UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE

PROGRAMA DE PÓS-GRADUAÇÃO EM NEUROCIÊNCIAS

PROVOCAÇÃO SOCIAL E COMPORTAMENTO AGRESSIVO: ENVOLVIMENTO DOS RECEPTORES 5-HT_{1A} E 5-HT_{1B} NO CÓRTEX PRÉ-FRONTAL

LÍGIA ALINE CENTENARO

Porto Alegre, 2008

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LÍGIA ALINE CENTENARO

Dissertação de mestrado apresentada ao Programa de Pós-graduação em Ciências Biológicas: Neurociências, da Universidade Federal do Rio Grande do Sul, como requisito parcial para a obtenção do título de mestre.

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Co-Orientador: Prof. Dr. Aldo Bolten Lucion

Porto Alegre, 2008

"Aos animais utilizados em experimentos,

os principais colaboradores na

busca do conhecimento".

AGRADECIMENTOS

Agradeço primeiramente ao ***meu Pai***, que me ensinou desde pequena a lutar por todos os meus sonhos. Agradeço pelos abraços carinhosos na hora da chegada e da despedida, por todo amor demonstrado e especialmente pelo seu imenso esforço na busca da nossa felicidade. Também agradeço à ***minha querida Mãe***, por sempre ter confiado em mim e pelo incentivo nessa caminhada. Obrigada pela incansável disponibilidade em ajudar e imensa generosidade. Saibas que senti tua presença em todos os momentos difíceis, apesar da distância. Tudo o que sou, devo inteiramente a vocês dois. Amo vocês do fundo do coração.

Aos meus irmãos, ***Idagel e Alberto***, que sempre me apoiaram e incentivaram em todos os momentos. Obrigada pelo carinho.

Ao ***Celo***, por estar sempre ao meu lado, me estendendo a mão em todos os momentos de dificuldades e se preocupando com a minha felicidade. Agradeço desde o início: pela ajuda em conhecer um orientador, pelos cafés no final da tarde, pelos risos, pelas flores, pela companhia aos sábados para ir até a Unisinos, pelo ombro amigo, pelos bilhetinhos, por cada demonstração de carinho. Obrigada pelo seu amor sincero. "Você é assim, um sonho pra mim..."

À minha irmã de coração, ***Lidiane***, pelo imenso carinho, por todo incentivo e, especialmente, por ser essa pessoa corajosa e sincera. Torço muito por você e agradeço a Deus pela dádiva da sua companhia. Também agradeço à ***Cristine*** e ***Angélica*** pela grande amizade e convivência nos momentos de risos e de angústias.

À professora ***Rosa Maria Martins de Almeida*** pela confiança, pelos ensinamentos e orientações, mas acima de tudo pela oportunidade concedida.

Ao professor ***Aldo Bolten Lucion*** por seu exemplo de profissionalismo, pela grande dedicação à vida acadêmica e por toda a transmissão de conhecimento.

A todos os ***professores*** do Programa de Pós-Graduação em Neurociências, agradeço pelos ensinamentos que me foram passados e que vou levar comigo na caminhada profissional.

À minha querida ***Nicole***, companheira leal e fiel durante a realização deste trabalho, mas também minha grande amiga e incentivadora.

À ***Karin*** e a ***Morgana***, minhas colegas de laboratório da Unisinos, pessoas especiais que me acompanharam durante essa trajetória.

Ao meu grande amigo ***Dirson***, pelo sorriso franco, pelo companheirismo, pelo exemplo de pessoa que és. Tenho orgulho de ter te conhecido.

Aos meus colegas de laboratório da UFRGS, ***Karin, Anelise, Rosana, Ana Raquel, Camila, Fabiana, Silvana, Marcelo H., Patrícia, Caroline, Charlis, Marcel, Ximena, Bruno, Marcinha e Vanise*** por todo auxílio e pelos bons momentos vividos. Em especial, gostaria de agradecer a ***Ana Lúcia Cecconelo***, pela disponibilidade em ajudar e também por todo o ensinamento.

Aos amigos ***Osni e Natália***, também colegas do laboratório da UFRGS, pela amizade sincera e pelo carinho. Vocês são pequeninos, mas tem um coração enorme. Talvez nem tenham percebido, mas aprendi muito com as suas atitudes.

Aos colegas de Pós-graduação, ***Jocemar, Patrícia, Rafaela, Simone e Márcio*** pela convivência, conselhos e especialmente pela amizade verdadeira nos momentos de dificuldade.

Ao pessoal da secretaria, ***Andréa, Beti e Fábio***, sempre dispostos a ajudar.

Ao ***CNPq*** pelo auxílio econômico que tornou possível a realização deste trabalho.

Por fim, agradeço em especial a ***Deus***, pelas pessoas maravilhosas que colocou em minha vida e principalmente por ter me dado força e coragem para alcançar mais essa conquista.

"A calma é o caminho."

Marcelo Alves de Souza

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DISSERTAÇÃO

CP-93,129 – 1,4 – Dihydro - 3 - [1,2,3,6 - tetrahydro - 4 - pyridinyl] - SH – pyrrolo [3,2 - b] pyridin - 5 - one dihydrochloride

LCR – Líquido céfalo-raquidiano

MAO – Monoamina oxidase

PAG – Substância cinzenta periaquedutal

pCPA – *para*-clorofenilalanina

SB-224,289 – (2,3,6,7 - Tetra - hydro - 1´- methyl - 5 - [2´- methyl - 4´ - [(5 - methyl - 1,2,4 - oxadiazole - 3 - yl) biphenyl - 4 - yl)] carbonyl) furo [2,3 - f] - indole - 3 - spiro- 4´- piperidine oxalate

TFMPP – (1 - (3 - trifluoromethylphenyl) piperazine)

WAY-100,635 – (N - [2 - [4 - (2 - methoxyphenyl) - 1 - piperazinyl] ethyl] - N - (2, pyridinyl) cyclohexanecarboxamide trihydrochloride

5,7-DHT – 5,7 - diidroxitriptamina

5-HIAA – Ácido 5 - hidroxiindoleacético

5-HT – Serotonina ou 5 - Hidroxitriptamina

5-HTP – 5 - hidroxi - triptofano

5-HTT – Transportador de serotonina

8-OH-DPAT – 8 - hydroxy - 2 - (di-*n*-propylamino) tetralin hydrobromide

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ANOVA – Analisys of Variance

CP-93,129 – 1,4 – Dihydro - 3 - [1,2,3,6 - tetrahydro - 4 - pyridinyl] - SH – pyrrolo [3,2 - b] pyridin - 5 - one dihydrochloride

CP-94,253 – (5 - propoxy - 3 - (1,2,3,6 - tetrahydro - 4 - pyridinyl) - 1H - pyrrolo [3,2 - b] pyridine)

CSF – Cerebrospinal fluid

GR-127,935 – (*N* - [4 - methoxi - 3 - (4,methyl - 1 - piperazinyl) phenyl] - 2 - methyl - 4′- (methyl - 1,2,4 - oxadiozol - 3 - yl) - [1,1 - biphenyl] - 4 - carboxamide)

PAG – Gray periaquedutal substance

PFA – Paraformaldehyde

S-15535 – (4 - (benzodioxan - 5- yl) l - (indan - 2 - yl) piperazine)

SB-224,289 – (2,3,6,7 - Tetra - hydro - 1′- methyl - 5 - [2′- methyl - 4′ - [(5 - methyl - 1,2,4 - oxadiazole - 3 - yl) biphenyl - 4 - yl)] carbonyl) furo [2,3 - f] - indole - 3 - spiro- 4′- piperidine oxalate

SEM – Standard error of the mean

VO PFC – Ventral orbitofrontal cortex

WAY-100,635 – (N - [2 - [4 - (2 - methoxyphenyl) - 1 - piperazinyl] ethyl] - N - (2, pyridinyl) cyclohexanecarboxamide trihydrochloride

5-HIAA – Ácid 5 - hidroxiindoleacetic

5-HT – Serotonin ou 5 – Hidroxitriptamine

8-OH-DPAT – 8 - hydroxy - 2 - (di-*n*-propylamino) tetralin hydrobromide

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RESUMO

A provocação social é um método utilizado em animais de laboratório para a indução de elevados níveis de agressividade, produzindo padrões comportamentais semelhantes ao de indivíduos violentos. Estudos prévios utilizando drogas que atuam seletivamente sobre os receptores 5-HT_{1A} e 5-HT_{1B} demonstraram uma redução pronunciada no comportamento agressivo. Um dos mais importantes sítios de ação para esses agentes serotonérgicos é o córtex pré-frontal, uma região cerebral particularmente relevante no controle inibitório da agressividade e da impulsividade. O objetivo do presente estudo foi verificar os possíveis efeitos anti-agressivos da microinjeção de 8-OH-DPAT e CP-93,129 (agonistas específicos dos receptores 5-HT_{1A} e 5-HT_{1B}, respectivamente) na região ventro orbital do córtex pré-frontal de camundongos machos provocados socialmente. Para confirmar a especificidade do receptor, antagonistas 5-HT_{1A} e 5-HT_{1B} (WAY-100,635 e SB-224,289, respectivamente) também foram microinjetados na mesma região cerebral. 8-OH-DPAT na dose de 1.0 μg reduziu significativamente a freqüência de mordidas. A menor dose de CP-93,129 (0.1 μg) também diminuiu o número de mordidas e de ataques laterais. Tais efeitos anti-agressivos não foram acompanhados por alterações no restante do repertório comportamental. A participação específica desses receptores foi verificada pela reversão dos efeitos com a utilização de WAY-100,635 (10.0 μg) e SB-224,289 (1.0 μg). Nossos resultados confirmam o envolvimento da região VO CPF e dos receptores 5-HT_{1A} e 5-HT_{1B} na modulação de altos níveis de agressividade, sem conseqüentes alterações em outras rotinas motoras.

PALAVRAS CHAVE

Agressão; Provocação social; Serotonina; Receptores 5-HT_{1A}; Receptores 5-HT_{1B}, Região ventro orbital do córtex pré-frontal.

ABSTRACT

Social instigation is used in rodents to induce high levels of aggression, a pattern of behavior similar to that of violent individuals. This procedure consists of a brief exposure to a provocative stimulus male, before direct confrontation with an intruder. Studies using 5-HT_{1A} and 5-HT_{1B} agonist receptors show a reduction in aggressive behavior. An important site of action for these drugs is the ventral orbito frontal cortex (VO PFC), an area of the brain which is particularly relevant in the inhibitory control of aggressiveness and impulsiveness. The objective of the present study was to assess the anti-aggressive effects of 5-HT_{1A} and 5-HT_{1B} agonist receptors (8-OH-DPAT and CP-93,129) on the VO PFC of socially provoked male mice. To confirm the specificity of the receptor, 5-HT_{1A} and 5-HT_{1B} antagonist receptors (WAY-100,635 and SB-224,289) were microinjected into the same area, in order to reverse the agonist effects. 8-OH-DPAT at 1.0 μg dose reduced the frequency of attack bites. The lowest dose of CP-93,129 (0.1 μg) also decreased the number of attack bites and lateral threats. The anti-aggressive effects were not accompanied by impairment of non-aggressive activities. Specific participation of the 1A and 1B receptors was verified by reversal of anti-aggressive effects using selective antagonists WAY-100,635 (10.0 μg) and SB-224,289 (1.0 μg). In conclusion, the decrease in aggressiveness observed with microinjections of 5-HT_{1A} and 5-HT_{1B} receptor agonists into the VO PFC of socially provoked mice, supports the hypothesis that activation of these receptors modulates high levels of aggression in a behaviorally specific manner.

