting in a longer duration of this phase. In addition, a decrease in the contact area and the length and width of the paw print of these animals was observed, suggesting dysfunctions in the gait of the animals of the CP group. These findings are similar to those found in children with cerebral palsy, such as incoordination of locomotion, lack of precision in foot positioning, and reduced walking ability (Cappellini et al., 2016; Prosser et al., 2010). A previous study using the same PC model found similar results, in addition to altered gait cadence, with interlimb incoordination, and an increased base of support for the forepaws during active locomotion (Visco et al., 2023). The use of sensorimotor restriction was able to change the gait pattern, reducing the maximum contact area of the rodents' paws (Delcour et al., 2018), corroborating the findings of this study. A recent review showed that models that associate perinatal anoxia and sensorimotor restriction lead to a greater impairment of locomotion and motor coordination in animals, similar to what happens in children with CP (Pereira et al., 2021b). Animals with CP develop locomotor characteristics similar to the equinus foot, possibly because they undergo ankle and knee extension during sensorimotor restriction (Delcour et al., 2018), which explains the abnormal gait pattern observed in our results.

In an unprecedented way, this is the first study to evaluate the postural outcomes present in an experimental model of CP. From these results, postural alterations evidenced by the misalignment of the head and trunk were found. However, neonatal treatment with RES attenuated these damages, where the animals in the CPR group had better postural control, similar to the pups in the control group. Regarding the

evaluation of the muscular strength of the front paws, the animals submitted to the CP model showed a reduction in strength. Treatment with resveratrol was able to reverse these losses, where the animals in the CPR group demonstrated an increase in strength, similar to the animals in the control group. Here, we demonstrate that neonatal treatment with resveratrol prevented the impact of CP on postural development and strength, suggesting that this polyphenol may act by modifying motor function.

It is already well established that muscle weakness is the predominant negative characteristic in people with cerebral palsy (Multani et al., 2019), as observed in the experimental model used in our study. Children with CP have skeletal muscle abnormalities such as weakness and decreased muscle thickness and volume (Elder et al., 2003; Graham and Selber, 2003; Multani et al., 2019). Studies are currently being carried out with natural compounds that can act on skeletal muscle, such as polyphenols (resveratrol, curcumin, urolithin A) and flavonoids (quercetin, apigenin) (Hil et al., 2015; Nikawa et al., 2021; Yadav et al., 2022). These are well known for increasing muscle strength and mass, promoting muscle stem cell differentiation and mitochondrial biogenesis, and reducing hydrogen peroxide production as well as inflammation in skeletal muscles (Yadav et al., 2022). Suggesting possible ways of action of resveratrol in the musculoskeletal system, which may explain the improvement in posture and muscle strength of the animals.

In addition to motor function outcomes, we further investigate the effects of resveratrol on the somatosensory cortex. It is well known in the literature that several injuries are associated to the oxidative stress, because of this we evaluate several parameters, and we notice a significant increase in carbonyls levels, an important OS biomarker, in rats submitted to CP model. Furthermore, we continue analyzing the effect of CP and we observed a significant decrease in MDA and carbonyls in CP rats treated with resveratrol. There are no reports in the literature that show the neuroprotective and antioxidant effects of resveratrol in our model of cerebral palsy. Using a different models, previous authors showed results that indicated that RES had an antioxidant effect, being able to decrease MDA in brain tissue as we observed in CR group (Gao et al., 2018; Orsu et al., 2013). These results are similar to those of our study and support the antioxidant role of resveratrol as evidenced by significantly reducing the levels of MDA and carbonyls. Gao et al. (2018) suggested that RES can scavenge a variety of free radicals owing to its phenol rings and three free hydroxyl group, and thus RES treatment can concomitantly abrogate the ROS production under ischemic conditions. After the promising finding observed in MDA and carbonyls levels in the CPR group, we further analyzed the antioxidant enzymes, but to our surprise we observed a decrease in SOD and CAT activity in CR group, compared to the control animals. Raising the hypothesis that the antioxidant enzymes had a decrease activities, due to the potent antioxidant effect of RES. Resveratrol is a remarkable antioxidant (Miguel et al., 2021) and here it can act in the healthy body; we hypothesized that it acts preventively against the insults. In this context, we suggest that future studies be carried out to elucidate the molecular and biochemical mechanisms whereby resveratrol beneficially affects the nervous system of healthy animals.

In sequence, with the intent to understand more the mechanistic effect of RES we evaluated mitochondrial biogenesis markers, measuring the levels of transcription factors and mitochondrial complexes subunits. Our data showed that PGC-1α expression is reduced in CPR animals when compared to the CPS group. However, resveratrol-treated CP animals showed upregulation of mitochondrial transcription factor A (TFAM) compared to the CPS group. PGC-1α is mainly expressed in energy-demanding tissues, such as the brain and skeletal muscle (Di et al., 2018), and is an important coordinator of mitochondrial metabolism and function (Finck and Kelly, 2006; Gabrielson et al., 2014). In addition, it participates in the regulation and TFAM transcription (Gabrielson et al., 2014). These data suggest that PGC-1α and TFAM were positively modulated by RES treatment in animals submitted to CP. Therefore, at mRNA levels, we can speculate that RES treatment in CP animals might increase the number of mitochondria. As a result, the increase in the number may decrease the levels of free radicals released by mitochondria that CP damaged. Corroborating our results, Zhou et al. (2021) highlight that RES increased the expression of PGC-1α, NRF1, and TFAM in the temporal cortex in animals submitted to early brain injury after subarachnoid hemorrhage (Zhou et al., 2021), improving the mitochondrial biogenesis.

Oxidative stress is closely linked to mitochondrial function, with the mitochondrial respiratory chain being the main source of ROS (Chen et al., 2021). Dysfunctions in the mitochondrial respiratory chain, such as changes in the protein complexes, which are responsible for the functionality of this system, prevents electrons from being transferred, resulting in increased production of reactive oxygen species (Chen et al., 2021). The lack of oxygen in the developing brain leads to the depletion of cellular energy stores and triggers various pathophysiological responses, including suppression of mitochondrial respiration (Jacobsson and Hagberg, 2004; Thornton and Hagberg, 2015). Here, we observed a marked decrease in complex V subunits and a tendency to reduce the complex II subunits in the CPR group compared to the animals of the CPS group. With our results, RES treatment decreases the levels of these mRNA, which might lead to a decrease in the electron leak and ROS production.

On the other hand, in healthy animals, RES treatment affects the cells differently. In the control group treated with resveratrol, we observed an increase in complexes II and V subunits, compared the CS group, which suggest that RES treatment increase the flow of electrons at mitochondrial respiratory chain, improving the mitochondrial function as a whole. Due to these results, we decided to analyze the activity of the citrate synthase, the first enzyme in Krebs cycle and highly used as an indicator of mitochondrial function. Indeed, in CR group, the citrate synthase activity increased significantly compared to CS, corroborating with our data related to the complex subunits. However as expected in CPS the activity of the citrate synthase was decreased compared to CS, and the treatment with RES could restore the activity capacity in CPR similarly to the levels of CS. Citrate synthase is an important component of energy metabolism: it is necessary for the catalysis and condensation of Acetyl CoA with oxaloacetate for the formation of citrate (Fernandes et al., 2020; Meng et al., 2021). With the increase in citrate synthase activity in the groups treated with resveratrol, we can speculate that this polyphenol was able to substantially improve the mitochondrial function of cells after CP injury.

Finally, our study demonstrated that resveratrol improved posture and muscle strength, reduced carbonyls and MDA, the main markers of oxidative stress, and increased the expression of the TFAM gene and the enzyme citrate synthase in animals submitted to cerebral palsy. The findings of this work give a new perspective on the use of RES in the treatment of cerebral palsy, through its antioxidant properties. More studies are needed to determine the mechanisms of action involved and to establish the best form of administration to obtain a beneficial and prolonged effect.

CRediT authorship contribution statement

Vanessa da Silva Souza: Conceptualization, Formal analysis, Investigation, Writing – original draft. **Raul Manha˜es-de-Castro:** Resources, Writing – original draft. **Sabrina da Conceiça˜o Pereira:** Formal analysis, Investigation, Writing – original draft. **Caio Matheus Santos da Silva Calado:** Formal analysis, Investigation, Writing – original draft. **Beatriz Souza de Silveira:** Formal analysis, Investigation, Writing – original draft. **Eula´lia Rebeca da Silva Araújo:** Formal analysis, Investigation, Writing – original draft. **Severina Cassia de Andrade Silva:** Formal analysis, Investigation, Writing – original draft. **Osmar Henrique dos Santos Junior:** Formal analysis, Investigation, Writing – original draft. **Claudia Jacques Lagranha:** Formal analysis, Investigation, Writing – original draft, Resources. **Luan Kelwyny Thaywa˜ Marques da Silva:** Formal analysis, Investigation, Writing –

original draft. **Ana Elisa Toscano:** Supervision, Conceptualization.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Data availability

Data will be made available on request.

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APÊNDICE B — LOCOMOTION IS IMPACTED DIFFERENTLY ACCORDING TO THE PERINATAL BRAIN INJURY MODEL: META-ANALYSIS OF PRECLINICAL STUDIES WITH IMPLICATIONS FOR CEREBRAL PALSY

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Locomotion is impacted differently according to the perinatal brain injury model: Meta-analysis of preclinical studies with implications for cerebral palsy

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ABSTRACT

Background: Different approaches to reproduce cerebral palsy (CP) in animals, contribute to the knowledge of the pathophysiological mechanism of this disease and provide a basis for the development of intervention strategies. Locomotion and coordination are the main cause of disability in CP, however, few studies highlight the quantitative differences of CP models, on locomotion parameters, considering the methodologies to cause brain lesions in the perinatal period.

Methods: Studies with cerebral palsy animal models that assess locomotion parameters were systematically retrieved from Medline/PubMed, SCOPUS, LILACS, and Web of Science. Methodological evaluation of included studies and quantitative assessment of locomotion parameters were performed after eligibility screening.

Results: CP models were induced by hypoxia-ischemia (HI), Prenatal ischemia (PI), lipopolysaccharide inflammation (LPS), intraventricular haemorrhage (IVH), anoxia (A), sensorimotor restriction (SR), and a combination of different models. Overall, 63 studies included in qualitative synthesis showed a moderate quality of evidence. 16 studies were included in the quantitative meta-analysis. Significant reduction was observed in models that combined LPS with HI related to distance traveled (SMD -7.24 95 % CI [-8.98, -5.51], $Z = 1.18$, $p < 0.00001$) and LPS with HI or anoxia with sensory-motor restriction (SMD -6.01, 95 % CI [-7.67, -4.35], $Z = 7.11$), or IVH (SMD -4.91, 95 % CI [-5.84, -3.98], $Z = 10.31$, $p < 0.00001$) related to motor coordination.

Conclusion: The combination of different approaches to reproduce CP in animals causes greater deficits in locomotion and motor coordination from the early stages of life to adulthood. These findings contribute to methodological refinement, reduction, and replacement in animal experimentation, favoring translational purposes.

1. Introduction

Over the past few years, a variety of experimental models of early brain injury with implications for cerebral palsy (CP) have been proposed. These preclinical studies have revealed fundamental importance in elucidating the pathophysiological aspects related to CP and they are based on the ability to reproduce neurofunctional changes observed in CP in humans (Cavarsan et al., 2019; Silva et al., 2016). CP is a multifactorial pathology that arises from insults on the brain and it can occur prenatally, perinatally, or postnatally (Graham et al., 2019). It is considered a non-progressive neurodevelopmental disorder that permanently impairs motor functions, and it is considered a more

Abbreviations: CP, cerebral palsy; HI, hypoxia-ischemia; LPS, lipopolysaccharide inflammation; IVH, intraventricular haemorrhage; A, anoxia; SR, sensorimotor restriction.

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common motor disability in childhood (Gulati and Sondhi, 2017; Stavsky et al., 2017).

