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RODRIGO MORENO KLEIN

AVALIAÇÃO COMPORTAMENTAL E DO SISTEMA ENDOCANABINOIDE EM FILHOTES DE RATOS EXPOSTOS AO PARACETAMOL DURANTE A GESTAÇÃO

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Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde da Universidade Estadual de Londrina como requisito para obtenção do título de Doutor em Ciências.

Orientador: Profa. Dra. Estefânia Gastaldello Moreira

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BANCA EXAMINADORA

Orientadora: Profª. Drª. Estefânia G. Moreira
Universidade Estadual de Londrina - UEL

Profª. Drª. Sabrina Francesca de Souza Lisboa
Universidade de São Paulo – USP

Prof. Dr. Célio Roberto Estanislau
Universidade Estadual de Londrina – UEL

Prof. Dr. Thiago Berti Kirsten
Universidade Paulista – UNIP

Prof. Dr. Victorio Bambini-Junior
Lancaster University

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“Enquanto o cérebro for um mistério, o universo permanecerá um mistério.”

Santiago Ramon y Cajal

KLEIN, RM. **Avaliação comportamental e do sistema endocanabinoide em filhotes de ratos expostos ao paracetamol durante a gestação.** 2023. 131 p. Tese (Doutorado em Ciências da Saúde) – Universidade Estadual de Londrina, Londrina, 2023.

RESUMO

O paracetamol é um fármaco de venda livre utilizado como analgésico/antipirético por aproximadamente 40-50% das gestantes mundialmente. Embora estudos epidemiológicos venham sugerindo que a exposição uterina ao paracetamol seja um fator de risco para transtornos do neurodesenvolvimento, causalidade e modos de ação de sua possível ação neurotoxicante do desenvolvimento são incertas. Entre as hipóteses levantadas, mas ainda não testadas, estão disfunção do sistema endocanabinoide (eCB) e ativação imune-inflamatória. Desta forma, objetivamos avaliar os efeitos da exposição gestacional ao paracetamol sobre comportamento e efeitos comportamentais de um fármaco canabinoide, além de possíveis alterações sobre marcadores neuroquímicos relevantes para sinalização endocanabinoide e inflamação. Para isto, ratas prenhes receberam paracetamol (350 mg/kg) ou água por gavage, do dia gestacional 6 até o nascimento da prole. Medidas de toxicidade geral e reprodutiva incluíram peso corporal das progenitoras e dos filhotes até o desmame. Avaliamos o comportamento de filhotes com 10 (busca pelo ninho), 24 (campo aberto), 25 (estereotipia comportamental induzida pela apomorfina, enterramento das bolinhas de gude) 30 (teste das três câmaras) dias de idade 30 min após injeção do agonista canabinoide WIN 55,212-2 (0.3 mg/kg, via intraperitoneal) ou veículo. Reconhecimento do objeto novo e placa quente e comportamento social de brincar foram avaliados em filhotes com 25 ou 30 dias, respectivamente. Quantificamos as concentrações do eCB 2-araquidonoilglicerol (2-AG) e do lipídio pró-inflamatório fator ativador de plaquetas (PAF) bem como a expressão genica das principais enzimas de síntese e degradação de 2-AG, diacilglicerol lipase alfa e beta (DAGL-α e DAGL-β) e monoacilglicerol lipase, além dos marcadores inflamatórios ciclooxigenase-2 e interleucina 1 beta em regiões encefálicas de ratos com 22 dias de idade. O protocolo de exposição não afetou as medidas de toxicidade geral. Entretanto, filhotes expostos apresentaram hiperatividade no campo aberto e maior enterramento de bolinhas de gude independentemente do sexo. Fêmeas expostas apresentaram maior estereotipia comportamental induzida por apomorfina e passaram mais tempo na área central do campo aberto. Injeção prévia de agonista canabinoide modificou a resposta comportamental no teste de busca pelo ninho, induzindo efeitos opostos em fêmeas expostas e não expostas. Além disso, aumento nas concentrações de 2-AG no córtex pré-frontal e de PAF no córtex-prefrontal, estriado e cerebelo foi observado em machos e fêmeas expostos. A exposição diminuiu a expressão gênica cerebelar de DAGL-β, uma sintase de 2-AG. Os resultados apontam para alterações no sistema eCB e inflamação como modos de ação pelo qual o paracetamol prejudica o neurodesenvolvimento e altera o comportamento da prole em decorrência da exposição gestacional.

Palavras-chave: Acetaminofeno. Sistema endocanabinoide. Neurodesenvolvimento. Transtornos do neurodesenvolvimento.

KLEIN, RM. **Behavioral and endocannabinoid system evaluation in rat pups exposed to paracetamol during gestation.** 2023. 131 p. Thesis (Ph.D. in Health Sciences) – Universidade Estadual de Londrina, Londrina, 2023.

ABSTRACT

Acetaminophen is an over-the-counter medicine used as an analgesic/antipyretic by approximately 40-50% of pregnant women worldwide. Although epidemiological studies have pointed uterine exposure to paracetamol as a risk factor for neurodevelopmental disorders, causality and modes of action of its possible developmental neurotoxicant action are uncertain. Among the raised hypotheses are dysfunction of the endocannabinoid system (eCB) and immune-inflammatory activation, but they were not directly tested. Thus, we aimed to evaluate the effects of gestational exposure to paracetamol on behavior and on behavioral effects of a cannabinoid drug, in addition to possible alterations in neurochemical markers relevant to endocannabinoid signaling and inflammation. For this, pregnant rats received paracetamol (350 mg/kg) or water by gavage, from gestational day 6 until the birth of the offspring. Measures of general and reproductive toxicity included body weight of dams and pups until weaning. We evaluated offspring behavior of 10- (nest seeking), 24- (open field), 25- (apomorphine-induced stereotypy, marble burying) and 30- (three-chamber test) day-old rats 30 min after injection of the cannabinoid agonist WIN 55,212-2 (0.3 mg/kg, intraperitoneally) or vehicle. Novel object recognition and hot plate and social play behavior were evaluated in 25- or 30-day-old rats. We quantified the concentrations of the eCB 2-arachidonoylglycerol (2-AG) and of the pro-inflammatory lipid platelet activating factor (PAF) in the prefrontal cortex, hippocampus, striatum and cerebellum of 22-day-old rats. Additionally, we evaluated gene expression of the main 2-AG synthesis and degradation enzymes, diacylglycerol alpha and beta (DAGL-α and DAGL-β) and monoacylglycerol lipase, in addition to the inflammatory markers cyclooxygenase-2 and interleukin 1 beta. Exposure regimen did not affect overall toxicity measures. However, hyperactivity in the open field and increased marble burying were observed in exposed male and female rats. Exposed females presented increased apomorphine-induced behavioral stereotypy and spent more time in the central area of the open field. Previous cannabinoid agonist injection modified the behavioral response in the nest seeking test, inducing opposite effects in exposed and unexposed females. Furthermore, increased concentrations of 2-AG in the prefrontal cortex and of PAF in the prefrontal cortex, striatum and cerebellum were observed in exposed males and females. Exposure decreased cerebellar gene expression of DAGL-β, a 2-AG synthase. The results point to alterations in the eCB system and inflammation as modes of action by which paracetamol impairs neurodevelopment and alters offspring behavior as a result of gestational exposure.

Key words: Acetaminofen. Endocannabinoid system. Neurodevelopment. Neurodevelopmental disorders.

LISTA DE ABREVIATURAS

2-AG	2-Araquidonoilglicerol
ADHD	Transtorno de déficit de atenção e hiperatividade
AEA	N-araquidonoiletanolamida
ASD	Transtorno do espectro autista
AM404	N-araquidonoilfenolamina
BDNF	Neurotrofina derivada do encéfalo
CB1	Receptor canabinoide tipo 1
CB2	Receptor canabinoide tipo 2
CON	Grupo controle
COX-2	Ciclooxigenase-2
DAGL-α	Diacilglicerol lipase alfa
DAGL-β	Diacilglicerol lipase beta
DG	Dia gestacional
DPN	Dia pós-natal
eCB	Endocanabinoide
FAAH	Enzima amida hidrolase de ácidos graxos
GD	Dia gestacional
IL-1β	Interleucina 1 beta
LC-MS	Cromatografia líquida acoplada à espectrometria de massas
MAGL	Monoacilglicerol lipase
NAPQI	N-acetil-p-benzoquinona imina
NOR	Reconhecimento do objeto novo
PAF	Fator ativador de plaquetas
PAR	Paracetamol
PND	Dia pós-natal
qPCR	PCR quantitativo
SNC	Sistema nervoso central
TDAH	Transtorno de déficit de atenção e hiperatividade
TEA	Transtorno do espectro autista
TRPV1	Receptor vaniloide de potencial transitório tipo 1
VEI	Veículo
WIN	WIN 55,212-2
η_p^2	Eta parcial quadrado

SUMÁRIO

1. INTRODUÇÃO

O desenvolvimento do sistema nervoso central (SNC) permite que um pequeno grupo de células não diferenciadas resulte na formação de um encéfalo funcional. Para que este grupo de células se torne um encéfalo, é necessário que elas se proliferem, se diferenciem, migrem para zonas específicas, que estabeleçam sinapses (caso sejam neurônios), além de outros processos (Heyer & Meredith, 2017). É por haver uma série de processos, que dependem da ocorrência prévia ou simultânea um do outro, que o SNC nos estágios inicias da vida é especialmente vulnerável a injúrias e aos efeitos de neurotoxicantes (Heyer & Meredith, 2017; Rice & Barone, 2000). A exposição a substâncias químicas durante esse período pode resultar em alterações no neurodesenvolvimento e em danos que podem ser distintos e mais duradouros que os observados se a exposição ocorresse em adultos (Ross et al., 2015). A área de estudo que investiga os efeitos de toxicantes sobre o sistema nervoso em desenvolvimento é a neurotoxicologia do desenvolvimento.

Os medicamentos podem induzir neurotoxicidade do desenvolvimento e gestantes comumente relatam utilizá-los, especialmente aqueles de venda livre (Haas et al., 2018). O mais utilizado por essa população é o paracetamol (Hussain & Ashmead, 2017). De fato, aproximadamente 50% das gestantes ingerem esse medicamento e uma parte delas repete o uso por várias semanas durante a gravidez (Bandoli et al., 2019). Índice de uso parecido foi encontrado em um estudo com brasileiras de uma cidade do sul do país (Bertoldi et al., 2020). Os altos índices de uso são possivelmente decorrentes de o paracetamol ser um dos únicos analgésicos e antipiréticos não contraindicados para gestantes, devido à sua presumida (e não suficientemente testada) segurança para essa população e para o feto (Cendejas-Hernandez et al., 2022).

Número crescente de estudos epidemiológicos vêm associando o uso do paracetamol durante a gestação como fator de risco para alterações neurocomportamentais na prole (Brandlistuen et al., 2013; Liew, Ritz, et al., 2016; Lye et al., 2022; Tovo-Rodrigues et al., 2020). Especial atenção foi dada para o potencial do medicamento em aumentar as chances de ocorrência de transtorno de déficit de atenção e hiperatividade (TDAH) e transtorno do espectro autista (TEA). Meta-análises sugerem que o uso materno de paracetamol durante a gestação é, de fato, fator de risco para a presença sintomas de TDAH (Alemany et al., 2021; Gou et al., 2019; Masarwa et al., 2018) e TEA (Alemany et al., 2021; Masarwa et al., 2018) na prole.

O TEA e o TDAH fazem parte de um conjunto maior de transtornos chamados transtornos do neurodesenvolvimento e são caracterizados pelo aparecimento nos primeiros anos de vida (American Psychiatric Association, 2014). O TEA é um grupo de psicopatologias

caracterizadas por prejuízos na interação e comunicação social recíproca, além da presença de padrões repetitivos de comportamento (estereotipias) ou de interesses. Alterações na sensibilidade a estímulos sensoriais, em ritmos biológicos e ansiedade podem ser sintomas associados (American Psychiatric Association, 2014). Os principais sintomas do TDAH são desatenção, impulsividade e hiperatividade persistentes e inapropriadas para a idade (American Psychiatric Association, 2014). Características associadas ao TDAH são baixa tolerância à frustração, irritabilidade e labilidade do humor, além de prejuízos em processos cognitivos em testes de atenção, função executiva e memória (American Psychiatric Association, 2014).

Mesmo que os resultados descrevendo associação entre uso materno de paracetamol e alterações no comportamento da progênie sejam preocupantes, eles devem ser interpretados com cautela. Estudos observacionais possuem conhecidas limitações (Wood, 2020). Variáveis de confusão como indicação de uso, ocorrência de febre e/ou infecções e histórico familiar de doenças psiquiátricas são importantes para os desfechos avaliados e dificultam o estabelecimento de relações de causalidade nestes estudos (Leppert et al., 2019). Cuidados adicionais devem ser tomados quando os estudos avaliam desfechos sem definição clara, com etiologia multifatorial e que avaliam associações sem mecanismo biológico estabelecido, como é o caso dos estudos de associação com exposição gestacional ao paracetamol (Laue et al., 2019; Masarwa et al., 2020). Os estudos em modelos animais são importantes porque permitem investigar a causalidade e os modos de ação pelos quais o paracetamol pode prejudicar o desenvolvimento do SNC (Zucker, 2017).

Estudos em roedores encontraram efeitos sobre comportamentos relevantes para transtornos do neurodesenvolvimento e neuroquímica como consequência da exposição ao paracetamol durante o neurodesenvolvimento. Grupos de pesquisa publicaram estas investigações utilizando diferentes protocolos de exposição materna, tratamento com paracetamol durante o desenvolvimento ou combinação de ambos. Baker et al. (2023) expôs maternalmente camundongos ao paracetamol (150 mg/kg/dia, gomas de gelatina comestíveis) do dia gestacional 4 até o dia pós-natal 14 (DPN 14). O protocolo resultou em alterações na vocalização ultrassônica de neonatos, no comportamento exploratório e na expressão de genes relacionados à metabolização do paracetamol, à danos ao DNA e ao sistema endócrino e imune no córtex pré-frontal (Baker et al., 2023). O estudo de Saad et al. (2016), no entanto, demonstrou que a administração de paracetamol (150 mg/kg, gavage) do dia gestacional 7 até o nascimento em camundongos não alterou o comportamento da prole no teste do campo aberto ou o volume de 29 regiões encefálicas (Saad et al., 2016). A combinação da exposição materna com tratamento prolongado com paracetamol (5 ou 15 mg/kg, diluído na água do bebedouro) em ratos, desde o dia de diagnóstico de prenhez até a

data de testagem da prole (60 dias de idade), alterou: atividades motora e padrão exploratório (Blecharz-Klin et al., 2017); comportamento social e concentração de neurotrofina derivada do encéfalo (BDNF) no estriado (Blecharz-Klin et al., 2018); neurotransmissão monoaminérgica e de aminoácidos no bulbo (Blecharz-Klin et al., 2015a) e medula espinhal (Blecharz-Klin et al., 2015b); neurotransmissão monoaminérgica no cerebelo (Blecharz-Klin et al., 2016) e hipotálamo (Blecharz-Klin et al., 2019) de ratos machos expostos. Injeções de paracetamol (2 doses de 30 mg/kg, subcutânea) em camundongos com 10 dias de vida resultaram em alterações no padrão de habituação (Philippot et al., 2017, 2022), na memória espacial (Philippot et al., 2022) e em esperadas respostas analgésicas e ansiolíticas ao paracetamol na idade adulta (Viberg et al., 2014). Adicionalmente, alteraram concentrações de BDNF no córtex frontal e parietal (Viberg et al., 2014) e de marcadores de estresse oxidativo no hipocampo (Philippot et al., 2022) horas depois das administrações no dia 10. Injeções repetidas de paracetamol (103,9 mg/kg/dia, subcutânea) nos dias pós-natais (DPN) 5, 8 e 11 aumentaram evitação social e estereotipias comportamentais de camundongos com pelo menos 45 dias de vida (Harshaw & Warner, 2022).

Nosso grupo de pesquisa estabeleceu modelos de exposição materna a doses relevantes para humanos de paracetamol nos quais não há toxicidade geral materna e reprodutiva (Klein et al., 2020; Rigobello et al., 2021). Filhotes pré-púberes expostos ao paracetamol (350 mg/kg, gavage) do dia gestacional 6 ao dia do nascimento apresentaram prejuízos na discriminação olfativa e aumento de comportamentos estereotipados induzidos por apomorfina. Filhotes fêmeas expostas apresentaram hiperatividade no campo aberto. Marcadores de estresse oxidativo ou concentração de BDNF no córtex pré-frontal e hipocampo não foram afetados (Klein et al., 2020). Filhotes machos pré-púberes expostos ao paracetamol (35 ou 350 mg/kg, gavage) do dia gestacional 6 ao dia do desmame (DPN 21) apresentaram hiperatividade no campo aberto e aumento de estereotipia comportamental induzida por apomorfina, expostos às doses menor e maior, respectivamente. A exposição à dose de 35 mg/kg diminuiu as concentrações de glutationa reduzida no hipocampo e superóxido dismutase no estriado de machos expostos (Rigobello et al., 2021).

Potenciais modos de ação pelos quais o paracetamol pode prejudicar o desenvolvimento do SNC foram hipotetizados na literatura e incluem: formação excessiva de seu metabólito reativo N-acetil-p-benzoquinona imina (NAPQI); alteração nas concentrações do BDNF; inibição da ciclooxigenase-2 (COX-2); alteração hormonal; estresse oxidativo e ativação imune-inflamatória; e disfunção do sistema endocanabinoide (eCB) (Bauer et al., 2018).

O sistema eCB é uma via de sinalização lipídica quase onipresente no reino animal, com surgimento evolutivo coincidente com o aparecimento de SNCs multicelulares e de maior complexidade (Silver, 2019). Os constituintes do sistema são seus mediadores endógenos, os eCBs, as enzimas responsáveis pelas suas sínteses e degradações, e os receptores canabinoides tipo 1 (CB1) e tipo 2 (CB2) (Lu & Mackie, 2021). Os eCBs com melhor caracterização são o 2-araquidonoilglicerol (2-AG) e a N-araquidonoiletanolamida (AEA), mediadores lipídicos derivados do ácido araquidônico. Esses mediadores possuem distinta afinidade de ligação e eficácia com os receptores canabinoides, e o 2-AG é ~170 x mais disponível no encéfalo que o AEA (Cristino et al., 2020). Assim como com outros mediadores lipídicos, há redundância nas vias de síntese e degradação desses mediadores e promiscuidade para alvos de ligação. Por exemplo, a enzima amida hidrolase de ácidos graxos (FAAH) pode hidrolisar tanto AEA quanto 2-AG, inativando-os (Lu & Mackie, 2021). Os mediadores podem se ligar à vários outros alvos além de CB1 e CB2, como o receptor vaniloide de potencial transitório tipo 1 (TRPV1), receptores nucleares e outros (Cristino et al., 2020). Este complexo sistema regula o desenvolvimento do SNC, a liberação de neurotransmissores, a plasticidade sináptica e outros importantes processos neurobiológicos (Cristino et al., 2020; Lu & Mackie, 2021). Devido a isso, a exposição gestacional a fármacos que alteram a sinalização eCB foi associada com alterações comportamentais e no neurodesenvolvimento da prole (Harkany & Cinquina, 2021).

O paracetamol pode interagir com o sistema eCB de várias maneiras. Primeiramente, pode inibir a oxidação de AEA e 2-AG pela COX-2, o que pode resultar em maior disponibilidade desses mediadores (Patel & Windsor, 2015). Em segundo lugar, o paracetamol pode interagir com o sistema eCB por meio de seu metabólito N-araquidonoilfenolamina (AM404). Esse é o produto da conjugação do metabólito desacetilado do paracetamol, o para-aminofenol, com ácido araquidônico mediado pela FAAH no SNC. Há evidências de conversão *in vivo* desse metabólito no encéfalo de ratos (Högestätt et al., 2005; Muramatsu et al., 2016) e no líquido cefalorraquidiano humano (Sharma et al., 2017) após a administração de paracetamol. O AM404 inibe enzimas envolvidas no carreamento intracelular (Kaczocha et al., 2012) e no transporte transmembrana (Beltramo et al., 1997) de AEA, além de inibir a COX-2 em micróglias ativadas (Perazzo et al., 2017). Todas essas ações podem resultar no aumento da disponibilidade de eCBs. O AM404 também é um potente agonista do receptor TRPV1 (Zygmunt et al., 2000) e um fraco agonista CB1 (Beltramo et al., 1997). O TRPV1 e CB1 são receptores colocalizados (Cristino et al., 2006) que frequentemente têm ações opostas sobre respostas celulares e comportamento (Cristino et al., 2020; Moreira et al., 2012). Em conjunto, estes dados sugerem que o paracetamol pode interagir com o sistema eCB em desenvolvimento por variados mecanismos.

Philippot e colaboradores (2018) investigaram os efeitos da coadministração de paracetamol (2 doses de 30 mg/kg, subcutâneo) com o agonista não específico de receptores canabinoides WIN 55,212-2 (WIN, 1 mg/kg, subcutâneo) em camundongos com 10 dias de vida. Essa administração combinada induziu alterações mais demarcadas na motricidade e no padrão de habituação que as induzidas em animais que receberam apenas injeções de paracetamol no período neonatal. Sugere-se que esses efeitos possam ser atribuíveis à redução na expressão de genes envolvidos na neurotransmissão, sinalização de BDNF e metabolismo de eCB pela FAAH observada no hipocampo e córtex frontal (Philippot et al., 2018).

Outro possível modo pelo qual o paracetamol pode prejudicar o desenvolvimento do SNC é a ativação imune-inflamatória. O paracetamol é conhecido pela hepatotoxicidade em altas doses, cujos mecanismos envolvem estresse oxidativo e respostas inflamatórias induzidos pela excessiva formação de NAPQI (Athersuch et al., 2018). No entanto, mesmo quando usado em doses terapêuticas, o paracetamol (500 mg a cada 6 h por 3 dias) alterou a expressão de genes relacionados a citocinas, ao estresse oxidativo e ao metabolismo de lipídios em uma avaliação transcriptômica em amostras de sangue de voluntários saudáveis (Jetten et al., 2012). Da mesma forma, injeções de paracetamol (15 mg/kg, 2x / dia) em ratas entre o meio e o final da gestação afetaram a transcrição de genes relacionados à imunidade inata e adaptativa na placenta e, em menor grau, no encéfalo fetal (Koehn et al., 2020). Estes dados indicam ocorrência de respostas inflamatórias como consequência do uso de ou exposição fetal ao paracetamol.

Considerando que (a) o paracetamol é amplamente utilizado por gestantes; (b) estudos epidemiológicos sugerem possíveis efeitos negativos da exposição durante a gestação sobre o neurodesenvolvimento de crianças; (c) esses estudos dificultam o estabelecimento de relações de causalidade devido ao fraco controle de variáveis; (d) estudos com animais possibilitam avaliar causalidade e modos de ação envolvidos; (e) disfunção no sistema eCB e alteração imune-inflamatória foram ambos sugeridos como modos de ação pelos quais o paracetamol pode afetar o desenvolvimento do SNC; esse estudo se justifica pela avaliação de possíveis alterações comportamentais, funcionais e neuroquímicas decorrentes da exposição gestacional ao paracetamol.

2. OBJETIVOS

2.1. Objetivo geral

Investigar os efeitos da exposição gestacional ao paracetamol em filhotes machos e fêmeas de ratas sobre parâmetros comportamentais e neuroquímicos relevantes para sinalização eCB e inflamação.

2.2. Objetivos específicos

Avaliar em filhotes expostos ao paracetamol durante a gestação:

a) discriminação olfativa, atividade motora, comportamentos ansiedade-símile, estereotipia comportamental induzida por apomorfina, comportamento repetitivo e sociabilidade;
b) se injeção prévia de um agonista canabinoide não específico modularia os efeitos comportamentais sobre discriminação olfativa, atividade motora e comportamentos ansiedade-símile, estereotipia comportamental induzida por apomorfina, comportamento repetitivo e sociabilidade;
c) memória de reconhecimento, nocicepção e comportamento social de brincar;
d) a concentração de 2-AG e PAF em regiões encefálicas (córtex pré-frontal, hipocampo, estriado e cerebelo);
e) a expressão gênica de DAGL-α, DAGL-β, MAGL, COX-2 e IL-1β em regiões encefálicas (córtex pré-frontal, estriado e cerebelo).

3. MATERIAL E MÉTODOS

3.1. Composição do estudo

Esta tese foi elaborada com dois estudos com metodologias distintas descritas a seguir. A primeira metodologia descrita (item 3.2) é relacionada à elaboração do primeiro artigo científico apresentado. A segunda (item 3.3) refere-se ao segundo artigo científico apresentado.

3.2. Artigo 1: *Gestational paracetamol exposure induces core behaviors of neurodevelopmental disorders in infant rats and modifies response to a cannabinoid agonist in females*

3.2.1. Animais, exposição materna e procedimentos perinatais

Ratos Wistar ingênuos machos e fêmeas foram acasalados durante a noite (1 macho e 2 fêmeas em cada gaiola), e o dia gestacional 0 (DG 0) foi determinado por esfregaço vaginal na manhã seguinte, pela presença de espermatozoides. No DG 6 as fêmeas foram alocadas individualmente em gaiolas viveiro. Os animais ficaram em ambiente com temperatura controlada de 21 ± 2 ºC e ciclo claro escuro de 12h (luzes acesas às 6:00 A.M.), com água e ração sempre disponíveis.

O paracetamol (Tylenol®, Johnson & Johnson, Brasil), foi diluído em água e administrado, diariamente, do DG 6 até o DPN 0, na dose de 350mg/kg por gavage (n= 56). O grupo controle (n= 48) recebeu água por gavage. As ratas foram pesadas de 3 em 3 dias do DG 6 ao desmame e o volume ajustado de acordo com o peso. A dose representa o ajuste dosimétrico da dose de 500 mg/dia em humanos (65 Kg) (USEPA, 2006) com adição de um fator de segurança de 10 para considerar a variabilidade intra-espécie (Nielsen et al., 2008), e está de acordo com a dose sistêmica efetiva para analgesia em ratos (Hoshijima et al., 2021). O início do tratamento no DG6 foi escolhido para se evitar possíveis efeitos do paracetamol no período de implantação (OECD, 2007).

No DPN 4, as ninhadas foram ajustadas para 10 filhotes, mantendo-se, sempre que possível, 5 animais para cada sexo. Ninhadas com menos que 8 filhotes foram descartadas. O peso corporal da prole foi registrado nos DPN 0, 4, 7, 14 e 21, dia no qual os filhotes foram desmamados e separados por sexo até a data de avaliação comportamental ou eutanásia. A ninhada foi considerada a unidade experimental, de modo que irmãos do mesmo sexo não foram utilizados para a mesma finalidade. A distribuição dos filhotes para cada um dos desfechos avaliados está descrita na Tabela Suplementar 1 referente a este artigo. Os animais excedentes foram utilizados em outros experimentos ou submetidos à eutanásia. Os protocolos experimentais foram aprovados pela Comissão de Ética no Uso de Animais da Universidade Estadual de Londrina (protocolo nº 8522.2019.35) (Anexo A).

3.2.2. Injeção e dosagem de WIN 55,212-2

Trinta minutos antes dos testes comportamentais um filhote macho e uma fêmea de cada ninhada receberam injeção intraperitoneal (i.p.) do agonista não específico de receptores canabinoides WIN 55,212-2 (WIN, 0,3 mg/kg, Sigma, EUA) enquanto outro casal da mesma ninhada recebeu injeção de veículo (VEI: DMSO 10% em solução salina). A dosagem do WIN foi baseada em um estudo que descreveu alterações comportamentais em ratos Wistar púberes (Trezza & Vanderschuren, 2008).

3.2.3. Testes comportamentais

Todos os testes comportamentais, com exceção do teste de enterramento de bolinhas de gude, foram gravados por uma câmera de vídeo, ligada a um computador em uma sala adjacente. Os vídeos foram analisados por observador treinado, mascarado as condições experimentais.

Os machos foram testados antes das fêmeas para evitar a influência de possível odor sexual no comportamento do animal. Os experimentos foram realizados no período da manhã (8h00 - 12h00) ou da tarde (13h00 - 18h00) conforme descrito abaixo.

3.2.3.1. Teste de busca pelo ninho (discriminação olfativa)

Na manhã do DPN 10 a latência dos filhotes para alcançar a maravalha do ninho com base em pistas olfativas foi avaliada (Schneider & Przewłocki, 2005). Os animais foram colocados individualmente no centro de uma gaiola retangular de acrílico com dimensões 20 x 8 x 8 cm (comprimento x largura x altura) divididos em 3 compartimentos iguais delimitados por tinta permanente e avaliados por 3 min. Um dos compartimentos laterais continha 10 g de maravalha da gaiola moradia do filhote enquanto no lado oposto havia 10 g de maravalha limpa. Uma entrada positiva foi considerada quando as patas dianteiras e a cabeça cruzaram a linha em direção a um dos compartimentos.

3.2.3.2. Teste do campo aberto (atividade motora e comportamentos ansiedade-símile)

Na tarde do DPN 24 animais foram colocados individualmente em uma arena circular de madeira (72 cm de diâmetro) dividida em 12 quadrantes iguais, e deixados 5 min para explorar livremente. O comportamento de levantar sobre as patas traseiras e o número de quadrantes cruzados foram avaliados para estimar a atividade motora. Um cruzamento foi considerado quando o rato colocou as quatro patas em outro quadrante. O tempo despendido nos quadrantes centrais da arena e em autolimpeza rostral (limpeza do focinho e cabeça) e corporal (limpeza do tronco, cauda e região anogenital) foram avaliados como medidas

relevantes para ansiedade (Estanislau et al., 2013). O aparelho foi limpo com solução de etanol a 5% antes de cada teste.

3.2.3.3. Estereotipia comportamental induzida por apomorfina

Na manhã do DPN 25 animais receberam uma injeção subcutânea de apomorfina (0,3 mg/kg, Sigma, EUA). Em seguida, foram colocados individualmente em uma gaiola de acrílico transparente e o comportamento estereotipado foi avaliado a cada 10 min (10 segundos de observação) por 120 min. Escores atribuídos variaram de 0 (dormindo ou parado) a 6 (contínuo roer ou lamber da grade ou da maravalha), conforme descrito (Setler et al., 1976). A intensidade das estereotipias foi avaliada pela soma cumulativa dos 12 escores obtidos.

3.2.3.4. Enterramento de bolinhas de gude (comportamento repetitivo)

Na manhã do DPN 25 animais foram colocados individualmente em uma gaiola de polipropileno (47 x 27 x 15) coberta com 5 cm de espessura de maravalha limpa sobre a qual estavam distribuídas 16 bolinhas de gude de vidro, dispostas em 4 fileiras com 4 bolinhas cada. O animal foi deixado para explorar livremente por 30 min e então foi devolvido à gaiola moradia. Fotos de alta definição das gaiolas de teste foram obtidas e o número de bolinhas enterradas (> 50% da bolinha coberta) foram contadas por dois observadores independentes e treinados (Thomas et al., 2009).

3.2.3.5. Teste das três câmaras (sociabilidade)

Na tarde do DPN 30 animais foram submetidos ao teste de três câmaras para avaliar a preferência social em relação a um objeto novo. Cada animal foi colocado na área central de um aparato de madeira (120 x 40 x 40) dividido em três câmaras de iguais proporções. As câmaras laterais eram inicialmente fechadas para que o animal fosse habituado apenas à área central. Dez minutos depois o animal podia ter acesso às câmeras laterais. Uma delas continha um rato desconhecido, pareado em sexo e idade ao animal de testagem dentro de uma cesta de plástico com orifícios (área social). Na câmara oposta, havia uma cesta semelhante vazia (área de objeto novo). O animal era deixado livre para explorar todas as três câmaras por 10 min. O tempo gasto em cada uma das três áreas foi registrado. Uma entrada é contabilizada quando o rato coloca todas as quatro patas em uma das três câmaras e termina apenas depois de colocá-las em outra. O índice de sociabilidade foi calculado por meio da seguinte fórmula: [(tempo na área social - tempo na área do novo objeto) / (tempo na área social + tempo na área do novo objeto)] * 100. Este índice permite a avaliação da preferência social sobre o novo objeto. O tempo gasto em cheirar perto da cesta contendo o

outro animal também foi contado. O aparato foi limpo com solução de etanol a 70% e seco com toalhas de papel antes de cada teste (Bambini-Junior et al., 2014).

3.2.4. Análise estatística

Inicialmente foi checada a normalidade (teste de Shapiro-Wilk) e homogeneidade de variâncias (teste de Levene) dos dados. Dados brutos ou após transformação aritmética atingiram os critérios (p> 0,01) para testes paramétricos e foram analisados por ANOVA fatorial com exposição gestacional ao paracetamol, sexo e injeção de WIN como fatores (modelo 2 x 2 x 2). Na ocorrência de interações significativas entre os três fatores, os dados foram divididos por sexo e ANOVAs de duas vias foram usadas para descrever as diferenças. No caso de nova interação entre fatores, gráficos de interação foram criados para interpretar os resultados. Os tamanhos de efeito foram determinados por eta quadrado parcial (η_p^2) para todos os parâmetros avaliados. Adicionalmente, o d de Cohen foi calculado para parâmetros afetados pela exposição a fim de comparar tamanhos de efeito com literatura relevante (efeitos de manipulações pré-natais em variáveis dependentes semelhantes). Enquanto os valores de p indicam a probabilidade de diferenças estatísticas terem ocorrido pelo acaso, os tamanhos de efeito determinam quão grandes são as diferenças médias padronizadas (por exemplo, d de Cohen) ou quanto um fator influencia na variância dos dados (por exemplo, η_p^2). IBM-SPSS Windows versão 26 foi usado para analisar os dados e as conclusões foram estabelecidas em p≤ 0,05.

3.3. **Artigo 2: *Paracetamol exposure alters markers of endocannabinoid signaling and increases PAF levels of infant rat's brain***

3.3.1. Animais, exposição materna e procedimentos perinatais

Os procedimentos relacionados ao manejo dos animais, exposição materna ao paracetamol e procedimentos perinatais foram idênticos aos descritos na seção 3.2.1. Para este estudo utilizamos 17 ninhadas do grupo controle (n= 39 filhotes) e 21 ninhadas do grupo paracetamol (n= 45 filhotes). Filhotes pré-púberes foram distribuídos em casais para as seguintes avaliações: (1) cromatografia líquida acoplada a espectrometria de massas (LC-MS); (2) PCR quantitativo (qPCR); (3) comportamento social de brincar; (4) reconhecimento de objeto novo e placa quente. Animais excedentes foram utilizados em outros experimentos ou eutanasiados. Todos os protocolos experimentais foram aprovados pelo Comissão de Ética no Uso de Animais da Universidade Estadual de Londrina (protocolo nº 8522.2019.35) (Anexo A).

3.3.2. Testes comportamentais

Todos os testes comportamentais foram gravados por câmera de vídeo ligada a um computador em uma sala adjacente. Os vídeos foram analisados por um observador treinado, mascarado à exposição.

Os machos sempre foram testados antes das fêmeas para evitar possíveis influências de odor sexual no comportamento dos animais. Os experimentos foram todos conduzidos entre 13:00 PM e 18:00 PM.

3.3.2.1. Reconhecimento do objeto novo (memória de reconhecimento)

No DPN 25 animais foram submetidos ao teste de reconhecimento do objeto novo para avaliar a discriminação de animais por um objeto novo em relação a um objeto previamente explorado (objeto familiar) (Bevins & Besheer, 2006). O teste tem duas fases: (1) fase de familiarização e (2) fase de testagem ou teste de objeto novo. Durante a fase de familiarização (1 h antes da fase de testagem) os animais foram colocados individualmente por 10 min em uma arena quadrada de madeira com dimensões de 40 x 40 x 40 cm contendo dois objetos de cor, tamanho, formato e material diferentes. Após a familiarização de 10 minutos, os animais foram devolvidos às suas gaiolas e deixados sem serem incomodados até o teste, que ocorreu uma hora depois. Na fase de testagem, um dos objetos foi substituído por um novo (com cor, tamanho, forma e material diferentes) e o animal foi deixado para explorar livremente a arena por 3 min. O tempo em contato direto (boca, nariz ou patas) com objetos ou comportamento de cheirar em direção a eles foi contado como exploração dos objetos. Contatos indiretos ou acidentais não foram computados. O índice de discriminação foi calculado da seguinte forma: tempo explorando objeto novo / tempo explorando todos os objetos. Os objetos e a arena foram limpos com álcool 70% após cada sessão (Parrini et al., 2017).