KEYWORDS

Aggression; Social instigation; Serotonin; 5-HT_{1A} receptor; 5-HT_{1B} receptor; Prefrontal cortex

1 INTRODUÇÃO

Do ponto de vista biológico, a agressividade pode ser considerada como um comportamento adaptativo, quando empregada para uma função específica como, por exemplo: para assegurar o acesso à comida, garantir lugares seguros para a sobrevivência, proteger a prole ou em um contexto reprodutivo (TINBERGEN, 1951). Entretanto, a agressão em humanos é vista como um problema de saúde pública e para o sistema criminal, pois resulta em prejuízos e danos a outras pessoas, sendo esse problema bastante agravado pelo fato de que não existem opções adequadas de tratamento (VOLAVKA, 1995).

Dessa forma, o dilema das pesquisas envolvendo o comportamento agressivo baseia-se em dois objetivos opostos: evitar danos e lesões tanto quanto for possível e, ao mesmo tempo, estudar os fenômenos comportamentais que representam de forma válida a essência biológica do comportamento agressivo (MICZEK, 2001).

Sugere-se que existem pelo menos dois tipos de agressão: 1) a premeditada ou instrumental e 2) a agressão impulsiva ou reativa (BARRAT *et al.*, 1999; BLAIR, 2004). A agressão premeditada envolve um planejamento antes da ação e tem um propósito definido. Esse comportamento tipicamente produz um ganho para o agressor, como por exemplo: um aumento do "status" em uma hierarquia social (BERKOWITZ, 1993; BLAIR, 2004). Na agressão impulsiva, eventos ameaçadores, frustrações ou provocações geram o comportamento agressivo, produzindo também raiva e irritação (BLAIR, 2004,). Esse segundo tipo de agressão é iniciado sem qualquer objetivo ou recompensa (BLAIR, 2004).

A impulsividade é um conceito multidimensional que incorpora a falha na inibição da resposta, o processamento rápido da informação, busca de novidades e intolerância ao atraso de um reforço (BARRAT, 1985, 1994; HORN, 2003). Esse termo é usualmente empregado para descrever comportamentos mal-adaptados que se caracterizam por serem

arriscados, prematuramente concebidos, impróprios para a situação e que geralmente resultam em conseqüências desagradáveis (DARUNA e BARNES, 1993; EVENDEN, 1999). Em contrapartida, atitudes impulsivas que culminam em resultados positivos podem ser vistas como indicadores de atrevimento, rapidez, espontaneidade, e coragem (DARUNA e BARNES, 1993; EVENDEN, 1999).

Em geral, comportamento agressivo, tentativas de suicídio e violência são relacionados com um prejuízo no controle impulsivo (PLUTCHIK e VAN PRAAG, 1989, 1995; HORN, 2003).

Uma parte considerável do conhecimento atual sobre a etiologia, neurobiologia e, particularmente, farmacologia da agressividade e impulsividade em humanos é baseado em modelos experimentais de agressão em animais (DE BOER e KOOLHAAS, 2005).

Ratos e primatas são espécies que vivem socialmente e formam uma hierarquia social (BARNETT, 1975, 2005; DE ALMEIDA *et al.*, 2005). O comportamento agressivo nesses animais é freqüentemente observado durante a formação dessa dominância hierárquica, principalmente durante o período pré-puberdade (STEINIGER, 1950; BERNSTEIN e GORDON, 1974; DE ALMEIDA *et al.*, 2005). Além disso, as fêmeas também podem tornar-se agressivas especificamente no período pós-parto. A agressividade nas fêmeas lactantes tem como objetivo defender a prole e é similar a dos machos dominantes em colônias (MOS *et al.,* 1989; BLANCHARD e BLANCHARD, 1990; LUCION e DE ALMEIDA, 1996).

O comportamento agressivo de camundongos é usualmente conhecido como territorial. Esses animais demarcam, patrulham e guardam seu ambiente, iniciando confrontos na presença de um intruso (VON HOLST, 1969; CRAWLEY *et al*., 1975; DE ALMEIDA *et al.*, 2005). Devido a esse fato, pesquisas experimentais realizadas com esses animais utilizam frequentemente um protocolo experimental baseado no confronto direto entre machos

residentes contra intrusos (MICZEK e O´DONNEL, 1978; CRAWLEY *et al.,* 1975; DE ALMEIDA *et al.*, 2005).

A análise das freqüências, duração, latência e padrões temporais e seqüenciais de todos os comportamentos e posturas observados nos diferentes tipos de animais fornece um quadro detalhado do comportamento agressivo ofensivo e defensivo (MICZEK e O´DONNEL, 1978; MICZEK *et al*., 2004).

De acordo com OLIVIER e OORSCHOT (2005), devido ao grande interesse no desenvolvimento de drogas que atuam seletivamente sobre a agressividade, vários paradigmas comportamentais relativos à agressão foram criados. Níveis excessivos de comportamento agressivo em animais de laboratório são obtidos principalmente através de quatro modelos experimentais: 1) farmacológicos (aumento da agressão pelo álcool), 2) comportamentais (agressão induzida por frustração), 3) ambientais (instigação ou provocação social) ou 4) por seleção genética (DE BOER e KOOLHAAS, 2005).

Em relação à utilização do álcool, sabe-se que a administração em doses baixas a moderadas promovem um aumento significativo da agressividade, sendo esse efeito observado somente em alguns indivíduos (MICZEK et al., 1992, 1998; MICZEK e DE ALMEIDA, 2001). Em camundongos, por exemplo, ocorre um aumento do comportamento agressivo em 20% dos animais após o tratamento com uma dose de 1.0 g/kg de etanol (VAN ERP e MICZEK, 1997; FISH, FACCIDOMO e MICZEK, 1999). Porém, quando administrado em altas doses, o álcool pode reduzir a agressão em muitas espécies animais devido aos seus efeitos sedativos (KRSIAK e BORGESOVA, 1973; SMOOTHY e BERRY, 1983; DE ALMEIDA et al., 2005).

Uma frustração gerada pela descontinuidade de um reforço também é um método eficiente para motivar e aumentar o comportamento agressivo (DOLARD et al., 1939; AMSEL e ROUSSEL, 1952; AZRIN et al., 1966; THOMPSON e BLOOM, 1966; KELLY,

1974; DE ALMEIDA e MICZEK, 2002). Diferentes espécies de animais podem exibir surtos de agressividade após a extinção de uma recompensa programada, incluindo ratos, camundongos, pombos e macacos (THOMPSON e BLOOM, 1966; CHEREK e PICKENS, 1970; CAPRARA, 1982; EVENDEN e RYAN, 1996; DE ALMEIDA e MICZEK, 2002). Indivíduos diferem na maneira de aceitar a interrupção de um reforço esperado. Essa "tolerância relativa" determina as quantidades variáveis de agressão observadas após uma frustração (CHEREK e PICKENS, 1970; DE ALMEIDA e MICZEK, 2002).

Por fim, a provocação social também é um protocolo experimental utilizado em roedores para aumentar os níveis de agressividade. Para a realização desse modelo, machos são mantidos em pares com uma fêmea, dentro de uma caixa residência por um determinado período. Nessas condições, o macho torna-se o "territorial ou residente" (OLIVIER e OORSCHOT, 2005). Para a indução de níveis elevados de agressão é realizada uma exposição prévia do animal residente a um oponente "instigador", que permanece protegido atrás de um anteparo. Depois disso, um intruso é colocado na caixa do animal residente, sem nenhuma proteção, permitindo o confronto direto entre eles (POTEGAL, 1991). Em geral, hamsters, camundongos e ratos iniciam os ataques com uma latência muito curta e com alta freqüência quando são testados com um intruso em suas caixas ou em um ambiente não-familiar, depois de terem sido previamente provocados na presença de um instigador (POTEGAL, 1991; FISH *et al.,*1999; DE ALMEIDA e MICZEK, 2002).

Níveis elevados de agressão são observados em relação ao "instigador" ou a outro oponente, presumivelmente devido ao "despertar da agressão" ou "prontidão do ataque" (DE ALMEIDA *et al.,* 2005). Além disso, a provocação social não interfere sobre outras atividades como a locomoção, comportamento sexual e alimentação (LAGERSPETZ e HAUTOJARVI, 1967; POTEGAL e TENBRINK, 1984; POTEGAL, 1991).

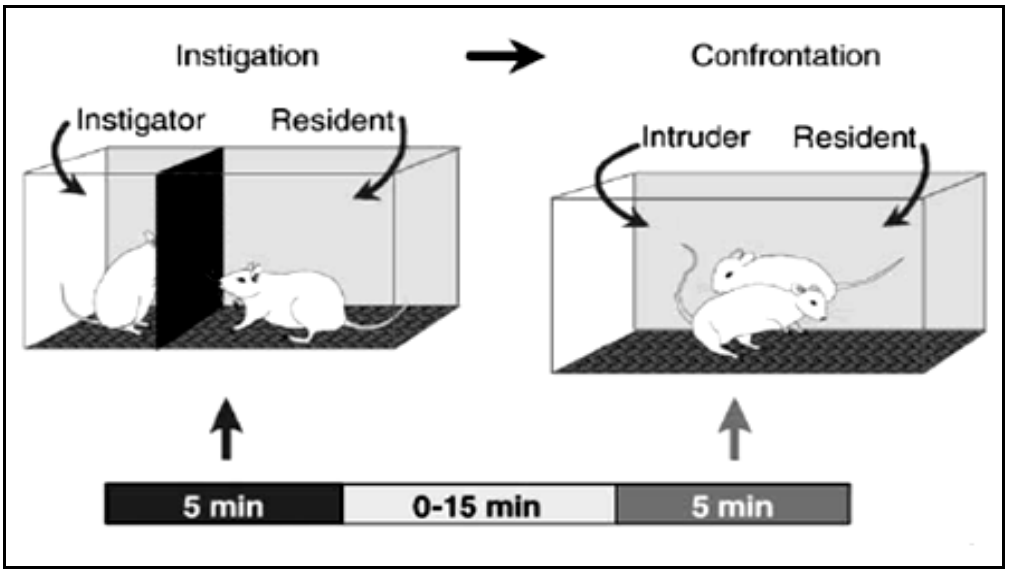

Figura 1. Protocolo da provocação social (DE ALMEIDA *et al*., 2005).