Experimental models of brain injury with pathophysiology related to cerebral palsy use early insults during the critical period of development too, at the time there is greater temporal and regional vulnerability to brain damage (Jensen, 2002; Jiang and Nardelli, 2016). These models are methodologically diverse, varying as to the time of the injury. or the mechanism of injury. There are models of prenatal inflammation, intrauterine or perinatal oxygen deprivation, prematurity, perinatal intraventricular haemorrhage, and postnatal sensorimotor restriction. In this sense, the injury period is an essential factor for the establishment of functional phenotypes (Marret et al., 2013). Methods and age of the evaluation of locomotion from experimental studies also determine the observation of motor disability according to the developmental milestones to be evaluated (Westerga and Gramsbergen, 1990). Thus, several animal models can represent the neuromotor alterations related to PC induction.

Depending on the objective of the experimental study and the phenotype to be reproduced with a specific model, it may cause more repercussions on motor development than other models, however, this is not elucidated in the literature. The child with CP is characterized by disorders of muscle tone and movement, in addition to the delay in the acquisition of motor skills (Gulati and Sondhi, 2017; Stavsky et al., 2017). Among the outcomes of CP, impairments on locomotion are the most explored in the literature as a striking feature to be reproduced. CP models usually propose to demonstrate how much this neuromotor disease compromises the locomotor functional ability (Aoi and Funato, 2016; Hoppeler and Flück, 2002).

The acquisition of locomotion follows the milestones of the development of the nervous system and depends on its maturation and integration among other systems (Westerga and Gramsbergen, 1990). Preclinical studies demonstrate that early brain injury triggers an inflammatory cascade in the central nervous system that promotes deregulation of the neurogenic niche and, consequently, impairs normal motor development (Dada et al., 2014; Stolp et al., 2012). After perinatal brain damage, neurogenesis response and brain repair mechanism are altered, and this occurrence may contribute to the retardation of development and acquisition of motor skills (Visco et al., 2021). The performance of movements of the animal that was submitted to experimental CP is impaired, including locomotor activity, gait pattern, which is related to impaired motor coordination, balance, and decreased physical capacity (Peterson et al., 2013; Silva et al., 2016). Thus, it is expected that a CP model will be able to reproduce the damage in this fundamental motor task for survival, the locomotion.

Although several CP models are demonstrated in the literature, there is a need for further clarification on which model of cerebral palsy most influences locomotor parameters concerning the relationship between different approaches to reproduce cerebral palsy and the motor damage that is reproduced in animals. In addition to the motor repercussions of the experimental models when combined still derived from greater elucidation. Thus, the objective of this systematic review was to determine the effects of different experimental models of cerebral palsy on locomotion and motor coordination in the short and long term. Also, providing evidence through a systematic review of animal models is valuable to achieve a better translational correlation to human health. This might serve as a guide for the development of the future intervention, contribute to “replacement” “reduction” and “refinement” in animal experimentation, and improve the rigor of the conduction and reporting of preclinical research.

2. Methods

2.1. Systematic review reporting and protocol registration

This systematic review was carried out following the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyzes (PRISMA). The protocol is registered in the Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies (CAMARADES) and its full text is available at http://syrf.org.uk/protocols.

2.2. Search strategy

The search in the scientific literature was carried out between July and August 2020 in the databases Medline/PubMed (National Library of Medicine / Medical Literature Analysis and Retrieval System Online: 1946 - Aug / 2020), SCOPUS (2004 - Aug / 2020), LILACS (Latin American and Caribbean Literature in Health Sciences: 1985 - Aug / 2020) and Web of Science (1900 - Aug / 2020). Appropriate terms were used in each database to cover the experimental models of cerebral palsy and locomotion, according to the MeSH (Medical Subject Headings) and DeCS (Health Sciences Descriptors) descriptors (Table 1). The detailed search strategy is available in supplementary data.

During the research from the databases, there was no linguistic restriction, year or, journal publication. The selection of studies took place in two phases, initially by reading the title and abstract, followed by reading the full text of the selected articles, and studies were included according to the eligibility criteria. Besides, the reference lists of the included articles were inspected and the relevant articles were selected. This process was carried out by two independent reviewers (PEREIRA and VISCO) in which any disagreement was resolved by discussion and consensus between the two reviewers, and when necessary, through a third reviewer (TOSCANO).

2.3. Eligibility

The selected studies followed the inclusion criteria: experimental studies with animals that were submitted to any model of CP; that CP has been induced during the prenatal, perinatal, or early postnatal period; which contained a control group in its study design; who evaluated short and/or long-term locomotion after induction of experimental CP. The exclusion criteria were: articles in which the animals were subjected to some treatment or insult concomitant with the CP model were excluded; which included genetically modified animals; that did not describe in detail the methods of the induced experimental CP model, which do not allow replication (Table 2). Discrepancies were resolved after discussion between the two authors (PEREIRA and VISCO) or were referred to a third author (TOSCANO).

2.4. Data extraction

The extraction of data from the included studies was carried out by two independent reviewers using a standardized form developed to collect relevant information. The following details were extracted from each study: 1- the year of publication and name of the first author; 2- Characteristics of the animals used (strain, sex, sample size); 3-

Table 1
Standard terms used in the search strategy.

Search strategy Component	Terms / Booleans operators
Animal model with implications for cerebral palsy	(Cerebral Palsy) OR (Hypoxia-Ischemia, Brain) OR (Lipopolysaccharides/toxicity) OR (Asphyxia Neonatorum) OR (Immobilization) OR (perinatal asphyxia) OR (sensory-motor restriction) OR (leukomalacia, periventricular)
	AND
Locomotor activity	(Gait) OR (Locomotion) OR (Motor Skills Disorders)
	AND
Animals	Laboratory animals search filters and terms (Hooijmans et al., 2010)

Note: The terms used could vary according to the requirement of each database.

Table 2
Inclusion and exclusion criteria.

Inclusion Criteria	EXclusion Criteria
Participants: o Animals.	Participants: o Humans. o Genetically modified animals.
EXposure: o Animal model with implications for cerebral palsy	EXposure: o Brain injury after perinatal/ neonatal period. o Any other intervention.
Control: o Sham.	Control: o Studies without a control group.
Outcomes: o Locomotion; o Motor coordination.	Outcomes o Drug-induced locomotion
Study type: o Original data. o Full-text was available.	Study type: o No original data (e.g reviews, editorial).

Characteristics of the cerebral palsy model (age and type of insult); 4- Analysed Sample; 5- Locomotion assessment (method and age at testing); 6- Outcomes and for each comparison between control and exposed groups we extracted data of mean value and standard deviation; 7- Statistical method used and descriptive results. As a secondary result, the neurodevelopment parameters were extracted like motor activity and postural changes on gait to qualitative descriptions. In the absence of data, we tried to contact the author for more specific information. Otherwise, we performed only qualitative analysis. The characteristics of the studies included in this systematic review are described in Table 3.

2.5. Methodological quality analysis

Methodological evaluation of the included studies was carried out using the SYRCLE risk of bias tool, which is an adapted version of the Cochrane Collaboration risk of bias tool (Hooijmans et al., 2014a, 2014b). It assesses specific issues that may interfere with the methodological quality of experimental animal studies, across 10 domains. These entries are related to selection bias (sequence generation, baseline characteristics, and allocation concealment), performance bias (random housing and blinding of caregivers and researchers), detection bias (random outcome assessment and blind of outcome assessor), attrition bias (incomplete outcome data), reporting bias (selective outcome reporting) and other sources of biases (Hooijmans et al., 2014a, 2014b). A “yes” judgment indicates a low risk of bias; a “no” judgment indicates a high risk of bias; the judgment will be “unclear” if insufficient details have been reported to assess the risk of bias properly (Hooijmans et al., 2014a, 2014b). The entire process was carried out by two independent reviewers in which the discrepancies were resolved after discussion between the two authors (PEREIRA and VISCO) or were referred to a third author (TOSCANO). The Review Manager Software Package 5.3 (RevMan v.5.3) was used to create the risk of bias summary figure and risk of bias graph.

2.6. Statistical analysis

The Kappa statistic for the interobserver agreement of inclusion criteria was performed using GraphPad QuickCalcs Web. The software RevMan v.5.3 was used to calculate separate meta-analytic comparisons were made for each locomotor parameter assessed and reported in > 3 studies. Cross-sectional comparisons between exposed and control animals assessed standardized mean differences (SMDs) calculated from mean and variance data computed into Cohen’s d pooled effect sizes (ES) and 95 % confidence intervals (95 % CI). These were weighted for sample size, using a random-effects model (Hooijmans et al., 2014a, 2014b). Sensitivity analyses were conducted to test the effects of removing potentially incomparable studies, comprising: those with insufficiently matched exposed and control animals, factors that may modify the relationship between Cerebral Palsy and locomotion (e.g. age analysis). These data are subsequently reported as “potentially incomparable”. The extent of heterogeneity between studies was inspected using I^2, with I^2 values exceeding 60 % indicating important heterogeneity (Hooijmans et al., 2014a, 2014b). Potential sources of statistical heterogeneity were explored; depending on sufficient data availability, variability, and the number of studies, meta-regression was planned. In comparisons that contained at least ten studies, the likelihood of publication bias was also assessed using the observation of funnel plots. Thus, a subgroup analysis was performed referring to different models of CP on each outcome: motor coordination in rotarod, distance traveled, and average speed in the open field test.

3. Results

3.1. Selection of studies

Initially, by searching the databases, 2,020 articles were found, 293 duplicates were excluded, leaving 1,727 articles for analysis of the eligibility criteria in the screening by title and abstract. 90 articles were selected for the complete reading, including 31 eligible articles based on the inspection of the reference list of the articles included in this phase. Of these 90 eligible studies, 27 were excluded and 63 remained for final qualitative analysis and 16 went to meta-analysis (Fig. 1).

3.2. Characteristics of studies included

A systematic narrative synthesis was carried out that summarizes the characteristics of the included studies and their main locomotor results according to the CP models used (Table 3). The animals predominantly used were mammals of the rodent strain. 21 studies included Wistar rats (Alexander et al., 2014; Arteni et al., 2010; Alles et al., 2010; Van De Berg et al., 2003; Bona et al., 1997; Buratti et al., 2019; Dura´n-Carabali et al., 2016; Jansen and Low, 1996; Lubics et al., 2005; Marcuzzo et al., 2010; Miguel et al., 2015; Misumi et al., 2016; Quinzan˜os-Fresnedo et al., 2008; Rousset et al., 2013; Sanches et al., 2013, 2015a, 2015b; Sanches et al., 2019; dos Santos et al., 2017; Stigger et al., 2013, 2011; Ueda et al., 2018), 23 studies used Sprague-Dawley Rats (Baharnoori et al., 2012; Balasubramaniam et al., 2006; Balduini et al., 2000; Delcour et al., 2011, 2018a; Delcour et al., 2012b, a, 2018b; Fan et al., 2011; Grow et al., 2003; Hermans et al., 1992; Hoeger et al., 2000; Jantzie et al., 2014; Lekic et al., 2011, 2012; Ohshima et al., 2016; Poggi et al., 2005; Robinson et al., 2005; Strata et al., 2004; Tashima et al., 2001; Wallace et al., 2010; Wang et al., 2013; Weitzdoerfer et al., 2002), 1 study used Lewis Rats (Girard et al., 2009), another study used Long Evans Rats (Ruff et al., 2017), 1 used mice (Zaghloul et al., 2017), 4 used CD-1 mice (Dada et al., 2014; Kadam et al., 2009; Makinson et al., 2017; Wang et al., 2010), 2 used guinea pigs (Hoeger et al., 2003; Windle and Becker, 1943) and 9 used New Zealand white rabbits (Balakrishnan et al., 2013; Chua et al., 2009; Derrick et al., 2004; Georgiadis et al., 2008; Kannan et al., 2011; Saadani-Makki et al., 2008; Shi et al., 2018; Tan et al., 2005; Zhang et al., 2016). Only one study used the piglet as an experimental animal (Aquilina et al., 2007). Of these studies, 18 included only male animals in their sample, 3 used only females and 28 studies included both sexes. Besides, 18 studies do not make clear the sex of the animals studied.

