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

YNGRID MICKAELLI OLIVEIRA DOS SANTOS

ALTERAÇÕES ONTOGÊNICAS, IMUNOLÓGICAS E COMPORTAMENTAIS DA EXPOSIÇÃO AO CRACK DURANTE O PERÍODO GESTACIONAL

Maceió 2023

YNGRID MICKAELLI OLIVEIRA DOS SANTOS

ALTERAÇÕES ONTOGÊNICAS, IMUNOLÓGICAS E COMPORTAMENTAIS DA EXPOSIÇÃO AO CRACK DURANTE O PERÍODO GESTACIONAL

Defesa de Doutorado ao Programa de Pós-Graduação em Ciências da Saúde da Universidade Federal de Alagoas, como requisito parcial para obtenção do grau de Doutora em Ciências da Saúde.

Orientador: Prof. Dr. Olagide Wagner de Castro

Coorientador: Prof. Dr. Alexandre Urban Borbely

Maceió 2023

Catalogação na Fonte
Universidade Federal de Alagoas
Biblioteca Central
Divisão de Tratamento Técnico
Bibliotecário: Marcelino de Carvalho Freitas Neto – CRB-4 – 1767

S237a Santos, Yngrid Mickaelli Oliveira dos.
Alterações ontogênicas, imunológicas e comportamentais da exposição ao crack durante o período gestacional / Yngrid Mickaelli Oliveira dos Santos. – 2023.
83 f. : il., grafs., tabs. color.

Orientador: Olagide Wagner de Castro.
Coorientador: Alexandre Urban Borbely.
Tese (doutorado em ciências da saúde) – Universidade Federal de Alagoas. Instituto de Ciências Biológicas e da Saúde. Programa de Pós-Graduação em Ciências da Saúde. Maceió, 2023.

Bibliografia: f. 55-81.
Anexo: f. 83.

1. Cocaína crack. 2. Comportamento materno. 3. Gravidez. 4. Baço. 5. Morte celular. I. Título.

CDU: 615.32:618.2/.3

DEDICATÓRIA

Dedico este trabalho primeiramente a minha mãe Mª Quitéria de Vieira de Oliveira, a ela devo tudo que sou e que tenho.

Às minhas irmãs, Noemi e Francielly, por existirem e darem o real sentido de família.

Ao meu companheiro, Arnon Almeida, principalmente pela paciência e compreensão nesses anos, nos quais naturalmente o pós graduando passa por altos e baixos, e tantas vezes precisa da força que o amor fornece para superar os desafios e seguir atrás dos sonhos. Aos meus sogros, cunhados pelo incentivo e apoio. Aos meus sobrinho Miguel, Mateus e Layla, pela leveza que sempre trouxeram a minha vida.

A um anjo em forma de cachorro, Luna, que sempre me recebe com amor imutável e incondicional.

A todos os meus amigos pelas conversas e saídas divertidas renovando meu ânimo. A todos amigos dos laboratórios pelos quais passei, pelo trabalho em equipe. Uma ênfase para Maisa e Jucilene por ouvirem meus desabafos e compartilharem situações em comum.

A toda minha família por ser a base de tudo.

AGRADECIMENTOS

Para que a concretização deste estudo se efetivasse: agradeço às inúmeras pessoas que participaram neste processo, seja conduzindo os experimentos juntamente comigo, seja pela troca de conhecimento e experiências, essenciais em minha caminhada pessoal e profissional. Por estes exemplos, expresso meus reais agradecimentos.

Ao Profº Dr. Olagide Wagner de Castro, pela inteligência e confiança ao aceitar a orietação.

Ao Profº Dr. Alexandre Borbely pela coorientação, disponibilidade e ensinamentos.

Aos professores mestres e doutores, que passaram durante toda minha jornada de construção profissional e pessoal.

As colegas de laboratório Maisa, Amanda, Fernanda e Jucilene, por dividirem o espaço, tempo, experiências, conhecimento, alegrias e tristezas, assim como todos do laboratório LNFI pela disposição de contribuir como equipe na realização de todas as etapas. A Milenna, aluna de iniciação científica, que se mostrou mais do que preparada para abraças o projeto. Ao Igor, amigo e companheiro, hoje professor da UFAL, por sempre acompanhar todo meu trajeto, e por esse último ano me impulsionar a finalizar esse ciclo, muitas vezes quando me faltavam forças, e pela colaboração nas análises dos resultados e na escrita do artigo.

Aos meus colegas de curso da graduação, pós-graduação e disciplinas que compartilharam comigo seus conhecimentos e momentos de alegrias.

Aos Laboratórios de Biologia celular (LBC), Laboratório de biologia celular e Molecular (LBCM) e Laboratório de metabolômica.

A todos os funcionários do ICBS-UFAL e às instituições CAPES e CNPQ pelo apoio financeiro.

A todos aqueles que de alguma forma contribuíram ou torceram pela concretização desta pesquisa.

“Todos temos luz e trevas dentro de nós. O que nos define é o lado com o qual escolhemos agir.”

Harry Potter e a ordem da fenix

“Pois onde estiver o seu tesouro, aí também estará o seu coração”
Harry Potter e as Relíquias da morte e Mateus 6:21

RESUMO

O crack é a forma cristalina da cocaína que possui alto poder viciante e estimula o sistema nervoso central (SNC). O crack tem ações potencializadas devido às substâncias geradas por sua pirólise, aumentando a biodisponibilidade, a velocidade do metabolismo e a dependência pela droga. O aumento do consumo de drogas psicoativas por mulheres em idade reprodutiva tem gerado muitos desafios nas áreas de saúde e social. O uso de crack por gestantes tem sido correlacionado com maior incidência de fetos prematuros, malformações do SNC e dano celular, bem como alterações no sistema imunológico. O objetivo do nosso estudo foi avaliar os efeitos da exposição gestacional ao crack em ratas grávidas sobre as células da placenta, órgãos imunológicos, comportamento materno e ansioso e desenvolvimento do reflexo sensório-motor. Ratas prenhes foram expostas ao ar ou ao crack (200 mg, por 10 min) do 5º ao 9º dia ou até o final da prenhes. A exposição gestacional ao crack aumentou a morte das células trofoblásticas e reduziu as células viáveis, associada à redução do crescimento dos cones ectoplacentários em ensaios de morte celular por citometria de fluxo e outgrowt. Além disso, a exposição ao crack durante a gravidez reduziu o peso relativo do baço, bem como o número de esplenócitos totais e subpopulações de linfócitos T CD8+ pela técnica de citomtria de fluxo. Por fim, a exposição ao crack levou a comportamento do tipo ansiogênico e negligência no cuidado materno, analisado por observações de comportamentos estereotipados. Tomados em conjunto, nossos achados fornecem informações sobre as mudanças gestacionais promovidas após a exposição ao crack e dão suporte a futuras intervenções e tratamentos clínicos.

PALAVRAS-CHAVE: crack; comportamento materno; gravidez; baço; morte celular.

ABSTRACT

Crack is the crystal form of cocaine that has a high addictive power and stimulates the central nervous system (CNS). Crack has potentiated actions due to the substances generated by its pyrolysis, increasing the bioavailability, the speed of metabolism and drug addiction. The increased consumption of psychoactive drugs by women of childbearing age has generated many challenges in the health and social areas. Crack use by pregnant women has been correlated with a higher incidence of premature fetuses, CNS malformations and cell damage, as well as changes in the immune system. Our study aimed to evaluate the effects of gestational exposure to crack cocaine in pregnant rats on placental cells, immune organs, maternal and anxiety-like behavior, and sensorimotor reflex development. Pregnant rats were exposed to air or crack cocaine (200 mg, for 10 min) from the 5^{th} to the 9^{th} day or until the end of pregnancy. Gestational exposure to crack cocaine increased trophoblastic cell death and reduced viable cells, associated with reduced growth of ectoplacental cones in cell death assays by flow cytometry and outgrowth. In addition, exposure to crack during pregnancy reduced the relative weight of the spleen, as well as the number of total splenocytes and CD8+ T lymphocyte subpopulations by the flow cytometry technique. Finally, exposure to crack led to anxiogenic-like behavior and negligence in maternal care. Taken together, our findings provide insight into gestational changes promoted following exposure to crack cocaine and support future clinical interventions and treatments.

KEYWORDS: crack; maternal behavior; pregnancy; spleen; cell death.

LISTA DE FIGURAS

SUMÁRIO

LISTA DE ABREVIATURAS

ASR: resposta de sibressalto auditivo

CAE: closed arm entries (entrada nos braços fechados)

CD: grupo de diferenciação

CNS: central nervous system

EPM: elevated plus-maze (labirinto em cruz elevado)

GD: dia gestacional

GN: geotaxia negativa

GRO: grooming (autolimpeza)

PBS: solução salina fosfato

OAE: open arm entries (entradas no braço aberto)

OAT: time spent in open arms (tempo nos braços abertos)

OF: open field (campo aberto)

PDN: dia pós natal

PG: palmar-grasp (prensão palmar)

pSAP: protected stretch attend postures (estiramento com as patas dianteiras)

RD: recovery of the decubitus (recuperação de decúbito)

FBS: foetal bovine serum (soro fetal bovino)

1 INTRODUÇÃO

Conceitos e dados epidemiológicos

A cocaína tem sido apontada como uma droga ilícita, cujo consumo e venda são ilegais. Dentre as drogas que tem sua venda proibida a mais frequentemente utilizada é a cocaína, segundo atendimento em departamentos de emergência ou centros de desintoxicação de drogas (Rodrigues e Silveira, 2022). O Benzoilmetilecgonina, popularmente conhecido como cocaína, é uma substância presente nas folhas da planta Erythroxylon coca. A partir dessas folhas, é obtida a pasta base, que ao final do processo de isolamento e refino gera o cloridrato de cocaína, o qual pode ser inalado ou injetado. Quando é adicionado outros produtos a essa pasta base, principalmente o bicarbonato de sódio, o crack é formado (Stewart apud Kintz et al., 1995; Goldstein, 2009.).

A cocaína e o crack são consumidos por 0,3% da população mundial (UNODCCP, 2001). A maior parte dos usuários concentra-se nas Américas (70%) (Negrete, 1991). O crack foi identificado em 1980 a partir de relatórios de uso que emergiram de Los Angeles, San Diego, Houston e no Caribe. Alguns anos depois, na mesma década, o número de pessoas que usavam cocaína rotineiramente aumentou de 4,2 milhões para 5,8 milhões (Reinerman e Levine, 1997). No Brasil, o crack surgiu no início da década de 90 (Donato, 2011).

Segundo a Organização das Nações Unidas sobre drogas e crimes, a cocaína é a droga ilícita mais vendida e consumida nas Américas do Norte e Sul, bem como em regiões da Europa Ocidental e Central. Além disso, o consumo na América do Sul em 2010 era estimado em 1,84 milhões de usuários e passou para 3,54 milhões de usuários em 2012, com o Brasil liderando o maior mercado do continente (UNODC, 2015).

O último levantamento sobre os usuários de crack no Brasil, realizado por um grupo de pesquisa da Fiocruz, indicou cerca de 370 mil usuários regulares de crack e/ou de formas similares de cocaína fumada (pasta-base, merla e oxi), nas 26 capitais brasileiras e no Distrito Federal. Esse quantitativo representa 0,8% população do Brasil, sendo 140 mil desses usuários pertencentes à região do nordeste. Considerada uma população oculta e de difícil acesso, e o crack representa 35% do total de consumidores de drogas ilícitas, com exceção da maconha. Destes usuários de crack, 40% são mulheres no qual metade das participantes da pesquisa, engravidaram durante o uso da droga (Bastos; Bertoni et al, 2014).

Fisiologia da cocaína e crack

A cocaína quando consumida é absorvida e convertida em metabólitos, tais como a benzoilecgonina e o cocaetileno (Parks et al, 2010). Esses metabólitos podem alcançar rapidamente o sistema nervoso central (SNC) e exercer suas principais ações de hiperestimulação no sistema dopaminérgico (Ritz et al., 1990 e Azevedo, 2014), impulsionando a liberação e aumentando o tempo de ação dos neurotransmissores noradrenalina e serotonina (Ferreira, et al 2017).

Em situações normais, os neurotransmissores são secretados para a realização da sinapse, sendo novamente conduzidos para o interior dos neurônios por meio de transportadores. No entanto, na presença de cocaína, esses transportadores são inibidos, o que aumenta o pool do neurotransmissor, como a dopamina, na fenda sináptica, consequentemente, o consumo dessa droga leva ao aumento da concentração e duração desses neurotransmissores (Rego, 2010). Essa situação acarreta nos sintomas de euforia e agitação, aumentando também a autoestima de quem faz uso da droga além das habilidades do indivíduo (Azevedo, 2014). Entretanto, com a frequência do consumo, a confiança é transformada em necessidade de recompensa que culmina no uso de quantidades cada vez maiores para alcançar o efeito desejado (Ferreira et al 2017).

De uma forma geral, os efeitos fisiológicos e psicoativos da cocaína são semelhantes independentemente da forma apresentada. A farmacocinética da cocaína depende de múltiplos fatores, tais como a forma física/química, a via de administração, a genética do usuário e o consumo concomitante de outras drogas lícitas/ilícitas, como o álcool (Dinis-Oliveira, 2015). No entanto, existem evidências que demonstram as diferenças e particularidades de cada forma de consumo, como a duração e a magnitude do efeito, além de maior propensão à dependência o imediatismo, e consequências mais graves acontecem quando a cocaína é fumada (crack) (Hatsukami e Fischman, 1996).

Além disso, há um produto formado apenas quando a base da cocaína é fumada, a metilecgonidina ou éster metílico de anidroecgonina (AEME), identificada em fluidos biológicos de fumantes de crack. A ecgonina, um metabólito da metilecgonidina formado através da atividade da esterase, também foi identificada em amostras semelhantes coletadas de fumantes de crack. Um estudo in vitro mostrou que esse composto, ao contrário dos efeitos

estimulantes cardiovasculares da cocaína, possui efeitos de diminuir a contratilidade e estimular a produção de óxido nítrico em células e tecidos cardíacos (Scheidweiler et al, 2003).

O metabólito do crack, AEME, também tem sido apontado como um produto neurotóxico, causando maior neurotoxicidade do que após o uso de cocaína isoladamente (Garcia, 2012). Essa suscetibilidade a neurotoxicidade pode estar sendo influenciada pela melatonina, um hormônio pineal neuroprotetor (Mesquita, et al 2017). Outro efeito divergente dos produzidos pela cocaína, a AEME diminui a pressão arterial e leva a alterações neuroquímicas nos sistemas dopaminérgico e endocanabinóide (Erzouki, 1995 e Gomes, 2018). Esses processos são notados apenas na pirólise do crack, o que pode sugerir que tal metabólito pode estar influenciando o padrão mais intenso de dependência ao crack (Areal, 2015).

Crack na gestação

O uso de drogas de abuso representa um importante problema de saúde pública, atingindo todas as classes sociais, idades e gêneros, inclusive gestantes (Siliquini et al., 2005). Pesquisas epidemiológicas e estudos de coorte mostraram um aumento da dependência de drogas ilícitas em mulheres grávidas em diferentes regiões do mundo (Kassada et al., 2013).

Cocaína, crack e metabólitos de combustão atravessam a barreira placentária (Bell e Lau, 1995), o que pode promover complicações e efeitos prolongados para embriões e fetos. Portanto, é crescente o número de crianças afetadas pela exposição ao crack (Duailibi et al., 2008).

O consumo de crack pode apresentar diversas complicações em humanos e modelos experimentais. O crack pode levar a síndromes associadas à placenta (ou seja, descolamento prematuro da placenta, pré-eclâmpsia e infarto da placenta), parto prematuro e crescimento fetal prejudicado (dos Santos et al., 2018; Legido et al., 1992).

Em um estudo com ratas grávidas expostas ao crack, foram observados déficits neuropsicomotores e cognitivos de longo prazo na prole, incluindo prejuízo no processo de aprendizagem e memória, problemas comportamentais e efeitos adversos na função executiva (Pacheco et al., 2021). Além disso, o uso de cocaína e seus derivados durante a gravidez pode comprometer os estágios mais avançados do desenvolvimento: 1) as crianças podem apresentar déficits cognitivos, dificuldade de verbalização, agressividade, comprometimento de regiões

cerebrais envolvidas em funções de memória declarativa, ansiedade e depressão; 2) os adultos podem ter suscetibilidade alterada a crises epilépticas (Pacheco et al., 2021; Slamberová, 2003).

Os efeitos do uso de crack durante a gravidez também podem ser indiretos, associados à desnutrição induzida por drogas, falta exame de pré-natal, uso concomitante de outras substâncias tóxicas e estresse perinatal (de Moraes Costa et al., 2012).

Estudos em animais sugerem que o ambiente pré-natal pode ter um efeito profundo na prole, principalmente associado ao estresse desafiador da homeostase durante a gravidez e ao sexo da prole (Howerton e Bale, 2012; Weinstock, 2005). Animais machos e fêmeas são afetados pelo estímulo estressor de forma diferente e apresentam diferentes padrões de mudanças comportamentais após o estresse materno (Babb et al., 2013).

Nos mamíferos, as mães são as principais cuidadoras e programadas pelos hormônios produzidos durante a gravidez. No período que antecede o parto, há flutuações nos níveis de hormônio esteroide, que modulam a expressão do comportamento materno (Kohl et al., 2017). Além disso, a produção e a liberação de progesterona e estrogênio aumentam lentamente durante a gestação. Conforme os níveis de progesterona chegam no limiar e logo em sequência caem rapidamente, controlam a liberação pulsátil de ocitocina, que irão desencadear as contrações uterinas. No parto, a prolactina também aumenta para estimular a produção de leite e ocitocina para a ejeção do leite em resposta à sucção (Numan, 1977).

Os cuidados maternos são essenciais para a sobrevivência e saúde ao longo da vida da prole. Além disso, fatores ambientais, incluindo temperatura e luz, têm efeitos dramáticos na saúde e bem-estar animal durante o final da gestação e após o parto (Kohl et al., 2017). O comportamento materno compreende atividades comportamentais estereotipadas e específicas da espécie, como construção de ninho, agachamento e alimentação (incluindo amamentação), adotadas pela mãe desde o período pré-parto imediato até quando a prole pode sobreviver sozinha sem atenção e provisão materna (Curley e Champagne, 2016; Kohl e outros, 2017).

A compreensão dos mecanismos que modulam o comportamento materno pode ter diferentes enfoques em relação às consequências para a mãe e a prole. O uso de drogas de abuso durante o período gestacional pode comprometer o comportamento materno. O efeito da exposição à fumaça do crack no comportamento materno e no desenvolvimento embrionário da prole nunca foi medido em um modelo animal. Portanto, avaliamos a viabilidade celular de locais de implantação, crescimento de cones ectoplacentários, reflexologia, bem como comportamentos maternos, não maternos e de ansiedade de ratas grávidas sob a influência da

exposição pré-natal ao crack. A partir disso, testamos a hipótese de que a exposição materna ao crack afeta o desenvolvimento embrionário da prole e seus reflexos, bem como o comportamento materno, ansioso e órgãos linfóides de ratas prenhes.

2 OBJETIVOS

2.1 Geral

Avaliar a influência da exposição maternal aos produtos da pirólise do crack na gestação, no desenvolvimento embrionário da prole e seus reflexos, bem como o comportamento materno, ansioso e órgãos linfóides de ratas prenhes.