3.3.2.2. Placa quente (nocicepção)

Logo após o teste de reconhecimento de objeto novo os animais foram avaliados em uma placa quente (Ugo Basile, Brasil) a 55ºC. A latência para o tempo de reação (lamber ou levantar a pata traseira ou pular da superfície da placa) foi determinada (Klein et al., 2020). Um tempo limite de 30s foi estabelecido para evitar danos aos tecidos.

3.3.2.3. Comportamento social de brincar

Na manhã do DPN 30, os animais foram isolados por 3,5 horas com ração e água disponíveis. O comportamento de brincar atinge o pico no DPN 30 (Pletnikov et al., 1999) e o isolamento social de 3,5 h aumenta a motivação para engajar-se em brincadeira social

(Niesink & Van Ree, 1989). Após o isolamento, um par de animais do mesmo sexo e mesma exposição gestacional, mas não da mesma ninhada, foi colocado em uma gaiola de polipropileno (47 x 27 x 15) coberta com maravalha limpa e deixado para explorar e interagir livremente por 15 min. Foram analisados os seguintes comportamentos dos pares, e não de apenas um animal: frequência e duração de *pinning* (um animal deita em decúbito dorsal com o outro em cima); frequência e duração de pouncing (um animal toca o outro no pescoço com o focinho); duração de perseguição (seguir ou perseguir o outro animal) duração de cheirar ou lamber qualquer parte do corpo do outro (Olivier et al., 2011).

3.3.3. Avaliação neuroquímica

No DPN 22, animais foram guilhotinados, seus cérebros removidos e rapidamente dissecados sobre gelo. Córtex pré-frontal, hipocampo, corpo estriado e cerebelo foram isolados e congelados em nitrogênio líquido. Os tecidos foram pesados e armazenados em freezer a -80ºC até processamento posterior. Para tecidos destinados à quantificação de 2-AG e PAF, 1mL de MeOH puro foi adicionado aos tubos antes do armazenamento.

3.3.3.1. Cromatografia líquida acoplada a espectrometria de massa (LC-MS)

Córtex pré-frontal, hipocampo, estriado e cerebelo foram homogeneizados em MeOH, enriquecidos com padrões internos deuterados para 2-AG (2-AG-d5) e PAF (Lyso-PAFC-16-d4) (Cayman Chemical, EUA) e submetidos à extração lipídica com clorofórmio/MeOH/H2O (2:2:1,8), conforme descrito anteriormente (Bligh & Dyer, 1959). O conteúdo extraído foi seco, suspenso em 50 µl de MeOH e injetado no LC-MS. As fases móveis foram 0,1% ácido fórmico em (A) H2O ou (B) MeOH. A espectrometria de massa foi operada em modo positivo para análise de monitoramento de reação múltipla de alta resolução.

3.3.3.2. Extração de RNA, transcrição reversa e qPCR

O RNA total foi isolado do córtex pré-frontal, estriado e cerebelo por TRIzol (Invitrogen, EUA) e clorofórmio (2,5:1), de acordo com as instruções do fabricante. O cDNA foi sintetizado usando 2 µg de RNA seguindo as instruções do kit de transcrição reversa de cDNA de alta capacidade (Invitrogen, EUA). A quantificação foi realizada por PCR em tempo real (StepOne Plus, Applied Biosystems) usando matrizes TaqMan® para os seguintes genes de rato: Dagla (DAGL-α, Rn01454303_m1); Daglb (DAGL-β, Rn01453770_m1); Mgll (MAGL, Rn00593297_m1); Ptgs2 (COX-2, Rn01483828_m1); Il1b (IL-1β, Rn00580432_m1); e o gene de referência Gapdh (Rn01775763_g1). Testes estatísticos foram executados sobre valores de ΔCt (Ct do gene interesse – Ct de GAPDH) (Vogel-Ciernia et al., 2018) mas apresentados

em -ΔCt para facilitar a interpretação intuitiva da expressão gênica (valores menores refletem menor expressão e o oposto também é verdadeiro).

3.3.4. Análise estatística

Os dados foram inicialmente explorados quanto à normalidade (teste de Shapiro-Wilk) e homogeneidade de variâncias (teste de Levene). Caso os critérios fossem atingidos ($p > 0,01$) testes paramétricos foram realizados. Dados que não atingiram homogeneidade ou normalidade passaram por transformação aritmética e foram novamente testados. Os dados foram analisados por ANOVA de duas vias com sexo e exposição gestacional como fatores. Os tamanhos de efeito foram determinados por eta quadrado parcial (η_p^2) para todos os parâmetros avaliados. O d de Cohen foi calculado para parâmetros afetados pela exposição a fim de, sempre que possível, comparar os tamanhos de efeito com a literatura (por exemplo, efeitos de manipulações perinatais em variáveis dependentes semelhantes). Utilizou-se o IBM-SPSS Windows versão 26 para análise dos dados. As conclusões foram estabelecidas com $p \leq 0,05$.

4. RESULTADOS E DISCUSSÃO

De acordo com as normas do Programa de Pós-Graduação em Ciências da Saúde, a seção Resultados e Discussão serão apresentados em forma de artigos científicos, mantendo-se a formatação exigida pela revista à qual foi ou será submetida.

Artigo 1: *Gestational paracetamol exposure induces core behaviors of neurodevelopmental disorders in infant rats and modifies response to a cannabinoid agonist in females*

Submetido para a revista *Neurotoxicology and Teratology* (FI= 4,1). O guia para autores que descreve as regras para escrita do manuscrito pode ser encontrado em [https://www.elsevier.com/journals/neurotoxicology-and-teratology/0892-0362/guide-for-authors].

Artigo 2: *Paracetamol exposure alters markers of endocannabinoid signaling and increases PAF levels of infant rat's brain*

Será submetido para a revista *Food and Chemical Toxicology* (FI= 5,6). O guia para autores que descreve as regras para escrita do manuscrito pode ser encontrado em [https://www.elsevier.com/journals/food-and-chemical-toxicology/0278-6915/guide-for-authors].

ARTIGO 1

GESTATIONAL PARACETAMOL EXPOSURE INDUCES CORE BEHAVIORS OF NEURODEVELOPMENTAL DISORDERS IN INFANT RATS AND MODIFIES RESPONSE TO A CANNABINOID AGONIST IN FEMALES

Rodrigo Moreno Klein[a], Vanessa Nishikawa Motomura[b], Juliana Diosti Debiasi[b], Estefânia Gastaldello Moreira[a, b]*

[a] Graduation Program in Health Sciences, State University of Londrina, Londrina, PR, 86047-610, Brazil

[b] Department of Physiological Sciences, State University of Londrina, Londrina, PR, 86047-610, Brazil

*Corresponding author at:

Departamento de Ciências Fisiológicas

Centro de Ciências Biológicas

Universidade Estadual de Londrina

Rodovia PR 445, Km 380

Londrina – PR

Email adress: egmoreira@uel.br (E.G. Moreira)

ABSTRACT

Paracetamol (PAR) is an over-the-counter analgesic/antipyretic used by pregnant women worldwide. Epidemiological studies have been associating gestational PAR exposure with neurobehavioral alterations in the progeny resembling autism spectrum disorders and attention-deficit hyperactivity disorder symptoms. Endocannabinoid (eCB) dysfunction was previously hypothesized as one of the modes of action by which PAR may harm the developing nervous system. We aimed to evaluate possible effects of gestational exposure to PAR on male and female rat's offspring behavior and if an acute injection of WIN 55,212-2 (WIN, 0.3 mg/kg), a non-specific cannabinoid agonist, prior to behavioral tests, would induce different effects in PAR exposed and non-exposed animals. Pregnant Wistar rats were gavaged with PAR (350mg/kg/day) or water from gestational day 6 until delivery. Nest-seeking, open field, apomorphine-induced stereotypy, marble burying and three-chamber tests were conducted in 10-, 24-, 25- or 30-days-old rats, respectively. PAR exposure resulted in increased apomorphine-induced stereotyped behavior and time spent in the central area of the open field in exposed female pups. Additionally, it induced hyperactivity in the open field and increased marble burying behavior in both male and female pups. WIN injection modified the behavioral response in the nest seeking test, and opposite effects were observed in control and PAR-exposed neonate females. Reported alterations are relevant for the neurodevelopmental disorders that have been associated with maternal PAR exposure and suggest that eCB dysfunction may play a role in the action by which PAR may harm the developing brain.

Key words: Acetaminophen. Endocannabinoid system. Neurodevelopment. Neurodevelopmental disorders.

1.0 INTRODUCTION

Paracetamol (PAR, the recommended nonproprietary name of acetaminophen) is an over-the-counter analgesic and antipyretic and possibly the most commonly utilized medicine worldwide (Athersuch et al., 2018; McCrae et al., 2018). It is recommended for treating pain and fever in pregnant women (Chambers, 2015). Approximately 40-50% of pregnant women use PAR at least once during gestation worldwide (Bandoli et al., 2019; Rifas-Shiman et al., 2020; Sznajder et al., 2022) and part of them repeat the use for weeks during pregnancy (Bandoli et al., 2019). Considering PAR and some of its metabolites cross the placental barrier (Conings et al., 2019; Nitsche et al., 2017) there is fetal exposure as a result of maternal intake of this medicine.

There is great interest for the effects of *in utero* PAR exposure on the human developing central nervous system (CNS). Several epidemiological studies have been associating PAR maternal intake with neurobehavioral alterations in the progeny (e.g. (Avella-Garcia et al., 2016; Liew, Bach, et al., 2016; Tovo-Rodrigues et al., 2020). Meta-analyses of the epidemiological studies reported gestational exposure to PAR as a risk factor for autism spectrum disorders (ASD) (Alemany et al., 2021; Masarwa et al., 2018) and attention-deficit hyperactivity disorder (ADHD) symptoms (Alemany et al., 2021; Gou et al., 2019; Masarwa et al., 2018) in the progeny. Although these results are concerning, they should be interpreted with caution considering the known limitations of observational studies (Wood, 2020). For instance, a bias analysis suggests that previously reported meta-analysis association between gestational PAR exposure and ADHD diagnosis in offspring may be better explained by unmeasured confounding variables (Masarwa et al., 2020). Additional care must be taken when the studies measure outcomes with no clear definition, multifactorial etiology and assess associations without established biological mechanism, which is the case of association studies with gestational exposure to PAR (Laue et al., 2019; Masarwa et al., 2020). Animal studies can

investigate both causality and biological mechanisms and are necessary for the study of the potential effects of PAR on neurodevelopment (Zucker, 2017).

Potential modes of action by which PAR may harm neurodevelopment have been hypothesized, and include: excessive formation of its toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI); oxidative stress and immune-inflammatory activation; altered brain-derived neurotrophic factor; cyclooxygenase-2 (COX-2) inhibition; endocrine disruption; and endocannabinoid (eCB) system dysfunction (Bührer et al., 2021).

eCB system comprises its endogenous mediators, the eCBs, the enzymes responsible for the synthesis and degradation of eCBs and the cannabinoid receptors type 1 (CB1) and type 2 (CB2) (Lu & Mackie, 2021). The most well studied eCBs are N-arachidonoylethanolamide (anandamide, AEA) and 2-arachidonoylglycerol (2-AG), arachidonic acid-derived lipid mediators. CB1 are abundant in the cortex, basal ganglia, hippocampus and cerebellum (Cristino et al., 2006). CB2 are much less expressed in the CNS than the first type, and are more present in vascular element cells and microglia than neurons (Komorowska-Müller & Schmöle, 2021). The eCB system develops early in life and regulate multiple steps of CNS development, including cell proliferation, differentiation, migration, axonal guidance and neuronal survival (MacCarrone et al., 2014).

PAR may interact with the eCB system in several manners. First, it may inhibit the COX-2 oxygenation of AEA and 2-AG (Duggan et al., 2011). This process alters the structure of these eCBs and terminates their actions (Urquhart et al., 2015). By inhibiting this metabolic process, PAR may increase the availability of these mediators. Secondly, PAR can interact with the eCB system through its metabolite, N-arachidonoylphenolamine (AM404). This metabolite is the conjugation product from the deacetylated metabolite of PAR, p-aminophenol, with arachidonic acid by the enzyme fatty acid amide hydrolase (FAAH) in the CNS. There is evidence of *in vivo* conversion of this metabolite in the rat brain (Högestätt et al., 2005;

Muramatsu et al., 2016) and human cerebrospinal fluid (Sharma et al., 2017) after PAR administration. AM404 inhibit: an intracellular carrier that deliver AEA to FAAH leading to its hydrolysis (Kaczocha et al., 2012); the putative AEA membrane transporter (Beltramo et al., 1997); and COX-2 in activated microglia (Perazzo et al., 2017). All these actions may result in increased availability of eCBs. AM404 is also a potent vanilloid type-1 receptor (TRPV1) agonist (Zygmunt et al., 2000) and a weak CB1 agonist (Beltramo et al., 1997). TRPV1 and CB1 are co-localized receptors (Cristino et al., 2006) that often have opposite actions on cellular responses and behavior (Cristino et al., 2020; Moreira et al., 2012). Altogether these suggests PAR may interact with the developing eCB system through multiple mechanisms.

Therefore, the main objectives of this study were to evaluate in infant rats exposed to PAR during gestation: 1) possible effects of gestational exposure to PAR on rat´s offspring behavior and 2) the functionality of eCB system through investigating whether an acute injection of a non-specific cannabinoid agonist prior to behavioral tests would induce different effects in PAR exposed and non-exposed animals. The behavioral parameters included olfactory discrimination, locomotion and emotionality, drug-induced behavioral stereotypy, repetitive behavior and sociability.

2.0 MATERIAL AND METHODS

2.1 Animals, maternal exposure and perinatal procedures

Male and female naive Wistar rats, derived from the Central Vivarium of the State University of Londrina, were mated overnight (1 male and 2 females per cage), and gestational day 0 (GD 0) was determined through vaginal smear the next morning, by the presence of spermatozoa. On GD 6 females were individually allocated to separate cages. Animals were kept under controlled temperature (21 ± 2 ºC), 12h light/dark cycle (lights on at 6:00 AM) and with free access to tap water and rat chow (Nuvital™, Nuvilab CR1, Paraná, Brazil).

PAR (Tylenol®, Johnson & Johnson, Brazil) was diluted in tap water and daily administered, from GD 6 (to avoid possible effects on implantation) until delivery (post-natal day 0, or PND 0) by gavage in the dose of 350mg/kg (n = 56). Control group (CTL, n = 48) was gavaged with tap water. Dams were weighted every 3 days during gestation, starting at GD 6 and administration volume adjusted according to body weight. PAR dose represents a dosimetric adjustment of the dose of 500mg/day in humans (65 kg) (USEPA, 2006) with an added security factor of 10 considering intraspecies variability (Nielsen et al., 2008).

At PND 4 litters were culled to 10 pups, keeping 5 animals from each sex whenever possible. Litters smaller than 8 pups were discarded. Litter weight was registered on PND 0, 4, 7, 14 and 21, the day in which pups were weaned and separated by sex until behavioral evaluation or euthanasia. In the present study, litter was the experimental unit (i.e., same-sex siblings were not used for the same experimental group/procedure). To check the distribution of pups to each of the endpoints evaluated readers may refer to the Supplementary Table 1. Exceeding animals were utilized in other experiments or euthanized. All experimental protocols were approved by the State University of Londrina Ethics Committee for Animal Research (protocol nº 8522.2019.35).

2.2 WIN 55,212-2 injection and dosage

Thirty minutes prior to all behavioral tests one male and one female pup from each litter received an intraperitoneal (i.p.) injection of the non-specific cannabinoid receptors agonist WIN 55,212-2 (WIN, 0.3 mg/kg, Sigma, USA) whereas another couple from the same litter received a vehicle injection (VEH: 10% DMSO in saline). The WIN dosage was based on a study which described behavioral alterations in juvenile Wistar rats (Trezza & Vanderschuren, 2008).

2.3 *Behavioral tests*

All behavioral tests with exception of the marble burying test were recorded by a video camera, linked to a computer in an adjacent room. Videos were analyzed by a trained observer who was masked for the experimental conditions.

Males were always tested before the females in order to avoid the influence of possible sexual odor on the animal's behavior. Experiments were either conducted on the morning (8:00 AM – 12:00 PM) or the afternoon (13:00 PM – 18:00 PM) as described below.

2.3.1 Nest-seeking test (olfactory discrimination)

On the morning of the PND 10, the latency of neonates to reach nest bedding based on olfactory cues was evaluated as described by Schneider & Przewłocki (2005). Animals were individually placed in the center of a rectangular acrylic cage with dimensions (length/width/height in cm) 20 x 8 x 8 divided into 3 equal compartments delimited by permanent ink and evaluated for 3 min. One of the side compartments contained 10g of bedding from testing pup home cage, while on the opposite side there was 10g of fresh clean bedding. A positive entry was considered when forepaws and head crossed the line towards one of the compartments.

2.3.2 Open-field (motor activity and anxiety-like behavior)

On the afternoon of PND 24, animals were placed individually in a circular wooden arena (72 cm in diameter), divided into 12 equal quadrants, and left 5 min to freely explore. Rearing behavior (standing on hind legs) and the number of crossed quadrants were evaluated to estimate motor activity. A crossing was considered when the rat placed all four paws into another quadrant. Time spent in central quadrants of the arena and in rostral (self-grooming of nose and head region) and body grooming (trunk, tail and anogenital regions) were evaluated as measures of anxiety-like behavior (Estanislau et al., 2013). The apparatus was cleaned with a 5% ethanol solution and dried with paper towels between trials.

2.3.3 Apomorphine-induced stereotyped behavior

On the morning of PND 25, animals received a subcutaneous (s.c.) injection of apomorphine (0.3 mg/kg, Sigma, USA). Immediately after, they were individually placed in a transparent acrylic cage and stereotyped behavior was scored every 10 min (10 seconds of observation) for 120 min. Scores ranged from 0 (asleep or stationary) to 6 (continual licking and/or gnawing) as described by Setler et al. (Setler et al., 1976). The intensity of stereotypies was evaluated by cumulatively summing the 12 obtained scores.

2.3.4 Marble burying (repetitive behavior)

On the morning of PND 25, pups were individually placed in a polypropylene cage (47 x 27 x 15) covered with 5 cm of clean wood shavings and with 16 glass marbles distributed in 4 rows with 4 marbles each on shavings' surface. The animal was left to freely explore the apparatus for 30 min. High definitions photos of the testing cages were taken at the end of the session and the number of buried marbles (>50% marble covered by shavings) was counted by two independent and trained observers (Thomas et al., 2009).

2.3.5 Three-chamber test (sociability)

On the afternoon of PND 30, animals were submitted to the three-chamber test to evaluate social preference over a novel object. Each animal was placed in a central area of a wooden apparatus (120 x 40 x 40) divided into three chambers of equal proportions. Lateral chambers were initially closed by dividers, so the animal would be habituated only to the central area. Ten min later, an age- and sex-matched unfamiliar rat was placed in one of the side chambers, inside a plastic basket with holes (social area). On the opposite chamber, a similar empty basket was placed (novel object area). The dividers were then opened so the animal could explore all three chambers for 10 min. Time spent in each of the three areas was recorded. An entry began when the rat placed all four paws into one of the three chambers and finished only after placing them into another. The sociability index as follows [(time in the social area – time

in the novel object area) / (time in the social area + time in the novel object area)] * 100. This index allows the evaluation of social preference over new object. Time spent in sniffing near the basket containing the other animal was also counted. The apparatus was cleaned with a 70% ethanol solution and dried with paper towels between trials (Bambini-Junior et al., 2014).

2.4. Statistical analysis

Data were initially explored for normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test). Raw or transformed data reached the criteria ($p > 0.01$) for parametric tests and were analyzed by a factorial ANOVA with gestational exposure to PAR, sex and WIN injection as factors (2 x 2 x 2 model). In the occurrence of significant interactions among the three terms, data were split into sex and two-way ANOVAs were used to describe the significant differences. In the case of two-way interaction effects, interaction plots were created to interpret the results. Effect sizes were determined by partial eta squared (η_p^2) for all endpoints. Cohen's d was calculated for endpoints affected by exposure in order to compare effect sizes with relevant literature (e.g., effects of prenatal manipulations on similar dependent variables). While p values indicate the probability of differences to have occurred by random chance, effect sizes determine how large are standardized mean differences (e.g., Cohen's d) or how much a factor influence in variance (e.g., η_p^2). IBM-SPSS Windows version 26 was used to analyze the data and conclusions were established at $p \leq 0.05$.

3. RESULTS

3.1. General toxicity

From 113 litters, 9 were excluded (6 CON and 3 PAR) because they contained less than 8 pups. The 104 litters used for the present study contained 8-16 pups including stillborn, which occurred in 1 out of 48 CON litters (1 stillborn) and in 2 out of 56 PAR litters (1 stillborn in

each of the 2 affected litters). Student's t test indicated that the number of pups per litter did not differ between groups ($t_{(102)}$= 1.68, p= 0.096) and the means ± SD for the CON and PAR groups were, respectively 12.71 ± 1.56 and 12.18 ± 1.64 pups.

There was no treatment effect on dams' body weight profiles [$F_{(3.98, 374.09)}$= 1.90, p= 0.11] during the gestational and lactational periods (Fig. 1A) with PAR treatment explaining only 2% of data variance of dam's body weight (η_p^2= 0.02). On the other hand, RMANOVA indicated an effect of time on body weight [$F_{(3.98, 374.09)}$= 989.08, p< 0.001], reflecting the changes that are expected to occur in these periods. Time explains 91% of data variance (η_p^2= 0.913). There were 8 missing values (3 CON and 5 PAR) for GD21 because dams were at parturition at the moment of weighing. Considering RMANOVA cannot handle missing values at any timepoint, these subjects were not included in this statistical test. For pups' body weight in the first three weeks of life, RMANOVA also indicated a time effect [$F_{(1.63, 166.46)}$= 3600.71, p< 0.001] but PAR exposure did not affect it [$F_{(3.98, 374.09)}$= 8.49, p= 0.48] (Fig. 1B). Time explained 97% of variance in groups (η_p^2= 0.972) while PAR exposure accounted for less than 1% (η_p^2= 0.007).

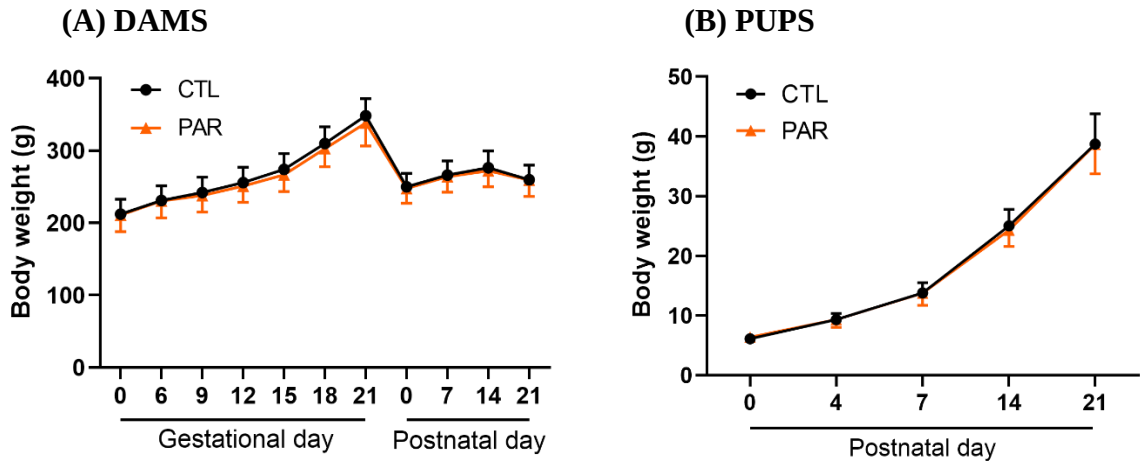

Fig. 1. Body weight of dams during gestation and lactation (A) and pups (average per litter) during lactation (B). Dams were treated (PAR) or not (CTL) with paracetamol from gestational day 6 to post-natal day 0. Data are means ± SD of 45-56 dams and litters in each group. RMANOVA, $p > 0.05$ for PAR treatment/exposure effect.

3.2.Behavioral tests

Data were graphed to reflect the statistically significant differences on three- or two-way ANOVA models. Means and 95% confidence intervals (CI) for all the analyzed parameters, including non-transformed values, are exhibited in Supplementary Table 2 and statistical values (F, df, p and η_p^2) from all the behavioral endpoints in Supplementary Table 3.

3.2.1. Nest-seeking test (olfactory discrimination)

None of the pups exhibited eye opening at the time of testing. Values of latency to reach nest bedding, in seconds, passed the criteria for parametric analysis only after transformation into natural logarithm (Ln), therefore Fig. 2 shows transformed values. Three-way ANOVA indicated an interaction effect among the three factors (exposure, sex and injection) over latency to reach nest bedding [$F_{(1;\,94)} = 5.593$, $p = 0.020$]. The split of the data into sex followed by two-way ANOVAs indicated that the exposure by injection interaction was not significant in males [$F_{(1;\,48)} = 1.001$, $p = 0.322$] but it was in females [$F_{(1;\,46)} = 5.641$, $p = 0.022$]. In females, exposure

by injection interaction explained 11% of data variance ($\eta_p^2 = 0.109$) and there was a difference of 0.96 standard deviations in latency values among groups (Cohen's d = 0.961). As shown in Fig. 2, there was a cross-over interaction between the factors in females. In VEH-injected females, PAR exposure increased latency to reach nest bedding in comparison to CTL group. However, WIN injection reversed the response profiles, reducing latency values for PAR-exposed females and increasing for CTL group.

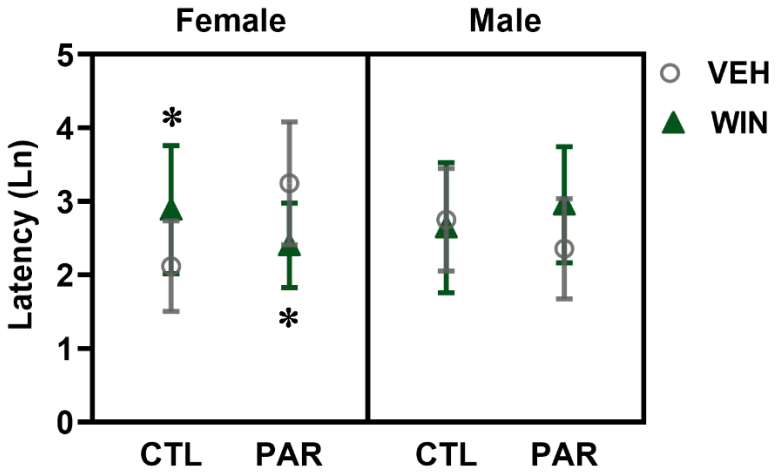

Fig. 2. Evaluation of latency to reach nest bedding by female and male rats exposed or not to PAR during gestation and injected with WIN or VEH 30 min prior to the nest-seeking test on PND 10. ✻ $p < 0.05$ exposure x injection interaction effect only in females in a two-way ANOVA. Data are means ± 95% CI of 11-14 animals per group. CTL: control; PAR: paracetamol; WIN: WIN 55,212-2; VEH: vehicle.

3.2.2 Open field (motor activity and anxiety-like behavior)

Independently of sex or injection, PAR-exposed rats crossed more quadrants (ambulation), as indicated by a significant exposure effect in the three-way ANOVA [$F_{(1; 96)} = 4.429$, $p = 0.038$] (Fig. 3A). Exposure factor accounted for 4% of ambulation data variance ($\eta_p^2 = 0.044$) and average activity of PAR-exposed animals is 0.43 standard deviations greater than

in CTL animals (Cohen's d = 0.429). Three-way ANOVA also indicated an interaction of exposure by sex on time spent in the central area [$F_{(1; 96)}$ = 6.301, p = 0.014]. The split of the data into sex followed by two-way ANOVAs indicated that the exposure effect was significant in females [$F_{(1; 52)}$ = 5.067, p = 0.029] but not in males [$F_{(1; 44)}$ = 1.845, p = 0.181] (Fig 3B). The exposure effect in females was associated with a 9% of data variability (η_p^2 = 0.089). Average time in central area was 0.62 standard deviations higher than in CTL females (Cohen's d = 0.625). Therefore, independently of WIN injection, PAR-exposed females spent more time in the central area of the open field. No other effects of exposure, sex, WIN injection or interaction among them were observed (Supplementary Table 2).

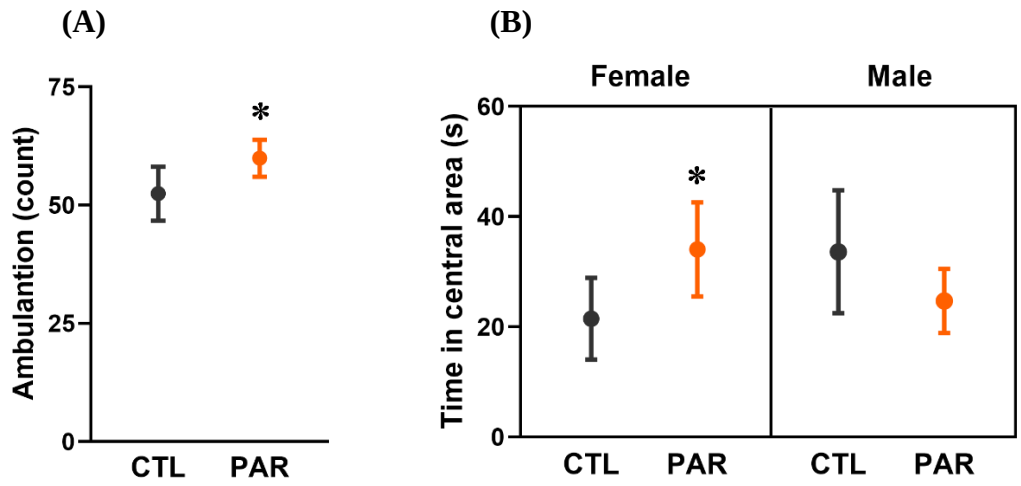

Fig. 3. Behavioral evaluation in the open field of rats exposed or not to PAR during gestation and injected with WIN or VEH 30 min prior to the test on PND 24. (A) Ambulation of 52 animals per group. ✻ p < 0.05 exposure effect in three-way ANOVA. (B) Time spent in central area of the arena of 22-30 animals per group. ✻ p < 0.05 exposure effect in two-way ANOVA. Data are means ± 95% CI. CTL: control group; PAR: paracetamol.

3.2.3. Apomorphine-induced stereotyped behavior

Three-way ANOVA indicated an interaction effect of exposure by sex on stereotypy intensity [$F_{(1;\ 124)}$ = 4.407, p = 0.038]. The split of the data into sex followed by two-way ANOVAs indicated that the exposure effect was significant only in females [$F_{(1;\ 66)}$ = 17.808, p = 0.000] but not in males [$F_{(1;\ 58)}$ = 0.618, p = 0.435] (Fig 4). Exposure factor in females accounted for 21% of data variability (η_p^2 = 0.212) and average stereotypy intensity of PAR-exposed females was 1.04 standard deviations greater than CTL females (Cohen's d = 1.037). In this way, independently of injection, PAR exposure resulted in increased stereotypy intensity in female pups.

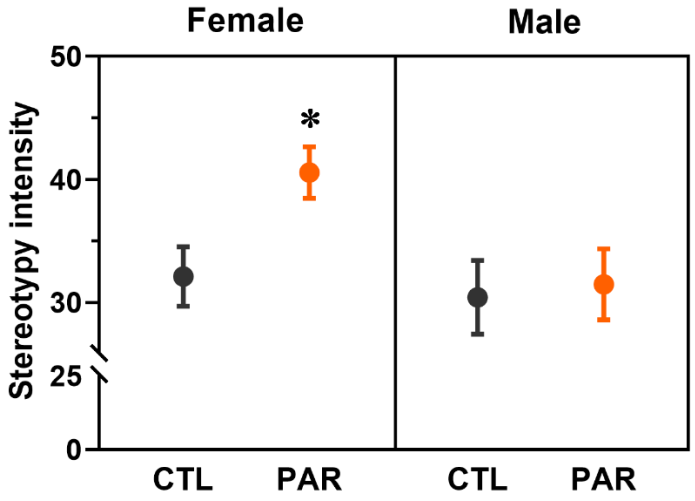

Fig. 4. Assessment of behavioral stereotypy induced by apomorphine at PDN 25 in female and male rats exposed or not to PAR during gestation and injected with WIN or VEH 30 min prior to apomorphine. ✻ p <0.05 exposure effect only in females in a two-way ANOVA. Data are means ± 95% CI of 15-19 animals per group. CTL: control, PAR: paracetamol.

3.2.4. Marble burying (spontaneous repetitive behavior)

The number of marbles buried passed the criteria for parametric analysis only after transformation into squared root. Independently of sex or injection, PAR-exposed pups buried

more marbles, as indicated by a significant exposure effect in the three-way ANOVA [$F_{(1, 118)}$ = 5.21, p = 0.024] (Fig. 5A). PAR exposure accounted for 4% of data variance (η_p^2 = 0.042). Average buried marbles by exposed animals were 0.42 standard deviations greater than by CTL animals (Cohen's d = 0.4188). Moreover, independently of exposure or injection, females buried more marbles than males, as indicated by a significant sex effect [$F_{(1,118)}$ = 5.07, p = 0.026] (Fig 5B). Similar to exposure, sex effect is also associated with 4% of variance (η_p^2 = 0.041).

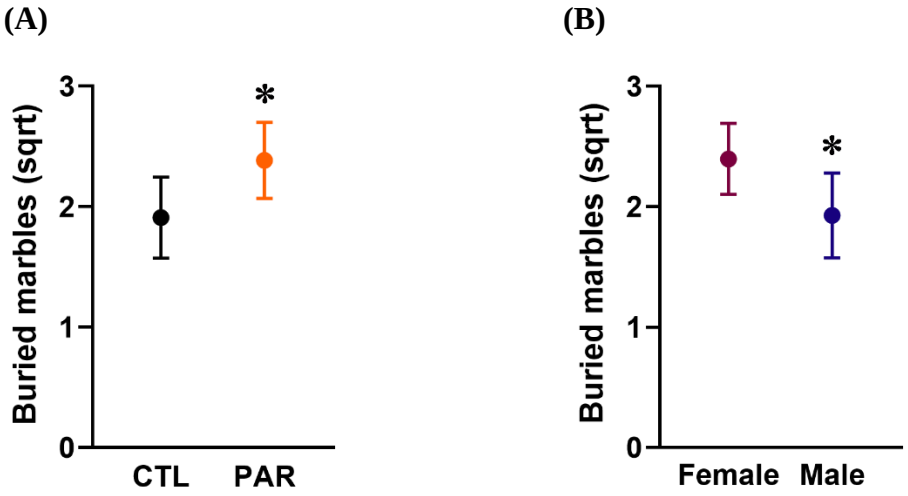

Fig. 5. Evaluation of marble burying in PDN 25 rats exposed or not to PAR during gestation and injected with WIN or VEH 30 min prior to test. (A) ✻ p < 0.05 exposure effect in three-way ANOVA (B) ✻ p < 0.05 sex effect in three-way ANOVA. Data are means ± 95% CI of 60-66 animals per group.

3.2.5. Three-chamber test (sociability)

No effects of exposure, sex, WIN injection or interaction term among them were observed in the parameters evaluated in this test. The results are displayed in Supplementary Table 2.

3. DISCUSSION

The major finding of our study was that PAR-exposure altered the response to a non-specific cannabinoid receptors agonist, WIN, in an olfactory discrimination task in female neonates. Additionally, PAR-exposed infant females presented increased apomorphine-induced stereotypies and spent more time in the central area of an open field. Exposed male and female infant rats presented hyperactivity in the open field and increased spontaneous repetitive behavior in the marble burying test, but normal sociability in the three-chamber test. Females seems to be more susceptible to PAR exposure than males.

