Programa de Pós Graduação em Ciências da Saúde

AUTOADMINISTRAÇÃO ORAL DE COCAINA EM RATOS MACHOS E FÊMEAS COM TRANSTORNO DE DÉFICIT DE ATENÇÃO E HIPERATIVIDADE (TDAH) INDUZIDO POR 6-HIDROXIDOPAMINA

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Dissertação de Mestrado
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SUMÁRIO

RESUMO

O Transtorno de déficit de atenção e hiperatividade (TDAH) é um dos transtornos psiquiátricos mais comuns da infância, que pode persistir na fase adulta. Em adultos, a comorbidade mais comum do TDAH é o transtorno por Uso de Substâncias (SUD), sendo a cocaína uma das principais. Nestes indivíduos, o uso e a dependência são mais precoces. Sabe-se que mulheres são mais sensíveis aos efeitos da droga, enfrentando um padrão mais grave de dependência .Nosso objetivo foi avaliar o consumo oral de cocaína em ratos machos e fêmeas em caixas de condicionamento operante em um modelo de indução ao TDAH com 6 hidroxidopamina (6-OHDA). Ratos Wistar foram divididos em grupos de machos e fêmeas, alocados aleatoriamente nos 4 dias pós-natal (PND4) foram induzidos por injeção intracistenal da neurotoxina 6-OHDA e *sham* receberam o veículo (0,1% ácido ascórbico). Aos 30 dias de idade os ratos foram colocados diariamente em caixas de condicionamento operante para a auto-administração oral de cocaína de razão fixa 1 (FR1) durante 28 dias. Nos resultados, observa-se que as fêmeas têm um comportamento maior de busca pela droga do que os machos, corroborando com a literatura. Tendo em consideração que os animais lesionados tiveram menor número de reforços de cocaína na autoadministração por via oral a possível explicação é baseada na lesão dopaminérgico induzido por 6-OHDA.

Mais estudos são necessários para entender melhor os resultados comportamentais da cocaína na autoadministração, em ratos lesionados pela 6-OHDA.

Palavras-chave: Autoadministração, TDAH, cocaína, fêmea, macho.

ABSTRACT

The attention deficit hyperactivity disorder (ADHD) is one of the most common psychiatric disorders of childhood and it can persist into adulthood. In adults, the most common comorbidity of ADHD is Substance Use Disorder (SUD). Cocaine abuse is a main problem associated to adulthood ADHD. In ADHD patients, cocaine use and dependence are more precocious. It is known that females are more sensitive to the effects of cocaine, facing a more serious pattern of dependence. Our objective was to evaluate the oral consumption of cocaine in male and female rats in a model to ADHD with 6-hydroxydopamine (6-OHDA). *Wistar* rats were divided into groups of males and females rats, were allocated randomly at postnatal day 4 (PND4) to intrathecal injection of the neurotoxin 6-hydroxydopamine (6-OHDA) or *sham* vehicle (0.1% ascorbic acid) procedure. When 30 days old the rats were daily placed in boxes of operant conditioning for oral self-administration of cocaine reinforcement (FR1) for 28 days. It is observed that females show more drug search behavior than males, corroborating the literature. Taking into consideration that the lesioned animals performed less in the acquisition of oral cocaine self-administration the possible explanation relies on the dopaminergic lesion induced by 6-OHDA.

More studies are needed to understand better the behavioral outcomes of cocaine self-administration in 6-OHDA lesioned rats.

Key words: Self-administration, ADHD, cocaine, female, male.

1.1 *Transtorno de Déficit de Atenção e Hiperatividade (TDAH)*

1.1.1 Definição e Sintomatologia

O Transtorno do Déficit de Atenção e Hiperatividade (TDAH) é considerado um dos transtornos psiquiátricos mais comuns na infância, podendo persistir na idade adulta com consequentes implicações no âmbito familiar, acadêmico e social (BARKLEY, 2002).

O TDAH é caracterizado principalmente por sintomas de hiperatividade, impulsividade e desatenção, podendo se manifestar na maioria dos casos antes dos sete anos de idade (ROHDE & HALPERN, 2004). Segundo descrito no Manual de Diagnóstico e Estatística de Doenças mentais (DSM-IV-TR,APA, 2000) o TDAH se subdivide em três tipos: TDAH com predomínio de sintomas de desatenção, sendo prevalente em meninas, demonstrando déficit de atenção mais grave, distração. Com isso refletindo em prejuízos acadêmicos e causando problemas profissionais na idade adulta (ROHDE, 2004) ;TDAH com predomínio de sintomas de hiperatividade/impulsividade,prevalente em meninos (TAYLOR,1998) apresentam comportamento mais agressivo e impulsivo, podendo gerar problemas no âmbito social e desvios de conduta, e principalmente a propensão ao abuso de drogas (BARKLEY, 2003); TDAH combinado, os sintomas combinados do transtorno causam um dano

elevado na maioria dos casos, gerando sérios problemas principalmente na vida social e acadêmica (ROHDE,2004) .

O diagnóstico do TDAH deve obedecer aos critérios estabelecidos pelo DSM-IV-TR, onde o indivíduo apresenta pelo menos seis dos sintomas de desatenção e/ou seis sintomas de hiperatividade que são avaliados pelo especialista, demonstrando prejuízos em pelo menos dois ambientes diferentes (familiar e escolar), por um período mínimo de seis meses.

1.1.2 Epidemiologia

De acordo com alguns estudos, o TDAH é uma das psicopatologias mais comuns e mostram uma prevalência de 3 a 12% do transtorno em criança em idade escolar (APA, 2000; FARAONE, SERGEANT, GILBERG, & BIEDERMAN, 2003). Podendo persistir na fase adulta em 30 a 50% dos casos (BÁLINT *et* al., 2008).

Estudos demonstram que o diagnóstico do TDAH é mais frequente em meninos que meninas na infância, numa proporção de 2:1em estudos populacionais e até 9:1 em estudos clínicos(GAUB e CARLSON ,1997; BIEDERMAN e FARAONE, 2005) . Essa diferença parece estar relacionada com o fato de meninas apresentarem menos sintomas de conduta, demonstrando menos problemas no ambiente familiar e escolar, com isso não sendo corretamente diagnosticadas (GAUB e CARLSON ,1997; BIEDERMAN e FARAONE, 2005 ; SADOCK e SADOCK, 2007).

1.1.3 Neurobiologia

De acordo com alguns estudos, as alterações envolvidas no surgimento dos sintomas do TDAH, parecem estar relacionadas não somente com um único sistema de neurotransmissores, diferentes teorias relacionam o envolvimento de outros sistemas na patofisiologia do TDAH, tendo por base as projeções neuronais do córtex pré frontal (CPF) (POSTNER & PETERSON, 1990;). Segundo esses autores, parece estar relacionado com alterações dos Sistemas Dopaminérgico, GABAérgico e Glutamatérgico (PLISZKA, 1992,2000). As teorias bioquímicas se referem principalmente no envolvimento do sistema dopaminérgico (BIEDERMAN *et* al., 2002; CASTELLANOS, 1997; VOLKOW *et* al.;1998) . Segundo essa teoria, os neurônios do CPF enviam projeções excitatórias glutamatérgicas para neurônios do estriado e NAc. Essas estruturas enviam projeções inibitórias GABAérgicas para neurônios ativos da substância nigra, que inibem os núcleos talâmicos. O tálamo, por sua vez, completa o circuito enviando projeções excitatórias glutamatérgicas para neurônios corticais. O circuito pré-frontal parece estar envolvido em funções planejamento de comportamentos futuros, memória de curto prazo e direcionamento de atenção (POSTNER & PETERSON, 1990).

Mesmo com as hipóteses dopaminérgicas e glutamatérgicas tendo prevalecido inicialmente, a contribuição de mecanismos noradrenérgicos na patofisiologia do TDAH parece ser relevante. Segundo Faraone e Biederman, 1998, demonstrou que os circuitos frontosubcorticais possivelmente envolvidos no TDAH, apresentaram uma quantidade significativa de dopamina e noradrenalina. Algumas regiões cerebrais primariamente moduladas por conexões noradrenérgicas, como o lócus

ceruleus, parecem estar envolvidas em processos de atenção seletiva (AZEREDO,2007).Além disso, a eficácia clinica dos psicoestimulantes, dentre eles o metilfenidato, provavelmente depende de alterações em funções dopaminérgicas e noradrenérgicas (AZEREDO, 2007). Pliszka e cols. (2000) teorizaram o envolvimento de circuitos noradrenérgicos no TDAH, através da verificação que os sintomas de TDAH teriam origem em um excesso de noradrenalina no *locus ceruleus,* que alteraria funções cognitivas e relacionadas com a atenção. Uma hiperfuncionalidade do sistema noradrenérgico nessa região também seria responsável por uma diminuição da inibição comportamental, através de inputs noradrenérgicos aumentados na região cortical. Dessa forma, essas disfunções noradrenérgicas interfeririam ainda com a transmissão dopaminérgica, GABAérgica e glutamatérgica em outras áreas cerebrais. Mesmo que não definitivos, esses dados são coerentes e consistentes com a ideia de que alterações dopaminérgicas, GABAérgicas, glutamatérgicas e noradrenérgicas têm um papel fundamental no TDAH. Até o momento , não existem estudos integrando todos esses achados em uma única teoria, podendo explicar a neurobiologia do TDAH de forma mais completa (AZEREDO, 2007).