2.2 Específicos

1) Avaliar a morte das células trofoblásticas da placenta, bem como possíveis alterações de viabilidade;

2) Avaliar o comportamento ansioso nas ratas grávidas;

3) Identificar o efeito do uso do crack no comportamento materno;

4) Verificar o desenvolvimento sensório motor na geração F1 durante período neonatal;

5) Avaliar o padrão de neurodegeneração, processos necróticos e apoptóticos nas mães

6) Verificar o efeito do uso do crack nos órgãos linfóides;

3 ARTIGO

ONTOGENIC, IMMUNOLOGICAL AND BEHAVIORAL CHANGES OF CRACK EXPOSURE DURING THE GESTATIONAL PERIOD

Yngrid Mickaelli Oliveira dos Santos[1], Igor Santana de Melo[1], Amanda Larissa Dias Pacheco[1], Milenna Maria Jatoba Hasten Reiter[1], Maisa de Araújo Costa[1], Keyla Silva Nobre Pires[1], Fernanda Maria Araújo de Souza[1], Jucilene Freitas-Santos[1], Mayara Rodrigues Barbosa[1], Marvin Paulo Lins[1], Amanda de Barros Coutinho[1], Eloisa Bortolote Oliosi[1], Bianca Rodrigues Melo[1], Keylla Lavínia da Silva Oliveira[1], Marcelo Duzzioni[1], Alexandre Urban Borbely[1], Olagide Wagner de Castro[1*].

[1]Institute of Biological Sciences and Health, Federal University of Alagoas (UFAL), Maceio, AL, Brazil.

*Author's correspondence:

Olagide Wagner de Castro

Institute of Biological Sciences and Health, Federal University of Alagoas (UFAL), Av. Lourival de Melo Mota, km 14, Campus A. C. Simões, Cidade Universitária, Maceió/AL, Brazil; CEP 57072-970, e-mail: olagidewww@gmail.com

ABSTRACT

Crack cocaine is the crystal form of cocaine that has a high addictive power and stimulates the central nervous system (CNS). Crack cocaine has potentiated actions due to the substances generated by its pyrolysis, increasing the bioavailability, the speed of metabolism and drug addiction. The increased consumption of psychoactive drugs by women of childbearing age has generated many challenges in the health and social areas. Crack cocaine use by pregnant women has been correlated with a higher incidence of premature fetuses, CNS malformations and cell damage, as well as changes in the immune system. Our studyaimed to evaluate the effects of gestational exposure to crack cocaine in pregnant rats on placental cells, immune organs, maternal and anxiety-like behavior, and sensorimotor reflex development. Pregnant rats were exposed to air or crack cocaine (200 mg, for 10 min) from the 5^{th} to the 9^{th} day or until the end of pregnancy. Gestational exposure to crack cocaine increased trophoblastic cell death and reduced viable cells, associated with reduced growth of ectoplacental cones. In addition, exposure to crack cocaine during pregnancy reduced the relative weight of the spleen, as well as the number of total splenocytes and CD8+ T lymphocyte subpopulations. Finally, exposure to crack cocaine led to anxiogenic-like behavior and negligence in maternal care. Taken together, our findings provide insight into gestational changes promoted following exposure to crack cocaine and support future clinical interventions and treatments.

KEYWORDS: crack; maternal behavior; pregnancy; spleen; cell death.

3.1 INTRODUCTION

Psychoactive substances can modify the proper functioning of the central nervous system (CNS), promoting a stimulating, depressing or disturbing effect (Duailibi et al., 2008). The use of drugs of abuse represents a major public health problem, reaching all social classes, ages and genders, including pregnant women (Siliquini et al., 2005). Epidemiological surveys and cohort studies have shown an increase in illicit drug dependence in pregnant women in different regions of the world (Kassada et al., 2013).

Cocaine and crack cocaine are among the main drugs of abuse used that cause dependence, especially in women with an abundance of adipose tissue, which makes it difficult to eliminate the drug and increases vulnerability to consumption (Hoang et al., 2020; Philipsen et al., 2020).

Cocaine is considered a psychotropic drug, characterized by its sympathomimetic effect, drug addiction and overdose deaths (Baumann and Pescatore, 2018). Cocaine is in the form of powder (cocaine hydrochloride), while crack cocaine is the free base form of cocaine hydrochloride, a potent CNS stimulant. Smoked crack cocaine results in immediate and pleasurable effects with different duration and intensities. Because of this, crack cocaine is one of the most worrying drugs due to its addictive potential, low price for manufacture and sale, high availability, and easy consumption (Kessler and Pechansky, 2008).

Cocaine, crack cocaine and combustion metabolites cross the placental barrier (Bell and Lau, 1995), which can promote complications and prolonged effects for embryos and fetuses. Therefore, the number of children affected by exposure to crack cocaine is growing (Duailibi et al., 2008).

Crack cocaine consumption can present several complications in humans and experimental models. Crack cocaine can lead to placenta-associated syndromes (i.e. placental

abruption, pre-eclampsia and placental infarction), premature birth, and impaired fetal growth (dos Santos et al., 2018; Legido et al., 1992).

In a study with pregnant rats exposed to crack, long-term neuropsychomotor and cognitive deficits were observed in the offspring, including impairment in the learning and memory process, behavioral problems and adverse effects on executive function (Pacheco et al., 2021). In addition, the use of cocaine and its derivatives during pregnancy can compromise the most advanced stages of development: 1) children may have cognitive deficits, difficulty in verbalization, aggressiveness, impairment of brain regions involved in declarative memory functions, anxiety and depression; 2) adults may have altered susceptibility to epileptic seizures (Pacheco et al., 2021; Slamberová, 2003).

The effects of crack cocaine use during pregnancy can be direct or indirect, associated with drug-induced malnutrition, lack of prenatal care, concomitant use of other toxic substances and perinatal stress (de Moraes Costa et al., 2012).

Animal studies suggest that the prenatal environment can have a profound effect on the offspring, primarily associated with homeostasis-challenging stress during pregnancy and the sex of the offspring (Howerton and Bale, 2012; Weinstock, 2005). Male and female animals are affected by the stressor stimulus differently and show different patterns of behavioral changes after maternal stress (Babb et al., 2013).

In mammals, mothers are the primary caregivers, programmed by the hormones produced during pregnancy. Maternal care is essential for the survival and lifelong health of the offspring. In addition, environmental factors, including temperature and light, have dramatic effects on animal health and well-being during late gestation and after parturition (Kohl et al., 2017). Maternal behavior comprises stereotyped, species-specific behavioral activities such as nest building, squatting, and feeding (including nursing), adopted by the mother from the

immediate antepartum period until when the offspring can survive alone without maternal attention and provision (Curley and Champagne, 2016; Kohl et al., 2017).

The understanding of the mechanisms that modulate maternal behavior may have different focuses about the consequences on the mother and offspring. The use of drugs of abuse during the gestational period can compromise maternal behavior.

The effect of exposure to crack cocaine smoke on maternal behavior and embryonic development of the offspring has never been measured in an animal model. Therefore, we evaluated the cell viability of implantation sites, growth of ectoplacental cones, reflexology, as well as maternal, non-maternal and anxiety-like behaviors of pregnant rats under the influence of prenatal exposure to crack cocaine.

Starting from this, we tested the hypothesis that maternal exposure to crack cocaine affects the embryonic development of the offspring and its reflexes, as well as the maternal and anxiety-like behavior, and lymphoid organs of pregnant rats.

3.2 METHODS

3.2.1 Animals and mating protocol

Female Wistar rats (*Rattus norvegicus* [n= 29, 200-300g, 2-3 months]) of reproductive age were obtained from the main breeding stock of the Federal University of Alagoas (UFAL), with a 12h/12h light/dark cycle at 21 ± 2°C and water and food *ad libitum*. All experimental procedures were performed to reduce animal suffering and to limit the number of rats used, as well as approved by the Ethics Committee of the UFAL (Protocol #28/2021), Crack cocaine samples were obtained by the Civil Police Department of the State of Alagoas, AL-Brazil. For mating, females were placed in cages with sexually experienced male rats, and pregnancy was

confirmed by the presence of sperm in the vaginal wash. Confirmation of sperm indicated day zero of pregnancy (D0) (Salas-Ramirez et al., 2010).

3.2.2 Gestational exposure to crack cocaine

Pregnant rats were randomly assigned to a control group (Control, exposed to air, n=11) or exposed to crack cocaine (200 mg, n=14). All animals were weighed and handled equally in the apparatus. The exposures were made between 8 am and 1 pm from the 5th gestational day (5thGD) to the 9th GD or until the end of pregnancy (21st GD), according to the protocol (Pacheco et al., 2021) (Fig. 1A-B). To expose to crack cocaine, we used a device with a smoke generator pump attached at one end to a tube (where the substance was burned) and the other to an acrylic chamber (animal housing) with a fan configured to generate 150 ml /min (Pacheco et al., 2021; Ypsilantis et al., 2012). The animals were placed individually in an acrylic box while the crack cocaine was burning for 1 min. When the burning was interrupted, the rats were exposed to the smoke for 10 minutes, as described in detail by Pacheco et al., 2021.

3.2.3 Collection of Ectoplacental Cones

Female rats on the 9th day of gestation (DG9) were used for the experiment. They were euthanized and the uterus and ovaries were aseptically removed. The myometrium was discarded, and the endometrial implantation sites were carefully isolated. Subsequently, using a stereomicroscope, each endometrial implantation site was dissected, separating the mesometrial decidua regions, containing the ectoplacental cone (Träger), and the antimesometrial decidua regions, containing the embryo. The ectoplacental cones were collected and immediately placed in phosphate saline solution (PBS) at 37º C. After washing in PBS, the ectoplacental cones were cultured in a culture dish containing DMEM medium with

1mM glutamine, 1% antibiotics and 10% FBS in a humid oven at 37ºC, with 5% carbon dioxide for 24 and 48 hours (Fig. 1A).

3.2.4 Cell Viability Assay

The MTT colorimetric test evaluates the activity of enzymes that reduce MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolim bromide) to formazan crystals. Using a spectrophotometer for microplates, these eluted crystals were measured. Briefly, trophoblastic cells were transferred to 96-well microplates and cell viability was evaluated after adding 20 µL of MTT (5 µg/mL) for 4h at 37ºC. The precipitate was discarded and the formazan crystals were eluted with 150 µL of dimethyl sulfoxide. After analysis with a wavelength adjusted to 540 nm and a reference filter of 630 nm, the results expressed the percentage of viable cells.

3.2.5 Annexin V/Propidium Iodide Cell Death Assay

Cultured material was analyzed by flow cytometry with FITC Annexin V/Propidium Iodide (BD) apoptosis detection Kit I, where 1 x 10 6 cells/well were removed from the substrate with a 0.5% trypsin solution. The enzymatic action was neutralized with goat serum (Vector Laboratories) at 5% in PBS for 20 min. After washing in PBS, the cells were incubated with the kit and labeled according to the manufacturer's instructions. The analysis was performed on a FACS CantoTM II (BD) flow cytometer with the FACSDiva (BD) program. A total of 10000 events were acquired/sample. The data obtained were analyzed using the program FlowJo version 8.7.

3.2.6 Outgrowth Assay

Ectoplacental cones were plated in 12-well plates, a total of 3 ectoplacental cones per well. The photomicrographs were acquired through an inverted optical microscope (Nikon Eclipse TS100) at 200x magnification at points 0h, 24h, 48h, 72h and 96. The outgrowth area of the ectoplacental cone was measured and analyzed using an image analysis program Image J.

3.2.7 Behavioral tests

We evaluated the effect of gestational crack cocaine exposure on anxiety in pregnant rats (Control, n = 12; Crack, n = 13) using elevated plus-maze (EPM) and open field (OF). The physical dimensions of the EPM and OF, as well as the environmental conditions where the sessions were held are like those described by Pacheco et al., 2021. All behavioral tests were performed at the end of gestation in pregnant rats (Fig. 1B).

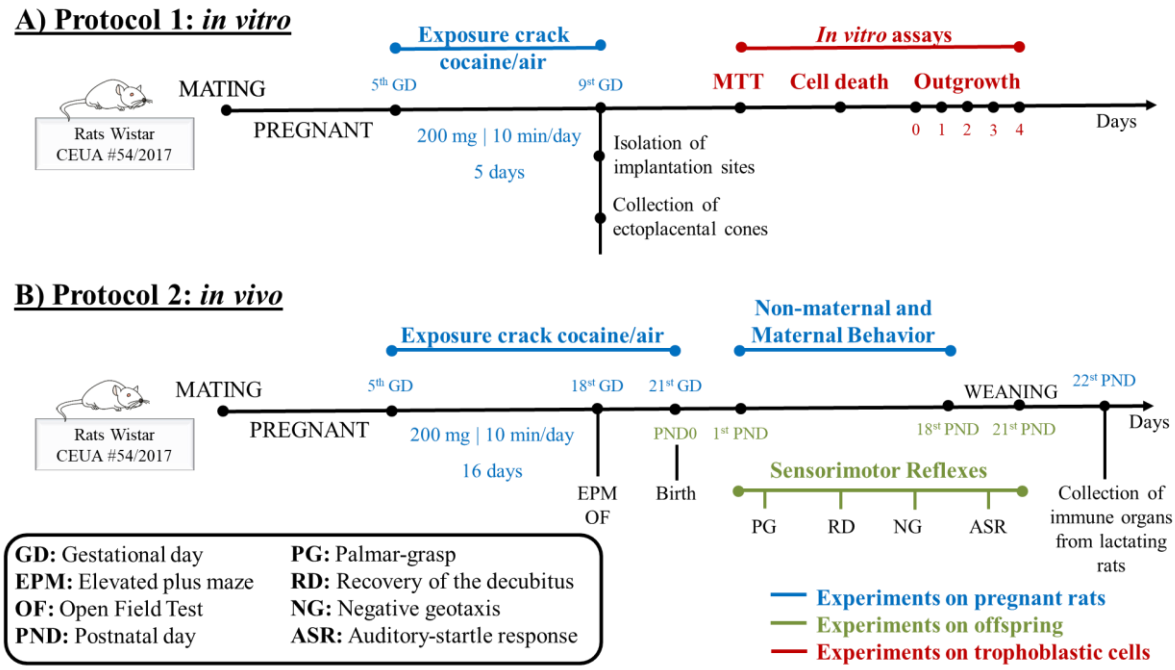

Fig. 1 Experimental design of gestational exposure to crack cocaine. Once the pregnancy is confirmed, considering day zero, exposure begins on the fifth day. (A) On the 9th day of gestation, surgeries were performed to obtain the ectoplacental cones, cultured and subjected to cell viability assays by MTT, cell death assay by flow cytometry for annexin V/propidium iodide, and cell growth assay of the ectoplacental cones. (B) In the group exposed to crack cocaine until the end of pregnancy, analysis of immune organs, maternal and anxiety-like behavior, and sensorimotor reflex development was performed.

3.2.8 Elevated plus maze (EPM)

Pregnant rats were placed individually in the central area of the maze, with their heads directed towards an enclosed arm. The animals were allowed to move freely through the maze for 5 minutes. The number of entries and time spent in both arms were recorded. EPM data were shown as the percentage of open arm entries (%OAE, number of entries in open arms/[number of entries in open + closed arms]∗100), percentage of time spent in open arms (%OAT, time spent in the open arms/[time spent in the open + closed arms]∗100), and number of closed arm entries (CAE). Furthermore, ethological parameters were evaluated, such as protected stretch attend postures (pSAP) and grooming (GRO; self-cleaning). The set of these data represented an index of anxiety-like behavior. The EPM was recorded using a video

camera connected to a computer, located 1m above the center of the EPM. All test sessions were performed at controlled room temperature, at the same time of day. At the end of the test, the EPM was properly cleaned with a 10% ethanol solution (Handley and Mithani, 1984).

3.2.9 Open field test (OF)

To evaluate the anxious-type behavior, the pregnant rats were placed individually in the center of the circular arena of the OF (60 cm × 60 cm × 50 cm), with the white acrylic floor divided into twelve parts. The OF test was recorded in the top view for 5 min (1m above OF's center) (Gáll et al., 2020). After each test, the apparatus was thoroughly cleaned with 10% ethanol. The measured parameters were as follows: (1) number of entries in the center; (2) time spent in the central disc; (3) number of entries in the corners; (4) time spent in the corners; (5) grooming activity; (6) rearing activity; and (7) total fecal bolli (Holubová-Kroupová and Šlamberová, 2021).

3.2.10 Birth and maternal care

After the birth of the offspring, considered as postnatal day (PND0), all the litters were adjusted to eight pups for each mother to have the same number of pups (4 males and 4 females). The analysis of maternal behavior was performed daily from the 1st to the 18th postnatal day (1^{st} PND1 – 18^{st} PND) for 1 h, between 8 am and 9 am. During this period, a new observation was made every 3 min, totaling 360 observations. The following variables were analyzed: non-maternal behavior (feeding, exploring, and self-grooming) and maternal behavior (licking pups, blanket and passive nursing, and nest building) (Costa et al., 2013).

3.2.11 Development of sensorimotor reflexes

A series of reflex tests were performed daily from the 1st to the 21st postnatal day or until confirmation of the consolidation of each reflex to evaluate the sensorial and motor development of the offspring exposed to air (Control, n = 16) or crack (n = 16) during the gestational period. Each animal of generation 1 was observed between 11 am and 1 pm. The following reflexes were analyzed: palmar-grasp (PG), righting (recovery of the decubitus – RD), negative geotaxis (NG) and auditory-startle response (ASR) (Fig. 1B). The day of consolidation of each reflex was considered the first day of the sequence of three consecutive days in which the reflex response appears within a maximum period of 10 seconds (Deiró et al., 2006; Fox, 1965; Smart and Dobbing, 1971).

3.2.12 Palmar-grasp

A light percussion was performed on the left fore paw of the rat with a 5 cm long, 1 mm diameter rod. The positive response was rapid finger flexion after two attempts (Deiró et al., 2008).

3.2.13 Righting (recovery of the decubitus)

The rat is placed in a supine position on a flat surface. The positive response was turning the body to the prone position. A reflex of muscle strength and subcortical maturation is assessed from this test (Abramova et al., 2021; Deiró et al., 2008; Fan et al., 2008).

3.2.14 Negative geotaxis

Upside down, the rat was placed in the middle of a ramp with a 45° inclination, and covered with non-slip material. The positive response was the body turning at least 140° to position the head upwards. Cerebellar integration, vestibular labyrinth, reflex development, and motor skills are evaluated from this test. (Abramova et al., 2021; Deiró et al., 2008; Fan et al., 2008).

3.2.15 Auditory-startle response

The rat was exposed to a high-pitched sound produced by the percussion of metallic sticks The positive response was the retraction and immobilization of the animal, typical of fright (Deiró et al., 2008).