A agressividade é um comportamento primitivo, mas altamente conservado, e é razoável esperar que os mecanismos moleculares relacionados com a agressão como neurotransmissores, hormônios, citocinas, enzimas, fatores de crescimento e moléculas sinalizadoras, sejam similares entre os vertebrados (NELSON e CHIAVEGATTO, 2001). Assim, aspectos específicos do comportamento agressivo de cada espécie poderiam resultar da inserção de moléculas novas em um circuito neural pré-existente (NELSON e CHIAVEGATTO, 2001).

Postula-se que o sistema responsável pela cognição social e pela modulação da agressão envolva o córtex pré-frontal, além de áreas da amígdala medial que enviam projeções através da estria terminal para o hipotálamo e dele para a porção dorsal da substância cinzenta periaquedutal (PAG) (BLAIR E CIPOLOTTI, 2000). Esse sistema é organizado de uma maneira hierárquica, sendo que a agressão evocada na amígdala é dependente da integridade funcional do hipotámalo medial e da PAG, enquanto a agressão evocada pela PAG não é dependente da integridade funcional da amígdala (GREGG e SIEGEL, 2001; BLAIR, 2004).

O circuito descrito é responsável pela resposta comportamental dos animais frente a uma ameaça (BLAIR, 2004). Baixos níveis de estimulação advindos de uma ameaça distante geram um comportamento de congelamento; níveis mais altos de intimidação fazem com que o animal tente escapar do ambiente; finalmente, níveis intensos de uma atitude ameaçadora, em uma ambiente onde a fuga é impossível, levam o animal a exibir uma agressão reativa (BLANCHARD, BLANCHARD E TAKAHASHI, 1977).

Tanto a amígdala quanto o córtex pré-frontal desempenham diferentes papéis na modulação da circuitaria neural envolvida na agressão (BLAIR, 2004). A amígdala pode aumentar ou diminuir a responsividade do sistema sub-cortical relacionado com a agressividade, dependendo do ambiente social em que o animal está inserido; portanto, lesões na amígdala podem aumentar ou diminuir a probabilidade de uma agressão reativa (DAVIS, 2000; FUNAYAMA *et al.,* 2001; RAMAMURTHI, 1988; VAN ELST *et al.,* 2000; ZAGRODZKA *et al.,* 1998). Por outro lado, o controle pelo córtex pré-frontal ocorre em função de indícios sociais e emocionais, além do conhecimento de normas culturais e da posição hierárquica dos outros indivíduos (BLAIR, 2004).

Além do seu papel no controle do comportamento inibitório em geral, principalmente do comportamento agressivo e impulsivo (BLAIR, 2001; CARDINAL *et al.,* 2004; SEGUIN, 2004; SPINELLA, 2004; KHERAMIN *et al.,* 2005), o córtex pré-frontal também regula outras classes de comportamentos afetivos e motivacionais em roedores, primatas e humanos (MORGAN e LEDOUX, 1995; WALL e MESSIER, 2000; WALL *et al.,* 2003). Mais especificamente, estudos sugerem que as regiões medial e orbital do córtex pré-frontal estão mais relacionadas com a modulação da agressão, enquanto o córtex pré-frontal dorsolateral parece ter apenas um pequeno envolvimento (GRAFMAN *et al.*, 1996; BLAIR, 2004). Em contrapartida, a área infralímbica do córtex pré-frontal está associada com os

efeitos da ansiedade e com funções cognitivas, como a atenção e memória (WALL e MESSIER, 2002; DALLEY *et al.,* 2004).

Crianças e adultos com lesões no córtex pré-frontal medial e orbital demonstram pouco controle sobre suas emoções, apresentam um risco maior de expressar comportamentos agressivos e não se preocupam com as conseqüências de suas ações (GRAFMAN *et al.*, 1996; ANDERSON *et al.* 1999; DAVIDSON, 2000; BEST, WILLIANS E COCCARO, 2002). Além disso, várias condições psiquiátricas são relacionadas com um prejuízo geral do córtex pré-frontal, especialmente devido ao fato desses indivíduos serem incapazes de inibir atitudes agressivas e impulsivas. Assassinos mostram uma redução geral do metabolismo de glicose no córtex pré-frontal (RAINE *et al.*, 1994; BEST, WILLIANS E COCCARO, 2002). Pacientes com transtorno de personalidade que apresentam agressão impulsiva mostram uma redução de 11% no volume de substância cinzenta no córtex pré-frontal (RAINE, 2000; BEST, WILLIANS E COCCARO, 2002).

A agressão impulsiva pode estar relacionada com uma disfunção das projeções inibitórias do córtex pré-frontal, mais especificamente das regiões orbital e medial para a amígdala, que pode ser resultante de uma anormalidade neuroquímica envolvendo a serotonina (5-HT) (DAVIDSON, 2000; BEST, WILLIANS e COCCARO, 2002). Essa afirmação baseia-se no fato de que indivíduos exibindo um comportamento agressivo impulsivo possuem uma atividade serotonérgica pré- e pós-sináptica reduzida (LINNOILA *et al.*, 1983; COCCARO *et al.*, 1989; VIRKKUMEN *et al.*, 1994), sendo geralmente tratados para a redução desses sintomas com fármacos que inibem a recaptação de 5-HT (COCCARO e KAVOUSSI, 1997; BEST, WILLIANS e COCCARO, 2002). Além disso, sabe-se que o córtex pré-frontal contém uma alta densidade de receptores serotonérgicos, mais especificamente o 5-HT_{1A} e 5-HT_{1B}, e é um dos locais de ação mais importantes para ação de drogas serotonérgicas (BEST, WILLIANS e COCCARO, 2002).

Há mais de 40 anos postula-se que o sistema serotonérgico é essencial no controle da agressividade (BOER e KOOLHAAS, 2005). O grande dogma na relação entre a serotonina e a agressão consiste na idéia de que baixos níveis desse neurotransmissor estariam associados com o aumento no comportamento agressivo (OLIVIER e OORSCHOT, 2005). Essa relação inversa é baseada principalmente em estudos anteriores, nos quais as concentrações de serotonina no encéfalo foram reduzidas por agentes neurotóxicos, como a *para*-clorofenilalanina (pCPA) ou 5,7-diidroxitriptamina (5,7-DHT). Além disso, mensurações da atividade serotonérgica no líquido céfalo-raquidiano (LCR) de humanos baseadas nos níveis do principal metabólito da serotonina, o ácido 5-hidróxiindoleacetico (5-HIAA), confirmaram esse conceito e, apesar de várias críticas a respeito dos parâmetros utilizados nesses estudos, essa medida foi por muitos anos à única que refletia (indiretamente) o estado funcional do sistema serotonérgico (OLIVIER e OORSCHOT, 2005).

Estudos posteriores baseados nas diferenças entre indivíduos altamente agressivos e indivíduos pouco agressivos, mostraram uma correlação positiva entre o nível de agressão (alto ou baixo) e a concentração basal de 5-HT e 5-HIAA, contrariando uma possível relação inversa entre a propensão a ter um comportamento agressivo e déficits de serotonina (YODYINGYUAD *et al*., 1985; VOLAVKA *et al.*, 1990; VAN DER VEGT *et al.*, 2003; DE ALMEIDA *et al.,* 2005).

Acredita-se também que exista uma forte influência do sistema serotonérgico sobre a impulsividade, particularmente quando associada à agressão (EVENDEN, 1999; VAN ERP e MICZEK, 2000; FAIRBANKS et al., 2001; DALLEY, 2002). Muitos estudos têm correlacionado tendências impulsivas com uma deficiência na função serotonérgica e, semelhantemente, essa conclusão é baseada em medidas relativamente indiretas, como concentrações de 5-HIAA no LCR, atividade serotonérgica plaquetária, e efeitos

comportamentais e neuroendócrinos relativamente seletivos para agentes serotonérgicos (DALLEY, 2002).

Antidepressivos como o inibidor não seletivo da monoaminoxidase (enzima que degrada a serotonina – Monoamina oxidase MAO), tranicipromina e inibidores seletivos da recaptação de serotonina como a fluoxetina (COCCARO et al., 1990; CORNELIUS et al., 1991; MARKOVITZ, 1995; EVENDEN, 1999) e sertralina (KAVOUSSI et al., 1994; EVENDEN, 1999) são utilizados clinicamente no controle da agressividade e da impulsividade. O mecanismo de ação desses fármacos baseia-se na estabilização dos níveis de serotonina (EVENDEN, 1999).

O aminoácido essencial triptofano é o substrato para a produção da serotonina. A síntese desse neurotransmissor ocorre em duas etapas; primeiramente, o triptofano é convertido em 5-hidroxi-triptofano (5-HTP) pela enzima triptofano hidroxilase. Essa etapa é considerada o passo limitante para sua síntese, pois depende dos níveis de triptofano encontrados no fluido extracelular que banha os neurônios. Subseqüentemente, o 5-HTP é rapidamente descarboxilado pela enzima 5-HTP descarboxilase, produzindo-se então a serotonina (5-Hidroxitriptamina ou 5-HT). Após ser liberada na fenda sináptica, a serotonina é rapidamente removida pelo seu transportador específico (5-HTT), sendo transportada para uma vesícula sináptica ou degradada pela enzima monoamina oxidase (MAO) (BOULLOSA E LOPEZ-MATO, 1995; BEAR, CONNORS e PARADISO, 2002).

A serotonina ocupa um lugar de destaque entre os diferentes tipos de neurotransmissores por estar envolvida em numerosas funções fisiológicas, incluindo a ingestão alimentar, termorregulação, regulação do ciclo circadiano, locomoção, comportamento sexual, memória, vigília, nocicepção e migração neuronal (WILKINSON e DOURISH, 1991; PARENT *et al.,*1981; STEINBUSCH, 1981; SARI, 2004). Além disso,

esse neurotransmissor também está relacionado com muitas doenças psiquiátricas como a depressão, ansiedade e agressividade (SLEIGHT *et al.,* 1991; SARI, 2004).

O sistema serotonérgico contém um limitado, mas bem definido número de células (OLIVIER E OORSCHOT, 2005). Os corpos celulares dos neurônios serotonérgicos (soma) estão localizados principalmente na região do tronco cerebral, mais especificamente nos núcleos da Rafe, e se projetam para todas as regiões encefálicas (PARENT, DESCARRIES E BEAUDET, 1981; STEINBUSCH, 1981).

A multiplicidade de funções fisiológicas e comportamentais nas quais a serotonina está envolvida é ligada, em parte, a sua larga distribuição no sistema nervoso central (SNC) e pela sua diversidade de receptores (SARI, 2004). Mais de 14 subtipos de receptores serotonérgicos têm sido determinados por técnicas moleculares e farmacológicas, sendo classificados em 5-HT_{1A}, 5-HT_{1B}, (citado anteriormente), 5-HT_{1D}, 5-$ht1_{1E}$, 5-ht_{1F}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_3, 5-HT_4, 5-ht_{5A}, 5-HT_6, e 5-HT_7. Os receptores 5-HT fazem parte de duas famílias extensas - receptores acoplados à proteína G e canais iônicos dependentes de ligantes. Os receptores 5-$HT_{1,2,4,5,6,\ e\ 7}$ são acoplados à proteína G e ativam as cascatas da adenilato ciclase e fosfatidiinositol, enquanto os receptores 5-HT_3 são canais iônicos (SARI, 2004; OLIVIER e OORSCHOT, 2005).

