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AMANDA LARISSA DIAS PACHECO

ALTERAÇÕES COMPORTAMENTAIS E NA SUSCEPTIBILIDADE DE CRISES EPILÉPTICAS DECORRENTES DA EXPOSIÇÃO GESTACIONAL À FUMAÇA DE *CRACK*

Maceió
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Trabalho apresentado ao Programa de Pós-Graduação em Ciências da Saúde da Universidade Federal de Alagoas, em cumprimento às exigências para a obtenção do título de Mestre em Ciências da Saúde.

Orientador: Prof. Dr. Olagide Wagner de Castro

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Alterações comportamentais e na susceptibilidade de crises epilépticas decorrentes da exposição gestacional à fumaça do crack

Dissertação submetida ao corpo docente do Programa de Pós-Graduação em Ciências da Saúde da Universidade Federal de Alagoas e aprovada em 22 de Fevereiro de 2019.

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Dedico este trabalho a todos os cientistas e professores que buscam caminhos e um universo de possibilidades todos os dias.

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“Ao fim do dia, podemos aguentar muito mais do que pensamos que podemos".

Frida Kahlo

RESUMO

O *crack*, cocaína em sua forma fumada, é uma droga de abuso estimulante do sistema nervoso central. Usuários de *crack* sofrem os efeitos da cocaína presente na droga, bem como de outros compostos ativos produzidos em sua pirólise, o que tem sido associado a um maior risco de neurotoxicidade e maior poder de dependência. O consumo desta droga é atualmente um problema de saúde pública associado ao aumento das taxas de criminalidade. Outro fator alarmante é o aumento do número de usuárias de *crack* grávidas, cujos filhos são conhecidos como "*crack babies"*. A cocaína, o crack e os metabólitos de sua combustão atravessam a placenta promovendo maior incidência de restrição de crescimento intrauterino, pré-eclâmpsia, vasoconstrição, taquicardia, hipertensão e aborto espontâneo. Estudos com *crack babies* indicam a presença de prematuridade, febre, irritabilidade, sudorese e convulsões. Em crianças, os efeitos da cocaína e seus derivados têm sido associados a déficits cognitivos, dificuldade de verbalização, agressividade e depressão, além de modificar a susceptibilidade a crises epilépticas na vida adulta. No entanto, as alterações comportamentais e na susceptibilidade das crises devido à exposição gestacional ao crack são pouco descritas na literatura. Nesse estudo, testamos a hipótese de que a exposição ao *crack* durante a gravidez afeta a função cognitiva, acarreta comportamentos tipo-ansiosos e depressivos e aumenta a susceptibilidade para crises e padrões de *Status Epilepticus* (SE). Através de uma bateria de testes comportamentais e microinjecção de pilocarpina intra-hipocampal (H-PILO) em doses subconvulsivas (sH-PILO) e convulsivas (cH-PILO) em modelo animal, nós demonstramos que a exposição ao *crack* durante o estágio embrionário leva ao comportamento tipo-ansioso e ao comprometimento da memória de longo prazo em ambos os sexos; bem como ao desenvolvimento de comportamento tipo-depressivo em fêmeas. Animais expostos à dose de sH-PILO apresentaram maior susceptibilidade ao desenvolvimento do SE, maior frequência de crises e tempo total de convulsão, enquanto animais com cH-PILO não apresentaram alteração na gravidade do SE. Dessa forma, nossos dados sugerem que a exposição ao *crack* durante o período gestacional compromete o desenvolvimento da prole, com a presença de comorbidades, maior propensão à ansiedade e depressão, déficit de memória de longo prazo e redução do limiar de crises epilépticas, retratando a importância de nosso estudo científico sobre os efeitos da exposição ao *crack* em usuárias grávidas.

Palavras-chave: Crack, Comportamento ansiogênico, Comportamento depressivo, Epilepsia

ABSTRACT

Crack cocaine, cocaine in its smoked form, is an abuse drug that stimulates the central nervous system. Crack cocaine users suffer the effects of cocaine present in the drug, as well as the affects others active compounds due to its pyrolysis, what has been associated with a higher risk of neurotoxicity and greater addictive power. The consumption of this drug is currently a public health problems associated with the increase in crime rates. Another alarming fact is the increase in the number of pregnant crack cocaine users, whose children are known as crack babies. Cocaine, crack cocaine and its combustion products cross the placenta promoting higher incidence of fetal intrauterine growth restriction, preeclampsia, vasoconstriction, tachycardia, hypertension and miscarriage. Studies with crack babies indicate the presence of prematurity, fever, irritability, sweating and seizures. In children, the effects of cocaine and its derivatives have been associated with cognitive deficits, difficulty in verbalization, aggressiveness and depression, besides modifying the susceptibility to epileptic seizures in adulthood. However, behavior alteration and seizures susceptibility due to gestational experience to crack cocaine have never been measured in animal models. Here, we tested the hypothesis that crack cocaine exposure during pregnancy affects cognitive function, mood, seizure susceptibility and patterns of Epilepticus Status (SE). Through a battery of behavioral tests and intrahippocampal pilocarpine (H-PILO) microinjection at subconvulsive and convulsive doses in a rat model, we demonstrate that exposure to crack cocaine during the embryonic stage leads to anxiogenic-like behavior and long-term memory impairment in both genders, and depressive-like behavior in female. Animals exposed to subconvulsive dose of PILO showed greater susceptibility to SE, increased seizure frequency and total seizure time, while animals with convulsive PILO had no alteration in SE severity. Take together, our data suggest that crack cocaine exposure during the gestational period lead to the involvement of offspring with the presence of comorbidities such as increased propensity to anxiety and depression, long-term memory deficit and reduction of the threshold of epileptic seizures, which may predispose crack cocaine-addicted patients to severe clinical outcomes.

Key-words: Crack Cocaine. Gestational. Anxiety. Depression. Epilepsy.

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µg	Microgram
µL	Microliter
5-HT	Serotonin
ACTH	Adrenocorticotropic hormone
AIR	Group exposed to air
AEME	Anhydroecgonine Methyl Ester
ANOVA	Analysis of Variance
AP	Antero-posterior
BZE	Benzoylecgonine
CEUA	Ethics Committee on the Use of Animals
CNS	Central Nervous System
CTR	Group exposed to air
CRK	Crack
dH2O	Distilled water
DA	Dopamine
DAT	Dopamine transporter
DG	Dentate gyrus
DV	Dorso-ventral
DZP	Diazepam
ECA	Entries Closed Arm
EPM	Elevated Plus Maze
EOA	Entries Open Arm
EXP	Group exposed to crack
FST	Forced Swim Test
GD	Gestacional day
GRO	Gromming
HPA	Hypothalamic-Hypophysis-Adrenal Shaft
H-PILO	Pilocarpina intrahipocampal
sH-PILO	Subvulsive intrahipocampal pilocarpine
cH-PILO	Convulsive intrahipocampal pilocarpine

IAT	Inhibitory Avoidannce Test
I.P	Intraperitoneal
ML	Medial–lateral
OFT	Open Field Test
OAT	Open Arms Time
P	Postnatal life day
PBS	Phosphate Buffered Saline
PFA	Paraformaldehyde
PFC	Prefrontal Cortex
PN	Prenatal period
PN-Crack	Prenatal period exposed to crack
PILO	Pilocarpine
PPE	Personal protective equipment
pSAP	Streched attend posture
REA	Rearing
SE	*Status Epilepticus*
uHD	Head-dipping no protected
VHE	Vehicle Solution (0,9% Sodium Chloride)
WDS	Wet Dog Shake

SUMÁRIO

1 INTRODUÇÃO

1.1 *Crack*: definição e epidemiologia

Crack, cocaína em sua forma fumada, é um subproduto da pasta base da cocaína processada com bicarbonato de sódio ou amônia e transformada em "pedra". O *crac*k é consumido por via pulmonar, produzindo rápida absorção, efeitos mais intensos e maior potencial de causar dependência quando comparado às vias intranasal e endovenosa (Fischman, 1988; National Institute on Drug Abuse, 2018). O *crack* recebeu esse nome devido ao som crepitante ouvido quando a pedra é aquecida e fumada (Haim et al., 1995). Em contraposição à cocaína, utilizada como medicamento por muito tempo, o crack foi produzido com a finalidade de entorpecer, sendo a forma mais potente em que a droga já apareceu. Devido ao seu alto potencial de adicção, baixo custo unitário e facilidade de manuseio, a utilização do *crack* se expandiu mundialmente, entre todas as idades, sexos e classes sociais (Kunjwal, 2017; Washton et al., 1986).

O uso do *crack* ascendeu na década de 80 na Colômbia e Peru, transformando outros países sul americanos, tais como, Brasil, Chile, Argentina e Uruguai em locais de trânsito para o seu tráfico ilícito (Pascale Antonio, Hynes Marya, Cumsille Franciasco, 2014). Atualmente, os maiores indicadores da América do Norte sugerem que o consumo de cocaína em suas diferentes formas continua em ascendência entre os anos de 2013 a 2016, e está relacionado ao aumento da morte de usuários de drogas com aproximadamente 10.000 mortes por ano (World Drug Report, 2018). A cocaína é a segunda droga mais apreendida em todo o mundo, estimando que 18,2 milhões de pessoas usaram cocaína em 2016, sendo 7,28 milhões usuárias de crack, permanecendo sua concentração na América do Norte e na América do Sul (World Drug Report, 2018).

Como descrito, a dependência do consumo de *crack* vem ao longo dos anos se tornando um grave problema de saúde pública. O *crack* é rapidamente absorvido pela circulação pulmonar, no sangue o princípio ativo começa a ser biotransformado devido ao pH do meio aquoso e segue para ser hidrolisado no fígado por colinesterases que produzem seus metabólitos principais, a benzoilecgonina (BZE) e a metil-éster de ecgnonina (AEME), sendo transmitido ao cérebro em menos de dez segundos (Chaudhary et al., 2009; Rennert, 1975). Acredita-se que sua propriedade psicotrópica aumenta a estimulação dopaminérgica em locais críticos de "recompensa" do cérebro (Cressman et al., 2012).

Dessa forma, o *crack* produz efeitos farmacológicos sistêmicos e psicotrópicos. Entre os efeitos do seu uso se encontram: bem-estar, euforia, aumento do estado de alerta e da concentração, diminuição do apetite e fadiga, hiperatividade motora, verbal e ideativa, aumento da libido e desejo de consumir a substância novamente (Lam et al., 2004; Washton et al., 1986). As respostas fisiológicas incluem dilatação das pupilas, vasoconstrição e aumento da pressão arterial, frequência cardíaca e respiratória, temperatura corporal e atividade motora. Em longo prazo, esses efeitos são substituídos por um consumo compulsivo e repetido em períodos curtos de tempo e em doses cada vez maiores, induzindo a fissura e consequentemente a dependência (Hatsukami and Fischman, 1996; Pierce and Vanderschuren, 2010).