The experimental CP models included in this systematic review were based on hypoXic-ischemia (HI), Prenatal ischemia (PI), lipopolysaccharide inflammation (LPS), intraventricular hemorrhage (IVH), anoXia (A), sensorimotor restriction (SR), and the combination of the different models. HI was the one of most frequent among the CP models being performed in17 studies in the postnatal period through unilateral common carotid artery occlusion followed by a period of anoXia at P3 or P7 (Alexander et al., 2014; Arteni et al., 2010; Balduini et al., 2000; Bona et al., 1997; Dura´n-Carabali et al., 2016; Grow et al., 2003; Jansen and Low, 1996; Lubics et al., 2005; Miguel et al., 2015; Quinzan˜os-Fresnedo

Table 3
Characteristics of included studies.

CP MODEL	AUTHOR (YEAR)	ANIMAL	SEX	EXPERIMENTAL GROUPS (n)	CP INDUCTION PERIOD			LOCOMOTION AND MOTOR COORDINATION OUTCOMES
					Prematurity	Antenatal	Postnatal	
COMBINED MODELS	Strata et al. (2004)	Sprague-Dawley Rats	Not described	C (n = 17); A (n = 18); SR (n = 22); A + RS (n = 22).	–	–	A (P0 e P1) + SR (P2 - P28)	↓ Fall latency in rotarod (P24, P31, and P38) ↓ The open-field score (P17, P 24, and P 31) ↓ Suspended bar score (P17, P24, P31, P38, and P45) ↓ Stride, swing, and vertical displacement in Plexiglas walkway (P17, P24, P31, P38, and P45)
	Girard et al. (2009)	Lewis Rats	Both sexes	C; HI; LPS; LPS + HI. *Total: 106 pups	–	LPS (E17 until the end of pregnancy)	HI (P1)	↓ Fall latency in rotarod (P30, P35, and P40) ↓ Distance traveled, Crossings, ↑ time of immobility open-field (P15, P20, and P25)
	Marcuzzo et al. (2010)	Wistar Rats	Male	C (n = 14); A (n = 16); SR (n = 14); A + SR (n = 12).	–	–	A (P0) + SR (P1 - P30)	↑ Step error mean Horizontal ladder walking (P31 and P52) ↓ Beam walking score (P31, P38, P45, and P52)
	Stigger et al. (2011)	Wistar Rats	Male	C; LPS; A; LPS + A; RS; LPS + SR; A + SR; LPS + A + SR. *Total: 57 pups (n = 6–8/group)	–	LPS (E17 until the end of pregnancy)	A (P0) + SR (P2 - P28	↓ Fall latency in rotarod (P29) ↑Step error mean on Horizontal ladder (P29) ↑Step error mean on the suspended bar (P29)
	Stigger et al. (2013)	Wistar Rats	Male	C (n = 13); LPS (n = 14); A (n = 14); LPS + A (n = 13).	–	LPS (E17 until the end of pregnancy)	A (P0)	↓ Fall latency in rotarod (P29) ↓ Distance traveled and Average time in locomotor activity in open-field (P29)
	Jantzie et al. (2014)	Sprague-Dawley Rats	Both sexes	C (n = 18); LPS (n = 13); PI (n = 14); PI + LPS (n = 21).	–	PI + LPS (E18)	–	↓ Motor activity score (P1 - 15) ↓ Stride length, time in the propel phase, paw area at peak stride, consistent with toe-walking on Digigait analyses (P28) ↑Stride variation, stride frequency, and ataxia coefficient on Digigait analyses (P28)
	dos Santos et al. (2017)	Wistar Rats	Male	C (n = 12) LPS + A + SR (n = 12)	–	LPS (E17)	A (P0) + SR (P2 – P30)	↓ Hindlimb motor function on Basso, Beattie e Bresnahan score (BBB score) in adapted open-field (P29 and P45)
LIPOPOLYSACCHARIDE INFLAMM	Poggi et al. (2005)	Sprague-Dawley Rats Male	Not described	Sham (n = 30) LPS (n = 45)	–	LPS (E15)	–	There was no difference between groups in Rotarod and the open-field test (2.5 months old)
	Saadani-Makki et al. (2008)	New Zealand white rabbits	Not described	Sham (n = 30) LPS (n = 18)	–	LPS (E28)	–	↓ Motor activity score (P1)
	Wallace et al. (2010)	Sprague-Dawley Rats	Male	Sham (n = 25) LPS (n = 25)	PT (E20-21)	LPS (E17)	–	↓ Fall latency in rotarod (P30, P60-62)

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Table 3 (*continued*)

CP MODEL	AUTHOR (YEAR)	ANIMAL	SEX	EXPERIMENTAL GROUPS (n)	CP INDUCTION PERIOD			LOCOMOTION AND MOTOR COORDINATION OUTCOMES
					Prematurity	Antenatal	Postnatal	
	Wang et al. (2010)	Mice CD-1	Both sexes	Sham (n = 10) LPS (n = 10)	–	LPS (E15)	–	There was no difference between groups in locomotor parameters evaluated by the open-field (P30- P60) ↑ Peripheral activity and crossings in the open-field (female on P200) ↓Latency Beam walking (female on P200) ↑ Tightrope score (male on P400)
	Kannan et al. (2011)	New Zealand white rabbits	Not described	C (n = 4) Sham (n = 6) LPS (n = 8)	–	LPS (E28)	–	↓ Motor activity score (P1)
	Fan et al. (2011)	Sprague-Dawley Rats	Not described	Sham (n = 20) LPS (n = 20)	–	–	LPS (P5)	↑ Distance traveled (P14, P35, and P42) and rearings (P14 - P49 or P56) in the open-field ↑ Latency in movement initiation test (P21 - P56) ↑ Latency in pole test (P28 - P56) ↑ Slip step ratio and latency in tapered / ledged beam walking test (P21 - P56)
	Baharnoori et al. (2012)	Sprague-Dawley Rats Male	Not described	Sham (n = 5) LPS (n = 5)	–	LPS (E15 e E16)	–	There was no difference between groups in locomotor parameters evaluated by the open-field (P6-P10)
	Wang et al. (2013)	Sprague-Dawley Rats	Female	Sham (n = 18) LPS (n = 18)	–	–	LPS (P5)	↑ Rearing in the open-field (P21 and 49) There was no difference between groups in distance traveled in the open field (P21, P49, and P70)
	Rousset et al. (2013)	Wistar Rats	Both sexes	Sham (n = 12) LPS (n = 10)	–	LPS (E19 e E20)	–	↓ Gait efficiency (P14) There was no difference between groups in Grid walking and foot fault test, Rotarod and Cyclotron Test
	Balakrishnan et al. (2013)	New Zealand white rabbits	Not described	C (n = 10) Sham (n = 6) LPS (n = 13)	–	LPS (E28)	–	↓ Motor activity score (P1)
	Dada et al. (2014)	CD-1 outbred mouse strain	Both sexes	Sham (N = 11) LPS (N = 16)	–	LPS (E17)	–	↑ time to complete the ambulation test (P9) ↑ Peripheral activity in the open-field (P60)
	Zhang et al. (2016)	New Zealand white rabbits	Not described	Sham (n = 8) LPS (n = 8)	–	LPS (E28)	–	↓ Motor activity score (P1)
	Shi et al. (2018)	New Zealand white rabbits	Not described	Sham P1 (n = 18) Sham P1 (n = 128) LPS P1 (n = 128) LPS P11 (n = 33)	–	LPS (E28)	–	↓ Motor activity score (P1 and P11)
	Makinson et al. (2017)	Mice CD-1	Both sexes	Sham (n = 10) LPS (n = 10)	–	LPS (E15)	–	↑ Peripheral activity and rearing in the open-field (12-14 weeks old)

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Table 3 (*continued*)

CP MODEL	AUTHOR (YEAR)	ANIMAL	SEX	EXPERIMENTAL GROUPS (n)	CP INDUCTION PERIOD			LOCOMOTION AND MOTOR COORDINATION OUTCOMES
					Prematurity	Antenatal	Postnatal	
HYPOXIA-ISCHEMIA	Jansen and Low (1996)	Wistar Rats	Both sexes	C (n = 15) HI (n = 19)	–	–	HI (P7)	↓ Fall latency in rotarod (3 - 9 weeks old) ↓ Motor activity score (P42)
	Bona et al. (1997)	Wistar Rats	Both sexes	C (n = 11) Sham operated without hypoXia (n = 5) Ischemia without hypoXia (n = 4) HI (n = 11)	–	–	HI (P7)	Foot fault asymmetric on-grid walking (P42)
	Balduini et al. (2000)	Sprague–Dawley Rats	Male	Sham (n = 8) HI (n = 8)	–	–	HI (P7)	There was no difference between groups on parameters evaluated by the open-field (P90) or in rotarod (P35) ↑ Spontaneous activity in automated motor activity monitor (P21)
	Grow et al. (2003)	Sprague-Dawley Rats	Both sexes	C (n = 8) HI (n = 16)	–	–	HI (P7)	Asymmetry of the paws in cylinder test (5 weeks old) ↓ Gait efficiency (P1 – P9) ↑ Foot fault and on ↓ number of steps on-grid walking (2 and 4 weeks old)
	Lubics et al. (2005)	Wistar Rats	Both sexes	C (n = 12) HI (n = 12)	–	–	HI (P7)	↓ Time to remain a square bridge (2 weeks old) ↑ Distance traveled, speed (2 and 6 weeks old), rearing (6 weeks old), peripheral activity in the open field speed (2 weeks old) There was no difference between groups in rotarod (2 and 5 weeks old)
	Quinzan˜os-Fresnedo et al. (2008)	Wistar Rats Both sexes	Not described	24 pups distributed in: C Sham HI	–	–	HI (P7)	↓ Crossings in the open field (P42)
	Kadam et al. (2009)	Mice CD-1	Both sexes	Sham (n = 15, 8 Male, 7 Female) HI (n = 15, 7 Male, 8 Female)	–	–	HI (P12)	↓ Distance traveled in the open-field (P40) There was no difference between groups in Rotarod and Cylinder test (P40)
	Arteni et al. (2010)	Wistar Rats	Both sexes	C HI-L HI-R (n = 11-13/ group)	–	–	HI (P7)	↑ Crossings in the open-field (13 weeks old)
	Sanches et al. (2013)	Wistar Rats	Both sexes	Sham HI-R HI-L (n = 8 a 16 / group)	–	–	HI (P3)	There was no difference between groups in locomotor parameters evaluated by the open-field (17 weeks old)
	Alexander et al. (2014)	Wistar Rats	Male	Sham (n = 6) HI P3 (n = 14) HI P7 (n = 14)	–	–	HI (P3 ou P7)	↓ Fall latency in rotarod (HI P7 group on P30-31) There was no difference between the Sham and HI P3 group in the rotarod
	Misumi et al. (2016)	Wistar Rats	Male	Sham(n = 12)	–	–	HI (P3)	

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Table 3 (*continued*)