Os receptores 5-HT_{1A} são chamados de auto-receptores somatodendríticos quando localizados pré-sinapticamente como, por exemplo, nos neurônios serotonérgicos dos núcleos da Rafe (ZIFA e FILLION, 1992). Da mesma forma, os receptores 5-HT_{1B} também são considerados auto-receptores quando estão presentes no neurônio pré-sináptico, mas eles diferem dos receptores 5-HT_{1A} por serem encontrados no terminal axonal e não na região do corpo celular (ZIFA e FILLION, 1992; BOSCHERT *et al*., 1994). Adicionalmente, tanto os receptores 5-HT_{1A} quanto os receptores 5-HT_{1B} são encontrados pós-sinapticamente, sendo considerados heteroreceptores devido ao fato de estarem presentes em terminais axonais de

neurônios não-serotonérgicos. Além disso, os receptores 5-HT_{1B} encontrados em ratos e camundongos são funcionalmente homólogos aos receptor 5-$HT_{D\beta}$ encontrado em humanos, diferindo apenas por um único aminoácido (asparagina versus treonina) no sétimo domínio transmembrana do receptor (SCHLICKER, 1997; SARI, 2004). Os demais receptores serotonérgicos são encontrados pós-sinapticamente (BONAVENTURA *et al.,* 1998).

Acredita-se que a atividade de um neurônio serotonérgico é regulada pelos auto-receptores 5-HT_{1A} e 5-HT_{1B}, além dos transportadores de serotonina 5-HTT (PIÑEYRO e BLIER, 1999). Os auto-receptores somatodendríticos 5-HT_{1A} quando ativados inibem o disparo do neurônio e, conseqüentemente, a liberação de serotonina. Além disso, a ativação de auto-receptores 5-HT_{1B} nos terminais sinápticos também contribui para a regulação do disparo celular, levando a uma inibição direta da liberação de serotonina. Os transportadores 5-HTT, encontrados nos terminais axonais, corpos celulares e dendritos são os responsáveis pela recaptação da serotonina liberada na fenda sináptica, um mecanismo muito importante para restaurar as condições neuronais de repouso, permitindo um novo disparo e evitando uma superestimulação dos receptores (OLIVIER E OORSCHOT, 2005).

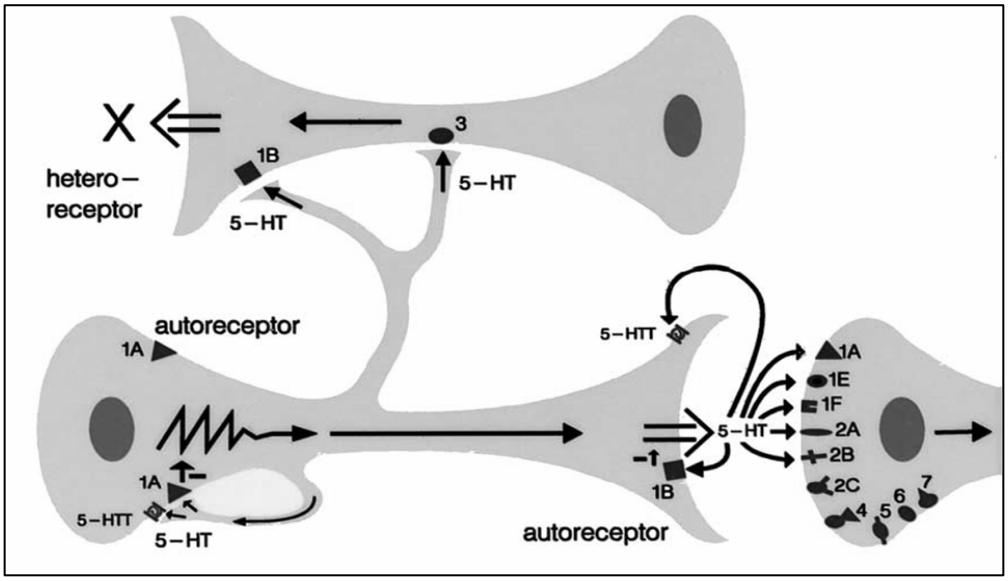

Figura 2. Subtipos de receptores serotonérgicos (autoreceptores e heteroreceptores) e sua possível localização neuronal (OLIVIER e OORSCHOT, 2005).

A interação desses processos modulatórios leva a um padrão de disparo finamente regulado dos neurônios serotonérgicos. Todavia, é indispensável levar em consideração o fato de que outros sistemas (GABA-érgico, noradrenérgico, colinérgico, glutamatérgico, dopaminégico e outros) também influenciam os neurônios serotonérgicos e que é a interação de todos que determina os resultados funcionais (PIÑEYRO e BLIER, 1999; ADELL *et al*., 2002; OLIVIER E OORSCHOT, 2005).

Estudos farmacológicos utilizando agonistas dos receptores 5-$HT_{1A/1B}$ e antagonistas dos receptores e 5-$HT_{2A/C}$ mostraram uma redução do comportamento agressivo em várias espécies animais, incluindo humanos (TOMPKINS *et al*., 1980; BENTON *et al*., 1983; FLANNELLY *et al*., 1985; PARMIGIANI *et al*., 1989; COCCARO, GABRIEL e SIEVER, 1990; NIKULINA, 1991; OLIVIER e MOS, 1992; SANCHEZ *et al*., 1993; BELL e HOBSON, 1994; SANCHEZ e HYTTEL, 1994; OLIVIER *et al*., 1995; SANCHEZ, ARNT e MOLTZEN, 1996; DE ALMEIDA e LUCION, 1997; JOPPA, ROWE e MEISEL, 1997; LOPEZ-MENDOZA, GUILAR-BRAVO e SWANSON, 1998; DE ALMEIDA *et al*., 2001; OLIVIER, 2001; DE ALMEIDA e MICZEK, 2002; OLIVIER, 2004; KNYSHEVSKI *et al*., 2005). Todavia, de maneira semelhante aos tratamentos farmacológicos utilizados atualmente na prática clínica, esses agonistas dos receptores 5-$HT_{1A/1B}$ e antagonistas dos receptores e 5-$HT_{2A/C}$ possuíam efeitos anti-agressivos em doses que induzem sedação, inatividade motora e comportamentos estereotipados (DE BOER e KOOLHAAS, 2005).

Pesquisas posteriores empregando análises etiológicas detalhadas revelaram que agonistas mais seletivos para os receptores 5-HT_{1A} (alnespirona, S-15535, 8-OH-DPAT; DE ALMEIDA e LUCION, 1997; DE BOER *et al.,* 1999, 2000), uma mistura de agonistas dos receptores 5-$HT_{1A/B}$ (eltoprazine; OLIVIER *et al.,* 1995) e vários agonistas específicos dos receptores 5-HT_{1B} (CP-93, 129, CP-94, 253, anpirtolina, TFMPP e zolmitriptan; BELL e HOBSON, 1994; DE ALMEIDA *et al.,* 2001; MICZEK *et al.,* 2004; VEIGA *et al*., 2007)

promovem uma notável inibição do comportamento agressivo sem alterar o restante do repertório comportamental (DE BOER e KOOLHAAS, 2005). Em particular, agonistas dos receptores 5-HT_{1B} mostram efeitos mais seletivos sobre o comportamento agressivo de camundongos quando comparados àqueles que agem sobre os receptores 5-HT_{1A} (MICZEK *et al.,* 2004; OLIVIER, 2004). Camundongos *knock-out* para o gene que codifica o receptor 5-HT_{1B} confirmam que esses animais possuem níveis elevados de agressividade (BOUWKNECHT *et al*., 2001), consumindo também mais álcool e cocaína (ROCHA *et al*., 1998). Isto sugere que este receptor, além de estar envolvido no aumento do comportamento agressivo, também está relacionado com a impulsividade (LESCH e MERSCHDORF, 2000).

O problema da especificidade comportamental, isto é, reduzir o comportamento agressivo sem comprometer outros elementos não-agressivos, bem como, o mecanismo neurobiológico exato no qual os agentes farmacológicos atuam para reduzir o comportamento agressivo (localização e sítio específico do receptor) ainda são alvos de pesquisas (OLIVIER *et al.,* 1995; MICZEK *et al.,* 1998a,b; DE BOER *et al.,* 1999; DE BOER e KOOLHAAS, 2005).

2 HIPÓTESE

O estabelecimento de uma possível correlação entre os níveis de serotonina e a ativação de receptores serotonérgicos específicos com uma redução da agressividade, requer o entendimento de mudanças locais em regiões cerebrais responsáveis pela modulação do comportamento agressivo. Além disso, agentes terapêuticos possivelmente eficazes no manejo da violência em humanos precisam ser avaliados mediante o emprego de paradigmas experimentais que provocam níveis excessivos de agressão em animais de experimentação. Desse modo, este trabalho pretende verificar se agonistas dos receptores 5-HT_{1A} e 5-HT_{1B}, quando microinjetados especificamente no córtex pré-frontal, promovem efeitos anti-agressivos em camundongos submetidos ao protocolo de provocação social (caracterizado por elevar o comportamento agressivo). Pretende-se também confirmar a especificidade dos receptores envolvidos na modulação da agressão com a microinjeção de antagonistas seletivos dos receptores 5-HT_{1A} e 5-HT_{1B} na mesma região encefálica.

3 OBJETIVOS

3.1 Objetivo geral

Analisar os efeitos da microinjeção de agonistas e antagonistas dos receptores 5-HT_{1A} e 5-HT_{1B}, especificamente na região ventro orbital do córtex pré-frontal, sobre o comportamento agressivo de camundongos machos submetidos ao protocolo de provocação social.

3.2 Objetivos específicos

- Estabelecer uma curva dose-efeito sobre o comportamento agressivo de camundongos provocados socialmente a partir da microinjeção de diferentes doses de 8-OH-DPAT e CP-93,129 (agonistas específicos dos receptores 5-HT_{1A} e 5-HT_{1B}, respectivamente) na região ventro orbital do córtex pré-frontal.
- Confirmar a especificidade dos receptores possivelmente envolvidos na modulação do comportamento agressivo através do pré-tratamento com WAY-100,635 e SB-224,289, antagonistas seletivos dos receptores 5-HT_{1A} e 5-$HT_{1B,}$ respectivamente.
- Verificar alterações em outras atividades motoras com os tratamentos empregados.

4 MÉTODOS E RESULTADOS

4.1 Artigo - Lígia Aline Centenaro, Karin Vieira, Nicole Zimmemann, Klaus Alexander Miczek, Aldo Bolten Lucion, Rosa Maria Martins de Almeida. Social instigation and aggressive behavior in mice: role of 5-HT_{1A} and 5-HT_{1B} receptors in the prefrontal cortex. **Psychopharmacology** (submitted).