Além disso, o uso do *crack* está associado ao aumento do risco de doenças infecciosas, desnutrição, complicações gastrointestinais e eventos cardiovasculares agudos (Glauser and Queen, 2007; Kuo et al., 2014). O consumo de *crack* também está aumentando entre as mulheres em idade reprodutiva gerando maiores problemas de saúde pública (Yamaguchi et al., 2008). A exposição ao *crack* em gestantes não apenas afeta a saúde materna, mas também a do feto. Estas crianças intoxicadas pelo uso do *crack* no período gestacional são reconhecidas na literatura como "*crack babies*" (Aghamohammadi and Zafari, 2016; D'Avila et al., 2016b; Duailibi et al., 2008).

1.2 *Crack* e gestação

Embora um a cada três usuários de drogas seja mulher, as mulheres continuam representando apenas uma em cada cinco pessoas em tratamento. Dificilmente os usuários procuram centros de apoio ou buscam tratamento para dependência, o que dificulta a obtenção de dados epidemiológicos com estimativas do uso de drogas entre homens e mulheres. Dados disponíveis no World Drug Report (2018) informam essa diferença de gênero em alguns países em desenvolvimento, como Argentina, Bolívia, Chile e Colômbia, que chegam a marcar aproximadamente 2% da população de usuárias apenas de cocaína em suas diferentes formas. No Brasil, a parcela de mulheres usuárias de *crack* representa 22% de um total de 370 mil usuários de *crack* (Bastos and Bertoni, 2014). O alto índice de uso de drogas por mulheres pode interferir de modo relevante na saúde sexual e reprodutiva, além de consequências adversas com relação à morbimortalidade materno-fetal e infantil.

O consumo de *crack* durante a gravidez vem sendo relacionado com maior risco de aborto espontâneo, morte fetal, retardo de crescimento intra-uterino (Chasnoff and Griffith,

1989; Svensson, 1978), parto prematuro, pré-eclâmpsia (Mbah et al., 2012), edema pulmonar (bronquiolite do uso pulmonar da droga), arritmias, convulsões e morte súbita (Fox, 1994; Martins-costa et al., 2013). Já é conhecido que o *crack* e seus metabólitos atravessam a barreira placentária e se acumulam nos tecidos fetais (mecônio, cordão umbilical), cerca de 3 a 5% da cocaína atinge a circulação fetal através do líquido amniótico em concentrações maiores que aquelas observadas no plasma materno e funcionam como uma reserva apresentando uma maior retenção placentária do que materna (Bell and Lau, 1995; Cavalli et al., 2006; De Giovanni and Marchetti, 2012; Pichini et al., 2005; Slutsker, 1992). Estudos com cocaína mostram que as concentrações plasmáticas da droga no cérebro fetal chegam a ser cerca de quatro vezes maiores que as concentrações plasmáticas máximas (Farrar and Kearns, 1989). Dessa forma, o desenvolvimento fetal é exposto potencialmente a altos níveis de cocaína que possivelmente continuam após o nascimento, alterando a proliferação, migração e agregação celular, maturação neuronal, sinaptogênese e os mecanismos de morte celular (Cowan, 1979; Kosofsky, 1991). Essas mudanças ocorrem durante toda a fase gestacional, constituindo uma organização e modelamento dos circuitos cerebrais contínuos, persistindo nos primeiros três a quatro anos após o nascimento, sendo a mielinização cortical perdurada por toda a vida e de forma desregulada após a exposição à droga (Goldman-Rakic, 1987; Nowakowski, 1987).

Ao nascer, o neonato pode apresentar comumente perda excessiva de peso, anormalidades no tônus e postura muscular, quadros febris, irritabilidade, sudorese e vômitos, que podem ser associados às alterações no conteúdo cerebral de dopamina e serotonina, caracterizando a síndrome de abstinência (Bell and Lau, 1995; Kessler and Pechansky, 2008; Legido et al., 1992). Além disso, pode ser desencadeada uma série de anormalidades neurológicas e alterações comportamentais significativas: nervosismo, irritabilidade, déficits cognitivos de longo prazo, como baixo desenvolvimento de linguagem e verbalização, raciocínio perceptivo lentificado; comprometimentos das regiões cerebrais envolvidas nas funções de memória declarativa, ansiedade e depressão, bem como convulsões em casos mais graves (Epstein and Volpe, 1992; Mardini et al., 2017; Morrow et al., 2006; Pastor et al., 2018; Slamberová, 2003).

1.3 Comorbidades associadas à exposição pré-natal de *crack*

Não existem dados satisfatórios em relação ao uso de *crack*/cocaína durante o período pré-natal que confirmem as mudanças no desenvolvimento infantil, ressaltando que as variáveis

sociais, ambientais e psicossociais das gestantes (poliuso de drogas, educação, estado nutricional materno e outros) desempenham papel decisivo na ocorrência do dano comportamental e físico (Ackerman et al., 2010; Messinger et al., 2004). Algumas características psicossociais da mãe, juntamente com a exposição à droga, parecem influenciar a gestação e o feto, (Behnke et al., 2002); além de não descartar completamente a possibilidade de que diferentes neuroadaptações venham a surgir após a exposição de *crack* durante todo o período gestacional. Vários estudos mostram que a falta de cuidado materno e de amamentação na prole podem acarretar o desenvolvimento alterado do eixo hipotálamo-hipófise-adrenal (HPA), levando ao surgimento de características como ansiedade, estresse, pânico e déficit na consolidação de memórias (Kaffman and Meaney, 2007; Szyf et al., 2005). Outros estudos incluem retardo mental, atraso no desenvolvimento, coordenação motora deficiente, dificuldades de aprendizagem, hiperatividade, déficits de atenção, podendo a prole se tornar deprimida, suicida e ansiosa (Mayes et al., 1997; Scherling, 1994; Van Dyke and Fox, 1990).

As monoaminas influenciam a proliferação celular e o crescimento neural, desempenhando um papel crítico no desenvolvimento do cérebro (Lauder, 1988) e na ontogenia dos sistemas de neurotransmissores, modificando-se na presença de drogas como cocaína/*crack*, sendo a fase organizacional um momento crítico para a modelagem final dos circuitos cerebrais (Mattson, 1988). Algumas alterações podem incluir redução de espessuras corticais, aumento na densidade de fibras catecolaminérgicas em áreas cerebrais selecionadas, incluindo lobo parietal, hipocampo e giro do cíngulo, além de uma afinidade aumentada dos receptores D2 de dopamina no estriado (Akbari and Azmitia, 1992; Feo and Neurologiche-i, 1995). A diminuição na quantidade de neurotransmissores liberados, como a serotonina (5-HT), pode ocasionar mudanças emocionais envolvidas na depressão e levar à neurogênese mal interpretada entre as áreas afetadas e não afetadas do cérebro com alterações nas conexões sinápticas (Mayes et al., 1997), o que possivelmente explica a suscetibilidade da gênese de manifestações epilépticas em diferentes idades (Feo and Neurologiche-i, 1995).

Desse modo, a exposição pré-natal ao *crack* pode afetar o indivíduo não somente durante a gestação ou infância, mas também na idade adulta, na qual é possível detectar alterações nas respostas neurocomportamentais e ainda observar diferentes alterações entre gêneros, estímulos externos e no funcionamento cerebral. Baseado nestas informações, nosso estudo investiga a relação causa-consequência da administração pré-natal de *crack* e o desenvolvimento de comorbidades relacionadas à ansiedade, depressão, memória em longo prazo, além da susceptibilidade e gravidade de crises convulsivas. Acredita-se que a compreensão destas alterações possibilitará um significativo avanço científico, que permitirá o

desenvolvimento de novas estratégias para o monitoramento, tratamento e intervenções que poderão aumentar a sobrevivência e a qualidade de vida das crianças e adultos expostos à fumaça do *crack* durante o período gestacional.

Artigo. *Gestational effects of crack cocaine use: behavioral and seizure susceptibility alteration in F1 generation*

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1 INTRODUCTION

Cocaine stimulates the central nervous system (CNS) and is on the rise worldwide, becoming a serious public health problem (Kunjwal, 2017). About 275 million people worldwide (5.6% of the global population) have used a psychotropic drug at least once a year, being 18.2 million cocaine users. In 2018, global cocaine production reached the highest level

ever recorded, totaling approximately 1410 tons (United Nations Office on Drugs and Crime, 2018). Crack, cocaine in its smoked form, became popular in the mid-1980s and continues as one of the most commonly used drugs in developing countries. Currently, crack addiction represents a major public health problem for providing violent acts, increased psychosocial and environmental risks, depression and anxiety (Moreira et al., 2015). Crack cocaine users are more likely to be dependent (62.8%) compared to intranasal cocaine use, since pulmonary absorption promotes greater bioavailability and a decrease in drug metabolism time (Fischman, 1988; Hatsukami and Fischman, 1996). Moreover, continuous use of crack produces intense and fast effects, which leads the individual to craving, fissure and and consequently dependence (Falck et al., 2008).

Another alarming point in the society is the increased number of women crack users followed by their marginalization, being considered "hypersexual deviants" (Boyd, 2004; D'Avila et al., 2016b). This case has been reported as an "epidemy" because of its correlation with HIV, psychiatric disorders, low educational level, lack of family structure, extreme poverty, violence and pregnancy (Buchanan et al., 2006; Bungay et al., 2010; Cunha et al., 2001; Galduróz et al., 2005; Hobden and Cunningham, 2006). Therefore, the number of children intoxicated by crack use during the gestational period, called crack babies, has been increased exponentially (Aghamohammadi and Zafari, 2016; D'Avila et al., 2016b; Duailibi et al., 2008). Cocaine, crack cocaine, and combustion metabolites cross the placental barrier and cause prolonged effects on the embryo or fetus (Bell and Lau, 1995; D'Avila et al., 2016a). Throughout the gestational period, crack cocaine acts in maternal and fetal CNS, making it difficult to reuptake dopamine, noradrenaline and serotonin through the presynaptic terminals, accentuating the effects on the effector organs (Bell and Lau, 1995; Kessler and Pechansky, 2008; Legido et al., 1992). In addition, gestational exposure may lead to morphological and neurochemical changes in the prefrontal cortex (PFC) and other cortical areas involved in cognition (Harvey et al., 2001; Levitt et al., 1997; Salas-Ramirez et al., 2010).