Cannabinoid drugs administration induces dose- (Sañudo-Peña et al., 2000), sex- (Wiley et al., 2021) and age-dependent (Marco et al., 2004) behavioral alterations in rodents. Generally, CB1 agonists induce stimulating effects in low doses and sedative and cataleptic effects in higher doses (Sañudo-Peña et al., 2000). In peripubertal rats, WIN, a CB1/CB2 full agonist, induced: hyperlocomotion on the open field in the lower doses but no locomotor alterations in the highest (0.25, 1.25 and 2.5 mg/kg, i.p., male Wistar rats, PND 28-35) (Pandolfo et al., 2007); reduction in social play behaviors but no alterations in locomotion (0.3 mg/kg, i.p., male Wistar rats, PND 28-30) (Trezza & Vanderschuren, 2008); impaired object and social recognition, reduction in social interaction and increased spontaneous social behavior (1.2 mg/kg, i.p. male Wistar rats, PND 40) (Schneider et al., 2008); hypolocomotion and analgesia (3 and 10 mg/kg, s.c., male and female Wistar rats PND 20 and 25) (Marco et al., 2004); and catalepsy-like immobility (5 mg/kg, i.p., male Sprague-Dawley rats, PND 20) (Rudenko et al., 2012). We selected the 0.3 mg/kg because it selectively altered social play behavior without altering locomotion (Trezza & Vanderschuren, 2008). Considering that even lower doses than 0.3 induce behavioral alterations in prepubertal (Pandolfo et al., 2007) and adult rats (Polissidis et al., 2009) the dose selected in the present study seems appropriate to the objective of investigating possible PAR-exposure modulation of cannabinoid agonism effects.

Effect sizes are arguably the most important outcome of empirical studies. They can inform about the practical significance of the results (Ellis, 2010) and facilitate cumulative science (Lakens, 2013). Although there are several ways to asses effect sizes, its final interpretation is accomplished by comparison to relevant and compatible data and not according to arbitrary categorical values (Lakens, 2013). The comparison of effect sizes obtained in the present study with calculated effect sizes from other published developmental neurotoxicity studies demonstrates that effects observed in the nest-seeking test and in apomorphine-induced stereotypy are in the range of other studies (Supplementary Table 4). Other endpoints presented effect sizes bellow the range based in other three publications. However, important part of the studies do not follow general indications for conducting DNT studies (e.g. controlling for litter effects), which may inflate effect sizes (Golub & Sobin, 2020). Still, the subtle effects observed in our study are very relevant considering the high frequency of prenatal PAR use and the existence of epidemiological data suggesting its neurodevelopmental toxicity. The implications of PAR to have subtle neurotoxic effects in brain development is that experimental studies require larger number of subjects to detect these effects and it may take longer for scientists to have a clear picture of its possible impacts on society.

Sex is known to modulate neurodevelopment and how toxicants affect it. In the present study, females were specially affected by gestational exposure to PAR. Association studies do not clearly indicate if prenatal PAR exposure differently affects girls and boys (Alemany et al., 2021), with contradictory reports regarding sex-dependent effects (Golding et al., 2020; Liew et al., 2014). Unfortunately, the few experimental studies investigating the behavioral effects of PAR exposure during neurodevelopment majorly utilized male animals only (e.g., Blecharz-Klin et al., 2018; Philippot et al., 2018; Viberg et al., 2014), making generalizations difficult. However, Philippot et al. (2017) demonstrated that neonatal PAR injections similarly affected habituation of adult males and females (Philippot et al., 2017). One possible explanation for the

increased vulnerability of females is that, in comparison to males, they express more CYP2E1 in different tissues since the fetal period (Penaloza et al., 2020), which could increase PAR oxidation/NAPQI synthesis resulting in oxidative damage.

The nest-seeking (or homing behavior) test evaluates the capability of pups to discriminate between fresh and home bedding trough olfactory cues (Gregory & Pfaff, 1971). Interestingly, this was the only behavior influenced by WIN injection, inducing opposite effects in CTL and PAR-exposed female neonates. Similarly, the injection of a FAAH inhibitor previous to the nest-seeking test attenuated the increased latency induced by gestational exposure to valproic acid in rats, an established animal model of autism, but did not affect the behavior of CTL rats (Servadio et al., 2016). Both results could be attributable to a direct/indirect activation of CB1 and CB2 in olfactory structures, especially expressed in the olfactory bulb and piriform cortex, and which seem to play a role in olfactory processing (Terral et al., 2020). Given the higher efficiency of CB1 receptor in females, they are more sensitive than males to most effects of cannabinoids (Sholler et al., 2021). This could explain why females but not males were affected by WIN injection in this task. Importantly, this was the earliest behavioral endpoint evaluated in pups (at PND10), which suggest age-dependent alterations in the endocannabinoid system functionality as a consequence of PAR exposure in females.

Apomorphine induces stereotypies through the activation of dopaminergic receptors mainly in the substantia nigra-striatum pathway (Ernst & Smelik, 1966). Quantification of behavioral responses to apomorphine has been used in different species as an indicative of dopaminergic system functionality and to model movement disorders involving behavioral stereotypies (Langen et al., 2011). In this way, the increased apomorphine-induced behavioral stereotypies in females exposed to PAR indicates enhanced dopaminergic signaling. Due to its

reproducibility across studies conducted in our laboratory (Klein et al., 2020; Rigobello et al., 2021) this result may be a behavioral footprint of maternal PAR exposure in infant rats.

Burying of non-aversive objects, such as glass marbles, is a naturally occurring behaviors in rodents. There is an intense and yet inconclusive debate about the validity of the marble burying test for investigating either anxiety or compulsive symptoms (de Brouwer et al., 2019; Dixit et al., 2020). In the anxiety perspective, the higher the number of marbles buried, the higher the anxiety. For the compulsive perspective, increased burying indicates increased repetitive digging. Considering PAR-exposed females spent more time in the central area of the open field, which is indicative of a decreased anxiety state (Prut & Belzung, 2003), altered emotionality is less likely to explain the result observed in marble burying test. Increased marble burying in PAR-exposed male and female infant rats is in alignment with the enhanced stereotypy in response to apomorphine in PAR-exposed females, which point to a phenotype of repetitive and stereotyped behaviors. Considering stereotyped/repetitive behavior are one of the core symptoms of ASD (American Psychiatric Association, 2014), this may be relevant for the previously described risk for ASD as a consequence of gestational PAR exposure.

PAR during gestation induced hyperactivity in infant male and female rats. Previous research reported altered locomotion as a result of neurodevelopmental PAR exposure (Blecharz-Klin et al., 2017; Klein et al., 2020; Philippot et al., 2017, 2018; Rigobello et al., 2021; Viberg et al., 2014), although there is also a report of lack of effect (Saad et al., 2016). It is noteworthy that hyperactivity is the most consistently observed outcome in human association studies considering gestational PAR exposure as a factor (Bauer et al., 2018).

Some behavioral alterations induced by gestational PAR exposure observed in this study may share some neurobiological similarities (e.g., stereotyped and repetitive behavior and hyperactivity). Albeit various brain regions govern repetitive behavior, basal ganglia seem to play a central role in regulating these (Kim et al., 2016; Wilkes & Lewis, 2018). The basal

ganglia, which comprises the striatum, globus pallidus, subthalamic nucleus and substantia nigra, could also be involved in the pathophysiology of other movement disorders, such as hyperactivity (Spagnolo et al., 2021). Dopamine is a major neurotransmitter in basal ganglia neuronal synapses (Vicente et al., 2020) and altered dopaminergic neurotransmission was reported as a consequence of developmental exposure to PAR (Blecharz-Klin et al., 2017). These points to basal ganglia (striatal) dopaminergic system as an especially vulnerable target of the potential developmental neurotoxic effects of PAR.

Complementarily to our study, Philippot et al. (2018) investigated the effects of co-injection of PAR (2 doses of 30 mg/kg, s.c.) and WIN (1 mg/kg, s.c.) in 10-day-old mice. This protocol resulted in reduced locomotion and habituation capability in adult mice, which may be at least partially attributable to reduced transcript levels of synaptophysin and TrkB in hippocampus and FAAH in frontal cortex (Philippot et al., 2018). The objective of Philippot`s study (to investigate the developmental neurotoxicity of combined PAR and WIN administration) and ours (to investigate the mechanisms involved in the behavioral alterations induced by PAR) were different but taken together, both studies support a role for eCB system dysfunction in the potential mode by which PAR may harm neurodevelopment and induce behavioral alterations.

Summing up, our study demonstrates altered response to a CB1/CB2 agonist in neonates, enhanced induced-stereotypy and reduced anxiety state in females but not males gestationally exposed to PAR. Exposed males and females presented hyperactivity and increased repetitive behavior. Observed behavioral alterations are similar to some of the core symptoms of ASD (stereotyped/repetitive behaviors) and ADHD (hyperactivity), and this may suggest a role for gestational PAR exposure in increasing risk for these neurodevelopmental disorders. Data point to increased susceptibility of females for the neurotoxic effects of PAR during development and to eCB dysfunction as a mode of action by which PAR may harm the developing brain.

Conflict of interest statement

Nothing declared.

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REFERENCES

Aagaard, S. K., Larsen, A., Andreasen, M. F., Lesnikova, I., Telving, R., Vestergaard, A. L., Tørring, N., Uldbjerg, N., & Bor, P. (2018). Prevalence of xenobiotic substances in first-trimester blood samples from Danish pregnant women: A cross-sectional study. *BMJ Open*, *8*(3), 1–7.

Aguiar, D. C., Moreira, F. A., Terzian, A. L., Fogaça, M. V., Lisboa, S. F., Wotjak, C. T., & Guimaraes, F. S. (2014). Modulation of defensive behavior by Transient Receptor Potential Vanilloid Type-1 (TRPV1) channels. *Neuroscience and Biobehavioral Reviews*, *46*(P3), 418–428.

Alemany, S., Avella-García, C., Liew, Z., García-Esteban, R., Inoue, K., Cadman, T., … Sunyer, J. (2021). Prenatal and postnatal exposure to acetaminophen in relation to autism spectrum and attention-deficit and hyperactivity symptoms in childhood: meta-analysis in six european population-based cohorts. *European Journal of Epidemiology*, (0123456789).

American Psychiatric Association. (2014). *Diagnostic and Statistical Manual of Mental Disorders* (5th ed.). Washington DC: American Psychiatric Association.

Athersuch, T. J., Antoine, D. J., Boobis, A. R., Coen, M., Daly, A. K., Possamai, L., Nicholson, J. K., & Wilson, I. D. (2018). Paracetamol metabolism, hepatotoxicity, biomarkers and therapeutic interventions: A perspective. *Toxicology Research*, *7*(3), 347–357.

Avella-Garcia, C. B., Julvez, J., Fortuny, J., Rebordosa, C., García-Esteban, R., Galán, I. R., Tardón, A., Rodríguez-Bernal, C. L., Iñiguez, C., Andiarena, A., Santa-Marina, L., &

Sunyer, J. (2016). Acetaminophen use in pregnancy and neurodevelopment: attention function and autism spectrum symptoms. *International Journal of Epidemiology*, *45*(6), 1987–1996.

Baker, B. H., Rafikian, E. E., Hamblin, P. B., Strait, M. D., Yang, M., & Pearson, B. L. (2023). Sex-specific neurobehavioral and prefrontal cortex gene expression alterations following developmental acetaminophen exposure in mice. *Neurobiology of Disease*, *177*(December 2022), 105970.

Bambini-Junior, V., Zanatta, G., Della Flora Nunes, G., Mueller de Melo, G., Michels, M., Fontes-Dutra, M., Nogueira Freire, V., Riesgo, R., & Gottfried, C. (2014). Resveratrol prevents social deficits in animal model of autism induced by valproic acid. *Neuroscience Letters*, *583*, 176–181.

Bandoli, G., Palmsten, K., & Chambers, C. (2019). Acetaminophen use in pregnancy: examining prevalence, timing, and indication of use in a prospective birth cohort. *Paediatric and Perinatal Epidemiology*, (August), 1–10.

Bauer, A. Z., Kriebel, D., Herbert, M. R., Bornehag, C. G., & Swan, S. H. (2018). Prenatal paracetamol exposure and child neurodevelopment: A review. *Hormones and Behavior*, *101*, 125–147.

Bauer, A. Z., Swan, S. H., Kriebel, D., Liew, Z., Taylor, H. S., Bornehag, C.-G., Andrade, A. M., Olsen, J., Jensen, R. H., Mitchell, R. T., Skakkebaek, N. E., Jégou, B., & Kristensen, D. M. (2021). Paracetamol use during pregnancy — a call for precautionary action. *Nature Reviews Endocrinology*, *0123456789*.

Beltramo, M., Stella, N., Calignano, A., Lin, S. Y., Makriyannis, A., & Piomelli, D. (1997). Functional role of high-affinity anandamide transport, as revealed by selective inhibition. *Science*, *277*(August), 1094–1097.

Berdyshev, E. V., Schmid, P. C., Krebsbach, R. J., & Schmid, H. H. O. (2001). Activation of PAF receptors results in enhanced synthesis of 2-arachidonoylglycerol (2-AG) in immune cells. *The FASEB Journal*, *15*(12), 2171–2178.

Bertoldi, A. D., Rifas-Shiman, S. L., Boing, A. C., da Silva Dal Pizzol, T., Miranda, V. I. A., Silveira, M. P. T., Freitas Silveira, M., Domingues, M. R., Santos, I. S., Bassani, D. G., Tovo-Rodrigues, L., & Oken, E. (2020). Associations of acetaminophen use during pregnancy and the first year of life with neurodevelopment in early childhood. *Paediatric*

and Perinatal Epidemiology, *34*(3), 267–277.

Bevins, R. A., & Besheer, J. (2006). Object recognition in rats and mice: A one-trial non-matching-to-sample learning task to study "recognition memory." *Nature Protocols*, *1*(3), 1306–1311.

Bitanihirwe, B. K., Peleg-Raibstein, D., Mouttet, F., Feldon, J., & Meyer, U. (2010). Late prenatal immune activation in mice leads to behavioral and neurochemical abnormalities relevant to the negative symptoms of schizophrenia. *Neuropsychopharmacology*, *35*(12), 2462–2478.

Blecharz-Klin, K., Joniec-Maciejak, I., Jawna-Zboinska, K., Pyrzanowska, J., Piechal, A., Wawer, A., & Widy-Tyszkiewicz, E. (2016). Cerebellar level of neurotransmitters in rats exposed to paracetamol during development. *Pharmacological Reports*, *68*(6), 1159–1164.

Blecharz-Klin, K., Joniec-Maciejak, I., Jawna, K., Pyrzanowska, J., Piechal, A., Wawer, A., & Widy-Tyszkiewicz, E. (2015a). Developmental exposure to paracetamol causes biochemical alterations in medulla oblongata. *Environmental Toxicology and Pharmacology*, *40*, 369–374.

Blecharz-Klin, K., Joniec-Maciejak, I., Jawna, K., Pyrzanowska, J., Piechal, A., Wawer, A., & Widy-Tyszkiewicz, E. (2015b). Effect of prenatal and early life paracetamol exposure on the level of neurotransmitters in rats - focus on the spinal cord. *International Journal of Developmental Neuroscience*, *47*, 133–139.

Blecharz-Klin, K., Piechal, A., Jawna-Zboińska, K., Pyrzanowska, J., Wawer, A., Joniec-Maciejak, I., & Widy-Tyszkiewicz, E. (2017). Paracetamol − effect of early exposure on neurotransmission, spatial memory and motor performance in rats. *Behavioural Brain Research*, *323*, 162–171.

Blecharz-Klin, K., Wawer, A., Jawna-zboi, K., Pyrzanowska, J., Piechal, A., Mirowska-guzel, D., & Widy-tyszkiewicz, E. (2018). Early paracetamol exposure decreases brain-derived neurotrophic factor (BDNF) in striatum and affects social behaviour and exploration in rats. *Pharmacology, Biochemistry and Behavior*, *168*, 25–32.

Blecharz-Klin, K., Wawer, A., Pyrzanowska, J., Piechal, A., Jawna-Zboińska, K., & Widy-Tyszkiewicz, E. (2019). Hypothalamus – response to early paracetamol exposure in male rats offspring. *International Journal of Developmental Neuroscience*, *76*(January), 1–5.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*(8), 911–917.

Brandlistuen, R. E., Ystrom, E., Nulman, I., Koren, G., & Nordeng, H. (2013). Prenatal paracetamol exposure and child neurodevelopment: A sibling-controlled cohort study. *International Journal of Epidemiology*, *42*(6), 1702–1713.

Briones, M. R. S., Snyder, A. M., Ferreira, R. C., Neely, E. B., Connor, J. R., & Broach, J. R. (2018). A possible role for platelet-activating factor receptor in amyotrophic lateral sclerosis treatment. *Frontiers in Neurology*, *9*(FEB), 5–9.

Bührer, C., Endesfelder, S., Scheuer, T., & Schmitz, T. (2021). Paracetamol (acetaminophen) and the developing brain. *International Journal of Molecular Sciences*, *22*(20), 11156.

Cendejas-Hernandez, J., Sarafian, J. T., Lawton, V. G., Palkar, A., Anderson, L. G., Larivière, V., & Parker, W. (2022). Paracetamol (acetaminophen) use in infants and children was never shown to be safe for neurodevelopment: a systematic review with citation tracking. *European Journal of Pediatrics*, *181*(5), 1835–1857.

Chambers, C. (2015). Over-the-counter medications: risk and safety in pregnancy. *Seminars in Perinatology*, *39*(7), 541–544.

Conings, S., Tseke, F., Broeck, A. Van Den, Qi, B., Paulus, J., Amant, F., Annaert, P., & Calsteren, K. Van. (2019). Transplacental transport of paracetamol and its phase II metabolites using the ex vivo placenta perfusion model. *Toxicology and Applied Pharmacology*, *370*(November 2018), 14–23.

Cristino, Bisogno, T., & Di Marzo, V. (2020). Cannabinoids and the expanded endocannabinoid system in neurological disorders. *Nature Reviews Neurology*, *16*(1), 9–29.

Cristino, L., de Petrocellis, L., Pryce, G., Baker, D., Guglielmotti, V., & Di Marzo, V. (2006). Immunohistochemical localization of cannabinoid type 1 and vanilloid transient receptor potential vanilloid type 1 receptors in the mouse brain. *Neuroscience*, *139*(4), 1405–1415.

de Brouwer, G., Fick, A., Harvey, B. H., & Wolmarans, D. W. (2019). A critical inquiry into marble-burying as a preclinical screening paradigm of relevance for anxiety and obsessive–compulsive disorder: Mapping the way forward. *Cognitive, Affective and Behavioral Neuroscience*, *19*(1), 1–39.

Dixit, P. V., Sahu, R., & Mishra, D. K. (2020). Marble-burying behavior test as a murine model

of compulsive-like behavior. *Journal of Pharmacological and Toxicological Methods*, *102*(August 2019), 106676.

Duggan, K. C., Hermanson, D. J., Musee, J., Prusakiewicz, J. J., Scheib, J. L., Carter, B. D., Banerjee, S., Oates, J. A., & Marnett, L. J. (2011). (R)-Profens are substrate-selective inhibitors of endocannabinoid oxygenation by COX-2. *Nature Chemical Biology*, *7*(11), 803–809.

Ellis, P. D. (2010). *The essential guide to effect sizes*. Cambridge: Cambridge University Press. Retrieved from https://www.ptonline.com/articles/how-to-get-better-mfi-results

Ernst, A. M., & Smelik, P. G. (1966). Site of action of dopamine and apomorphine on compulsive gnawing behaviour in rats. *Experientia*, *22*(12), 837–838.

Estanislau, C., Díaz-Morán, S., Cañete, T., Blázquez, G., Tobeña, a., & Fernández-Teruel, a. (2013). Context-dependent differences in grooming behavior among the NIH heterogeneous stock and the Roman high- and low-avoidance rats. *Neuroscience Research*, *77*(4), 187–201.

Fagundes, B. H. F., Nascimento, P. C., Aragão, W. A. B., Chemelo, V. S., Bittencourt, L. O., Eiró-Quirino, L., Silva, M. C. F., Freire, M. A. M., Fernandes, L. M. P., Maia, C. do S. F., Crespo-Lopez, M. E., & Lima, R. R. (2022). Methylmercury exposure during prenatal and postnatal neurodevelopment promotes oxidative stress associated with motor and cognitive damages in rats: an environmental-experimental toxicology study. *Toxicology Reports*, *9*, 563–574.

Favaro, P. d N., Costa, L. C., & Moreira, E. G. (2008). Maternal fluoxetine treatment decreases behavioral response to dopaminergic drugs in female pups. *Neurotoxicology and Teratology*, *30*(6), 487–494.

FDA. (2016). FDA Drug Safety Communication: FDA has reviewed possible risks of pain medicine use during pregnancy. Retrieved from http://www.fda.gov/Drugs/DrugSafety/ucm429117.htm%5Cnhttp://www.medscape.com/viewarticle/837858?src=wnl_edit_newsal

Foley, K. A., MacFabe, D. F., Vaz, A., Ossenkopp, K. P., & Kavaliers, M. (2014). Sexually dimorphic effects of prenatal exposure to propionic acid and lipopolysaccharide on social behavior in neonatal, adolescent, and adult rats: Implications for autism spectrum disorders. *International Journal of Developmental Neuroscience*, *39*(C), 68–78.

Golding, J., Gregory, S., Clark, R., Ellis, G., Iles-Caven, Y., & Northstone, K. (2020). Associations between paracetamol (acetaminophen) intake between 18 and 32 weeks gestation and neurocognitive outcomes in the child: A longitudinal cohort study. *Paediatric and Perinatal Epidemiology*, *34*(3), 257–266.

Golub, M. S., & Sobin, C. A. (2020). Statistical modeling with litter as a random effect in mixed models to manage "intralitter likeness." *Neurotoxicology and Teratology*, *77*, 106841.

Gonsiorek, W., Lunn, C., Fan, X., Narula, S., Lundell, D., & Hipkin, R. W. (2000). Endocannabinoid 2-arachidonyl glycerol is a full agonist through human type 2 cannabinoid receptor: Antagonism by anandamide. *Molecular Pharmacology*, *57*(5), 1045–1050.

Gou, X., Wang, Y., Tang, Y., Qu, Y., Tang, J., Shi, J., Xiao, D., & Mu, D. (2019). Association of maternal prenatal acetaminophen use with the risk of attention deficit/hyperactivity disorder in offspring: A meta-analysis. *Australian and New Zealand Journal of Psychiatry*, *53*(3), 195–206.

Gregory, E. H., & Pfaff, D. W. (1971). Development of olfactory-guided behavior in infant rats. *Physiology & Behavior*, *6*, 573–576.

Haas, D. M., Marsh, D. J., Dang, D. T., Parker, C. B., Wing, D. A., Simhan, H. N., … Reddy, U. M. (2018). Prescription and other medication use in pregnancy. *Obstetrics and Gynecology*, *131*(5), 789–798.

Harkany, T., & Cinquina, V. (2021). Physiological rules of endocannabinoid action during fetal and neonatal brain development. *Cannabis and Cannabinoid Research*, *6*(5), 381–388.

Harshaw, C., & Warner, A. G. (2022). Interleukin-1β-induced inflammation and acetaminophen during infancy: Distinct and interactive effects on social-emotional and repetitive behavior in C57BL/6J mice. *Pharmacology Biochemistry and Behavior*, *220*(September).

Hershkowitz, M., & Adunsky, A. (1996). Binding of platelet-activating factor to platelets of Alzheimer's disease and multiinfarct dementia patients. *Neurobiology of Aging*, *17*(6), 865–868.

Heyer, D. B., & Meredith, R. M. (2017). Environmental toxicology: Sensitive periods of development and neurodevelopmental disorders. *NeuroToxicology*, *58*, 23–41.

Högestätt, E. D., Jönsson, B. A. G., Ermund, A., Andersson, D. A., Björk, H., Alexander, J. P., Cravatt, B. F., Basbaum, A. I., & Zygmunt, P. M. (2005). Conversion of acetaminophen to the bioactive N-acylphenolamine AM404 via fatty acid amide hydrolase-dependent arachidonic acid conjugation in the nervous system. *The Journal of Biological Chemistry*, *280*(36), 31405–31412.

Hoshijima, H., Hunt, M., Nagasaka, H., & Yaksh, T. (2021). Systematic review of systemic and neuraxial effects of acetaminophen in preclinical models of nociceptive processing. *Journal of Pain Research, Volume 14*, 3521–3552.

Hsu, K.-L., Tsuboi, K., Adibekian, A., Pugh, H., Masuda, K., & Cravatt, B. F. (2012). DAGLβ inhibition perturbs a lipid network involved in macrophage inflammatory responses. *Nature Chemical Biology*, *8*(12), 999–1007.

Hussain, F. N., & Ashmead, G. G. (2017). The safety of over-the-counter medications in pregnancy. *Topics in Obstetrics & Gynecology*, *37*(14), 1–8.

Ishihara, Y., Honda, T., Ishihara, N., Namba, K., Taketoshi, M., Tominaga, Y., Tsuji, M., Vogel, C. F. A., Yamazaki, T., Itoh, K., & Tominaga, T. (2022). A CCR5 antagonist, maraviroc, alleviates neural circuit dysfunction and behavioral disorders induced by prenatal valproate exposure. *Journal of Neuroinflammation*, *19*(1), 195.

Jetten, M. J. A., Gaj, S., Ruiz-Aracama, A., de Kok, T. M., van Delft, J. H. M., Lommen, A., van Someren, E. P., Jennen, D. G. J., Claessen, S. M., Peijnenburg, A. A. C. M., Stierum, R. H., & Kleinjans, J. C. S. (2012). 'Omics analysis of low dose acetaminophen intake demonstrates novel response pathways in humans. *Toxicology and Applied Pharmacology*, *259*(3), 320–328.

Kaczocha, M., Vivieca, S., Sun, J., Glaser, S. T., & Deutsch, D. G. (2012). Fatty acid-binding proteins transport N-acylethanolamines to nuclear receptors and are targets of endocannabinoid transport inhibitors. *Journal of Biological Chemistry*, *287*(5), 3415–3424.

Kim, H., Lim, C. S., & Kaang, B. K. (2016). Neuronal mechanisms and circuits underlying repetitive behaviors in mouse models of autism spectrum disorder. *Behavioral and Brain Functions*, *12*(1), 1–13.

Kim, S.-A., Chai, J.-H., & Jang, E.-H. (2021). Prenatal trimethyltin exposure induces long-term DNA methylation changes in the male mouse hippocampus. *International Journal of*

Molecular Sciences, *22*(15), 8009.

Klein, R. M., Rigobello, C., Vidigal, C. B., Moura, K. F., Barbosa, D. S., Gerardin, D. C. C., Ceravolo, G. S., & Moreira, E. G. (2020). Gestational exposure to paracetamol in rats induces neurofunctional alterations in the progeny. *Neurotoxicology and Teratology*, *77*(September 2019), 106838.

Koehn, L. M., Huang, Y., Habgood, M. D., Kysenius, K., Crouch, P. J., Dziegielewska, K. M., & Saunders, N. R. (2020). Effects of paracetamol (acetaminophen) on gene expression and permeability properties of the rat placenta and fetal brain. *F1000Research*, *9*.

Komorowska-Müller, J. A., & Schmöle, A. C. (2021). CB2 receptor in microglia: The guardian of self-control. *International Journal of Molecular Sciences*, *22*(1), 1–27.

Lakens, D. (2013). Calculating and reporting effect sizes to facilitate cumulative science: A practical primer for t-tests and ANOVAs. *Frontiers in Psychology*, *4*(NOV), 1–12.

Langen, M., Durston, S., Kas, M. J. H., van Engeland, H., & Staal, W. G. (2011). The neurobiology of repetitive behavior: of mice... *Neuroscience and Biobehavioral Reviews*, *35*(3), 356–365.

Laue, H. E., Cassoulet, R., Abdelouahab, N., Serme-Gbedo, Y. K., Desautels, A. S., Brennan, K. J. M., Bellenger, J. P., Burris, H. H., Coull, B. A., Weisskopf, M. G., Takser, L., & Baccarelli, A. A. (2019). Association between meconium acetaminophen and childhood neurocognitive development in GESTE, a canadian cohort study. *Toxicological Sciences : An Official Journal of the Society of Toxicology*, *167*(1), 138–144.

Leppert, B., Havdahl, A., Riglin, L., Jones, H. J., Zheng, J., Davey Smith, G., Tilling, K., Thapar, A., Reichborn-Kjennerud, T., & Stergiakouli, E. (2019). Association of maternal neurodevelopmental risk alleles with early-life exposures. *JAMA Psychiatry*.

Levine, A., Liktor-Busa, E., Lipinski, A. A., Couture, S., Balasubramanian, S., Aicher, S. A., Langlais, P. R., Vanderah, T. W., & Largent-Milnes, T. M. (2021). Sex differences in the expression of the endocannabinoid system within V1M cortex and PAG of Sprague Dawley rats. *Biology of Sex Differences*, *12*(1), 60.

Liew, Z., Bach, C. C., Asarnow, R. F., Ritz, B., & Olsen, J. (2016). Paracetamol use during pregnancy and attention and executive function in offspring at age 5 years. *International Journal of Epidemiology*, *45*(6), 2009–2017.

Liew, Z., Ritz, B., Rebordosa, C., Lee, P.-C., & Olsen, J. (2014). Acetaminophen use during pregnancy, behavioral problems, and hyperkinetic disorders. *JAMA Pediatrics*, *168*(4), 313–320.

Liew, Z., Ritz, B., Virk, J., & Olsen, J. (2016). Maternal use of acetaminophen during pregnancy and risk of autism spectrum disorders in childhood: a Danish national birth cohort study. *Autism Research*, *9*(9), 951–958.

Liu, J., Bátkai, S., Pacher, P., Harvey-White, J., Wagner, J. A., Cravatt, B. F., Gao, B., & Kunos, G. (2003). Lipopolysaccharide induces anandamide synthesis in macrophages via CD14/MAPK/phosphoinositide 3-kinase/NF-κB independently of platelet-activating factor. *Journal of Biological Chemistry*, *278*(45), 45034–45039.

Liu, Y., Shields, L. B. E., Gao, Z., Wang, Y., Zhang, Y. P., Chu, T., Zhu, Q., Shields, C. B., & Cai, J. (2017). Current understanding of platelet-activating factor signaling in central nervous system diseases. *Molecular Neurobiology*, *54*(7), 5563–5572.

Lordan, R., Tsoupras, A., Zabetakis, I., & Demopoulos, C. A. (2019). Forty years since the structural elucidation of platelet-activating factor (PAF): Historical, current, and future research perspectives. *Molecules*, *24*(23).

Lu, H. C., & Mackie, K. (2021). Review of the endocannabinoid system. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*, *6*(6), 607–615.

Lye, J. M., Knight, J. A., Arneja, J., Seeto, R. A., Wong, J., Adel khani, N., Brooks, J. D., Levitan, R. D., Matthews, S. G., Lye, S. J., & Hung, R. J. (2022). Maternal acetaminophen use and cognitive development at 4 years: the Ontario Birth Study. *Pediatric Research*, (June 2021), 1–5.

MacCarrone, M., Guzmán, M., MacKie, K., Doherty, P., & Harkany, T. (2014). Programming of neural cells by (endo)cannabinoids: From physiological rules to emerging therapies. *Nature Reviews Neuroscience*, *15*(12), 786–801.

Marco, E. M., Viveros, M.-P., Borcel, E., Pérez-Alvarez, L., Guaza, C., Ramirez, B. G., de Ceballos, M. L., Rubio, M., & Fernández, B. (2004). Functional responses to the cannabinoid agonist WIN 55,212-2 in neonatal rats of both genders: influence of weaning. *Pharmacology Biochemistry and Behavior*, *78*(3), 593–602.

Masarwa, R., Levine, H., Gorelik, E., Reif, S., Perlman, A., & Matok, I. (2018). Prenatal

exposure to acetaminophen and risk for attention deficit hyperactivity disorder and autistic spectrum disorder: A systematic review,meta-analysis, and meta-regression analysis of cohort studies. *American Journal of Epidemiology*, *187*(8), 1817–1827.

Masarwa, R., Platt, R. W., & Filion, K. B. (2020). Acetaminophen use during pregnancy and the risk of attention deficit hyperactivity disorder: A causal association or bias? *Paediatric and Perinatal Epidemiology*, *34*(3), 309–317.

McCrae, J. C., Morrison, E. E., MacIntyre, I. M., Dear, J. W., & Webb, D. J. (2018). Long-term adverse effects of paracetamol – a review. *British Journal of Clinical Pharmacology*, *84*(10), 2218–2230.

McLemore, G. L., & Richardson, K. A. (2016). Data from three prospective longitudinal human cohorts of prenatal marijuana exposure and offspring outcomes from the fetal period through young adulthood. *Data in Brief*, *9*, 753–757.

Mehta, T. R., Monegro, A., Nene, Y., Fayyaz, M., & Bollu, P. C. (2019). Neurobiology of ADHD: A Review. *Current Developmental Disorders Reports*, *6*(4), 235–240.

Melancia, F., Schiavi, S., Servadio, M., Cartocci, V., Campolongo, P., Palmery, M., Pallottini, V., & Trezza, V. (2018). Sex-specific autistic endophenotypes induced by prenatal exposure to valproic acid involve anandamide signalling. *British Journal of Pharmacology*, *175*(18), 3699–3712.

Moreira, F. A., Aguiar, D. C., Terzian, A. L. B., Guimarães, F. S., & Wotjak, C. T. (2012). Cannabinoid type 1 receptors and transient receptor potential vanilloid type 1 channels in fear and anxiety - two sides of one coin? *Neuroscience*, *204*, 186–192.

Muramatsu, S., Shiraishi, S., Miyano, K., Sudo, Y., Toda, A., Mogi, M., Hara, M., Yokoyama, A., Kawasaki, Y., Taniguchi, M., & Uezono, Y. (2016). Metabolism of AM404 from acetaminophen at human therapeutic dosages in the rat brain. *Anesthesiology and Pain Medicine*, *6*(1), 1–5.

Murataeva, N., Straiker, A., & MacKie, K. (2014). Parsing the players: 2-arachidonoylglycerol synthesis and degradation in the CNS. *British Journal of Pharmacology*, *171*(6), 1379–1391.

Nielsen, E., Ostergaard, G., & Larsen, J. (2008). *Toxicological risk assessment of chemicals: a practical guide.* New York: Informa Healthcare USA,.

Niesink, R. J. M., & Van Ree, J. M. (1989). Involvement of opioid and dopaminergic systems in isolation-induced pinning and social grooming of young rats. *Neuropharmacology*, *28*(4), 411–418.

Nitsche, J. F., Patil, A. S., Langman, L, J., Penn, H. J., Derleth, D., Watson, W. J., & Brost, B. C. (2017). Transplacental passage of acetaminophen in term pregnancy. *American Journal of Perinatology*, *34*(6), 541–543.

Nomura, D. K., Morrison, B. E., Blankman, J. L., Long, J. Z., Kinsey, S. G., Marcondes, M. C. G., Ward, A. M., Hahn, Y. K., Lichtman, A. H., Conti, B., & Cravatt, B. F. (2011). Endocannabinoid hydrolysis generates brain prostaglandins that promote neuroinflammation. *Science*, *334*(6057), 809–813.

OECD. (2007). OECD guideline for the testing of chemicals 426 - Developmental Neurotoxicity Study, (September), 1–38.

Olivier, J. D. a, Vallès, a., Van Heesch, F., Afrasiab-Middelman, A., Roelofs, J. J. P. M., Jonkers, M., Peeters, E. J., Korte-Bouws, G. a H., Dederen, J. P., Kiliaan, A. J., Martens, G. J., Schubert, D., & Homberg, J. R. (2011). Fluoxetine administration to pregnant rats increases anxiety-related behavior in the offspring. *Psychopharmacology*, *217*(3), 419–432.

Palmer, A. A., Brown, A. S., Keegan, D., Siska, L. D., Susser, E., Rotrosen, J., & Butler, P. D. (2008). Prenatal protein deprivation alters dopamine-mediated behaviors and dopaminergic and glutamatergic receptor binding. *Brain Research*, *1237*, 62–74.

Pandolfo, P., Pamplona, F. A., Prediger, R. D. S., & Takahashi, R. N. (2007). Increased sensitivity of adolescent spontaneously hypertensive rats, an animal model of attention deficit hyperactivity disorder, to the locomotor stimulation induced by the cannabinoid receptor agonist WIN 55,212-2. *European Journal of Pharmacology*, *563*(1–3), 141–148.

Panikashvili, D., Simeonidou, C., Ben-Shabat, S., Hanuš, L., Breuer, A., Mechoulam, R., & Shohami, E. (2001). An endogenous cannabinoid (2-AG) is neuroprotective after brain injury. *Nature*, *413*(6855), 527–531.