SOCIAL INSTIGATION AND AGGRESSIVE BEHAVIOR IN MICE: ROLE OF 5-HT_{1A} AND 5-HT_{1B} RECEPTORS IN THE PREFRONTAL CORTEX

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Abstract

Rationale: Social instigation is used in rodents to induce high levels of aggression, a pattern of behavior similar to that of violent individuals. This procedure consists of a brief exposure to a provocative stimulus male, before direct confrontation with an intruder. Studies using 5-HT_{1A} and 5-HT_{1B} receptor agonists show an effective reduction in aggressive behavior. An important site of action for these drugs is the ventral orbito frontal cortex (VO PFC), an area of the brain which is particularly relevant in the inhibitory control of aggressive and impulsive behavior.

Objectives: To assess the anti-aggressive effects of 5-HT_{1A} and 5-HT_{1B} agonist receptors (8-OH-DPAT and CP-93,129) in the VO PFC of socially provoked male mice. To confirm the specificity of the receptor, 5-HT_{1A} and 5-HT_{1B} antagonist receptors (WAY-100,635 and SB-224,289) were microinjected into the same area, in order to reverse the agonist effects.

Results: 8-OH-DPAT (1.0 μg) reduced the frequency of attack bites. The lowest dose of CP-93,129 (0.1 μg) also decreased the number of attack bites and lateral threats. 5-HT1A and 5-HT1B receptor agonists differed in their effects on non-aggressive activities, the former decreasing rearing and grooming and the latter increasing these acts. Specific participation of the 1A and 1B receptors was verified by reversal of anti-aggressive effects using selective antagonists WAY-100,635 (10.0 μg) and SB-224,289 (1.0 μg).

Conclusions: The decrease in aggressiveness observed with microinjections of 5-HT_{1A} and 5-HT_{1B} receptor agonists into the VO PFC of socially provoked mice, supports the hypothesis that activation of these receptors modulates high levels of aggression in a behaviorally specific manner.

Keywords: aggression--social instigation--serotonin--5-HT_{1A} receptor--5-HT_{1B} receptor--prefrontal cortex

Introduction

Aggression can be a biologically adaptive behavior, important in acquiring resources, maintaining dominance and establishing social hierarchy (Miczek et al. 2002). However, in human and veterinary clinics, aggression can be a significant problem, because escalated aggression may result in serious damage to others. This situation is aggravated by the fact that there are no selective pharmacological treatment options (Volavka 1995).

Under specific conditions, laboratory animals can exhibit high levels of aggression, a pattern of behavior with certain aspects that are similar to those of violent individuals (Fish et al. 1999; de Boer and Koolhaas 2005). Social instigation is one of the procedures used in rodents to increase aggressive behavior. It consists of exposing the resident animal to a rival that can be seen, heard and smelled, but it is protected by a screen and cannot be expelled from the home cage of the resident (Potegal 1991; De Almeida et al. 2005). Hamsters, mice and rats start attacks with a very short latency and high frequency after social instigation (Potegal 1991; Fish et al. 1999; De Almeida and Miczek 2002).

Pharmacological agents that act on 5-HT_{1A} and 5-HT_{1B} autoreceptors may induce a more pronounced reduction in aggressive behavior than the current treatments with antipsychotic medications, β-adrenergic blockers and selective serotonin reuptake inhibitors (Volavka 1995; Fish et al. 1999; Caldwell and Miczek 2008). Selective 5-HT_{1A} receptor agonists such 8-OH-DPAT, alnespirone, S-15535 (Joppa et al. 1997; De Boer et al. 2000; De Boer and Koolhaas 2005) and some specific 5-HT_{1B} receptor agonists such as CP-93,129, CP-94,253 and zolmitriptan (Fish et al. 1999; De Almeida et al. 2001; Veiga et al. 2007) have efficacious anti-aggressive activity, both on species-typical and in escalated aggression. The role of these receptors in aggressive behavior was convincingly confirmed with selective 5-HT_{1A} receptor antagonist WAY-100,635 and 5-$HT_{1D/B}$ receptor antagonist GR-127935, that

effectively blocked the anti-aggressive effects of the agonists (Lopez-Mendoza et al. 1998; Miczek et al. 1998b; Fish et al. 1999; De Boer et al. 1999; 2000; De Almeida et al. 2001). However, it is important to note that 5-HT_{1A} and 5-HT_{1B} agonists have been shown to exert contrasting effects on drug taking, sexual behavior and food intake (Simansky and Vaidya, 1990; Crabbe et al. 1996; Parsons et al. 1998; Ahlenius et al. 2001).

The prefrontal cortex is a brain region that contains a high density of 5-HT_{1A} and 5-HT_{1B} receptors and it has been identified as particularly important in the inhibitory control of the sub-cortical circuit mediating aggressive and impulsive behavior (De Almeida et al. 2005; Blair 2004). Patients with orbital and medial prefrontal cortex lesions exhibit impulsive and aggressive behavior, showing lack of control and unawareness of the implications of their actions (Grafman et al. 1996; Anderson et al. 1999; Davidson 2000). Thus, impulsive aggression may be related to a dysfunction of inhibitory projections from the orbital/medial prefrontal cortex to the amygdala, which can result from a neurochemical abnormality that may involve serotonin (Davidson 2000; Best et al. 2002).

The current study aims to verify whether 5-HT_{1A} and 5-HT_{1B} agonists microinjected specifically into ventral orbitofrontal cortex may reduce the escalated aggressive behavior in socially provoked mice. Besides, the study intends to confirm the specificity of the receptor involved in the modulation of the aggressive behavior with the microinjection of selective 5-HT_{1A} and 5-HT_{1B} antagonist receptors (WAY-100,635 and SB-224,289, respectively) into the same brain area.

Materials and method

Animals

Subjects were adult male mice CF1, *Mus musculus*, (acquired from FEEPS, Porto Alegre, RS, Brazil), weighing from 40 to 50 g. The mice were cared for accordance with the guidelines of the National Institute of Health (NIH) and Colégio Brasileiro de Experimentação Animal (COBEA). Firstly, the animals were divided into "residents" (*n=110*), "intruders" (*n=110*) and "instigators" (*n=50*). Each resident was housed in clear polycarbonate cages (28 x 17 x 14 cm) with a female (*n=110***)** from the same strain. Intruders and instigators were maintained in groups of ten, in standard polycarbonate boxes (46 x 24 x 15 cm). All mice were in the same room under 12:12 h light/dark cycle (lights on at 6:00), in a temperature controlled environment (20 ± 2 ºC) with food and water available *ad libitum*. The animals were tested during the light phase of the photo cycle from 9:00 to 16:00 h.

Resident-intruder confrontations

Resident males were allowed to acclimate for 21 days with their female cagemate in the laboratory environment. After this period, each resident was submitted to successive confrontations with a male intruder (3 times a week with intervals of at least 24 h) to establish the baseline of aggressive behavior. In these tests, female and pups were removed and a male intruder was placed into the home cage of residents. Each behavioral test lasted 5 minutes and if no attack bite occurred, the experimental session was terminated at 5 minutes (Miczek and O'Donnell 1978). Only the animals that delivered more than 10 bites, were included in the experiment. If the resident was attacked, the intruder was substituted immediately. According to Winslow and Miczek (1984) during the first confrontations the levels of aggression from

the resident mice were more variable than after some tests (usually six or seven confrontations with the same intruder).

Social instigation

Once aggressive behavior towards the intruder was reliably displayed at stable levels, the first social instigation was performed. This procedure consisted of removing the female and pups and placing a clear perforated cylinder (18 x 6 cm) containing an instigator in the center of resident's cage for 5 minutes (Fish et al. 1999), followed by an interval of additional 5 minutes. At the end of this period, a male intruder was inserted in the resident's box, without any protection, allowing direct contact between the two animals. Each behavioral test lasted 5 minutes and was videotaped.

Surgery

The next day, each resident mouse was anesthetized with ketamine and xylazine (100mg/kg and 10mg/kg body weight intramuscularly, respectively), placed in a stereotaxic frame and implanted with a 26-gauge guide cannula (Plastics One Inc., USA). The cannula was aimed at the ventral orbitofrontal cortex (VO PFC) in the left hemisphere using the following coordinates: 2.3 mm anterior to bregma, 0.6 mm lateral to the mid-sagittal line and 1.0 mm below the dura mater, based on Paxinos and Franklin (1997). After surgery, an obdurator (33-gauge; Plastics One Inc., USA) was inserted to prevent cannula blockage and the animals were properly warmed until recovery from the surgery.

Microinjections and behavioral analysis

After 72 h of recovery from surgery, microinjections of 5-HT_{1A} and 5-HT_{1B} agonists and antagonists or vehicle were accomplished. For the microinjections, the obdurator

was removed and a 33-gauge injector (Plastics One Inc., USA) was inserted through the guide cannula. The injector was 1.0 mm longer than the guide cannula, allowing for the introduction of the drug 2 mm below the dura mater, into the target area. Solutions were slowly infused for a period of 2 minutes, using the injector connected via a polyethylene tube (P20) to a Hamilton syringe fitted into a pump (WPI - Sp 210 iv syringe pump, model 210, USA). The injector was left *in situ* for 30 additional seconds after microinjection, allowing the complete diffusion of the solution and preventing backflow.

8-OH-DPAT or CP-93,129 in different doses (0.1, 0.56 and 1.0 μg/0.2 μl) or vehicle (saline solution 0.9%) was administered 15 minutes before the behavioral tests. Likewise, for a 5-minute period a protected instigator was placed in the center of the resident's cage, followed by an interval of 5-min. After that, an intruder was inserted (without any protection) in the resident's cage, and the behavior of the animals was recorded for 5 min. Each animal received only one of the doses.

In another group of animals, WAY-100,635 (10.0 μg/0.2 μl dose) or SB-224,289 (1.0 μg/0.2 μl dose) was microinjected 30 minutes before 8-OH-DPAT (1.0 μg/0.2 μl), CP-93,129 (0.1 μg/0.2 μl) or vehicle, respectively. Again, residents were submitted to a social confrontation 5 minutes after the last injection. In this experiment, the animals received two microinjections, the first microinjection with the specific antagonist and the other with the agonist. The vehicle used for the two antagonists was physiological saline solution.

The resident-intruder confrontations after microinjections were videotaped and later analyzed by a trained investigator using The Observer software (version 3.0, Noldus, The Netherlands). The behavioral repertoire, previously defined for Miczek and O'Donnell (1978), included frequency of aggressive elements such as sniffing the intruder, sideways threat, attack bite, pursuit, tail rattle and duration of non-aggressive elements such as grooming, rearing and walking.