Prenatal exposure to crack cocaine (PN-Crack) can cause several problems at each stage of development: prenatal, postnatal, childhood, puberty and adults. In the fetus, adrenergic effects may lead to reduced blood placental flow, premature birth, placental associated syndromes (eg, placental abruption, preeclampsia and placental infarction) and impaired fetal growth (Freitas-Santos et al., 2018; Legido et al., 1992; Mbah et al., 2012). The newborns of women crack users may not respond adequately to environmental stimuli, presenting febrile, irritability, sweating, seizures and vomiting, which may be associated with changes in brain

content of dopamine and serotonin, characterizing withdrawal syndrome (Bell and Lau, 1995; Legido et al., 1992). Children exposed to PN-Crack may have long-term neuropsychomotor and cognitive deficits including: low language development, difficulty in verbalization and learning, slowed perceptual reasoning, adverse effects on memory, aggression, impairments of brain regions involved in declarative memory functions, anxiety and depression, besides presenting seizures (Bandstra et al., 2010; Cunha et al., 2001; Epstein and Volpe, 1992; Mardini et al., 2017; Morrow et al., 2006; Pastor et al., 2018; Slamberová, 2003). Finally, in adolescence and adulthood, little is known about the effects of NP-crack exposure, with only a few changes in decision-making skills (Lambert and Bauer, 2012) and an increase in susceptibility to epileptic seizures (Slamberová, 2003).

There is evidence of seizures that suggest the correlation between cocaine use and progressive sensitization in the genesis of epileptic seizures through a special mechanism called kindling (Slamberová, 2003). This cocaine-induced sensitization is produced when a constant dose of cocaine is intermittently and repeatedly administered over time (Macêdo et al., 2004; Meehan and Schechter, 1996). Besides epileptic seizures, cocaine use may lead to Status Epilepticus (SE), characterized as self-sustaining long-term seizure or short seizures without recovery between them (Dhuna et al., 1991; Gasior et al., 1999; Spivey and Euerle, 1990).

It is important to emphasize that the user, as well as the offspring exposed to crack, suffers not only the effects of cocaine present in the sample, but also the active compound anhydroecgonine methyl ester (AEME) due to its pyrolysis, which has been associated with a higher risk of neurotoxicity and greater power of addiction in users (Areal et al., 2015; Garcia et al., 2012; Gomes et al., 2018). In previous studies of our group we have already confirmed the existence of active principle (benzoylecgonine [BZE] and AEME) by mass spectrometry gas chromatography (Araújo et al., 2018). Longitudinal surveys are needed to better understand and explain the interactive role of neurobiological and environmental factors of these children's early behaviors as they enter adolescence and early adulthood. Here, we provide a new animal model of gestational crack cocaine exposure that can help in the understanding of these alterations, which will allow the development of new strategies for monitoring, treatment and interventions that may increase the survival and quality of life in crack-exposed children and mothers during the gestational period.

2 EXPERIMENTAL PROCEDURES

All the experimental procedures were conducted in strict accordance with the guidelines established by the Animal Research Report: *In Vivo* and were approved by the Ethics Committee of the Federal University of Alagoas (UFAL; CEUA: #54/2017). The animals were housed in the Laboratory of Neuropharmacology and Integrative Physiology (LNFI) of the Institute of Biological Sciences and Health (ICBS/UFAL). Crack samples were obtained by the Civil Police Department of the State of Alagoas.

2.1 Animals and mating protocol

Wistar rats (n = 30, females; n = 15, males; 3 months), from the Central Animal Laboratory UFAL (BIOCEN/UFAL), were housed in groups of five animals per cage under controlled temperature, submitted to the cycle clear-dark, with water and *ad libitium*.

Animals were mated in a ratio of 2: 1, using the Poiley method, which consists of the rigid crossing scheme with predetermined groups (Poiley, 1960), thereby for the maintaining the heterozygosity of the descendent population. Initially, males were distributed in individual cages. After 24 hours, males were removed and females placed in these same cages, without being cleaned, aiming to obtain the Whitten effect, caused by the action of the pheromones produced by the male rodents (Santos, 2002). Control of the beginning of the estrous cycle will be done, defining when ovulation will occur, usually described between the fourth and fifth day after the beginning of this period. Subsequently, males were housed in conviviality with the females, four days after the beginning of stimulation, being removed 24 hours later. To confirm that there was mating, female vaginal canal was washed with 20μL of 9% sodium chloride solution (VHE; NaCl 9%). The collected material was placed on glass slides for optical microscopy verification of the presence of spermatozoa (Salas-Ramirez et al., 2010). If positive, it was considered as day 0 of gestation (GD 0).

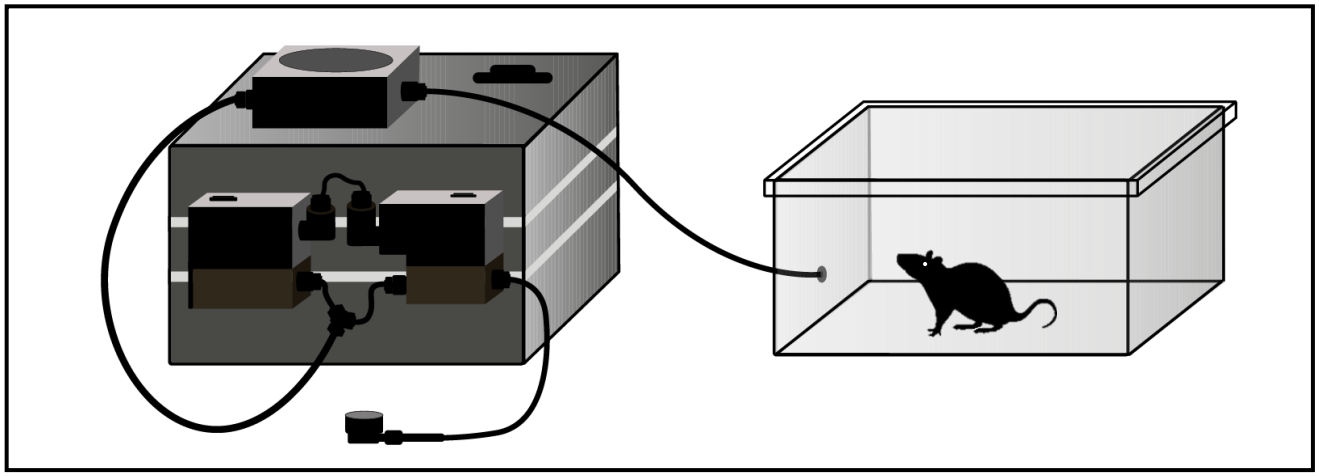
2.2 Prenatal exposure to crack cocaine (PN-Crack)

The pregnant rats were randomized into two treatment groups: control group (CTR; exposed to air, n=15) and exposed group (EXP; exposed to the products of crack pyrolysis during the whole gestation period, n=15). Both groups were exposed during the prenatal period (PN) from the 5^{th} (embryonic implantation) to the 21^{st} GD (Tung and Parr, 1987). For the exposure of the animals to the drug, a modified model of the system was used Ypsilantis et al.,

2012. Briefly, the apparatus consists of a smoke-generating pump coupled at one end to a tube (the place where the substance was burned) and the other to an acrylic chamber (animal housing location) with a fan configured for generation of 150 ml/min (Fig. 1).

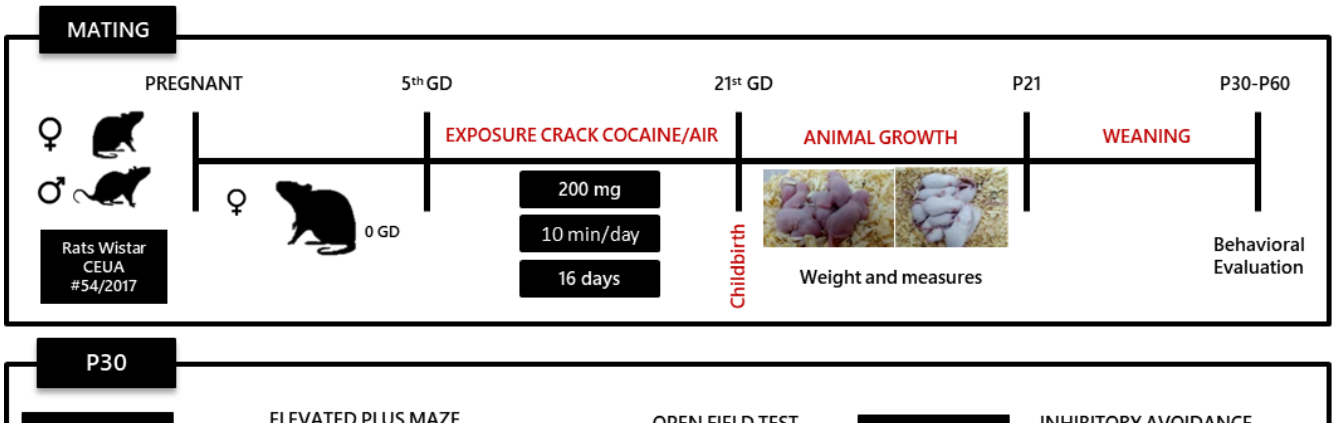
The pregnant rats were exposed to 200 mg of crack cocaine daily. The entire experiment was performed in a room suitable for exposure in a chapel with filter, exhaust fans and all necessary personal protective equipment (PPE). After the birth of offspring, considered as postnatal day (P) 0, all litters were adjusted to eight pups, so that each mother had the same number of pups (4 males and 4 females). Body weights of offspring were evaluated every two days, as well as cephalic perimeters, animal length and tail length. Animals were weaned in P21 and housed in groups (EXP, exposed; CTR, control) of five males and five females until reaching puberty (P30) and adulthood (P60) to be divided into the experimental protocols (Fig. 2).

Figure 1- Animal model for gestational crack cocaine exposure.



Source: Author

Figure 2- Experimental Design



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2.3 Behavioral tests

2.3.1 Elevated plus maze (EPM)

P30 animals (females n = 28, males n = 32) were submitted to elevated plus maze test (EPM), consisting of two open (50 x 10 cm) and two closed arms (50 x 10 x 40 cm), opposite in cross-shaped and elevated 60 cm above the floor level. Open arms were surrounded by lateral bars of 0.25 cm in height to avoid the fall of animal. The platform and side walls of the closed arms were made of clear acrylic and the gray acrylic floor. Animals were submitted to EPM, being placed individually in central platform of the apparatus with the head turned towards one of the closed arms and its behavior was evaluated for five minutes. The following behavioral parameters were recorded: percentage of residence time (% OAT) and number of entries open (% EOA) and percentage of closed arms (ECA). In addition, ethological parameters were also

evaluated: unprotected head-dipping (uHD), protected stretch-attend (pSAP) rearing (REA) and grooming (GRO). At the end of the evaluation time, EPM was cleaned with 10% alcohol. The entire procedure was filmed with a camera positioned approximately one meter above the center of the EPM.