1.1.4 Comorbidades

Evidências clínicas apontam, que cerca de 70% dos indivíduos com TDAH, apresentam algum outro transtorno psiquiátrico , sendo em sua maioria transtornos de humor (depressão maior, transtorno bipolar), transtornos de ansiedade e transtornos de conduta. (BIEDERMAN, 1998 ;ROHDE *et al*, 2004).

Os transtornos de ansiedade são mais prevalentes na infância e na adolescência com taxas de 6 a 18%, onde podemos observar agitação, irritabilidade e problemas afetivos (BERNSTEIN,1997).

Uma das comorbidades associadas ao TDAH é o uso de substâncias (SUD – *Substance use disorder*). Evidências clínicas apontam um risco aumentado do uso inicial de substâncias psicoativas em pacientes diagnosticados com TDAH (BIEDERMAN, 1999). Esse risco aumenta quando o TDAH esta associado a outro transtorno bipolar ou de conduta, havendo uma predisposição ao abuso de substâncias (GUARDIOLA, 1990).

Os pacientes diagnosticados com TDAH tem um inicio mais precoce ao abuso de drogas, chegando a idade adulta com padrões mais graves de dependência a cocaína (WILENS, 1997). Com isso tendo um aumento no envolvimento a acidentes de veículos e problemas familiares (WILENS; BIERDERMAN;MICK, 1998).

De acordo com a literatura, cerca de 35% dos adultos em tratamento de dependência à cocaína apresentam TDAH (WILENS et al.;2003). Os adolescentes com TDAH em tratamento de dependência química apresentam menor tempo de adesão ao tratamento e maiores taxas de recaídas quando comparados a usuários sem TDHA (BIEDERMAN e cols.; 1998; WILENS e cols.;1998).). Em um estudo de caso-

controle com adolescentes internados por abuso de álcool , observou-se uma prevalência de 25% do TDAH (ERCAN *et* al., 2003).

Estudos mostram que o tratamento do TDAH, diminuem os riscos desses pacientes tornarem-se usuários de drogas (BIERDERMAN, 1999, WILENS, 2003). De acordo com Biederman et al (1999), o tratamento farmacológico do TDAH esteve associado a uma redução em 85% no risco de uso problemático do uso de substâncias psicoativas em comparação a pacientes com TDAH sem tratamento. Os medicamentos no tratamento do TDAH incluem estimulantes (metilfenidato), agentes noradrenérgicos (atomoxetina), bupropiona, indutores de vigília (modafinil) (WILENS, 2006). No Brasil o estimulante disponível para o tratamento do TDAH, é o metilfenidato, encontrado em três apresentações comerciais distintas,uma formulação de ação imediata e duas formulações de liberação controlada (SZOBOT; ROMANO, 2007). . Em revisão da literatura, Wilens et al. (2003) demonstraram que, quando utilizado para fins do tratamento do TDAH desde a infância, o metilfenidato diminuiu o risco do uso de drogas.

1.1.5 Diferença entre os gêneros no TDAH

De acordo com vários autores, O TDAH é mais prevalente em meninos que meninas (GAUB & CARLSON, 1997 LAHEY,1999; BIEDERMAN e FARAONE, 2005). No entanto, as taxas de prevalência variam de 3 a 5% para o TDAH entre meninas, tendo um número significativo de meninas diagnosticadas com a doença, ainda são pouco estudadas em relação ao TDAH a(GERSHON, 2002; APA, 2000).

Atualmente, o diagnostico do TDAH, como qualquer transtorno psiquiátrico é baseado em sintomas, sem testes biológicos para decisões clínicas. O DSM-VI é baseado em sintomas que devem ser analisados para confirmar o diagnóstico.

De acordo com o DSM-VI, meninos tem frequentemente mais sintomas de impulsividade e hiperatividade, podendo explicar um possível subdiagnostico por meninas apresentarem mais sintomas cognitivos e de desatenção, podendo estar relacionado com o subdiagnostico do gênero (FARAONE, 2006). Alguns estudos têm demonstrado que meninas apresentam sintomas de hiperatividade e impulsividade, não havendo diferenças entre os gêneros (GAUB & CARLSON, 1997; BIEDERMAN,1999).

1.1.5 Modelos Animais de TDAH

Afim de compreender os mecanismos envolvidos na hiperatividade, inúmeros modelos animais têm sido usados para mimetizar o TDAH em animais (AZEREDO et al, 2010). Um modelo clássico, consegue reproduzir sintomas de hiperatividade , contornando os fatores limitantes dos roedores, lesionando neurônios dopaminérgicos por 6-hidroxidopamina(6-OHDA) em ratos neonatos (GUARDIOLA & BARROS, 1990).

Em estudos realizados na década de 70, revelaram que a desregulação do sistema catecolaminérgico de ratos neonatos induzidos por 6-OHDA acarretaria a característicos efeitos na neurotransmissão catecolaminérgica e subsequente comportamento destes animais (Shaywitz e cols., 1976; Shaywitz e cols., 1978). A 6-OHDA é uma neurotoxina, causando injúria nas projeções dopaminérgicas e noradrenérgicas, por ligação tóxica irreversível aos receptores catecolaminérgicos pós-sinápticos (Pearson e cols., 1990). Após ser injetada intracisternalmente, a 6-OHDA lesiona as projeções dopaminérgicas e noradrenérgicas no Sistema nervoso central (Shaywitz e cols., 1976).

A lesão dopaminérgica ocasionada por 6-OHDA de ratos neonatos ocasiona uma hiperatividade motora característica do TDAH, demonstrada quando os animais atingem a maioridade em correspondência intrínseca do período em humanos (Shaywitz e cols .,1976a, 1976b; Erinoff e cols., 1979; Heffner & Seiden, 1982; Davids e cols., 2002).

A indução neonatal de 6-OHDA resulta em déficits de memória, normalmente de aquisição de tarefas e formulação de respostas (Whishaw e cols., 1988). Déficits de aprendizagem podem ser detectados em 48horas após a administração de 6-OHDA, sendo que ocorre um aumento do déficit logo após o desmame e atinge o pico máximo na idade adulta (WOOL e cols.,1987). Apesar de perdas de projeções dopaminérgicas, ratos com lesão neonatal exibem uma hiperatividade motora robusta, que é atenuada por psicoestimulantes (GAINETNOV e cols., 1999). Em nosso laboratório houve uma diminuição da hiperatividade na administração oral de cocaína em ratos machos (AZEREDO *el* al 2010).

1.2 Cocaína

1.2.1 Epidemiologia

Nos últimos anos, o abuso de cocaína teve um aumento assustador. Nos Estados Unidos, os problemas relativamente pequenos associados à cocaína deram lugar a problemas que tornaram-se temas centrais das politicas de saúde, legislativas e policiais (ROUSE,1996). Esse crescimento desencadeou um grande número de pesquisas acerca do tema na literatura mundial, buscando maior entendimento sobre os transtornos de uso de psicoestimulantes e os mecanismos neurobiológicos dos transtornos mentais e de comportamento em comorbidade, principalmente o TDAH (CHILCOAT *et* al.;1999).

De acordo com o relatório mundial sobre drogas da Organização das Nações Unidas (ONU) realizado em 2012, o uso mundial de cocaína se manteve estável

em 0,3 e 0,4% da população entre 15 e 64 anos . Embora não existam dados recentes do uso de drogas ilícitas no Brasil, especialistas apontam para um aumento do uso de cocaína. Em apreensões federais tiveram um aumento até 2010 (27 toneladas). Outro ponto importante no relatório é que o uso ilícito de drogas entre mulheres,é aproximadamente um terço do uso entre homens no país (UNODC, 2012).