3.2.16 Assessment of immune organs

After euthanasia of lactating rats, the main immune organs, thymus, and spleen, were extracted. These organs were weighed and physically disrupted in phosphate buffer solution (PBS) with 2% fetal bovine serum (FBS) (both from Invitrogen, Carlsbad, CA, USA) in a 24-well plate. Then, thymocytes and splenocytes in suspension were counted with a Neubauer chamber using the exclusion method with 0.02% Trypan blue solution (Sigma-Aldrich, St. Louis, MO, USA). To lyse the red blood cells from the spleen, Turk's solution (composed of 1% glacial acetic acid and 0.1% violet crystal – Dinamica Química, Indaiatuba, SP, Brazil – in distilled water) was used. Finally, cells were evaluated by flow cytometer, to the expression of surface glycoproteins applying the following fluorochrome-conjugated antibodies: anti-CD4/APC, anti-CD8/PE, and anti-CD45R (B220)/PE-Cy7 (all from eBioscience, San Diego,

CA, USA). After 20 minutes of staining, at 4°C and protected from light, cells were washed with PBS and fixed with 2% formaldehyde (VETEC, Duque de Caxias, RJ, Brazil) in PBS. Immunostaining was evaluated using a flow cytometer (FACSCanto II, BD Biosciences) and data were analyzed by Flowing software version 2.0.

3.2.17 Histological processing and FJ-C staining procedure

After the lactation period, lactating rats were transcardially perfused with phosphate-buffered saline (PBS, 0.1 M, pH 7.4), followed by paraformaldehyde solution (4%, diluted in PBS). Then, the brains were collected, cryoprotected (sucrose 20%) and frozen (-20 ºC). Furthermore, using the cryostat (Leica CM 1850), brains were cut into sections (30 μm thickness) at a temperature ranging from -18 to -22°C. Thereafter, brain sections were placed onto slides and Fluoro-Jade C (FJ-C) staining procedure was performed as described (de Melo et al., 2020; Melo et al., 2016; Schmued et al., 1997). Finally, the sections were analyzed, and images were captured using a fluorescence microscope (Nikon DS RI1). Qualitative analysis of the occurrence of Fluoro-Jade positive (FJ+) neurons was performed in the hilus of the dentate gyrus, CA3 and CA1 of the hippocampus, as well as in the amygdaloid nucleus (lateral, dorsolateral part [LaDL]), according to the following coordinates: hippocampus (AP − 2.56 mm; AP − 3.30 mm; AP −6.30 mm) and amygdala (AP − 2.92 mm; AP − 3.48 mm; AP − 3.84 mm) (Castro et al., 2011; de Melo et al., 2021; Paxinos and Watson, 2007). As a positive control, photomicrographs of rodents submitted to convulsive doses of intrahippocampal pilocarpine (1.2 mg/uL) were used as described (de Melo et al., 2021; Melo et al., 2016).

3.2.18 Statistical analyzes

All experimental data were described as mean ± SEM, assuming a significance level of 5% ($P < 0.05$) in all statistical tests. Most of the results were compared by unpaired t-test, using the GraphPad Prism program (version 8.00 for Windows, GraphPad Software, San Diego, CA, USA). Only outgrowth assay and maternal and non-maternal behaviors were analyzed by two-way ANOVA followed by Bonferroni's post-hoc test, when two variables were considered (i.e., the exposure to crack cocaine and the days). In the legend of the figures, the number of animals was described.

3.3 RESULTS

3.3.1 Gestational exposure to crack cocaine potentiates trophoblastic cell death and affects the growth of ectoplacental cones

To assess the viability of trophoblastic cells after exposure to crack cocaine, the implantation sites were dissected and the ectoplacental cones isolated and counted. Exposure to crack cocaine smoke did not interfere with the number of implantation sites (Fig. 2A). We used the MTT cell viability assay to assess whether gestational exposure to crack cocaine is toxic to trophoblastic cells contained in the ectoplacental cones. Our results showed that gestational exposure to crack cocaine did not alter the viability of trophoblastic cells compared to the control group (*t test*, $t_6 = 0.298$, $P = 0.776$; Fig. 2B).

Although the cell viability assay showed no change, we performed annexin V/propidium iodide cell death assay by flow cytometry to assess with more specificity and sensitivity the effect of gestational crack cocaine exposure on trophoblastic cells. We observed an increase in the % dead cells (*t test*, $t_{13} = 2.57$, $P = 0.023$; Fig. 2C) and a reduction in % viable cells (*t test*, $t_{16} = 4.63$, $P = 0.0003$; Fig. 2C) after prenatal exposure to crack cocaine.

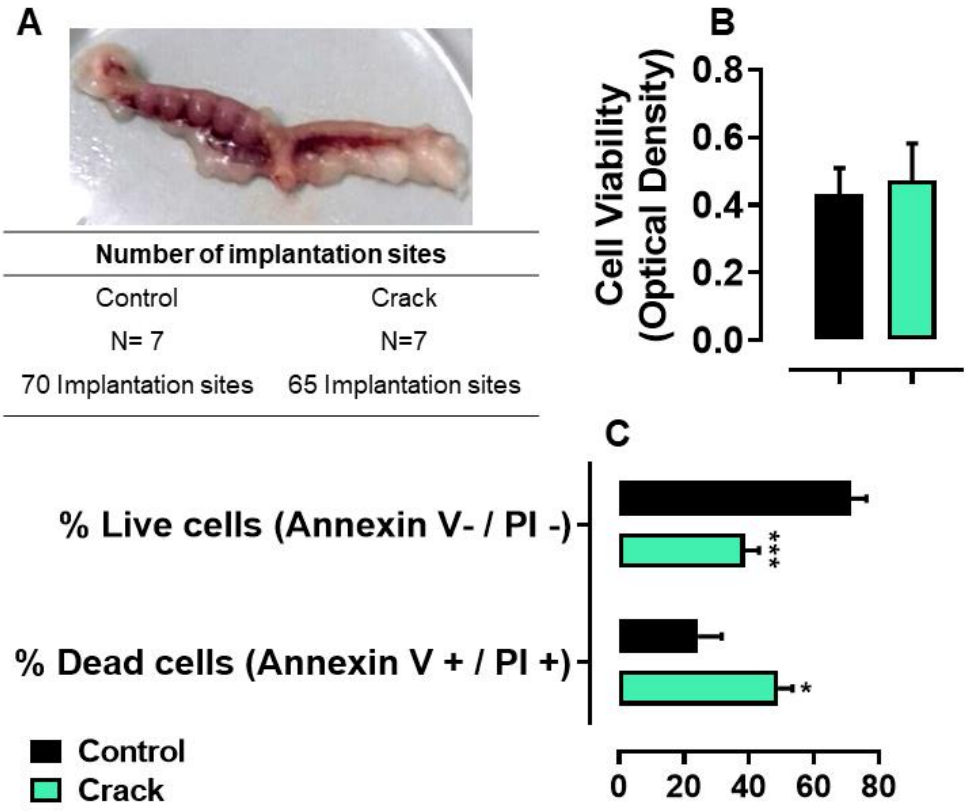

Fig. 2 Effect of gestational exposure to crack cocaine on the viability and death of trophoblastic cells. (A) Rat uterus and ovaries on the 9th day of pregnancy. To analyze resorption, the number of implantation sites was observed, but there was no significant difference between groups. (B) The cell viability test by MTT did not show any difference in trophoblastic cells from control and crack-exposed rats. (C) Crack cocaine exposure increased trophoblastic cell death while reducing the number of viable cells. *$p < 0.05$; **$p < 0.01$ when compared with the control group; unpaired t-test.

In cell culture, we analyzed the effect of crack cocaine exposure on trophoblastic cell growth from 0 to 96h. Although the control group did not show changes in growth over time (Fig. 3J, $P > 0.05$), the ectoplacental cones remained stable (Fig. 3A-E). In the group exposed to crack cocaine, the ectoplacental cones stopped growing and fell apart in the culture medium (Fig. 3F-I). We observed that prenatal exposure to crack cocaine (two-way ANOVA, $F (1, 120) = 179.7$, $P < 0.0001$; Fig. 3J) promoted a sudden reduction in the growth of ectoplacental cones after 48 hours (two-way ANOVA, $F (4, 120) = 10.83$, $P < 0.0001$; Fig. 3J), indicating an interaction between growth time and exposure to crack cocaine (two-way ANOVA, $F (4, 120) = 13.51$, $P < 0.0001$; Fig. 3J).

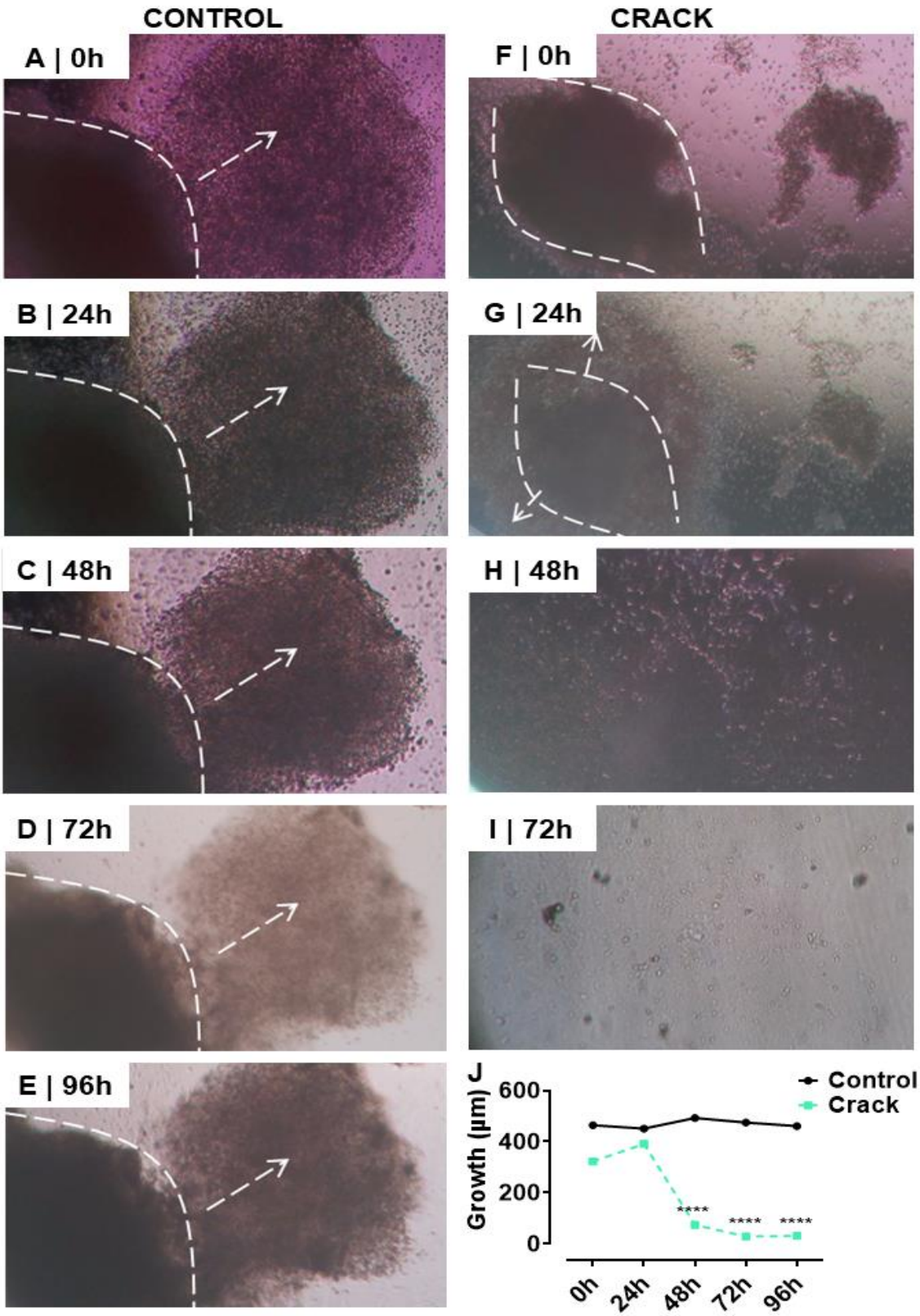

Fig. 3 Effect of gestational exposure to crack cocaine on ectoplacental cone growths. Initially, the outgrowth assay was performed at different times of the ectoplacental cones in culture. (A-E) As

expected, ectoplacental cones grow normally in the control group over the four days of assay. (F-J) However, exposure to crack cocaine dramatically stopped the growth of ectoplacental cones after 48h of assay. **** $p < 0.0001$ when compared with the control group; two-way ANOVA with Bonferroni's post-hoc test.

3.3.2 Gestational exposure to crack cocaine promotes anxiety-like behavior and increases the locomotor activity

To assess whether gestational exposure to crack cocaine promotes anxiogenic-like behavior in pregnant rats, EPM was performed. The %OAE (*t test*, $t_{20} = 1.15$, $P = 0.26$; Fig. 4A) and %OAT (*t test*, $t_{21} = 0.66$, $P = 0.52$; Fig. 4B) remained unchanged when comparing the exposed and control groups. However, exposure of pregnant rats to crack cocaine reduced the number of CAE compared to the control group (*t test*, $t_{20} = 4.32$, $P = 0.0003$; Fig. 4C).

Regarding the ethological parameters evaluated, the number of GRO (*t test*, $t_{17} = 2.76$, $P = 0.01$; Fig. 4D) and pSAP (*t test*, $t_{17} = 2.54$, $P = 0.02$; Fig. 4E) increased in pregnant rats exposed to crack cocaine. In other words, the increase in CAE and ethological parameters together may indicate an anxiogenic-like behavior in pregnant rats exposed to crack cocaine.

To assess whether gestational exposure to crack cocaine alters the locomotor activity of pregnant rats, the OF was performed. The time in the center (t-*test*, $t_{20} = 0.47$, $P = 0.64$; Fig. 4F) and in the corners (*t test*, $t_{21} = 0.06$, $P = 0.95$; Fig. 4G), as well as the number of entries in the center (*t-test*, $t_{22} = 0.62$, $P = 0.54$; Fig. 4H) remained unchanged in rats exposed to crack cocaine. However, there was an increase in the number of entries into the corners in pregnant rats exposed to crack cocaine when compared to the control (*t test*, $t_{19} = 2.39$, $P = 0.03$; Fig. 4I). In the OF, this behavior is related to anxiety and fear. In other words, gestational exposure to crack cocaine can lead to locomotor hyperactivity, which may support the anxiogenic-like behavior observed in EPM.

Figure 4

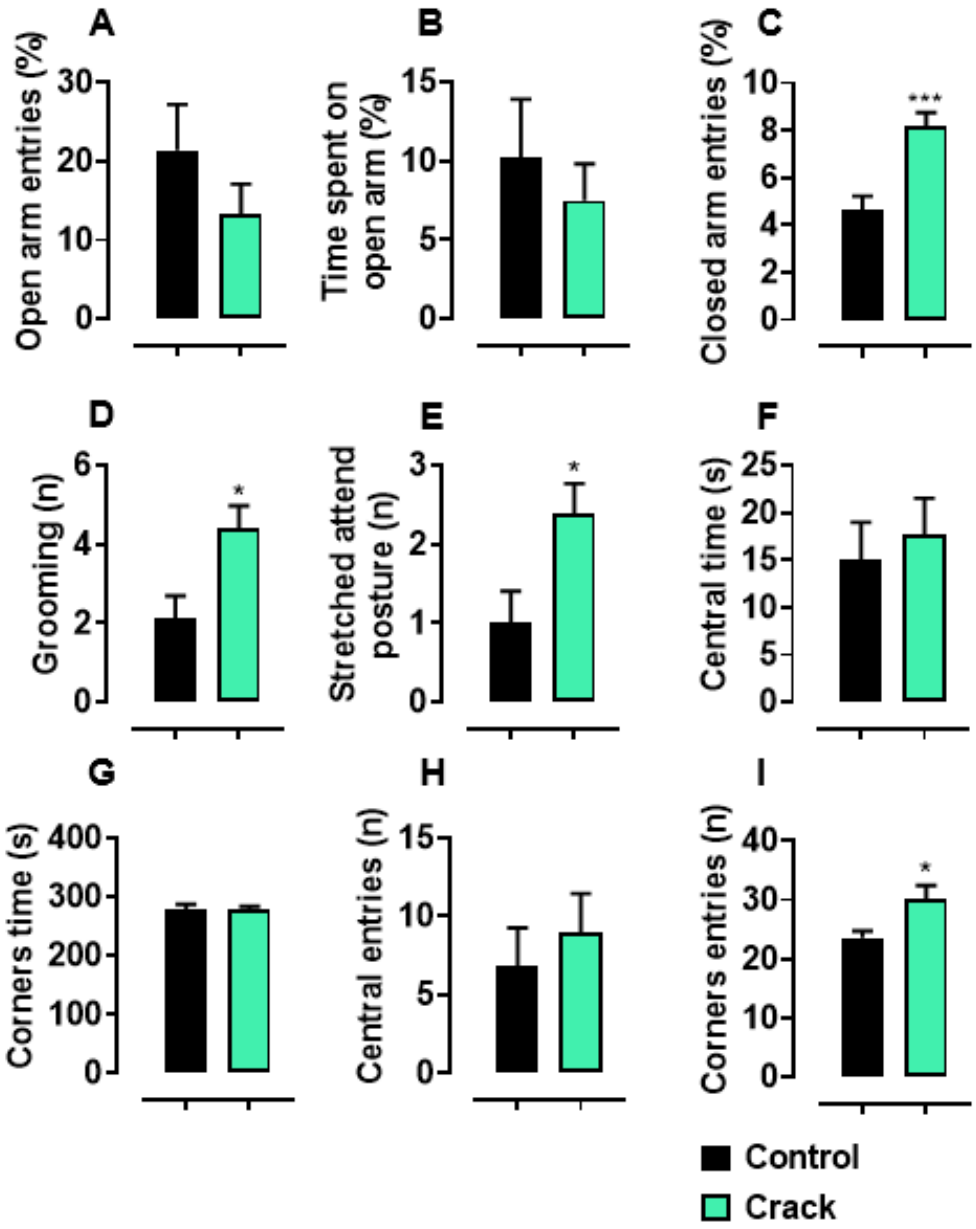

Fig. 4 Effects of gestational exposure to crack cocaine on anxiety-like behavior and locomotor activity. Gestational exposure to crack cocaine in pregnant rats increased the % closed arm entries (C), grooming (D) and stretched attended posture (E) of EPM, as well as corners entries of OF (I). Other EPM (A-B) and OF (F-H) parameters remained unchanged after exposure to crack cocaine. Data represent the mean ± SEM of 11-13 rats in each group. *$p < 0.05$; *** $p = 0.0003$ when compared with the control group; unpaired t-test. Elevated plus-maze, EPM; open field, OF.

3.3.3 Gestational exposure to crack cocaine compromises maternal behavior

To observe whether gestational exposure to crack cocaine induces changes in non-maternal and maternal behavior, we analyzed maternal care for offspring daily over 18 days of lactation.

When analyzing non-maternal behaviors individually, we observed that feeding and exploring behaviors remained unchanged when comparing the exposed and control groups (*P > 0.05*; Fig. 5A and B). However, exposure to crack cocaine increased self-grooming behavior (*t-test*, t_{19} = 2.16, *P* = 0.04; Fig. 5C) compared to the control group. Similarly, gestational exposure to crack cocaine increased the sum of non-maternal behaviors over the 18 days (two-way ANOVA, F (4, 14) = 10.21, P = 0.006; Fig. 5D and E).

Regarding the individual analysis of maternal behavior, we observed that licking pups, blanket and passive nursing remained similar when comparing the exposed and control groups (*P > 0.05*, Fig. 6F-H). However, nest building was reduced in rats that were exposed to crack cocaine during pregnancy (*t-test*, t_{18} = 2.20, *P* = 0.04; Fig. 5I). Similarly, gestational exposure to crack cocaine decreased the sum of maternal behaviors over the 18 days (two-way ANOVA, F (1, 14) = 9.10, *P = 0.009*; Fig. 5J and k). The observed changes in maternal and non-maternal behaviors together suggest that exposure to crack cocaine may increase maternal negligence in the care of offspring.