Parker, W., Hornik, C. D., Bilbo, S., Holzknecht, Z. E., Gentry, L., Rao, R., Lin, S. S., Herbert, M. R., & Nevison, C. D. (2017). The role of oxidative stress, inflammation and acetaminophen exposure from birth to early childhood in the induction of autism. *Journal of International Medical Research*, *45*(2), 407–438.

Parrini, M., Ghezzi, D., Deidda, G., Medrihan, L., Castroflorio, E., Alberti, M., Baldelli, P., Cancedda, L., & Contestabile, A. (2017). Aerobic exercise and a BDNF-mimetic therapy rescue learning and memory in a mouse model of Down syndrome. *Scientific Reports*, *7*(1), 1–22.

Patel, S., & Windsor, M. A. (2015). Patent application publication (10) Pub. No.: US 2015/0183737 A1.

Penaloza, C. G., Cruz, M., Germain, G., Jabeen, S., Javdan, M., Lockshin, R. A., & Zakeri, Z. (2020). Higher sensitivity of female cells to ethanol: Methylation of DNA lowers Cyp2e1, generating more ROS. *Cell Communication and Signaling*, *18*(1), 1–15.

Perazzo, J. C., Fiebich, B. L., Fortwängler, E., Muñoz, E., Marcotegui, A. R., Saliba, S. W., de Oliveira, A. C. P., & Ditrich, J. (2017). AM404, paracetamol metabolite, prevents prostaglandin synthesis in activated microglia by inhibiting COX activity. *Journal of Neuroinflammation*, *14*(1), 1–11.

Philippot, G., Gordh, T., Fredriksson, A., & Viberg, H. (2017). Adult neurobehavioral alterations in male and female mice following developmental exposure to paracetamol (acetaminophen): characterization of a critical period. *Journal of Applied Toxicology*, *37*(10), 1174–1181.

Philippot, G., Hallgren, S., Gordh, T., Fredriksson, A., Fredriksson, R., & Viberg, H. (2018). A cannabinoid receptor type 1 (CB1R) agonist enhances the developmental neurotoxicity of acetaminophen (Paracetamol). *Toxicological Sciences*, *166*(1), 203–212.

Philippot, G., Hosseini, K., Yakub, A., Mhajar, Y., Hamid, M., Buratovic, S., & Fredriksson, R. (2022). Paracetamol (acetaminophen) and its effect on the developing mouse brain. *Frontiers in Toxicology*, *4*(March), 1–11.

Pinto, B., Morelli, G., Rastogi, M., Savardi, A., Fumagalli, A., Petretto, A., Bartolucci, M., Varea, E., Catelani, T., Contestabile, A., Perlini, L. E., & Cancedda, L. (2020). Rescuing over-activated microglia restores cognitive performance in juvenile animals of the Dp(16) mouse model of Down syndrome. *Neuron*, (16), 1–18.

Pletnikov, M. V., Rubin, S. A., Vasudevan, K., Moran, T. H., & M. Carbone, K. (1999). Developmental brain injury associated with abnormal play behavior in neonatally Borna disease virus-infected Lewis rats: A model of autism. *Behavioural Brain Research*, *100*(1–2), 43–50.

Polissidis, A., Chouliara, O., Galanopoulos, A., Marselos, M., Papadopoulou-Daifoti, Z., & Antoniou, K. (2009). Behavioural and dopaminergic alterations induced by a low dose of WIN 55,212-2 in a conditioned place preference procedure. *Life Sciences*, *85*(5–6), 248–254.

Polli, F. S., Scharff, M. B., Ipsen, T. H., Aznar, S., Kohlmeier, K. A., & Andreasen, J. T. (2020). Prenatal nicotine exposure in mice induces sex-dependent anxiety-like behavior, cognitive deficits, hyperactivity, and changes in the expression of glutamate receptor associated-genes in the prefrontal cortex. *Pharmacology Biochemistry and Behavior*, *195*, 172951.

Prut, L., & Belzung, C. (2003). The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: A review. *European Journal of Pharmacology*, *463*(1–3), 3–33.

Przybyła, G. W., Szychowski, K. A., & Gmiński, J. (2021). Paracetamol – An old drug with new mechanisms of action. *Clinical and Experimental Pharmacology and Physiology*, *48*(1), 3–19.

Ray-Griffith, S. L., Morrison, B., & Stowe, Z. N. (2019). Chronic Pain Prevalence and Exposures during Pregnancy. *Pain Research and Management*, *2019*.

Rice, D., & Barone, S. (2000). Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environmental Health Perspectives*, *108*(3), 511–533.

Rifas-Shiman, S. L., Cardenas, A., Hivert, M. F., Tiemeier, H., Bertoldi, A. D., & Oken, E. (2020). Associations of prenatal or infant exposure to acetaminophen or ibuprofen with mid-childhood executive function and behaviour. *Paediatric and Perinatal Epidemiology*, *34*(3), 287–298.

Rigobello, C., Klein, R. M., Debiasi, J. D., Ursini, L. G., Michelin, A. P., Matsumoto, A. K., Barbosa, D. S., & Moreira, E. G. (2021). Perinatal exposure to paracetamol: dose and sex-dependent effects in behaviour and brain's oxidative stress markers in progeny. *Behavioural Brain Research*, *408*(March).

Rivera, P., Tovar, R., Ramírez-López, M. T., Navarro, J. A., Vargas, A., Suárez, J., & Fonseca, F. R. de. (2020). Sex-specific anxiety and prefrontal cortex glutamatergic dysregulation are long-term consequences of pre-and postnatal exposure to hypercaloric diet in a rat model. *Nutrients*, *12*(6), 1829.

Ross, E. J., Graham, D. L., Money, K. M., & Stanwood, G. D. (2015). Developmental consequences of fetal exposure to drugs: What we know and what we still must learn. *Neuropsychopharmacology*, *40*(1), 61–87.

Rudenko, V., Rafiuddin, A., Leheste, J. R., & Friedman, L. K. (2012). Inverse relationship of cannabimimetic (R+)WIN 55, 212 on behavior and seizure threshold during the juvenile period. *Pharmacology Biochemistry and Behavior*, *100*(3), 474–484.

Saad, A., Hegde, S., Kechichian, T., Gamble, P., Rahman, M., Stutz, S. J., Anastasio, N. C., Alshehri, W., Lei, J., Mori, S., Kajs, B., Cunningham, K. A., Saade, G., Burd, I., & Costantine, M. (2016). Is there a causal relation between maternal acetaminophen administration and ADHD? *PLoS ONE*, *11*(6), 1–10.

Sañudo-Peña, M. C., Romero, J., Seale, G. E., Fernandez-Ruiz, J. J., & Walker, J. M. (2000). Activational role of cannabinoids on movement. *European Journal of Pharmacology*, *391*(3), 269–274.

Schneider, M., Schömig, E., & Leweke, F. M. (2008). Acute and chronic cannabinoid treatment differentially affects recognition memory and social behavior in pubertal and adult rats. *Addiction Biology*, *13*(3–4), 345–357.

Schneider, T., & Przewłocki, R. (2005). Behavioral alterations in rats prenatally exposed to valproic acid: animal model of autism. *Neuropsychopharmacology*, *30*(1), 80–89.

Schultz, S., Gould, G. G., Antonucci, N., Brigida, A. L., & Siniscalco, D. (2021). Endocannabinoid system dysregulation from acetaminophen use may lead to autism spectrum disorder: could cannabinoid treatment be efficacious? *Molecules*, *26*(7), 1845.

Schultz, S. T., & Gould, G. G. (2016). Acetaminophen use for fever in children associated with autism spectrum disorder. *Autism Open Access*, *6*(2), 1000170.

Scipioni, L., Ciaramellano, F., Carnicelli, V., Leuti, A., Lizzi, A. R., De Dominicis, N., Oddi, S., & Maccarrone, M. (2022). Microglial endocannabinoid signalling in AD. *Cells*, *11*(7), 1237.

Servadio, M., Melancia, F., Manduca, A., Di Masi, A., Schiavi, S., Cartocci, V., Pallottini, V., Campolongo, P., Ascenzi, P., & Trezza, V. (2016). Targeting anandamide metabolism rescues core and associated autistic-like symptoms in rats prenatally exposed to valproic acid. *Translational Psychiatry*, *6*(9), 1–11.

Servadio, Michela, Manduca, A., Melancia, F., Leboffe, L., Schiavi, S., Campolongo, P., Palmery, M., Ascenzi, P., di Masi, A., & Trezza, V. (2018). Impaired repair of DNA damage is associated with autistic-like traits in rats prenatally exposed to valproic acid. *European Neuropsychopharmacology*, *28*(1), 85–96.

Setler, P., Sarau, H., & McKenzie, G. (1976). Differential attenuation of some effects of haloperidol in rats given scopolamine. *European Journal of Pharmacology*, *39*, 117–126.

Sharma, Jamie, H., Shah, S., Perrett, D., & Ayoub, S. S. (2017). First evidence of the conversion of paracetamol to AM404 in human cerebrospinal fluid. *Journal of Pain Research*, *10*, 2703–2709.

Sholler, D. J., Strickland, J. C., Spindle, T. R., Weerts, E. M., & Vandrey, R. (2021). Sex differences in the acute effects of oral and vaporized cannabis among healthy adults. *Addiction Biology*, *26*(4), 1–12.

Silver, R. J. (2019). The endocannabinoid system of animals. *Animals*, *9*(9), 686.

Spagnolo, P. A., Garvey, M., & Hallett, M. (2021). A dimensional approach to functional movement disorders: Heresy or opportunity. *Neuroscience and Biobehavioral Reviews*, *127*(November 2020), 25–36.

Stark, T., Iannotti, F. A., Di Martino, S., Di Bartolomeo, M., Ruda-Kucerova, J., Piscitelli, F., Wotjak, C. T., D'Addario, C., Drago, F., Di Marzo, V., & Micale, V. (2022). Early blockade of CB1 receptors ameliorates schizophrenia-like alterations in the neurodevelopmental MAM model of schizophrenia. *Biomolecules*, *12*(1), 108.

Stasiulewicz, A., Znajdek, K., Grudzień, M., Pawiński, T., & Sulkowska, A. J. I. (2020). A guide to targeting the endocannabinoid system in drug design. *International Journal of Molecular Sciences*, *21*(8), 1–46.

Subbanna, S., Psychoyos, D., Xie, S., & Basavarajappa, B. S. (2015). Postnatal ethanol exposure alters levels of 2-arachidonylglycerol-metabolizing enzymes and pharmacological inhibition of monoacylglycerol lipase does not cause neurodegeneration in neonatal mice. *Journal of Neurochemistry*, *134*(2), 276–287.

Sznajder, K. K., Teti, D. M., & Kjerulff, K. H. (2022). Maternal use of acetaminophen during pregnancy and neurobehavioral problems in offspring at 3 years: A prospective cohort study. *PLoS ONE*, *17*(9 September), 1–17.

Taylor, P. V., Veenema, A. H., Paul, M. J., Bredewold, R., Isaacs, S., & De Vries, G. J. (2012). Sexually dimorphic effects of a prenatal immune challenge on social play and vasopressin expression in juvenile rats. *Biology of Sex Differences*, *3*(1), 1.

Terral, G., Marsicano, G., Grandes, P., & Soria-Gómez, E. (2020). Cannabinoid control of olfactory processes: the where matters. *Genes*, *11*(431), 1–8.

Thomas, A., Burant, A., Bui, N., Graham, D., Yuva-Paylor, L. A., & Paylor, R. (2009). Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety. *Psychopharmacology*, *204*(2), 361–373.

Thompson, J. M. D., Waldie, K. E., Wall, C. R., Murphy, R., & Mitchell, E. A. (2014). Associations between acetaminophen use during pregnancy and ADHD symptoms measured at ages 7 and 11 years. *PLoS ONE*, *9*(9), 1–6.

Thornton, A. M., Humphrey, R. M., Kerr, D. M., Finn, D. P., & Roche, M. (2021). Increasing endocannabinoid tone alters anxiety-like and stress coping behaviour in female rats prenatally exposed to valproic acid. *Molecules*, *26*(12), 3720.

Tong, N., Sanchez, J. F., Maggirwar, S. B., Ramirez, S. H., Guo, H., Dewhurst, S., & Gelbard, H. A. (2001). Activation of glycogen synthase kinase 3 beta (GSK-3β) by platelet activating factor mediates migration and cell death in cerebellar granule neurons. *European Journal of Neuroscience*, *13*(10), 1913–1922.

Tovo-Rodrigues, L., Carpena, M. X., Martins-Silva, T., Santos, I. S., Anselmi, L., Barros, A. J. D., Barros, F. C., Bertoldi, A. D., & Matijasevich, A. (2020). Low neurodevelopmental performance and behavioural/emotional problems at 24 and 48 months in Brazilian children exposed to acetaminophen during foetal development. *Paediatric and Perinatal Epidemiology*, *34*(3), 278–286.

Trezza, V., & Vanderschuren, L. J. M. J. (2008). Bidirectional cannabinoid modulation of social behavior in adolescent rats. *Psychopharmacology*, *197*(2), 217–227.

Urquhart, P., Nicolaou, A., & Woodward, D. F. (2015). Endocannabinoids and their oxygenation by cyclo-oxygenases, lipoxygenases and other oxygenases. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids*, *1851*(4), 366–376.

USEPA. (2006). Harmonization in interspecies extrapolation: use of BW 3/4 as default method in derivation of the oral RfD, (68), 1–34.

Van Der Stelt, M., Mazzola, C., Esposito, G., Matias, I., Petrosino, S., De Filippis, D., Micale, V., Steardo, L., Drago, F., Iuvone, T., & Di Marzo, V. (2006). Endocannabinoids and β-amyloid-induced neurotoxicity in vivo: Effect of pharmacological elevation of endocannabinoid levels. *Cellular and Molecular Life Sciences*, *63*(12), 1410–1424.

Viberg, H., Eriksson, P., Gordh, T., & Fredriksson, A. (2014). Paracetamol (acetaminophen) administration during neonatal brain development affects cognitive function and alters its analgesic and anxiolytic response in adult male mice. *Toxicological Sciences*, *138*(1), 139–147.

Vicente, A. M., Martins, G. J., & Costa, R. M. (2020). Cortico-basal ganglia circuits underlying dysfunctional control of motor behaviors in neuropsychiatric disorders. *Current Opinion in Genetics and Development*, *65*, 151–159.

Vogel-Ciernia, A., Careaga, M., LaSalle, J. M., & Ashwood, P. (2018). Microglia from offspring of dams with allergic asthma exhibit epigenomic alterations in genes dysregulated in autism. *Glia*, *66*(3), 505–521.

Wiley, J. L., Barrus, D. G., Farquhar, C. E., Lefever, T. W., & Gamage, T. F. (2021). Sex, species and age: effects of rodent demographics on the pharmacology of Δ9-tetrahydrocanabinol. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *106*(April 2020).

Wilkerson, J. L., Ghosh, S., Bagdas, D., Mason, B. L., Crowe, M. S., Hsu, K. L., Wise, L. E., Kinsey, S. G., Damaj, M. I., Cravatt, B. F., & Lichtman, A. H. (2016). Diacylglycerol lipase β inhibition reverses nociceptive behaviour in mouse models of inflammatory and neuropathic pain. *British Journal of Pharmacology*, *173*(10), 1678–1692.

Wilkes, B. J., & Lewis, M. H. (2018). The neural circuitry of restricted repetitive behavior: Magnetic resonance imaging in neurodevelopmental disorders and animal models. *Neuroscience and Biobehavioral Reviews*, *92*(May), 152–171.

Witchey, S. K., Al Samara, L., Horman, B. M., Stapleton, H. M., & Patisaul, H. B. (2020). Perinatal exposure to FireMaster® 550 (FM550), brominated or organophosphate flame retardants produces sex and compound specific effects on adult Wistar rat socioemotional behavior. *Hormones and Behavior*, *126*, 104853.

Wood, M. E. (2020). Associations between prenatal acetaminophen exposure and child neurodevelopment: Truth, bias, or a bit of both? *Paediatric and Perinatal Epidemiology*,

34(3), 233–236.

Xi, C., Yang, Z., Yu, Y., Li, S., He, J., El-Aziz, T. M. A., Zhao, F., & Cao, Z. (2022). Influence of perinatal deltamethrin exposure at distinct developmental stages on motor activity, learning and memory. *Ecotoxicology and Environmental Safety*, *236*, 113460.

Ystrom, E., Gustavson, K., Brandlistuen, R. E., Knudsen, G. P., Magnus, P., Susser, E., Davey Smith, G., Stoltenberg, C., Surén, P., Håberg, S. E., Hornig, M., Lipkin, W. I., Nordeng, H., & Reichborn-Kjennerud, T. (2017). Prenatal exposure to acetaminophen and risk of ADHD. *Pediatrics*, *140*(5), 1–9.

Zou, M., Liu, Y., Xie, S., Wang, L., Li, D., Li, L., Wang, F., Zhang, Y., Xia, W., Sun, C., & Wu, L. (2021). Alterations of the endocannabinoid system and its therapeutic potential in autism spectrum disorder. *Open Biology*, *11*(2).

Zucker, I. (2017). Risk mitigation for children exposed to drugs during gestation: A critical role for animal preclinical behavioral testing. *Neuroscience and Biobehavioral Reviews*, *77*, 107–121.

Zygmunt, P. M., Chuang, H. H., Movahed, P., Julius, D., & Högestätt, E. D. (2000). The anandamide transport inhibitor AM404 activates vanilloid receptors. *European Journal of Pharmacology*, *396*(1), 39–42.

Supplementary Table 1. Litters' and pups' distribution to testing.

litter nº	litter id	group	test 1	test 2	test 3	test 4	test 5	other experiments
1st mating								
1	2PT	CTL	NST	APO	OF			
2	4AZ	CTL	NST	APO	OF			
3	4PT	CTL	NST	APO	OF			
4	4VM	CTL	NST	APO	OF			
5	7PT	PAR	NST	APO	OF			
6	8VM	CTL	NST	APO	OF			
7	10AZ	PAR	NST	APO	OF			
8	11VD	PAR	NST	APO	OF			
9	12VD	PAR	NST	APO	OF			
10	10VM	CTL		APO				
11	12VM	PAR		APO	OF			
12	2VD	CTL		APO	OF			
13	11VM	PAR		APO	OF			
14	3PT	PAR		APO				
2nd mating								
15	5AZ	CTL						other experiments
16	10az	PAR						other experiments
17	1vm	PAR						other experiments
18	2vm	PAR						other experiments
19	8az	CTL						other experiments
20	5vd	CTL						other experiments
21	7vd	PAR						other experiments
22	9vd	PAR						other experiments
23	9vm	CTL						other experiments
24	6vd	PAR						other experiments
25	1az	CTL						other experiments
26	3vm	PAR						other experiments
27	3az	PAR						other experiments
28	7vm	PAR						other experiments
29	4vm	PAR						other experiments
30	6az	CTL						other experiments
31	4vd	CTL						other experiments
32	3vd	CTL						other experiments
3rd mating								
33	1AZ	CTL			OF	3CT	MB	
34	2AZ	CTL		APO	OF			
35	3AZ	PAR			OF	3CT	MB	
36	4AZ	CTL		APO	OF		MB	
37	8AZ	PAR		APO	OF			
38	9AZ	CTL		APO	OF		MB	

39	1PT	CTL	APO	OF	3CT		
40	2PT	CTL		OF	3CT	MB	
41	3PT	PAR		OF	3CT	MB	
42	6PT	CTL	APO	OF			
43	7PT	CTL		OF	3CT	MB	
44	8PT	PAR	APO	OF		MB	
45	10PT	CTL		OF	3CT	MB	
46	1VD	CTL	APO	OF		MB	
47	3VD	PAR	APO	OF		MB	
48	1VM	PAR		OF	3CT	MB	
49	2VM	PAR		OF	3CT	MB	
50	3VM	PAR	APO	OF		MB	
51	4VM	PAR	APO	OF	3CT		
52	5VM	PAR		OF	3CT	MB	
53	6VM	PAR		OF		MB	
54	7VM	CTL	APO	OF	3CT		
55	9VM	CTL		OF	3CT	MB	
56	10VM	PAR		OF	3CT	MB	
57	4VD	PAR	APO		3CT	MB	
4th mating							
58	2AZ	PAR			3CT	MB	other experiments
59	2PT	CTL			3CT	MB	other experiments
60	2VD	PAR			3CT	MB	other experiments
61	3PT	PAR			3CT	MB	other experiments
62	3VD	CTL			3CT	MB	other experiments
63	4PT	CTL			3CT	MB	other experiments
64	4VD	CTL			3CT	MB	other experiments
65	5PT	CTL			3CT	MB	other experiments
66	5VD	PAR			3CT	MB	other experiments
67	6PT	PAR			3CT	MB	other experiments
68	7VD	CTL			3CT	MB	other experiments
69	8PT	CTL			3CT	MB	other experiments
70	8VD	PAR			3CT	MB	other experiments
71	9PT	PAR			3CT	MB	other experiments
72	3AZ	CTL				MB	other experiments
73	6AZ	PAR				MB	other experiments
74	1VD	PAR				MB	other experiments
75	6VD	CTL				MB	other experiments
76	9AZ	CTL				MB	other experiments
77	8AZ	PAR				MB	other experiments
78	3VM	CTL				MB	other experiments
79	9VD	PAR				MB	other experiments
80	2VM	PAR				MB	other experiments

5th mating					
81	6AZ	PAR		APO	other experiments
82	2AZ	CTL		APO	other experiments
83	7AZ	PAR		APO	other experiments
84	7VM	CTL	NST	APO	other experiments
85	5VM	PAR	NST	APO	other experiments
86	5AZ	CTL	NST	APO	other experiments
87	4AZ	PAR	NST	APO	other experiments
88	3VM	PAR		APO	other experiments
89	10VM	PAR	NST	APO	other experiments
90	6VM	PAR	NST	APO	other experiments
91	1PT	PAR	NST	APO	other experiments
92	8PT	CTL		APO	other experiments
93	6PT	PAR	NST	APO	other experiments
94	1VM	PAR	NST		other experiments
95	2PT	CTL	NST		other experiments
96	2VM	PAR	NST		other experiments
97	10AZ	CTL	NST		other experiments
98	5PT	CTL	NST		other experiments
99	7PT	PAR	NST		other experiments
100	8AZ	PAR	NST		other experiments
101	9VM	CTL	NST		other experiments
102	10PT	CTL	NST		other experiments
103	2VD	PAR			other experiments
104	3VD	CTL			other experiments

CTL: control group
PAR: paracetamol-exposed group
NST: nest-seeking test
APO: apomorphine-induced stereotyped behavior
OF: open field
3CT: three-chamber test
MB: marble burying

The listing of one test means that one pair of same-sex siblings, from either or both sexes, were destined to that analysis. One received a previous injection of WIN,55212-2 and the other, vehicle.

Supplementary Table 2. Behavioral evaluation of infant rats exposed or not to PAR during gestation injected with WIN or VEH prior to the tests.

	CTL				**PAR**			
	Females		Males		Females		Males	
	VEH	WIN	VEH	WIN	VEH	WIN	VEH	WIN
Nest-seeking test	(n = 11)	(n = 11)	(n = 12)	(n = 12)	(n = 14)	(n = 14)	(n = 14)	(n = 14)
Latency (s)	12.9 ± 10.1	43.6 ± 45.6	32.6 ± 33.5	38.7 ± 40.5	62.3 ± 44.8	22.7 ± 26.4	24.5 ± 26.8	48.7 ± 41.3
Latency (Ln)	2.1 ± 0.6	2.9 ± 0.9	2.7 ± 0.7	2.6 ± 0.9	3.2 ± 0.9	2.4 ± 0.6	2.3 ± 0.7	2.9 ± 0.8
Open-field	(n = 13)	(n = 13)	(n = 13)	(n = 13)	(n = 15)	(n = 15)	(n = 11)	(n = 11)
Ambulation (count)	51.7 ± 13.8	46.1 ± 11.2	58.8 ± 12.1	53.1 ± 12.4	63.9 ± 7.4	57.8 ± 7.3	62.5 ± 8.7	54.6 ± 11.2
Time in central area (s)	27.5 ± 13.1	15.4 ± 7.5	33.7 ± 16.4	33.5 ± 17.6	32.4 ± 12.2	35.7 ± 13.5	26.1 ± 7.3	23.3 ± 10.4
Rearing (count)	32.8 ± 10.2	31.8 ± 8.0	39.9 ± 10.9	30.1 ± 9.2	41.9 ± 5.8	40.9 ± 6.3	38.4 ± 9.4	32.2 ± 8.3
Rostral grooming (s)	15.2 ± 7.4	11.1 ± 5.8	10.8 ± 5.4	13.6 ± 8.8	12.6 ± 6.0	9.7 ± 4.5	10.9 ± 3.7	20.2 ± 17.6
Body grooming (s)	2.6 ± 2.8	1.4 ± 1.5	0.3 ± 0.5	3.7 ± 2.7	3.2 ± 2.0	1.0 ± 1.0	1.5 ± 2.0	0.8 ± 1.0
Apomorphine-induced stereotyped behavior	(n = 19)	(n = 19)	(n = 17)	(n = 17)	(n = 15)	(n = 15)	(n = 15)	(n = 15)
Stereotypy intensity	32.0 ± 3.0	32.2 ± 4.1	30.5 ± 4.8	30.3 ± 4.8	41.4 ± 3.0	39.7 ± 3.2	32.4 ± 4.3	30.5 ± 4.0
Marble burying	(n = 16)	(n = 16)	(n = 14)	(n = 14)	(n = 15)	(n = 15)	(n = 18)	(n = 18)
Buried marbles (count)	6.4 ± 2.3	5.3 ± 2.6	4.1 ± 2.9	5.3 ± 3.0	9.9 ± 2.9	6.8 ± 2.6	8.0 ± 2.7	4.9 ± 2.5
Burried marbles (sqrt)	2.4 ± 0.5	1.9 ± 0.7	1.4 ± 0.9	1.9 ± 0.8	3.0 ± 0.6	2.4 ± 0.6	2.5 ± 0.7	1.7 ± 0.7
Three-chamber test	(n = 11)	(n = 8)	(n = 13)	(n =11)	(n = 12)	(n = 12)	(n = 10)	(n = 10)
Time in social area (s)	267. 0 ± 69.4	356.3 ± 128.6	339.4 ± 57.3	303.9 ± 106.5	312.7 ± 91.7	277.4 ± 97.8	297.3 ± 47.4	253.2 ± 104.7
Time in novel object area (s)	119.6 ± 37.8	79.1 ± 42.5	82.8 ± 60.6	98.9 ± 57.0	83.6 ± 33.1	150.7 ± 80.2	100.7 ± 49.1	139.3 ± 110.5
Time in central area (s)	213.4 ± 62.4	283.1 ± 145.7	177.7 ± 60.7	230.4 ± 104.0	260.3 ± 109.3	171.8 ± 111.9	202.0 ± 67.8	207.4 ± 130.3
Sniffing time (s)	41.3 ± 12.7	28.9 ± 16.0	59.5 ± 20.5	45.6 ± 18.8	41.8 ± 22.3	44.9 ± 19.9	53.6 ± 20.3	52.7 ± 33.6
Sociability index	30.0 ± 33.9	54.4 ± 25.0	61.8 ± 13.8	52.3 ± 33.8	49.6 ± 17.2	28.5 ± 34.2	53.6 ± 20.6	34.3 ± 33.2

Data are means ± 95% confidence intervals. Number of animals are indicated in parenthesis. CTL: control; PAR: paracetamol; WIN: WIN 55,212-2; VEH: vehicle.

Supplementary Table 3. Statistical values from the behavioral evaluation of infant rats exposed or not to PAR during gestation injected with WIN or VEH prior to the tests.

Behavioral test	Parameter		Factors	F	df	p	η_p^2
Nest seeking test	Latency to nest bedding (Ln)		Exposure	0.320	1/94	0.573	0.003
			Sex	0.002	1/94	0.961	0.000
			Injection	0.179	1/94	0.673	0.002
			Exposure x Sex	0.544	1/94	0.463	0.006
			Exposure x Injection	0.856	1/94	0.357	0.009
			Sex x Injection	0.331	1/94	0.566	0.004
			Exposure x Sex x Injection	5.593	1/94	0.020*	0.056
		Split by sex: Females	Exposure	0.885	1/46	0.352	0.019
			Injection	0.012	1/46	0.913	0.000
			Exposure x Injection	5.641	1/46	0.022*	0.109
		Split by sex: Males	Exposure	0.014	1/48	0.906	0.000
			Injection	0.482	1/48	0.491	0.010
			Exposure x Injection	1.001	1/48	0.322	0.020
Open field	Ambulation (count)		Exposure	4.429	1/96	0.038*	0.044
			Sex	0.464	1/96	0.497	0.005
			Injection	3.358	1/96	0.070	0.034
			Exposure x Sex	1.814	1/96	0.181	0.019
			Exposure x Injection	0.033	1/96	0.857	0.000
			Sex x Injection	0.019	1/96	0.890	0.000
			Exposure x Sex x Injection	0.016	1/96	0.899	0.000
	Time in central area(s)		Exposure	0.183	1/96	0.669	0.002
			Sex	0.108	1/96	0.743	0.001
			Injection	0.474	1/96	0.493	0.005
			Exposure x Sex	6.301	1/96	0.014*	0.062
			Exposure x Injection	0.550	1/96	0.460	0.006
			Sex x Injection	0.109	1/96	0.742	0.001

		Exposure x Sex x Injection	1.099	1/96	0.297	0.011
	Split by sex: Females	Exposure	5.067	1/96	0.029*	0.089
		Injection	0.609	1/96	0.439	0.012
		Exposure x Injection	1.880	1/96	0.176	0.035
	Split by sex: Males	Exposure	1.845	1/96	0.181	0.040
		Injection	0.054	1/96	0.817	0.001
		Exposure x Injection	0.040	1/96	0.842	0.001
Rearing (count)		Exposure	2.810	1/96	0.097	0.028
		Sex	0.373	1/96	0.543	0.004
		Injection	2.600	1/96	0.110	0.026
		Exposure x Sex	2.491	1/96	0.118	0.025
		Exposure x Injection	0.104	1/96	0.748	0.001
		Sex x Injection	1.605	1/96	0.208	0.016
		Exposure x Sex x Injection	0.113	1/96	0.738	0.001
Rostral grooming (s)		Exposure	0.023	1/96	0.879	0.000
		Sex	0.311	1/96	0.578	0.003
		Injection	0.060	1/96	0.808	0.001
		Exposure x Sex	0.661	1/96	0.418	0.007
		Exposure x Injection	0.118	1/96	0.732	0.001
		Sex x Injection	1.825	1/96	0.180	0.019
		Exposure x Sex x Injection	0.002	1/96	0.964	0.000
Body grooming (s)		Exposure	0.196	1/96	0.659	0.002
		Sex	0.283	1/96	0.596	0.003
		Injection	0.049	1/96	0.825	0.001
		Exposure x Sex	0.296	1/96	0.587	0.003
		Exposure x Injection	2.259	1/96	0.136	0.023
		Sex x Injection	3.065	1/96	0.083	0.031
		Exposure x Sex x Injection	0.762	1/96	0.385	0.008

Apomorphine-induced stereotyped behavior	Stereotypy intensity		Exposure	11.030	1/124	0.001*	0.082
			Sex	11.196	1/124	0.001*	0.083
			Injection	0.399	1/124	0.529	0.003
			Exposure x Sex	4.407	1/124	0.038*	0.034
			Exposure x Injection	0.444	1/124	0.507	0.004
			Sex x Injection	0.108	1/124	0.966	0.000
			Exposure x Sex x Injection	0.370	1/124	0.938	0.000
		Split by sex: Females	Exposure	17.808	1/66	0.000*	0.212
			Injection	0.211	1/66	0.648	0.003
			Exposure x Injection	0.336	1/66	0.564	0.005
		Split by sex: Males	Exposure	0.618	1/58	0.435	0.011
			Injection	0.188	1/58	0.666	0.003
			Exposure x Injection	0.143	1/58	0.707	0.002
Marble burying	Buried marbles (sqrt)		Exposure	5.213	1/118	0.024*	0.042
			Sex	5.070	1/118	0.026*	0.041
			Injection	2.276	1/118	0.134	0.019
			Exposure x Sex	0.002	1/118	0.964	0.000
			Exposure x Injection	2.270	1/118	0.135	0.019
			Sex x Injection	0.544	1/118	0.462	0.005
			Exposure x Sex x Injection	1.341	1/118	0.249	0.011
Three-chamber sociability test	Social area (s)		Exposure	0.856	1/88	0.357	0.010
			Sex	0.893	1/88	0.347	0.010
			Injection	1.898	1/88	0.172	0.021
			Exposure x Sex	0.348	1/88	0.557	0.004
			Exposure x Injection	0.058	1/88	0.810	0.001
			Sex x Injection	0.563	1/88	0.455	0.006
			Exposure x Sex x Injection	0.017	1/88	0.896	0.000
	Novel object area (s)		Exposure	0.977	1/88	0.326	0.011
			Sex	0.085	1/88	0.772	0.001

	Injection	0.139	1/88	0.710	0.002
	Exposure x Sex	0.147	1/88	0.702	0.002
	Exposure x Injection	3.086	1/88	0.082	0.034
	Sex x Injection	0.238	1/88	0.627	0.003
	Exposure x Sex x Injection	1.669	1/88	0.200	0.019
Central area (s)	Exposure	0.061	1/88	0.805	0.001
	Sex	1.011	1/88	0.317	0.011
	Injection	1.008	1/88	0.318	0.011
	Exposure x Sex	0.090	1/88	0.765	0.001
	Exposure x Injection	0.647	1/88	0.423	0.007
	Sex x Injection	0.146	1/88	0.703	0.002
	Exposure x Sex x Injection	0.399	1/88	0.529	0.005
Sniffing (s)	Exposure	0.220	1/88	0.640	0.002
	Sex	3.245	1/88	0.075	0.036
	Injection	1.084	1/88	0.301	0.012
	Exposure x Sex	0.530	1/88	0.469	0.006
	Exposure x Injection	0.742	1/88	0.391	0.008
	Sex x Injection	0.141	1/88	0.709	0.002
	Exposure x Sex x Injection	0.070	1/88	0.792	0.001
Sociability index	Exposure	0.829	1/88	0.365	0.009
	Sex	1.226	1/88	0.271	0.014
	Injection	0.509	1/88	0.477	0.006
	Exposure x Sex	0.308	1/88	0.580	0.003
	Exposure x Injection	2.401	1/88	0.125	0.027
	Sex x Injection	0.812	1/88	0.370	0.009
	Exposure x Sex x Injection	0.995	1/88	0.321	0.011

*$p<0.05$

Supplementary Table 4. Effect sizes comparison with three other developmental neurotoxicity studies examining similar dependent variables.

	Present study		Literature range	Other developmental neurotoxicity studies		
Test (parameter)	**η_p^2**	**d**	**d**	**d**		
Nest-seeking test (latency)	0.109	0.961	0.288 – 1.362	1.362 [1]	1.226 [2]	0.288 [3]
Open field (time in central area)	0.089	0.625	0.742 – 1.191	1.191 [4]	0.742 [5]	0.843 [6]
Open field (motor activity)	0.044	0.429	0.707 – 1.218	1.218 [4]	0.707 [7]	0.930 [6]
Apomorphine-induced stereotyped behavior (scores)	0.212	1.037	0.859 - 1.279	1.279 [8]	2.286 [9]	0.859 [10]
Marble burying (buried marbles)	0.042	0.419	1.271 – 1.976	1.976 [11]	1.480 [12]	1.271 [13]

REFERENCES

1. Servadio, M. *et al.* Impaired repair of DNA damage is associated with autistic-like traits in rats prenatally exposed to valproic acid. *Eur. Neuropsychopharmacol.* **28**, 85–96 (2018).

2. Melancia, F. *et al.* Sex-specific autistic endophenotypes induced by prenatal exposure to valproic acid involve anandamide signalling. *Br. J. Pharmacol.* **175**, 3699–3712 (2018).

3. Foley, K. A., MacFabe, D. F., Vaz, A., Ossenkopp, K. P. & Kavaliers, M. Sexually dimorphic effects of prenatal exposure to propionic acid and lipopolysaccharide on social behavior in neonatal, adolescent, and adult rats: Implications for autism spectrum disorders. *Int. J. Dev. Neurosci.* **39**, 68–78 (2014).