Segundo o Levantamento Domiciliar sobre o Uso de Drogas Psicotrópicas no Brasil realizado em 2005 , que envolveu as 108 maiores cidades do país, 2,9% da população (1.459.000 pessoas) já fez uso de cocaína pelo menos uma vez na vida. A cocaina é a segunda droga ilícita mais utilizada,depois da maconha (CARLINI *et al., 2007*).

Nos Estados Unidos, aproximadamente 30% dos usuários de cocaína são mulheres (SAMSHA, 2007). Esses dados apontam a um aumento do uso de psicoestimulantes por mulheres, podendo estar relacionado a fatores biológicos, sociais e comportamentais, demonstrando a importância de estudar as diferenças entre os gêneros.

1.2.2 Farmacologia

De acordo com o Instituto Nacional de Drogas de Abuso, NIDA (2005) a cocaína pode ser encontrada na forma de cloridrato de cocaína, um pó cristalino altamente solúvel em água. A administração de cocaína é normalmente por via nasal, na forma de pó; oral, dissolvido em água; intravenosa e fumada, na forma de crack.

A purificação da cocaína é feita a partir das folhas de coca, utilizando um solvente orgânico, resultando em uma pasta de coca contendo aproximadamente 80% de cocaína (DICKERSON e JANDA,2005).

A cocaína tem quatro formas diferentes de apresentação : basuco (ou pasta base) que é fumada em alguns países (FIGLIE *et* al.;2004), a forma de pasta de coca; merla, a forma pastosa; cloridrato de cocaína, a forma de pó cristalino; crack, a forma de base livre, sendo as duas últimas formas de uso mais populares no sul do país. A forma de cloridrato de cocaína é obtida através do tratamento da pasta de coca purificada com ácido clorídrico. A dissolução do alcalóide no ácido forma um pó hidrossolúvel, que precisa ser recristalizado, através de aquecimento, para que o produto final seja o pó de cloridrato de cocaína, o qual pode ser ingerido via oral, aspiração nasal ou via intravenosa. Esta forma não pode ser fumada, pois, com o aumento da temperatura, ocorre a volatilização e conseqüente decomposição do composto (CHASIN et al., 2008; DICKERSON & JANDA, 2005).

O *crack*, base livre, é preparado através do aquecimento da solução aquosa do cloridrato com uma substância básica, como o bicarbonato ou o hidróxido de sódio, neutralizando o bicarbonato ou a parte ácida. . Esta forma é estável a altas

temperaturas, permitindo ser fumado, e é conhecida como crack, devido ao som que faz quando aquecido (EGRED e DAVIS, 2005; DICKERSON & JANDA, 2005; FIGLIE et al.;2004).

1.2.3 Farmacocinética

A velocidade de absorção e a máxima concentração plasmática atingida são dependentes das vias de introdução pelas quais ocorre a autoadministração de cocaína que, por suas características de absorção, podem ser divididas em intranasal, oral, intravenosa e respiratória (CHASIN et al., 2008) .

A via de administração pulmonar (crack e Merla) é a via que causa o início dos efeitos mais rapidamente. Estes se iniciam de 10 a 15 segundos; enquanto que na via intranasal o tempo é de 10 a 15 minutos e na intravenosa 3 a 5 minutos. O tempo de duração dos efeitos na via pulmonar (crack) é em média 5 minutos, enquanto na forma injetável 20 minutos e na intranasal 45 minutos. Devido a essas propriedades, o crack leva à dependência muito mais rapidamente que o uso de cocaína por outras vias (EGRED & DAVIS, 2005). A droga se acumula nos tecidos adiposos e no Sistema Nervoso Central em função de sua lipossolubilidade, vezes maiores que a plasmática, com posterior liberação desses sítios se concentrando no baço, rins, fígado e cérebro, permitindo que a substância atravesse a barreira hematoencefálica. Após a absorção, a cocaína (benzoilmetilecgonina) é biotransformada por hidrólise em éster metilecgonina e

benzoilecgonina, que correspondem a 95% da metabolização da cocaína (FIGLIE et al., 2004; CHASIN et al., 2008).

A excreção é feita pelos rins, sendo que apenas 10% da cocaína é excretada na sua forma inalterada. Cerca de 75 a 90% é excretada na forma de benzoilecgonina (29 a 45% da excreção urinária) e éster metilecgonina (32 a 49% da excreção urinária).

1.2.4 Farmacodinâmica

A cocaína é considerada um potente agente simpaticomimético, por sua capacidade de bloquear a recaptação nos neurônios pré-sinápticos, aumentando os níveis de catecolaminas (dopamina, noradrenalina e serotonina), produzindo elevação temporária da concentração desses neurotransmissores nos receptores pós-sinápticos, justificando os efeitos estimulantes SNC (EGRED & DAVIS, 2005).

Os neurônios dopaminérgicos do tronco encefálico localizam-se no mesencéfalo, em duas regiões muito próximas: a área tegmentar ventral, que pertence por sua vez à formação reticular, e a substância negra. Nessa última, tem origem a via nigro-estriatal, onde está presente aproximadamente 75% da dopamina presente no cérebro e cujos corpos celulares se localizam na substância nigra e os axônios terminam no corpo estriado. A via nigro-estriatal é imprescindível no controle da atividade motora somática. Já na área tegmentar ventral, origina-se a via mesolímbica, a qual projeta-se para o corpo estriado ventral, para o sistema límbico e para o córtex pré-frontal, sendo responsável pela regulação do comportamento emocional. Uma terceira via envolvida é a do sistema túbero-hipofisário, cujo grupo de neurônios partem do núcleo arqueado do

hipotálamo e terminam na eminência mediana e na hipófise, tendo está ultima suas secreções controladas por determinada via. Além dessas três vias neuronais, existem ainda vários neurônios dopaminérgicos localizados no córtex olfatório, no bulbo e na retina (HERNANDESZ, 2010).

Neurônios dopaminérgicos estão relacionados ao efeito de recompensa e à tomada de decisões. Um elemento de suporte é que a cocaína, assim como outras drogas de abuso, aumenta a neurotransmissão dopaminérgica, potencializando os efeitos de recompensa e de busca pela droga.

De acordo com pesquisas atuais, comprovou-se a existência de duas famílias de receptores dopaminérgicos: uma composta pelos receptores D1 e D5 e a outa pelos receptores D2, D3 e D4. Dentre estes, o receptor D2 exibe maior concentração no corpo estriado e no núcleo accumbens, tendo sua estimulação associada ao comportamento de caráter psicótico (HUANG, 2011).

Com o uso de cocaína, independente da forma utilizada (inalada, intravenosa ou fumada), ocorre um aumento temporário das concentrações de dopamina e de norepinefrina com subsequente redução a valores abaixo do considerado normal. Tal alternância das concentrações dessas duas catecolaminas explica os estados de euforia e depressão induzidos pelo uso dessa droga (VENTON, 2006, EGRED & DAVIS, 2005).

1.2.5 Efeitos da cocaína

Os efeitos da cocaína tem ações periféricas, como centrais (LARANJEIRA *et al*., 2003) Sendo os efeitos por intoxicação aguda: intensa euforia e bem estar, aumento do estado de vigília, autoconfiança elevada , os sintomas físicos são aumento da frequência cardíaca e respiratória, aumento da sudorese, midríase , tremor nas extremidades e um comportamento estereotipado. O abuso de cocaína em pacientes sem desordens psiquiátricas tem sido associado com ataques de pânico, depressão, e aumento da ideação suicida (LARANJEIRA *et al*., 2003).

A Dependência de Cocaína pode estar associada com Transtorno de Estresse Pós-Traumático, Transtorno da Personalidade Anti-Social, Transtorno de Déficit de Atenção / Hiperatividade e Jogo Patológico. O DSM-IV- (APA,2000) lista muitos transtornos relacionados à cocaína, além dos conhecidos processos de abstinência, intoxicação, abuso e dependência. Dentre eles estão o *delirium* por intoxicação por cocaína; o transtorno psicótico induzido por cocaína, com delírios; o transtorno psicótico induzido por cocaína, com alucinações; o transtorno do humor induzido por cocaína; o transtorno de ansiedade induzido por cocaína; a disfunção sexual induzida por cocaína; o transtorno do sono induzido por cocaína; além do transtorno relacionado à cocaína sem outra especificação. Os psicoestimulantes da cocaína consegue ser representado em testes de locomoção e campo aberto, e seu efeito reforçador em caixas de condicionamento operante (UMPIERREZ, 2013).