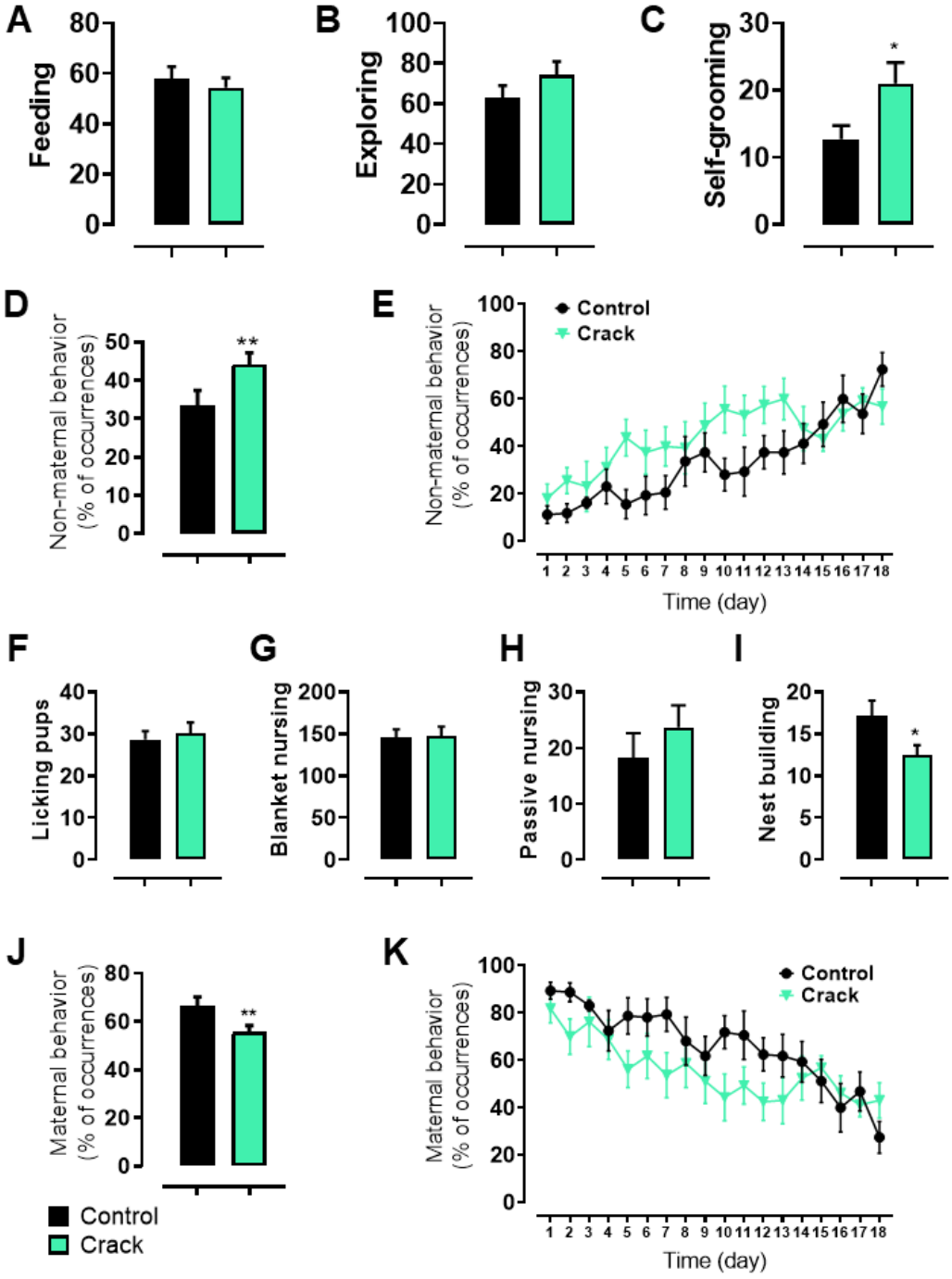

Fig. 5 Effects of maternal exposure to crack cocaine on maternal behavior. Feeding (A) and exploring (B) remained unchanged. On the other hand, self-grooming (C) and total non-maternal behavior (D-E) increased after gestational exposure to crack cocaine. Furthermore, licking pups (F), blanket (G) and passive nursing (H) remained unchanged. However, nest Building (I) and total maternal behavior (J-K) decreased after gestational exposure to crack cocaine. Data represent the mean ± SEM of the percentage of episodes in 20 observations per day, totaling 360 during the lactation period (n=12-13 in each group). *$p < 0.05$; **$p < 0.01$ when compared with the control group; unpaired t-test or two-way ANOVA with Bonferroni's post-hoc test.

3.3.4 Gestational exposure to crack cocaine does not interfere development of sensorimotor reflexes

To observe whether gestational exposure to crack cocaine affects the maturation of the offspring's physical features, the development of sensorimotor reflexes was assessed. The palmar-grasp, righting, negative geotaxis, and auditory-startle response reflexes remained unchanged when comparing the crack cocaine and control groups (*P > 0.05*; Fig. 6A-D). In other words, gestational exposure to crack cocaine did not promote any delay in the development of sensorimotor reflexes.

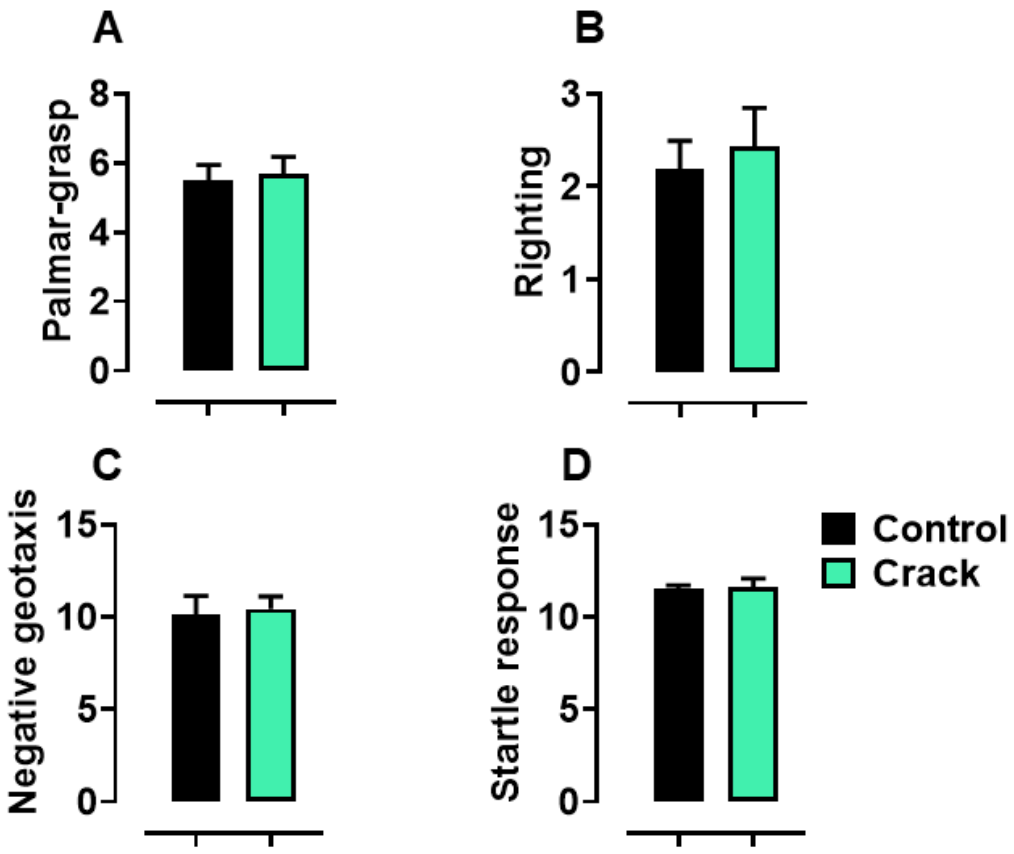

Fig. 6 Effect of gestational exposure to crack cocaine on the development of sensorimotor reflexes. Palmar-grasp, righting, negative geotaxis, and startle response were not altered in the offspring of rats exposed to crack cocaine during pregnancy. Data represent the mean ± SEM of 16 rats in each group. *p*

> 0.05; unpaired t-test.

3.3.5 Gestational exposure to crack cocaine modulates spleen cellularity

Continuing with the set of experiments, and after evaluating the reproductive (placenta) and behavioral alterations of pregnant rats exposed to crack cocaine, it was of our interest to evaluate the possible modulations that the immune system presented. To address this, relative weights of thymus and spleen were observed (Figure 7A and D). No changes were observed in the thymus of these animals ($P > 0.05$; Fig. 7A-C). Only the spleen exhibited a lower relative weight compared to the control group (*t-test*, $t_8 = 2.61$, $P = 0.03$; Fig. 7D). This reduction was reflected in the lower total cellularity of the organ (fewer splenocytes) (*t-test*, $t_7 = 2.14$, $P = 0.06$; Fig. 7E) and mainly in the CD8+ T lymphocytes subset (*t-test*, $t_8 = 2.57$, $P = 0.03$; Fig. 7F). Figure 7G and H show the immunostaining by representative dot plots for CD4 and CD8 molecules, identifying T lymphocytes subsets in thymus and spleen.

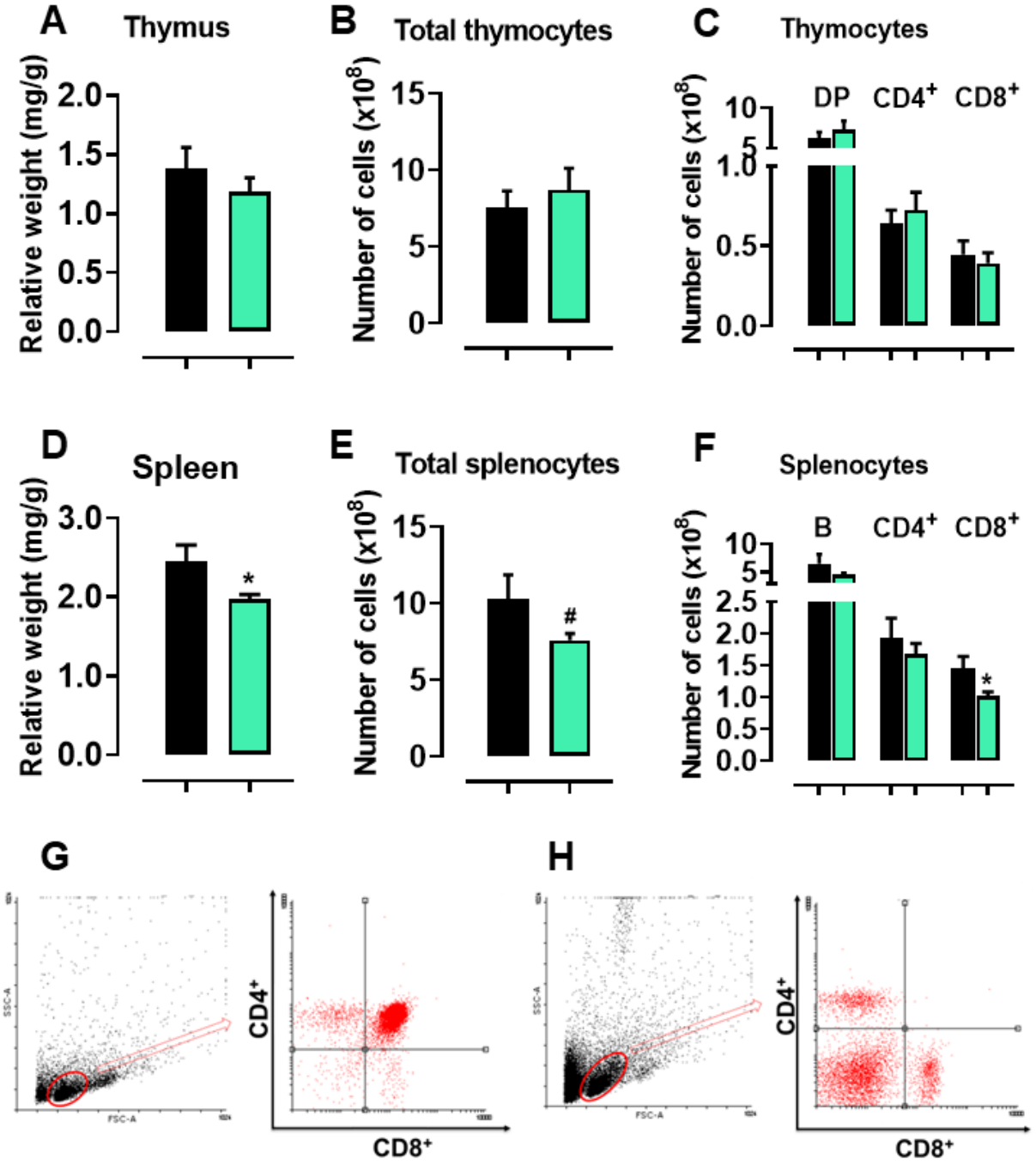

Fig. 7 Effects of gestational exposure to crack cocaine in lymphoid organs. After surgical removal, the thymus (A) and spleen (D) were weighed and correlated with the animal's body weight to assess their relative weight. Then, these organs were macerated, and their cells were counted (B) and (E), respectively. Only the spleen exhibited a lower relative weight and a lower number of splenocytes. In (C), numbers indicate the cell absolute number ($x10^8$), of $CD4^+/CD8^+$ thymic subsets. In (F), numbers indicate splenic subsets. There was a decrease in $CD8^+$ T cells in the spleen. (G) and (H) show the immunostaining by representative dot plots for CD4 and CD8 molecules, identifying T lymphocyte subsets in thymus and spleen. Data represent the mean ± SEM of 4-7 rats in each group. *$p \leq 0.05$; # $p \leq 0.06$ when compared with the control group; unpaired t-test**.**

3.3.6 Gestational exposure to crack cocaine promotes neuronal death in the amygdala but not in the hippocampus

To assess whether gestational exposure to crack cocaine leads to neuronal death in the hippocampus and amygdala of pregnant rats, the process of neurodegeneration was qualitatively assessed by FJ histochemistry. Pregnant rats exposed to crack cocaine did not have neuronal death in the subareas of the hippocampus (hilus, CA3, and CA1; Fig. 8I-K), but neurodegeneration was observed in the LaDL amygdaloid nucleus (Fig. 8L) when compared to control (Fig. 8E-H). Figure 8A-D, especially at digital zoom, represents positive control for neurodegeneration in the hippocampus and amygdala after infusion of convulsive doses of pilocarpine.

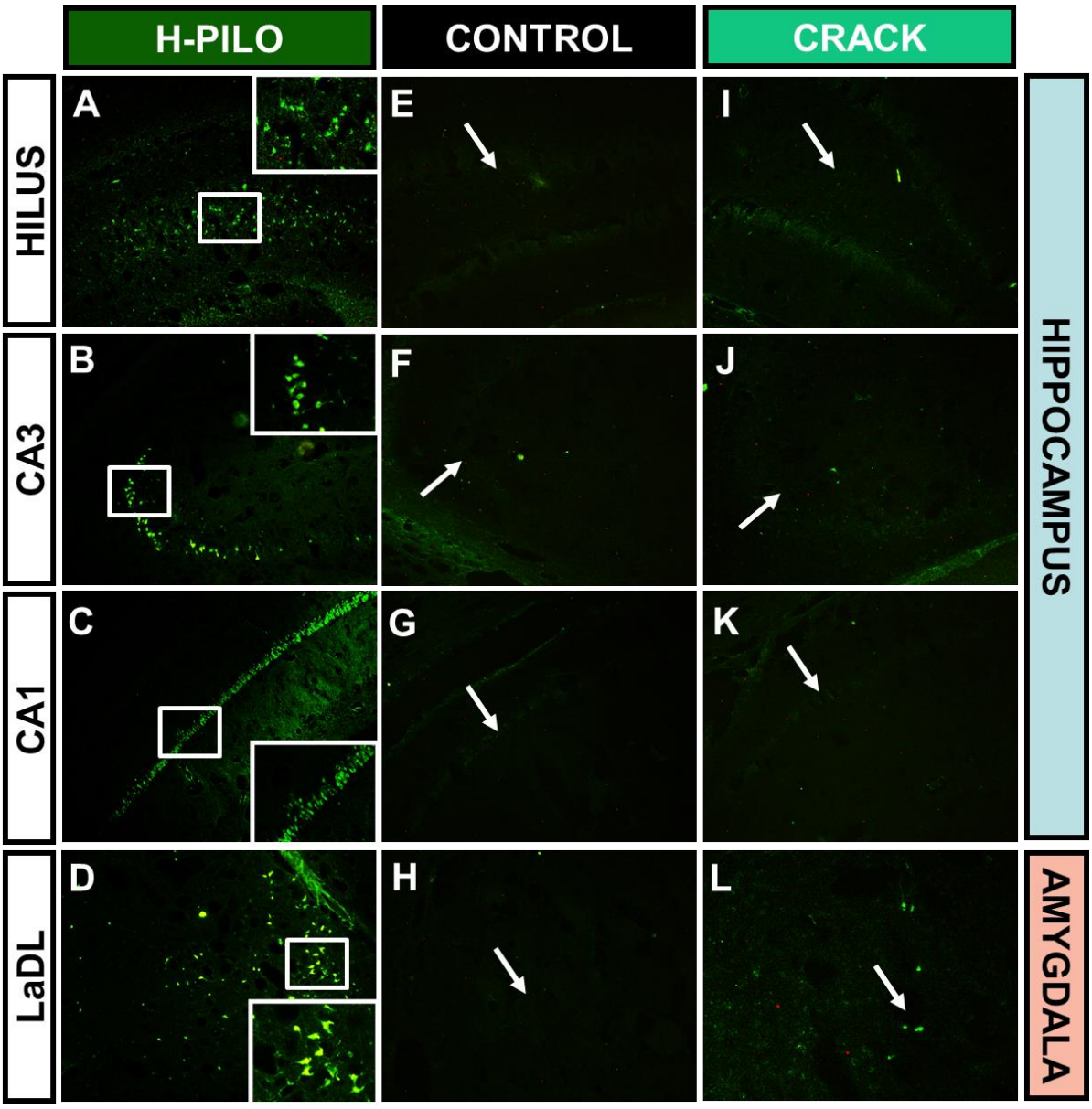

Fig. 8 Effects of gestational exposure to crack cocaine in the neurodegenerative process of the hippocampus and amygdala. The neurons of the hilus, CA3, and CA1 of the hippocampus, and the amygdaloid nucleus (LaDL) were stained with FJ (FJ+, green). H-PILO positive control shows FJ+ neurons after intrahippocampal pilocarpine infusion (A-D). Representative digital zoom was made on the photomicrographs of the H-PILO group (A-D; see squares). Gestational exposure to crack cocaine did not promote neuronal death in the hilus (I), CA3 (J) and CA1 (K) of the hippocampus, when compared to the control (E-G). However, FJ+ neurons were detected in the LaDL amygdaloid nucleus (L) of pregnant rats exposed to crack cocaine compared to the control group (H). Arrows represent the hilus, CA3, CA1, and LaDL regions. Magnification, 100×; scale bar, 100 μm.