Drugs

8-OH-DPAT (8 - hydroxy - 2 - (di-*n*-propylamino) tetralin hydrobromide; Sigma, St. Louis, MO, USA), CP-93,129 (1,4 - Dihydro - 3 - [1,2,3,6 - tetrahydro - 4 - pyridinyl] -SH - pyrrolo [3,2 - b] pyridine - 5 - one dihydrochloride; Pfizer, Groton, CT, USA) and WAY-100,635 (N - [2 - [4 - (2 - methoxyphenyl) - 1 - piperazinyl] ethyl] - N -(2, pyridinyl) cyclohexanecarboxamide trihydrochloride; Wyeth-Ayerst, Princeton, NJ, USA) were dissolved in 0.9% saline. SB-224,289 (2,3,6,7 - Tetra - hydro - 1′ - methyl - 5 - {2′ - methyl - 4′ - [(5 - methyl - 1,2,4 - oxadiazole - 3 - yl) biphenyl - 4 - yl)] carbonyl} furo [2,3 - f] - indole - 3 - spiro - 4′ - piperidine oxalate; Pfizer, Groton, CT, USA) was dissolved with the aid of sonication in 0.9% saline and was gently warmed.

Histological analysis

At the end of the experiment, all resident mice were deeply anesthetized (ketamine and xylazine) and intracardially perfused with 0.9% saline and 0.4% paraformaldehyde (PFA). Brains were removed and fixed in 0.4% PFA until sliced on a vibratome in 60 μm thick coronal sections. The slices were placed on gelatinized slides and examined by a microscope interfaced to a computer. The location of the guide cannula was verified through images of brain slices captured with specific software (Neuro Image Pró 1.0). Mice with placements outside VO PFC served as anatomical controls and were analyzed separately.

Statistical analysis

All data were expressed as mean ± SEM. The effect of social instigation on aggressive behavior was analyzed using a paired *Student t* test, comparing species-typical baseline aggression versus aggression after social instigation.

Dose-effect data from each agonist (8-OH-DPAT and CP-93,129) and antagonist (WAY-100,635 and SB-224,289) were separately analyzed using a one-way analysis of variance (ANOVA). When there were statistically significant F values ($p \leq 0.05$), Bonferroni post hoc tests were conducted comparing drug treatments with the corresponding vehicle group.

Regarding non-aggressive motor behaviors, the data from all groups with agonist and antagonist treatments were compared with those from their respective controls using ANOVA. When significant differences were found, Bonferroni post hoc tests were accomplished.

Results

Heightened aggression after social instigation

Social instigation significantly increased the aggressive behavior of all mice when compared to their species-typical aggression baseline, as indicated by the increase in frequency of attack bites (t (109)=2.92; p=0.004; Fig. 1). The baseline of the aggressive behaviors was expressed by the average of the last three resident-intruder confrontations tests.

Histological verification

Histological analysis showed that among all the animals studied, 96 presented injections correctly positioned in the target area (Fig. 2). The remaining animals ($n=14$) were included in the anatomical control group (not on target).

Dose-effects of 8-OH-DPAT and CP-93,129 on instigation-heightened aggression

8-OH-DPAT microinjected into VO PFC reduced the frequency of attack bites at the 1.0 μg dose (F (4,32)=4.26; p=0.007; Fig. 3a and Table 1) as compared to the control

group. The frequency of sideways threat (Fig. 3b), sniffing the intruder and tail rattling were unchanged as compared to the control group (Table 1). Moreover, the effective dose of 8-OH-DPAT (1.0 μg) microinjected outside of VO PFC did not alter aggressive behaviors (Table 1).

The lowest dose of CP-93,129 (0.1 μg) exerted significant anti-aggressive effects when microinjected into VO PFC. Specifically, CP-93,129 reduced the frequency of attack bites (F (4,35)=2.92; p=0.03; Fig. 4a and Table 1) and sideways threat (F (4,35)=3.03; p=0.02; Fig. 4b and Table 1) as compared to the control group. The frequency of sniffing the intruder and tail rattling was unchanged as compared to the control group (Table 1). Aggressive elements remained unaltered with the dose (0.1 μg) microinjected outside of VO PFC (Table 1).

Dose-effects of 8-OH-DPAT and CP-93,129 on non-aggressive behaviors

The frequency and duration of walking, grooming and rearing were not significantly different with any of the 8-OH-DPAT and CP-93,129 doses studied when compared to the control group (Table 1). The results of 5-HT_{1A} and 5-HT_{1B} receptor agonists showed only subtle alterations in some elements of motor activity when compared to the vehicle group, the former decreasing rearing and grooming and the latter increasing these acts. However, the two drugs differ significantly from each other when the duration measures from animals microinjected with 8-OH-DPAT were compared to those from the animals microinjected with CP-93,129 (Fig. 5).

Antagonism/Combination studies

Pretreatment with WAY-100,635, a selective 5-HT_{1A} receptor antagonist (10 μg), antagonized the reduction in the frequency of attack bites produced by 8-OH-DPAT (F

(2,17)=0.29; *p*=0.74; Fig. 3a and table 2). Sideways threat (Fig. 3b), sniffing the intruder and tail rattling were not significantly modified when compared to the values from the control group (Table 2). No significant effects on aggressive and non-aggressive behaviors were found after microinjections of vehicle or WAY-100,635 plus vehicle in VO PFC (Table 2). The duration of walking, grooming and rearing remained unaltered by pretreatment with WAY-100,635 and 8-OH-DPAT as compared to the control group (Table 2).

Pretreatment with selective 5-HT_{1B} receptor antagonist SB-224,289 (1.0 μg/0.2 μl) completely blocked the decrease in the frequency of attack bites and sideways threat produced by CP-93,129 as compared to the control group (*F* (2,17)=1.71; *p*=0.21; Fig. 4a and Table 2; *F* (2,17)=1.52; *p*=0.24; Fig. 4b and table 2). Sniffing the intruder and tail rattling were unchanged as compared to the control group (Table 2). No significant effects on aggressive and non-aggressive behaviors were found after microinjections of vehicle or SB-224,289 plus vehicle into VO PFC (Table 2). Moreover, pretreatment with SB-224,289 and CP-93,129 did not reduce the duration of non-aggressive elements, such as walking, grooming and rearing when compared to the control group (Table 2).

Discussion

The present study confirms that social instigation is an experimental procedure that increases robustly and significantly the levels of aggression. In addition, the results demonstrate that 8-OH-DPAT and CP-93,129 exert significant anti-aggressive effects when microinjected into VO PFC of instigated animals. The 1.0 μg dose of 8-OH-DPAT significantly reduced the frequency of attack bites. The lowest dose of CP-93,129 (0.1 μg) decreased the frequency of attack bites and sideways threats as compared to the control group, showing a more potent anti-aggressive effect than 8-OH-DPAT. Importantly the specific

participation of these receptors was confirmed by reversal of anti-aggressive effects using selective antagonists WAY-100,635 and SB-224,289. In relation to motor activities, there was no significant effect with 5-HT_{1A} receptor agonist or 5-HT_{1B} receptor agonist when compared to control group, but just a trend. However, the two compounds differ from each other in their effects on rearing and grooming. The current results on these behavioral elements are in concordance with the contrasting effects of these 5-HT receptor agonists on sexual behavior (Ahlenius et al. 2001), food intake (Simansky and Vaidya, 1990) and drug taking (Parsons et al. 1998; Crabbe et al. 1996) were found.

Aggressive confrontations induce *c-fos* activation in the prefrontal cortex, and this activation was especially strong in mice selected for high aggressive behavior (Halász et al. 2006; Haller et al. 2006). Medial and orbital regions of prefrontal cortex have been clearly related with the modulation of impulsive aggression, while dorsolateral region appears to play a minor role (Grafman et al. 1996; Blair 2004). In humans, anatomical evidence exits for a general deficit of prefrontal cortex in several psychiatric conditions, which are characterized by the inability to inhibit aggressive and impulsive behavior (Best et al. 2002). For example, patients with antisocial personality disorder who displayed impulsive aggression showed an 11% reduction in the volume of prefrontal cortex, and murderers exhibit a general reduction in glucose metabolism within the prefrontal cortex (Raine et al. 1994; Raine et al. 2000). Furthermore, prefrontal cortex contains a high density of 5-HT_{1B} receptors and it is one of the most important sites of action for serotonergic drugs (De Almeida et al. 2006).

8-OH-DPAT (*pKi* = 8.7) is consistently used in studies characterizing the behavioral effects of 5-HT_{1A} receptors, including also those on aggressive behavior. WAY-100,635, the 5-HT_{1A} receptor antagonist, has higher specificity for this receptor subtype (*pKi* = 9.0). When given systemically, 8-OH-DPAT, flesinoxan, and partial agonists such buspirone and ipsapirone effectively and potently decrease aggressive behavior in various

animal species ranging from invertebrates, rodents, guinea pigs, primates to humans (Tompkins et al. 1980; Olivier et al. 1989; Mos et al. 1993; Olivier et al. 1994; Bell and Hobson 1994; Joppa et al. 1997; De Boer et al. 1999; 2000; Van Der Vegt et al. 2001). However, the most effective doses of these agonists compromise several non-aggressive elements of the behavioral repertoire (Olivier et al. 1989; Mos et al. 1993; Sanchez et al. 1993; Olivier et al. 1994; Miczek et al. 1998a; Miczek et al. 1998b; Fish et al. 1999; De Almeida et al. 2001).

Previous studies using systemic injections of selective 5-HT_{1B} receptor agonists, zolmitriptan, CP-94,253 and CP-93,129, demonstrate a reduction in the frequency of attacks bites and lateral threats similar to 5-HT_{1A} receptor agonists. The decrease of aggressive behavior after administration of 5-HT_{1B} receptor agonists was observed in species-typical aggression and in several models of heightened aggression (for example, social instigation, frustration procedures or consumption of moderate doses of alcohol). Importantly, those anti-aggressive effects were highly specific, that is, not accompanied by an increase in behavioral inactivity or sedation (Fish et al. 1999; De Almeida et al. 2001; Miczek and De Almeida 2001; De Almeida and Miczek 2002; Bannai et al. 2007; Fish et al. 2008). CP-93,129 is the agonist with the highest selectivity for 5-HT_{1B} (pKi = 8.1), as well as the antagonist SB-224,289 (pKi = 8.2), when compared to other 5-HT receptor subtypes (Roberts et al. 2001). In the current experiments, CP-93,129 decreased aggressive behavior at the lowest dose, suggesting that 5-HT_{1B} agonist receptors have more selective and potent anti-aggressive effects than agonists acting on 5-HT_{1A} receptors (Miczek et al. 2004; Olivier 2004). Furthermore, we could infer that microinjections of 5-HT_{1B} agonist receptors in VO PFC reduce aggressiveness only in a narrow range of activation, without a systematic dose-effect relation (De Almeida and Lucion 1997).

The participation of serotonin in adaptive aggressive behavior could differ from its role in violence defined as the pathological form of aggression (De Boer and Koolhaas 2005). The decrease in aggressive behavior after social instigation with 5-HT_{1A} and 5-HT_{1B} agonist receptors in the VO PFC can be hypothesized to be due to stimulation of post-synaptic receptors, although activation of pre-synaptic autoreceptor may also exert anti-aggressive effects (Mos et al. 1993; De Almeida and Lucion 1997; Bannai et al. 2007).