1.2.6 Diferença entre os gêneros no uso de cocaína

O abuso de drogas tem sido considerado um problema mais frequente entre os indivíduos do sexo masculino. No entanto, o consumo de cocaína por mulheres tem aumentado rapidamente nas ultimas décadas, cerca de 600.000 dos 2 milhões de usuários de cocaína nos Estados Unidos sejam mulheres (SAMHSA,2007). Com isso, vem crescendo o a preocupação em verificar essas diferenças entre homens e mulheres na aquisição da dependência química (PERROTTI et al., 2000).

Em estudos com animais, as respostas agudas aos psicoestimulantes são diferentes entre machos e fêmeas (PERROTTI et al., 2000; QUINONES-JENAB et al., 1999). Como descrito na mulher, ratas são mais sensíveis aos efeitos psicomotores da cocaína do que machos e adquirem comportamento de autoadminstração de drogas mais rápidos (CARROL et al 2004, LYNCH, 2002). Esses achados nos levam a ter um interesse maior em estudar as diferenças entre os gêneros, num contexto comportamental e biológico.

3. Objetivos

3.1. Objetivo geral

O objetivo geral deste estudo será avaliar as diferenças de uso de cocaína entre machos e fêmeas e o efeito da autoadministração oral de cocaína sobre as alterações comportamentais em um modelo de ratos com TDAH por lesão neonatal por 6-OHDA.

3.2. Objetivos específicos

- Reproduzir a sintomatologia clássica de hiperatividade, desatenção e memória induzida por lesão neonatal com 6-OHDA em ratos machos e fêmeas;

- Avaliar o consumo de cocaína por autoadministração oral em caixas de condicionamento operante em ratos machos e fêmeas com lesão neonatal por 6-OHDA;

- Avaliar os efeitos comportamentais após o uso de cocaína ao longo dos 28 dias de autoadministração oral em ratos machos e fêmeas com lesão neonatal por 6-OHDA no teste de locomoção.

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UMPIERREZ, L.S . AUTO-ADMINISTRAÇÃO ORAL DE COCAÍNA EM RATOS MACHOS E FÊMEAS COM TRANSTORNO DE DÉFICIT DE ATENÇÃO E HIPERATIVIDADE (TDAH) INDUZIDOS POR 6-HIDROXIDOPAMINA (6-OHDA). Trabalho de conclusão de curso – Biomedicina – Universidade Federal de Ciências da Saúde de Porto Alegre – 2013.

5. ARTIGO

Oral Self-administration of cocaine by male and female rats with Attention Deficit Hyperactivity Disorder induced by 6-hydroxydopamine.

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Keywords: self-administration, ADHD, cocaine, female, male.

Abstract

The attention deficit hyperactivity disorder (ADHD) is one of the most common psychiatric disorders of childhood and it can persist into adulthood. In adults, the most common comorbidity of ADHD is Substance Use Disorder (SUD). Cocaine abuse is a main problem associated to adulthood ADHD. In ADHD patients, cocaine use and dependence are more precocious. It is known that females are more sensitive to the effects of cocaine, facing a more serious pattern of dependence. Our objective was to evaluate the oral consumption of cocaine in male and female rats in a model to ADHD with 6-hydroxydopamine (6-OHDA). Wistar rats were divided into groups of males and females rats, were allocated randomly at PN day 4 to intrathecal injection of the neurotoxin 6-hydroxydopamine (6-OHDA) or *sham* vehicle (0.1% ascorbic acid) procedure. When 30 days old the rats were daily placed in boxes of operant conditioning for oral self-administration of cocaine reinforcement (FR1) for 28 days. It is observed that females show more drug search behavior than males, corroborating the literature. Taking into consideration that the lesioned animals performed less in the acquisition of oral cocaine self-administration the possible explanation relies on the dopaminergic lesion induced by 6-OHDA.

More studies are needed to understand better the behavioral outcomes of cocaine self-administration in 6-OHDA lesioned rats.

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is among the most common psychiatric disorders of childhood. It persists into adulthood in the majority of cases (Barkley RA et al.,2002). ADHD is a complex and heterogeneous disorder and its etiology is not yet completely understood (Genro et.al.,2010). Neurobiologic research has shown lack of connectivity in key brain regions, delayed brain maturation, and noradrenergic and dopaminergic dysfunction in multiple brain regions of patients (Dopheide JA and Plizka SR,2009) .ADHD is currently diagnosed based on three core behaviors: inatention, hyperactivity, and impulsivity (Diagnostic and Statistical Manual of Mental Disorders; 4th ed.text rev.; DSM-IV-TR; American Psychiatric Association [APA],2000).

A prevalence rate of 9.5% was reported by the center for Diase Control and Prevension(Visser, Bitsko,Danielson,& Perou,2010) and is typically more prevalent in boys than girls, with sex ratio estimates typically ranging from 2:1 to 9:1 (Gaub e Carlson, 1997; Biederman and Faraone, 2005; Sadock and Sadock, 2007). This difference can result from difficulty in diagnosing the disorder in females, possibly by cause the girls present fewer symptoms of hyperactivity and impulsivity, causing less trouble to the family and school, thereby are less referrals to treatment (Baumgaertel et al, 1995; Golfeto & Barbosa, 2003).

Specifically, boys are likely to be more hyperactive and impulsive and to have more comorbid externalizing disorders (such as conduct disorder and oppositional defiant disorder), whereas girls are more likely to be inattentive and to have comorbid internalizing desorders (such as anxiety and depression) (APA,2000; Gaud & Carlson 1997).

A common comorbidity of ADHD is Substance Use Disorder (SUD). The lifetime risk for SUD is approximately 50% in subjects whose childhood ADHD persists into adulthood (Bukstein, 2008). Cocaine is a main concern, because adults with ADHD have been reported to have earlier onset of cocaine dependence and engaged in more frequent and more severe cocaine dependence relative to adults without ADHD [Wilens, 2003]. Individual vulnerability to drug dependence may be related to a particular functional state of biological substrates that interact with the drug, inducing the individual to experience singular effects after drug use and promoting continuous use, leading to abuse or dependence [Piazza 1996, Charney 2004, Gordon 2002, Sutton 2000]. Sex differences in the profile of cocaine dependence, for example, have indicated that women, relative to men, transition faster from first use to entering treatment (Westermeyer et al, 1997), which may be explained by women abusing cocaine via more addictive routes more quickly and reporting a more rapid progression of drug dependence (Hu e Becker, 2008; Kerstetter & Kippin, 2011; Samhsa, 2011). In animals, studies with rats show that females acquire self-administration (Lynch and Carroll, 1999) and develop conditioned place preference (Russo et al., 2003) to cocaine in fewer sessions than males. Females also show a greater locomotor response and stronger sensitization to repeated cocaine exposure (Sircar and Kim, 1999; Festa & Quinones-Jenab, 2004). The clinical literature lacks information and different on cocaine abuse between men and woman.

This study aims to reproducing or not the animal model of ADHD and evaluate the behavior of males and females rats after cocaine self-administration.

Aims to evaluate oral compare cocaine self-adminstration of male and female rats submitted or not to 6-OHDA lesion to model ADHD.

Methods

Subjects

The *Wistar* rats dams, bred at the animal house of UFCSPA. Each dams was housed in polypropylene cages (40 × 33 × 17 cm) under standard environmental conditions, temperature-controlled rooms (22±2 ◦C) and a 12-h light/dark cycle from 7:00 a.m to 7:00 p.m. The animals received Nuvilab CR-1 commercial chow phytoestrogens free Nuvital® (Curitiba, Paraná, Brazil) and tap drinking water ad libitum, except during the testing period.

All experiments followed the guidelines of the International Council for Laboratory Animal Science and were approved by Ethics Committee for Research of (12-075) UFCSPA, Porto Alegre, Brazil.

When pups were born they were kept in the most until 21 days old, when they were weaned and transferred to cages with serve sex only cages.

Drugs

Desipramine HCl and 6-hydroxydopamine hydrobromide (6-OHDA.HBr), and ascorbic acid (vehicle) were obtained from Sigma Chemical Co. (St Louis, Missouri, USA). The solution Desipramine 2mg/mL was prepared in saline and of 6-OHDA 10µg/mL prepared in Ringer solution containing ascorbic acid(0.1%) .

Cocaine hydrochloride (Merck Germany) was prepared in solutions of 0.2mg/mL; 0.3mg/mL or 0.4mg/mL in sucrose 15mg/mL. Sucrose 15mg/mL was used during the training session and for the baseline sessions have designated as cocaine 0mg.