3.4 DISCUSSION

Crack cocaine is smoked cocaine and its consumption has more harmful effects than intravenous or nasal routes (Garcia et al., 2012). Cocaine and its products are neurotoxic (Garcia et al., 2012; Lin and Leskawa, 1994) and cause toxicity to the cardiovascular, neuromuscular, and central nervous systems, as well as infectious complications, kidney and lung injuries, hepatotoxicity, and reproductive disorders (Glauser and Queen, 2007). Furthermore, a large amount of cocaine can be deposited in the placenta (Simone et al., 1994), leading to maternal-fetal complications. Given this, understanding the repercussions of exposure to crack cocaine during pregnancy is important for maternal-fetal care. In the present study, we evaluated the effect of gestational exposure to crack cocaine on the embryonic development of trophoblastic cells, on maternal anxiety, maternal behavior and sensorimotor maturation of the offspring, and on immune organs and brain areas. Overall, our findings showed *in vitro* changes in trophoblastic cells, psychiatric and behavioral problems, and alterations in the spleen and amygdala after exposure of pregnant rats to crack cocaine.

Several studies have reported that cocaine compromises cellular viability and promotes cell death in various biological tissues (Cerretani et al., 2012; Kovacic and Cooksy, 2005; Lepsch et al., 2009; Macêdo et al., 2010; Nassogne et al., 1997; Pacheco et al., 2021; Varga et al., 2015). Cocaine leads to activation of caspases (Cunha-Oliveira et al., 2006; Dey and Snow, 2007; IMAM et al., 2005; Mitchell and Snyder-Keller, 2003; Oliveira et al., 2003), mitochondrial dysfunction, including damage to the respiratory chain, mitochondrial enzymes and membrane (Cerretani et al., 2012; Varga et al., 2015), as well as release of cytochrome c within the cytoplasm (Cunha-Oliveira et al., 2006; Oliveira et al., 2003), and increased oxidative stress (De Oliveira and Jardim, 2016; Dietrich et al., 2005; Georgieva et al., 2021; Kovacic and Cooksy, 2005; Macêdo et al., 2010, 2005; Malacarne et al., 2021; Numa et al., 2008; Souza-Silva et al., 2020). Typically, all these factors together can culminate in cocaine

induced cell death. Despite several reports regarding the effect of cocaine on many biological tissues, studies on the action of this drug on the placenta remain scarce. Our results indicated that gestational exposure to crack cocaine intensified the death of trophoblastic cells, even without altering cell viability. Similarly to ours, a recent study exposed pregnant rats to crack cocaine smoke for 10 minutes, but twice a day with 250mg of crack cocaine, from the 7th to the 20th day of pregnancy (Souza-Silva et al., 2020). These authors showed that gestational exposure to crack cocaine intensified lipid peroxidation and the activity of catalase and glutathione peroxidase antioxidant enzymes in the placenta, indicating placental oxidative stress induced by crack cocaine. These findings together support crack cocaine-induced placental oxidative stress, which can culminate in the increase in cell death observed in our study. However, in an in vitro study with human trophoblastic cells (HTR-8/SVneo cells), cell viability, growth, proliferation, and migration remained unchanged after exposure to cocaine (Correia-Branco et al., 2019).

It is already well established from previous studies that cocaine inhibits the presynaptic dopamine transporter, preventing the reuptake of dopamine back into the presynaptic neuron (Adinoff, 2004; Baik, 2013). Dopamine is a crucial neurotransmitter for signaling the reward system, modulating emotional behaviors through the mesolimbic dopaminergic pathway, which begins in the ventral tegmental area and sends projections to the nucleus accumbens and the limbic system (Baik, 2013; Enoch, 2008; McBride, 2002). Disturbances in the mesolimbic dopaminergic pathway can lead to behavioral changes, i.e. anxiety and depression (Kleinridders and Pothos, 2019; Nikolaus et al., 2019; Raghav et al., 2021). In clinical studies with patients chronically exposed to crack cocaine, we observed sensitivity to anxiety and depression (Lejuez et al., 2008; McDermott et al., 2009; Naifeh et al., 2012; Zubaran et al., 2013).

In our study, gestational exposure to crack cocaine increased the number of CAE, GRO and pSAP in EPM and the number of entries into the periphery in OF, indicating an anxiogenic

like behavior (Choleris et al., 2001; Handley and Mithani, 1984; Lister, 1990; Pellow and File, 1986). Similarly, in a clinical study with cocaine-dependent pregnant or postpartum women, the authors observed an increased risk for an anxiety disorder (Killeen et al., 1995). In addition, a preclinical study chronically exposed adult female rats to cocaine (15 mg/kg; 10 days) prior to pregnancy to assess possible changes in anxiety-like behavior using EPM (Delgado et al., 2019). These authors observed that lactating rats exposed to cocaine during adulthood had an increase in anxiety-like response, which may be associated with increased glucose metabolism in the prefrontal cortex of these lactating dams. In contrast, using the same cocaine treatment for 10 days, another study noted the lack of an anxiogenic effect in postpartum female rats (Nephew and Febo, 2010). Possibly, these differences in results are associated with the different methods used to assess the anxiogenic effect: EPM in Delgado et al., (2019) and light/dark test (Nephew and Febo, 2010).

At the end of pregnancy, maternal behaviors are crucial for the survival of the offspring since they are born blind and unable to thermoregulate and protect themselves from attacks (Numan, 1994; Stern, 1997; Teodorov et al., 2002). In rodent models, maternal behavior has been extensively studied, which makes it possible to easily determine disturbances in maternal–offspring bonding (Li, 2020, 2015; López-Rodríguez et al., 2021; Numan and Stolzenberg, 2009; Pedersen et al., 1994, 1985, 1982; Uriarte et al., 2008). It has been reported that cocaine use compromises maternal behavior (Eiden et al., 2011b; Hawley et al., 1995; Johns et al., 2005a, 2007), however there are few studies on the effect of crack cocaine on maternal care (Hawley et al., 1995; Lam et al., 2004). After gestational exposure to crack cocaine, we observed that lactating rats exhibited reduced performance in maternal care of offspring. Our findings corroborate with several clinical (Eiden et al., 2011b, 2011a; Hawley et al., 1995; Tyler et al., 1997; Wasserman and Leventhal, 1993) and preclinical (Johns et al., 2007, 2005b, 1997; Kinsley et al., 1994; McMurray et al., 2008; Nephew and Febo, 2010; Williams and Johns,

2014) studies. Cocaine use by pregnant women has been shown to lead to child abuse (Hawley et al., 1995; Tyler et al., 1997), impaired maternal-infant bonding and interactions (Bauman and Dougherty, 1983; Howard et al., 1995; Johnson and Rosen, 1990), as well as neglect of maternal care (Wasserman and Leventhal, 1993). Similarly, pregnant Sprague-Dawley rats treated with cocaine (30mg/kg) throughout pregnancy exhibited early impairment of maternal behavior and a more aggressive profile (Johns et al., 1997). Furthermore, when administered during postpartum Sprague-Dawley female rats, cocaine (5, 10 or 20 mg/kg) also affects the initiation and maintenance of maternal behavior (Kinsley et al., 1994). Thus, the various findings have indicated that all forms of exposure to cocaine (acute, intermitente and chronic) disturb maternal behavior, with the severity of the drug dependent on the dose, time of analysis and route of exposure (Johns et al., 1998, 1994; JOHNS et al., 1998; Kinsley et al., 1994). In other words, animal and human research suggest that mothers who are addicted to substances such as cocaine, even when not actively using the drug, may be less able to adequately respond to their offspring's needs, finding such interactions less intrinsically rewarding or more stressful (Mayes et al., 1997).

Maternal care and bonding with the offspring are supported by a Maternal Brain Network (Swain et al., 2019; Wallin et al., 2021), composed of some brain areas, such as the medial preoptic area (Bridges, 2015; Numan and Woodside, 2010; Rosenblatt and Ceus, 1998), nucleus accumbens (Lonstein et al., 1997; Stack et al., 2002), ventral pallidum (Numan, 2007; Numan et al., 2006; Numan and Young, 2016), ventral tegmental area (Numan, 2016; Numan and Smith, 1984), and periaqueductal gray (Silva and McNaughton, 2019; Swain et al., 2019), which are associated with maternal defensive aggression and care behaviors (Swain et al., 2019). Amygdala, insular cortex, and orbitofrontal cortex also mediate maternal behavior. For the first time, our study showed that pregnant rats exposed to crack cocaine showed subtle

neuronal death in the amygdaloid nucleus. Such impairment in the amygdala may support the maternal neglect seen in these lactating dams.

It is known that dopamine and oxytocin are involved in these neural pathways and play an important role in maternal care, mediating social and reward-related behaviors and stress reactivity (Numan and Stolzenberg, 2009; Strathearn and Mayes, 2010; Wallin et al., 2021; Williams and Johns, 2014).

Oxytocin neurons from the paraventricular and supraoptic hypothalamic nuclei send their projections to the posterior pituitary, releasing oxytocin into the bloodstream (Williams and Johns, 2014; Wotjak et al., 1998). From the paraventricular hypothalamic nucleus, oxytocin neurons also send their fibers to the amygdala, hippocampus, ventral tegmental area, nucleus accumbens, and medial preoptic area, where there is oxytocin receptor expression (Gimpl and Fahrenholz, 2001). Chronic gestational cocaine treatment (15 mg/kg, sc) of Sprague-Dawley rats has been observed to reduce oxytocin levels in the medial preoptic area, ventral tegmental area and hippocampus on postpartum days 1 and 2 (Johns et al., 1997). However, acute cocaine treatment (30 mg/kg, sc) increased oxytocin levels in the amygdala of Sprague-Dawley rats on postpartum day 6 (Elliott et al., 2001). Furthermore, gestational cocaine treatment differently alters oxytocin receptor binding and affinity in the ventromedial hypothalamus, medial preoptic area, and amygdala of lactating rat dams (Jarrett et al., 2006; Johns et al., 2004). All of these data have been associated with disturbance in maternal behavior and maternal aggression in rat dams (Elliott et al., 2001; McMurray et al., 2008). Accordingly, studies have shown that infusion of oxytocin or oxytocin antagonists directly into the central amygdaloid nucleus reduced or increased the maternal aggressive behavior in lactating rats, respectively (Consiglio et al., 2005; Lubin et al., 2003).

The child's stimuli activate the mother's mesolimbic dopaminergic system during maternal behavior (Lorberbaum et al., 2002). Similarly, in preclinical studies, maternal care of offspring increases mesolimbic dopamine levels in the nucleus accumbens and ventral striatum (Champagne et al., 2004; Hansen et al., 1993; Lavi-Avnon et al., 2008). It has already been observed that the dopamine D1 and/or D2 receptor blockade disrupted ongoing maternal behavior, such as retrieval and nest-building behaviors (Silva et al., 2001). Gestational cocaine exposure can reduce transient dopamine levels in the nucleus accumbens (Shnitko et al., 2017), and D2 and D3 receptor binding in the striatum of maternal dams (Silvers et al., 2006) associated with disturbance in maternal behavior. Furthermore, the mesolimbic dopaminergic system of the offspring may be altered following gestational cocaine exposure. Male offspring of dams exposed to cocaine throughout pregnancy also exhibited reduced D2 receptor binding in nucleus accumbens and enhanced D3 receptor binding in the accumbens and striatum (Silvers et al., 2006). Additionally, exposure of female rats to cocaine prior to pregnancy displayed an upregulation of D1 dopamine receptor mRNA expression in the medial prefrontal cortex (Sasaki et al., 2014).

The reflex is a motor response to the CNS in the face of environmental stimuli, being a pre-established behavior that arises during ontogenetic development and continues as a predetermined sequence according to the age of the animals (Fox, 1965; Smart and Dobbing, 1971; Swerdlow and Geyer, 1998). The inadequate response to the sensorimotor reflex through ontogeny can also be a parameter to predict changes in brain structure and function (Soares et al., 2009). Acute or sustained cocaine administration promoted a dose-dependent decrease in the prepulse inhibition of the startle reflex, returning to the control level after 10 days of sustained drug administration (Martinez et al., 1999). Similarly, chronic cocaine treatment (8 weeks) reduced the startle response during withdrawal in rats (Adams et al., 2001). However, in our study, gestational exposure to crack cocaine did not affect the development of

sensorimotor reflexes. According to our study, chronic cocaine treatment (10 mg/kg/day, sc) throughout pregnancy did not change the righting reflex and negative geotaxis (Smith et al., 1989). The previously observed discrepancies may be associated with the type of drug, route of administration and time of exposure.

The evaluation of animal's immune system is essential, considering that it endows it with capabilities of defense against infections or tumors, as well as maintaining organic homeostasis (Chowdhury et al., 2020). Our attention turned to thymus, a primary lymphoid organ, and spleen, a secondary lymphoid organ, which contributes to the generation of T cells and the activation of the immune response, respectively (Nigam and Knight, 2020). Regarding the thymus, no macroscopic alterations of the organ (anatomical appearance and relative weight) were observed, neither in its total cellularity nor in their subsets (CD4/CD8 thymocytes). This result is controversial to that found by Unuma et al., (2022), in which animals exposed to cocaine, exhibited a reduction in thymic tissue, due to loss of thymic cells through apoptosis and suppression of cell proliferation. However, it was showed that at low doses cocaine did not reduce thymic weight (Kubera et al., 2004). The form of administration, time and dose seem to be modulating the stress response that these animal models present to cocaine.

Concermimg the spleen, our study demonstrated that relative weight, total splenocyte cellularity and $CD8^+$ T lymphocyte subpopulation were lower than the control group parameters. Interestingly, the acute action of cocaine in reducing spleen volume (on average by 20%) in adult men has been reported (Kaufman et al., 1998). Additionally, splenic rupture has been documented as a rare but severe complication of cocaine abuse (Khan et al., 2017). Cocaine use by adult women reduced the absolute number of naïve CD8 T cells in the blood and increased Th1/Th2/Th17 cytokine production in vitro (Zaparte et al., 2019), since it confirms our findings. Similarly, crack cocaine users exhibited signs of genotoxicity in blood lymphocytes (de Freitas et al., 2014) and in adult Wistar rats treated with cocaine, the reduction

in blood lymphocytes was accompanied/justified by an increase in plasma corticosterone (Jankowski et al., 2010). In short, studying the deleterious effects of crack cocaine on spleen and lymphocytes should also be the target of research and therapies to recover the health of drug' users undergoing treatment.

3.5 CONCLUSION

Gestational exposure to crack cocaine led to increased trophoblastic cell death and reduced viable cells, as well as reduced growth of ectoplacental cones. Also, we demonstrated a reduction in the relative weight of the spleen, in the number of total splenocytes and subpopulations of CD8+ T lymphocytes. Finally, crack cocaine influenced anxiogenic-type behavior and negligence in maternal care.

STUDY LIMITATIONS

The study had the limitation to quantify the amount of crack smoke that was inhaled by the animals during exposure.

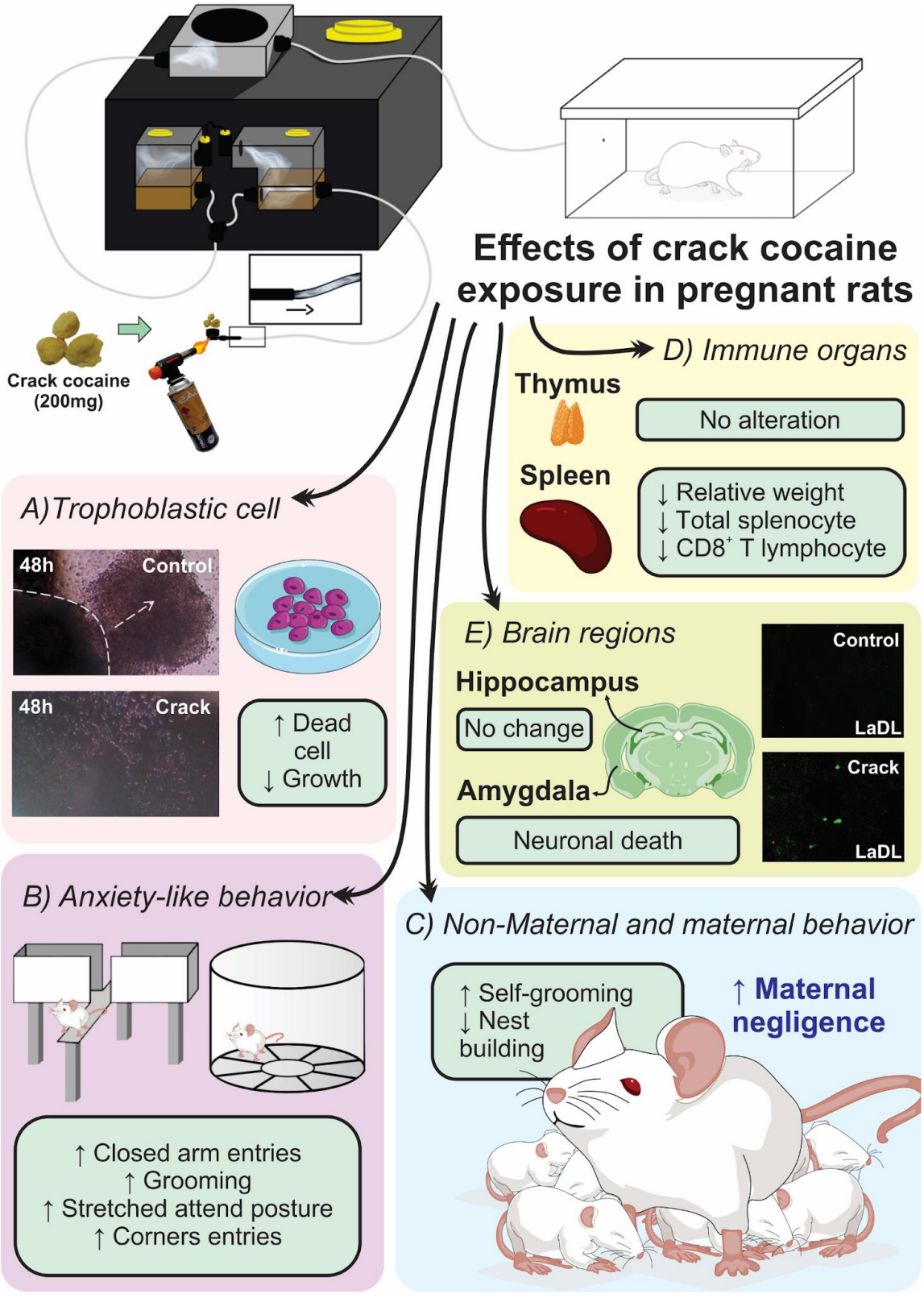

Fig. 9 The cartoon depicts the effects of crack cocaine on pregnant rats. We observed the death of trophoblastic cells and reduced growth of ectoplacental cones, indication of anxiety like behavior, increased maternal neglect, changes in the spleen, and neuronal death in the amygdala of pregnant rats exposed to crack cocaine.

Acknowledgments

OWC was supported by the Research Productivity Scholarship Program in Brazilian National Council for Scientific and Technological Development (CNPq). We thank CAPES-Brazil for PhD Research Fellowship to Y.M.O.S., A.L.D.P.