CP MODEL	AUTHOR (YEAR)	ANIMAL	SEX	EXPERIMENTAL GROUPS (n)	CP INDUCTION PERIOD			LOCOMOTION AND MOTOR COORDINATION OUTCOMES
					Prematurity	Antenatal	Postnatal	
PRENATAL ISCHEMIA	Sanches et al. (2015a, 2015b)	Wistar Rats	Both sexes	HI (n = 16) Sham HI-R HI-L (n = 12 a 18 / group)	–	–	HI (P3 ou P7)	↓ Fall latency of falls in rotarod (8 months old) ↑ Crossings in the open field in HI P7 group (P60)
	Miguel et al. (2015)	Wistar Rats	Male	HI (n = 9) Controle (n = 11)	–	–	HI (P7)	There was no difference between groups in locomotor parameters evaluated by the open-field (P60)
	Dur´an-Carabali et al. (2016)	Wistar Rats	Both sexes	C (n = 14) HI 120'(n = 11) HI 180' (n = 14) HI 210' (n = 10)	–	–	HI (P3)	↑Foot fault on ladder walking test (P35 and 45) Asymmetric use of the forelimb on cylinder test (P35 and 45)
	Ueda et al. (2018)	Wistar Rats	Male	C (n = 46) Sham (n = 10) HI (n = 62) Sham (n = 25)	–	–	HI (P3)	↓ Locomotor activity score (8–9 weeks old) Swing durations asymmetry on DigiGait (8–9 weeks old) There was no difference between groups on Horizontal Ladder Test (8–9 weeks old)
	Zaghloul et al. (2017)	Mice	Not described	HI (n = 25)	–	–	HI (P5)	↓ Fall latency in rotarod (P60) ↓ Beam break and rearing in the open-field (P60)
	Sanches et al. (2019)	Wistar Rats	Both sexes	Sham (n = 13) HI (n = 19)	–	–	HI (P3)	↑ Crossings in the open field (P45)
	Windle and Becker (1943)	Guinea pig	Not described	C (n = 90) PI (n = 103)	PT (E63 – E65)	PI	–	↓ Motor activity and loss of control of hind limbs
	Tashima et al. (2001)	Sprague- Dawley Rats	Both sexes	C (n = 15, 10 Male e 10 Female) PI (n = 12, 5 e 7 Female)	–	PI (E17 and E22)	–	↓ Crossings in the open-field in male (7 weeks old)
	Derrick et al. (2004)	New Zealand white rabbits	Male	C (n = 129) PI 30 min on E22 (n = 26) PI 37-40 min on E22 (n = 102) PI 37-40 min on E21 (n = 61)	–	PI (E21 ou 22)	–	↓ Motor activity score (P1)
	Tan et al. (2005)	New Zealand white rabbits	Both sexes	C (n = 26) PI (n = 16)	–	PI (E21, 22, ou 25)	–	↓ Motor activity score (P1)
	Robinson et al. (2005)	Sprague- Dawley Rats	Not described	C (n = 24) PI (n = 20) Sham (n = 42)	–	PI (E18)	–	↓ Squares visited and stride length in the open field (4 months old)
	Delcour et al. (2011)	Sprague-Dawley Rats	Both sexes	PI (n = 44)	–	PI (E17)	–	↓ Normalized swing length relative to the tibial size and normalized foot speeds during a swing on both the *x*-axis and the *z*-axis on treadmill 3D kinematics (P65) ↑ Squares visited in the open field (P65)
	Delcour et al. (2012a)	Sprague-Dawley Rats	Both sexes	Sham (n = 18) PI (n = 14)	–	PI (E17)	–	The angles of all joints were significantly different between HI and sham rats indicating both hips over flexion

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Table 3 (*continued*)

CP MODEL	AUTHOR (YEAR)	ANIMAL	SEX	EXPERIMENTAL GROUPS (n)	CP INDUCTION PERIOD			LOCOMOTION AND MOTOR COORDINATION OUTCOMES
					Prematurity	Antenatal	Postnatal	
								and knee and ankle overextension when the foot was at the highest point of the swing.
	Delcour et al. (2012b)	Sprague-Dawley Rats	Both sexes	Sham (n = 26) PI (n = 23)	–	PI (E17)	–	↑ Squares visited in the open-field (P65) ↑ square visiting in the open field (P40- P80)
	Oshima et al. (2016)	Sprague-Dawley Rats	Both sexes	Sham (n = 9 Female and 14 Male) C (n = 24 Female and 24 Male). PI (n = 17 Female and 23 Male)	–	PI (E17)	–	↑ Crossings in females in the open field (P15) ↓ Rearings in males in the open field (P15)
	Ruff et al. (2017)	Long Evans Rats	Both sexes	Sham (n = 12) PI / Growth restricted (mild n = 5; moderate, n = 9) C (n = 15)	–	PI (E20)	–	↓ Gait on Catwalk
	Hermans et al. (1992)	Sprague-Dawley Rats	Both sexes	A (n = 15)	–	A (E15 to E20)	–	↑ Crossings (P19 P44 e 80) and rearings (P19) in the open-field in males ↓ Crossings (P19 P44 e 80 and rearings (P19) in the open-field in females
	Hoeger et al. (2000)	Sprague-Dawley Rats	Female	C A 5' A 10' A 15' A 20' (n = 10/group)	–	–	A (P0)	There was no difference between groups in locomotor parameters evaluated by the open field or motor activity score (3 months old)
	Weitzdoerfer et al. (2002)	Sprague-Dawley Rats	Female	31 pups Female distributed in: C A 10' A 20'	–	–	A (P0)	There was no difference between groups in motor activity score or latency of falls in rotarod or locomotor score
ANOXIA	Hoeger et al. (2003)	Guinea pig	Not described	C A 2' A 3' A 4' A 5' (n = 10/group)	–	–	A (P0)	There was no difference between groups in locomotor parameters evaluated by the open-field or motor activity score (3 months old)
	Van de Berg et al. (2003)	Wistar Rats	Male	C (n = 15) A (n = 12) C (n = 13)	–	–	A (P0)	There was no difference between groups in locomotor parameters evaluated by the open-field or footprint test (P21 and P42)
	Ireland et al. (2010)	Spiny mouse	Not described	PI (n = 13)	–	PI (E38)	–	↑ Fall latency in rotatod (P1 and P2) ↓ Distance traveled and percentage of time stationary in the open field (P5 and P15)
INTRAVENTRICULAR HEMORRHAGE	Balasubramaniam et al. (2006)	Sprague-Dawley Rats	Both sexes	C (n = 17, 9 Male and 8 Female) Sham (n = 19, 6 Male and 13 Female) IVH (n = 21, 11 Male and 10 Female)	–	–	IVH (P0 – P1)	↓ Locomotor score (P4, 8, 11 e 15) ↑ Fall latency on the rotarod (6, 7 e 8 weeks old)
	Aquilina et al. (2007)	Piglet		C (n = 3)	–	–	IVH (P0)	

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Table 3 (*continued*)

CP MODEL	AUTHOR (YEAR)	ANIMAL	SEX	EXPERIMENTAL GROUPS (n)	CP INDUCTION PERIOD			LOCOMOTION AND MOTOR COORDINATION OUTCOMES
					Prematurity	Antenatal	Postnatal	
			Not described	14 pups distributed in: IVH autologous blood IVH autologous blood with elevated hematocrit IVH artificial CSF				↓ Locomotion only in the 1st week old
	Georgiadis et al. (2008)	New Zealand white rabbits	Not described	12 litters distributed in: C IVH	PT (E29)	–	IVH (P0)	↓ Locomotor score (P1 – P3)
	Chua et al. (2009)	New Zealand white rabbits	Not described	C (n = 15) C + Glicerol (n = 17) IVH + Glicerol (n = 20)	PT (E29)	–	IVH (P0)	↓ locomotor scores and distance traveled in 60 seconds
	Alles et al. (2010)	Wistar Rats	Male	C (n = 12) Salina-Uni (n = 12) Salina-Bi (n = 12) IVH Bi (n = 12) IVH Uni (n = 12)	–	–	IVH (P6)	↓ Locomotor score (P7 e 11) ↓ Crossings and rearings in the open-field (P30)
	Lekic et al. (2011)	Sprague-Dawley Rats	Not described	C (n = 8) Sham (n = 8) IVH (n = 8)	–	–	IVH (P7)	↑ Locomotor activity in the open-field (4 weeks old) ↓ Fall latency of falls in rotarod (4 weeks old)
	Lekic et al. (2012)	Sprague-Dawley Rats	Not described	C (n = 18) Sham (n = 41) IVH Colagenase-0,1 units (n = 10); IVH Colagenase-0,3 units (n = 84)	–	–	IVH (P7)	↑ Distance traveled in the open field (P8 and P9) ↓ Fall latency in rotarod (2 weeks old) ↑ Foot fault on-grid walking
SENSORIOMOTOR RESTRICTION	Delcour et al. (2018a)	Sprague-Dawley Rats	Both sexes	C (n = 19, 10 Female and 9 Male); SR (n = 16, 8 Female and 8 Male) C (n = 18)	–	–	RS (P1 -P28)	Somatosensory hind limb map features were highly correlated to locomotion kinematics impairments, to musculoskeletal histopathology and tended to correlate with neurotransmission findings based on principal components (PC) analysis (PCA) locomotion on a treadmill (P30 and P65) ↑ Swing duration (P30 and P65), ↓ normalized swing length relative to the tibial size, and normalized foot
	Delcour et al. (2018b)	Sprague-Dawley Rats	Both sexes	SR (n = 16)	–	–	RS (P1 -P28)	speeds during a swing on both the *x*-axis and the *z*-axis on treadmill 3D kinematics (P65), ↓ feet contact area during weight support (P30) in 3D kinematics during treadmill locomotion.

Abbreviations: P: Postnatal day; C - Control; A - Anoxia; RS - Sensorimotor restriction; LPS - Lipopolysaccharide inflammation; HI: Hypoxic-ischemia; PI: Prenatal Ischemia; L-Left injury; R-Right injury; CSF: cerebrospinal fluid; n - number of animals per group. **Symbols:** ↑- Increased locomotor parameter in the test; ↓- Reduced locomotor parameter in the test.

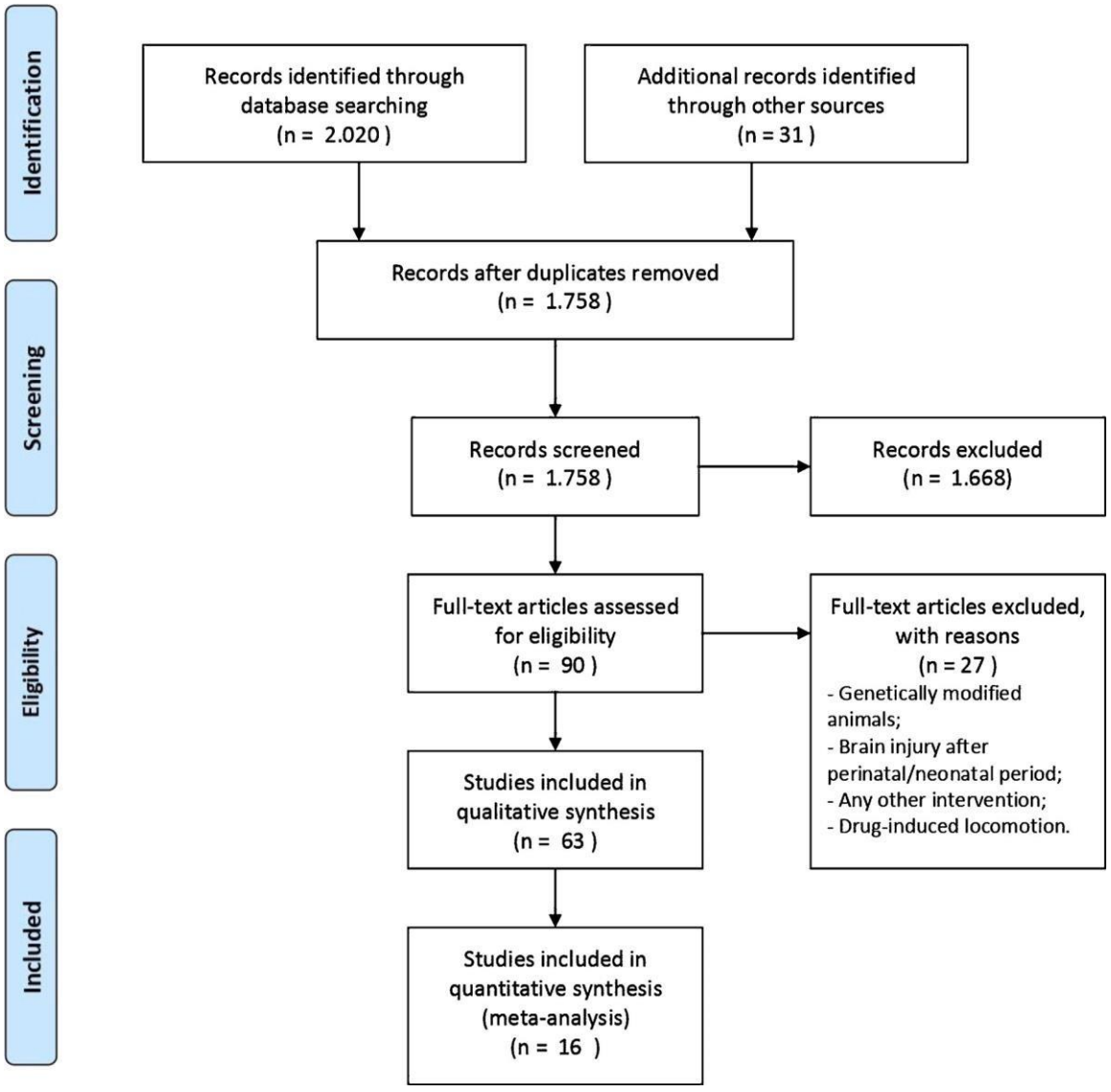

Fig. 1. PRISMA flow diagram of the study selection process.

et al., 2008; Sanches et al., 2013, 2015a, 2015b; Sanches et al., 2019; Ueda et al., 2018; Zaghloul et al., 2017), or it was performed at P12 as observed in only one study (Kadam et al., 2009). PI was performed in 10 studies that used the prenatal period through uterine ischemia (Delcour et al., 2011, 2012a; 2012b; Derrick et al., 2004; Ohshima et al., 2016; Robinson et al., 2005; Ruff et al., 2017; Tan et al., 2005; Tashima et al., 2001; Windle and Becker, 1943).