Procedures

Induction of ADHD by lesion 6-OHDA

On post natal day 4 (PND=4), pups of different dams were separated by gender and randomly assigned to 6-OHDA lesion or sham groups. The groups obtained from 10 dams (male *sham* n=20,male 6-OHDA n=22,female *sham* n=23and female 6-OHDA n=20). Lesion animals were given intraperitoneal injections (i.p) of desipramine hydrochloride (20 mg/kg), and sham pups were given intraperitoneal injection of saline. After 30 min, lesion animals received a 10µL intracisternal injection (i.c) the 6-OHDA (100 µg free base) and *sham* vehicle (0.1% ascorbic acid) . The animals were then returned to their mothers. After 21 days, the pups were weaned. In an earlier study, this procedure consistently produced a 98–99% depletion of dopamine, and desipramine effectively protected norepinephrine neurons from the destructive action of 6-OHDA (Shaywitz et al., 1976a).

Oral cocaine Self administration

Self-administration was performed in chambers 25x37x28cm made of acrylic and metal with stainless steel rod floor. Inside two the chambers, levers were located on the same wall, in which only one of them was active with lights signaling the ative lever with a green light, compartment in the between those two levers which delivers the solution . Was activated by responses on the right or 'active' lever, while responses on the left or 'inactive' lever where recorded but did not result in any programmed consequences. The chambers were placed inside a sound proof timber cubicles. The chamber was connected to a infusion pump containing a syringe. Active lever presses resulted in the delivery 0.2 mL of solution per 5 seconds on a fixed-ratio 1 (FR1) . Lever pressure during infusion did not produce a second injection, but lever press immediately after completion of the infusion produced another injection. An *software* (*Insight*® Brazil) controlled the delivery of de drinking solution and registered total number of responses on right and left lever presses.

Training for Self-administration

Was performed with 64 rats (PND= ±30). Rats were trained to lever-press on a FR1 schedule for sucrose0,2mL solution (15mg/mL) in operant chambers. Sucrose solution training sessions lasted for 3hours. For the first training sessions the animals were restricted or drinking water . The training sessions ended when the animal were able to hit 85% FR1or higher in the active lever, without water restriction. when the rats did not reach the 85% within 7 days were removed from the shaping.

Cocaine Self-administration

All experimental sessions took place from Monday through Saturday between 1 p.m and 4 p.m , for 28 days (male *sham*= 5, male 6-OHDA= 7, female *sham*=6, female 6-OHDA=7). Self-administration sessions on a FR1 schedule lasted for 3 hours or a maximum of 200 drug reinforcement. During the first 9 days, each active lever press resulted in a cocaine 0,2mg/mL reinforcement . The dose of cocaine solution was increased to 0.3 mg / mL in the 10 day of session. Session from days 19 cocaine 0.4mg/mL until 28 day was used for .

Behaviour analysis

Locomotor Activity

Locomotor Activity was used to measure the spontaneous activity of 6-OHDA lesion rats or *sham* rats. Each automated device, consisting of rodent test chambers (80 x 26 x 22 cm; Alsbarch, Porto Alegre, BR) each placed within 4 series of infra-red beams. It was registered by the low grid of infra-red beams. Each rat was allowed to remain in the test chamber for 20 min (Luthman et al., 1997).

Activity testing chambers was performed when the rats were PND±30 of age (cocaine 0mg). This tests were made to evaluate the induction hyperactivity with 6-OHDA. We also evaluated the behavior of animals in the test chambers on the 1st, 15 th and 28th days of drug self-administration which correspond to the 0.2mg, 0.3mg and 0.4mg respectively to verify if there was a decrease of hyperactive behaviors in rats, as previously observed in our laboratory with the oral cocaine in male rats (Azeredo et al., 2010). The estrous cycle stage of female rats was determined after the final activity testing chambers. The estrous cycle in rats is characterized as: proestrus, estrus, metestrus and diestrus, which may be determined according to the cell types observed in the vaginal smear.

Vaginal secretion was collected with a plastic pipette filled with saline (NaCl 0.9%) by inserting the tip into the rat vagina. Vaginal fluid was placed on glass slides. The material was observed under a light microscope, without the use of the condenser

lens, with 10 and 40 x objective lenses. Three types of cells could be recognized: round and nucleated ones are epithelial cells; irregular ones without nucleus are the cornified cells; and the little round ones are the leukocytes. The proportion among them was used for the determination of the estrous cycle phases (Long & Evans, 1922; Mandl, 1951).

Data analysis

For the analysis of locomotion activity, we used a Two Way RM ANOVA, considering the factors 6-OHDA lesion and cocaine dose. For the analysis of reinforcement, pressure of inactive lever and cumulative reinforcement, we used a Two Way ANOVA, considering factors group and dose (0.2mg – 0.3mg – 0.4mg) and group and day (1 to 28).

The Tukey test was used for post hoc comparisons when appropriate. All data were presented as means ± standard error of the mean. Differences were considered significant at $P < 0.05$.

Results

Effects 6-OHDA lesion and of cocaine self-administ on locomotor activity in male and female rats

The estrous cycle stage of female rats was determined after the final locomotor activity test, and is shows that females were evenly distributed in the different estrous cycle phases .

The male are differences significantly in locomotion of injured and not injured in 0 mg dose (MSHAM= 73.6 ± 16.7 ; M6-OHDA= 136.6 ± 15.2 , P= 0.041). The uninjured males had significant differences in the doses of 0 and 0.4 (MSHAM – 0mg= 73.6 ± 16.7 ; MSHAM – 0,4mg= 159.8 ± 23).

Females injured has no differences in locomotion of females not injured. The locomotor activity in female shows a significant difference between the doses, with increased locomotion 0.4 mg dose compared to the 0.2 mg dose ($F(3.33) = 3.695$, $P = 0.021$). As may be seen in figure 1.

Self-administration

The mean number of lever press in the active bar to achieve the 85% FR1 was (M6-OHDA= 68,5 ± 10,9 ; MSHAM= 197,6 ±13,8 ; F6-OHDA= 133,8 ± 10,9 ; FSHAM= 118,4 ± 13,8) .

In comparison between male and female injured, damaged females had higher reinforcements in the dose of 0.2 mg and 0.4 mg (M6-OHDA-0.2mg= 53.65 ± 6.47; F6-OHDA- 0.2mg= 104.37 ± 6.33; M6-OHDA- 0.4mg = 16.75 ± 6.83; F6-OHDA- 0.4 mg, 80.56±7.06,P<0.001). FSHAM group compared with the group MSHAM had more reinforcements at doses 0.3 mg and 0.4 mg (FSHAM 0,3mg 82, 37 ± 8.01; MSHAM-0,3mg 52 ± 8.01; FSHAM -0.4mg 68,89 ± 7.76; MSHAM-0, 4mg 31.44 ± 7.60, P <0.001).

We analyzed the number of pressure inactive lever in different doses. There was no difference between the groups (F (9.72) = 0.97 NS) .

In the analysis of reinforcement day, represented in the figure shows the daily reinforcement between groups. Represented in the table 2 shows the daily reinforcement between groups in 3 days of sucrose (15mg/mL). The group MSHAM within in 2 days and 0.2 mg of cocaine (247 ± 23.23) had the highest increase compared to other groups. During the days decreased the number of reinforcements. From 6 days cocaine 0.2 mg FSHAM group had an increase in reinforcements , showing peaks that can be seen during the day (day 6 cocaina0,2mg 164.60 ± 23.23; 2 days cocaine 0.3 mg

106, 40 ± 23.23, 4 day-cocaine 0.3mg 148.40 ± 23.23 ; 6 days cocaine- 0.3mg 111.60 ± 23.23 ; 9 days cocaine 0.4mg - 106.25 ± 25.98).

During the days males began to have a smaller number of reinforcements over females, showing the F6-OHDA group compared to the group M6-OHDA significant difference in days 8, 9 and 10 in the dose of 0.4 mg cocaine (F6 -OHDA - 114.66 ± 21.21, 123.83 ± 21.21, 130.75 ± 25.98; M6-OHDA 3.83 ± 21.21, 27.20 ± 23.23, 10.40 ± 23.23, P <0.001).

The total of reinforcements over the days shows that females consume more injured than males injured and not injured females than males consume more not injured (FSHAM - 1415.23 ± 32.86; MSHAM - 1180.84 ± 32.86 ; F6-OHDA 917.33 ± 28.17; M6-OHDA 538.12 ± 28.12, P <0.001).