Author Contributions

Conceptualization, Y.M.O.S., I.S.M. and O.W.C.; Methodology, Y.M.O.S., I.S.M., A.L.D.P., M.M.J.H.R., F.M.A.S, J.F.S., K.S.N.P., B.R.M., M.R.B., K.L.S.O., M.A.C., A.B.C., M.P.L., E.B.O., M.D., A.U.B., and O.W.C.; Investigation, Y.M.O.S., I.S.M., A.U.B., and O.W.C.; Formal Analysis, Y.M.O.S., I.S.M., A.L.D.P., M.M.J.H.R., F.M.A.S, J.F.S., K.S.N.P., B.R.M., M.R.B., K.L.S.O., M.A.C., A.B.C., M.P.L., E.B.O., M.D., A.U.B., and O.W.C.; Supervision and Fund Acquisition, O.W.C.; Writing – Review & Editing, I.S.M., A.U.B., and O.W.C.; Resources, A.U.B., and O.W.C.

Funding

This project was supported by FAPEAL, CNPq and CAPES.

Compliance with Ethical Standards

Conflict of Interests

The authors declare that they have no conflict of interest.

Ethics Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Experiments were performed in accordance with the NIH guidelines

for the care and use of laboratory animals, and with approval of the Federal University of Alagoas Animal Use Ethics Committee.

3.6 REFERENCES

Abramova, O., Ushakova, V., Zorkina, Y., Zubkov, E., Storozheva, Z., Morozova, A., Chekhonin, V., 2021. The Behavior and Postnatal Development in Infant and Juvenile Rats After Ultrasound-Induced Chronic Prenatal Stress. Front. Physiol. 12, 659366. https://doi.org/10.3389/FPHYS.2021.659366

Adams, J.U., Efferen, T.R., Duncan, E.J., Rotrosen, J., 2001. Prepulse inhibition of the acoustic startle response in cocaine-withdrawn rats. Pharmacol. Biochem. Behav. 68, 753–759. https://doi.org/10.1016/S0091-3057(01)00478-6

Adinoff, B., 2004. Neurobiologic Processes in Drug Reward and Addiction. Harv. Rev. Psychiatry 12, 305. https://doi.org/10.1080/10673220490910844

Babb, J.A., Masini, C. V., Day, H.E.W., Campeau, S., 2013. Sex differences in activated corticotropin-releasing factor neurons within stress-related neurocircuitry and hypothalamic-pituitary-adrenocortical axis hormones following restraint in rats. Neuroscience 234, 40–52. https://doi.org/10.1016/j.neuroscience.2012.12.051

Baik, J.H., 2013. Dopamine signaling in reward-related behaviors. Front. Neural Circuits 7. https://doi.org/10.3389/FNCIR.2013.00152

Bauman, P.S., Dougherty, F.E., 1983. Drug-addicted mothers' parenting and their children's development. Int. J. Addict. 18, 291–302. https://doi.org/10.3109/10826088309039348

Baumann, B.M., Pescatore, R.M., 2018. Hypertensive emergencies, Third Edit. ed, Cardiac Intensive Care. Elsevier Inc. https://doi.org/10.1016/B978-0-323-52993-8.00027-8

Bell, G.L., Lau, K., 1995. Perinatal and neonatal issues of substance abuse. Pediatr. Clin. North Am. 42, 261–81.

Bridges, R.S., 2015. Neuroendocrine regulation of maternal behavior. Front. Neuroendocrinol. 36, 178–196. https://doi.org/10.1016/J.YFRNE.2014.11.007

Castro, O.W., Furtado, M. a., Tilelli, C.Q., Fernandes, a., Pajolla, G.P., Garcia-Cairasco, N.,

2011. Comparative neuroanatomical and temporal characterization of FluoroJade-positive neurodegeneration after status epilepticus induced by systemic and intrahippocampal pilocarpine in Wistar rats. Brain Res. 1374, 43–55. https://doi.org/10.1016/j.brainres.2010.12.012

Cerretani, D., Fineschi, V., Bello, S., Riezzo, I., Turillazzi, E., Neri, M., 2012. Role of oxidative stress in cocaine-induced cardiotoxicity and cocaine-related death. Curr. Med. Chem. 19, 5619–5623. https://doi.org/10.2174/092986712803988785

Champagne, F.A., Chretien, P., Stevenson, C.W., Zhang, T.Y., Gratton, A., Meaney, M.J., 2004. Variations in nucleus accumbens dopamine associated with individual differences in maternal behavior in the rat. J. Neurosci. 24, 4113–4123. https://doi.org/10.1523/JNEUROSCI.5322-03.2004

Choleris, E., Thomas, A.W., Kavaliers, M., Prato, F.S., 2001. A detailed ethological analysis of the mouse open field test: Effects of diazepam, chlordiazepoxide and an extremely low frequency pulsed magnetic field. Neurosci. Biobehav. Rev. 25, 235–260. https://doi.org/10.1016/S0149-7634(01)00011-2

Chowdhury, M.A., Hossain, N., Kashem, M.A., Shahid, M.A., Alam, A., 2020. Immune response in COVID-19: A review. J. Infect. Public Health 13, 1619–1629. https://doi.org/10.1016/J.JIPH.2020.07.001

Consiglio, A.R., Borsoi, A., Pereira, G.A.M., Lucion, A.B., 2005. Effects of oxytocin microinjected into the central amygdaloid nucleus and bed nucleus of stria terminalis on maternal aggressive behavior in rats. Physiol. Behav. 85, 354–362. https://doi.org/10.1016/J.PHYSBEH.2005.05.002

Correia-Branco, A., Keating, E., Martel, F., 2019. Placentation-related processes in a human first-trimester extravillous trophoblast cell line (HTR-8/SVneo cells) are affected by several xenobiotics. Drug Chem. Toxicol. 42, 541–545.

https://doi.org/10.1080/01480545.2018.1463240

Costa, H.H.V., Vilela, F.C., Giusti-Paiva, A., 2013. Continuous central infusion of cannabinoid receptor agonist WIN 55,212-2 decreases maternal care in lactating rats: Consequences for fear conditioning in adulthood males. Behav. Brain Res. 257, 31–38. https://doi.org/10.1016/j.bbr.2013.09.022

Cunha-Oliveira, T., Rego, A.C., Cardoso, S.M., Borges, F., Swerdlow, R.H., Macedo, T., de Oliveira, C.R., 2006. Mitochondrial dysfunction and caspase activation in rat cortical neurons treated with cocaine or amphetamine. Brain Res. 1089, 44–54. https://doi.org/10.1016/J.BRAINRES.2006.03.061

Curley, J.P., Champagne, F.A., 2016. Influence of maternal care on the developing brain: Mechanisms, temporal dynamics and sensitive periods. Front. Neuroendocrinol. 40, 52–66. https://doi.org/10.1016/J.YFRNE.2015.11.001

de Freitas, T.A.B., Palazzo, R.P., de Andrade, F.M., Reichert, C.L., Pechansky, F., Kessler, F., de Farias, C.B., de Andrade, G.G., Leistner-Segal, S., Maluf, S.W., 2014. Genomic Instability in Human Lymphocytes from Male Users of Crack Cocaine. Int. J. Environ. Res. Public Health 11, 10003. https://doi.org/10.3390/IJERPH111010003

de Melo, I.S., dos Santos, Y.M.O., Pacheco, A.L.D., Costa, M.A., de Oliveira Silva, V., Freitas-Santos, J., de Melo Bastos Cavalcante, C., Silva-Filho, R.C., Leite, A.C.R., Gitaí, D.G.L., Duzzioni, M., Sabino-Silva, R., Borbely, A.U., de Castro, O.W., 2021. Role of Modulation of Hippocampal Glucose Following Pilocarpine-Induced Status Epilepticus. Mol. Neurobiol. 58, 1217–1236. https://doi.org/10.1007/S12035-020-02173-0

de Melo, I.S., dos Santos, Y.M.O., Pacheco, A.L.D., Costa, M.A., de Oliveira Silva, V., Freitas-Santos, J., de Melo Bastos Cavalcante, C., Silva-Filho, R.C., Leite, A.C.R., Gitaí, D.G.L., Duzzioni, M., Sabino-Silva, R., Borbely, A.U., de Castro, O.W., 2020. Role of Modulation of Hippocampal Glucose Following Pilocarpine-Induced Status Epilepticus.

Mol. Neurobiol. 58. https://doi.org/10.1007/s12035-020-02173-0

de Moraes Costa, G., Soibelman, M., Zanchet, D.L., de Moraes Costa, P., Salgado, C.A.I., 2012. Pregnant crack addicts in a psychiatric unit. J. Bras. Psiquiatr. 61, 8–12. https://doi.org/10.1590/s0047-20852012000100003

De Oliveira, M.R., Jardim, F.R., 2016. Cocaine and mitochondria-related signaling in the brain: A mechanistic view and future directions. Neurochem. Int. 92, 58–66. https://doi.org/10.1016/J.NEUINT.2015.12.006

Deiró, T.C.B.D.J., Carvalho, J., Do Nascimento, E., Medeiros, J.M.B., Cajuhi, F., Ferraz-Pereira, K.N., Manhães-de-Castro, R., 2008. Neonatal exposure to citalopram, a serotonin selective reuptake inhibitor, programs a delay in the reflex ontogeny in rats. Arq. Neuropsiquiatr. 66, 736–740. https://doi.org/10.1590/S0004-282X2008000500025

Deiró, T.C.B.J., Manhães-de-Castro, R., Cabral-Filho, J.E., Barreto-Medeiros, J.M., Souza, S.L., Marinho, S.M.O.C., Castro, F.M.M., Toscano, A.E., Jesus-Deiró, R.A., Barros, K.M.F.T., 2006. Sertraline delays the somatic growth and reflex ontogeny in neonate rats. Physiol. Behav. 87, 338–344. https://doi.org/10.1016/j.physbeh.2005.10.003

Delgado, H., Agrati, D., Machado, L., Reyes, L., Savio, E., Engler, H., Ferreira, A., 2019. Cocaine treatment before pregnancy differentially affects the anxiety and brain glucose metabolism of lactating rats if performed during adulthood or adolescence. Behav. Brain Res. 372. https://doi.org/10.1016/J.BBR.2019.112070

Dey, S., Snow, D.M., 2007. Cocaine exposure in vitro induces apoptosis in fetal locus coeruleus neurons through TNF-alpha-mediated induction of Bax and phosphorylated c-Jun NH(2)-terminal kinase. J. Neurochem. 103, 542–556. https://doi.org/10.1111/J.1471-4159.2007.04750.X

Dietrich, J.B., Mangeol, A., Revel, M.O., Burgun, C., Aunis, D., Zwiller, J., 2005. Acute or repeated cocaine administration generates reactive oxygen species and induces

antioxidant enzyme activity in dopaminergic rat brain structures. Neuropharmacology 48, 965–974. https://doi.org/10.1016/j.neuropharm.2005.01.018

dos Santos, J.F., de Melo Bastos Cavalcante, C., Barbosa, F.T., Gitaí, D.L.G., Duzzioni, M., Tilelli, C.Q., Shetty, A.K., de Castro, O.W., 2018. Maternal, fetal and neonatal consequences associated with the use of crack cocaine during the gestational period: a systematic review and meta-analysis. Arch. Gynecol. Obstet. https://doi.org/10.1007/s00404-018-4833-2

Duailibi, L.B., Ribeiro, M., Laranjeira, R., 2008. Profile of cocaine and crack users in Brazil. Cad. Saude Publica 24, s545–s557. https://doi.org/10.1590/S0102-311X2008001600007

Eiden, R.D., Schuetze, P., Colder, C.R., Veira, Y., 2011a. Maternal cocaine use and mother-toddler aggression. Neurotoxicol. Teratol. 33, 360–369. https://doi.org/10.1016/J.NTT.2011.03.001

Eiden, R.D., Schuetze, P., Coles, C.D., 2011b. Maternal cocaine use and mother-infant interactions: Direct and moderated associations. Neurotoxicol. Teratol. 33, 120–128. https://doi.org/10.1016/J.NTT.2010.08.005

Elliott, J.C., Lubin, D.A., Walker, C.H., Johns, J.M., 2001. Acute cocaine alters oxytocin levels in the medial preoptic area and amygdala in lactating rat dams: implications for cocaine-induced changes in maternal behavior and maternal aggression. Neuropeptides 35, 127–134. https://doi.org/10.1054/NPEP.2001.0854

Enoch, M.A., 2008. The role of GABA(A) receptors in the development of alcoholism. Pharmacol. Biochem. Behav. 90, 95–104. https://doi.org/10.1016/J.PBB.2008.03.007

Fan, L.W., Chen, R.F., Mitchell, H.J., Lin, R.C.S., Simpson, K.L., Rhodes, P.G., Cai, Z., 2008. alpha-Phenyl-n-tert-butyl-nitrone attenuates lipopolysaccharide-induced brain injury and improves neurological reflexes and early sensorimotor behavioral performance in juvenile rats. J. Neurosci. Res. 86, 3536–3547.

https://doi.org/10.1002/JNR.21812

Fox, W.M., 1965. Reflex-ontogeny and behavioural development of the mouse. Anim. Behav. 13. https://doi.org/10.1016/0003-3472(65)90041-2

Gáll, Z., Farkas, S., Albert, Á., Ferencz, E., Vancea, S., Urkon, M., Kolcsár, M., 2020. Effects of Chronic Cannabidiol Treatment in the Rat Chronic Unpredictable Mild Stress Model of Depression. Biomolecules 10. https://doi.org/10.3390/BIOM10050801

Garcia, R.C.T., Dati, L.M.M., Fukuda, S., Torres, L.H.L., Moura, S., de Carvalho, N.D., Carrettiero, D.C., Camarini, R., Levada-Pires, A.C., Yonamine, M., Negrini-Neto, O., Abdalla, F.M.F., Sandoval, M.R.L., Afeche, S.C., Marcourakis, T., 2012. Neurotoxicity of Anhydroecgonine Methyl Ester, a Crack Cocaine Pyrolysis Product. Toxicol. Sci. 128, 223–234. https://doi.org/10.1093/toxsci/kfs140

Georgieva, E., Karamalakova, Y., Miteva, R., Abrashev, H., Nikolova, G., 2021. Oxidative Stress and Cocaine Intoxication as Start Points in the Pathology of Cocaine-Induced Cardiotoxicity. Toxics 9. https://doi.org/10.3390/TOXICS9120317

Gimpl, G., Fahrenholz, F., 2001. The oxytocin receptor system: Structure, function, and regulation. Physiol. Rev. 81, 629–683. https://doi.org/10.1152/PHYSREV.2001.81.2.629/ASSET/IMAGES/LARGE/9J0210132006.JPEG

Glauser, J., Queen, J.R., 2007. An overview of non-cardiac cocaine toxicity. J. Emerg. Med. 32, 181–6. https://doi.org/10.1016/j.jemermed.2006.05.044

Handley, S.L., Mithani, S., 1984. Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of 'fear'-motivated behaviour. Naunyn. Schmiedebergs. Arch. Pharmacol. 327, 1–5. https://doi.org/10.1007/BF00504983

Hansen, S., Bergvall, Å.H., Nyiredi, S., 1993. Interaction with pups enhances dopamine release in the ventral striatum of maternal rats: a microdialysis study. Pharmacol.

Biochem. Behav. 45, 673–676. https://doi.org/10.1016/0091-3057(93)90523-V

Hawley, T.L., Halle, T.G., Drasin, R.E., Thomas, N.G., 1995. Children of addicted mothers: effects of the “crack epidemic” on the caregiving environment and the development of preschoolers. Am. J. Orthopsychiatry 65, 364–379. https://doi.org/10.1037/H0079693

Hoang, T., Czuzoj-Shulman, N., Abenhaim, H.A., 2020. Pregnancy outcome among women with drug dependence: A population-based cohort study of 14 million births. J. Gynecol. Obstet. Hum. Reprod. 49, 101741. https://doi.org/10.1016/j.jogoh.2020.101741

Holubová-Kroupová, A., Šlamberová, R., 2021. Perinatal Stress and Methamphetamine Exposure Decreases Anxiety-Like Behavior in Adult Male Rats. Front. Behav. Neurosci. 15. https://doi.org/10.3389/FNBEH.2021.648780

Howard, J., Beckwith, L., Espinosa, M., Tyler, R., 1995. Development of infants born to cocaine-abusing women: Biologic/maternal influences. Neurotoxicol. Teratol. 17, 403–411. https://doi.org/10.1016/0892-0362(94)00077-Q

Howerton, C.L., Bale, T.L., 2012. Prenatal programing: at the intersection of maternal stress and immune activation. Horm. Behav. 62, 237–242. https://doi.org/10.1016/J.YHBEH.2012.03.007

IMAM, S.Z., DUHART, H.M., SKINNER, J.T., ALI, S.F., 2005. Cocaine induces a differential dose-dependent alteration in the expression profile of immediate early genes, transcription factors, and caspases in PC12 cells: a possible mechanism of neurotoxic damage in cocaine addiction. Ann. N. Y. Acad. Sci. 1053, 482–490. https://doi.org/10.1111/J.1749-6632.2005.TB00058.X

Jankowski, M.M., Ignatowska-Jankowska, B., Glac, W., Swiergiel, A.H., 2010. Cocaine administration increases CD4/CD8 lymphocyte ratio in peripheral blood despite lymphopenia and elevated corticosterone. Int. Immunopharmacol. 10, 1229–1234. https://doi.org/10.1016/J.INTIMP.2010.07.003

Jarrett, T.M., McMurray, M.S., Walker, C.H., Johns, J.M., 2006. Cocaine treatment alters oxytocin receptor binding but not mRNA production in postpartum rat dams. Neuropeptides 40, 161–167. https://doi.org/10.1016/J.NPEP.2006.03.002

Johns, J.M., Elliott, D.L., Hofler, V.E., Joyner, P.W., McMurray, M.S., Jarrett, T.M., Haslup, A.M., Middleton, C.L., Elliott, J.C., Walker, C.H., 2005a. Cocaine treatment and prenatal environment interact to disrupt intergenerational maternal behavior in rats. Behav. Neurosci. 119, 1605–1618. https://doi.org/10.1037/0735-7044.119.6.1605

Johns, J.M., Elliott, D.L., Hofler, V.E., Joyner, P.W., McMurray, M.S., Jarrett, T.M., Haslup, A.M., Middleton, C.L., Elliott, J.C., Walker, C.H., 2005b. Cocaine treatment and prenatal environment interact to disrupt intergenerational maternal behavior in rats. Behav. Neurosci. 119, 1605–1618. https://doi.org/10.1037/0735-7044.119.6.1605

Johns, J.M., Lubin, D.A., Walker, C.H., Joyner, P., Middleton, C., Hofler, V., McMurray, M., 2004. Gestational treatment with cocaine and fluoxetine alters oxytocin receptor number and binding affinity in lactating rat dams. Int. J. Dev. Neurosci. 22, 321–328. https://doi.org/10.1016/j.ijdevneu.2004.03.002

Johns, J.M., McMurray, M.S., Hofler, V.E., Jarrett, T.M., Middleton, C.L., Elliott, D.L., Mirza, R., Haslup, A., Elliott, J.C., Walker, C.H., 2007. Cocaine disrupts pup-induced maternal behavior in juvenile and adult rats. Neurotoxicol. Teratol. 29, 634–641. https://doi.org/10.1016/J.NTT.2007.07.003

Johns, J.M., Nelson, C.J., Meter, K.E., Lubin, D.A., Couch, C.D., Ayers, A., Walker, C.H., 1998. Dose-dependent effects of multiple acute cocaine injections on maternal behavior and aggression in Sprague-Dawley rats. Dev. Neurosci. 20, 525–532. https://doi.org/10.1159/000017353