Another frequent model of CP was exposure to LPS inflammation occurring in the prenatal period through maternal injection of LPS as observed in 12 studies (Baharnoori et al., 2012; Balakrishnan et al., 2013; Dada et al., 2014; Kannan et al., 2011; Makinson et al., 2017; Nance et al., 2017; Poggi et al., 2005; Rousset et al., 2013; Saadani--Makki et al., 2008; Shi et al., 2018; Wallace et al., 2010; Wang et al., 2010; Zhang et al., 2016). The model of LPS inflammation in the postnatal period was performed in only 2 studies through intracerebral injection of LPS in pups at P5 (Fan et al., 2011; Wang et al., 2013).

Among the models that performed insults on the postnatal period to induce CP, the IVH model and the anoxia model occurred earlier. The IVH model occurred in the postnatal period between P0 and P7 by injection of glycerol intraperitoneally in 2 studies (Chua et al., 2009; Georgiadis et al., 2008), or by intracerebral infusion of collagenase in 3 studies (Alles et al., 2010; Lekic et al., 2011) or autologous blood in 2 studies (Aquilina et al., 2007; Balasubramaniam et al., 2006). The anoxia model was performed predominantly as a single event at P0 in 5 studies (Van De Berg et al., 2003; Ireland et al., 2010; Hoeger et al., 2000, 2003; Weitzdoerfer et al., 2002), but it was also performed as repeated events in the prenatal period from E15 to E20 in one study (Hermans et al., 1992). The sensorimotor restriction model was the one that extended the most throughout postnatal development, as observed in 2 studies that covered P1 to P28 (Delcour et al., 2018a; 2018b).

The combination of experimental models to induce CP was diverse and included the combination of prenatal LPS inflammation combined with postnatal HI (Girard et al., 2009), LPS inflammation and PI both in prenatal period (Jantzie et al. 2014), anoxia with sensorimotor restriction both in the postnatal period (Marcuzzo et al., 2010; Strata et al., 2004) and prenatal LPS inflammation combined with anoxia with the

post-sensorimotor restriction natal (Buratti et al., 2019; dos Santos et al., 2017; Stigger et al., 2013, 2011). In addition to these combinations between different CP models, 3 studies induced prematurity in a complementary insult, it was performed in the models of PI (Windle and Becker, 1943), LPS inflammation (Wallace et al., 2010), and IVH (Chua et al., 2009; Georgiadis et al., 2008). In all studies that used an injectable agent or surgical procedure as in the HI, LPS, IVH, or combination of these models, the authors included a simulated control group in which saline was administered or the same surgical procedure was performed, but without inducing CP.

3.3. Main results

3.3.1. Effects of different CP models on locomotion

In general, studies have shown a deficit in the global mobility of animals in which the hind limbs were more affected than the forelimbs in all models. HI impaired the quality of movement of the head, forelimbs, and hind limbs, and it leads to hypertonia and postural deficits impaired the animals' ability to swim (Tan et al., 2005). It was observed that in pups submitted to PI there were changes in the articular angles of the lower limbs, including a slight hyperflexion of the hip compensated by a slight overextension of both knees and ankles during gait, mainly in the maximum swing amplitude before the foot was projected for the front (Delcour et al., 2011). The LPS inflammation model also promoted postural changes in the extremities, such as joint stiffness (Saadani--Makki et al., 2008), the crossing of hind limbs combined with hypertonia leading to poor motor function (Zhang et al., 2016). In animals submitted exclusively to perinatal anoxia, changes in gait were subtle and reversible (Stigger et al., 2013, 2011; Strata et al., 2004), or were not even evident in the short term (Van De Berg et al., 2003; Hoeger et al., 2000, 2003; Weitzdoerfer et al., 2002).

In animals submitted to experimental model combinations, more severe and lasting functional deficits were observed, especially when the sensorimotor restriction was present. It was evidenced in animals submitted to sensorimotor restriction combined with HI, LPS, or Anoxia, reduced movements of the joints, irregular cycles of steps, inability to coordinate the anterior and posterior limbs, hip elevation, external turns of the feet, unstable standing posture with the base of extended support, in addition to that the forelimbs propelled the rats, while the hindlimbs were dragged (dos Santos et al., 2017; Stigger et al., 2011; Strata et al., 2004). Similarly, the combination between PI and LPS led to impairments in cadence, consistency, and coordination between limbs during gait, there was a decrease in the area of the hind legs at the peak of the step consistent with finger-walking (Jantzie et al., 2014).

In addition to the observational assessment of gait patterns and deficiencies in dynamic postural control, locomotor activity was analyzed using devices already established in the literature. The open-field test was the most used to evaluate the animals and it was carried out in 36 studies that included the parameters distance traveled, average speed, mobility and immobility time, and exploratory behavior. Another method frequently used was the gait and mobility score, it was performed in 23 studies from the earliest stages of postnatal development. This score points to the quality of the head, trunk, and limb movements during spontaneous locomotion. 6 studies used the cylinder test to analyze the use of forelimbs and asymmetries in locomotion, 6 studies performed the test on a horizontal ladder to assess the accuracy of placing paws on the steps, 4 carried out the test on a treadmill for capacity analysis of walking at different speeds, 3 performed the gait analysis in Catwalk that evaluates the kinematics in the gait phases and 2 performed the Suspended bar to assess the ability to walk on the bars.

As for the locomotion assessment period, in this review, short-term outcomes were considered for analyses carried out up to the 30th day of postnatal life, and for long-term analyses carried out after this period. Studies that used the LPS inflammation model found divergent effects. Some were able to reproduce the damage to locomotor activity resulting from this insult, but only in the short term in the bar walk test and gait and mobility score (Balakrishnan et al., 2013; Dada et al., 2014; Fan et al., 2011; Kannan et al., 2011; Rousset et al., 2013; Saadani-Makki et al., 2008; Shi et al., 2018; Zhang et al., 2016), others did not show dysfunction in the short or long term in the open field or the gait score and mobility (Baharnoori et al., 2012; Poggi et al., 2005; Wallace et al., 2010; Wang et al., 2013). In contrast, other studies observed long-term hyperactivity in the open field (Dada et al., 2014; Fan et al., 2011; Makinson et al., 2017; Wang et al., 2010).

Similarly, the hypoxic-ischemia e prenatal ischemia models also showed divergences in their results, including some studies that observed opposite effects due to the same model. Most studies have found short or long-term deficits in locomotor activity in the open field, in the horizontal ladder test, or in the treadmill test (Alexander et al., 2014; Bona et al., 1997; Delcour et al., 2011, 2012b; Derrick et al., 2004; Dura´n-Carabali et al., 2016; Grow et al., 2003; Ireland et al., 2010; Jansen and Low, 1996; Kadam et al., 2009; Lubics et al., 2005; Misumi et al., 2016; Quinzan˜os-Fresnedo et al., 2008; Robinson et al., 2005; Ruff et al., 2017; Tan et al., 2005; Ueda et al., 2018; Windle and Becker, 1943; Zaghloul et al., 2017). Other studies have observed hyperactivity mainly in the open field and in the bar walk test (Arteni et al., 2010; Delcour et al., 2011, 2012b; Ireland et al., 2010; Lubics et al., 2005; Sanches et al., 2015a, 2015b; Ueda et al., 2018). A PI study demonstrated effects depending on the sex of animals, in which the male pups showed hypoactivity in the open field and the female hyperactivity (Ohshima et al., 2016). Finally, other studies have not been able to show differences between the animals submitted to HI and the control animals in the open field analysis, the cylinder test, the bar walk test, and the horizontal stair test (Balduini et al., 2000; Kadam et al., 2009; Lubics et al., 2005; Miguel et al., 2015; Sanches et al., 2013, 2019).

On IVH model studies, most of them have evidenced the locomotor deficit resulting from this insult, mainly in the short term in the open field analysis, in the bar walk test, and the gait and mobility score (Alles et al., 2010; Balasubramaniam et al., 2006; Chua et al., 2009; Georgiadis et al., 2008). Only one study observed hyperactivity in the open field, occurring in juvenile animals (Lekic et al., 2012). In contrast, studies using perinatal anoxia have not been able to demonstrate deficits in locomotor parameters even in the short term through open field analysis, bar walk test, horizontal ladder test, and gait and mobility score (Van De Berg et al., 2003; Hoeger et al., 2000, 2003; Weitzdoerfer et al., 2002; Ireland et al., 2010). Only one study, which used anoxia as a prenatal insult, observed opposite effects between males and females, in which males showed hyperactivity and females hypoactivity in an open field in the short term (Hermans et al., 1992).

Combined models of CP showed disorders in several locomotor parameters evaluated. The combination of the models, regardless of the period of application of the insult, was effective in demonstrating the worst performance of the animals in the spontaneous locomotion tests in the open field, in the bar walk test, in the horizontal stair test, in the treadmill test, in the suspended bar and gait and mobility score. From the earliest stages of life, the combined models led to a reduction in locomotor activity that extended into adulthood. Thus, the method of insult to which the animals were subjected shows that the models of LPS combined with anoxia (Stigger et al., 2013) and LPS combined with HI or PI (Girard et al., 2009; Jantzie et al., 2014) promote less lasting effects than in models of sensorimotor restriction, where functional degradation remained for a long term (Buratti et al., 2019; Marcuzzo et al., 2010; dos Santos et al., 2017; Stigger et al., 2011; Strata et al., 2004). Studies with isolated sensorimotor restriction also observed a similar effect in the gait analysis in Catwalk (Delcour et al., 2018a; 2018b).

The distance covered and the average speed in the open field test are outcomes widely used in the assessment of spontaneous locomotor activity, and the effects of different models on these parameters are investigated in this review. Through the analysis of subgroups, there was a significant reduction in the distance traveled only in the model that combined LPS with anoxia with SR (Girard et al., 2009) (SMD -7.24 95 % CI [-8.98, -5.51], Z = 1.18) and in the anoxia model (Van De Berg et al.,

2003) (SMD -1.77 95 % CI [-2.66, -0.87], Z = 3.87). Despite this result, in the models in general, the distance traveled showed a high rate of heterogeneity without significant effect size (SMD 1.00, 95 % CI [-0.20, 2.19], Z = 1.64; p < .10; I^2 = 95 %) (Fig. 2). As for the speed meta-analysis, according to eligibility criteria and data availability, only the models of HI and anoxia were included. Similarly, the average speed also showed no significant effect resulting from these models, even with high heterogeneity (SMD 0.96 95 % CI [-1.31, 3.22], Z = 0.83; p < .41; I^2 = 96 %) (Fig. 4). Also, the funnel plots for the analysis of the distance traveled and average speed demonstrated good accuracy and symmetry consistent with low publication bias (Figs. 3 and 5).