Further supporting evidence comes from other studies using local intracerebral injection in resident male rats (Mos et al. 1992; 1993). Injection of eltoprazine (a mixed 5-$HT_{1A/1B}$ agonist) into the third ventricle, aimed at activating post-synaptic 5-HT receptors, led to anti-aggressive effects, whereas injection of eltoprazine into the raphe nuclei (activating somatodendritic autoreceptores) had no effects on aggressive behavior (Olivier and Oorschot 2005). On the other hand, microinjections of 8-OH-DPAT and CP-93,129 at raphe nuclei also decrease aggressive behavior, indicating a possible role for presynaptic receptors in these agonists effects (Mos et al. 1993; De Almeida and Lucion 1997; Bannai et al. 2007). Studies using the neurotoxin 5,7-dihydroxytryptamine to reduce the number of pre-synaptic neurons containing 5-HT into the median and dorsal raphé (but not post-synaptic) found that lesions of these neurons do not change the anti-aggressive effects of eltoprazine or zolmitriptan (Sijbesma et al. 1991; De Almeida et al. 2001). However, the neurotoxin technique is limited by incomplete destruction (approximately 60-80%) of ascending pre-synaptic 5-HT neurons (De Almeida et al. 2001) and possibly the depletion by 5,7-DHT causes receptor upregulation, altering the sensitivity of post-synaptic 5-HT receptors (Sijbesma et al. 1991; Frankfurt et al. 1993, 1994; Manrique et al. 1994; Dugar and Lakoski 1997; Van de Kar et al. 1998; De Almeida et al. 2001). Further studies are necessary to delineate the exact site of action of these receptor agonists for their anti-aggressive effects.

In conclusion, the anti-aggressive effects observed with microinjections of specific 5-HT_{1A} and 5-HT_{1B} receptor agonists in socially provoked male mice seem to be associated with an increase in 5-HT levels at the prefrontal cortex, most likely due to activation of post-synaptic sites.

Acknowledgements

The authors would like to thank Mr. Marcelo Grilo for his technical support and Mr. Dirson Stein for his important assistance and animal care. CNPq and FAPESP supported this research.

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Fig. 1 The effects of social instigation on aggression in the resident male mice. The aggressive behavior portrayed is the frequency of attack bites towards the male intruder. Vertical bars represent the mean ± SEM and asterisks indicate the statistical difference between the groups, $p \leq 0.05$.

Fig. 2 A Photomicrograph showing the correct placement of guide cannula and injection at VO PFC region.

B, C Schematic representation of successive coronal sections of the mouse brain showing the histological verification of injection placement (n=96) in the ventral orbital frontal cortex (rostral to caudal: 2.68, 2.58, 2.46, 2.34 and 2.22 mm anterior to the bregma). *VO* ventral orbital frontal cortex, *Cgl* Cingulate cortex, area 1, *Prl* prelimbic cortex, *MO* medial orbital cortex, *LO* lateral orbital cortex. All the images are from Paxinos and Franklin (1997).

B * represents the site of 8-OH-DPAT injection or vehicle and º represents the site of WAY-100,635 injection.

C * represents the site of CP-93,129 injection or vehicle and º the site of SB-224,289 injection or vehicle.

Fig. 3 Effects of 5-HT_{1A} receptor agonist and antagonist on instigation-heightened aggression.

A Frequency of attack bite after 8-OH-DPAT at different doses (0.1, 0.56 and 1.0 µg /0.2 µl) and after pretreatment with WAY-100,635 (10.0 µg/0.2 µl) plus vehicle and 8-OH-DPAT (1.0 µg/0.2 µl), respectively.

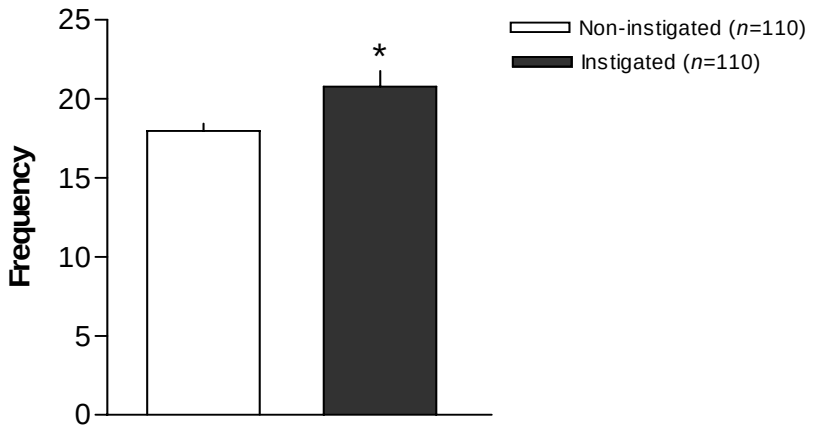
B Frequency of sideways threats after 8-OH-DPAT at different doses (0.1, 0.56 and 1.0 µg/0.2 µl, respectively) and after pretreatment with WAY-100,635 (10.0 µg/0.2 µl) plus vehicle and 8-OH-DPAT (1.0 µg/0.2 µl), respectively. Vertical bars represent the mean ± SEM and asterisks indicate $p \leq 0.05$.

Fig. 4 Effects of 5-HT_{1B} receptor agonist and antagonist on instigation-heightened aggression. **A** Frequency of attack bite after CP-93,129 at different doses (0.1, 0.56 and 1.0 μg/0.2 μl) and after pretreatment with SB-224,289 (1.0 μg/0.2 μl) plus vehicle and CP-93,129 (0.1 μg/0.2 μl), respectively.

B Frequency of sideways threats after CP-93,129 at different doses (0.1, 0.56 and 1.0 μg/0.2 μl) and after pretreatment with SB-224,289 (1.0 μg/0.2μl) plus vehicle and CP-93,129 (0.1 μg/0.2 μl), respectively. Vertical bars represent the mean ± SEM and asterisks indicate $p \leq 0.05$.

Fig. 5 Duration of motor activities (**A** rearing and **B** grooming) after 5-HT_{1A} and 5-HT_{1B} receptor agonist microinjections. Vertical bars represent the mean ± SEM. *****$p \leq 0.05$, ****** $p \leq 0.001$.

Figure 1


25
20
15
10
5
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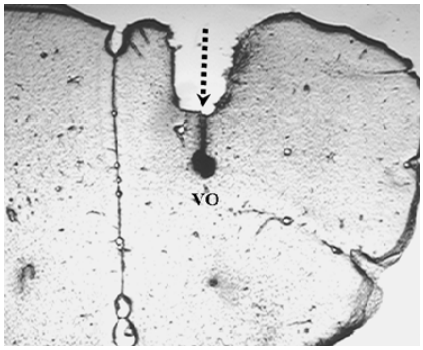
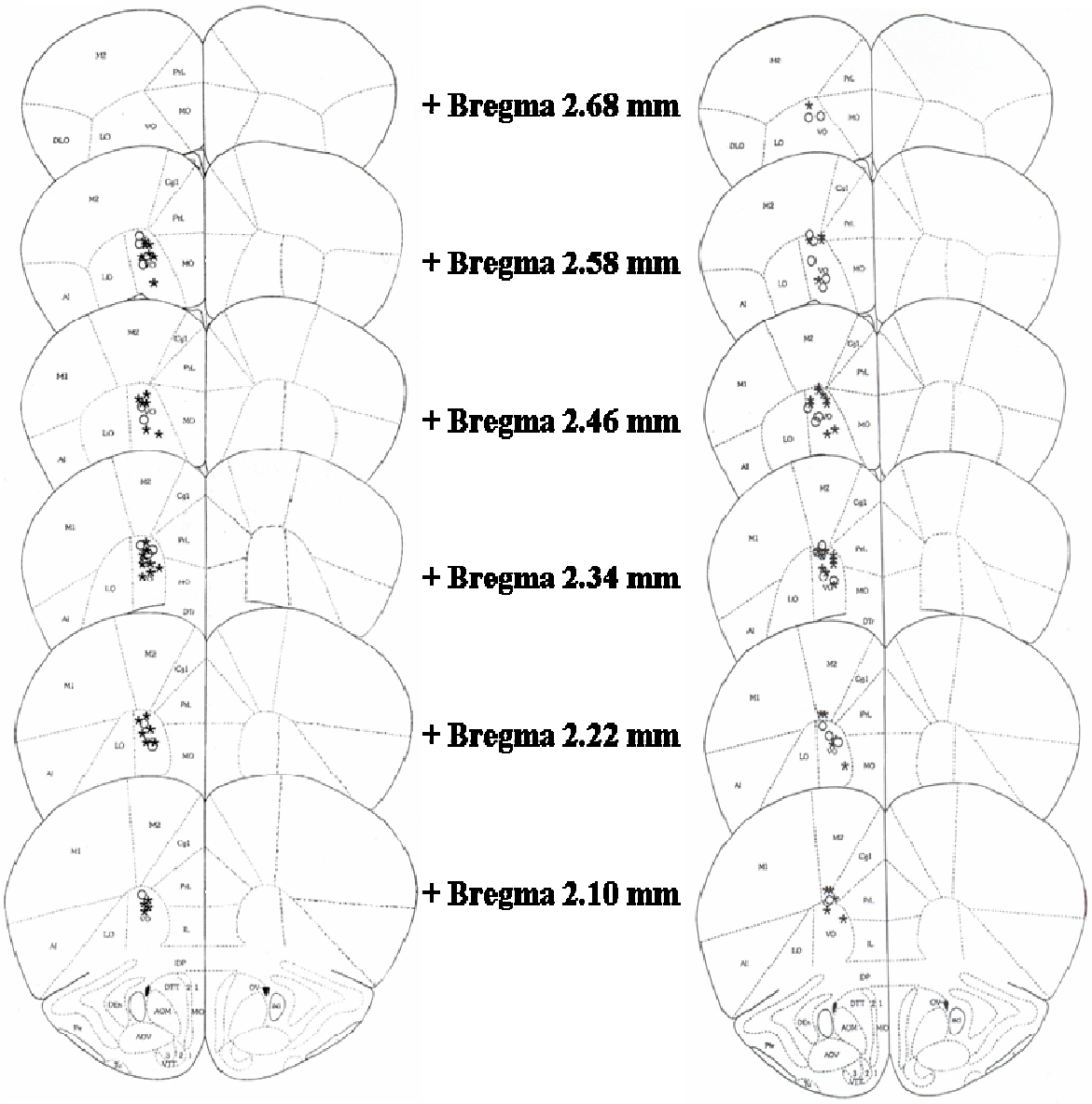
Frequency
*
Non-instigated (n=110)
Instigated (n=110)