Johns, J.M., Noonan, L.R., Zimmerman, L.I., Li, L., Pedersen, C.A., 1997. Effects of short- and long-term withdrawal from gestational cocaine treatment on maternal behavior and

aggression in Sprague-Dawley rats. Dev. Neurosci. 19, 368–374. https://doi.org/10.1159/000111234

Johns, J.M., Noonan, L.R., Zimmerman, L.I., Li, L., Pedersen, C.A., 1994. Effects of chronic and acute cocaine treatment on the onset of maternal behavior and aggression in Sprague-Dawley rats. Behav. Neurosci. 108, 107–112. https://doi.org/10.1037/0735-7044.108.1.107

JOHNS, J.M., NOONAN, L.R., ZIMMERMAN, L.I., McMILLEN, B.A., MEANS, L.W., WALKER, C.H., LUBIN, D.A., METER, K.E., NELSON, C.J., PEDERSEN, C.A., MASON, G.A., LAUDER, J.M., 1998. Chronic Cocaine Treatment Alters Social/Aggressive Behavior in Sprague-Dawley Rat Dams and in Their Prenatally Exposed Offspring a. Ann. N. Y. Acad. Sci. 846, 399–404. https://doi.org/10.1111/j.1749-6632.1998.tb09765.x

Johnson, H.L., Rosen, T.S., 1990. Mother-infant interaction in a multirisk population. Am. J. Orthopsychiatry 60, 281–288. https://doi.org/10.1037/H0079181

Kassada, D.S., Marcon, S.S., Pagliarini, M.A., Rossi, R.M., 2013. Prevalence of drug abuse among pregnant women. ACTA Paul. Enferm. 26, 467–471. https://doi.org/10.1590/s0103-21002013000500010

Kaufman, M.J., Siegel, A.J., Mendelson, J.H., Rose, S.L., Kukes, T.J., Sholar, M.B., Lukas, S.E., Renshaw, P.F., 1998. Cocaine administration induces human splenic constriction and altered hematologic parameters. J. Appl. Physiol. 85, 1877–1883. https://doi.org/10.1152/JAPPL.1998.85.5.1877

Kessler, F., Pechansky, F., 2008. A contemporary psychiatric view on the crack phenomenon. Rev. Psiquiatr. do Rio Gd. do Sul. https://doi.org/10.1590/S0101-81082008000300003

Khan, A.N., Casaubon, J.T., Regan, J.P., Monroe, L., 2017. Cocaine-induced splenic rupture. J. Surg. Case Reports 2017, 1–3. https://doi.org/10.1093/JSCR/RJX054

Killeen, T.K., Brady, K.T., Thevos, A., 1995. Addiction severity, psychopathology and treatment compliance in cocaine-dependent mothers. J. Addict. Dis. 14, 75–84. https://doi.org/10.1300/J069V14N01_08

Kinsley, C.H., Turco, D., Bauer, A., Beverly, M., Wellman, J., Graham, A.L., 1994. Cocaine alters the onset and maintenance of maternal behavior in lactating rats. Pharmacol. Biochem. Behav. 47, 857–864. https://doi.org/10.1016/0091-3057(94)90288-7

Kleinridders, A., Pothos, E.N., 2019. Impact of Brain Insulin Signaling on Dopamine Function, Food Intake, Reward, and Emotional Behavior. Curr. Nutr. Rep. 8, 83–91. https://doi.org/10.1007/S13668-019-0276-Z

Kohl, J., Autry, A.E., Dulac, C., 2017. The neurobiology of parenting: A neural circuit perspective. BioEssays 39, 1–11. https://doi.org/10.1002/bies.201600159

Kovacic, P., Cooksy, A.L., 2005. Unifying mechanism for toxicity and addiction by abused drugs: electron transfer and reactive oxygen species. Med. Hypotheses 64, 357–366. https://doi.org/10.1016/J.MEHY.2004.07.021

Kubera, M., Filip, M.S., Basta-Kaim, A., Nowak, E., Siwanowicz, J., Zajicova, A., Holan, V., Maes, M., Lasoń, W., 2004. The effect of cocaine sensitization on mouse immunoreactivity. Eur. J. Pharmacol. 483, 309–315. https://doi.org/10.1016/j.ejphar.2003.10.021

Lam, W.K.K., Wechsberg, W., Zule, W., 2004. African-American women who use crack cocaine: A comparison of mothers who live with and have been separated from their children. Child Abus. Negl. 28, 1229–1247. https://doi.org/10.1016/j.chiabu.2004.06.009

Lavi-Avnon, Y., Weller, A., Finberg, J.P.M., Gispan-Herman, I., Kinor, N., Stern, Y., Schroeder, M., Gelber, V., Bergman, S.Y., Overstreet, D.H., Yadid, G., 2008. The reward system and maternal behavior in an animal model of depression: a microdialysis study. Psychopharmacology (Berl). 196, 281–291. https://doi.org/10.1007/S00213-007-

0961-2

Legido, A., Clancy, R.R., Spitzer, A.R., Finnegan, L.P., 1992. Electroencephalographic and behavioral-state studies in infants of cocaine-addicted mothers. Am. J. Dis. Child. 146, 748–52.

Lejuez, C.W., Zvolensky, M.J., Daughters, S.B., Bornovalova, M.A., Paulson, A., Tull, M.T., Ettinger, K., Otto, M.W., 2008. Anxiety sensitivity: A unique predictor of dropout among inner-city heroin and crack/cocaine users in residential substance use treatment. Behav. Res. Ther. 46, 811–818. https://doi.org/10.1016/J.BRAT.2008.03.010

Lepsch, L.B., Munhoz, C.D., Kawamoto, E.M., Yshii, L.M., Lima, L.S., Curi-Boaventura, M.F., Salgado, T.M.L., Curi, R., Planeta, C.S., Scavone, C., 2009. Cocaine induces cell death and activates the transcription nuclear factor kappa-B in PC12 cells. Mol. Brain 2. https://doi.org/10.1186/1756-6606-2-3

Li, M., 2020. Psychological and neurobiological mechanisms underlying the decline of maternal behavior. Neurosci. Biobehav. Rev. 116, 164–181. https://doi.org/10.1016/J.NEUBIOREV.2020.06.009

Li, M., 2015. Antipsychotic drugs on maternal behavior in rats. Behav. Pharmacol. 26, 616–626. https://doi.org/10.1097/FBP.0000000000000168

Lin, Y., Leskawa, K.C., 1994. Cytotoxicity of the cocaine metabolite benzoylecgonine. Brain Res. 643, 108–114. https://doi.org/10.1016/0006-8993(94)90015-9

Lister, R.G., 1990. Ethologically-based animal models of anxiety disorders. Pharmacol. Ther. 46, 321–340. https://doi.org/10.1016/0163-7258(90)90021-S

Lonstein, J.S., Simmons, D.A., Swann, J.M., Stern, J.M., 1997. Forebrain expression of c-fos due to active maternal behaviour in lactating rats. Neuroscience 82, 267–281. https://doi.org/10.1016/S0306-4522(97)00283-2

López-Rodríguez, D., Aylwin, C.F., Delli, V., Sevrin, E., Campanile, M., Martin, M.,

Franssen, D., Gérard, A., Blacher, S., Tirelli, E., Noël, A., Lomniczi, A., Parent, A.S., López-Rodríguez, D., Aylwin, C.F., 2021. Multi- and Transgenerational Outcomes of an Exposure to a Mixture of Endocrine-Disrupting Chemicals (EDCs) on Puberty and Maternal Behavior in the Female Rat. Environ. Health Perspect. 129. https://doi.org/10.1289/EHP8795

Lorberbaum, J.P., Newman, J.D., Horwitz, A.R., Dubno, J.R., Lydiard, R.B., Hamner, M.B., Bohning, D.E., George, M.S., 2002. A potential role for thalamocingulate circuitry in human maternal behavior. Biol. Psychiatry 51, 431–445. https://doi.org/10.1016/S0006-3223(01)01284-7

Lubin, D.A., Elliott, J.C., Black, M.C., Johns, J.M., 2003. An oxytocin antagonist infused into the central nucleus of the amygdala increases maternal aggressive behavior. Behav. Neurosci. 117, 195–201. https://doi.org/10.1037/0735-7044.117.2.195

Macêdo, D.S., de Vasconcelos, S.M.M., dos Santos, R.S., Aguiar, L.M.V., Lima, V.T.M., Viana, G.S.B., de Sousa, F.C.F., 2005. Cocaine alters catalase activity in prefrontal cortex and striatum of mice. Neurosci. Lett. 387, 53–6. https://doi.org/10.1016/j.neulet.2005.07.024

Macêdo, D.S., Vasconcelos, S.M.M., Andrade-Neto, M., Belchior, L.D., Honório Júnior, J.E.R., Gonçalves, D.O., Fonteles, M.M.F., Silva, M.I.G., Aguiar, L.M.V., Viana, G.S.B., de Sousa, F.C.F., 2010. Cocaine-induced status epilepticus and death generate oxidative stress in prefrontal cortex and striatum of mice. Neurochem. Int. 56, 183–7. https://doi.org/10.1016/j.neuint.2009.10.001

Malacarne, I.T., De Souza, D.V., Rosario, B.D.A., Viana, M.D.B., Pereira, C.D.S., Estadella, D., dos Santos, J.N., Ribeiro, D.A., 2021. Genotoxicity, oxidative stress, and inflammatory response induced by crack-cocaine: relevance to carcinogenesis. Environ. Sci. Pollut. Res. Int. 28, 14285–14292. https://doi.org/10.1007/S11356-021-12617-2

Martinez, Z.A., Ellison, G.D., Geyer, M.A., Swerdlow, N.R., 1999. Effects of sustained cocaine exposure on sensorimotor gating of startle in rats. Psychopharmacology (Berl). 142, 253–260. https://doi.org/10.1007/S002130050887

Mayes, L.C., Feldman, R., Granger, R.H., Haynes, O.M., Bornstein, M.H., Schottenfeld, R., 1997. The effects of polydrug use with and without cocaine on mother-infant interaction at 3 and 6 months. Infant Behav. Dev. 20, 489–502. https://doi.org/10.1016/S0163-6383(97)90038-2

McBride, W.J., 2002. Central nucleus of the amygdala and the effects of alcohol and alcohol-drinking behavior in rodents. Pharmacol. Biochem. Behav. 71, 509–515. https://doi.org/10.1016/S0091-3057(01)00680-3

McDermott, M.J., Tull, M.T., Gratz, K.L., Daughters, S.B., Lejuez, C.W., 2009. The role of anxiety sensitivity and difficulties in emotion regulation in posttraumatic stress disorder among crack/cocaine dependent patients in residential substance abuse treatment. J. Anxiety Disord. 23, 591–599. https://doi.org/10.1016/J.JANXDIS.2009.01.006

McMurray, M.S., Joyner, P.W., Middleton, C.W., Jarrett, T.M., Elliott, D.L., Black, M.A., Hofler, V.E., Walker, C.H., Johns, J.M., 2008. Intergenerational effects of cocaine on maternal aggressive behavior and brain oxytocin in rat dams. Stress 11, 398–411. https://doi.org/10.1080/10253890701850239

Melo, I.S., Santos, Y.M.O., Costa, M.A., Pacheco, A.L.D., Silva, N.K.G.T., Cardoso-Sousa, L., Pereira, U.P., Goulart, L.R., Garcia-Cairasco, N., Duzzioni, M., Gitaí, D.L.G., Tilelli, C.Q., Sabino-Silva, R., Castro, O.W., 2016. Inhibition of sodium glucose cotransporters following status epilepticus induced by intrahippocampal pilocarpine affects neurodegeneration process in hippocampus. Epilepsy Behav. 61, 258–68. https://doi.org/10.1016/j.yebeh.2016.05.026

Mitchell, E.S., Snyder-Keller, A., 2003. Blockade of D1 dopaminergic transmission alleviates

c-fos induction and cleaved caspase-3 expression in the brains of rat pups exposed to prenatal cocaine or perinatal asphyxia. Exp. Neurol. 182, 64–74. https://doi.org/10.1016/S0014-4886(03)00026-8

Naifeh, J.A., Tull, M.T., Gratz, K.L., 2012. Anxiety Sensitivity, Emotional Avoidance, and PTSD Symptom Severity Among Crack/Cocaine Dependent Patients in Residential Treatment. Cognit. Ther. Res. 36, 247. https://doi.org/10.1007/S10608-010-9337-8

Nassogne, M.C., Louahed, J., Evrad, P., Courtoy, P.J., 1997. Cocaine induces apoptosis in cortical neurons of fetal mice. J. Neurochem. 68, 2442–2450. https://doi.org/10.1046/J.1471-4159.1997.68062442.X

Nephew, B.C., Febo, M., 2010. Effect of cocaine sensitization prior to pregnancy on maternal care and aggression in the rat. Psychopharmacology (Berl). 209, 127–135. https://doi.org/10.1007/S00213-010-1777-Z

Nigam, Y., Knight, J., 2020. The lymphatic system 2: structure and function of the lymphoid organs. Nurs. Times 116.

Nikolaus, S., Mamlins, E., Hautzel, H., Müller, H.W., 2019. Acute anxiety disorder, major depressive disorder, bipolar disorder and schizophrenia are related to different patterns of nigrostriatal and mesolimbic dopamine dysfunction. Rev. Neurosci. 30, 381–426. https://doi.org/10.1515/REVNEURO-2018-0037

Numa, R., Kohen, R., Poltyrev, T., Yaka, R., 2008. Tempol diminishes cocaine-induced oxidative damage and attenuates the development and expression of behavioral sensitization. Neuroscience 155, 649–58. https://doi.org/10.1016/j.neuroscience.2008.05.058

Numan, M., 2016. Hypothalamic Neural Circuits Regulating Maternal Responsiveness Toward Infants. https://doi.org/10.1177/1534582306288790 5, 163–190. https://doi.org/10.1177/1534582306288790

Numan, M., 2007. Motivational systems and the neural circuitry of maternal behavior in the rat. Dev. Psychobiol. 49, 12–21. https://doi.org/10.1002/DEV.20198

Numan, M., 1994. A neural circuitry analysis of maternal behavior in the rat. Acta Paediatr. Suppl. 397, 19–28.

Numan, M., Numan, M.J., Pliakou, N., Stolzenberg, D.S., Mullins, O.J., Murphy, J.M., Smith, C.D., 2006. The effects of D1 or D2 dopamine receptor antagonism in the medial preoptic area, ventral pallidum, or nucleus accumbens on the maternal retrieval response and other aspects of maternal behavior in rats. Behav. Neurosci. 119, 1588. https://doi.org/10.1037/0735-7044.119.6.1588

Numan, M., Smith, H.G., 1984. Maternal behavior in rats: evidence for the involvement of preoptic projections to the ventral tegmental area. Behav. Neurosci. 98, 712–727. https://doi.org/10.1037//0735-7044.98.4.712

Numan, M., Stolzenberg, D.S., 2009. Medial preoptic area interactions with dopamine neural systems in the control of the onset and maintenance of maternal behavior in rats. Front. Neuroendocrinol. 30, 46–64. https://doi.org/10.1016/J.YFRNE.2008.10.002

Numan, M., Woodside, B., 2010. Maternity: Neural mechanisms, motivational processes, and physiological adaptations. Behav. Neurosci. 124, 715. https://doi.org/10.1037/A0021548

Numan, M., Young, L.J., 2016. Neural mechanisms of mother–infant bonding and pair bonding: Similarities, differences, and broader implications. Horm. Behav. 77, 98–112. https://doi.org/10.1016/J.YHBEH.2015.05.015

Oliveira, M.T., Rego, A.C., Macedo, T.R.A., Oliveira, C.R., 2003. Drugs of abuse induce apoptotic features in PC12 cells. Ann. N. Y. Acad. Sci. 1010, 667–670. https://doi.org/10.1196/ANNALS.1299.121

Pacheco, A., de Melo, I., de Souza, F., Nicácio, D., Freitas-Santos, J., Oliveira Dos Santos, Y., Costa, M., Cavalcante, C., Gomes Dos Santos Neto, J., Gitaí, D., Sabino-Silva, R.,

Torres de Miranda, C., Borbely, A., Duzzioni, M., Shetty, A., de Castro, O., 2021. Maternal crack cocaine use in rats leads to depressive- and anxiety-like behavior, memory impairment, and increased seizure susceptibility in the offspring. Eur. Neuropsychopharmacol. 44, 34–50. https://doi.org/10.1016/J.EURONEURO.2020.12.011

Paxinos, G., Watson, C., 2007. The rat brain in stereotaxic coordinates, 6th ed. San Diego: Academic Press.

Pedersen, C.A., Ascher, J.A., Monroe, Y.L., Prange, A.J., 1982. Oxytocin induces maternal behavior in virgin female rats. Science 216, 648–650. https://doi.org/10.1126/SCIENCE.7071605

Pedersen, C.A., Caldwell, J.D., Johnson, M.F., Fort, S.A., Prange, A.J., 1985. Oxytocin antiserum delays onset of ovarian steroid-induced maternal behavior. Neuropeptides 6, 175–182. https://doi.org/10.1016/0143-4179(85)90108-8

Pedersen, C.A., Caldwell, J.D., Walker, C., Ayers, G., et al, 1994. Oxytocin activates the postpartum onset of rat maternal behavior in the ventral tegmental and medial preoptic areas. Behav. Neurosci. 108, 1163–1171. https://doi.org/10.1037//0735-7044.108.6.1163

Pellow, S., File, S.E., 1986. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: A novel test of anxiety in the rat. Pharmacol. Biochem. Behav. 24, 525–529. https://doi.org/10.1016/0091-3057(86)90552-6

Philipsen, M.H., Phan, N.T.N., Fletcher, J.S., Ewing, A.G., 2020. Interplay between Cocaine, Drug Removal, and Methylphenidate Reversal on Phospholipid Alterations in Drosophila Brain Determined by Imaging Mass Spectrometry. ACS Chem. Neurosci. 11, 806–813. https://doi.org/10.1021/acschemneuro.0c00014

Raghav, R., Jain, R., Jacob, T.G., Dhawan, A., Roy, T.S., 2021. Co-administration of nalbuphine attenuates the morphine-induced anxiety and dopaminergic alterations in

morphine-withdrawn rats. Psychopharmacology (Berl). 238, 1193–1211. https://doi.org/10.1007/S00213-021-05765-3

Rosenblatt, J.S., Ceus, K., 1998. Estrogen Implants in the Medial Preoptic Area Stimulate Maternal Behavior in Male Rats. Horm. Behav. 33, 23–30. https://doi.org/10.1006/HBEH.1997.1430

Salas-Ramirez, K.Y., Frankfurt, M., Alexander, A., Luine, V.N., Friedman, E., 2010. Prenatal cocaine exposure increases anxiety, impairs cognitive function and increases dendritic spine density in adult rats: influence of sex. Neuroscience 169, 1287–1295. https://doi.org/10.1016/j.neuroscience.2010.04.067

Sasaki, A., Constantinof, A., Pan, P., Kupferschmidt, D.A., McGowan, P.O., Erb, S., 2014. Cocaine exposure prior to pregnancy alters the psychomotor response to cocaine and transcriptional regulation of the dopamine D1 receptor in adult male offspring. Behav. Brain Res. 265, 163–170. https://doi.org/10.1016/J.BBR.2014.02.017

Schmued, L.C., Albertson, C., Slikker, W., 1997. Fluoro-Jade: A novel fluorochrome for the sensitive and reliable histochemical localization of neuronal degeneration. Brain Res. 751, 37–46. https://doi.org/10.1016/S0006-8993(96)01387-X

Shnitko, T.A., Mace, K.D., Sullivan, K.M., Martin, W.K., Andersen, E.H., Williams Avram, S.K., Johns, J.M., Robinson, D.L., 2017. Use of fast-scan cyclic voltammetry to assess phasic dopamine release in rat models of early postpartum maternal behavior and neglect. Behav. Pharmacol. 28, 648–660. https://doi.org/10.1097/FBP.0000000000000347

Siliquini, R., Morra, A., Versino, E., Renga, G., 2005. Recreational drug consumers: Who seeks treatment? Eur. J. Public Health 15, 580–586. https://doi.org/10.1093/eurpub/cki031

Silva, C., McNaughton, N., 2019. Are periaqueductal gray and dorsal raphe the foundation of

appetitive and aversive control? A comprehensive review. Prog. Neurobiol. 177, 33–72. https://doi.org/10.1016/J.PNEUROBIO.2019.02.001

Silva, M.R.P., Bernardi, M.M., Felicio, L.F., 2001. Effects of dopamine receptor antagonists on ongoing maternal behavior in rats. Pharmacol. Biochem. Behav. 68, 461–468. https://doi.org/10.1016/S0091-3057(01)00471-3

Silvers, J.M., Wallace, D.R., Harrod, S.B., Mactutus, C.F., Booze, R.M., 2006. Prenatal cocaine alters dopamine and sigma receptor binding in nucleus accumbens and striatum in dams and adolescent offspring. Neurotoxicol. Teratol. 28, 173–180. https://doi.org/10.1016/J.NTT.2006.01.009

Simone, C., Derewlany, L.O., Oskamp, M., Knie, B., Koren, G., 1994. Transfer of cocaine and benzoylecgonine across the perfused human placental cotyledon. Am. J. Obstet. Gynecol. 170, 1404–1410. https://doi.org/10.1016/S0002-9378(94)70171-7

Slamberová, R., 2003. [Drugs during pregnancy--effects on the mother and next generation]. Ceskoslov. Fysiol. / Ústrední ústav Biol. 52, 15–21.