4. Kim, S.-A., Chai, J.-H. & Jang, E.-H. Prenatal trimethyltin exposure induces long-term DNA methylation changes in the male mouse

hippocampus. *Int. J. Mol. Sci.* **22**, 8009 (2021).

5. Thornton, A. M., Humphrey, R. M., Kerr, D. M., Finn, D. P. & Roche, M. Increasing endocannabinoid tone alters anxiety-like and stress coping behaviour in female rats prenatally exposed to valproic acid. *Molecules* **26**, 3720 (2021).

6. Fagundes, B. H. F. *et al.* Methylmercury exposure during prenatal and postnatal neurodevelopment promotes oxidative stress associated with motor and cognitive damages in rats: an environmental-experimental toxicology study. *Toxicol. Reports* **9**, 563–574 (2022).

7. Xi, C. *et al.* Influence of perinatal deltamethrin exposure at distinct developmental stages on motor activity, learning and memory. *Ecotoxicol. Environ. Saf.* **236**, 113460 (2022).

8. Bitanihirwe, B. K., Peleg-Raibstein, D., Mouttet, F., Feldon, J. & Meyer, U. Late prenatal immune activation in mice leads to behavioral and neurochemical abnormalities relevant to the negative symptoms of schizophrenia. *Neuropsychopharmacology* **35**, 2462–2478 (2010).

9. Favaro, P. d N., Costa, L. C. & Moreira, E. G. Maternal fluoxetine treatment decreases behavioral response to dopaminergic drugs in female pups. *Neurotoxicol. Teratol.* **30**, 487–494 (2008).

10. Palmer, A. A. *et al.* Prenatal protein deprivation alters dopamine-mediated behaviors and dopaminergic and glutamatergic receptor binding. *Brain Res.* **1237**, 62–74 (2008).

11. Witchey, S. K., Al Samara, L., Horman, B. M., Stapleton, H. M. & Patisaul, H. B. Perinatal exposure to FireMaster® 550 (FM550), brominated or organophosphate flame retardants produces sex and compound specific effects on adult Wistar rat socioemotional behavior. *Horm. Behav.* **126**, 104853 (2020).

12. Ishihara, Y. *et al.* A CCR5 antagonist, maraviroc, alleviates neural circuit dysfunction and behavioral disorders induced by prenatal valproate exposure. *J. Neuroinflammation* **19**, 195 (2022).

13. Polli, F. S. *et al.* Prenatal nicotine exposure in mice induces sex-dependent anxiety-like behavior, cognitive deficits, hyperactivity, and changes in the expression of glutamate receptor associated-genes in the prefrontal cortex. *Pharmacol. Biochem. Behav.* **195**, 172951 (2020).

ARTIGO 2

PRENATAL PARACETAMOL EXPOSURE ALTERS MARKERS OF ENDOCANNABINOID SIGNALING AND INCREASES PAF LEVELS OF INFANT RAT'S BRAIN

Rodrigo Moreno Klein[a], Juliana Diosti Debiasi[b], Vanessa Nishikawa Motomura[b], Carlos Arterio Sorgi[c], Sabrina Francesca de Souza Lisboa[d], Estefânia Gastaldello Moreira[a,b,*]

[a] Graduation Program in Health Sciences, State University of Londrina, 86038-350, Londrina, PR, Brazil

[b] Department of Physiological Sciences, State University of Londrina, 86057-970, Londrina, PR, Brazil

[c] Department of Clinical, Toxicological and Bromatological Analysis, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, 14040-900, Ribeirão Preto, SP, Brazil

[d] Department of BioMolecular Sciences, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, 14040-900, Ribeirão Preto, São Paulo, Brazil

*Corresponding author: egmoreira@uel.br

Depto de Ciências Fisiológicas, CCB

UEL- Campus Universitário

86057-970 – Londrina-PR- Brasil

+55 43 3371-4307

ABSTRACT

Paracetamol (PAR) is an over-the-counter analgesic/antipyretic widely used by pregnant women worldwide. Although epidemiological studies have been suggesting that gestational PAR exposure increases the risk for neurodevelopmental alterations in the offspring, causality and mode of action for PAR possible developmental neurotoxicity are still uncertain. Endocannabinoid (eCB) dysfunction and immune-inflammatory activation were hypothesized as possible modes of action by which PAR might induce neurotoxicity, but they have not been tested. We aimed to evaluate possible effects of PAR prenatal exposure on male and female rat offspring in social behaviors, recognition memory and nociception and neurochemical markers of eCB signaling and inflammatory markers. Pregnant Wistar rats were gavaged with PAR (350mg/kg/day) or water from gestational day 6 until delivery. Novel object recognition, hot plate and social play behavior tests were evaluated in 25 or 30-day-old rats, respectively. We quantified the concentrations of the eCB, 2-arachidonoylglycerol (2-AG), and of the pro-inflammatory lipid, platelet activating factor (PAF), as well as the gene expression of the main 2-AG-synthesis and degradation enzymes, diacylglycerol lipase (DAGL-) α and β, and monoacylglycerol lipase, in addition to the inflammatory markers cyclooxygenase-2 and interleukin 1 beta in brain regions of 22-day-old rats. Gestational exposure to PAR increased both 2-AG levels in the prefrontal cortex and the pro-inflammatory lipid, platelet-activating factor (PAF) in the prefrontal cortex, striatum and cerebellum in male and female pups. DAGL-β, a 2-AG synthase, was downregulated in cerebellum. It is suggested a role for both alterations in eCB system and inflammation in PAR developmental neurotoxicity.

Keywords: Acetaminophen, developmental neurotoxicity, endocannabinoid system, inflammation.

1 INTRODUCTION

Paracetamol (PAR, acetaminophen) is an over-the-counter analgesic/antipyretic medication widely used by pregnant women worldwide. About half of pregnant women, from different countries, reported PAR usage at least once during this period (Aagaard et al., 2018; Ray-Griffith et al., 2019) and a significative percentage repeat its use along pregnancy (Bandoli et al., 2019). PAR is one of the few not contraindicated analgesics for pregnant women because of its presumed (and not sufficiently tested) safety for this population and the exposed fetus (Cendejas-Hernandez et al., 2022).

Epidemiological studies have been suggesting that gestational exposure to PAR increases risk for neurodevelopmental disorders. Special attention has been given for the potential of the drug to increase odds for autism spectrum disorders (ASD) (Liew, Ritz, et al., 2016; S. T. Schultz & Gould, 2016) and attention deficit hyperactivity disorder (ADHD) (Avella-Garcia et al., 2016; Liew et al., 2014; Thompson et al., 2014; Ystrom et al., 2017) symptoms or diagnosis. ASD core symptomatology are impaired social interaction and communication and presence of behavioral stereotypies, but altered sensorial sensitivity, anxiety and other symptoms are frequently observed (American Psychiatric Association, 2014). For ADHD, attention deficits, impulsivity and hyperactivity are central to diagnostics (American Psychiatric Association, 2014). Meta-analyses of observational data points prenatal PAR exposure as a risk factor for both (Alemany et al., 2021; Gou et al., 2019; Masarwa et al., 2018). However, observational studies do not allow implying causality or the investigation of the biological mechanisms and modes of action involved in the alterations. Due to these limitations, the examination of available studies by Food and Drug Administration (FDA) lead to no change in recommendation for PAR usage during pregnancy (FDA, 2016).

Animal studies can investigate causality and the modes of action by which PAR may harm the developing central nervous system (CNS) (Zucker, 2017). In fact, behavioral

alterations were observed as a consequence of neurodevelopmental exposure to PAR. Exposed rodents presented altered motricity (Blecharz-Klin et al., 2017; Klein et al., 2020; Philippot et al., 2017, 2018; Rigobello et al., 2021; Viberg et al., 2014), sociability (Blecharz-Klin et al., 2018), cognitive performance (Pinto et al., 2020), olfactory discrimination, emotionality and pharmacologically-induced stereotyped behavior (Klein et al., 2020).

Potential modes of action by which PAR may harm CNS development have been hypothesized in the literature, and include: excessive formation of its toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI); altered brain-derived neurotrophic factor (BDNF); cyclooxygenase-2 (COX-2) inhibition; endocrine disruption; oxidative stress and immune-inflammatory activation; and endocannabinoid (eCB) system dysfunction (Bauer et al., 2018). Interestingly, a recently published transcriptomic evaluation indicated altered expression of genes relating to glutathione and cytochrome p450 metabolism, DNA damage, and the endocrine and immune systems in the prefrontal cortex of PAR exposed offspring (Baker et al., 2023), all of which were previously discussed to be involved in PAR-induced developmental neurotoxicity. Philipot et al. (Philippot et al., 2018) reported a synergic effect of PAR (two doses of 30 mg/kg, subcutaneous) and a non-specific cannabinoid agonist, WIN 55,212-2 (1 mg/kg, subcutaneous), in impairing neurodevelopment of neonatal mice, reducing expression of genes involved in neurotransmission, BDNF signaling and eCB metabolism and altering motor behavior at adulthood (Philippot et al., 2018).

The eCB system coordinates a variety of physiological functions (Cristino et al., 2020) and is an important modulator of CNS development, regulating cell proliferation and differentiation, migration and axonal growth (Harkany & Cinquina, 2021). Early-life disturbance of eCBs and consequent dysfunction of the system can lead to sustained alterations in neurodevelopment and behavior (McLemore & Richardson, 2016). The system comprises its endogenous mediators (the eCBs), the enzymes responsible for the synthesis and degradation

of eCBs and the cannabinoid receptors type 1 (CB1) and type 2 (CB2) (Cristino et al., 2020). The most well studied eCBs are 2-arachidonoylglycerol (2-AG) and N-arachidonoylethanolamide (anandamide, AEA). 2-AG is the most abundant eCB in the brain and the bulk of it is synthesized from membrane phospholipids via enzymatic conversion by diacylglycerol lipase- (DAGL-) α and β (Murataeva et al., 2014). 2-AG breakdown occurs mainly by monoacylglycerol lipase (MAGL) and to a lesser stent by fatty acid amide hydrolase (FAAH) and cyclooxygenase-2 (COX-2) (Murataeva et al., 2014). 2-AG is a high-efficacy CB1 and CB2 agonist, whereas AEA is a low- and very-low-efficacy CB1 and CB2 agonist, respectively (Gonsiorek et al., 2000).

PAR may interact with the eCB system in several manners, most of which increase eCB tone. They are: (1) COX-2 inhibition by PAR and consequent increased availability of AEA and 2-AG (Przybyła et al., 2021); (2) Inhibition of enzymes involved in AEA intracellular careering (Kaczocha et al., 2012) and transmembrane uptake (Beltramo et al., 1997), besides inhibition of microglial COX-2 (Perazzo et al., 2017), all mediated by N-arachidonoylphenolamine (AM404), a PAR metabolite; (3) Agonism of CB1 (Beltramo et al., 1997) and transient receptor potential vanilloid type-1 channel (TRPV1) (Zygmunt et al., 2000). By inhibiting COX-2, its substrates became more available and these include AEA and 2-AG (Przybyła et al., 2021). Inhibiting careering or uptake of AEA can also increase this mediator. CB1 and TRPV1 are co-localized receptors (Cristino et al., 2006) that often have opposite actions on cellular response and behavior (Aguiar et al., 2014; Cristino et al., 2020).

Another possible mode of action for PAR developmental neurotoxicity is immune-inflammatory activation (Parker et al., 2017). Even when used in therapeutic doses (repeated 500 mg doses), PAR altered cytokines-, oxidative stress- and lipid metabolism-related gene expression in a transcriptomic evaluation in blood samples of healthy volunteers (Jetten et al., 2012). Similarly, prenatal PAR injections in mid- to late-gestation to rat dams (15 mg/kg,

2x/day) affect transcription of innate and adaptative immunity-related genes in the placenta and, to a lesser extent, in the fetal brain (Koehn et al., 2020). PAR injections (100 mg/kg/day, for 3 days, intraperitoneal) altered microglia morphology in peri-weaning but not adult mice (Pinto et al., 2020). These data are indicative of inflammatory responses as consequence of PAR usage or neurodevelopmental exposure.

In this study, we investigated behavioral parameters in infant rats exposed to PAR during gestation including social play behavior, recognition memory and nociception. These behaviors are relevant for ASD and ADHD, disorders whose occurrence were previously associated with prenatal PAR exposure in humans (Bauer et al., 2021). Moreover, considering some authors have been suggesting that both eCB dysfunction and immune-inflammatory activation are possible modes of action by which PAR might induce neurotoxicity (Bauer et al., 2018), we quantified markers for both in prefrontal cortex, hippocampus, striatum and cerebellum. These brain regions seem to be involved in the pathophysiology of neurodevelopmental disorders (Kim et al., 2016; Mehta et al., 2019). 2-AG and gene expression of its main synthesis and degradation enzymes, DAGL-α, DAGL-β and MAGL were evaluated as eCB signaling markers. Platelet-activating factor (PAF), a pro-inflammatory lipidic mediator, and gene expression of interleukin-1β (IL-1β), a pro-inflammatory cytokine, and COX-2 were evaluated as inflammatory markers.

2 MATERIALS AND METHODS

2.1 Animals, maternal exposure and perinatal procedures

Male and female naive Wistar rats, derived from the Central Vivarium of the State University of Londrina, were mated overnight (1 male and 2 females per cage), and gestational day 0 (GD 0) was determined through vaginal smear the next morning, by the presence of spermatozoa. On GD 6, females were individually allocated to separate cages. Animals were

kept under controlled temperature (21 ± 2 ºC), 12h light/dark cycle (lights on at 6:00 AM) and with free access to tap water and rat chow (Nuvital™, Nuvilab CR1, Paraná, Brazil).

PAR (Tylenol®, Johnson & Johnson, Brazil) was diluted into tap water and daily administered from gestational day (GD) 6 until delivery (post-natal day 0, or PND 0) by gavage in the dose of 350mg/kg (n= 21). The concentration of the solution was adjusted to administer a volume of approximately 0.3ml. Control group (CTL, n= 17) was gavaged with tap water. Administration volume was adjusted according to body weight. PAR dose represents a dosimetric adjustment of the dose of 500mg/day in humans (65 kg) (USEPA, 2006) with an added security factor of 10 accounting for intraspecies variability (Nielsen et al., 2008).

At PND 4, litters were culled to 10 and sex-balanced whenever possible. Litters smaller than 8 pups were discarded. We used 4 couples of infant rats from each litter in our 5-endpoint evaluation distributed as follows: (1) 2-AG and PAF evaluation; (2) gene expression evaluation; (3) social play behavior; (4) novel-object recognition and hot plate. Exceeding animals were utilized in other experiments or euthanized. The experimental protocols were approved by the State University of Londrina Ethics Committee for Animal Research (protocol nº 8522.2019.35).

2.2 Behavioral evaluation

All behavioral tests were recorded by a video camera linked to a computer in an adjacent room. Videos were analyzed by a trained observer who was masked to exposure. Males were always tested before the females in order to avoid the influence of possible sexual odor on the animal's behavior. Experiments were all conducted between 13:00 PM and 18:00 PM.

2.2.1 Novel object recognition (NOR, recognition memory)

On the PND 25 animals were submitted to the novel object recognition test to evaluate the discrimination of animals for a novel object over a previously explored object (a familiar object), and conducted as previously reported (Bevins & Besheer, 2006). Briefly, novel-object

test phase occurred 1 h after familiarization phase. In the familiarization phase, animals were individually placed for 10 min in a squared wooden arena with dimensions 40 x 40 x 40 cm (L x W x H) containing two objects of identical color, size, shape and material. After, animals were returned to their cages and left undisturbed until testing. In the testing phase, one of the objects was replaced with a new one (with different color, size, shape and material) and the animal was allowed to explore for 3 min. Time in direct contact (mouth, nose or paws) with objects or sniffing behavior towards them (nose within 1 cm of the object) were counted as exploration of the objects. Discrimination index was calculated as follows: discrimination index = time exploring novel object / time exploring all objects. The objects and the arena were cleaned with 70% alcohol (Parrini et al., 2017) after each session.

2.2.2 Hot plate (nociception)

Right after NOR test, animals were evaluated on a hot plate (Ugo Basile, Brazil) at 55ºC. The latency for a behavioral reaction (licking or lifting of the hind paw or jumping from the surface of the plate) was determined as adapted from (Klein et al., 2020). A cut-off time of 30s was established to avoid tissue damage.

2.2.3 Social play behavior

In the morning of the PND 30, animals were isolated for 3.5 h with chow and water available. Play behavior peaks at PND 30 (Pletnikov et al., 1999) and a social isolation of 3.5 h was shown to increase motivation to engage in social play (Niesink & Van Ree, 1989). After isolation, a pair of animals from the same sex and gestational exposure, but not the same litter, were placed in a polypropylene cage (47 x 27 x 15) covered with clean wood shavings and allowed to freely explore and interact for 15 min. The following behaviors from the pairs, and not from one animal alone, were analyzed: pinning frequency (one animal lies supine with the other animal on top of it); pouncing frequency (one animal touches the other neck with the

snout); and time spent in following (follow or chase the other animal) and sniffing and grooming (sniffing or grooming any part of the other animal's body) (Olivier et al., 2011).

2.3 Neurochemical evaluation

At PND 22 animals were guillotined, their brains removed and quickly dissected over ice. Prefrontal cortex, hippocampus, striatum and cerebellum were isolated and snap frozen in liquid nitrogen. Tissues were weighed and stored in -80º C freezer until further processing. For tissues destined to 2-AG and PAF quantification, 1mL of pure MeOH was added to tubes before storage.

2.3.1. Liquid chromatography tandem mass spectrometry (LC-MS)

Prefrontal cortex, hippocampus, striatum and cerebellum were homogenized in MeOH, spiked with deuterated internal standards for 2-AG (2-AG-d5) and PAF (Lyso-PAFC-16-d4) (Cayman Chemical, USA) and subjected to lipid extraction with chloroform/MeOH/H2O (2:2:1.8), as previously described (Bligh & Dyer, 1959). Extracted content was dried, suspended in 50 µl of MeOH and injected into the LC-MS (TripleTOF® 5600, Sciex, USA). Mobile phases were 0.1% formic acid in either (A) H2O or (B) MeOH. Mass spectrometry was operated in positive mode for high resolution multiple reaction monitoring analysis.

2.3.2. RNA extraction, reverse transcription and qPCR

Total RNA was isolated from prefrontal cortex, striatum and cerebellum by TRIzol (Invitrogen, USA) and chloroform (2.5:1), according to manufacturer's instructions. cDNA was synthesized using 2µg of RNA following instructions of utilized high-capacity cDNA reverse transcription kit (Invitrogen, USA). Quantification was performed by real-time PCR (StepOne Plus, Applied Biosystems) using TaqMan® arrays for the following rat genes: Dagla (DAGL-α, Rn01454303_m1); Daglb (DAGL-β, Rn01453770_m1); Mgll (MAGL, Rn00593297_m1); Ptgs2 (COX-2, Rn01483828_m1); Il1b (IL-1β, Rn00580432_m1); and the reference gene Gapdh (Rn01775763_g1). Statistical tests were run over ΔCt values (Ct gene of interest – Ct

Gapdh) (Vogel-Ciernia et al., 2018) but presented as -ΔCt to facilitate intuitive interpretation of relative expression (e.g., lower values reflect lower expression and the opposite is also true).

2.4 Statistical analysis

Data were initially explored for normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test). If the criteria were reached ($p>0.01$) parametric tests were conducted. Data that did not reach homogeneity or normality underwent arithmetic transformation, and again tested. Data were analyzed by two-way ANOVA with sex and gestational exposure as factors. Effect sizes were determined by partial eta squared (η_p^2) for all endpoints. Cohen's d was calculated for endpoints affected by exposure in order to, whenever possible, compare effect sizes with literature (e.g., effects of perinatal manipulations on similar dependent variables). We used the IBM-SPSS Windows version 26 to analyze the data. Conclusions were established with $p \leq 0.05$.

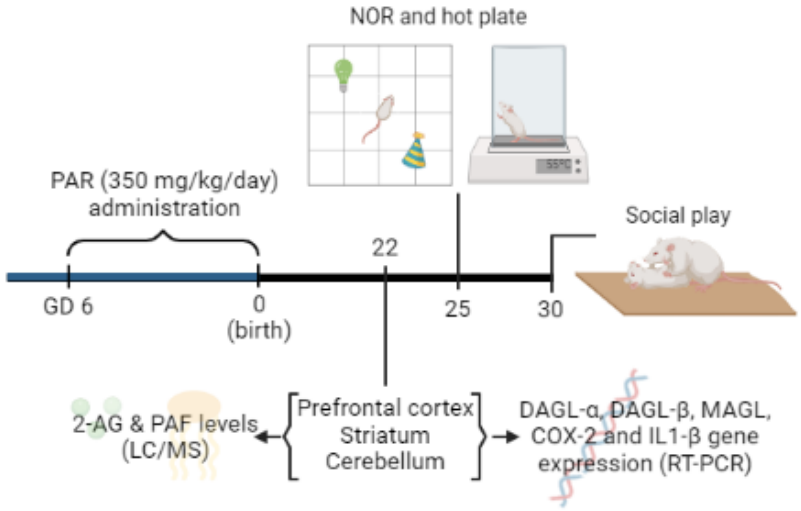

Fig 1. Experimental design for treatment and behavioral and neurochemical evaluations conducted in rats exposed or not to PAR during gestation. Blue line indicates gestational days (GD) and black line, postnatal days.

3 RESULTS

Data were graphed to reflect statistically significant differences on two-way ANOVA. Means and 95% confidence intervals (CI) for all the analyzed parameters, including non-transformed values, are exhibited in Supplementary Table 1 and their respective statistical values (F, df, p and η_p^2), presented in Supplementary Table 2.

3.1 Behavioral evaluation

3.1.1 NOR (memory recognition)

One male from the CTL group did not explore any of the objects and was excluded from the analyses. Gestational exposure to PAR did not affect the discrimination index or the exploration time of either the objects (Supplementary Table 1). Two-way ANOVA indicated a sex effect for time exploring the familiar object [$F_{(1, 42)}$= 4.93, p= .032], females exploring more than males (Fig. 2A).

3.1.2 Hot plate (nociception)

Two-way ANOVA showed no effect of exposure or sex on reaction time for nociception-related behaviors (Supplementary Table 1).

3.1.3 Social play behavior

Two-way ANOVA indicated no effect of exposure on social play parameters (Fig. 2B). However, a sex effect was observed in all analyzed parameters, as frequency of pinning [$F_{(1; 30)}$= 4.22, p= .049] and pouncing [$F_{(1; 30)}$= 15.54, p< .001], following time [$F_{(1; 30)}$= 13.67, p= .001] and sniffing and licking time [$F_{(1; 30)}$= 8.73, p= .006] (Fig. 2B).

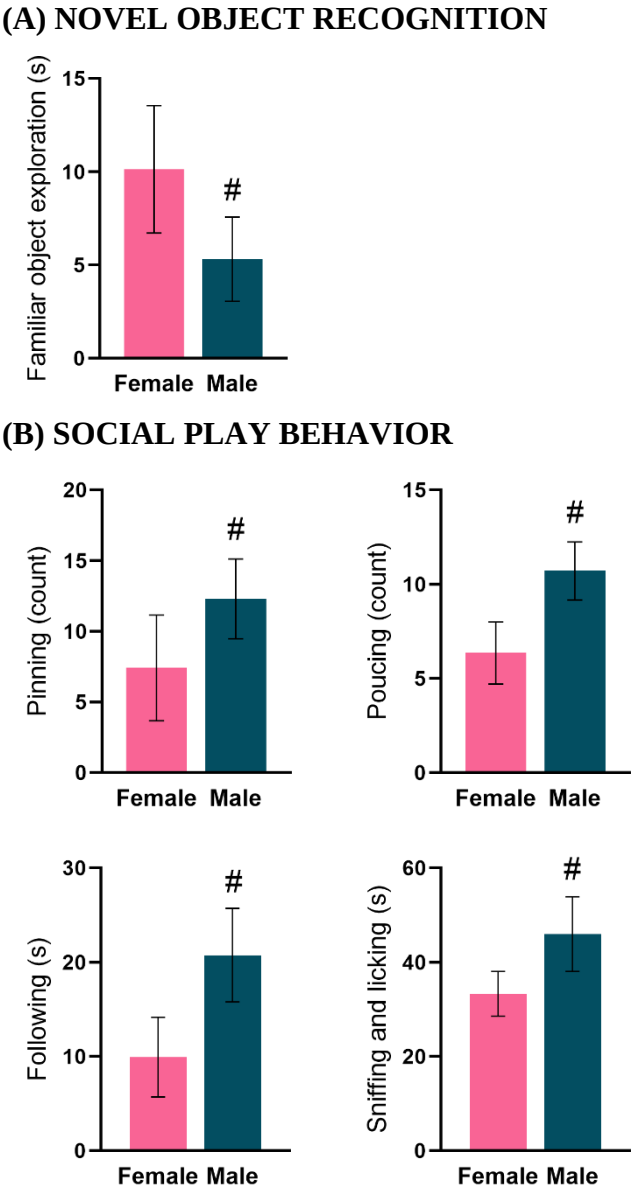

Fig 2. Behavioral evaluation of infant male and female rats exposed or not to PAR during gestation. (A) Sex effect in time exploring familiar object in the novel object recognition test. (B) Sex effect in different parameters in the social play behavior test. # p< .05 sex effect in two-way ANOVA. Data are means ± 95% CI of 17-24 animals per group.

3.2 Neurochemical evaluation

3.2.1 Prefrontal cortex

Gestational exposure to PAR increased the levels of 2-AG [$F_{(1;22)}$= 9.82, p= .005] and PAF [$F_{(1;22)}$= 6.46, p= .018] in the prefrontal cortex of both male and female animals, as indicated by a main exposure effect in the two-way ANOVA (Fig. 3). For 2-AG levels, exposure factor accounts for 30% of data variability (η_p^2= .299) and average levels of PAR-exposed animals are 1.3 standard deviations greater than of non-exposed (Cohen's d= 1.306). When compared to studies examining effect of prenatal manipulations on this variable, effect sizes

reported were higher (Cohen's d= 1.55 and 2.38 in (Stark et al., 2022) and (Zou et al., 2021), respectively). Exposure accounts for 22% of PAF data variability (η_p^2= .219) and its average levels are 1.1 standard deviations greater in PAR-exposed animals than in non-exposed (Cohen's d= 1.059). DAGL-α and β, MAGL, COX-2 and IL-1β gene expression were not affected by exposure, sex or interaction between them (Supplementary Table 1).

3.2.2 Hippocampus

No alterations in hippocampal 2-AG or PAF levels were observed in exposed animals (Supplementary Table 1). Therefore, gene expression analyses were not conducted in this tissue.

3.2.3 Striatum

Striatal PAF levels was increased in exposed male and females [$F_{(1;22)}$= 7.55, p= 0.011], as indicated by a main exposure effect in the two-way ANOVA. PAR exposure explains 24% of PAF levels data variability (η_p^2= .239) and average levels are 1.1 standard deviations greater in PAR-exposed in comparison to non-exposed animals (Cohen's d= 1.121). Exposure did not affect 2-AG levels in striatum and no sex differences were observed as well. No effects of exposure, sex or interaction between them were observed in gene expression of DAGL-α and β, MAGL, COX-2 and IL-1β (Supplementary Table 1).

3.2.4 Cerebellum

Gestational exposure to PAR increased PAF levels [$F_{(1;22)}$= 4.80, p= .038] and decreased DAGL-β gene expression [F(1;20)= 7.35, p= .013] in the cerebellum of both male and female rats, as indicated by a main exposure effect in the two-way ANOVA. Exposure accounts for 17% of PAF levels variability (η_p^2= .167) and average PAF levels are 0.9 standard deviations greater in PAR-exposed when compared to non-exposed animals (Cohen's d= .895). For DAGL-β expression, exposure factor explains 27% of data variability (η_p^2= .269) and its average levels are 1.2 standard deviations greater in PAR-exposed animals than in non-exposed

(Cohen's d= 1.213). This is in line with other prenatal manipulations that altered brain DAGL-β expression (Cohen's d= 1.17 and .82 in (Subbanna et al., 2015) and (Rivera et al., 2020), respectively). 2-AG levels were not affected by exposure, sex or interaction between these factors. DAGL-α, MAGL, COX-2 and IL-1β gene expression were not affected by exposure, sex or interaction between them (Supplementary Table 1).

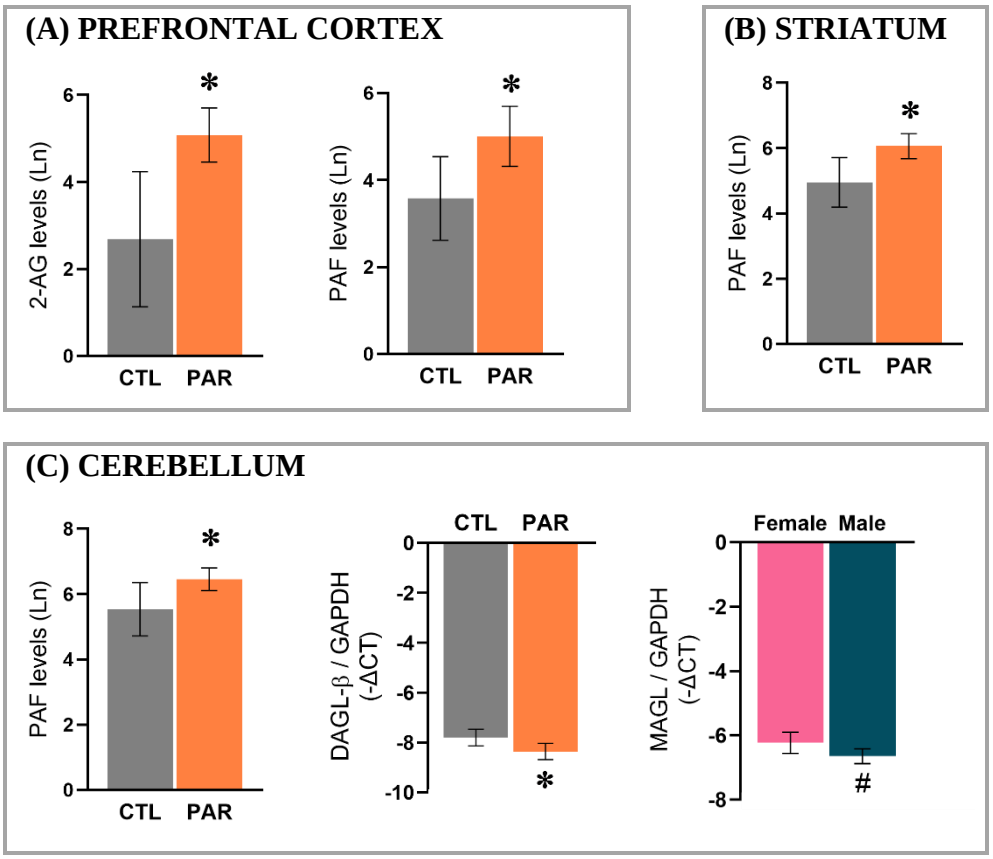

Fig 3. Neurochemical evaluation of infant male and female rats exposed or not to PAR during gestation. (A) PAR-exposure effect on prefrontal cortex 2-AG and PAF levels. (B) PAR-exposure effect on striatal PAF levels. (C) PAR-exposure effects on PAF and DAGL-β expression and sex effect on MAGL expression. ✻ $p < .05$ for exposure effect and # $p < .05$ for sex effect in a two-way ANOVA. Data are means ± 95% CI of 13-14 animals per group.

4 DISCUSSION

In the present study, gestational exposure to PAR increased 2-AG signaling in the prefrontal cortex and PAF levels in the prefrontal cortex, striatum and cerebellum of exposed males and females. DAGL-β expression was downregulated in the cerebellum of exposed rats independently of sex. Behavior in NOR, hot plate and play behavior tests were not altered by exposure. Longer exploration of familiar object by females in NOR test may either reflect increased motor behavior that is usually observed in females (Prut & Belzung, 2003) or decreased capability of memory-recognition in this paradigm. An expected sex effect was observed in social play behavior, with males interacting more with the conspecific (Taylor et al., 2012). Increased brain MAGL gene expression in females was previously reported (Levine et al., 2021) although cerebellar sex-differences seem underexplored. Compared to data reported in developmental studies from the literature, effect sizes obtained in the present study are either lower (2-AG) or similar (DAGL-β expression) to reported values. Comparison of PAF effect size was not possible due to lack of studies examining effect of prenatal manipulations on this variable.

It has been suggested that eCB dysfunction plays a role in the possible neurodevelopmental alterations induced by prenatal PAR exposure (Schultz et al., 2021). In the present study, increased 2-AG levels were observed in prefrontal cortex 3 weeks after prenatal exposure had been finished. Although PAR exposure did not alter gene expression of canonical enzymes involved in synthesis or degradation of 2-AG in the prefrontal cortex, DAGL-β was downregulated in the cerebellum. These data suggest region-specific alterations in eCB system as a consequence of PAR exposure. Although PAR exposure increased mean 2-AG levels in up to 76% in relation to non-exposed group, comparison with other prenatal manipulations indicate this to be a small effect. However, even slight fluctuations in 2-AG availability can have important physiological implications, especially during neurodevelopment, when its

concentrations are tightly regulated because it modulates important maturational processes (MacCarrone et al., 2014). Finally, eCBs (of which 2-AG is an important part) regulate sociability, attention, motor activity and emotion (Stasiulewicz et al., 2020) and alterations in these functions were previously associated with prenatal PAR exposure. These could, therefore, be related to eCB dysfunction.

It has also been hypothesized that immune-inflammatory activation could participate in the developmental neurotoxicity of PAR (Parker et al., 2017). Increased levels of PAF were observed in the prefrontal cortex, striatum and cerebellum, but not hippocampus, of rats exposed to PAR during gestation. PAF is a pro-inflammatory phospholipid involved in biological functions that range from neurodevelopmental roles (Tong et al., 2001) to neurotoxicity and apoptosis (Liu et al., 2017). Despite having varied functions, excessive PAF is linked to pathophysiological conditions where neurodegeneration occurs, like Alzheimer's (Hershkowitz & Adunsky, 1996) and amyotrophic lateral sclerosis (Briones et al., 2018). When PAF activates its mainly microglia-expressed receptor, increased intracellular calcium leads to lipid mobilization that can be used for further synthesis of PAF or other inflammatory-related lipids (Lordan et al., 2019). This can result in a positive feedback loop that modulates acute and chronic inflammation (Lordan et al., 2019). However, exposure did not alter gene expression of evaluated inflammatory markers (IL-1β and COX-2) in any brain region, which may indicate specificity of inflammatory pathways affected by exposure.

eCB system and inflammation stablish intricate bidirectional relationships (Scipioni et al., 2022). Of interest, a role for PAF in inducing 2-AG synthesis was described in mouse macrophages (Berdyshev et al., 2001; Liu et al., 2003) and human platelets (Berdyshev et al., 2001) by pathways not yet described. These mediators seem to have opposed pathophysiological roles in neurotoxicity and neuroprotection: while PAF is involved in neurotoxicity, 2-AG seems to mainly exert neuroprotective effects, attenuating

neuroinflammation and neurodegeneration after brain injury (Panikashvili et al., 2001) and neurotoxic insults (Van Der Stelt et al., 2006). Therefore, increased prefrontal 2-AG levels observed in our study could be a compensatory response to excessive PAF. It should be mentioned, however, that 2-AG hydrolysis by MAGL is a critical source of arachidonic acid for inflammatory eicosanoids synthesis, such as prostaglandins, in the brain, liver and lungs (Nomura et al., 2011). Indeed, reduced 2-AG, but also arachidonic acid and prostaglandin levels, were associated with attenuated peripheral inflammatory responses induced by preferential pharmacological inhibition of DAGL-β (Hsu et al., 2012) or its genetic deletion (Wilkerson et al., 2016). Taken together, alterations in eCB system and PAF may interact to regulate neuroinflammatory tone after gestational PAR exposure.