Discussion

As in some previous studies, including one conducted in our laboratory, we observed an increase in locomotion of male rats lesioned by 6-OHDA able to reproduce one of the symptoms of the ADHD disorder, hyperactivity (Shaywitz et al, 1976; Erinoff et al, 1979 , Guardiola et al, 1993; Azevedo et al, 2010). As yet unpublished results from our laboratory, conducted to evaluate the learning and memory deficit induced by 6-OHDA, females injured, showed less object recognition , suggesting a possible deficit of learning and memory. Other studies on induction of neonatal 6-OHDA result in memory deficit, usually lowering the task acquisition and formulating responses (Luthman et al. 1989; Whishaw et al. 1988). These results suggest that the classical model of induction ADHD with 6-OHDA can reproduce the disorder not only

in males but also in females. Further studies will be conducted to discriminate the differences between males and females in this ADHD model . According to clinical studies in ADHD girls, symptoms of inattention are more frequent (Rohde, 2000) while in boys higher prevalence of hyperactivity symptoms is described (Poet & Neto, 2004 Gaud and Carlson, 1997).

In previous study in our laboratory it was shown that cocaine decreased the hyperactivity of male ADHD rats. In this the locomotor activity report in 6-OHDA males, after self-administration of lower doses of was cocaine, was decreased, as described previously in our laboratory (Azeredo et al, 2010), it was also detected, as expected that higher cocaine doses induces hyperactivity, in both males and females, irrespective to the 6-OHDA lesion.

These sex differences in the acquisition of drug addiction demonstrates effective hormonal interference, as the action in neurochemical systems and behavioral effects (Perrotti et al., 2000; Saleh, 2003; Kouri et al., 2002, Lynch et al.,2002.) Our results are in agreement with studies that show that cycling females are usually more sensitive to the effects of psychostimulants. They acquire cocaine self-administration more rapidly than males (Becker and Hu, 2008; Lynch et al. 2001; Carroll et al., 2002; Sircar & Kim, 1999; Festa & Quinones-Jenab, 2004, Carroll et al 2004) and make more association between drug administration and the environment in a shorter time period in the conditioned place preference (Russo et al , 2003).Our results proved that female rats, with 6-OHDA lesion or not, presented more reinforcements and oral cocaine self-administration than the respective groups of male rats. Ovarectomized female rats showed lower behavioral sensitization than rats with endogenous hormones presence. (Souza et al, Submitted). During the estrous cycle, levels of estrogen and progesterone fluctuate, and behavioral effects induced by cocaine may be affected by

the stages of the cycle (Sell et al, 2000; Walker et al., 2002).In rats, the increase in the behavioral response to cocaine is directly related to the proestrus and estrus phases where hormone levels of estrogen and progesterone are higher (Sell et al, 2000; Quiñones-Jenab et al., 1999; Chin et al. 2002).

Individuals with ADHD appear to have greater drug abuse, when compared with normal subjects (Vendruscolo and Takahashi, 2010). In our results, the injured and females had a number of reinforcements than non lesioned rats; and, yet females lower had higher responses than males. Classically, more cocaine effects is associated to the decreased frequency of reinforcements (Pickens and Thompson, 1968). And one may consider that rats have more dopamine receptors available. On the other hand, other studies show that higher super sensitivity leads to more cocaine intake addition, cocaine sensitization. Increased rate of acquisition to cocaine self-administration and of the breaking point during a progressive ratio schedule (Covington and Miczek, 2001). Taking into consideration that the lesioned animals performed less in the acquisition of oral cocaine self-administration the possible explanation relies on the dopaminergic lesion induced by 6-OHDA, in which case cocaine has fewer pre-synaptic neurons to act upon.

More studies are needed to understand better the behavioral outcomes of cocaine self-administration in 6-OHDA lesioned rats.

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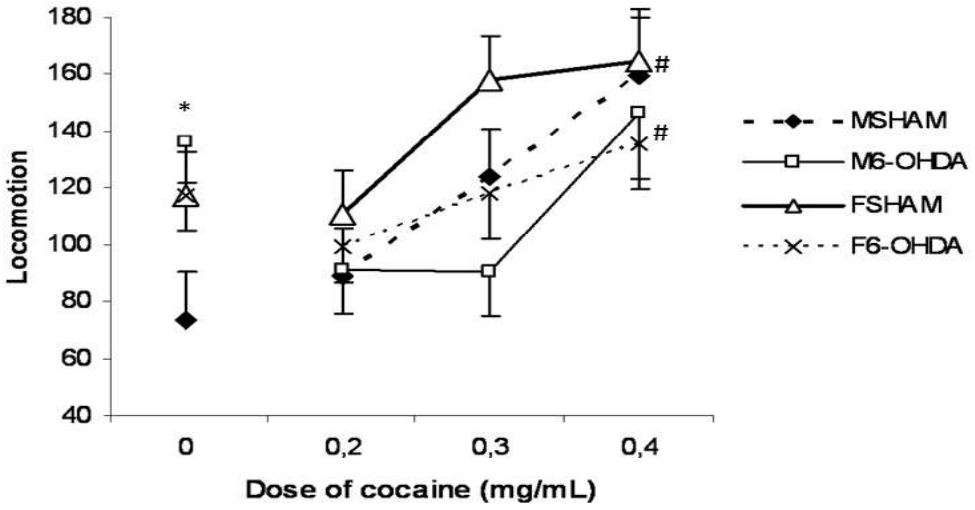

Figure 1. Locomotion (nº crosses of light beams) of *Sham* and 6-OHDA lesioned male and female rats. Rats were tested around 30 days old for baseline (cocaine 0mg/mL), the first day of drug oral self-administration (cocaine 0.2mg/mL), 15 days after drug administer (cocaine 0.3mg/mL) and 28 days after drug administer (cocaine 0.4mg/mL).

* represents differences male lesioned and male *sham* cocaine 0mg, # represents differences cocaine 0.2mg

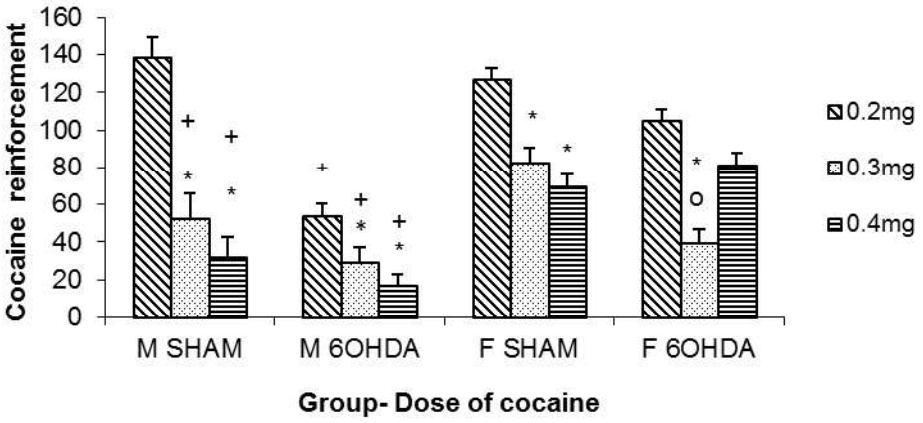

Figure 2: Mean number of daily reinforcements (FR1) with cocaine 0.2mg - 0.3mg - 0.4mg oral self-administration. * represents differences of 0.2mg, + differences of female, º difference of 0.4mg.