3.3.2. *Effects of different CP models on motor coordination*

Considering motor coordination and balance as an essential part of locomotion, many articles have evaluated this outcome. 19 studies performed the performance test on rotarod through the latency of falls, 6 studies the suspension rope test (Suspended bar) (Balasubramaniam et al., 2006; Lubics et al., 2005; Stigger et al., 2011; Strata et al., 2004; Wang et al., 2010; Weitzdoerfer et al., 2002), 4 used the analysis of the animal's ability to walk on a suspended bar (Beam Walking) (Marcuzzo et al., 2010; Misumi et al., 2016; Sanches et al., 2019; Wang et al., 2010), 4 assessed coordination through failure to place paws in the grid walk test (Grid walking and foot fault test) (Bona et al., 1997; Lekic et al., 2012; Lubics et al., 2005; Rousset et al., 2013) as well as in 4 studies on the ladder walk test (Dura´n-Carabali et al. 2016; Marcuzzo et al., 2010; Stigger et al., 2011; Ueda et al., 2018). Observational assessment of motor incoordination during spontaneous locomotion tests has also been reported in 5 studies (Jantzie et al., 2014; Marcuzzo et al., 2010; Stigger et al., 2013; Strata et al., 2004; Ueda et al., 2018).

The rotarod performance test assesses the latency of the animal's falls from the apparatus, that is, it determines the animal's ability to remain walking on a moving rod. Thus, studies that evaluated animals from P21 were considered for inclusion in the meta-analysis, since at this age the pups already have gait in the adult pattern. 16 studies were included in the meta-analysis, in which most of these were able to cause a decrease in performance during the test (Alexander et al., 2014; Balasubramaniam et al., 2006; Girard et al., 2009; Jansen and Low, 1996; Lekic et al., 2011; Misumi et al., 2016; Stigger et al., 2013, 2011; Strata et al., 2004; Wallace et al., 2010; Zaghloul et al., 2017). But 4 studies did not demonstrate significant differences between the group submitted to the CP model and the control group (Balduini et al., 2000; Kadam et al., 2009; Lubics et al., 2005; Rousset et al., 2013). This reduction in motor coordination in rotarod was confirmed through meta-analysis despite a high level of heterogeneity between studies (SMD -2.47, 95 % CI [-3.43, -1.51], Z = 5.05; p < .00001; I^2 = 97 %) (Fig. 6). Subgroup analysis shows the influence of the different CP models used in this outcome. We observed that the models that combined different insults stood out in reproducing the impairments in the animals' motor coordination (SMD -6.01, 95 % CI [-7.67, -4.35], Z = 7.11; p < .00001; I^2 = 89 %) (Girard et al., 2009; Stigger et al., 2013, 2011; Strata et al., 2004). As well as the IVH model, where considerable effect size with moderate heterogeneity was also observed between the studies (SMD -4.91, 95 % CI [-5.84, -3.98], Z = 10.31; p < .00001; I^2 = 44 %) (Balasubramaniam et al., 2006; Lekic et al., 2011, 2012) (Fig. 6). Also, the funnel plot demonstrates good accuracy of study results and symmetry consistent with low publication bias (Fig. 7).

3.4. *Methodological quality of included studies*

The methodological evaluation of the included studies was carried out through the SYRCLE risk of bias tool by two independent evaluators in which the Kappa test demonstrated agreement Kappa = 0.891 95 % CI

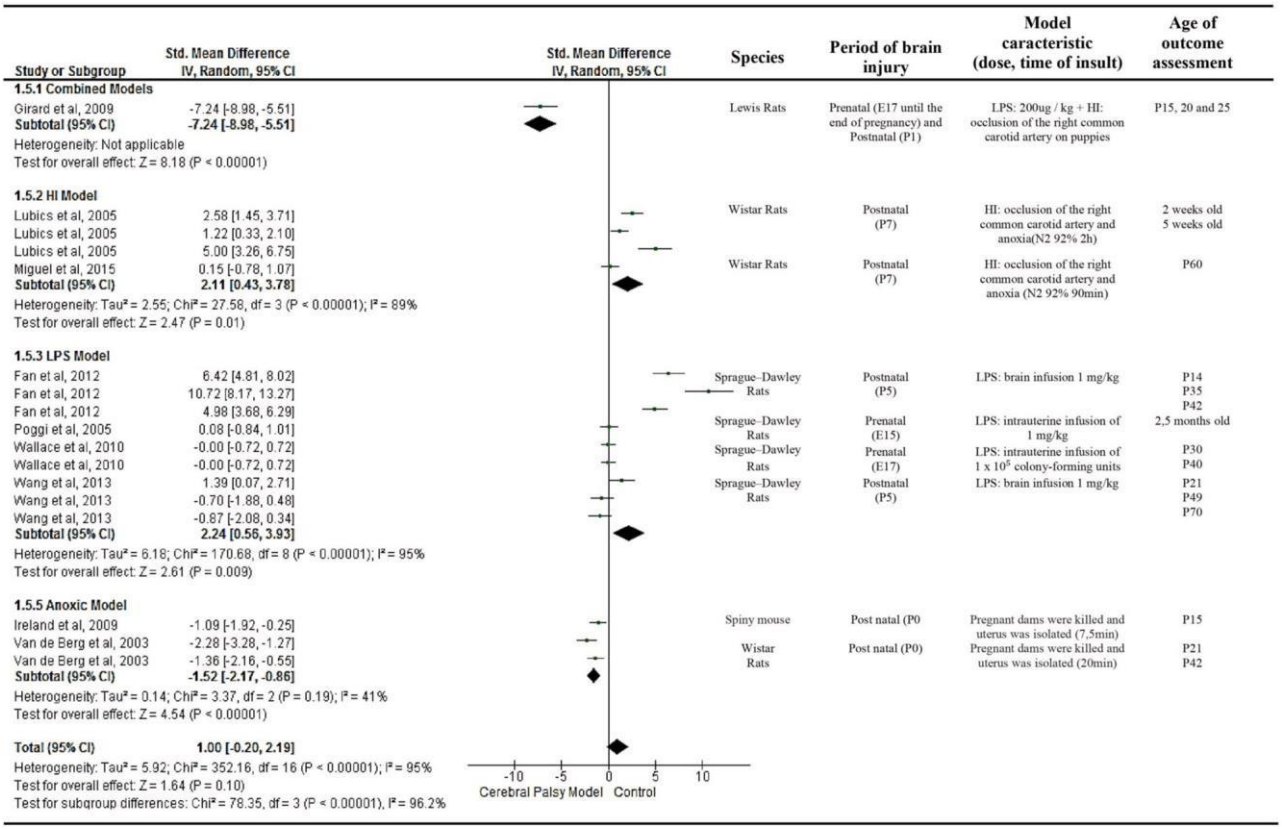

Fig. 2. Forest plot of included studies evaluating distance traveled in open-field (Subgroup: Experimental models of CP). Horizontal lines represent the effect size ± the confidence interval (95 %). The summary effect size is represented by the diamond. P: Postnatal day; C - Control; A - Anoxia; RS - Sensorimotor restriction; LPS - Lipopolysaccharide inflammation; HI: Hypoxic-ischemia.

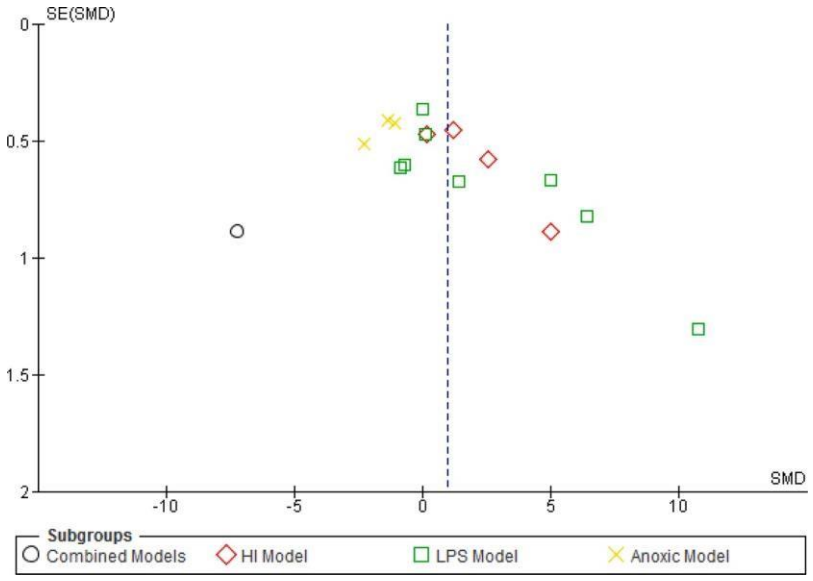

Fig. 3. Funnel plot of standardized mean differences (SMD) of distance traveled in open-field. *SE* = standard error.

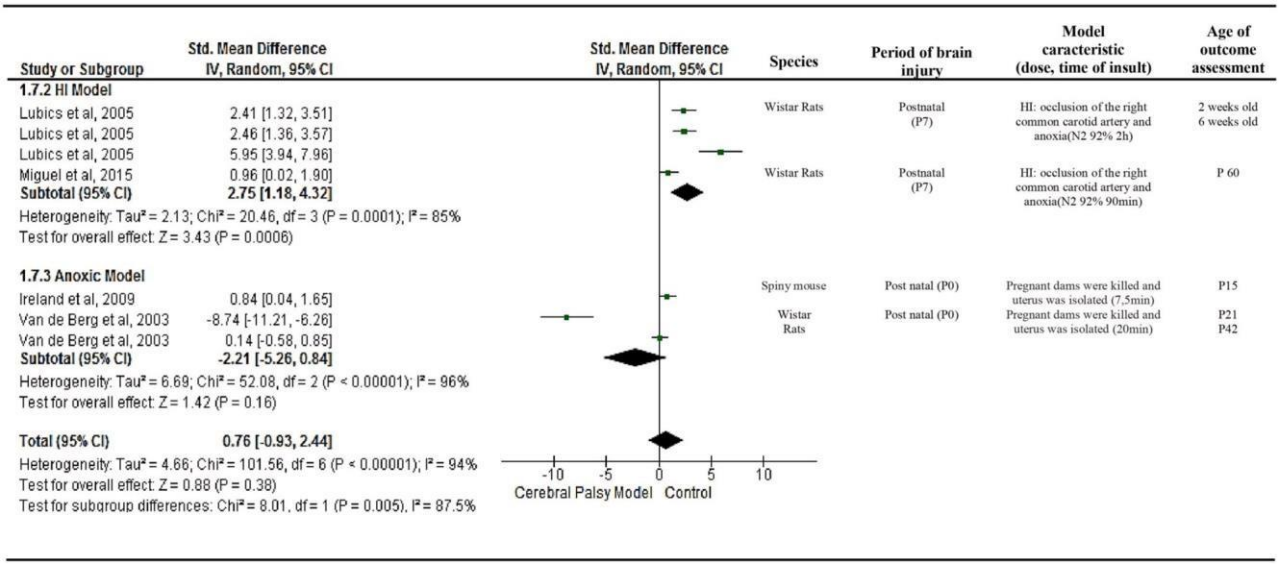

Fig. 4. Forest plot of included studies evaluating average speed in open-field (Subgroup: Experimental models of CP). Horizontal lines represent the effect size ± the confidence interval (95 %). The summary effect size is represented by the diamond. P: Postnatal day; C - Control; A - Anoxia; RS - Sensorimotor restriction; LPS - Lipopolysaccharide inflammation; HI: Hypoxic-ischemia.