2.3.2 Open Field Test (OFT)

The open field test (OFT) consists of a circular acrylic arena, with the floor divided into eight parts (60 cm x 50 cm) that allows to evaluate the spontaneous locomotor activity of the P30 animals (females n = 16; males n = 16) immediately after the EPM. Each animal remained in the test for five minutes and the number of squares crossed with the four legs was recorded, as well as the behavior of rearing under the hind legs (REA). A reduction in the number of crosses can be interpreted as impairment in spontaneous locomotor activity. On the other hand, an increase can be interpreted as an improvement in engine performance (Prut et al., 2003).

2.3.3 Inhibitory Avoidance Test (IAT)

In the inhibitory avoidance test (IAT), P30 animals (female n=14, male n=14) and P60 (female n=16, male n=12) were placed in an automatically operated box (40x25x25 cm) with a wall glass front, being the floor constituted by a steel grid coupled to an energy generating box. The test was divided: (1) learning session, animal was kept on the platform and received a shock (3.0s of 0.2 mA) after getting off with its four paws on the grid; (2) test session, 24 hours after training, animal was placed in the same apparatus, under the same environmental conditions, without the aversive stimulus.

2.3.4 Forced Swim Test (FST)

The forced swim test (FST) consists of one of the most used models in the literature for the screening of antidepressant substances (Porsolt, R. D., Le Pichon, M., & Jalfre, 1977). FST consists of two moments: training and testing (24 hours after training). P30 animals (female n=16; male n=16) were individually inserted into a cylinder (22x22x32cm) with water, previously heated and with a stabilized temperature around 25-26°C. After 24h of training, animals were again inserted in the cylinder with water and the parameters evaluated were:

latency for immobility and immobility in the last 4 minutes. Animals that remained immobile for the longest time were considered as depressive type behavior. At the end of the test, the animal was placed in a small box lined with paper towel and with the aid of a lamp, where it remained until the coat was completely dry.

2.4 Stereotactic surgery

P60 animals (males, n=48; females n=48) were anesthetized with ketamine (100 mg/kg), intraperitoneal) and xylazine (10 mg/kg), received 0.1 mL/100g veterinary pentabiotic (Fort Dodge®, subcutaneous) and had its head tricotomized before the surgery. After fixing on the stereotaxic apparatus, animals received local anesthetic (lidocaine with epinephrine, subcutaneous [Astra®]; 0,2 mL/100g). Subsequently, a cannula was implanted in the hilus of the dentate gyrus (DG) of the hippocampus, according to the following coordinates (Paxinos and Watson, 2007): -6.30 mm anterior–posterior (AP, reference: bregma); 4.50 mm medial–lateral (ML, reference: sagittal sinus); -4.50 mm dorsal–ventral (DV, reference: dura mater) (Castro et al., 2010; Furtado et al., 2011).

2.5 Intrahippocampal microinjections

Animals P60 were carefully immobilized to receive microinjections of 1 μL VEH (NaCl, 0.9%) and intrahippocampal pilocarpine [H-PILO; volume of 1 μL at the concentration of 0.6 mg/μL (sH-PILO) and 1.2 mg/μL (cH-PILO)], a muscarinic cholinergic agonist, to induce epileptic seizures and/or SE. Animals were subdivided into groups exposed in the prenatal period to air (AIR) or crack (CRK): AIR+VEH (N=8); AIR+sH-PILO (N=8); AIR+cH-PILO (N=8); CRK+VEH (N= 8); CRK+sH-PILO (N=8); CRK+cH-PILO (N=8). For microinjection, 5 μL syringe (Hamilton - Sigma) was coupled to a microinjection pump (Harvard Apparatus) at a speed of 0.5 μL/minute. After 90 minutes of microinjection, all animals received diazepam (5mg/kg; ip).

2.6 Behavioral analyzes

After epileptogenic insult, behavioral activity of animals was recorded by video camera (Digital Full HD camcorder Sony DCR-PJ6) for 90 minutes. During this period, animals were housed in acrylic cages, which allowed the simultaneous observation of six animals. Behavior-

based indices that indicate the severity of seizures were used according to the Racine (1972) scale (C0 – immobility; C1 – Facial Automatisms; C2 – Head and neck myoclonus; C3 – Forelimb myoclonus; C4 – Rearing; C5 – Rearing and falling), and the motor standard called wet dog shake (WDS), which is an indication of the severity of seizures, was analyzed.

2.7 Statistical analyzes

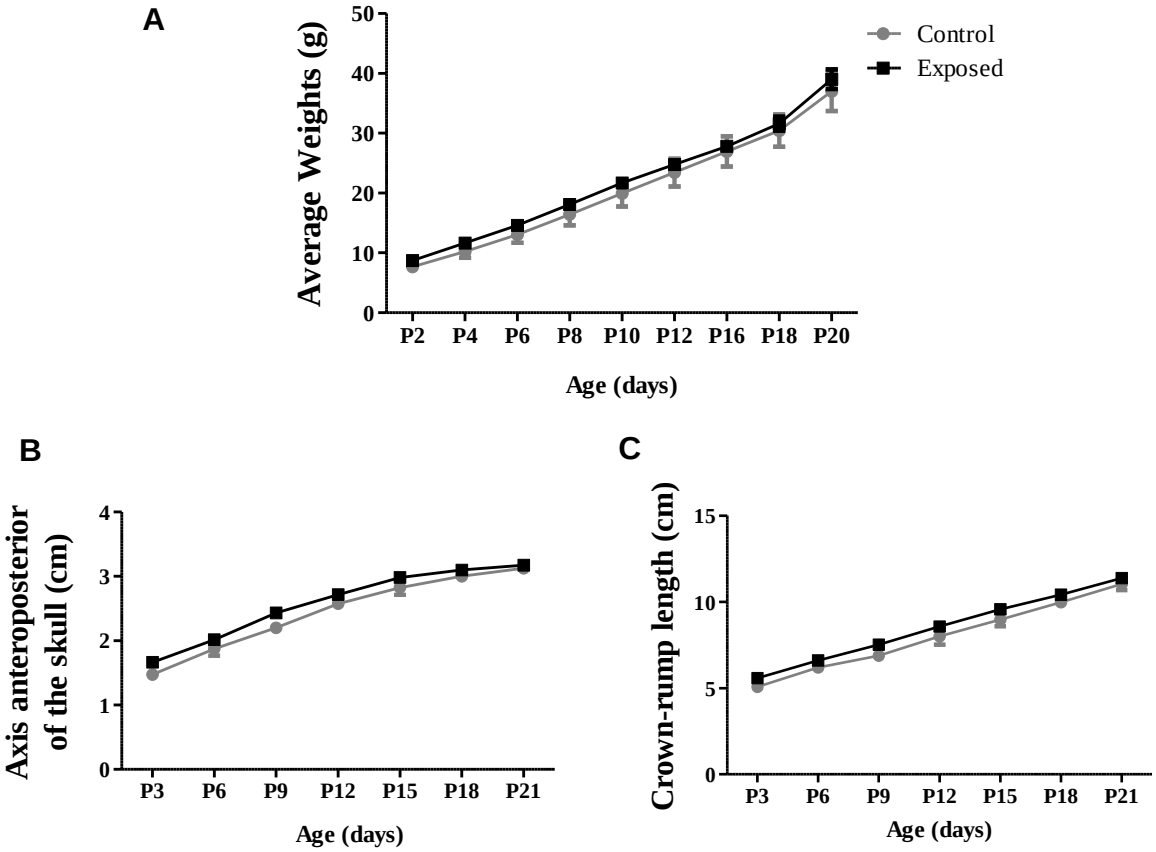
Statistical tests were conducted using GraphPad Prism (version 6.0). For comparison data were used Nonparametric test followed by Mann Whitney test and Two-Way ANOVA followed by Bonferroni posttest. Any difference with $p \leq 0.05$ was considered statistically significant.

3 RESULTS

3.1 Effect of PN-Crack exposure on the physical development of puppies

After PN-Crack, the weight of the animals of both groups remained unchanged throughout the development (p= 1.0000; Fig. 3A), as well as the circumference of the head (p = 0.9001; Fig. 3B) and body length (p= 0.9978; Fig. 3C).

Figure 3- Effect of PN-Crack exposure on the physical development of puppies



A) Total mean weight of animals during development until weaning (P21) weighed every two days. B) Total mean of the anteroposterior axis of the skull measured every three days until P21. C) Total mean of craniocaudal measured every three days up to P21. Results expressed as mean ± S.E.M. Compared to control (*Two-Way* ANOVA following by *Bonferroni posttest*).

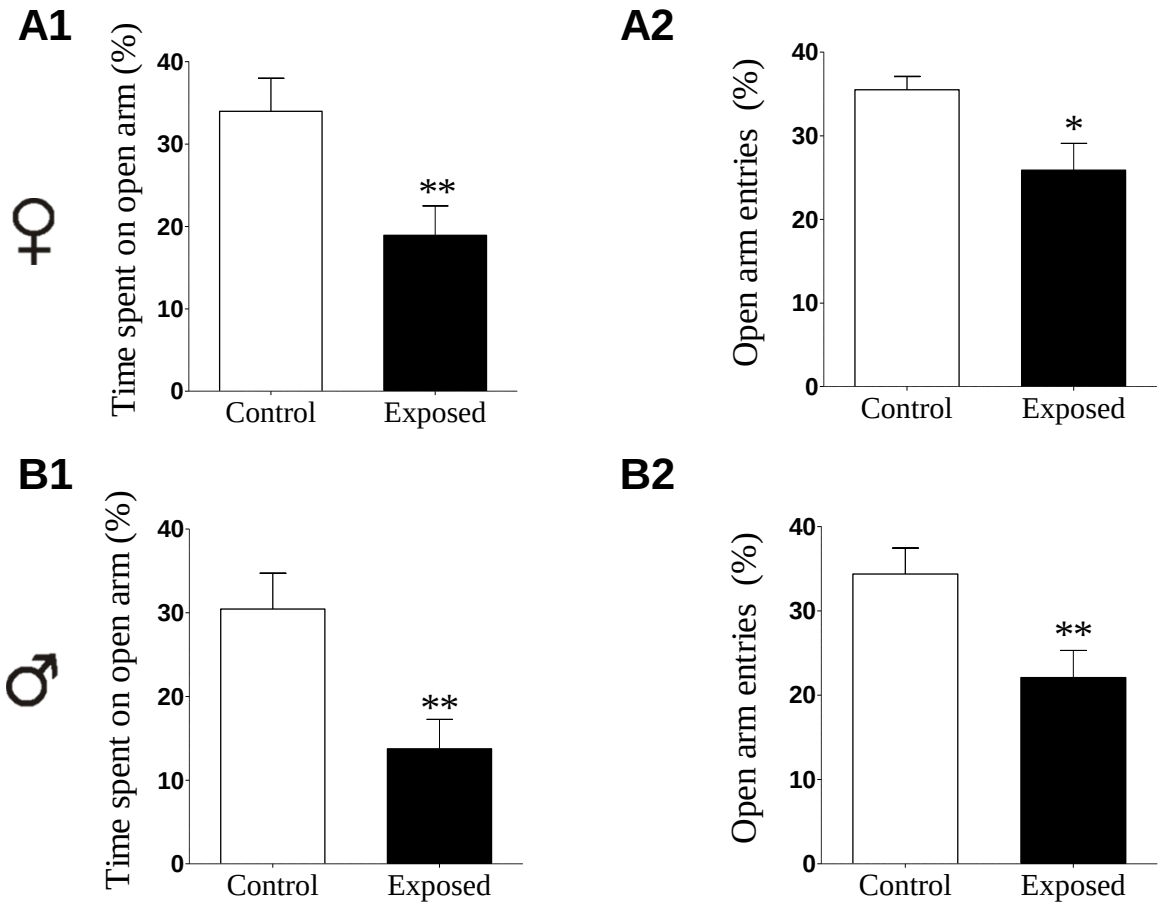
3.2 Effects of exposure to PN-Crack on anxiety and locomotor activity