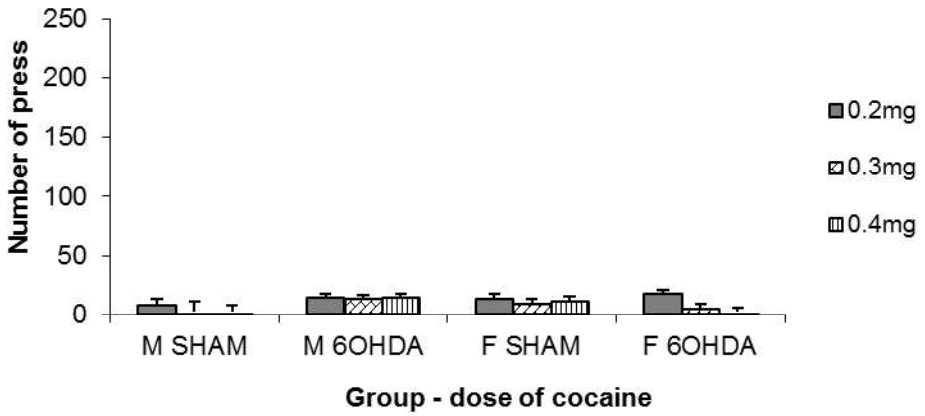

Figure 3. Pressure Inative . There was no difference between the groups

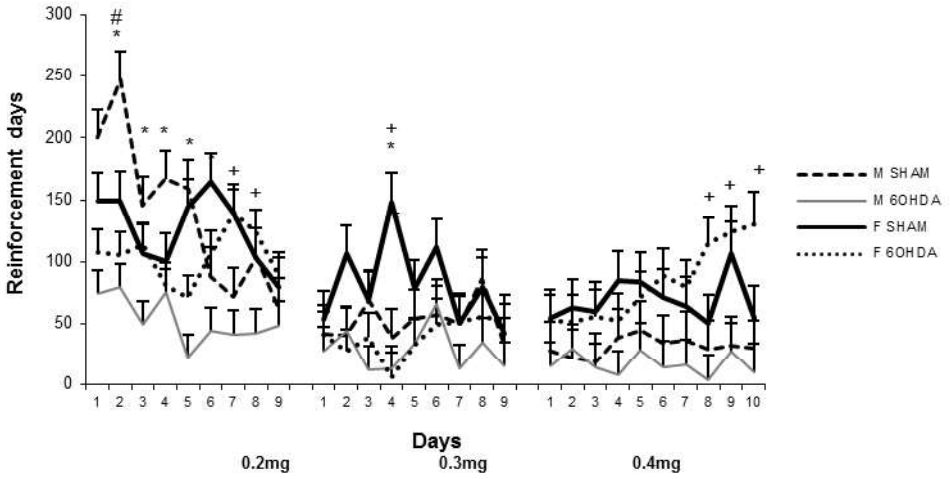

Figure 4. Daily mean number reinforcements of male and female in the doses 0.2mg - 0.3mg - 0.4mg. * represents differences of 6-ODHA, # differences of female, + differences of male.

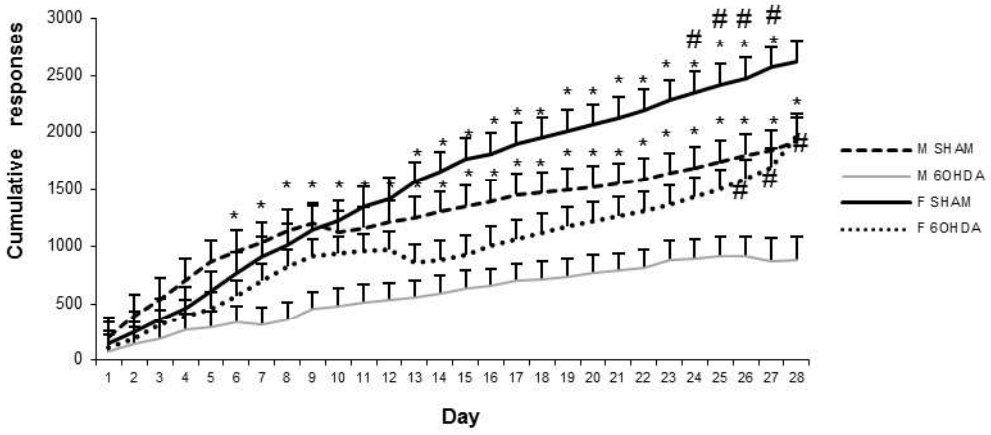

Figure 5. Total reinforcements of *sham* or 6-OHDA lesioned male and female rats, during the 28 days of oral cocaine self-adminstration. * represents differences of 6-OHDA, # differences of male.

6 ANEXO

Normas da Revista

Guidance for Authors on the Preparation and Submission of Manuscripts to

Behavioural Pharmacology

Note: These instructions comply with those formulated by the International Committee of Medical Journal Editors. For further details, authors should consult the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" www.icmje.org. The Journal is a member of the Committee on Publication Ethics (COPE) which aims to define best practice in the ethics of scientific publishing (www.publicationethics.org).

Scope

Behavioural Pharmacology accepts original full and short research reports in diverse areas ranging from ethopharmacology to the pharmacology of schedule-controlled operant behaviour, provided that their primary focus is behavioural. Suitable topics include drug, chemical and hormonal effects on behaviour, the neurochemical mechanisms under-lying behaviour, and behavioural methods for the study of drug action. Both animal and human studies are welcome; however, studies reporting neurochemical data should have a predominantly behavioural focus, and human studies should not consist exclusively of clinical trials or case reports. Preference is given to studies that demonstrate and develop the potential of behavioural methods, and to

papers reporting findings of direct relevance to clinical problems. Papers making a significant theoretical contribution are particularly welcome and, where possible and merited, space is made available for authors to explore fully the theoretical implications of their findings. Reviews of an area of the literature or at an appropriate stage in the development of an author's own work are welcome. Commentaries in areas of current interest are also considered for publication, as are Reviews and Commentaries in areas outside behavioural pharmacology, but of importance and interest to behavioural pharmacologists.

Points to consider before submission

Please think carefully about the following points and make the appropriate declarations.

Redundant or duplicate publication

We ask you to confirm that your paper has not been published in its current form or a substantially similar form (in print or electronically, including on a web site), that it has not been accepted for publication elsewhere, and that it is not under consideration by another publication. The International Committee of Medical Journal Editors has provided details of what is and what is not duplicate or redundant publication (www.icmje.org). If you are in doubt (particularly in the case of material that you have posted on a web site), we ask you to proceed with your submission but to include a copy of the relevant previously published work or work under consideration by other journals. In your covering letter to the editors, draw attention to any published work that concerns the same patients or subjects as the present paper.

Conflicts of interest

Authors must state all possible conflicts of interest in the manuscript, including financial, consultant, institutional and other relationships that might lead to bias or a conflict of interest. If there is no conflict of interest, this should also be explicitly stated as none declared. All sources of funding should be acknowledged in the manuscript. All relevant conflicts of interest and sources of funding should be included on the title page of the manuscript with the heading "Conflicts of Interest and Source of Funding:". For example:

Conflicts of Interest and Source of Funding: A has received honoraria from Company Z. B is currently receiving a grant (#12345) from Organization Y, and is on the speaker's bureau for Organization X – the CME organizers for Company A. For the remaining authors none were declared.

In addition, each author must complete and submit the journal's copyright transfer agreement, which includes a section on the disclosure of potential conflicts of interest based on the recommendations of the International Committee of Medical Journal Editors, "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (www.icmje.org/update.html). The form is readily available on the manuscript submission page www.editorialmanager.com/bpharm and can be completed and submitted electronically. Please note that authors may sign the copyright transfer agreement form electronically. For additional information about electronically signing this form, go tohttp://links.lww.com/ZUAT/A106.

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Authors should include with their submission copies of written permission to reproduce material published elsewhere (such as illustrations) from the copyright holder. Authors are responsible for paying any fees to reproduce material.

Patient consent forms

Patients have a right to privacy that should not be infringed without informed consent. Identifying details (written or photographic) should be omitted if they are not essential, but patient data should never be altered or falsified in an attempt to attain anonymity. Complete anonymity is difficult to achieve, and a consent form should be obtained if there is any doubt. For example, masking the eye region in photographs of patients is inadequate protection of anonymity. When informed consent has been obtained it should be indicated in the published article.

Ethics committee approval

You must state clearly in your submission in the Methods section that you conducted studies on human participants must with the approval of an appropriate named ethics committee. Please also look at the latest version of the Declaration of Helsinki. Similarly, you must confirm that experiments involving animals adhered to ethical standards and must state the care of animal and licensing guidelines under which the study was performed.

The editors will welcome the submission of work in any area of behavioural pharmacology. However, studies that subject animals to pain or stress require special consideration and three principles will be used to judge the ethics of such work. First, experiments of this kind will be required to reach a higher threshold of scientific quality in order to be publishable. Second, the utility of the study will be addressed, in terms of the balance between the distress inflicted and the likelihood of benefit. Third, it must be demonstrated that the objectives of the experiment could not have been achieved by the use if less stressful procedures; the fact that a particular procedure has been used in previously published work will not in itself suffice. The editors believe that behavioural pharmacologists apply strict ethical criteria to their work, and will have no difficulty in meeting these requirements.

Authorship

All authors must acknowledge that they have read and approved the paper, that they have met the criteria for authorship as established by the International Committee of Medical Journal Editors, that they believe that the paper represents honest work, and that they are able to verify the validity of the results reported. In addition to those from the ICJME the International Society for Medical Publication Professionals, ISMPP (www.ismpp.org) have produced some useful guidelines on authorship of studies sponsored by companies: Good Publication Practice (GPP2) (www.ismpp.org).

Compliance with NIH and Other Research Funding Agency Accessibility

Requirements

A number of research funding agencies now require or request authors to submit the post-print (the article after peer review and acceptance but not the final published article) to a repository that is accessible online by all without charge. As a service to our authors, LWW will identify to the National Library of Medicine (NLM) articles that require deposit and will transmit the post-print of an article based on research funded in whole or in part by the National Institutes of Health, Wellcome Trust, Howard Hughes Medical Institute, or other funding agencies to PubMed Central. The Copyright Transfer Agreement provides the mechanism.