(0.770–1.000) for the items of the tools. Kappa agreement was calculated using the GraphPad QuickCalcs Web site: http://www.graphpad.com/quickcalcs/ConfInterval1.cfm (accessed Jan/2021). It was observed that the baseline characteristics of the groups' experimental results were similar in the studies. Most of them did not perform adequate randomization, and of the 67 articles included, 18 performed randomization for groups (Aquilina et al., 2007; Balduini et al., 2000; Van De Berg et al., 2003; Dura´n-Carabali et al., 2016; Girard et al., 2009; Lekic et al., 2012; Marcuzzo et al., 2010; Miguel et al., 2015; Ohshima et al., 2016; Quinzan˜os-Fresnedo et al., 2008; Sanches et al., 2013, 2015a, 2015b; Stigger et al., 2013, 2011; Strata et al., 2004; Wallace et al., 2010; Wang et al., 2010; Zhang et al., 2016) and only 2 performed randomization to assess the outcome (Derrick et al., 2004; Kannan et al., 2011). No study has reported housing randomization, allocation concealment, or blinded the researchers. As for the blinding of the evaluators for the outcome, 19 studies performed it (Alles et al., 2010; Balasubramaniam et al., 2006; Chua et al., 2009; Delcour et al., 2018a; Derrick et al., 2004; Hermans et al., 1992; Hoeger et al., 2000, 2003; Jantzie et al., 2014; Kannan et al., 2011; Lekic et al., 2012; Makinson et al., 2017; Marcuzzo et al., 2010; Robinson et al., 2005; Saadani-Makki et al., 2008; Stigger et al., 2013, 2011; Strata et al., 2004; Zhang et al., 2016). Incomplete data and selective description of the outcome were observed in 2 studies (Aquilina et al., 2007; Weitzdoerfer et al., 2002). These data are presented in Fig. 8, which is a summary of the individual articles, and in Fig. 9, through a summary graph that shows the risk of bias in the studies included in percentage. Also, to detect publication bias, funnel plots were created for motor coordination outcomes in rotarod, distance traveled, and average speed in the open-field test. All of these demonstrated good precision and symmetry, consistent with low publication bias (Figs. 3,5, and 7).

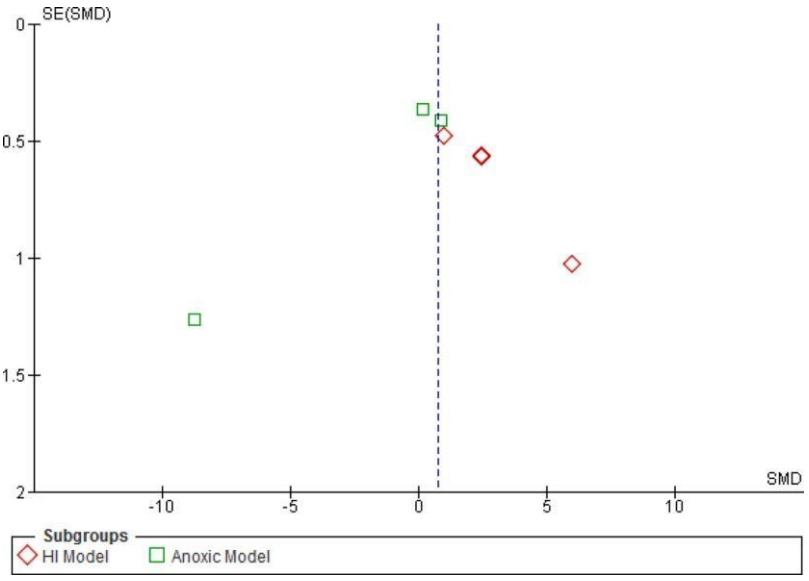

Fig. 5. Funnel plot of standardized mean differences (SMD) of average speed in open-field. *SE* = standard error.

4. Discussion

This systematic review demonstrated the effects of CP models in reproducing the damage to locomotion and motor coordination in animals. The combination of the different experimental models has been demonstrated as the CP model that leads to more severe changes and maintained over the long term, especially when the sensorimotor restriction was present. The combined models potentiated the functional repercussions of the individual models on locomotion and motor coordination, as confirmed through the meta-analysis of distance traveled and motor coordination. As a short-term locomotor dysfunction model, the IVH model stands out in this review. The LPS, PI and HI models were divergent in terms of locomotor outcomes, whereas the isolated anoxia model promoted subtle and reversible damage on locomotion. Additionally, this review evidenced that the hind limbs of the animals were the most affected, regardless of the model adopted in the studies, contributing strongly to the deterioration of the motor function, even if transiently in some models.

As observed in the present review, the combination of different experimental CP models has shown to be able to promote impairments in persistent locomotion and motor coordination. This can be elucidated by understanding the individual effects of each adopted experimental model. The sensorimotor restriction alone or combined with other models was the model that most contributed to the maintenance of motor damage in the long term due to the immobilization of the animals' hind limbs similar to the lack of movement as happens in humans (Buratti et al., 2019; Delcour et al., 2018a; 2018b; Marcuzzo et al., 2010; dos Santos et al., 2017; Stigger et al., 2011; Strata et al., 2004). This result can be elucidated by studies that show that reduction of sensory afferences leads to the degradation of the organization of the primary motor cortex in the representation of posterior limbs, reduction of the cross-sectional area of muscles important to locomotion, such as the anterior tibial and soleus, in addition to changes in types of muscle fibers, thus simulating a diplegic cerebral palsy, where there is a predominant involvement of hindlimbs (Stigger et al., 2011; Strata et al., 2004), leading to worse performance in locomotor outcomes and motor coordination as demonstrated in the meta-analyzes of the present study.

The greatest damage to the lower limbs resulting from experimental CP was not exclusive to SR and often occurred only in the short term. In models of HI, PI and LPS, deficiency in the control of lower limb movements is combined with the inflammatory process established in the central nervous system (Shi et al., 2018), which leads to a decrease in myelination (Saadani-Makki et al., 2008; Zaghloul et al., 2017), increased neuronal apoptosis (Saadani-Makki et al., 2008; Zaghloul et al., 2017) and reduced Purkinje cells in the cerebellum (Wallace et al., 2010), for example. Thus, among the observed changes are stiffness, deficiency in movement control, and postural compensations during gait that impair the animals' ability to move in a stable and coordinated manner (Delcour et al., 2011; Saadani-Makki et al., 2008; Tan et al., 2005; Zhang et al., 2016).

In this context, motor coordination was one of the outcomes that most demonstrated to be impaired in animals submitted to CP as found in our meta-analysis. Considering the neuromotor complexity required of animals, rotarod testing is an established and effective method for this type of assessment in rodents (Dunham and Miya, 1957; Kim et al., 2017). As with other functional tests, the analysis protocols were varied and we observed a high rate of heterogeneity, but it did not interfere with the significant size of the final effect. Thus, we can emphasize that the combination of the models obtained a larger effect size in this case, in which the models that combined LPS with HI (Girard et al., 2009), LPS with Anoxia (Stigger et al., 2013), LPS with Anoxia and SR (Stigger et al., 2011), and Anoxia with SR (Strata et al., 2004), reinforces the potential of this method in reproducing neurofunctional damage due to the confluence of the effects of individual models.

The animals' difficulty in maintaining a satisfactory gait pattern not only interferes with gait on a moving rod, as in the rotarod but also influences the simplest locomotion, that is, it affects spontaneous gait. As observed in the present study, a variety of tests were used in this analysis, including the open field test that allows us to quantify kinematic parameters of spontaneous locomotion such as the distance covered and the average speed (da Silva Araga˜o et al., 2011). In the meta-analysis of the distance traveled, once again it is evident that combination of models leads to a reduction in the ability to move, but as models of HI, PI and LPS were predominantly included, where the studies were divergent, we did not obtain a significant effect size (Lubics et al., 2005; Miguel et al., 2015; Poggi et al., 2005; Wallace et al., 2010; Wang et al., 2013). Similarly, in the analysis of average speed, only models of HI and anoxia were included, in which the latter has already revealed that it does not lead to motor deficits even in the short term (Van De Berg et al., 2003; Ireland et al., 2010; Lubics et al., 2005; Miguel et al., 2015). Thus, the absence of results models that limit the functionality of the animals contributed to not obtaining a significant effect size for the speed parameter.

We observed in this review that the variability of locomotor or motor

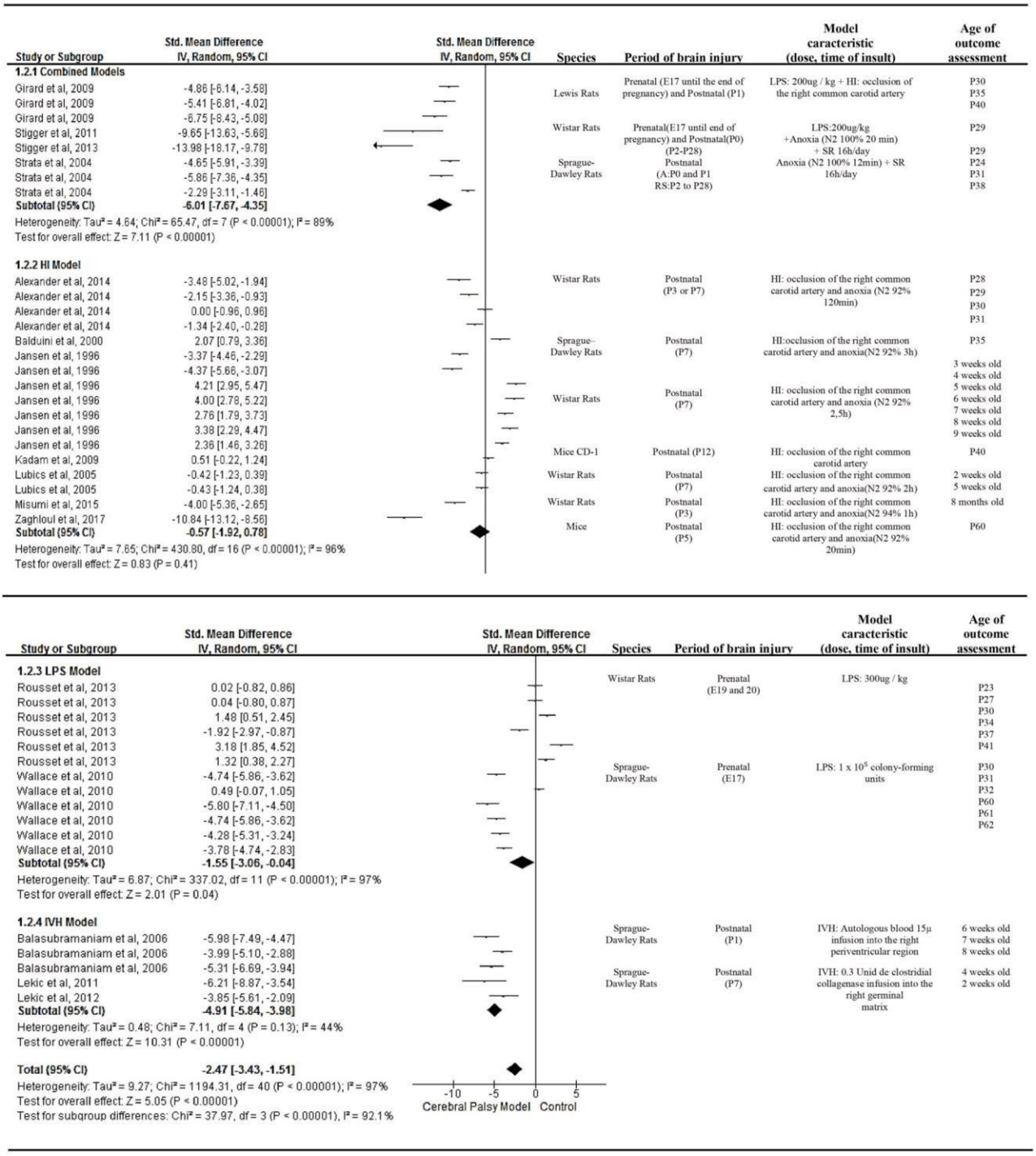

Fig. 6. Forest plot of included studies evaluating motor coordination in rotarod (Subgroup: Experimental models of CP). Horizontal lines represent the effect size ± the confidence interval (95 %). The summary effect size is represented by the diamond. P: Postnatal day; A - Anoxia; RS - Sensorimotor restriction; LPS - Lipopolysaccharide inflammation; HI: Hypoxic-ischemia.

coordination deficits related to the sex of the animals was discrete and punctual and points to the need for discussions on this topic, since the inclusion of animals of both sexes is still a controversial factor, diverging between the authors. Some studies have found no difference between the sexes regarding the parameters evaluated. Others who used only males in their sample seemed to be able to reproduce neurofunctional damage more significantly (Alexander et al., 2014; Marcuzzo et al., 2010; Misumi et al., 2016; Ohshima et al., 2016; dos Santos et al., 2017; Stigger et al., 2013, 2011; Tashima et al., 2001) which did not occur in some studies that used only females (Hoeger et al., 2000; Weitzdoerfer et al., 2002). Females submitted the LPS model (Wang et al., 2010) or HI (Arteni et al., 2010; Ohshima et al., 2016) showed greater evidence of hyperactivity in the open field, so we cannot rule out the effect of the model on these results, and it is necessary to raise the question of the real influence of sex on locomotion in CP models. Therefore, we suggest that further studies use both sexes in their experimental sample to elucidate the mechanisms involved in the particularities of the sexes.