Social interaction, memory and sensorial-related behaviors investigation in PAR-exposed offspring are outcomes of major interest in view of the associations with ASD and ADHD. Although some previous reports described altered social behaviors (Blecharz-Klin et al., 2018; Harshaw & Warner, 2022) and memory (Philippot et al., 2022; Pinto et al., 2020) in rodents exposed to PAR during neurodevelopment, others failed to do so (Baker et al., 2023; Blecharz-Klin et al., 2017, 2018; Klein et al., 2020; Rigobello et al., 2021). Decreased analgesic response to PAR was reported, but there were no alterations in basal nociception (Viberg et al., 2014). Those studies largely vary in methodologies applied, including dosage, exposure regimen and route, age and paradigm of testing and controlling for litter effects. Besides, mentioned studies used direct post-natal treatment in contrast to the maternal prenatal exposure utilized in the present study. Maternal responses to (Koehn et al., 2020) and metabolism of (Conings et al., 2019) PAR could affect exposure profile and, consequently, the outcome.

The strengths of the present study are to be the first to investigate markers of 2-AG signaling and inflammation in brain tissue and to evaluate play behavior at infant/juvenile age after prenatal exposure to PAR utilizing allometric dosage calculation, litter as experimental

unit and evaluating both males and females. An important limitation is that only one timepoint was evaluated for each endpoint, what does not allow tracking the alterations trough development. Restricted number of evaluated inflammatory markers could also be seen as a limitation, considering inflammatory status is dependent on the balance of different pro- and anti-inflammatory markers.

5 CONCLUSIONS

In conclusion, results suggest both alterations in eCB system and inflammation may be modes of action by which PAR alters CNS development. Additional work is needed to shed light on the specific molecular pathways that lead to observed alterations and their implications for neurodevelopment and behavior.

Declaration of competing interest

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

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REFERENCES

Aagaard, S. K., Larsen, A., Andreasen, M. F., Lesnikova, I., Telving, R., Vestergaard, A. L., Tørring, N., Uldbjerg, N., & Bor, P. (2018). Prevalence of xenobiotic substances in first-trimester blood samples from Danish pregnant women: A cross-sectional study. *BMJ Open*, *8*(3), 1–7.

Aguiar, D. C., Moreira, F. A., Terzian, A. L., Fogaça, M. V., Lisboa, S. F., Wotjak, C. T., & Guimaraes, F. S. (2014). Modulation of defensive behavior by Transient Receptor Potential Vanilloid Type-1 (TRPV1) channels. *Neuroscience and Biobehavioral Reviews*, *46*(P3), 418–428.

Alemany, S., Avella-García, C., Liew, Z., García-Esteban, R., Inoue, K., Cadman, T., … Sunyer, J. (2021). Prenatal and postnatal exposure to acetaminophen in relation to autism spectrum and attention-deficit and hyperactivity symptoms in childhood: meta-analysis in six european population-based cohorts. *European Journal of Epidemiology*, (0123456789).

American Psychiatric Association. (2014). *Diagnostic and Statistical Manual of Mental Disorders* (5th ed.). Washington DC: American Psychiatric Association.

Athersuch, T. J., Antoine, D. J., Boobis, A. R., Coen, M., Daly, A. K., Possamai, L., Nicholson, J. K., & Wilson, I. D. (2018). Paracetamol metabolism, hepatotoxicity, biomarkers and therapeutic interventions: A perspective. *Toxicology Research*, *7*(3), 347–357.

Avella-Garcia, C. B., Julvez, J., Fortuny, J., Rebordosa, C., García-Esteban, R., Galán, I. R., Tardón, A., Rodríguez-Bernal, C. L., Iñiguez, C., Andiarena, A., Santa-Marina, L., & Sunyer, J. (2016). Acetaminophen use in pregnancy and neurodevelopment: attention function and autism spectrum symptoms. *International Journal of Epidemiology*, *45*(6), 1987–1996.

Baker, B. H., Rafikian, E. E., Hamblin, P. B., Strait, M. D., Yang, M., & Pearson, B. L. (2023). Sex-specific neurobehavioral and prefrontal cortex gene expression alterations following developmental acetaminophen exposure in mice. *Neurobiology of Disease*, *177*(December 2022), 105970.

Bambini-Junior, V., Zanatta, G., Della Flora Nunes, G., Mueller de Melo, G., Michels, M., Fontes-Dutra, M., Nogueira Freire, V., Riesgo, R., & Gottfried, C. (2014). Resveratrol prevents social deficits in animal model of autism induced by valproic acid. *Neuroscience Letters*, *583*, 176–181.

Bandoli, G., Palmsten, K., & Chambers, C. (2019). Acetaminophen use in pregnancy: examining prevalence, timing, and indication of use in a prospective birth cohort. *Paediatric and Perinatal Epidemiology*, (August), 1–10.

Bauer, A. Z., Kriebel, D., Herbert, M. R., Bornehag, C. G., & Swan, S. H. (2018). Prenatal paracetamol exposure and child neurodevelopment: A review. *Hormones and Behavior*, *101*, 125–147.

Bauer, A. Z., Swan, S. H., Kriebel, D., Liew, Z., Taylor, H. S., Bornehag, C.-G., Andrade, A.

M., Olsen, J., Jensen, R. H., Mitchell, R. T., Skakkebaek, N. E., Jégou, B., & Kristensen, D. M. (2021). Paracetamol use during pregnancy — a call for precautionary action. *Nature Reviews Endocrinology*, *0123456789*.

Beltramo, M., Stella, N., Calignano, A., Lin, S. Y., Makriyannis, A., & Piomelli, D. (1997). Functional role of high-affinity anandamide transport, as revealed by selective inhibition. *Science*, *277*(August), 1094–1097.

Berdyshev, E. V., Schmid, P. C., Krebsbach, R. J., & Schmid, H. H. O. (2001). Activation of PAF receptors results in enhanced synthesis of 2-arachidonoylglycerol (2-AG) in immune cells. *The FASEB Journal*, *15*(12), 2171–2178.

Bertoldi, A. D., Rifas-Shiman, S. L., Boing, A. C., da Silva Dal Pizzol, T., Miranda, V. I. A., Silveira, M. P. T., Freitas Silveira, M., Domingues, M. R., Santos, I. S., Bassani, D. G., Tovo-Rodrigues, L., & Oken, E. (2020). Associations of acetaminophen use during pregnancy and the first year of life with neurodevelopment in early childhood. *Paediatric and Perinatal Epidemiology*, *34*(3), 267–277.

Bevins, R. A., & Besheer, J. (2006). Object recognition in rats and mice: A one-trial non-matching-to-sample learning task to study "recognition memory." *Nature Protocols*, *1*(3), 1306–1311.

Bitanihirwe, B. K., Peleg-Raibstein, D., Mouttet, F., Feldon, J., & Meyer, U. (2010). Late prenatal immune activation in mice leads to behavioral and neurochemical abnormalities relevant to the negative symptoms of schizophrenia. *Neuropsychopharmacology*, *35*(12), 2462–2478.

Blecharz-Klin, K., Joniec-Maciejak, I., Jawna-Zboinska, K., Pyrzanowska, J., Piechal, A., Wawer, A., & Widy-Tyszkiewicz, E. (2016). Cerebellar level of neurotransmitters in rats exposed to paracetamol during development. *Pharmacological Reports*, *68*(6), 1159–1164.

Blecharz-Klin, K., Joniec-Maciejak, I., Jawna, K., Pyrzanowska, J., Piechal, A., Wawer, A., & Widy-Tyszkiewicz, E. (2015a). Developmental exposure to paracetamol causes biochemical alterations in medulla oblongata. *Environmental Toxicology and Pharmacology*, *40*, 369–374.

Blecharz-Klin, K., Joniec-Maciejak, I., Jawna, K., Pyrzanowska, J., Piechal, A., Wawer, A., & Widy-Tyszkiewicz, E. (2015b). Effect of prenatal and early life paracetamol exposure on the level of neurotransmitters in rats - focus on the spinal cord. *International Journal of Developmental Neuroscience*, *47*, 133–139.

Blecharz-Klin, K., Piechal, A., Jawna-Zboińska, K., Pyrzanowska, J., Wawer, A., Joniec-

Maciejak, I., & Widy-Tyszkiewicz, E. (2017). Paracetamol − effect of early exposure on neurotransmission, spatial memory and motor performance in rats. *Behavioural Brain Research*, *323*, 162–171.

Blecharz-Klin, K., Wawer, A., Jawna-zboi, K., Pyrzanowska, J., Piechal, A., Mirowska-guzel, D., & Widy-tyszkiewicz, E. (2018). Early paracetamol exposure decreases brain-derived neurotrophic factor (BDNF) in striatum and affects social behaviour and exploration in rats. *Pharmacology, Biochemistry and Behavior*, *168*, 25–32.

Blecharz-Klin, K., Wawer, A., Pyrzanowska, J., Piechal, A., Jawna-Zboińska, K., & Widy-Tyszkiewicz, E. (2019). Hypothalamus – response to early paracetamol exposure in male rats offspring. *International Journal of Developmental Neuroscience*, *76*(January), 1–5.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*(8), 911–917.

Brandlistuen, R. E., Ystrom, E., Nulman, I., Koren, G., & Nordeng, H. (2013). Prenatal paracetamol exposure and child neurodevelopment: A sibling-controlled cohort study. *International Journal of Epidemiology*, *42*(6), 1702–1713.

Briones, M. R. S., Snyder, A. M., Ferreira, R. C., Neely, E. B., Connor, J. R., & Broach, J. R. (2018). A possible role for platelet-activating factor receptor in amyotrophic lateral sclerosis treatment. *Frontiers in Neurology*, *9*(FEB), 5–9.

Bührer, C., Endesfelder, S., Scheuer, T., & Schmitz, T. (2021). Paracetamol (acetaminophen) and the developing brain. *International Journal of Molecular Sciences*, *22*(20), 11156.

Cendejas-Hernandez, J., Sarafian, J. T., Lawton, V. G., Palkar, A., Anderson, L. G., Larivière, V., & Parker, W. (2022). Paracetamol (acetaminophen) use in infants and children was never shown to be safe for neurodevelopment: a systematic review with citation tracking. *European Journal of Pediatrics*, *181*(5), 1835–1857.

Chambers, C. (2015). Over-the-counter medications: risk and safety in pregnancy. *Seminars in Perinatology*, *39*(7), 541–544.

Conings, S., Tseke, F., Broeck, A. Van Den, Qi, B., Paulus, J., Amant, F., Annaert, P., & Calsteren, K. Van. (2019). Transplacental transport of paracetamol and its phase II metabolites using the ex vivo placenta perfusion model. *Toxicology and Applied Pharmacology*, *370*(November 2018), 14–23.

Cristino, Bisogno, T., & Di Marzo, V. (2020). Cannabinoids and the expanded endocannabinoid system in neurological disorders. *Nature Reviews Neurology*, *16*(1), 9–29.

Cristino, L., de Petrocellis, L., Pryce, G., Baker, D., Guglielmotti, V., & Di Marzo, V. (2006). Immunohistochemical localization of cannabinoid type 1 and vanilloid transient receptor

potential vanilloid type 1 receptors in the mouse brain. *Neuroscience*, *139*(4), 1405–1415.

de Brouwer, G., Fick, A., Harvey, B. H., & Wolmarans, D. W. (2019). A critical inquiry into marble-burying as a preclinical screening paradigm of relevance for anxiety and obsessive–compulsive disorder: Mapping the way forward. *Cognitive, Affective and Behavioral Neuroscience*, *19*(1), 1–39.

Dixit, P. V., Sahu, R., & Mishra, D. K. (2020). Marble-burying behavior test as a murine model of compulsive-like behavior. *Journal of Pharmacological and Toxicological Methods*, *102*(August 2019), 106676.

Duggan, K. C., Hermanson, D. J., Musee, J., Prusakiewicz, J. J., Scheib, J. L., Carter, B. D., Banerjee, S., Oates, J. A., & Marnett, L. J. (2011). (R)-Profens are substrate-selective inhibitors of endocannabinoid oxygenation by COX-2. *Nature Chemical Biology*, *7*(11), 803–809.

Ellis, P. D. (2010). *The essential guide to effect sizes*. Cambridge: Cambridge University Press. Retrieved from https://www.ptonline.com/articles/how-to-get-better-mfi-results

Ernst, A. M., & Smelik, P. G. (1966). Site of action of dopamine and apomorphine on compulsive gnawing behaviour in rats. *Experientia*, *22*(12), 837–838.

Estanislau, C., Díaz-Morán, S., Cañete, T., Blázquez, G., Tobeña, a., & Fernández-Teruel, a. (2013). Context-dependent differences in grooming behavior among the NIH heterogeneous stock and the Roman high- and low-avoidance rats. *Neuroscience Research*, *77*(4), 187–201.

Fagundes, B. H. F., Nascimento, P. C., Aragão, W. A. B., Chemelo, V. S., Bittencourt, L. O., Eiró-Quirino, L., Silva, M. C. F., Freire, M. A. M., Fernandes, L. M. P., Maia, C. do S. F., Crespo-Lopez, M. E., & Lima, R. R. (2022). Methylmercury exposure during prenatal and postnatal neurodevelopment promotes oxidative stress associated with motor and cognitive damages in rats: an environmental-experimental toxicology study. *Toxicology Reports*, *9*, 563–574.

Favaro, P. d N., Costa, L. C., & Moreira, E. G. (2008). Maternal fluoxetine treatment decreases behavioral response to dopaminergic drugs in female pups. *Neurotoxicology and Teratology*, *30*(6), 487–494.

FDA. (2016). FDA Drug Safety Communication: FDA has reviewed possible risks of pain medicine use during pregnancy. Retrieved from http://www.fda.gov/Drugs/DrugSafety/ucm429117.htm%5Cnhttp://www.medscape.com/viewarticle/837858?src=wnl_edit_newsal

Foley, K. A., MacFabe, D. F., Vaz, A., Ossenkopp, K. P., & Kavaliers, M. (2014). Sexually

dimorphic effects of prenatal exposure to propionic acid and lipopolysaccharide on social behavior in neonatal, adolescent, and adult rats: Implications for autism spectrum disorders. *International Journal of Developmental Neuroscience*, *39*(C), 68–78.

Golding, J., Gregory, S., Clark, R., Ellis, G., Iles-Caven, Y., & Northstone, K. (2020). Associations between paracetamol (acetaminophen) intake between 18 and 32 weeks gestation and neurocognitive outcomes in the child: A longitudinal cohort study. *Paediatric and Perinatal Epidemiology*, *34*(3), 257–266.

Golub, M. S., & Sobin, C. A. (2020). Statistical modeling with litter as a random effect in mixed models to manage "intralitter likeness." *Neurotoxicology and Teratology*, *77*, 106841.

Gonsiorek, W., Lunn, C., Fan, X., Narula, S., Lundell, D., & Hipkin, R. W. (2000). Endocannabinoid 2-arachidonyl glycerol is a full agonist through human type 2 cannabinoid receptor: Antagonism by anandamide. *Molecular Pharmacology*, *57*(5), 1045–1050.

Gou, X., Wang, Y., Tang, Y., Qu, Y., Tang, J., Shi, J., Xiao, D., & Mu, D. (2019). Association of maternal prenatal acetaminophen use with the risk of attention deficit/hyperactivity disorder in offspring: A meta-analysis. *Australian and New Zealand Journal of Psychiatry*, *53*(3), 195–206.

Gregory, E. H., & Pfaff, D. W. (1971). Development of olfactory-guided behavior in infant rats. *Physiology & Behavior*, *6*, 573–576.

Haas, D. M., Marsh, D. J., Dang, D. T., Parker, C. B., Wing, D. A., Simhan, H. N., … Reddy, U. M. (2018). Prescription and other medication use in pregnancy. *Obstetrics and Gynecology*, *131*(5), 789–798.

Harkany, T., & Cinquina, V. (2021). Physiological rules of endocannabinoid action during fetal and neonatal brain development. *Cannabis and Cannabinoid Research*, *6*(5), 381–388.

Harshaw, C., & Warner, A. G. (2022). Interleukin-1β-induced inflammation and acetaminophen during infancy: Distinct and interactive effects on social-emotional and repetitive behavior in C57BL/6J mice. *Pharmacology Biochemistry and Behavior*, *220*(September).

Hershkowitz, M., & Adunsky, A. (1996). Binding of platelet-activating factor to platelets of Alzheimer's disease and multiinfarct dementia patients. *Neurobiology of Aging*, *17*(6), 865–868.

Heyer, D. B., & Meredith, R. M. (2017). Environmental toxicology: Sensitive periods of development and neurodevelopmental disorders. *NeuroToxicology*, *58*, 23–41.

Högestätt, E. D., Jönsson, B. A. G., Ermund, A., Andersson, D. A., Björk, H., Alexander, J. P.,

Cravatt, B. F., Basbaum, A. I., & Zygmunt, P. M. (2005). Conversion of acetaminophen to the bioactive N-acylphenolamine AM404 via fatty acid amide hydrolase-dependent arachidonic acid conjugation in the nervous system. *The Journal of Biological Chemistry*, *280*(36), 31405–31412.

Hoshijima, H., Hunt, M., Nagasaka, H., & Yaksh, T. (2021). Systematic review of systemic and neuraxial effects of acetaminophen in preclinical models of nociceptive processing. *Journal of Pain Research, Volume 14*, 3521–3552.

Hsu, K.-L., Tsuboi, K., Adibekian, A., Pugh, H., Masuda, K., & Cravatt, B. F. (2012). DAGLβ inhibition perturbs a lipid network involved in macrophage inflammatory responses. *Nature Chemical Biology*, *8*(12), 999–1007.

Hussain, F. N., & Ashmead, G. G. (2017). The safety of over-the-counter medications in pregnancy. *Topics in Obstetrics & Gynecology*, *37*(14), 1–8.

Ishihara, Y., Honda, T., Ishihara, N., Namba, K., Taketoshi, M., Tominaga, Y., Tsuji, M., Vogel, C. F. A., Yamazaki, T., Itoh, K., & Tominaga, T. (2022). A CCR5 antagonist, maraviroc, alleviates neural circuit dysfunction and behavioral disorders induced by prenatal valproate exposure. *Journal of Neuroinflammation*, *19*(1), 195.

Jetten, M. J. A., Gaj, S., Ruiz-Aracama, A., de Kok, T. M., van Delft, J. H. M., Lommen, A., van Someren, E. P., Jennen, D. G. J., Claessen, S. M., Peijnenburg, A. A. C. M., Stierum, R. H., & Kleinjans, J. C. S. (2012). 'Omics analysis of low dose acetaminophen intake demonstrates novel response pathways in humans. *Toxicology and Applied Pharmacology*, *259*(3), 320–328.

Kaczocha, M., Vivieca, S., Sun, J., Glaser, S. T., & Deutsch, D. G. (2012). Fatty acid-binding proteins transport N-acylethanolamines to nuclear receptors and are targets of endocannabinoid transport inhibitors. *Journal of Biological Chemistry*, *287*(5), 3415–3424.

Kim, H., Lim, C. S., & Kaang, B. K. (2016). Neuronal mechanisms and circuits underlying repetitive behaviors in mouse models of autism spectrum disorder. *Behavioral and Brain Functions*, *12*(1), 1–13.

Kim, S.-A., Chai, J.-H., & Jang, E.-H. (2021). Prenatal trimethyltin exposure induces long-term DNA methylation changes in the male mouse hippocampus. *International Journal of Molecular Sciences*, *22*(15), 8009.

Klein, R. M., Rigobello, C., Vidigal, C. B., Moura, K. F., Barbosa, D. S., Gerardin, D. C. C., Ceravolo, G. S., & Moreira, E. G. (2020). Gestational exposure to paracetamol in rats induces neurofunctional alterations in the progeny. *Neurotoxicology and Teratology*,

77(September 2019), 106838.

Koehn, L. M., Huang, Y., Habgood, M. D., Kysenius, K., Crouch, P. J., Dziegielewska, K. M., & Saunders, N. R. (2020). Effects of paracetamol (acetaminophen) on gene expression and permeability properties of the rat placenta and fetal brain. *F1000Research*, *9*.

Komorowska-Müller, J. A., & Schmöle, A. C. (2021). CB2 receptor in microglia: The guardian of self-control. *International Journal of Molecular Sciences*, *22*(1), 1–27.

Lakens, D. (2013). Calculating and reporting effect sizes to facilitate cumulative science: A practical primer for t-tests and ANOVAs. *Frontiers in Psychology*, *4*(NOV), 1–12.

Langen, M., Durston, S., Kas, M. J. H., van Engeland, H., & Staal, W. G. (2011). The neurobiology of repetitive behavior: of mice... *Neuroscience and Biobehavioral Reviews*, *35*(3), 356–365.

Laue, H. E., Cassoulet, R., Abdelouahab, N., Serme-Gbedo, Y. K., Desautels, A. S., Brennan, K. J. M., Bellenger, J. P., Burris, H. H., Coull, B. A., Weisskopf, M. G., Takser, L., & Baccarelli, A. A. (2019). Association between meconium acetaminophen and childhood neurocognitive development in GESTE, a canadian cohort study. *Toxicological Sciences : An Official Journal of the Society of Toxicology*, *167*(1), 138–144.

Leppert, B., Havdahl, A., Riglin, L., Jones, H. J., Zheng, J., Davey Smith, G., Tilling, K., Thapar, A., Reichborn-Kjennerud, T., & Stergiakouli, E. (2019). Association of maternal neurodevelopmental risk alleles with early-life exposures. *JAMA Psychiatry*.

Levine, A., Liktor-Busa, E., Lipinski, A. A., Couture, S., Balasubramanian, S., Aicher, S. A., Langlais, P. R., Vanderah, T. W., & Largent-Milnes, T. M. (2021). Sex differences in the expression of the endocannabinoid system within V1M cortex and PAG of Sprague Dawley rats. *Biology of Sex Differences*, *12*(1), 60.

Liew, Z., Bach, C. C., Asarnow, R. F., Ritz, B., & Olsen, J. (2016). Paracetamol use during pregnancy and attention and executive function in offspring at age 5 years. *International Journal of Epidemiology*, *45*(6), 2009–2017.

Liew, Z., Ritz, B., Rebordosa, C., Lee, P.-C., & Olsen, J. (2014). Acetaminophen use during pregnancy, behavioral problems, and hyperkinetic disorders. *JAMA Pediatrics*, *168*(4), 313–320.

Liew, Z., Ritz, B., Virk, J., & Olsen, J. (2016). Maternal use of acetaminophen during pregnancy and risk of autism spectrum disorders in childhood: a Danish national birth cohort study. *Autism Research*, *9*(9), 951–958.

Liu, J., Bátkai, S., Pacher, P., Harvey-White, J., Wagner, J. A., Cravatt, B. F., Gao, B., & Kunos, G. (2003). Lipopolysaccharide induces anandamide synthesis in macrophages via

CD14/MAPK/phosphoinositide 3-kinase/NF-κB independently of platelet-activating factor. *Journal of Biological Chemistry*, *278*(45), 45034–45039.

Liu, Y., Shields, L. B. E., Gao, Z., Wang, Y., Zhang, Y. P., Chu, T., Zhu, Q., Shields, C. B., & Cai, J. (2017). Current understanding of platelet-activating factor signaling in central nervous system diseases. *Molecular Neurobiology*, *54*(7), 5563–5572.

Lordan, R., Tsoupras, A., Zabetakis, I., & Demopoulos, C. A. (2019). Forty years since the structural elucidation of platelet-activating factor (PAF): Historical, current, and future research perspectives. *Molecules*, *24*(23).

Lu, H. C., & Mackie, K. (2021). Review of the endocannabinoid system. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*, *6*(6), 607–615.

Lye, J. M., Knight, J. A., Arneja, J., Seeto, R. A., Wong, J., Adel khani, N., Brooks, J. D., Levitan, R. D., Matthews, S. G., Lye, S. J., & Hung, R. J. (2022). Maternal acetaminophen use and cognitive development at 4 years: the Ontario Birth Study. *Pediatric Research*, (June 2021), 1–5.

MacCarrone, M., Guzmán, M., MacKie, K., Doherty, P., & Harkany, T. (2014). Programming of neural cells by (endo)cannabinoids: From physiological rules to emerging therapies. *Nature Reviews Neuroscience*, *15*(12), 786–801.

Marco, E. M., Viveros, M.-P., Borcel, E., Pérez-Alvarez, L., Guaza, C., Ramirez, B. G., de Ceballos, M. L., Rubio, M., & Fernández, B. (2004). Functional responses to the cannabinoid agonist WIN 55,212-2 in neonatal rats of both genders: influence of weaning. *Pharmacology Biochemistry and Behavior*, *78*(3), 593–602.

Masarwa, R., Levine, H., Gorelik, E., Reif, S., Perlman, A., & Matok, I. (2018). Prenatal exposure to acetaminophen and risk for attention deficit hyperactivity disorder and autistic spectrum disorder: A systematic review,meta-analysis, and meta-regression analysis of cohort studies. *American Journal of Epidemiology*, *187*(8), 1817–1827.

Masarwa, R., Platt, R. W., & Filion, K. B. (2020). Acetaminophen use during pregnancy and the risk of attention deficit hyperactivity disorder: A causal association or bias? *Paediatric and Perinatal Epidemiology*, *34*(3), 309–317.

McCrae, J. C., Morrison, E. E., MacIntyre, I. M., Dear, J. W., & Webb, D. J. (2018). Long-term adverse effects of paracetamol – a review. *British Journal of Clinical Pharmacology*, *84*(10), 2218–2230.

McLemore, G. L., & Richardson, K. A. (2016). Data from three prospective longitudinal human cohorts of prenatal marijuana exposure and offspring outcomes from the fetal period through young adulthood. *Data in Brief*, *9*, 753–757.

Mehta, T. R., Monegro, A., Nene, Y., Fayyaz, M., & Bollu, P. C. (2019). Neurobiology of ADHD: A Review. *Current Developmental Disorders Reports*, *6*(4), 235–240.

Melancia, F., Schiavi, S., Servadio, M., Cartocci, V., Campolongo, P., Palmery, M., Pallottini, V., & Trezza, V. (2018). Sex-specific autistic endophenotypes induced by prenatal exposure to valproic acid involve anandamide signalling. *British Journal of Pharmacology*, *175*(18), 3699–3712.

Moreira, F. A., Aguiar, D. C., Terzian, A. L. B., Guimarães, F. S., & Wotjak, C. T. (2012). Cannabinoid type 1 receptors and transient receptor potential vanilloid type 1 channels in fear and anxiety - two sides of one coin? *Neuroscience*, *204*, 186–192.

Muramatsu, S., Shiraishi, S., Miyano, K., Sudo, Y., Toda, A., Mogi, M., Hara, M., Yokoyama, A., Kawasaki, Y., Taniguchi, M., & Uezono, Y. (2016). Metabolism of AM404 from acetaminophen at human therapeutic dosages in the rat brain. *Anesthesiology and Pain Medicine*, *6*(1), 1–5.

Murataeva, N., Straiker, A., & MacKie, K. (2014). Parsing the players: 2-arachidonoylglycerol synthesis and degradation in the CNS. *British Journal of Pharmacology*, *171*(6), 1379–1391.

Nielsen, E., Ostergaard, G., & Larsen, J. (2008). *Toxicological risk assessment of chemicals: a practical guide.* New York: Informa Healthcare USA,.

Niesink, R. J. M., & Van Ree, J. M. (1989). Involvement of opioid and dopaminergic systems in isolation-induced pinning and social grooming of young rats. *Neuropharmacology*, *28*(4), 411–418.

Nitsche, J. F., Patil, A. S., Langman, L, J., Penn, H. J., Derleth, D., Watson, W. J., & Brost, B. C. (2017). Transplacental passage of acetaminophen in term pregnancy. *American Journal of Perinatology*, *34*(6), 541–543.

Nomura, D. K., Morrison, B. E., Blankman, J. L., Long, J. Z., Kinsey, S. G., Marcondes, M. C. G., Ward, A. M., Hahn, Y. K., Lichtman, A. H., Conti, B., & Cravatt, B. F. (2011). Endocannabinoid hydrolysis generates brain prostaglandins that promote neuroinflammation. *Science*, *334*(6057), 809–813.

OECD. (2007). OECD guideline for the testing of chemicals 426 - Developmental Neurotoxicity Study, (September), 1–38.

Olivier, J. D. a, Vallès, a., Van Heesch, F., Afrasiab-Middelman, A., Roelofs, J. J. P. M., Jonkers, M., Peeters, E. J., Korte-Bouws, G. a H., Dederen, J. P., Kiliaan, A. J., Martens, G. J., Schubert, D., & Homberg, J. R. (2011). Fluoxetine administration to pregnant rats increases anxiety-related behavior in the offspring. *Psychopharmacology*, *217*(3), 419–

432.

Palmer, A. A., Brown, A. S., Keegan, D., Siska, L. D., Susser, E., Rotrosen, J., & Butler, P. D. (2008). Prenatal protein deprivation alters dopamine-mediated behaviors and dopaminergic and glutamatergic receptor binding. *Brain Research*, *1237*, 62–74.

Pandolfo, P., Pamplona, F. A., Prediger, R. D. S., & Takahashi, R. N. (2007). Increased sensitivity of adolescent spontaneously hypertensive rats, an animal model of attention deficit hyperactivity disorder, to the locomotor stimulation induced by the cannabinoid receptor agonist WIN 55,212-2. *European Journal of Pharmacology*, *563*(1–3), 141–148.

Panikashvili, D., Simeonidou, C., Ben-Shabat, S., Hanuš, L., Breuer, A., Mechoulam, R., & Shohami, E. (2001). An endogenous cannabinoid (2-AG) is neuroprotective after brain injury. *Nature*, *413*(6855), 527–531.

Parker, W., Hornik, C. D., Bilbo, S., Holzknecht, Z. E., Gentry, L., Rao, R., Lin, S. S., Herbert, M. R., & Nevison, C. D. (2017). The role of oxidative stress, inflammation and acetaminophen exposure from birth to early childhood in the induction of autism. *Journal of International Medical Research*, *45*(2), 407–438.

Parrini, M., Ghezzi, D., Deidda, G., Medrihan, L., Castroflorio, E., Alberti, M., Baldelli, P., Cancedda, L., & Contestabile, A. (2017). Aerobic exercise and a BDNF-mimetic therapy rescue learning and memory in a mouse model of Down syndrome. *Scientific Reports*, *7*(1), 1–22.

Patel, S., & Windsor, M. A. (2015). Patent application publication (10) Pub. No.: US 2015/0183737 A1.

Penaloza, C. G., Cruz, M., Germain, G., Jabeen, S., Javdan, M., Lockshin, R. A., & Zakeri, Z. (2020). Higher sensitivity of female cells to ethanol: Methylation of DNA lowers Cyp2e1, generating more ROS. *Cell Communication and Signaling*, *18*(1), 1–15.

Perazzo, J. C., Fiebich, B. L., Fortwängler, E., Muñoz, E., Marcotegui, A. R., Saliba, S. W., de Oliveira, A. C. P., & Ditrich, J. (2017). AM404, paracetamol metabolite, prevents prostaglandin synthesis in activated microglia by inhibiting COX activity. *Journal of Neuroinflammation*, *14*(1), 1–11.

Philippot, G., Gordh, T., Fredriksson, A., & Viberg, H. (2017). Adult neurobehavioral alterations in male and female mice following developmental exposure to paracetamol (acetaminophen): characterization of a critical period. *Journal of Applied Toxicology*, *37*(10), 1174–1181.

Philippot, G., Hallgren, S., Gordh, T., Fredriksson, A., Fredriksson, R., & Viberg, H. (2018). A cannabinoid receptor type 1 (CB1R) agonist enhances the developmental neurotoxicity of

acetaminophen (Paracetamol). *Toxicological Sciences*, *166*(1), 203–212.

Philippot, G., Hosseini, K., Yakub, A., Mhajar, Y., Hamid, M., Buratovic, S., & Fredriksson, R. (2022). Paracetamol (acetaminophen) and its effect on the developing mouse brain. *Frontiers in Toxicology*, *4*(March), 1–11.

Pinto, B., Morelli, G., Rastogi, M., Savardi, A., Fumagalli, A., Petretto, A., Bartolucci, M., Varea, E., Catelani, T., Contestabile, A., Perlini, L. E., & Cancedda, L. (2020). Rescuing over-activated microglia restores cognitive performance in juvenile animals of the Dp(16) mouse model of Down syndrome. *Neuron*, (16), 1–18.

Pletnikov, M. V., Rubin, S. A., Vasudevan, K., Moran, T. H., & M. Carbone, K. (1999). Developmental brain injury associated with abnormal play behavior in neonatally Borna disease virus-infected Lewis rats: A model of autism. *Behavioural Brain Research*, *100*(1–2), 43–50.

Polissidis, A., Chouliara, O., Galanopoulos, A., Marselos, M., Papadopoulou-Daifoti, Z., & Antoniou, K. (2009). Behavioural and dopaminergic alterations induced by a low dose of WIN 55,212-2 in a conditioned place preference procedure. *Life Sciences*, *85*(5–6), 248–254.

Polli, F. S., Scharff, M. B., Ipsen, T. H., Aznar, S., Kohlmeier, K. A., & Andreasen, J. T. (2020). Prenatal nicotine exposure in mice induces sex-dependent anxiety-like behavior, cognitive deficits, hyperactivity, and changes in the expression of glutamate receptor associated-genes in the prefrontal cortex. *Pharmacology Biochemistry and Behavior*, *195*, 172951.

Prut, L., & Belzung, C. (2003). The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: A review. *European Journal of Pharmacology*, *463*(1–3), 3–33.

Przybyła, G. W., Szychowski, K. A., & Gmiński, J. (2021). Paracetamol – An old drug with new mechanisms of action. *Clinical and Experimental Pharmacology and Physiology*, *48*(1), 3–19.

Ray-Griffith, S. L., Morrison, B., & Stowe, Z. N. (2019). Chronic Pain Prevalence and Exposures during Pregnancy. *Pain Research and Management*, *2019*.

Rice, D., & Barone, S. (2000). Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environmental Health Perspectives*, *108*(3), 511–533.

Rifas-Shiman, S. L., Cardenas, A., Hivert, M. F., Tiemeier, H., Bertoldi, A. D., & Oken, E. (2020). Associations of prenatal or infant exposure to acetaminophen or ibuprofen with mid-childhood executive function and behaviour. *Paediatric and Perinatal Epidemiology*, *34*(3), 287–298.

Rigobello, C., Klein, R. M., Debiasi, J. D., Ursini, L. G., Michelin, A. P., Matsumoto, A. K., Barbosa, D. S., & Moreira, E. G. (2021). Perinatal exposure to paracetamol: dose and sex-dependent effects in behaviour and brain's oxidative stress markers in progeny. *Behavioural Brain Research*, *408*(March).

Rivera, P., Tovar, R., Ramírez-López, M. T., Navarro, J. A., Vargas, A., Suárez, J., & Fonseca, F. R. de. (2020). Sex-specific anxiety and prefrontal cortex glutamatergic dysregulation are long-term consequences of pre-and postnatal exposure to hypercaloric diet in a rat model. *Nutrients*, *12*(6), 1829.

Ross, E. J., Graham, D. L., Money, K. M., & Stanwood, G. D. (2015). Developmental consequences of fetal exposure to drugs: What we know and what we still must learn. *Neuropsychopharmacology*, *40*(1), 61–87.

Rudenko, V., Rafiuddin, A., Leheste, J. R., & Friedman, L. K. (2012). Inverse relationship of cannabimimetic (R+)WIN 55, 212 on behavior and seizure threshold during the juvenile period. *Pharmacology Biochemistry and Behavior*, *100*(3), 474–484.

Saad, A., Hegde, S., Kechichian, T., Gamble, P., Rahman, M., Stutz, S. J., Anastasio, N. C., Alshehri, W., Lei, J., Mori, S., Kajs, B., Cunningham, K. A., Saade, G., Burd, I., & Costantine, M. (2016). Is there a causal relation between maternal acetaminophen administration and ADHD? *PLoS ONE*, *11*(6), 1–10.

Sañudo-Peña, M. C., Romero, J., Seale, G. E., Fernandez-Ruiz, J. J., & Walker, J. M. (2000). Activational role of cannabinoids on movement. *European Journal of Pharmacology*, *391*(3), 269–274.

Schneider, M., Schömig, E., & Leweke, F. M. (2008). Acute and chronic cannabinoid treatment differentially affects recognition memory and social behavior in pubertal and adult rats. *Addiction Biology*, *13*(3–4), 345–357.

Schneider, T., & Przewłocki, R. (2005). Behavioral alterations in rats prenatally exposed to valproic acid: animal model of autism. *Neuropsychopharmacology*, *30*(1), 80–89.

Schultz, S., Gould, G. G., Antonucci, N., Brigida, A. L., & Siniscalco, D. (2021). Endocannabinoid system dysregulation from acetaminophen use may lead to autism spectrum disorder: could cannabinoid treatment be efficacious? *Molecules*, *26*(7), 1845.