Smart, J.L., Dobbing, J., 1971. Vulnerability of developing brain. II. Effects of early nutritional deprivation on reflex ontogeny and development of behaviour in the rat. Brain Res. 28, 85–95. https://doi.org/10.1016/0006-8993(71)90526-9

Smith, R.F., Mattran, K.M., Kurkjian, M.F., Kurtz, S.L., 1989. Alterations in offspring behavior induced by chronic prenatal cocaine dosing. Neurotoxicol. Teratol. 11, 35–38. https://doi.org/10.1016/0892-0362(89)90082-2

Soares, A.K.F., Guerra, R.G.S., De Castro, M.L., Amancio-Dos-Santos, A., Guedes, R.C.A., Cabral-Filho, J.E., Costa, J.A., Medeiros, M.D.C., 2009. Somatic and reflex development in suckling rats: Effects of mother treatment with ketogenic diet associated with lack of protein. Nutr. Neurosci. 12, 260–266. https://doi.org/10.1179/147683009X423427

Souza-Silva, E.M., Alves, R.B., Simon, K.A., Hueza, I.M., 2020. Crack cocaine smoke on

pregnant rats: Maternal evaluation and teratogenic effect. Hum. Exp. Toxicol. 39, 411–422. https://doi.org/10.1177/0960327119891219

Stack, E.C., Balakrishnan, R., Numan, M.J., Numan, M., 2002. A functional neuroanatomical investigation of the role of the medial preoptic area in neural circuits regulating maternal behavior. Behav. Brain Res. 131, 17–36. https://doi.org/10.1016/S0166-4328(01)00370-9

Stern, J.M., 1997. Nurturance: Animal-Human Parallels. Dev Psychobiol 31, 19–37. https://doi.org/10.1002/(SICI)1098-2302(199707)31:1

Strathearn, L., Mayes, L.C., 2010. Cocaine addiction in mothers: Potential effects on maternal care and infant development. Ann. N. Y. Acad. Sci. 1187, 172–183. https://doi.org/10.1111/j.1749-6632.2009.05142.x

Swain, J.E., Ho, S.S., Fox, H., Garry, D., Brummelte, S., 2019. Effects of opioids on the parental brain in health and disease. Front. Neuroendocrinol. 54, 100766. https://doi.org/10.1016/J.YFRNE.2019.100766

Swerdlow, N.R., Geyer, M.A., 1998. Using an animal model of deficient sensorimotor gating to study the pathophysiology and new treatments of schizophrenia. Schizophr. Bull. 24, 285–301. https://doi.org/10.1093/oxfordjournals.schbul.a033326

Teodorov, E., Salzgeber, S.A., Felicio, L.F., Varolli, F.M.F., Bernardi, M.M., 2002. Effects of perinatal picrotoxin and sexual experience on heterosexual and homosexual behavior in male rats. Neurotoxicol. Teratol. 24, 235–245. https://doi.org/10.1016/S0892-0362(01)00213-6

Tyler, R., Howard, J., Espinosa, M., Doakes, S.S., 1997. Placement with substance-abusing mothers vs. placement with other relatives: Infant outcomes. Child Abuse Negl. 21, 337–349. https://doi.org/10.1016/S0145-2134(96)00175-5

Unuma, K., Kaga, H., Funakoshi, T., Nomura, M., Aki, T., Uemura, K., 2022. Thymic

involution caused by repeated cocaine administration includes apoptotic cell loss followed by ectopic adipogenesis. PLoS One 17, e0277032. https://doi.org/10.1371/JOURNAL.PONE.0277032

Uriarte, N., Ferreira, A., Rosa, X.F., Sebben, V., Lucion, A.B., 2008. Overlapping litters in rats: effects on maternal behavior and offspring emotionality. Physiol. Behav. 93, 1061–1070. https://doi.org/10.1016/J.PHYSBEH.2008.02.004

Varga, Z. V., Ferdinandy, P., Liaudet, L., Pacher, P., 2015. Drug-induced mitochondrial dysfunction and cardiotoxicity. Am. J. Physiol. Heart Circ. Physiol. 309, H1453–H1467. https://doi.org/10.1152/AJPHEART.00554.2015

Wallin, C.M., Bowen, S.E., Brummelte, S., 2021. Opioid use during pregnancy can impair maternal behavior and the Maternal Brain Network: A literature review. Neurotoxicol. Teratol. 86, 106976. https://doi.org/10.1016/J.NTT.2021.106976

Wasserman, D.R., Leventhal, J.M., 1993. Maltreatment of children born to cocaine-dependent mothers. Am. J. Dis. Child. 147, 1324–1328. https://doi.org/10.1001/ARCHPEDI.1993.02160360066021

Weinstock, M., 2005. The potential influence of maternal stress hormones on development and mental health of the offspring. Brain. Behav. Immun. 19, 296–308. https://doi.org/10.1016/j.bbi.2004.09.006

Williams, S.K., Johns, J.M., 2014. Prenatal and gestational cocaine exposure: Effects on the oxytocin system and social behavior with implications for addiction. Pharmacol. Biochem. Behav. 119, 10–21. https://doi.org/10.1016/J.PBB.2013.07.004

Wotjak, C.T., Ganster, J., Kohl, G., Holsboer, F., Landgraf, R., Engelmann, M., 1998. Dissociated central and peripheral release of vasopressin, but not oxytocin, in response to repeated swim stress: New insights into the secretory capacities of peptidergic neurons. Neuroscience 85, 1209–1222. https://doi.org/10.1016/S0306-4522(97)00683-0

Ypsilantis, P., Politou, M., Anagnostopoulos, C., Kortsaris, A., Simopoulos, C., 2012. A rat model of cigarette smoke abuse liability. Comp. Med. 62, 395–9.

Zaparte, A., Schuch, J.B., Viola, T.W., Baptista, T.A.S., Beidacki, A.S., do Prado, C.H., Sanvicente-Vieira, B., Bauer, M.E., Grassi-Oliveira, R., 2019. Cocaine Use Disorder Is Associated With Changes in Th1/Th2/Th17 Cytokines and Lymphocytes Subsets. Front. Immunol. 10, 2435. https://doi.org/10.3389/FIMMU.2019.02435

Zubaran, C., Foresti, K., Thorell, M.R., Franceschini, P.R., 2013. Anxiety symptoms in crack cocaine and inhalant users admitted to a psychiatric hospital in southern Brazil. Rev. Assoc. Med. Bras. 59, 360–367. https://doi.org/10.1016/J.RAMB.2013.01.008

Referências Bibliográficas

Areal, L.B.; Rodrigues, L.C.M.; Andrich, F.; Moraes, L.S.; Cicilini, M.A.; Mendonça, J.B.; Pelição, F.S.; Nakamura-Palacios, E.M.; Martins-Silva, C.; Pires, R.G.W. **Behavioural, biochemical and molecular changes induced by chronic crack-cocaine inhalation in mice: The role of dopaminergic and endocannabinoid systems in the prefrontal córtex.** Behav Brain Res. 2015.

Azevedo, L.B.A. **Alterações comportamentais, bioquímicas e moleculares em modelo animal de inalação crônica de "crack": papel dos sistemas dopaminérgicos e endocanabinoíde no cortéx pré-frontal**. Vitória, 2014.

Bastos; bertoni. **Pesquisa Nacional do Uso de Crack. Rio de janeiro**. ICICT/FIOCRUZ, 2014.

Dinis-Oliveira, R.J. **Metabolomics of cocaine: implications in toxicity**. Toxicol Mech Methods. 2015

Donato, E.M.; Rezende, E.P.; Ribeiro, M.; DA Silva, C.J**. Farmacologia e neurobiologia do consumo de crack. In: Ribeiro, M.; Laranjeira, R. (org). O Tratamento do Usuário de Crack: avaliação clínica, psicossocial e de risco, terapias psicológicas, farmacologia e reabilitação, ambientes de tratamento.** São Paulo: Casa Leitura Médica, 2010. p.49-59.

Erzouki, H.K; Allen, A.C ; Newman, A.H; Goldberg, S.R ; Schindler, C.W. **Effects of cocaine, cocaine metabolites and cocaine pyrolysis products on the hindbrain cardiac and respiratory centers of the rabbit**. Life Sciences, v. 57: 1861-1868,1995.

Ferreira, B.A.M.; Baía, L.V.M.; Alencar, I.P. Belo, M.H.L.; Alencar, S.M.P.; Fermoseli, A.F.O. **O uso e abuso da cocaína: efeitos neurofisiológicos**. Novembro 2017.

Garcia, R.C.T.; Dati, L.M.M.; Fukuda, S.; Torres, L.H.L.; Moura, S.; Carvalho, N.D.; Carrettiero, D.C.; Camarini, R.; Levada-Pires, A.C.; Yonamine, M.; Negrini-Neto, O.; Abdalla, F.M.F.; Sandoval, M.R.L.; Afeche, S.C.; Marcourakis, T. **Neurotoxicity of anhydroecgonine methyl ester, a crack cocaine pyrolysis product**. Toxicol Sci. 2012.

Gomes, E.F.; Lipaus, E.F.S.; Martins, C.W.; Araújo, A.M.; Mendonça, J.B.; Pelição, F.S.; Lebarch, E.C.; Rodrigues, L.C.M.; Nakamura-Palacios, E.M. **Anhydroecgonine Methyl Ester (AEME), a Product of Cocaine Pyrolysis, Impairs Spatial Working Memory and Induces Striatal Oxidative Stress in Rats**. Neurotox Res. 2018

Goldstein, R.A.; Derlauriers, C.; Burda, A.; Johnson-Arbor, K. **Cocaine: history, social implications, and toxicity: a review**. Semin Diagn Pathol, v. 26 : 10-7, 2009.

Hatsukami, D.K., E Fischman, M.W. **Crack cocaine and cocaine hydrochloride. Are the differences myth or reality?** JAMA, 1996.

Mesquita, L.S.M.; Garcia, R.C.T.; Amaral, F.G.; Peres, R.; Wood, S.M.; Lucena, R.V.L.; Frare, E.O.; Abrahão, M.V. Marcourakis, T.; Cipolla-Neto, J.; Afeche, S.C. **The muscarinic effect of anhydroecgonine methyl ester, a crack cocaine pyrolysis product, impairs melatonin synthesis in the rat pineal gland.** Toxicol Res (Camb), 2017.

Negrete JC. **Cocaine problems in the cocagrowing countries of South America. In: Bock GR, Whelan J, eds. Cocaine: scientific and social dimensions.** Ciba Foundation Symposium 166. Chichester:John Wiley & Sons;1992. pp. 40-9.

Numan M, Rosenblatt JS, Komisaruk BR. **Medial preoptic area and onset of maternal behavior in the rat.** J Comp Physiol Psychol. 1977; 91:146–64.

Parks, M.H., Greenberg, D.S.; Nickel, M.K.; Dietrich, M.S.; Rogers, B.P.; Martin, P.R. **Recruitment of additional brain regions to accomplish simple motor tasks in chronic alcohol-dependent patients**. Alcohol Clin Exp Res, v.34: 1098-109, 2010.

Rego, X.F.J. **Dependência química: os efeitos da cocaína no sistema nervoso central**. IPEMED, São Paulo, novembro de 2010.

Reinerman, C.; Levine, H.G. **The crack attack: politics and media in the crack scare. In: Reinerman C, Levine HG. Crack in America: demons, drugs and social justice**. Berkley: University of California Press; 1997.

Scheidweiler, K.B.; Plessinger, M.A.; Shojaie, J.; Wood, R.W.; Kwong, T.C. **Pharmacokinetics and pharmacodynamics of methylecgonidine, a crack cocaine pyrolyzate,** J Pharmacol Exp Ther. 2003.

Stewart, D.J.; Inaba, T.; Tang, B. K.; Kalow, W. **Hydrolysis of cocaine in human plasma by cholinesteras.** Life sciences, v. 20: 1557:1564, 1977.

UNODCCP. **United Nations Office for Drug Control and Crime Prevention (UNODCCP). Global illicit drug trends** 200[online]. Vienna:UNODCCP; 2001.

UNODC (**United Nations Office on Drugs and Crime**).World Drug Report. 2015.

Rodrigues, F.A E Silveira, F. M. **A neurotoxicidade no transtorno por uso de substância psicoativa derivados de cocaína**. RECISATEC, 2022.

Produtção Científica

1. Maternal crack cocaine use in rats leads to depressive- and anxiety-like behavior,memoryimpairment, and increased seizure susceptibility in the offspring. EUROPEANNEUROPSYCHOPHARMACOLOGY, v. 1, p. 1-17, 2021. PACHECO, A. L. D.; MELO, I. S. D.; SOUZA, F. M. A. ; NICACIO, D. C. S. P. ; FREITAS-SANTOS, J. ; SANTOS, Y. M. O. ;COSTA, M. D. A. ; CAVALCANTE, C. M. B. ; SANTOS NETO, J. G. ; GITAI, D. L. ; SABINO-SILVA, R. ; TORRES, C. M. ; BORBELY, A. U. ; DUZZIONI, M. ; SHETTY, A. K. ; CASTRO,O. W.

2. Role of Modulation of Hippocampal Glucose Following Pilocarpine-Induced Status Epilepticus.MOLECULAR NEUROBIOLOGY., v.1, p.1 - 20, 2020. DE MELO, IGOR SANTANA; DOSSANTOS, YNGRID MICKAELLI OLIVEIRA; PACHECO, AMANDA LARISSA DIAS;COSTA, MAISA ARAÚJO; DE OLIVEIRA SILVA, VANESSA; FREITAS-SANTOS,JUCILENE; DE MELO BASTOS CAVALCANTE, CIBELLE; SILVA-FILHO, REGINALDOCORREIA; LEITE, ANA CATARINA REZENDE; GITAÍ, DANIEL GÓES LEITE; DUZZIONI,MARCELO; SABINO-SILVA, ROBINSON; BORBELY, ALEXANDRE URBAN; DECASTRO, OLAGIDE WAGNER.

3. Modulation of glucose availability and effects of hypo- and hyperglycemia on status epilepticus:what we do not know yet?. Molecular Neurobiology. v.1, p.1 - 15, 2020. MELO, IGORSANTANA; PACHECO, A. L. D.; SANTOS, Y. M. O.; FIGUEIREDO, L. M.; NICACIO, D. C.S. P.; SOUSA, L. C.; GITAI, D. L.; TILELLI, CRISTIANE Q.; DUZZIONI, MARCELO;SABINO-SILVA, R.; CASTRO, O. W.

4. Neuroprotective Effect of Exogenous Galectin-1 in Status Epilepticus. Molecular Neurobiology, v. 59, p. 7354-7369, 2022. PACHECO, AMANDA LARISSA DIAS; DE MELO, IGOR SANTANA ; DE ARAUJO COSTA, MAISA ; AMARAL, MARIAH MORAIS

CELESTINO ; DE GUSMÃO TAVEIROS SILVA, NÍVEA KARLA ; SANTOS, YNGRID MICKAELLI OLIVEIRA ; GITAÍ, DANIEL LEITE GÓES ; DUZZIONI, MARCELO ; BORBELY, ALEXANDRE URBAN ; SILVA, ROBINSON SABINO ; DONATTI, ANA LUIZA FERREIRA ; MESTRINER, LUISA ; FUZO, CARLOS ALESSANDRO ; CUMMINGS, RICHARD D. ; GARCIA-CAIRASCO, NORBERTO ; DIAS-BARUFFI, MARCELO ; DE CASTRO, OLAGIDE WAGNER.

ANEXO

UNIVERSIDADE FEDERAL DE ALAGOAS
PRÓ-REITORIA DE PÓS-GRADUAÇÃO E PESQUISA
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CEUA Ufal

CERTIFICADO

Certificamos que a proposta intitulada *"Efeitos do canabidiol sobre as alterações neuropsicofarmacológicas em mães e filhotes expostos ao crack durante período gestacional"*, registrada com o **nº 28/2021**, sob a responsabilidade do pesquisador **Prof. Dr. Olagide Wagner de Castro**, que envolve a utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), **para fins de pesquisa científica,** 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 aprovada pela Comissão de Ética no Uso de Animais da Universidade Federal de Alagoas (CEUA/UFAL), em reunião de 30 de junho de 2022.

Vigência da autorização	16.08.2022 a 16.08.2026
Espécie/linhagem/raça	Rato Wistar
Nº de animais	200
Peso/idade	150g – 300 g / 60 – 90 dias
Sexo	Machos e fêmeas
Origem / Local de manutenção	Biotério Central da Ufal / LNFI - ICBS
Colaboradores	Jucilene Santos, Amanda Pacheco, Igor Melo, Maisa Costa, Yngrid Santos, Fernanda Souza, Bianca Silva, Keila Oliveira, Milenna Reiter, Kellyson Oliveira e Noemi Torres.

Maceió, 15 de agosto de 2022.

gov.br Documento assinado digitalmente
LAZARO WENDER OLIVEIRA DE JESUS
Data: 15/08/2022 19:05:56-0300
Verifique em https://verificador.iti.br

Lázaro Wender Oliveira de Jesus
Coordenador da CEUA
SIAPE 1265581