In addition to elucidating the objectives of reproducing neurofunctional damage with a CP model, it is essential to know the reproducibility of the models, including possible adverse events that may

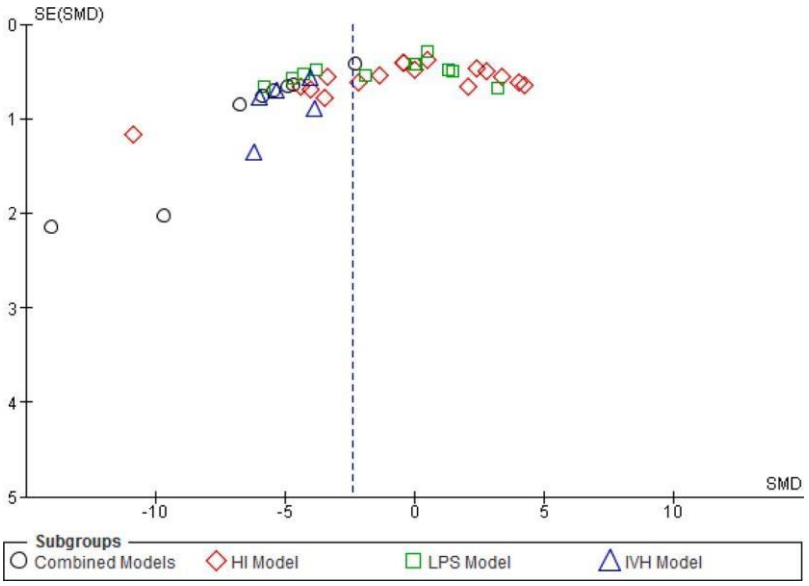

Fig. 7. Funnel plot of standardized mean differences (SMD) of motor coordination in rotarod. *SE* = standard error.

compromise the conduct of the study. In this sense, the principle of 3Rs (Replacement, Reduction, and Refinement) stands out as being a basic requirement for quality science (Kirk, 2018; Russell and Burch, 1959). The tendency is that experimental studies should be increasingly concerned with safer and reproducible methods, not only as an ethical issue but also as a financial one (Kirk, 2018; Percie du Sert et al., 2017). Relevant points to be considered when choosing an experimental model to be adopted: reducing the number of animals and resources needed to obtain information on the research question, and refinement, minimizing the suffering of animals during any experimental procedure (Kirk, 2018; Russell and Burch, 1959). Thus, careful planning is necessary for all phases of the animal care process, including accommodation, acclimatization, anesthesia, analgesia, permanent care of the litters, including mothers and pups, and the use of scales to assess the good.
-being of animals (Percie du Sert et al., 2017). In addition to training and awareness of the team about the importance of this care, they are indispensable for improving the quality of evidence from pre-clinical studies (Percie du Sert et al., 2017).

In this way, combined models, in addition to better efficiency in reproducing the damage on locomotion and motor coordination of CP, also demonstrated a low mortality rate of the pups during the application of the model. The mortality rate was below 5% in pups that were submitted to the combination of the postnatal anoxia model with sensorimotor restriction (Strata et al., 2004) and no mortality was observed in pups submitted to inflammation by prenatal LPS with postnatal HI (Girard et al., 2009). In contrast, in the model that associates LPS and PI both on prenatal, although there was no impact on the size of the litter, the early fetal loss was high, reaching 42 % (Jantzie et al., 2014). This suggests that insults combined with the prenatal phase favor more losses related to adverse events, requiring greater resources such as additional skills from researchers and effective preventive measures.

The quality of the studies included in this review was moderate. This can be explained by the lack of detailed information in the articles regarding the methods used to avoid risks of bias, such as, for example, describing the randomization method for experimental groups. Few studies have reported randomization for the groups, randomization for the outcome, and blinding for the assessment of outcomes. This may be due to the publication period of the included studies, in which a large part of them was published before understanding the importance of caring for biases in experimental studies (Hooijmans et al., 2014a, 2014b). Thus, we suggest the adoption of the SYRCLE risk of bias tool (Hooijmans et al., 2014a, 2014b) in future research as a guide from the initial stage of research development, so that better control of the risks of bias and consequently increase the level of available evidence. Additionally, the heterogeneity of the studies regarding the protocols for analyzing the repercussions on locomotion and coordination was one of the limiting points of the present study. This heterogeneity reflects the lack of standardization of methods in the use of the equipment and among research teams that investigate CP, which interferes with the crossing of data and reduces our capacity for inferences. A future alternative would be to homogenize the experimental analysis, for example, by the creation of an online platform for a consultation to protocols or, the strengthening of partnerships between the research groups through the construction of guides of evaluation methods. Finally, another limitation of our study was the generalization of motor impairments results, as they are very much dependent on the timing of insult and species used in the experimental model, and methods and age of the evaluation of locomotion. In this sense, we sought to facilitate the reader's understanding by performing the meta-analysis together with the study characteristics data.

Thus, as noted in this review, there are a variety of experimental CP models that are capable of reproducing neurofunctional changes similar to what happens in humans. Despite the limitations noted above it becomes necessary to reinforce the relevance of preclinical studies of good methodological quality since from them it is possible to clarify the mechanisms related to CP. Consequently, it is possible to outline a range of intervention strategies based on the motor phenotype that is produced with these models and to extrapolate within the ethical limits their implications for humans.

5. Conclusions

The results obtained in this study led us to the following conclusions: (a) The combination of perinatal brain injury models, regardless of the period of insults, enhances the damage on locomotion and motor coordination in the short and long term, especially when the sensorimotor restriction is performed; (b) The locomotor damage phenotype induced by the IVH model is temporary, whereas the isolated anoxia model does not lead to significant motor repercussions even in the short term; (c) New studies are needed to elucidate the effects of the LPS and HI models on locomotion, which presented divergent results in this review, in addition to the fact that new studies may clarify the influence of the sexes in the different models of CP.

Fig. 8. Risk of bias summary of included studies review authors' judgments about each risk of bias item for each included article.+ (green) low risk of bias; - (red) high risk of bias; (uncolored) unclear risk of bias.

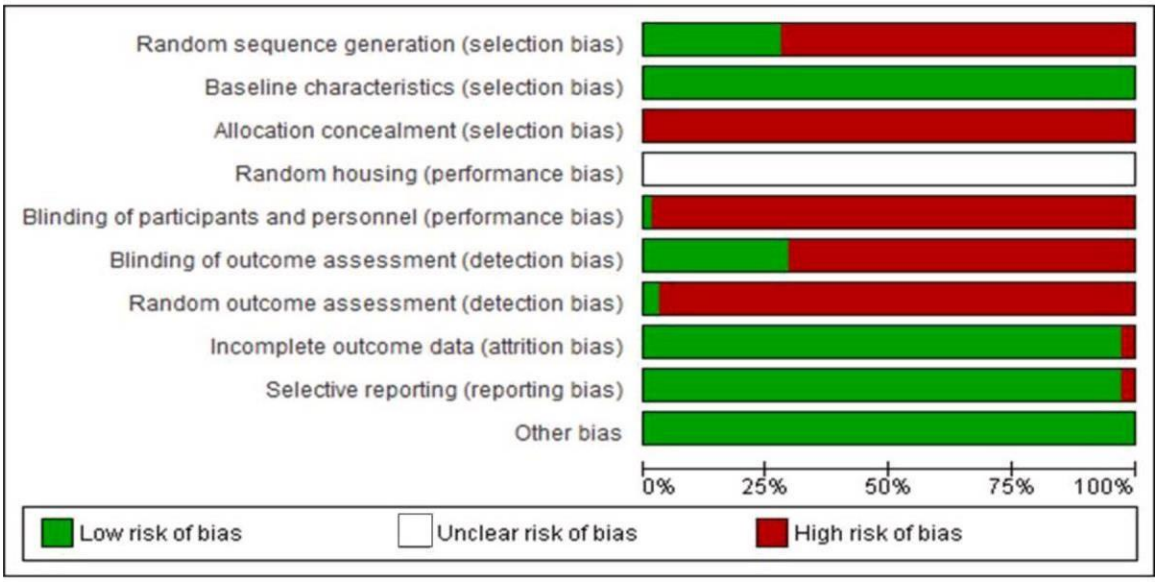

Fig. 9. Risk of bias graph of included studies. Review authors' judgments about each risk of bias item presented as percentages across all included studies. (green) low risk of bias; (red) high risk of bias; (white) unclear risk of bias.

CRediT authorship contribution statement

A.E. Toscano: is the supervisor. **S.C. Pereira, G.L. Albuquerque, C. M.S.S Calado, V.S. Souza, D.B. Visco**, and **A.E. Toscano:** carried out the manuscript preparation process, all of which participated in determining the eligibility criteria and standardizing the data collection forms and analyzing the risk of study bias. **D.B. Visco, R. Manha˜es-de-Castro**, and **A.E. Toscano:** participated in writing the review and editing for preparation. They contributed their experience regarding intellectual content and guidance. All authors reviewed and agreed with the final manuscript.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jneumeth.2021.109250.

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ANEXO A — COMITÊ DE ÉTICA

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Recife, 18 de março de 2022

Ofício nº 01/22

Da Comissão de Ética no Uso de Animais (CEUA) da UFPE
Para: **Prof. Ana Elisa Toscano Meneses da Silva Castro**
Centro Acadêmico de Vitória
processo **nº0032/2021**

Certificamos que a proposta intitulada "**Efeitos do tratamento com resveratrol sobre o desenvolvimento postural, função motora e balanço oxidativo no encéfalo de ratos submetidos a paralisia cerebral experimental**", registrado com o **nº0032/2021** sob a responsabilidade da **Prof Ana Elisa Toscano Meneses da Silva Castro** Que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo CONSELHO NACIONAL DE CONTROLE DE EXPERIMENTAÇÃO ANIMAL (CONCEA), e foi aprovada pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DA UNIVERSIDADE FEDERAL DE PERNAMBUCO (UFPE), em reunião de 22/02/2022

Finalidade	() Ensino (x) Pesquisa Científica
Vigência da autorização	22/02/2022 A 28/02/2023
Espécie/linhagem/raça	Ratos heterogênico
Nº de animais	105 animais
Peso/Idade	Ratos adultos progenitores: 220-250g / 90-120d Ratos filhotes: 5-80g / 1-29d
Sexo	Macho (75) e Femea (30)
Origem: Biotério de Criação	Biotério do Departamento de Nutrição da UFPE
Destino: Biotério de Experimentação	Biotério do Departamento de Nutrição da UFPE

Atenciosamente

[redacted]

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