Schultz, S. T., & Gould, G. G. (2016). Acetaminophen use for fever in children associated with autism spectrum disorder. *Autism Open Access*, *6*(2), 1000170.

Scipioni, L., Ciaramellano, F., Carnicelli, V., Leuti, A., Lizzi, A. R., De Dominicis, N., Oddi, S., & Maccarrone, M. (2022). Microglial endocannabinoid signalling in AD. *Cells*, *11*(7), 1237.

Servadio, M., Melancia, F., Manduca, A., Di Masi, A., Schiavi, S., Cartocci, V., Pallottini, V., Campolongo, P., Ascenzi, P., & Trezza, V. (2016). Targeting anandamide metabolism rescues core and associated autistic-like symptoms in rats prenatally exposed to valproic acid. *Translational Psychiatry*, *6*(9), 1–11.

Servadio, Michela, Manduca, A., Melancia, F., Leboffe, L., Schiavi, S., Campolongo, P., Palmery, M., Ascenzi, P., di Masi, A., & Trezza, V. (2018). Impaired repair of DNA damage is associated with autistic-like traits in rats prenatally exposed to valproic acid. *European Neuropsychopharmacology*, *28*(1), 85–96.

Setler, P., Sarau, H., & McKenzie, G. (1976). Differential attenuation of some effects of haloperidol in rats given scopolamine. *European Journal of Pharmacology*, *39*, 117–126.

Sharma, Jamie, H., Shah, S., Perrett, D., & Ayoub, S. S. (2017). First evidence of the conversion of paracetamol to AM404 in human cerebrospinal fluid. *Journal of Pain Research*, *10*, 2703–2709.

Sholler, D. J., Strickland, J. C., Spindle, T. R., Weerts, E. M., & Vandrey, R. (2021). Sex differences in the acute effects of oral and vaporized cannabis among healthy adults. *Addiction Biology*, *26*(4), 1–12.

Silver, R. J. (2019). The endocannabinoid system of animals. *Animals*, *9*(9), 686.

Spagnolo, P. A., Garvey, M., & Hallett, M. (2021). A dimensional approach to functional movement disorders: Heresy or opportunity. *Neuroscience and Biobehavioral Reviews*, *127*(November 2020), 25–36.

Stark, T., Iannotti, F. A., Di Martino, S., Di Bartolomeo, M., Ruda-Kucerova, J., Piscitelli, F., Wotjak, C. T., D'Addario, C., Drago, F., Di Marzo, V., & Micale, V. (2022). Early blockade of CB1 receptors ameliorates schizophrenia-like alterations in the neurodevelopmental MAM model of schizophrenia. *Biomolecules*, *12*(1), 108.

Stasiulewicz, A., Znajdek, K., Grudzień, M., Pawiński, T., & Sulkowska, A. J. I. (2020). A guide to targeting the endocannabinoid system in drug design. *International Journal of Molecular Sciences*, *21*(8), 1–46.

Subbanna, S., Psychoyos, D., Xie, S., & Basavarajappa, B. S. (2015). Postnatal ethanol exposure alters levels of 2-arachidonylglycerol-metabolizing enzymes and pharmacological inhibition of monoacylglycerol lipase does not cause neurodegeneration in neonatal mice. *Journal of Neurochemistry*, *134*(2), 276–287.

Sznajder, K. K., Teti, D. M., & Kjerulff, K. H. (2022). Maternal use of acetaminophen during pregnancy and neurobehavioral problems in offspring at 3 years: A prospective cohort study. *PLoS ONE*, *17*(9 September), 1–17.

Taylor, P. V., Veenema, A. H., Paul, M. J., Bredewold, R., Isaacs, S., & De Vries, G. J. (2012). Sexually dimorphic effects of a prenatal immune challenge on social play and vasopressin expression in juvenile rats. *Biology of Sex Differences*, *3*(1), 1.

Terral, G., Marsicano, G., Grandes, P., & Soria-Gómez, E. (2020). Cannabinoid control of olfactory processes: the where matters. *Genes*, *11*(431), 1–8.

Thomas, A., Burant, A., Bui, N., Graham, D., Yuva-Paylor, L. A., & Paylor, R. (2009). Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety. *Psychopharmacology*, *204*(2), 361–373.

Thompson, J. M. D., Waldie, K. E., Wall, C. R., Murphy, R., & Mitchell, E. A. (2014). Associations between acetaminophen use during pregnancy and ADHD symptoms measured at ages 7 and 11 years. *PLoS ONE*, *9*(9), 1–6.

Thornton, A. M., Humphrey, R. M., Kerr, D. M., Finn, D. P., & Roche, M. (2021). Increasing endocannabinoid tone alters anxiety-like and stress coping behaviour in female rats prenatally exposed to valproic acid. *Molecules*, *26*(12), 3720.

Tong, N., Sanchez, J. F., Maggirwar, S. B., Ramirez, S. H., Guo, H., Dewhurst, S., & Gelbard, H. A. (2001). Activation of glycogen synthase kinase 3 beta (GSK-3β) by platelet activating factor mediates migration and cell death in cerebellar granule neurons. *European Journal of Neuroscience*, *13*(10), 1913–1922.

Tovo-Rodrigues, L., Carpena, M. X., Martins-Silva, T., Santos, I. S., Anselmi, L., Barros, A. J. D., Barros, F. C., Bertoldi, A. D., & Matijasevich, A. (2020). Low neurodevelopmental performance and behavioural/emotional problems at 24 and 48 months in Brazilian children exposed to acetaminophen during foetal development. *Paediatric and Perinatal Epidemiology*, *34*(3), 278–286.

Trezza, V., & Vanderschuren, L. J. M. J. (2008). Bidirectional cannabinoid modulation of social behavior in adolescent rats. *Psychopharmacology*, *197*(2), 217–227.

Urquhart, P., Nicolaou, A., & Woodward, D. F. (2015). Endocannabinoids and their oxygenation by cyclo-oxygenases, lipoxygenases and other oxygenases. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids*, *1851*(4), 366–376.

USEPA. (2006). Harmonization in interspecies extrapolation: use of BW 3/4 as default method in derivation of the oral RfD, (68), 1–34.

Van Der Stelt, M., Mazzola, C., Esposito, G., Matias, I., Petrosino, S., De Filippis, D., Micale, V., Steardo, L., Drago, F., Iuvone, T., & Di Marzo, V. (2006). Endocannabinoids and β-amyloid-induced neurotoxicity in vivo: Effect of pharmacological elevation of endocannabinoid levels. *Cellular and Molecular Life Sciences*, *63*(12), 1410–1424.

Viberg, H., Eriksson, P., Gordh, T., & Fredriksson, A. (2014). Paracetamol (acetaminophen) administration during neonatal brain development affects cognitive function and alters its analgesic and anxiolytic response in adult male mice. *Toxicological Sciences*, *138*(1), 139–147.

Vicente, A. M., Martins, G. J., & Costa, R. M. (2020). Cortico-basal ganglia circuits underlying dysfunctional control of motor behaviors in neuropsychiatric disorders. *Current Opinion in Genetics and Development*, *65*, 151–159.

Vogel-Ciernia, A., Careaga, M., LaSalle, J. M., & Ashwood, P. (2018). Microglia from offspring of dams with allergic asthma exhibit epigenomic alterations in genes dysregulated in autism. *Glia*, *66*(3), 505–521.

Wiley, J. L., Barrus, D. G., Farquhar, C. E., Lefever, T. W., & Gamage, T. F. (2021). Sex, species and age: effects of rodent demographics on the pharmacology of Δ9-tetrahydrocanabinol. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *106*(April 2020).

Wilkerson, J. L., Ghosh, S., Bagdas, D., Mason, B. L., Crowe, M. S., Hsu, K. L., Wise, L. E., Kinsey, S. G., Damaj, M. I., Cravatt, B. F., & Lichtman, A. H. (2016). Diacylglycerol lipase β inhibition reverses nociceptive behaviour in mouse models of inflammatory and neuropathic pain. *British Journal of Pharmacology*, *173*(10), 1678–1692.

Wilkes, B. J., & Lewis, M. H. (2018). The neural circuitry of restricted repetitive behavior: Magnetic resonance imaging in neurodevelopmental disorders and animal models. *Neuroscience and Biobehavioral Reviews*, *92*(May), 152–171.

Witchey, S. K., Al Samara, L., Horman, B. M., Stapleton, H. M., & Patisaul, H. B. (2020). Perinatal exposure to FireMaster® 550 (FM550), brominated or organophosphate flame retardants produces sex and compound specific effects on adult Wistar rat socioemotional behavior. *Hormones and Behavior*, *126*, 104853.

Wood, M. E. (2020). Associations between prenatal acetaminophen exposure and child neurodevelopment: Truth, bias, or a bit of both? *Paediatric and Perinatal Epidemiology*, *34*(3), 233–236.

Xi, C., Yang, Z., Yu, Y., Li, S., He, J., El-Aziz, T. M. A., Zhao, F., & Cao, Z. (2022). Influence of perinatal deltamethrin exposure at distinct developmental stages on motor activity, learning and memory. *Ecotoxicology and Environmental Safety*, *236*, 113460.

Ystrom, E., Gustavson, K., Brandlistuen, R. E., Knudsen, G. P., Magnus, P., Susser, E., Davey Smith, G., Stoltenberg, C., Surén, P., Håberg, S. E., Hornig, M., Lipkin, W. I., Nordeng, H., & Reichborn-Kjennerud, T. (2017). Prenatal exposure to acetaminophen and risk of

ADHD. *Pediatrics*, *140*(5), 1–9.

Zou, M., Liu, Y., Xie, S., Wang, L., Li, D., Li, L., Wang, F., Zhang, Y., Xia, W., Sun, C., & Wu, L. (2021). Alterations of the endocannabinoid system and its therapeutic potential in autism spectrum disorder. *Open Biology*, *11*(2).

Zucker, I. (2017). Risk mitigation for children exposed to drugs during gestation: A critical role for animal preclinical behavioral testing. *Neuroscience and Biobehavioral Reviews*, *77*, 107–121.

Zygmunt, P. M., Chuang, H. H., Movahed, P., Julius, D., & Högestätt, E. D. (2000). The anandamide transport inhibitor AM404 activates vanilloid receptors. *European Journal of Pharmacology*, *396*(1), 39–42.

Supplementary Table 1. Behavioral and neurochemical evaluation of infant rats exposed or not to PAR during gestation.

	CTL		PAR	
	Females	Males	Females	Males
Novel object recognition	***(n= 10)***	***(n= 11)***	***(n= 14)***	***(n= 11)***
Familiar object exploration (s)	10.5 ± 4.6 #	5.9 ± 3.9	9.8 ± 5.4 #	5.2 ± 3.2
Novel object exploration (s)	10.1 ± 3.7	12.0 ± 7.5	10.4 ± 3.5	9.4 ± 4.8
Discrimination index (%)	48.8 ± 18	68.1 ± 17	60.5 ± 15	69.0 ± 16
Hot plate	***(n= 10)***	***(n= 12)***	***(n= 14)***	***(n= 11)***
Reaction latency (s)	9.46 ± 1.0	9.54 ± 0.8	11.8 ± 1.3	9.4 ± 0.9
Social play behavior	***(n= 7)***	***(n= 8)***	***(n= 10)***	***(n= 9)***
Pinning (count)	9.3 ± 8.9	13.0 ± 4.3	6.1 ± 3.7	11.7 ± 4.6
Pouncing (count)	6.6 ± 4.2 #	11.1 ± 2.2	6.2 ± 1.4 #	10.3 ± 2.7
Following (s)	7.5 ± 6.5 #	23.7 ± 7.8	11.6 ± 6.4 #	18.1 ± 7.4
Sniffing and licking (s)	32.4 ± 10.6 #	50.3 ± 16.1	33.9 ± 5.7 #	42.1 ± 8.3
Prefrontal cortex				
LC-MS	***(n = 6)***	***(n= 7)***	***(n= 7)***	***(n= 7)***
2-AG (pg/mg of tissue)	214.2 ± 357.8	89.1 ± 183.3	279.2 ± 179.4	256.0 ± 345.0
2-AG (Ln)	3.5 ± 2.8	2.0 ± 2.3	5.3 ± 0.9 *	4.9 ± 1.1 *
PAF (pmol/g of tissue)	98.8 ± 174.2	103.8 ± 129.9	239.6 ± 162.7	336.5 ± 371.6
PAF (Ln)	3.7 ± 1.4	3.5 ± 1.7	5.2 ± 0.8 *	4.8 ± 1.3 *
RT-qPCR	***(n = 6)***	***(n = 6)***	***(n = 6)***	***(n = 6)***
DAGL-α/GAPDH (-ΔCt)	-5.8 ± 0.4	-5.7 ± 0.7	-5.8 ± 0.4	-5.8 ± 0.5
DAGL-β/GAPDH (-ΔCt)	-7.4 ± 0.9	-7.3 ± 0.9	-7.6 ± 0.6	-7.6 ± 0.7
MAGL/GAPDH (-ΔCt)	-4.6 ± 0.3	-4.6 ± 0.7	-4.5 ± 0.4	-4.4 ± 0.6
COX-2/GAPDH (-ΔCt)	-7.3 ± 0.4	-7.6 ± 0.9	-7.2 ± 0.2	-7.6 ± 0.4
IL-1β/GAPDH (-ΔCt)	-15.2 ± 0.4	-14.8 ± 0.3	-14.7 ± 0.5	-15.0 ± 0.5
Hippocampus				
LC-MS	***(n = 7)***	***(n= 7)***	***(n= 7)***	***(n= 7)***
2-AG (pg/mg of tissue)	314.0 ± 426.1	307.5 ± 339.0	133.7 ± 132.6	284.7 ± 273.0
2-AG (Ln)	4.4 ± 4.8	5.2 ± 1.0	3.6 ± 5.9	4.2 ± 2.6
PAF (pmol/g of tissue)	392.6 ± 591.7	248.1 ± 355.9	311.4 ± 233.3	326.1 ± 480.2
PAF (Ln)	4.5 ± 1.8	4.8 ± 1.1	5.3 ± 1.2	5.6 ± 0.7
Striatum				
LC-MS	***(n = 7)***	***(n= 7)***	***(n= 6)***	***(n= 7)***
2-AG (pg/mg of tissue)	231.1 ± 283.6	467.4 ± 522.0	1038.6 ± 1245.6	283.3 ± 270.4
2-AG (Ln)	3.8 ± 2.4	5.2 ± 1.5	5.7 ± 2.4	4.7 ± 2.1
PAF (pmol/g of tissue)	328.9 ± 428.8	322.7 ± 411.5	624.0 ± 367.4	408.9 ± 163.0
PAF (Ln)	4.8 ± 1.4	5.1 ± 1.1	6.2 ± 0.7 *	5.9 ± 0.5 *
RT-qPCR	***(n = 6)***	***(n = 6)***	***(n = 6)***	***(n = 6)***
DAGL-α/GAPDH (-ΔCt)	-5.9 ± 0.3	-6.1 ± 0.4	-5.9 ± 0.5	-6.0 ± 0.2
DAGL-β/GAPDH (-ΔCt)	-7.9 ± 0.2	-8.0 ± 0.3	-7.7 ± 0.3	-8.0 ± 0.3
MAGL/GAPDH (-ΔCt)	-5.1 ± 0.6	-4.8 ± 0.6	-4.8 ± 0.8	-5.3 ± 0.5
COX-2/GAPDH (-ΔCt)	-8.8 ± 1.0	-9.8 ± 1.6	-9.4 ± 1.6	-9.8 ± 1.4
IL-1β/GAPDH (-ΔCt)	-14.9 ± 0.5	-15.3 ± 0.3	-15.1 ± 0.6	-15.3 ± 0.5

Cerebellum				
LC-MS	***(n = 7)***	***(n= 7)***	***(n= 7)***	***(n= 6)***
2-AG (pg/mg of tissue)	392.5 ± 293.1	405.7 ± 462.0	347.9 ± 342.4	767.4 ± 657.6
2-AG (Ln)	5.8 ± 0.6	5.6 ± 0.8	5.4 ± 1.4	6.4 ± 0.7
PAF (pmol/g of tissue)	747.7 ± 847.9	431.6 ± 472.3	647.1 ± 372.3	876.0 ± 645.6
PAF (Ln)	5.7 ± 1.4	5.4 ± 1.2	6.3 ± 0.6 *	6.6 ± 0.7 *
RT-qPCR	***(n = 6)***	***(n = 6)***	***(n = 6)***	***(n = 6)***
DAGL-α/GAPDH (-ΔCt)	-5.7 ± 0.5	-5.8 ± 0.6	-6.0 ± 0.8	-6.2 ± 0.6
DAGL-β/GAPDH (-ΔCt)	-7.9 ± 0.5	-7.6 ± 0.6	-8.5 ± 0.6 *	-8.2 ± 0.4 *
MAGL/GAPDH (-ΔCt)	-6.1 ± 0.5 #	-6.6 ± 0.4	-6.3 ± 0.6 #	-6.6 ± 0.4
COX-2/GAPDH (-ΔCt)	-11.0 ± 0.4	-11.0 ± 0.7	-10.6 ± 0.7	-11.2 ± 0.8
IL-1β/GAPDH (-ΔCt)	-15.7 ± 0.6	-15.7 ± 0.9	-15.3 ± 0.4	-15.2 ± 0.8

* $p < .05$ for exposure effect and # $p < .05$ for sex effect in a two-way ANOVA.

Supplementary Table 2. Statistical values for the behavioral and neurochemical evaluation of infant rats exposed or not to PAR during gestation.

Behavioral test / brain region	Parameter	Factors	F	df	p	η_p^2
Behavioral evaluation						
Novel object recognition	Familiar object exploration (s)	Exposure	0.119	1/42	0.732	0.003
		Sex	4.934	1/42	0.032 #	0.105
		Interaction	0.000	1/42	0.997	0.000
	Novel object exploration (s)	Exposure	0.252	1/42	0.618	0.006
		Sex	0.038	1/42	0.845	0.001
		Interaction	0.432	1/42	0.515	0.010
	Discrimination index (%)	Exposure	0.656	1/42	0.422	0.015
		Sex	3.996	1/42	0.052	0.087
		Interaction	0.509	1/42	0.479	0.012
Hot plate	Reaction latency (s)	Exposure	1.110	1/43	0.298	0.025
		Sex	1.167	1/43	0.286	0.026
		Interaction	1.339	1/43	0.254	0.030
Social play behavior	Pinning (count)	Exposure	1.001	1/30	0.325	0.032
		Sex	4.220	1/30	0.049 #	0.123
		Interaction	0.168	1/30	0.685	0.006
	Pouncing (count)	Exposure	0.279	1/30	0.602	0.009
		Sex	15.538	1/30	0.000 #	0.341
		Interaction	0.036	1/30	0.850	0.001
	Following (s)	Exposure	0.063	1/30	0.804	0.002
		Sex	13.672	1/30	0.001 #	0.313
		Interaction	2.537	1/30	0.122	0.078
	Sniffing and licking (s)	Exposure	0.559	1/30	0.461	0.018
		Sex	8.731	1/30	0.006 #	0.225
		Interaction	1.208	1/30	0.280	0.039

Neurochemical evaluation						
Prefrontal cortex	2-AG (Ln)	Exposure	9.822	1/23	0.005 *	0.299
		Sex	1.835	1/23	0.189	0.074
		Interaction	0.564	1/23	0.460	0.024
	PAF (Ln)	Exposure	6.458	1/23	0.018 *	0.219
		Sex	0.370	1/23	0.549	0.016
		Interaction	0.010	1/23	0.919	0.000
	DAGL-α expression (-ΔCt)	Exposure	0.045	1/20	0.835	0.002
		Sex	0.000	1/20	0.987	0.000
		Interaction	0.029	1/20	0.866	0.001
	DAGL-β expression (-ΔCt)	Exposure	0.718	1/20	0.407	0.035
		Sex	0.009	1/20	0.925	0.000
		Interaction	0.002	1/20	0.969	0.000
	MAGL expression (-ΔCt)	Exposure	0.423	1/20	0.523	0.021
		Sex	0.012	1/20	0.913	0.001
		Interaction	0.220	1/20	0.644	0.011
	COX-2 expression (-ΔCt)	Exposure	0.021	1/20	0.887	0.001
		Sex	2.389	1/20	0.138	0.107
		Interaction	0.009	1/20	0.927	0.000
	IL-1β expression (-ΔCt)	Exposure	0.429	1/20	0.520	0.021
		Sex	0.031	1/20	0.861	0.002
		Interaction	2.979	1/20	0.100	0.130
Hippocampus	2-AG (Ln)	Exposure	1.131	1/24	0.298	0.045
		Sex	0.679	1/24	0.418	0.027
		Interaction	0.012	1/24	0.912	0.001
	PAF (Ln)	Exposure	2.721	1/24	0.112	0.102
		Sex	0.372	1/24	0.547	0.015
		Interaction	0.000	1/24	0.986	0.000
Striatum	2-AG (Ln)	Exposure	0.542	1/24	0.469	0.022
		Sex	0.051	1/24	0.824	0.002
		Interaction	1.795	1/24	0.193	0.070

	PAF (Ln)	Exposure	7.552	1/24	0.011 *	0.239
		Sex	0.001	1/24	0.981	0.000
		Interaction	0.566	1/24	0.459	0.023
	DAGL-α expression (-ΔCt)	Exposure	0.144	1/20	0.709	0.007
		Sex	1.078	1/20	0.311	0.051
		Interaction	0.001	1/20	0.977	0.000
	DAGL-β expression (-ΔCt)	Exposure	1.169	1/20	0.293	0.055
		Sex	3.616	1/20	0.072	0.153
		Interaction	0.855	1/20	0.366	0.041
	MAGL expression (-ΔCt)	Exposure	0.118	1/20	0.735	0.006
		Sex	0.324	1/20	0.575	0.016
		Interaction	1.876	1/20	0.186	0.086
	COX-2 expression (-ΔCt)	Exposure	0.392	1/20	0.538	0.019
		Sex	1.663	1/20	0.212	0.077
		Interaction	0.231	1/20	0.636	0.011
	IL-1β expression (-ΔCt)	Exposure	0.393	1/20	0.538	0.019
		Sex	1.868	1/20	0.187	0.085
		Interaction	0.315	1/20	0.581	0.016
Cerebellum	2-AG (Ln)	Exposure	0.416	1/24	0.526	0.019
		Sex	1.208	1/24	0.284	0.052
		Interaction	2.550	1/24	0.125	0.104
	PAF (Ln)	Exposure	4.800	1/24	0.038 *	0.167
		Sex	0.002	1/24	0.962	0.000
		Interaction	0.561	1/24	0.461	0.023
	DAGL-α expression (-ΔCt)	Exposure	2.143	1/20	0.159	0.097
		Sex	0.496	1/20	0.489	0.024
		Interaction	0.001	1/20	0.980	0.000
	DAGL-β expression (-ΔCt)	Exposure	6.909	1/20	0.016 *	0.257
		Sex	1.607	1/20	0.219	0.074
		Interaction	0.069	1/20	0.795	0.003

MAGL expression (-ΔCt)	Exposure	0.304	1/20	0.588	0.015
	Sex	4.748	1/20	0.041 #	0.192
	Interaction	0.242	1/20	0.628	0.012
COX-2 expression (-ΔCt)	Exposure	0.058	1/20	0.812	0.003
	Sex	1.315	1/20	0.265	0.062
	Interaction	1.462	1/20	0.241	0.068
IL-1β expression (-ΔCt)	Exposure	3.192	1/20	0.089	0.138
	Sex	0.042	1/20	0.840	0.002
	Interaction	0.032	1/20	0.860	0.002

✻ $p < .05$ for exposure effect and # $p < .05$ for sex effect in a two-way ANOVA.

5. CONCLUSÕES

O presente estudo descreveu alterações comportamentais à resposta comportamental a um agonista canabinoide decorrentes da exposição gestacional ao paracetamol, além de alterações neuroquímicas relevantes para sinalização endocanabinoide e inflamação. Alterações comportamentais observadas são semelhantes a alguns dos sintomas centrais do TEA e TDAH, o que implica a exposição gestacional ao paracetamol como fator de risco para esses transtornos. As fêmeas parecem ser mais afetadas pelos efeitos neurotóxicos do paracetamol, ao menos em relação aos parâmetros comportamentais. A disfunção do sistema eCB e ativação imune-inflamatória podem participar dos modos de ação pelos quais o paracetamol prejudica o SNC em desenvolvimento e altera o comportamento do feto exposto. A exposição parece ter variados, mas sutis efeitos sobre neurodesenvolvimento e comportamento da prole, o que dificulta a detecção destes em indivíduos expostos.

6. REFERENCIAS

Aagaard, S. K., Larsen, A., Andreasen, M. F., Lesnikova, I., Telving, R., Vestergaard, A. L., Tørring, N., Uldbjerg, N., & Bor, P. (2018). Prevalence of xenobiotic substances in first-trimester blood samples from Danish pregnant women: A cross-sectional study. *BMJ Open*, *8*(3), 1–7.

Aguiar, D. C., Moreira, F. A., Terzian, A. L., Fogaça, M. V., Lisboa, S. F., Wotjak, C. T., & Guimaraes, F. S. (2014). Modulation of defensive behavior by Transient Receptor Potential Vanilloid Type-1 (TRPV1) channels. *Neuroscience and Biobehavioral Reviews*, *46*(P3), 418–428.

Alemany, S., Avella-García, C., Liew, Z., García-Esteban, R., Inoue, K., Cadman, T., … Sunyer, J. (2021). Prenatal and postnatal exposure to acetaminophen in relation to autism spectrum and attention-deficit and hyperactivity symptoms in childhood: meta-analysis in six european population-based cohorts. *European Journal of Epidemiology*, (0123456789).

American Psychiatric Association. (2014). *Diagnostic and Statistical Manual of Mental Disorders* (5th ed.). Washington DC: American Psychiatric Association.

Athersuch, T. J., Antoine, D. J., Boobis, A. R., Coen, M., Daly, A. K., Possamai, L., Nicholson, J. K., & Wilson, I. D. (2018). Paracetamol metabolism, hepatotoxicity, biomarkers and therapeutic interventions: A perspective. *Toxicology Research*, *7*(3), 347–357.

Avella-Garcia, C. B., Julvez, J., Fortuny, J., Rebordosa, C., García-Esteban, R., Galán, I. R., Tardón, A., Rodríguez-Bernal, C. L., Iñiguez, C., Andiarena, A., Santa-Marina, L., & Sunyer, J. (2016). Acetaminophen use in pregnancy and neurodevelopment: attention function and autism spectrum symptoms. *International Journal of Epidemiology*, *45*(6), 1987–1996.

Baker, B. H., Rafikian, E. E., Hamblin, P. B., Strait, M. D., Yang, M., & Pearson, B. L. (2023). Sex-specific neurobehavioral and prefrontal cortex gene expression alterations following developmental acetaminophen exposure in mice. *Neurobiology of Disease*, *177*(December 2022), 105970.

Bambini-Junior, V., Zanatta, G., Della Flora Nunes, G., Mueller de Melo, G., Michels, M., Fontes-Dutra, M., Nogueira Freire, V., Riesgo, R., & Gottfried, C. (2014). Resveratrol prevents social deficts in animal model of autism induced by valproic acid. *Neuroscience Letters*, *583*, 176–181.

Bandoli, G., Palmsten, K., & Chambers, C. (2019). Acetaminophen use in pregnancy:

examining prevalence, timing, and indication of use in a prospective birth cohort. *Paediatric and Perinatal Epidemiology*, (August), 1–10.

Bauer, A. Z., Kriebel, D., Herbert, M. R., Bornehag, C. G., & Swan, S. H. (2018). Prenatal paracetamol exposure and child neurodevelopment: A review. *Hormones and Behavior*, *101*, 125–147.

Bauer, A. Z., Swan, S. H., Kriebel, D., Liew, Z., Taylor, H. S., Bornehag, C.-G., Andrade, A. M., Olsen, J., Jensen, R. H., Mitchell, R. T., Skakkebaek, N. E., Jégou, B., & Kristensen, D. M. (2021). Paracetamol use during pregnancy — a call for precautionary action. *Nature Reviews Endocrinology*, *0123456789*.

Beltramo, M., Stella, N., Calignano, A., Lin, S. Y., Makriyannis, A., & Piomelli, D. (1997). Functional role of high-affinity anandamide transport, as revealed by selective inhibition. *Science*, *277*(August), 1094–1097.

Berdyshev, E. V., Schmid, P. C., Krebsbach, R. J., & Schmid, H. H. O. (2001). Activation of PAF receptors results in enhanced synthesis of 2-arachidonoylglycerol (2-AG) in immune cells. *The FASEB Journal*, *15*(12), 2171–2178.

Bertoldi, A. D., Rifas-Shiman, S. L., Boing, A. C., da Silva Dal Pizzol, T., Miranda, V. I. A., Silveira, M. P. T., Freitas Silveira, M., Domingues, M. R., Santos, I. S., Bassani, D. G., Tovo-Rodrigues, L., & Oken, E. (2020). Associations of acetaminophen use during pregnancy and the first year of life with neurodevelopment in early childhood. *Paediatric and Perinatal Epidemiology*, *34*(3), 267–277.

Bevins, R. A., & Besheer, J. (2006). Object recognition in rats and mice: A one-trial non-matching-to-sample learning task to study “recognition memory.” *Nature Protocols*, *1*(3), 1306–1311.

Bitanihirwe, B. K., Peleg-Raibstein, D., Mouttet, F., Feldon, J., & Meyer, U. (2010). Late prenatal immune activation in mice leads to behavioral and neurochemical abnormalities relevant to the negative symptoms of schizophrenia. *Neuropsychopharmacology*, *35*(12), 2462–2478.

Blecharz-Klin, K., Joniec-Maciejak, I., Jawna-Zboinska, K., Pyrzanowska, J., Piechal, A., Wawer, A., & Widy-Tyszkiewicz, E. (2016). Cerebellar level of neurotransmitters in rats exposed to paracetamol during development. *Pharmacological Reports*, *68*(6), 1159–1164.

Blecharz-Klin, K., Joniec-Maciejak, I., Jawna, K., Pyrzanowska, J., Piechal, A., Wawer, A., & Widy-Tyszkiewicz, E. (2015a). Developmental exposure to paracetamol causes

biochemical alterations in medulla oblongata. *Environmental Toxicology and Pharmacology*, *40*, 369–374.

Blecharz-Klin, K., Joniec-Maciejak, I., Jawna, K., Pyrzanowska, J., Piechal, A., Wawer, A., & Widy-Tyszkiewicz, E. (2015b). Effect of prenatal and early life paracetamol exposure on the level of neurotransmitters in rats - focus on the spinal cord. *International Journal of Developmental Neuroscience*, *47*, 133–139.

Blecharz-Klin, K., Piechal, A., Jawna-Zboińska, K., Pyrzanowska, J., Wawer, A., Joniec-Maciejak, I., & Widy-Tyszkiewicz, E. (2017). Paracetamol − effect of early exposure on neurotransmission, spatial memory and motor performance in rats. *Behavioural Brain Research*, *323*, 162–171.

Blecharz-Klin, K., Wawer, A., Jawna-zboi, K., Pyrzanowska, J., Piechal, A., Mirowska-guzel, D., & Widy-tyszkiewicz, E. (2018). Early paracetamol exposure decreases brain-derived neurotrophic factor (BDNF) in striatum and affects social behaviour and exploration in rats. *Pharmacology, Biochemistry and Behavior*, *168*, 25–32.

Blecharz-Klin, K., Wawer, A., Pyrzanowska, J., Piechal, A., Jawna-Zboińska, K., & Widy-Tyszkiewicz, E. (2019). Hypothalamus – response to early paracetamol exposure in male rats offspring. *International Journal of Developmental Neuroscience*, *76*(January), 1–5.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*(8), 911–917.

Brandlistuen, R. E., Ystrom, E., Nulman, I., Koren, G., & Nordeng, H. (2013). Prenatal paracetamol exposure and child neurodevelopment: A sibling-controlled cohort study. *International Journal of Epidemiology*, *42*(6), 1702–1713.

Briones, M. R. S., Snyder, A. M., Ferreira, R. C., Neely, E. B., Connor, J. R., & Broach, J. R. (2018). A possible role for platelet-activating factor receptor in amyotrophic lateral sclerosis treatment. *Frontiers in Neurology*, *9*(FEB), 5–9.

Bührer, C., Endesfelder, S., Scheuer, T., & Schmitz, T. (2021). Paracetamol (acetaminophen) and the developing brain. *International Journal of Molecular Sciences*, *22*(20), 11156.

Cendejas-Hernandez, J., Sarafian, J. T., Lawton, V. G., Palkar, A., Anderson, L. G., Larivière, V., & Parker, W. (2022). Paracetamol (acetaminophen) use in infants and children was never shown to be safe for neurodevelopment: a systematic review with citation tracking. *European Journal of Pediatrics*, *181*(5), 1835–1857.

Chambers, C. (2015). Over-the-counter medications: risk and safety in pregnancy. *Seminars in Perinatology*, *39*(7), 541–544.

Conings, S., Tseke, F., Broeck, A. Van Den, Qi, B., Paulus, J., Amant, F., Annaert, P., & Calsteren, K. Van. (2019). Transplacental transport of paracetamol and its phase II metabolites using the ex vivo placenta perfusion model. *Toxicology and Applied Pharmacology*, *370*(November 2018), 14–23.

Cristino, Bisogno, T., & Di Marzo, V. (2020). Cannabinoids and the expanded endocannabinoid system in neurological disorders. *Nature Reviews Neurology*, *16*(1), 9–29.

Cristino, L., de Petrocellis, L., Pryce, G., Baker, D., Guglielmotti, V., & Di Marzo, V. (2006). Immunohistochemical localization of cannabinoid type 1 and vanilloid transient receptor potential vanilloid type 1 receptors in the mouse brain. *Neuroscience*, *139*(4), 1405–1415.

de Brouwer, G., Fick, A., Harvey, B. H., & Wolmarans, D. W. (2019). A critical inquiry into marble-burying as a preclinical screening paradigm of relevance for anxiety and obsessive–compulsive disorder: Mapping the way forward. *Cognitive, Affective and Behavioral Neuroscience*, *19*(1), 1–39.

Dixit, P. V., Sahu, R., & Mishra, D. K. (2020). Marble-burying behavior test as a murine model of compulsive-like behavior. *Journal of Pharmacological and Toxicological Methods*, *102*(August 2019), 106676.

Duggan, K. C., Hermanson, D. J., Musee, J., Prusakiewicz, J. J., Scheib, J. L., Carter, B. D., Banerjee, S., Oates, J. A., & Marnett, L. J. (2011). (R)-Profens are substrate-selective inhibitors of endocannabinoid oxygenation by COX-2. *Nature Chemical Biology*, *7*(11), 803–809.

Ellis, P. D. (2010). *The essential guide to effect sizes*. Cambridge: Cambridge University Press. Retrieved from https://www.ptonline.com/articles/how-to-get-better-mfi-results

Ernst, A. M., & Smelik, P. G. (1966). Site of action of dopamine and apomorphine on compulsive gnawing behaviour in rats. *Experientia*, *22*(12), 837–838.

Estanislau, C., Díaz-Morán, S., Cañete, T., Blázquez, G., Tobeña, a., & Fernández-Teruel, a. (2013). Context-dependent differences in grooming behavior among the NIH heterogeneous stock and the Roman high- and low-avoidance rats. *Neuroscience Research*, *77*(4), 187–201.

Fagundes, B. H. F., Nascimento, P. C., Aragão, W. A. B., Chemelo, V. S., Bittencourt, L. O., Eiró-Quirino, L., Silva, M. C. F., Freire, M. A. M., Fernandes, L. M. P., Maia, C. do S. F., Crespo-Lopez, M. E., & Lima, R. R. (2022). Methylmercury exposure during prenatal and postnatal neurodevelopment promotes oxidative stress associated with motor and cognitive damages in rats: an environmental-experimental toxicology study. *Toxicology*

Reports, *9*, 563–574.

Favaro, P. d N., Costa, L. C., & Moreira, E. G. (2008). Maternal fluoxetine treatment decreases behavioral response to dopaminergic drugs in female pups. *Neurotoxicology and Teratology*, *30*(6), 487–494.