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tracking system at http://www.editorialmanager.com/bpharm/. The site contains instructions and advice on how to use the system. Authors should NOT in addition then post a hard copy submission to the editorial office, unless you are supplying artwork, letters or files that cannot be submitted electronically, or have been instructed to do so by the editorial office. For those authors who have no option but to submit by mail please send one copy of the article, plus an electronic version on disk or CD-ROM to one of the editors. Manuscripts originating in the USA should be sent to Jack Bergman, those from Europe to Louk Vanderschuren, and those from elsewhere to Paul Willner. Members of the European Behavioural Pharmacology Society may submit manuscripts to Louk Vanderschuren irrespective of geographical location. Editor addresses: Jack Bergman, PhD, Harvard Medical School, McLean Hospital, 115 Mill Street ,Belmont, MA 02478-9106, USA. E-mail: jbergman@hms.harvard.edu; Dr. Louk Vanderschuren, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, Pharmacology and Anatomy, Universiteitsweg 100, Utrecht 3584 CG, Netherlands E-mail: l.j.m.j.vanderschuren@umcutrecht.nl. Professor Paul Willner, Department of Psychology, University of Wales Swansea, Singleton Park, Swansea SA2 8PP, UK. Tel: (+44) 1792 295844; Fax: (+44) 1792 295679; E-mail: p.willner@swansea.ac.uk

Double spacing should be used throughout the manuscript, which should include the following sections, each starting on a separate page: Title Page, abstract and keywords, text, acknowledgements, references, individual tables and captions. Margins should be not less than 3 cm. Pages should be numbered consecutively, beginning with the Title Page, and the page number should be placed in the top right hand corner of each page. Abbreviations should be defined on their first appearance in the text; those not accepted by international bodies should be avoided. Submit the required number of paper copies and keep copies of everything submitted.

Presentation of papers

Title Page

The Title Page should carry the full title of the paper and a short title, of no more than 45 characters and spaces, to be used as a ‘running head’ (and which should be so identified). The first name, middle initial and last name of each author should appear. If the work is to be attributed to a department or institution, its full name should be included. Any disclaimers should appear on the Title Page, as should the name and address of the author responsible for correspondence concerning the manuscript and the name and address of the author to whom requests for reprints should be made. Finally, the Title Page should state conflicts of interest and source of funding, and when none state “none declared”.

Abstracts

The second page should carry a structured abstract of no more than 200 words. The abstract should state the Objective(s) of the study or investigation, basic Methods (selection of study subjects or laboratory animals; observational and analytical methods), main Results (giving specific data and their statistical significance, if possible), and the principal Conclusions. It should emphasise new and important aspects of the study or observations.

Key Words

The abstract should be followed by a list of 3–10 keywords or short phrases which will assist the cross-indexing of the article and which may be published. When possible, the terms used should be from the Medical Subject Headings list of the National Library of Medicine (http:// www.nlm.nih.gov/mesh/meshhome.html).

Text

Full papers of an experimental or observational nature may be divided into sections headed Introduction, Methods (including ethical and statistical information), Results and Discussion (including a conclusion), although reviews may require a different format.

Acknowledgements

Acknowledgements should be made only to those who have made a substantial contribution to the study. Authors are responsible for obtaining written permission from people acknowledged by name in case readers infer their endorsement of data and conclusions.

References

References are to be cited in the text by author and year, as Black and White (1991) or (Black and White, 1991). A series of references in the text should appear in chronological order, e.g. White and Black, 1989; Black and White, 1991. References having three or more authors are cited Black et al., (1991). References to papers by the same authors in the same year are distinguished by letters a, b, etc. (e.g. 1988a,b). Publications having no obvious authors are cited as Anon, (1991) in the text and bibliography. References with three or more authors should be placed in chronological order after taking account of the names of the first and second authors. References should also include full title and source information. Journal names should be abbreviated as in MEDLINE (NLM Catalog, http://www.ncbi.nlm.nih.gov/nlmcatalog). Some sample reference styles follow:

Articles in journals

Standard journal article*:*

Sanger D, Willner P, Bergman J (2003). Transgenic mouse models of Alzheimer's disease: phenotype and application. *Behav Pharmacol* 14: 363-7.

More than seven authors:

Sahraei H, Poorheidari G, Foadaddini M, Khoshbaten A, Asgari A, Noroozzadeh A, et al. (2004). Effects of nitric oxide on morphine self-administration in rat. *Pharmacol Biochem Behav* 77:111-6.

Books

Book:

Perry PJ, Alexander B, Liskow B, DeVane CL (2006). *Psychotropic drug handbook*. 8th ed. Philadelphia: Lippincott Williams & Wilkins.

Chapter in a book:

Flaisher-Grinberg S, Einat H (2009). Mice models for the manic pole of bipolar disorder. In: *Mice models for mood and anxiety disorders*. Gould TD (editor). Berlin: Springer Press; pp. 297-326.

Personal communications and unpublished work should not feature in the reference list but should appear in parentheses in the text. Unpublished work accepted for publication but not yet released should be included in the reference list with the words 'in press' in parentheses beside the name of the journal concerned. References must be verified by the author(s) against the original documents.

Either British or American spellings are acceptable, but please be consistent.

Tables

Each table should be typed on a separate sheet in double spacing. Tables should not be submitted as photographs. Each table should be assigned an Arabic numeral, e.g. (Table 3) and a brief title. Vertical rules should not be used. Place explanatory matter in footnotes, not in the heading. Explain in footnotes all non-standard abbreviations that are used in each table. Identify statistical measures of variations, such as standard deviation and standard error of the mean.

Be sure that each table is cited in the text. If you use data from another published or unpublished source, obtain permission and acknowledge the source fully.

Illustrations

A) Creating Digital Artwork

1. Learn about the publication requirements for Digital
2. Artwork: http://links.lww.com/ES/A42
3. Create, Scan and Save your artwork and compare your final figure to the Digital Artwork Guideline Checklist (below).
4. Upload each figure to Editorial Manager in conjunction with your manuscript text and tables.

B) Digital Artwork Guideline Checklist

Here are the basics to have in place before submitting your digital artwork:

- Artwork should be saved as TIFF, EPS, or MS Office (DOC, PPT, XLS) files. High resolution PDF files are also acceptable.
- Crop out any white or black space surrounding the image.
- Diagrams, drawings, graphs, and other line art must be vector or saved at a

resolution of at least 1200 dpi. If created in an MS Office program, send the native (DOC, PPT, XLS) file.

- Photographs, radiographs and other halftone images must be saved at a resolution of at least 300 dpi.
- Photographs and radiographs with text must be saved as postscript or at a resolution of at least 600 dpi.
- Each figure must be saved and submitted as a separate file. Figures should not be embedded in the manuscript text file.

Remember:

- References to figures and tables should be made in order of appearance in the text and should be in Arabic numerals in parentheses, e.g. (Fig. 2).
- Number figures in the figure legend in the order in which they are discussed.
- Upload figures consecutively to the Editorial Manager web site and enter figure numbers consecutively in the Description field when uploading the files.
- If hard copies are submitted they should have a label pasted to the back bearing the figure number, the title of the paper, the author's name and a mark indicating the top of the figure. Illustrations should be presented to a width of 82 mm or, when the illustration demands it, to a width of 166 mm.
- Photomicrographs must have internal scale markers.
- If photographs of people are used, their identities must be obscured or the picture must be accompanied by written consent to use the photograph.
- If a figure has been published before, the original source must be acknowledged and written permission from the copyright holder for both print and electronic formats should be submitted with the material. Permission is required regardless of authorship or publisher, except for documents in the public domain.

- Figures may be reduced, cropped or deleted at the discretion of the editor.
- Colour illustrations are acceptable but authors will be expected to cover the extra reproduction costs (for current charges, contact the publisher).

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Captions should be typed in double spacing, beginning on a separate sheet of paper. Each one should have an Arabic numeral corresponding to the illustration to which it refers. Internal scales should be explained and staining methods for photomicrographs should be identified.

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Measurements of length, height, weight, and volume should be reported in metric units (metre, kilogram, or litre) or their decimal multiples. Temperatures should be given in degrees Celsius. Blood pressures should be given in millimetres of mercury.

All haematologic and clinical chemistry measurements should be reported in the metric system in terms of the International System of Units (SI). Editors may request that alternative or non-SI units be added by the authors before publication.

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Use only standard abbreviations. Avoid abbreviations in the title and abstract. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement. Drug names should be generic, although authors may add brand names in parentheses if they wish.

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