Animals of EXP group (males and females) had a reduction of %OAT (females p=0.0031 and males p=0.0051; Fig.4, A1-B1) and %EOA (females p = 0.0146 and males p = 0.0098, Fig. 4, A2-B2) when compared to CTR, indicating an anxiogenic-like behavior in both sexes. Regarding the ethological parameters analyzed, only male EXP group presented a reduction in the number of uHD (p=0.015; Table 1). ECA number of EPM was not altered in both groups (p>0.05, Table 1), indicating that there was no change in the locomotor activity.

Corroborating these results, EXP group did not present any alteration in the locomotor activity when submitted to the OFT (Fig. 5, A1-B2).

Anxiety test	**EPM**					
Group/M or F	**N**	ECA	REA	uHD	pSAP	GRO
Control/Female	16	10.86±0.8824	9.375±0.9214	5.438±0.5843	4.250±1.074	2.375±0.3750
Exposed/Female	16	9.571±0.8498	9.500±0.7958	4.500±0.6258	3.813±1.314	1.938±0.4028
Control/Male	18	9.938±0.8035	6.500±1.130	4.167±0.5438	2.444±0.7058	2.389±0.4792
Exposed/Male	18	8.125±0.8107	5.444±0.9675	2.389±0.4364*****	3.111± .8200	1.667±0.3025

Figure 4- Effects of PN-crack exposure on EPM

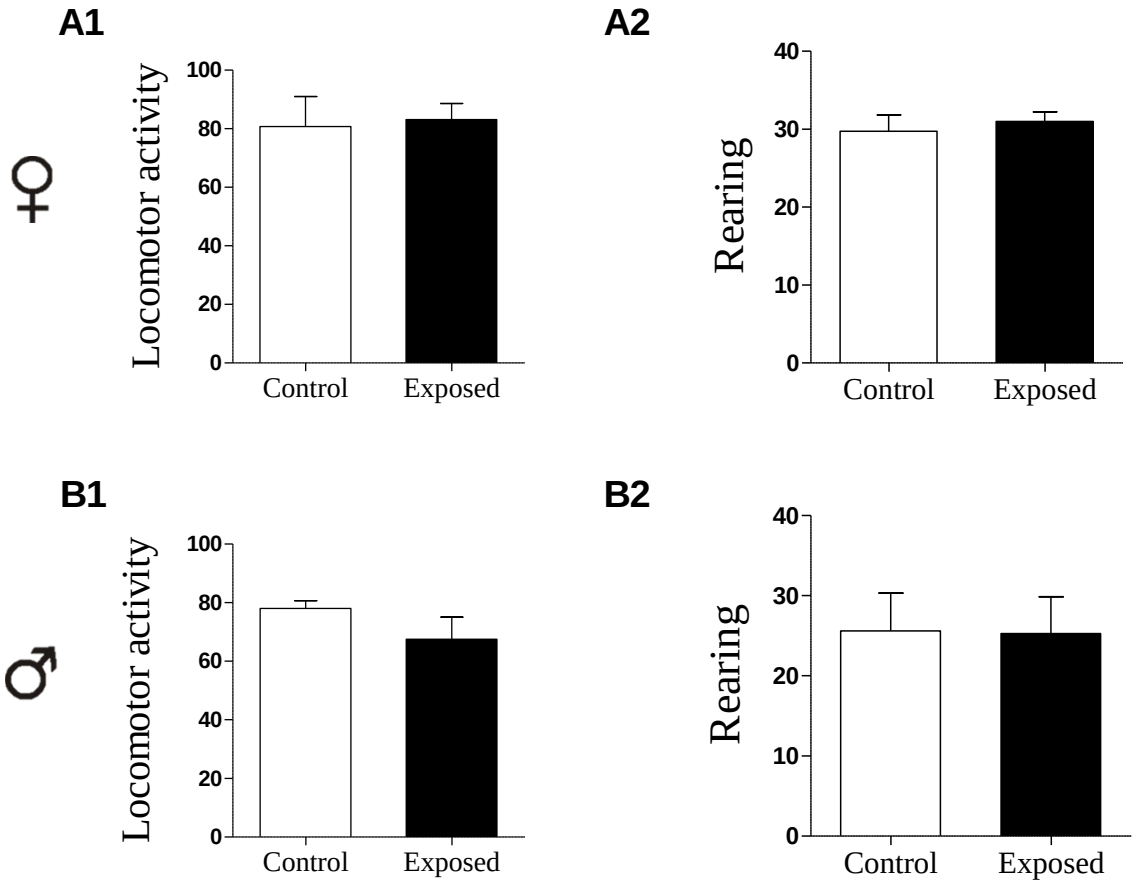


Percentage of time and entries in open arms (female rats, A1-A2; males, B1-B2). Results expressed as mean ± S.E.M. ** P < 0,05 compared to control (*Nonparametric test* following by *Mann Whitney test*).

Table 1- Effects of PN-crack exposure on the ethological parameters and number of entries in the closed arms of EPM

uHD= unprotected head-dipping, pSAP= protected stretch-attend posture, REA= rearings, GRO= groomings, ECA= entries closed arms. Results expressed as mean ± S.P.M. * p < 0,05 compared to control (*Nonparametric test* following by *Mann Whitney test*).

Figure 5- PN-crack exposure effects on the total number of OP crossings in OFT

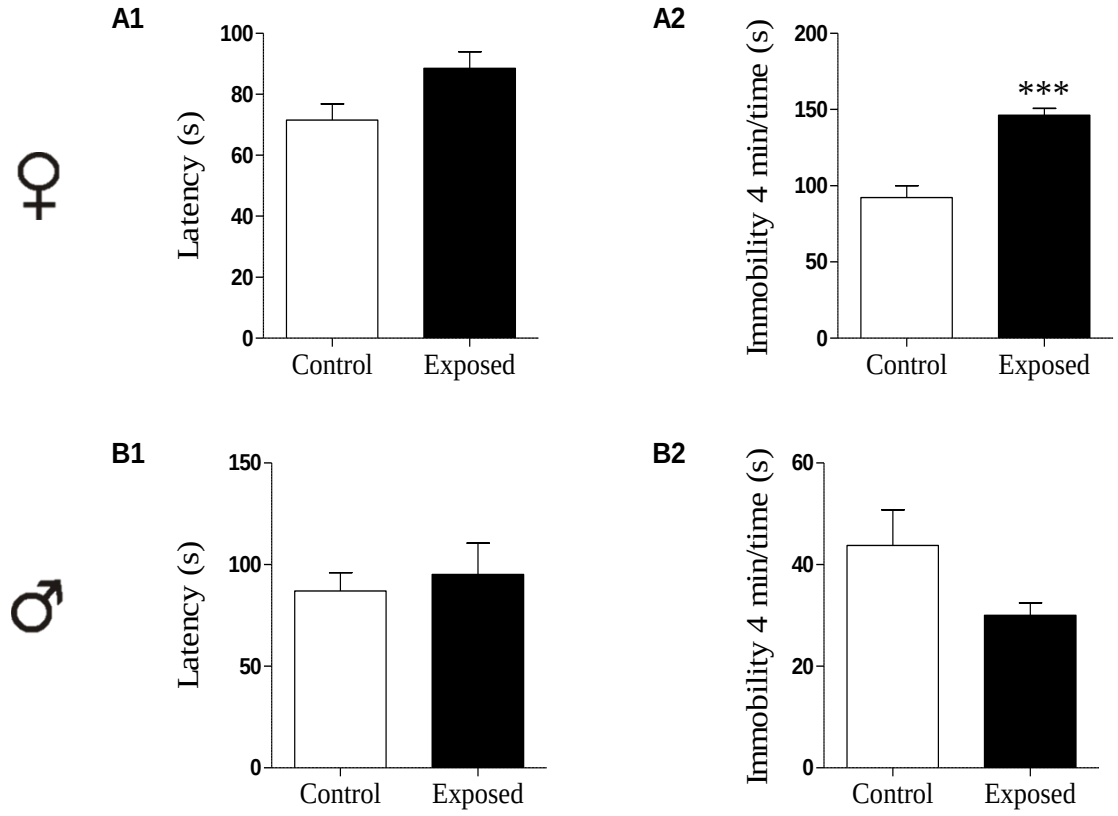


Total number of crosses (A1, females, B1, males) and number of rearings (A2, females; B2, males) in OFT. Results expressed as mean ± S.E.M. *Nonparametric test* following by *Mann Whitney test*.

3.3 Effects of PN-Crack exposure on depressive behavior

In FST, female EXP group had an increase in the immobility time in the last 4 minutes (p=0.0009 [Fig. 6 A2; B2]) compared to CTR group, indicating a depressive-like behavior.