FDA. (2016). FDA Drug Safety Communication: FDA has reviewed possible risks of pain medicine use during pregnancy. Retrieved from http://www.fda.gov/Drugs/DrugSafety/ucm429117.htm%5Cnhttp://www.medscape.com/viewarticle/837858?src=wnl_edit_newsal

Foley, K. A., MacFabe, D. F., Vaz, A., Ossenkopp, K. P., & Kavaliers, M. (2014). Sexually dimorphic effects of prenatal exposure to propionic acid and lipopolysaccharide on social behavior in neonatal, adolescent, and adult rats: Implications for autism spectrum disorders. *International Journal of Developmental Neuroscience*, *39*(C), 68–78.

Golding, J., Gregory, S., Clark, R., Ellis, G., Iles-Caven, Y., & Northstone, K. (2020). Associations between paracetamol (acetaminophen) intake between 18 and 32 weeks gestation and neurocognitive outcomes in the child: A longitudinal cohort study. *Paediatric and Perinatal Epidemiology*, *34*(3), 257–266.

Golub, M. S., & Sobin, C. A. (2020). Statistical modeling with litter as a random effect in mixed models to manage “intralitter likeness.” *Neurotoxicology and Teratology*, *77*, 106841.

Gonsiorek, W., Lunn, C., Fan, X., Narula, S., Lundell, D., & Hipkin, R. W. (2000). Endocannabinoid 2-arachidonyl glycerol is a full agonist through human type 2 cannabinoid receptor: Antagonism by anandamide. *Molecular Pharmacology*, *57*(5), 1045–1050.

Gou, X., Wang, Y., Tang, Y., Qu, Y., Tang, J., Shi, J., Xiao, D., & Mu, D. (2019). Association of maternal prenatal acetaminophen use with the risk of attention deficit/hyperactivity disorder in offspring: A meta-analysis. *Australian and New Zealand Journal of Psychiatry*, *53*(3), 195–206.

Gregory, E. H., & Pfaff, D. W. (1971). Development of olfactory-guided behavior in infant rats. *Physiology & Behavior*, *6*, 573–576.

Haas, D. M., Marsh, D. J., Dang, D. T., Parker, C. B., Wing, D. A., Simhan, H. N., … Reddy, U. M. (2018). Prescription and other medication use in pregnancy. *Obstetrics and Gynecology*, *131*(5), 789–798.

Harkany, T., & Cinquina, V. (2021). Physiological rules of endocannabinoid action during fetal and neonatal brain development. *Cannabis and Cannabinoid Research*, *6*(5), 381–388.

Harshaw, C., & Warner, A. G. (2022). Interleukin-1β-induced inflammation and acetaminophen during infancy: Distinct and interactive effects on social-emotional and repetitive behavior in C57BL/6J mice. *Pharmacology Biochemistry and Behavior*, *220*(September).

Hershkowitz, M., & Adunsky, A. (1996). Binding of platelet-activating factor to platelets of Alzheimer's disease and multiinfarct dementia patients. *Neurobiology of Aging*, *17*(6), 865–868.

Heyer, D. B., & Meredith, R. M. (2017). Environmental toxicology: Sensitive periods of development and neurodevelopmental disorders. *NeuroToxicology*, *58*, 23–41.

Högestätt, E. D., Jönsson, B. A. G., Ermund, A., Andersson, D. A., Björk, H., Alexander, J. P., Cravatt, B. F., Basbaum, A. I., & Zygmunt, P. M. (2005). Conversion of acetaminophen to the bioactive N-acylphenolamine AM404 via fatty acid amide hydrolase-dependent arachidonic acid conjugation in the nervous system. *The Journal of Biological Chemistry*, *280*(36), 31405–31412.

Hoshijima, H., Hunt, M., Nagasaka, H., & Yaksh, T. (2021). Systematic review of systemic and neuraxial effects of acetaminophen in preclinical models of nociceptive processing. *Journal of Pain Research*, *Volume 14*, 3521–3552.

Hsu, K.-L., Tsuboi, K., Adibekian, A., Pugh, H., Masuda, K., & Cravatt, B. F. (2012). DAGLβ inhibition perturbs a lipid network involved in macrophage inflammatory responses. *Nature Chemical Biology*, *8*(12), 999–1007.

Hussain, F. N., & Ashmead, G. G. (2017). The safety of over-the-counter medications in pregnancy. *Topics in Obstetrics & Gynecology*, *37*(14), 1–8.

Ishihara, Y., Honda, T., Ishihara, N., Namba, K., Taketoshi, M., Tominaga, Y., Tsuji, M., Vogel, C. F. A., Yamazaki, T., Itoh, K., & Tominaga, T. (2022). A CCR5 antagonist, maraviroc, alleviates neural circuit dysfunction and behavioral disorders induced by prenatal valproate exposure. *Journal of Neuroinflammation*, *19*(1), 195.

Jetten, M. J. A., Gaj, S., Ruiz-Aracama, A., de Kok, T. M., van Delft, J. H. M., Lommen, A., van Someren, E. P., Jennen, D. G. J., Claessen, S. M., Peijnenburg, A. A. C. M., Stierum, R. H., & Kleinjans, J. C. S. (2012). 'Omics analysis of low dose acetaminophen intake demonstrates novel response pathways in humans. *Toxicology and Applied Pharmacology*, *259*(3), 320–328.

Kaczocha, M., Vivieca, S., Sun, J., Glaser, S. T., & Deutsch, D. G. (2012). Fatty acid-binding proteins transport N-acylethanolamines to nuclear receptors and are targets of endocannabinoid transport inhibitors. *Journal of Biological Chemistry*, *287*(5), 3415–

3424.

Kim, H., Lim, C. S., & Kaang, B. K. (2016). Neuronal mechanisms and circuits underlying repetitive behaviors in mouse models of autism spectrum disorder. *Behavioral and Brain Functions*, *12*(1), 1–13.

Kim, S.-A., Chai, J.-H., & Jang, E.-H. (2021). Prenatal trimethyltin exposure induces long-term DNA methylation changes in the male mouse hippocampus. *International Journal of Molecular Sciences*, *22*(15), 8009.

Klein, R. M., Rigobello, C., Vidigal, C. B., Moura, K. F., Barbosa, D. S., Gerardin, D. C. C., Ceravolo, G. S., & Moreira, E. G. (2020). Gestational exposure to paracetamol in rats induces neurofunctional alterations in the progeny. *Neurotoxicology and Teratology*, *77*(September 2019), 106838.

Koehn, L. M., Huang, Y., Habgood, M. D., Kysenius, K., Crouch, P. J., Dziegielewska, K. M., & Saunders, N. R. (2020). Effects of paracetamol (acetaminophen) on gene expression and permeability properties of the rat placenta and fetal brain. *F1000Research*, *9*.

Komorowska-Müller, J. A., & Schmöle, A. C. (2021). CB2 receptor in microglia: The guardian of self-control. *International Journal of Molecular Sciences*, *22*(1), 1–27.

Lakens, D. (2013). Calculating and reporting effect sizes to facilitate cumulative science: A practical primer for t-tests and ANOVAs. *Frontiers in Psychology*, *4*(NOV), 1–12.

Langen, M., Durston, S., Kas, M. J. H., van Engeland, H., & Staal, W. G. (2011). The neurobiology of repetitive behavior: of mice... *Neuroscience and Biobehavioral Reviews*, *35*(3), 356–365.

Laue, H. E., Cassoulet, R., Abdelouahab, N., Serme-Gbedo, Y. K., Desautels, A. S., Brennan, K. J. M., Bellenger, J. P., Burris, H. H., Coull, B. A., Weisskopf, M. G., Takser, L., & Baccarelli, A. A. (2019). Association between meconium acetaminophen and childhood neurocognitive development in GESTE, a canadian cohort study. *Toxicological Sciences : An Official Journal of the Society of Toxicology*, *167*(1), 138–144.

Leppert, B., Havdahl, A., Riglin, L., Jones, H. J., Zheng, J., Davey Smith, G., Tilling, K., Thapar, A., Reichborn-Kjennerud, T., & Stergiakouli, E. (2019). Association of maternal neurodevelopmental risk alleles with early-life exposures. *JAMA Psychiatry*.

Levine, A., Liktor-Busa, E., Lipinski, A. A., Couture, S., Balasubramanian, S., Aicher, S. A., Langlais, P. R., Vanderah, T. W., & Largent-Milnes, T. M. (2021). Sex differences in the expression of the endocannabinoid system within V1M cortex and PAG of Sprague Dawley rats. *Biology of Sex Differences*, *12*(1), 60.

Liew, Z., Bach, C. C., Asarnow, R. F., Ritz, B., & Olsen, J. (2016). Paracetamol use during pregnancy and attention and executive function in offspring at age 5 years. *International Journal of Epidemiology*, *45*(6), 2009–2017.

Liew, Z., Ritz, B., Rebordosa, C., Lee, P.-C., & Olsen, J. (2014). Acetaminophen use during pregnancy, behavioral problems, and hyperkinetic disorders. *JAMA Pediatrics*, *168*(4), 313–320.

Liew, Z., Ritz, B., Virk, J., & Olsen, J. (2016). Maternal use of acetaminophen during pregnancy and risk of autism spectrum disorders in childhood: a Danish national birth cohort study. *Autism Research*, *9*(9), 951–958.

Liu, J., Bátkai, S., Pacher, P., Harvey-White, J., Wagner, J. A., Cravatt, B. F., Gao, B., & Kunos, G. (2003). Lipopolysaccharide induces anandamide synthesis in macrophages via CD14/MAPK/phosphoinositide 3-kinase/NF-κB independently of platelet-activating factor. *Journal of Biological Chemistry*, *278*(45), 45034–45039.

Liu, Y., Shields, L. B. E., Gao, Z., Wang, Y., Zhang, Y. P., Chu, T., Zhu, Q., Shields, C. B., & Cai, J. (2017). Current understanding of platelet-activating factor signaling in central nervous system diseases. *Molecular Neurobiology*, *54*(7), 5563–5572.

Lordan, R., Tsoupras, A., Zabetakis, I., & Demopoulos, C. A. (2019). Forty years since the structural elucidation of platelet-activating factor (PAF): Historical, current, and future research perspectives. *Molecules*, *24*(23).

Lu, H. C., & Mackie, K. (2021). Review of the endocannabinoid system. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*, *6*(6), 607–615.

Lye, J. M., Knight, J. A., Arneja, J., Seeto, R. A., Wong, J., Adel khani, N., Brooks, J. D., Levitan, R. D., Matthews, S. G., Lye, S. J., & Hung, R. J. (2022). Maternal acetaminophen use and cognitive development at 4 years: the Ontario Birth Study. *Pediatric Research*, (June 2021), 1–5.

MacCarrone, M., Guzmán, M., MacKie, K., Doherty, P., & Harkany, T. (2014). Programming of neural cells by (endo)cannabinoids: From physiological rules to emerging therapies. *Nature Reviews Neuroscience*, *15*(12), 786–801.

Marco, E. M., Viveros, M.-P., Borcel, E., Pérez-Alvarez, L., Guaza, C., Ramirez, B. G., de Ceballos, M. L., Rubio, M., & Fernández, B. (2004). Functional responses to the cannabinoid agonist WIN 55,212-2 in neonatal rats of both genders: influence of weaning. *Pharmacology Biochemistry and Behavior*, *78*(3), 593–602.

Masarwa, R., Levine, H., Gorelik, E., Reif, S., Perlman, A., & Matok, I. (2018). Prenatal

exposure to acetaminophen and risk for attention deficit hyperactivity disorder and autistic spectrum disorder: A systematic review,meta-analysis, and meta-regression analysis of cohort studies. *American Journal of Epidemiology*, *187*(8), 1817–1827.

Masarwa, R., Platt, R. W., & Filion, K. B. (2020). Acetaminophen use during pregnancy and the risk of attention deficit hyperactivity disorder: A causal association or bias? *Paediatric and Perinatal Epidemiology*, *34*(3), 309–317.

McCrae, J. C., Morrison, E. E., MacIntyre, I. M., Dear, J. W., & Webb, D. J. (2018). Long-term adverse effects of paracetamol – a review. *British Journal of Clinical Pharmacology*, *84*(10), 2218–2230.

McLemore, G. L., & Richardson, K. A. (2016). Data from three prospective longitudinal human cohorts of prenatal marijuana exposure and offspring outcomes from the fetal period through young adulthood. *Data in Brief*, *9*, 753–757.

Mehta, T. R., Monegro, A., Nene, Y., Fayyaz, M., & Bollu, P. C. (2019). Neurobiology of ADHD: A Review. *Current Developmental Disorders Reports*, *6*(4), 235–240.

Melancia, F., Schiavi, S., Servadio, M., Cartocci, V., Campolongo, P., Palmery, M., Pallottini, V., & Trezza, V. (2018). Sex-specific autistic endophenotypes induced by prenatal exposure to valproic acid involve anandamide signalling. *British Journal of Pharmacology*, *175*(18), 3699–3712.

Moreira, F. A., Aguiar, D. C., Terzian, A. L. B., Guimarães, F. S., & Wotjak, C. T. (2012). Cannabinoid type 1 receptors and transient receptor potential vanilloid type 1 channels in fear and anxiety - two sides of one coin? *Neuroscience*, *204*, 186–192.

Muramatsu, S., Shiraishi, S., Miyano, K., Sudo, Y., Toda, A., Mogi, M., Hara, M., Yokoyama, A., Kawasaki, Y., Taniguchi, M., & Uezono, Y. (2016). Metabolism of AM404 from acetaminophen at human therapeutic dosages in the rat brain. *Anesthesiology and Pain Medicine*, *6*(1), 1–5.

Murataeva, N., Straiker, A., & MacKie, K. (2014). Parsing the players: 2-arachidonoylglycerol synthesis and degradation in the CNS. *British Journal of Pharmacology*, *171*(6), 1379–1391.

Nielsen, E., Ostergaard, G., & Larsen, J. (2008). *Toxicological risk assessment of chemicals: a practical guide.* New York: Informa Healthcare USA,.

Niesink, R. J. M., & Van Ree, J. M. (1989). Involvement of opioid and dopaminergic systems in isolation-induced pinning and social grooming of young rats. *Neuropharmacology*, *28*(4), 411–418.

Nitsche, J. F., Patil, A. S., Langman, L, J., Penn, H. J., Derleth, D., Watson, W. J., & Brost, B. C. (2017). Transplacental passage of acetaminophen in term pregnancy. *American Journal of Perinatology*, *34*(6), 541–543.

Nomura, D. K., Morrison, B. E., Blankman, J. L., Long, J. Z., Kinsey, S. G., Marcondes, M. C. G., Ward, A. M., Hahn, Y. K., Lichtman, A. H., Conti, B., & Cravatt, B. F. (2011). Endocannabinoid hydrolysis generates brain prostaglandins that promote neuroinflammation. *Science*, *334*(6057), 809–813.

OECD. (2007). OECD guideline for the testing of chemicals 426 - Developmental Neurotoxicity Study, (September), 1–38.

Olivier, J. D. a, Vallès, a., Van Heesch, F., Afrasiab-Middelman, A., Roelofs, J. J. P. M., Jonkers, M., Peeters, E. J., Korte-Bouws, G. a H., Dederen, J. P., Kiliaan, A. J., Martens, G. J., Schubert, D., & Homberg, J. R. (2011). Fluoxetine administration to pregnant rats increases anxiety-related behavior in the offspring. *Psychopharmacology*, *217*(3), 419–432.

Palmer, A. A., Brown, A. S., Keegan, D., Siska, L. D., Susser, E., Rotrosen, J., & Butler, P. D. (2008). Prenatal protein deprivation alters dopamine-mediated behaviors and dopaminergic and glutamatergic receptor binding. *Brain Research*, *1237*, 62–74.

Pandolfo, P., Pamplona, F. A., Prediger, R. D. S., & Takahashi, R. N. (2007). Increased sensitivity of adolescent spontaneously hypertensive rats, an animal model of attention deficit hyperactivity disorder, to the locomotor stimulation induced by the cannabinoid receptor agonist WIN 55,212-2. *European Journal of Pharmacology*, *563*(1–3), 141–148.

Panikashvili, D., Simeonidou, C., Ben-Shabat, S., Hanuš, L., Breuer, A., Mechoulam, R., & Shohami, E. (2001). An endogenous cannabinoid (2-AG) is neuroprotective after brain injury. *Nature*, *413*(6855), 527–531.

Parker, W., Hornik, C. D., Bilbo, S., Holzknecht, Z. E., Gentry, L., Rao, R., Lin, S. S., Herbert, M. R., & Nevison, C. D. (2017). The role of oxidative stress, inflammation and acetaminophen exposure from birth to early childhood in the induction of autism. *Journal of International Medical Research*, *45*(2), 407–438.

Parrini, M., Ghezzi, D., Deidda, G., Medrihan, L., Castroflorio, E., Alberti, M., Baldelli, P., Cancedda, L., & Contestabile, A. (2017). Aerobic exercise and a BDNF-mimetic therapy rescue learning and memory in a mouse model of Down syndrome. *Scientific Reports*, *7*(1), 1–22.

Patel, S., & Windsor, M. A. (2015). Patent application publication (10) Pub. No.: US

2015/0183737 A1.

Penaloza, C. G., Cruz, M., Germain, G., Jabeen, S., Javdan, M., Lockshin, R. A., & Zakeri, Z. (2020). Higher sensitivity of female cells to ethanol: Methylation of DNA lowers Cyp2e1, generating more ROS. *Cell Communication and Signaling*, *18*(1), 1–15.

Perazzo, J. C., Fiebich, B. L., Fortwängler, E., Muñoz, E., Marcotegui, A. R., Saliba, S. W., de Oliveira, A. C. P., & Ditrich, J. (2017). AM404, paracetamol metabolite, prevents prostaglandin synthesis in activated microglia by inhibiting COX activity. *Journal of Neuroinflammation*, *14*(1), 1–11.

Philippot, G., Gordh, T., Fredriksson, A., & Viberg, H. (2017). Adult neurobehavioral alterations in male and female mice following developmental exposure to paracetamol (acetaminophen): characterization of a critical period. *Journal of Applied Toxicology*, *37*(10), 1174–1181.

Philippot, G., Hallgren, S., Gordh, T., Fredriksson, A., Fredriksson, R., & Viberg, H. (2018). A cannabinoid receptor type 1 (CB1R) agonist enhances the developmental neurotoxicity of acetaminophen (Paracetamol). *Toxicological Sciences*, *166*(1), 203–212.

Philippot, G., Hosseini, K., Yakub, A., Mhajar, Y., Hamid, M., Buratovic, S., & Fredriksson, R. (2022). Paracetamol (acetaminophen) and its effect on the developing mouse brain. *Frontiers in Toxicology*, *4*(March), 1–11.

Pinto, B., Morelli, G., Rastogi, M., Savardi, A., Fumagalli, A., Petretto, A., Bartolucci, M., Varea, E., Catelani, T., Contestabile, A., Perlini, L. E., & Cancedda, L. (2020). Rescuing over-activated microglia restores cognitive performance in juvenile animals of the Dp(16) mouse model of Down syndrome. *Neuron*, (16), 1–18.

Pletnikov, M. V., Rubin, S. A., Vasudevan, K., Moran, T. H., & M. Carbone, K. (1999). Developmental brain injury associated with abnormal play behavior in neonatally Borna disease virus-infected Lewis rats: A model of autism. *Behavioural Brain Research*, *100*(1–2), 43–50.

Polissidis, A., Chouliara, O., Galanopoulos, A., Marselos, M., Papadopoulou-Daifoti, Z., & Antoniou, K. (2009). Behavioural and dopaminergic alterations induced by a low dose of WIN 55,212-2 in a conditioned place preference procedure. *Life Sciences*, *85*(5–6), 248–254.

Polli, F. S., Scharff, M. B., Ipsen, T. H., Aznar, S., Kohlmeier, K. A., & Andreasen, J. T. (2020). Prenatal nicotine exposure in mice induces sex-dependent anxiety-like behavior, cognitive deficits, hyperactivity, and changes in the expression of glutamate receptor

associated-genes in the prefrontal cortex. *Pharmacology Biochemistry and Behavior*, *195*, 172951.

Prut, L., & Belzung, C. (2003). The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: A review. *European Journal of Pharmacology*, *463*(1–3), 3–33.

Przybyła, G. W., Szychowski, K. A., & Gmiński, J. (2021). Paracetamol – An old drug with new mechanisms of action. *Clinical and Experimental Pharmacology and Physiology*, *48*(1), 3–19.

Ray-Griffith, S. L., Morrison, B., & Stowe, Z. N. (2019). Chronic Pain Prevalence and Exposures during Pregnancy. *Pain Research and Management*, *2019*.

Rice, D., & Barone, S. (2000). Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environmental Health Perspectives*, *108*(3), 511–533.

Rifas-Shiman, S. L., Cardenas, A., Hivert, M. F., Tiemeier, H., Bertoldi, A. D., & Oken, E. (2020). Associations of prenatal or infant exposure to acetaminophen or ibuprofen with mid-childhood executive function and behaviour. *Paediatric and Perinatal Epidemiology*, *34*(3), 287–298.

Rigobello, C., Klein, R. M., Debiasi, J. D., Ursini, L. G., Michelin, A. P., Matsumoto, A. K., Barbosa, D. S., & Moreira, E. G. (2021). Perinatal exposure to paracetamol: dose and sex-dependent effects in behaviour and brain's oxidative stress markers in progeny. *Behavioural Brain Research*, *408*(March).

Rivera, P., Tovar, R., Ramírez-López, M. T., Navarro, J. A., Vargas, A., Suárez, J., & Fonseca, F. R. de. (2020). Sex-specific anxiety and prefrontal cortex glutamatergic dysregulation are long-term consequences of pre-and postnatal exposure to hypercaloric diet in a rat model. *Nutrients*, *12*(6), 1829.

Ross, E. J., Graham, D. L., Money, K. M., & Stanwood, G. D. (2015). Developmental consequences of fetal exposure to drugs: What we know and what we still must learn. *Neuropsychopharmacology*, *40*(1), 61–87.

Rudenko, V., Rafiuddin, A., Leheste, J. R., & Friedman, L. K. (2012). Inverse relationship of cannabimimetic (R+)WIN 55, 212 on behavior and seizure threshold during the juvenile period. *Pharmacology Biochemistry and Behavior*, *100*(3), 474–484.

Saad, A., Hegde, S., Kechichian, T., Gamble, P., Rahman, M., Stutz, S. J., Anastasio, N. C., Alshehri, W., Lei, J., Mori, S., Kajs, B., Cunningham, K. A., Saade, G., Burd, I., & Costantine, M. (2016). Is there a causal relation between maternal acetaminophen

administration and ADHD? *PLoS ONE*, *11*(6), 1–10.

Sañudo-Peña, M. C., Romero, J., Seale, G. E., Fernandez-Ruiz, J. J., & Walker, J. M. (2000). Activational role of cannabinoids on movement. *European Journal of Pharmacology*, *391*(3), 269–274.

Schneider, M., Schömig, E., & Leweke, F. M. (2008). Acute and chronic cannabinoid treatment differentially affects recognition memory and social behavior in pubertal and adult rats. *Addiction Biology*, *13*(3–4), 345–357.

Schneider, T., & Przewłocki, R. (2005). Behavioral alterations in rats prenatally exposed to valproic acid: animal model of autism. *Neuropsychopharmacology*, *30*(1), 80–89.

Schultz, S., Gould, G. G., Antonucci, N., Brigida, A. L., & Siniscalco, D. (2021). Endocannabinoid system dysregulation from acetaminophen use may lead to autism spectrum disorder: could cannabinoid treatment be efficacious? *Molecules*, *26*(7), 1845.

Schultz, S. T., & Gould, G. G. (2016). Acetaminophen use for fever in children associated with autism spectrum disorder. *Autism Open Access*, *6*(2), 1000170.

Scipioni, L., Ciaramellano, F., Carnicelli, V., Leuti, A., Lizzi, A. R., De Dominicis, N., Oddi, S., & Maccarrone, M. (2022). Microglial endocannabinoid signalling in AD. *Cells*, *11*(7), 1237.

Servadio, M., Melancia, F., Manduca, A., Di Masi, A., Schiavi, S., Cartocci, V., Pallottini, V., Campolongo, P., Ascenzi, P., & Trezza, V. (2016). Targeting anandamide metabolism rescues core and associated autistic-like symptoms in rats prenatally exposed to valproic acid. *Translational Psychiatry*, *6*(9), 1–11.

Servadio, Michela, Manduca, A., Melancia, F., Leboffe, L., Schiavi, S., Campolongo, P., Palmery, M., Ascenzi, P., di Masi, A., & Trezza, V. (2018). Impaired repair of DNA damage is associated with autistic-like traits in rats prenatally exposed to valproic acid. *European Neuropsychopharmacology*, *28*(1), 85–96.

Setler, P., Sarau, H., & McKenzie, G. (1976). Differential attenuation of some effects of haloperidol in rats given scopolamine. *European Journal of Pharmacology*, *39*, 117–126.

Sharma, Jamie, H., Shah, S., Perrett, D., & Ayoub, S. S. (2017). First evidence of the conversion of paracetamol to AM404 in human cerebrospinal fluid. *Journal of Pain Research*, *10*, 2703–2709.

Sholler, D. J., Strickland, J. C., Spindle, T. R., Weerts, E. M., & Vandrey, R. (2021). Sex differences in the acute effects of oral and vaporized cannabis among healthy adults. *Addiction Biology*, *26*(4), 1–12.

Silver, R. J. (2019). The endocannabinoid system of animals. *Animals*, *9*(9), 686.

Spagnolo, P. A., Garvey, M., & Hallett, M. (2021). A dimensional approach to functional movement disorders: Heresy or opportunity. *Neuroscience and Biobehavioral Reviews*, *127*(November 2020), 25–36.

Stark, T., Iannotti, F. A., Di Martino, S., Di Bartolomeo, M., Ruda-Kucerova, J., Piscitelli, F., Wotjak, C. T., D'Addario, C., Drago, F., Di Marzo, V., & Micale, V. (2022). Early blockade of CB1 receptors ameliorates schizophrenia-like alterations in the neurodevelopmental MAM model of schizophrenia. *Biomolecules*, *12*(1), 108.

Stasiulewicz, A., Znajdek, K., Grudzień, M., Pawiński, T., & Sulkowska, A. J. I. (2020). A guide to targeting the endocannabinoid system in drug design. *International Journal of Molecular Sciences*, *21*(8), 1–46.

Subbanna, S., Psychoyos, D., Xie, S., & Basavarajappa, B. S. (2015). Postnatal ethanol exposure alters levels of 2-arachidonylglycerol-metabolizing enzymes and pharmacological inhibition of monoacylglycerol lipase does not cause neurodegeneration in neonatal mice. *Journal of Neurochemistry*, *134*(2), 276–287.

Sznajder, K. K., Teti, D. M., & Kjerulff, K. H. (2022). Maternal use of acetaminophen during pregnancy and neurobehavioral problems in offspring at 3 years: A prospective cohort study. *PLoS ONE*, *17*(9 September), 1–17.

Taylor, P. V., Veenema, A. H., Paul, M. J., Bredewold, R., Isaacs, S., & De Vries, G. J. (2012). Sexually dimorphic effects of a prenatal immune challenge on social play and vasopressin expression in juvenile rats. *Biology of Sex Differences*, *3*(1), 1.

Terral, G., Marsicano, G., Grandes, P., & Soria-Gómez, E. (2020). Cannabinoid control of olfactory processes: the where matters. *Genes*, *11*(431), 1–8.

Thomas, A., Burant, A., Bui, N., Graham, D., Yuva-Paylor, L. A., & Paylor, R. (2009). Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety. *Psychopharmacology*, *204*(2), 361–373.

Thompson, J. M. D., Waldie, K. E., Wall, C. R., Murphy, R., & Mitchell, E. A. (2014). Associations between acetaminophen use during pregnancy and ADHD symptoms measured at ages 7 and 11 years. *PLoS ONE*, *9*(9), 1–6.

Thornton, A. M., Humphrey, R. M., Kerr, D. M., Finn, D. P., & Roche, M. (2021). Increasing endocannabinoid tone alters anxiety-like and stress coping behaviour in female rats prenatally exposed to valproic acid. *Molecules*, *26*(12), 3720.

Tong, N., Sanchez, J. F., Maggirwar, S. B., Ramirez, S. H., Guo, H., Dewhurst, S., & Gelbard, H. A. (2001). Activation of glycogen synthase kinase 3 beta (GSK-3β) by platelet activating factor mediates migration and cell death in cerebellar granule neurons. *European Journal of Neuroscience*, *13*(10), 1913–1922.

Tovo-Rodrigues, L., Carpena, M. X., Martins-Silva, T., Santos, I. S., Anselmi, L., Barros, A. J. D., Barros, F. C., Bertoldi, A. D., & Matijasevich, A. (2020). Low neurodevelopmental performance and behavioural/emotional problems at 24 and 48 months in Brazilian children exposed to acetaminophen during foetal development. *Paediatric and Perinatal Epidemiology*, *34*(3), 278–286.

Trezza, V., & Vanderschuren, L. J. M. J. (2008). Bidirectional cannabinoid modulation of social behavior in adolescent rats. *Psychopharmacology*, *197*(2), 217–227.

Urquhart, P., Nicolaou, A., & Woodward, D. F. (2015). Endocannabinoids and their oxygenation by cyclo-oxygenases, lipoxygenases and other oxygenases. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids*, *1851*(4), 366–376.

USEPA. (2006). Harmonization in interspecies extrapolation: use of BW 3/4 as default method in derivation of the oral RfD, (68), 1–34.

Van Der Stelt, M., Mazzola, C., Esposito, G., Matias, I., Petrosino, S., De Filippis, D., Micale, V., Steardo, L., Drago, F., Iuvone, T., & Di Marzo, V. (2006). Endocannabinoids and β-amyloid-induced neurotoxicity in vivo: Effect of pharmacological elevation of endocannabinoid levels. *Cellular and Molecular Life Sciences*, *63*(12), 1410–1424.

Viberg, H., Eriksson, P., Gordh, T., & Fredriksson, A. (2014). Paracetamol (acetaminophen) administration during neonatal brain development affects cognitive function and alters its analgesic and anxiolytic response in adult male mice. *Toxicological Sciences*, *138*(1), 139–147.

Vicente, A. M., Martins, G. J., & Costa, R. M. (2020). Cortico-basal ganglia circuits underlying dysfunctional control of motor behaviors in neuropsychiatric disorders. *Current Opinion in Genetics and Development*, *65*, 151–159.

Vogel-Ciernia, A., Careaga, M., LaSalle, J. M., & Ashwood, P. (2018). Microglia from offspring of dams with allergic asthma exhibit epigenomic alterations in genes dysregulated in autism. *Glia*, *66*(3), 505–521.

Wiley, J. L., Barrus, D. G., Farquhar, C. E., Lefever, T. W., & Gamage, T. F. (2021). Sex, species and age: effects of rodent demographics on the pharmacology of ∆9-tetrahydrocanabinol. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*,

106(April 2020).

Wilkerson, J. L., Ghosh, S., Bagdas, D., Mason, B. L., Crowe, M. S., Hsu, K. L., Wise, L. E., Kinsey, S. G., Damaj, M. I., Cravatt, B. F., & Lichtman, A. H. (2016). Diacylglycerol lipase β inhibition reverses nociceptive behaviour in mouse models of inflammatory and neuropathic pain. *British Journal of Pharmacology*, *173*(10), 1678–1692.

Wilkes, B. J., & Lewis, M. H. (2018). The neural circuitry of restricted repetitive behavior: Magnetic resonance imaging in neurodevelopmental disorders and animal models. *Neuroscience and Biobehavioral Reviews*, *92*(May), 152–171.

Witchey, S. K., Al Samara, L., Horman, B. M., Stapleton, H. M., & Patisaul, H. B. (2020). Perinatal exposure to FireMaster® 550 (FM550), brominated or organophosphate flame retardants produces sex and compound specific effects on adult Wistar rat socioemotional behavior. *Hormones and Behavior*, *126*, 104853.

Wood, M. E. (2020). Associations between prenatal acetaminophen exposure and child neurodevelopment: Truth, bias, or a bit of both? *Paediatric and Perinatal Epidemiology*, *34*(3), 233–236.

Xi, C., Yang, Z., Yu, Y., Li, S., He, J., El-Aziz, T. M. A., Zhao, F., & Cao, Z. (2022). Influence of perinatal deltamethrin exposure at distinct developmental stages on motor activity, learning and memory. *Ecotoxicology and Environmental Safety*, *236*, 113460.

Ystrom, E., Gustavson, K., Brandlistuen, R. E., Knudsen, G. P., Magnus, P., Susser, E., Davey Smith, G., Stoltenberg, C., Surén, P., Håberg, S. E., Hornig, M., Lipkin, W. I., Nordeng, H., & Reichborn-Kjennerud, T. (2017). Prenatal exposure to acetaminophen and risk of ADHD. *Pediatrics*, *140*(5), 1–9.

Zou, M., Liu, Y., Xie, S., Wang, L., Li, D., Li, L., Wang, F., Zhang, Y., Xia, W., Sun, C., & Wu, L. (2021). Alterations of the endocannabinoid system and its therapeutic potential in autism spectrum disorder. *Open Biology*, *11*(2).

Zucker, I. (2017). Risk mitigation for children exposed to drugs during gestation: A critical role for animal preclinical behavioral testing. *Neuroscience and Biobehavioral Reviews*, *77*, 107–121.

Zygmunt, P. M., Chuang, H. H., Movahed, P., Julius, D., & Högestätt, E. D. (2000). The anandamide transport inhibitor AM404 activates vanilloid receptors. *European Journal of Pharmacology*, *396*(1), 39–42.

ANEXOS

ANEXO A

Parecer da Comissão de Ética (nº8522.2019.35)

UNIVERSIDADE ESTADUAL DE LONDRINA

COMISSÃO DE ÉTICA NO USO DE ANIMAIS

OF. CIRC. CEUA Nº 74/2019 **Londrina, 30 de maio de 2019.**

Prezado (a) professor (a)

Certificamos que o projeto de pesquisa intitulado: **"Avaliação comportamental e do sistema canabinóide em ratos expostos ao paracetamol durante a gestação e lactação"** protocolo CEUA nº **8522.2019.35** sob a responsabilidade de **Estefânia Gastaldello Moreira,** que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem) 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 da Experimentação Animal (CONCEA), e foi **aprovado** pela Comissão de Ética no Uso de Animais da Universidade Estadual de Londrina (CEUA/UEL), em **28/05/2019.**

Este projeto tem por objetivo avaliar, na prole masculina e feminina de ratas tratadas com paracetamol durante a gestação e lactação, o comportamento em testes de função sensorial, motora, estereotipia comportamental e comportamento social na presença e ausência de agonista canabinoide. Serão também quantificadas as concentrações de receptores, enzimas e mediadores que compõem o sistema canabinóide ou vias associadas. Grau de invasividade: GI2

Finalidade	( ) Ensino (x) Pesquisa científica
Vigência da autorização	10/01/2020 a 20/12/2022
Espécie/ linhagem/ raça	Rato heterogênico
Nº de animais	1113 ratos. Sendo 51 machos e 102 fêmeas (75 dias) oriundos do Biotério Central. Prole estimada: 480 machos e 480 fêmeas (22-30 dias).
Peso/ Idade	75 dias e 22-30 dias
Sexo	Machos e Fêmeas
Origem	Biotério Central da UEL
Amostras a serem coletadas	Regiões encefálicas (córtex pré-frontal, hipocampo, estriado e cerebelo)

Cumpre orientar que caso pretendam-se quaisquer alterações no protocolo experimental aprovado, deve-se submeter o novo protocolo à apreciação da CEUA/UEL anteriormente à execução das modificações.

Coloco-me à disposição para quaisquer esclarecimentos que se fizerem necessários. Sem mais para o momento, subscrevo-me, cordialmente.

Maria Fernanda R. Graciano

Profa. Dra. Maria Fernanda Rodrigues Graciano
Coordenadora da CEUA/UEL

Profª Drª Maria Fernanda Rodrigues Graciano
Coordenadora do Comitê de Ética no Uso de Animais
Universidade Estadual de Londrina
/ (43) 3371-5454

Ilmo.(a) Sr.(a)
Prof. (a) Dr (a). Estefânia Gastaldello Moreira
Responsável pelo projeto
Departamento de Ciências Fisiológicas
C/C para a Chefia do Depto. de Ciências Fisiológicas /CCB
C/C para a Direção de Centro do CCB
C/C para o Biotério Central/CCB