Figure 6- Effects of PN-crack exposure on latency for immobility and immobility time in FST



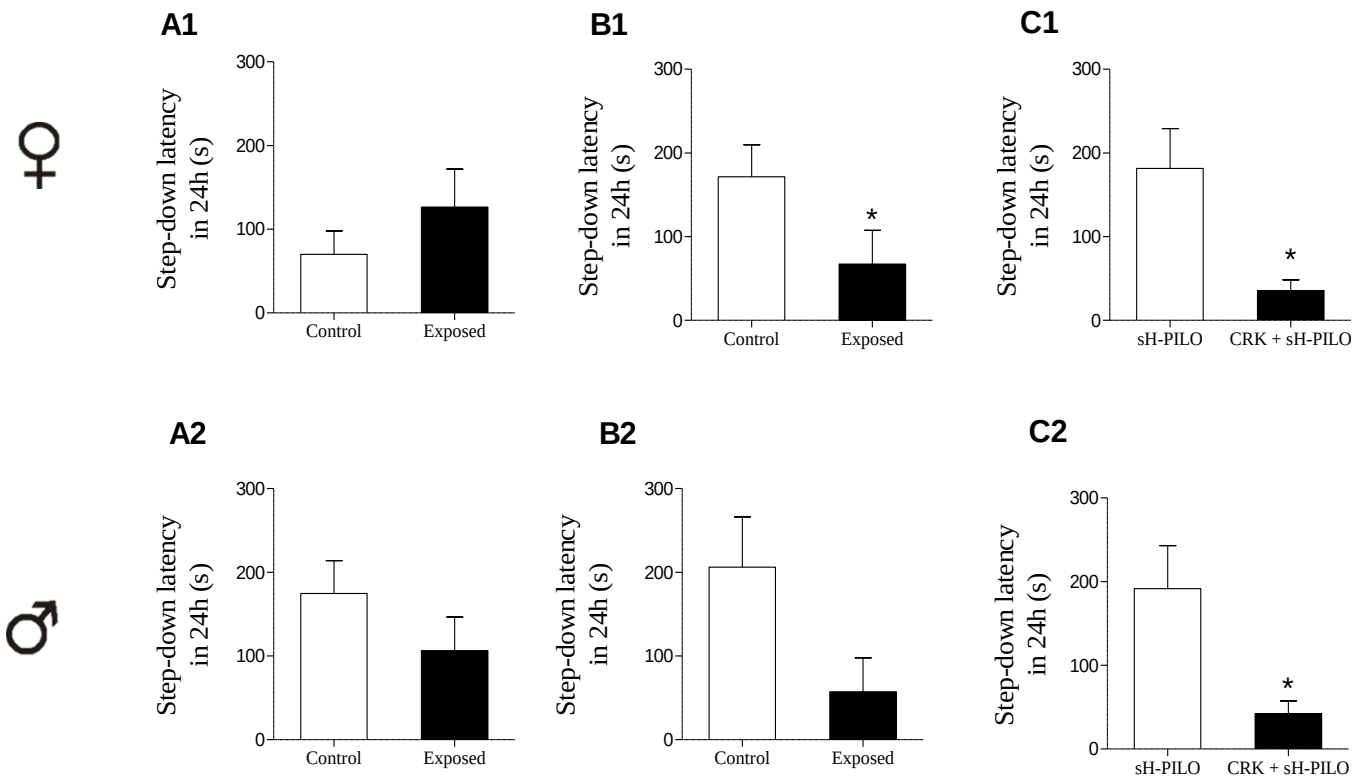
Latency for immobility (A1, females; B1, males) and immobility in the last 4 minutes (A2, females; B2, males). Results expressed as mean ± S.E.M. ***p<0,001compared to control (*Nonparametric test* following by *Mann Whitney test*).

3.4 Effects of exposure PN-Crack in long-term memory

After the learning session, all P30 animals had no impairment in memory consolidation (p> 0,05; Fig.7, A1; A2). However, only female EXP group (P60) had a lower latency time in the platform (p=0.034), indicating an impairment in memory consolidation (Fig.7, B1). Regarding male EXP animals (P60), no significant values were observed (p> 0,05; Fig. 7, B2). In addition, both genders exposed to PN-crack (P60) had impairment in memory consolidation after insult with low doses of sH-PILO (females, p=0.0391; males, p=0.0391; Fig.7, C1-C2).

Figure 7- Effects of PN-crack exposure on long-term memory consolidation in young animals

P30 **P60** **P60 + sH-PILO**



Platform latency time 24h after aversive stimulus in P30 (A1, females, A2 males) and P60 (B1, females, B2, males) animals. Results expressed as mean ± S.E.M. * P < 0,05 compared to control (*Nonparametric test* following by *Mann Whitney test*).

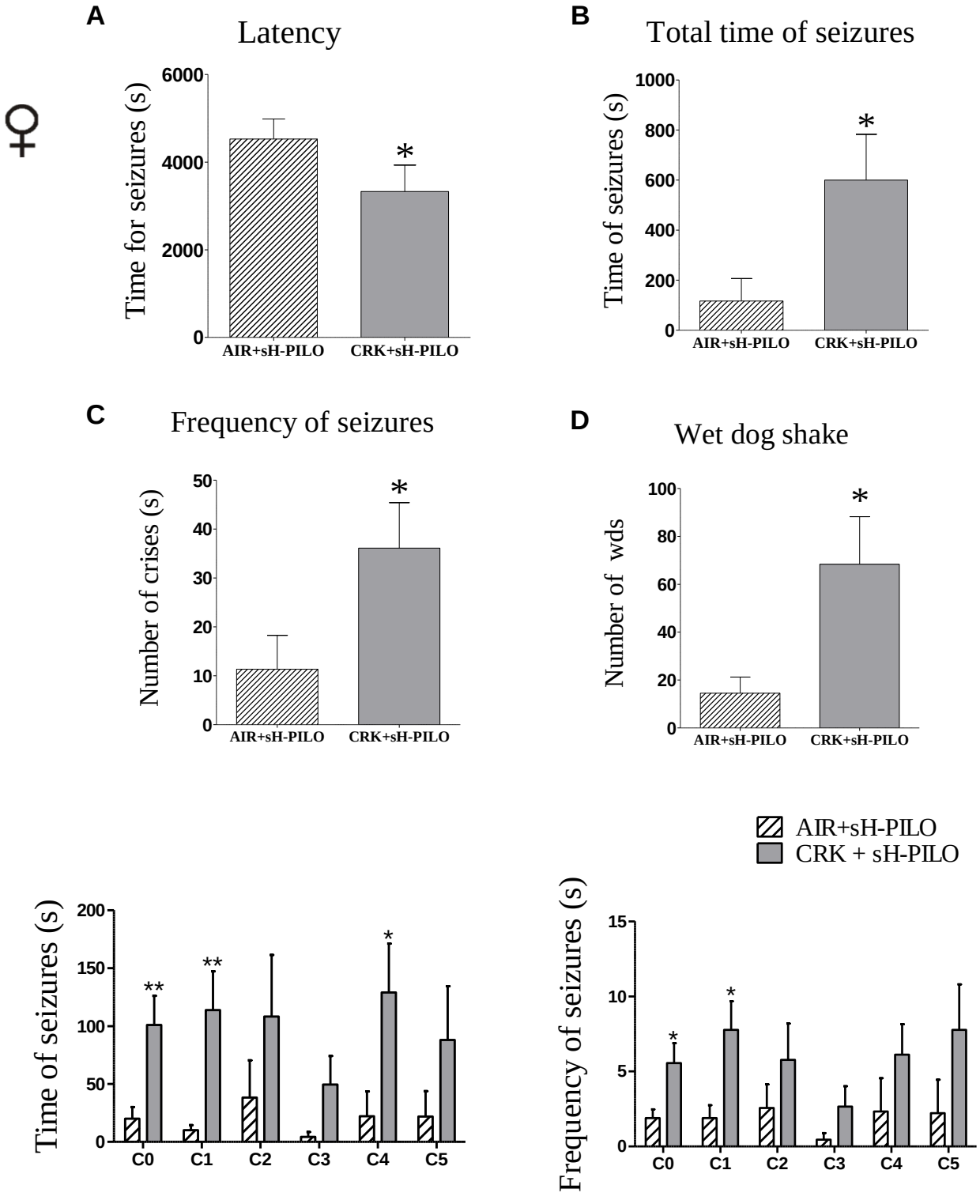
3.5 Effects of exposure to PN-Crack on susceptibility to epileptic seizures

EXP animals (P60) were induced with low doses of sH-PILO (0.6 mg/μL) for analysis of the threshold for epileptic seizures. It is known that low doses of pilocarpine are not able to induce animals to SE (Castro et al., 2011). Females CRK+sH-PILO had a reduction in latency for seizures (p=0.0465; Fig.8, A1). In the VEH+sH-PILO group, only two animals had isolated seizures, while six animals in the CRK+sH-PILO group evolved into SE. The time (p=0.0307, Fig.8, B1) and total frequency (p = 0.0485; Fig.8, C1) of seizures were higher in females CRK+sH-PILO. The number of WDS was higher in CRK+sH-PILO animals (p=.0212) when compared to VEH+sH-PILO, indicating an increase in the severity of seizures (Figure 8; D1).

The analysis of limbic seizures was performed in both groups, CRK+sH-PILO and VEH+sH-PILO, for 90 minutes after seizures induction (Fig. 8; E1; F1). During the first few minutes of SE, the frequency and time of classes 0 and 1 (Fig. 8; E1) increased in CRK+sH-PILO, as well as the time of class 4, already considered a severe class (Fig. 8; F1). However,

males CRK+sH-PILO had no significant difference in any of these behavioral parameters (supplementary data).

Figure 8- Effects of PN-crack susceptibility to epileptic seizures in female

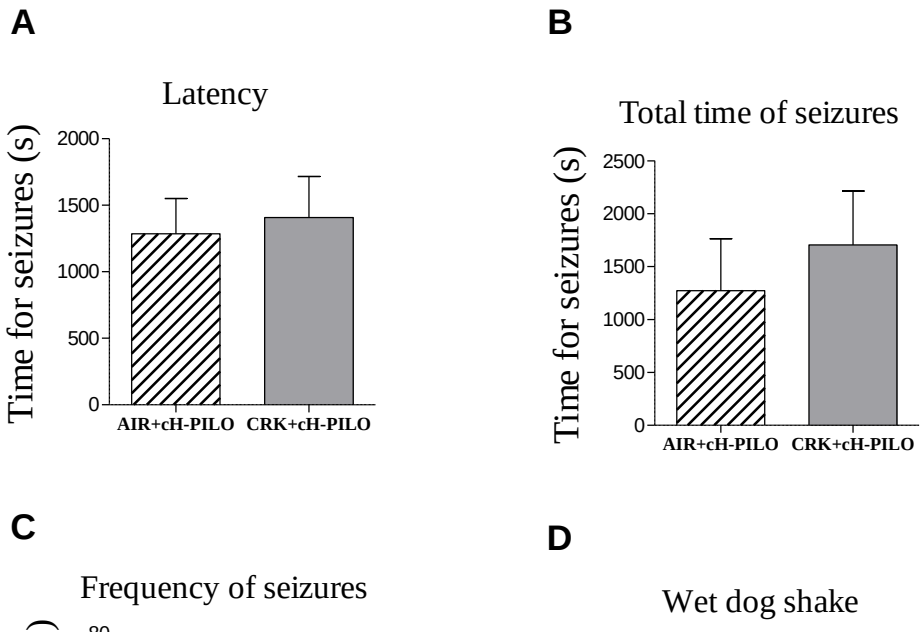


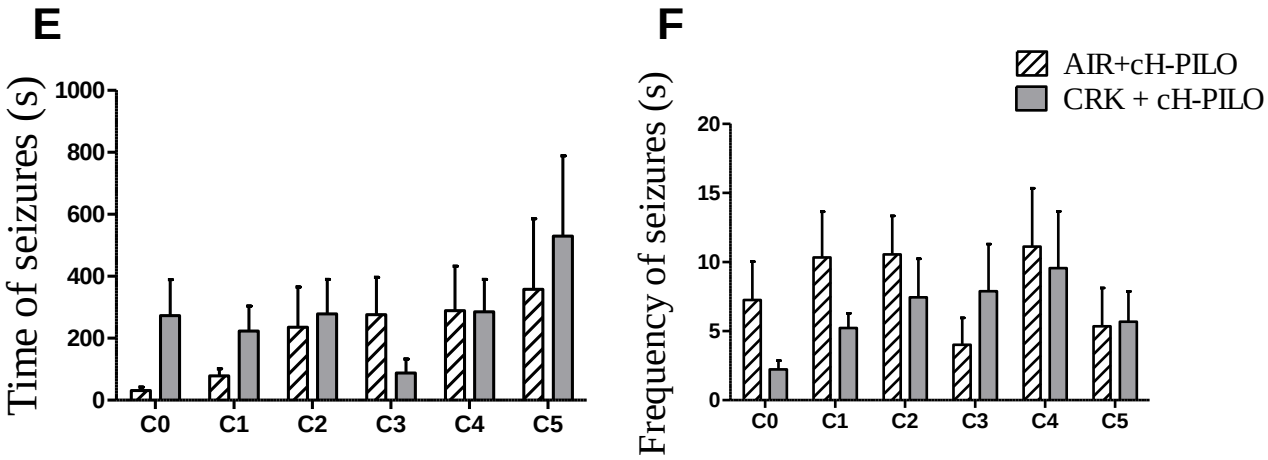
Latency time for onset of seizures (A); Total time of the attacks during the observed 90 min (B); Frequency of attacks during 90 min (C); Number of WDS (D), *Nonparametric test* following by *Mann Whitney test*; Total frequency in the classes of the Racine Scale (E) and total number of classes (F), *Two-Way* ANOVA followed by *Bonferroni posttest*. Results expressed as mean ± S.E.M. * P <0.05 compared to control.

3.6 Effects of PN-Crack exposure on the severity of epileptic seizures

Another group of animals (P60), exposed PN-Crack, was induced with convulsive doses of cH-PILO (1.2 mg/μL) to analyze the severity of epileptic seizures and SE. The latency time for the onset of epileptic seizures (Fig.9, A1, A2), as well as the total time and frequency of seizures (Fig.9, B1, C1) remained unchanged when compared to CRK+cH-PILO and VEH+cH-PILO groups. In addition, the amount of WDS remained similar between groups (Fig. 9, D1). During the 90 minutes of the SE, the PN-Crack exposure did not interfere in the severity of the seizures (Fig.9 E1; F1). As several animals of both groups that had spontaneous recurrent seizures (SRS), the IAT was not feasible.

Figure 9- Effects of PN-crack susceptibility to severity of epileptic seizures in female





Latency time for SE initiation (A); Total seizure time during the observed 90 min of SE (B); Frequency of seizures during SE (C); Number of WDS (D), Nonparametric test following by Mann Whitney test; Total frequency in the classes of the Racine Scale (E) and total number of classes (F). *Two-Way ANOVA* followed by *Bonferroni posttest*. Results expressed as mean ± S.E.M comparing cH-PILO and CRK + cH-PILO.

4 DISCUSSION

The present study demonstrated that PN-crack exposure in rats promoted alterations in offspring behavior. In neonates, the exposure did not alter body weight, brain measurements or length of animals (both gender). On the other hand, contributed to the development of anxiety-like behavior (both gender) and depressive-like behavior in females. In addition, the consolidation of long-term memory was not changed (P30), in contrast to the adult phase (P60) that had memory deficit and a reduction in the threshold for epileptic seizures. Additionally,

after induction of low-doses of pilocarpine, males and females of the EXP group (P60) presented memory impairment.

PN-crack exposure in humans is associated with low birth weight, premature birth, reduction in head circumference and short stature (Bauer et al., 2005; Freitas- Santos et al., 2018), which corroborates with the characteristics detected in cocaine-exposed children during the gestational period (Aghamohammadi and Zafari, 2016; Modernel Xavier et al., 2017). However, in our study, we observed that crack cocaine exposure in rats during embryonic period did not alter the time of gestation, body weight, body length and head circumference in females and males over first days of life (P0-P21). Some studies are controversial about the crack cocaine use in relation to premature birth, weight and body measurements, showing the implication of mothers' lack of nutrition during the gestational period due to drug use (Overstreet et al., 2000; Salas-Ramirez et al., 2010; Wood et al., 1994). In addition, the amount of substance used and the presence of congenital diseases may lead to the change in offspring (Bauer et al., 2005; Zickler, 1999). It is possible that in animal model with controlled variables during pregnancy, some of these changes may not occur. In this study, there was no multiuse drug, food remained *ad libitium* and the pregnant rats were free of congenital diseases.

Some behavioral impairments resulting from exposure to PN-crack were observed in this study. It is important to emphasize that behavioral changes in the offspring with the use of gestational crack cocaine in animal model have been few explored, indicating that our study one of the first to show this aspect. In the EPM, P30 animals reduced the %OAT and the %EOA, parameters that characterize an anxiogenic-like behavior in offspring, being observed in both gender. Similarly, prepubertal and adult animals (P90-P365) exposed to cocaine in the gestational period had the same behavioral pattern in EPM (Salas-Ramirez et al., 2010; Sithisarn et al., 2011). Age seems to influence behavior in EPM and be a relevant factor in the test. Patterns of drug-induced responses in the gestational period appear inappropriately during adolescence of the offspring, increasing the frequency of conditions such as fear and social isolation, and may lead the individual to aggressive behavior in adulthood (Johns et al., 1992; Johns and Noonan, 1995). It is known that brain maturation is highly sensitive in adolescence and may occur neurobiological changes induced by external stimuli (Paule, 2005; Realini et al., 2009; Zhu et al., 2016). Cocaine stress in the gestational period increases adrenocorticotrophic hormone (ACTH) and corticosterone levels in the mother, which affects the fetus by reducing HPA axis activity, as well as corticosterone in exposed offspring (Magnano et al., 1992; Moldow and Fischman, 1987; Pollard, 1984; Scafidi et al., 1996). Another association with behavioral changes in the offspring can be explained by the low interaction and maternal

neglect, as observed in animal and human models (Johns et al., 2005; Mayes et al., 1997). Previous reports have shown that lack of maternal care in offspring can lead to altered development of HPA axis (Kaffman and Meaney, 2007; Szyf et al., 2005). This may be associated with the inhibition of dopamine neural activity that may reduce the emotional and conditioned responses due to the blockade of dopaminergic receptors in amygdala during the presence of cocaine and alteration in development of CNS in offspring (Adinoff, 2010; Hearing et al., 2012).

In our study, exposure to PN-crack did not result in motor activity changes in offspring in the OFT (P30), complementing the EPM results (ECA), which confirms that the anxiety-like behavior did not occur due to altered locomotor activation. Similarly, in another study, P28 animals (females and males) did not show differences in the proportion of time spent on locomotor activity after gestational cocaine exposure, as well as P96-136 animals (Sithisarn et al., 2011). Although female rats are generally more active than males, prenatal treatment did not produce locomotor differences (Brunzell et al., 2002; Malanga et al., 2007; Salas-Ramirez et al., 2010).

Among the most prevalent mental disorders affecting crack users, depression stands out, affecting almost half of the users (47,8%), and being one of the most frequent psychiatric comorbidities (Paim Kessler et al., 2012). However, little is known about depression associated with PN-Crack exposure in offspring. Previous studies with humans have shown that cocaine-exposed children present higher risk characteristics of emotional and behavioral problems (Warner et al., 2006). In our study, female rats (P30) exposed to PN-Crack had increased immobility in the OFT, which can be considered as a sign of fear or maybe an inability to calm down when excited, characterizing a depressive-like behavior (Bilitzke and Church, 1992). Similarly, cocaine exposure in prenatal period increased immobility in P60-120 animals (males and females) tested by FST (Overstreet et al., 2000). One of the possible explanations for only females presenting a depressive-like behavior may be associated with changes in serotonergic function in prenatal period (Henderson and McMillen, 1993; JOHNS et al., 1998). In addition, the maturation of dopaminergic neurons in female rats prior to male is a factor to be considered, as well as the fact that females have more dopamine transporter (DAT) in the *striatum* (Beyer et al., 1991). These differences occur during the gestational period and continue until the postnatal period with females decreasing the number of 5-HT in P30, whereas males do not show this change until P60 (Dow-edwards, 2010; Johns et al., 2002). Thus, this set of gender differences that occur in the ontogeny of the nervous system and brain hormonal activation effects provides a range of factors in which crack cocaine and its metabolites can act.

Consequently, PN-Crack exposure in females may result in alterations of striatal cells and serotonergic function, mediating the behavioral consequences observed.

We also observed that there was no impairment in the long-term memory consolidation at P30, while in the adult phase (P60) females showed memory deficits and a reduction in threshold for epileptic seizures. In addition, after induction of low-dose pilocarpine, males and female exposure to PN-crack (P60) presented memory damage. Among the neurological complications that occur with crack/cocaine consumption are changes in the ability to retain, manipulate, and store new information (Cunha et al., 2004). It is known that memory in cocaine dependent users during early life interrupts many learning processes when adults (Frazer et al., 2018; Mendez et al., 2008; Tractenberg et al., 2015). When the gestational exposition, it is known that there is a relationship between exposure and memory and learning impairment, leading to greater attention problems or delayed cognitive development in children, especially when associated with cocaine and alcohol (Bandstra et al., 2001; Coles and Black, 2006; Heffelfinger et al., 1997). However, is unknown about the pubertal, adolescent or adult period, particularly in relation to the PN-Crack exposure.

Our results showed that P30 animals had no impairment in long-term memory consolidation in both gender, the same occurred for short-term memory (data not shown). Previous study has showed that cocaine-exposed male mice (P120) had no change in short-term memory (Lu et al., 2012), corroborating with our finding that males would be less susceptible to memory deficits. In contrast, we observed that adult female rats (P60) had long-term memory deficit after PN-Crack. This observation regarding gender differences may be associated with the large amount of estrogen in females. Estrogen promotes neurogenesis and synaptogenesis during development (Miranda et al., 1994), which may provide a communicative network of the drug to stimulate synaptic retention, leading to increased neuroplasticity in females. Theories differ regarding to neurogenesis, not sure whether its increase will benefit or impair the formation of memory. Therefore, exposure affects females for a longer period. Long-term memory impairment in animals exposed to cocaine or crack during pregnancy not concisely described in the literature. The findings described different results on spatial, visual and operational memory, varying between age and gender (Gomes et al., 2018; Mendez et al., 2008; Morrow et al., 2006; Salas-Ramirez et al., 2010). Thus, our hypothesis is that long-term memory in exposed PN-Crack offspring is maintained during the period of neuroplasticity (from childhood to adolescence), at least partially, and after remodeling of synapses in the prefrontal cortex and dopaminergic neurons are mediated in different ways in males and females, which

regulate memory in adulthood (Bhatt et al., 2009; Morrow et al., 2002; Parducz et al., 2006; Salas-Ramirez et al., 2010).

Another alarming finding is the incidence of neonatal seizures during cocaine and crack cocaine exposure that has already been discussed in the literature (Chasnoff and Griffith, 1989; Dusick et al., 1993; Slamberová, 2003), but few studies mention how exposure can lead to problems in the adult individual. Our findings show the relevance of observing long-term individuals who were exposed in gestational period, since despite the recovery of epileptogenicity as children, abnormal brain abnormalities during this period can lead to seizures under stress or insults throughout life. These symptoms may be silenced by transient interruptions in neurochemical balances, or because they are caused by structural changes in the brain for which neurochemical compensations occur at the same time (Snyder-Keller and Keller, 1998). Our results showed that female rats exposed to PN-Crack had a reduction in the convulsive threshold, becoming more susceptible to epileptic seizures. Similarly, in another study with maternal exposure to cocaine and PTZ-induced seizures, male and female rats (P60-90) were more susceptible to seizures, but severity of the seizures was greater only in exposed females (Snyder-Keller and Keller, 1998). Increased susceptibility in these animals exposed to crack/cocaine with a chemical insult suggests that there are changes in neurotransmitter systems in CNS that can be disturbed in animal brains during prenatal exposure. Seizures during cocaine use are already well evidenced, either after a single exposure or in a chronic way, because even in low doses can lead to an effect called kindling (Meehan and Schechter, 1996). Kindling may be characterized by repeated administrations using lower doses of the drug (Kaminski et al., 2011), producing lasting changes in sensitivity to seizures, which may occur during prenatal exposure. Other study compared the incidence of seizures using low doses of cocaine in kindling and high doses acutely, with animals (P30) exposed until the onset of seizures. The authors observed that prenatal exposure to cocaine differentially affected genders, being males more susceptible to cocaine-induced seizures in kindling, while females to acute cocaine, which indicates that exposure differentially affects the developing brain depending on gender (Snyder-Keller and Keller, 1995). Based on these observations, neuroplasticity in childhood and adolescence contributes to normal neuronal excitability in adult brain, but brain becomes more susceptible to modifications and rearrangements in neurotransmitter system in presence of insults, making it capable of generating spontaneous epileptic seizures.

Taken together, our data present a new animal model for gestational crack cocaine exposure, supporting the importance of understanding changes and consequences of offspring exposure to PN-Crack, and the need to study different ages and genders. For the first time the

long-term implications of crack use are being shown during pregnancy and how the consequences can lead to serious health problems innately in subsequent generation. Despite our findings, new studies are needed to elucidate the neurochemical mechanisms that justify the repercussions of PN-crack exposure, generating new perspectives to treatment and clinical interventions that may increase the children's survival and quality of life of individuals exposed to crack.

5 CONTRIBUTORS

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6 CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

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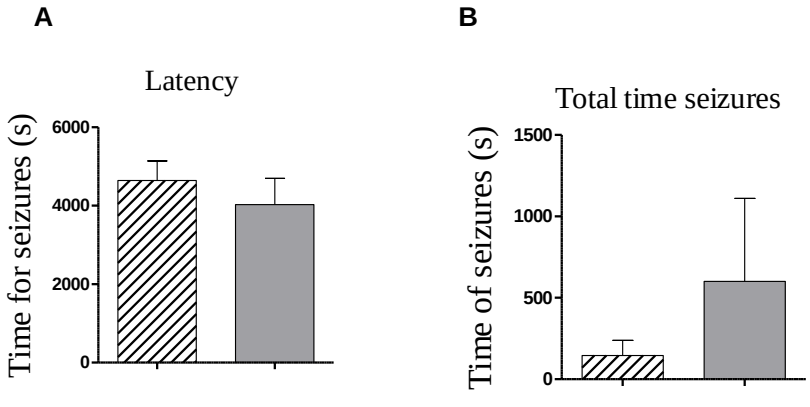
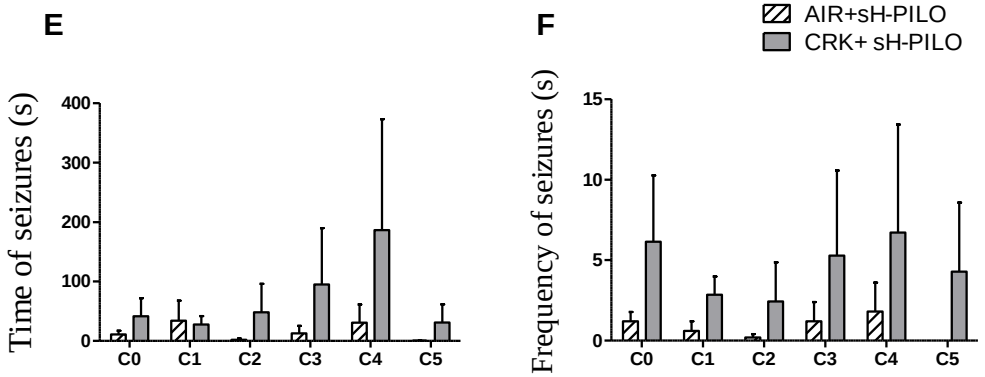
SUPPLEMENTARY DATA

RESULTS

3.5 Effects of exposure to PN-Crack on susceptibility to epileptic seizures and long-term memory.

Figure 10- Effects of PN-crack susceptibility to epileptic seizures in male

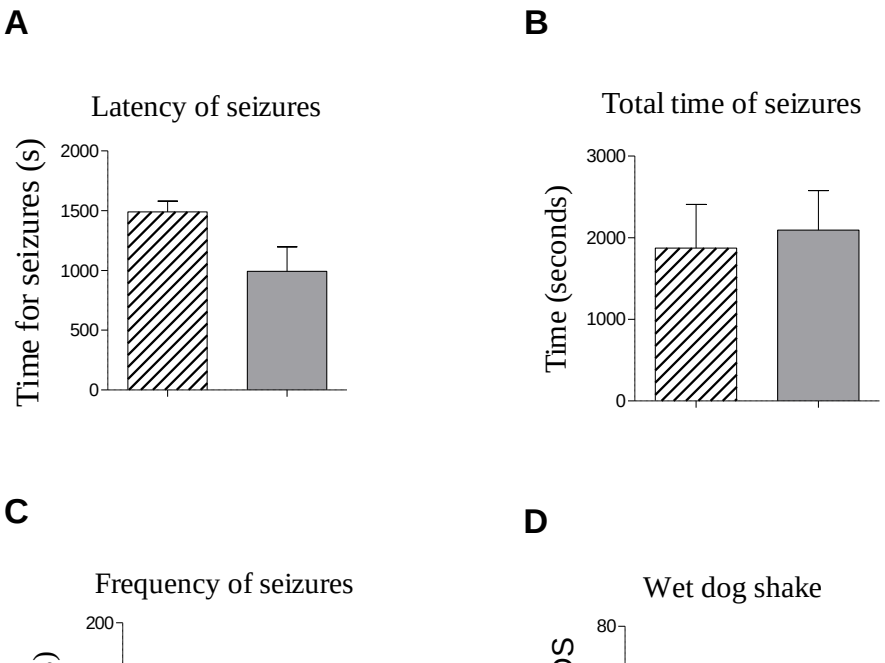


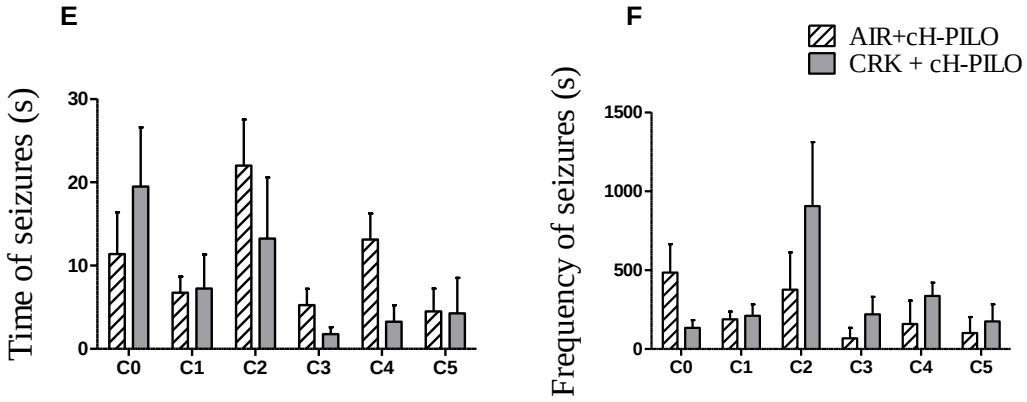


Latency time for onset of seizures (A); Total time of the attacks during the observed 90 min (B); Frequency of attacks during 90 min (C); Number of WDS (D), *Nonparametric test* following by *Mann Whitney test*; Total frequency in the classes of the Racine Scale (E) and total number of classes (F), *Two-Way* ANOVA followed by *Bonferroni posttest*. Results expressed as mean ± S.E.M. Comparing cH-PILO and CRK + cH-PILO.

3.6 Effects of PN-Crack exposure on the severity of epileptic seizures

Figure 11- Effects of PN-crack susceptibility to severity of epileptic seizures in male





Latency time for SE initiation (A); Total seizure time during the observed 90 min of SE (B); Frequency of seizures during SE (C); Number of WDS (D), *Nonparametric test* following by *Mann Whitney test*; Total frequency in the classes of the Racine Scale (E) and total number of classes (F), *Two-Way* ANOVA followed by *Bonferroni posttest*. Results expressed as mean ± S.E.M. Comparing cH-PILO and CRK + cH-PILO.