Figura 2

A


VO

B **C**


+ Bregma 2.68 mm
+ Bregma 2.58 mm
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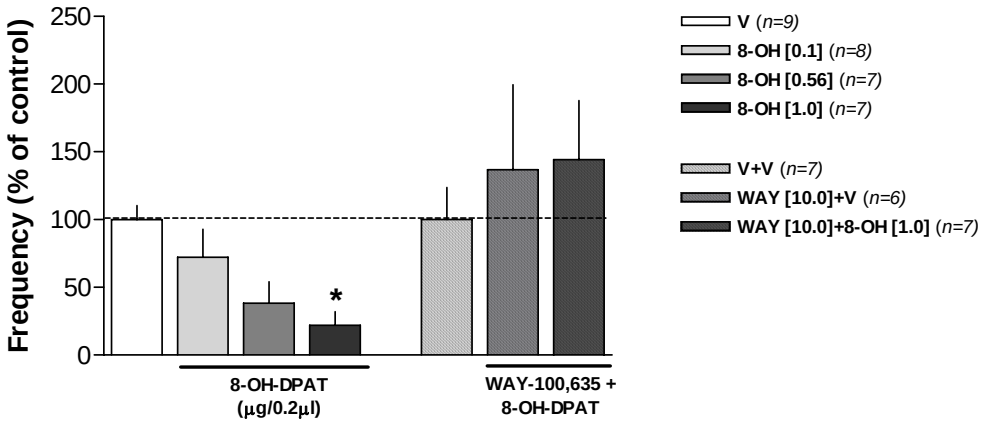
+ Bregma 2.22 mm
+ Bregma 2.10 mm

Figure 3

A Attack Bites



B Sideways Threats

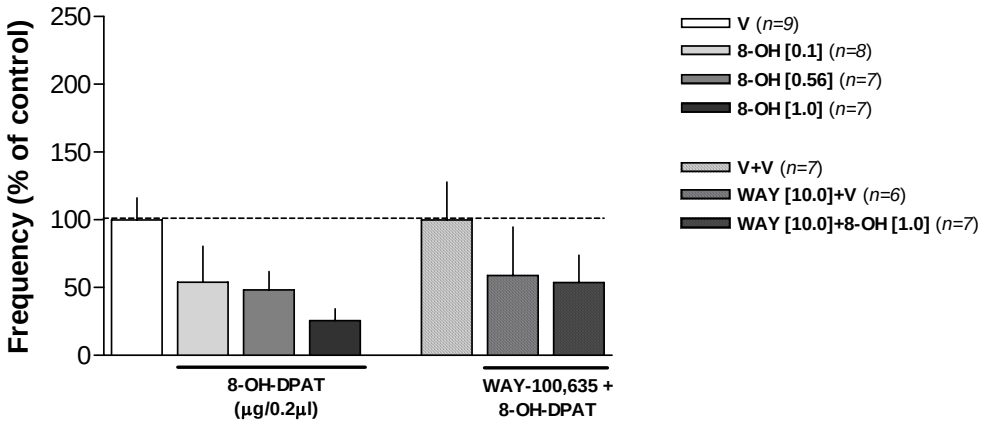
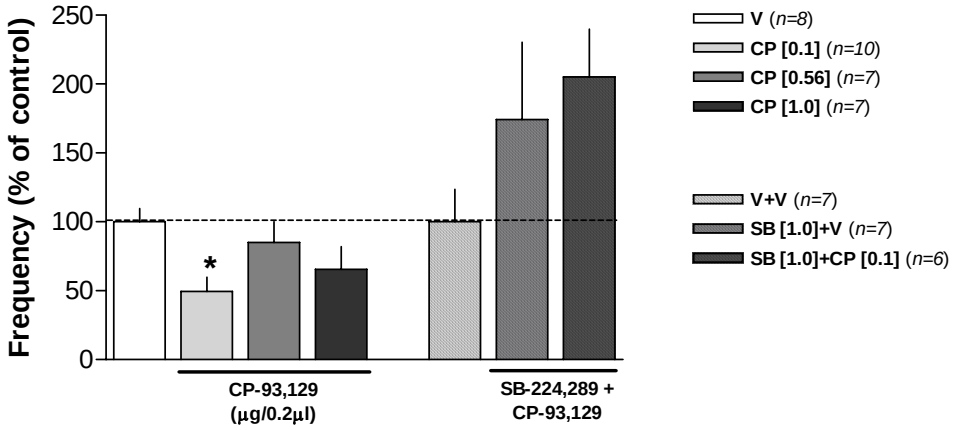


Figure 4

A Attack Bites



B Sideways Threats

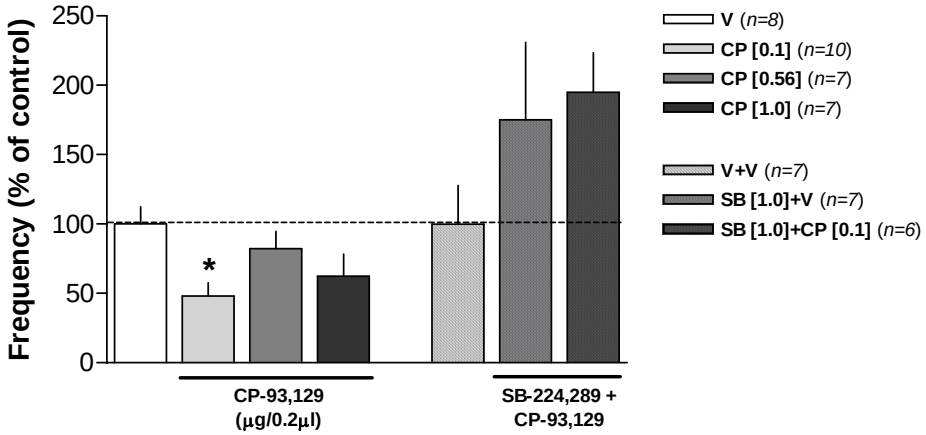
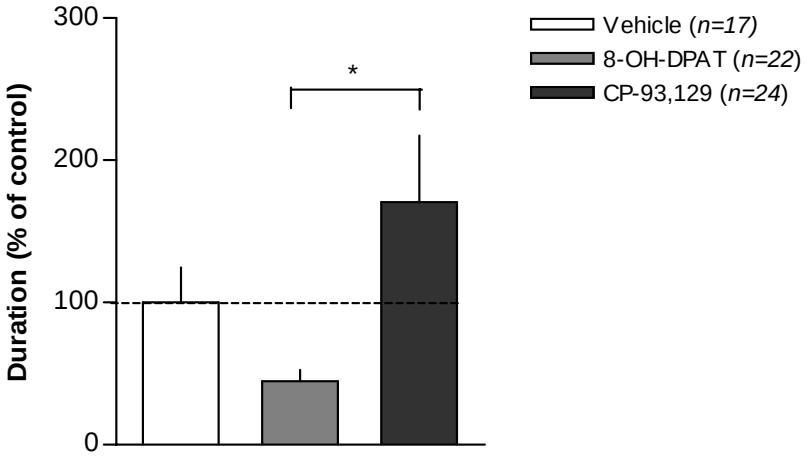


Figure 5

A Rearing



B Grooming

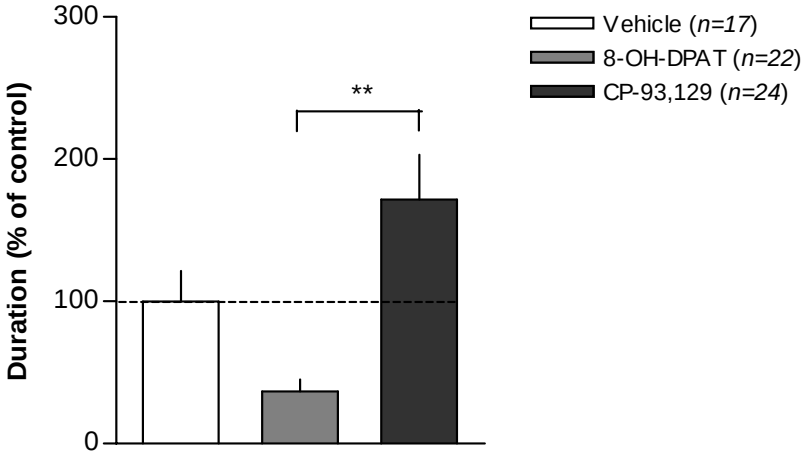


Table 1. Frequency of aggressive and duration of non-aggressive behaviors (in seconds) after microinjection of 5-HT_{1A} and 5-HT_{1B} receptor agonists (8-OH-DPAT and CP-93,129 respectively). Data expressed in mean ± SEM.

	8-OH-DPAT doses (µg/0.2 µl)					CP-93,129 doses (µg/0.2 µl)				
Frequency	***V*[a]*(n=9)***	***No*[b]*(n=6)***	***0.1(n=8)***	***0.56(n=7)***	***1.0(n=7)***	***V*[a]*(n=8)***	***No*[b]*(n=8)***	***0.1(n=10)***	***0.56(n=7)***	***1.0(n=7)***
Attack bites	22.0±2.2	12.1±3.6	15.8±4.5	8.4±3.4	4.8±2.1*	24.0±2.3	23.5±3.7	11.9±2.4*	20.4±3.6	15.7±3.9
Sideways threat	10.6±1.9	6.6±2.8	5.7±2.8	5.1±1.4	2.7±0.9	30.2±3.7	26.6±3.8	14.5±2.9*	24.8±3.8	18.8±4.8
Sniff	6.3±1.4	12.6±2.5	14.3±2.7	11.7±2.9	13.5±2.0	11.1±3.2	4.7±0.8	13.5±3.6	6.4±3.1	5.1±2.0
Tail rattle	11.8±1.5	5.1±2.0	8.1±2.0	9.5±2.6	18.1±5.1	14.5±3.4	17.1±4.1	20.9±4.0	23.5±6.7	15.8±3.9
Duration										
Groom (s)	6.6±1.3	2.3±1.2	4.1±0.8	3.2±0.9	5.3±2.9	17.1±4.3	21.3±5.2	26.3±7.2	13.0±4.4	17.5±5.2
Rear (s)	13.9±4.7	5.6±1.5	9.0±2.6	3.0±0.8	10.1±2.2	20.3±7.1	22.1±10.5	12.2±3.0	40.7±21.0	40.6±15.7
Walk (s)	69.2±11.7	74.2±5.4	81.2±20.1	47.6±12.2	63.1±6.6	35.2±3.4	34.3±5.4	32.7±5.1	34.3±5.3	35.8±10.3

[a] Vehicle; [b] No-target; * $p<0,05$

Table 2. Frequency of aggressive and duration of non-aggressive behaviors (in seconds) after microinjection of 5-HT_{1A} and 5-HT_{1B} receptor antagonists (WAY-100,635 and SB-224,289, respectively) into VO PFC, before microinjection of vehicle or 5-HT_{1A} and 5-HT_{1B} receptor agonists (8-OH-DPAT and CP-93,129, respectively). Data expressed in mean ± SEM.

BEHAVIOR	VEHICLE	WAY-100,635 (µg/0.2 µl)		SB-224,289 (µg/0.2 µl)	
Frequency	***+Vehicle (n=7)***	***+Vehicle (n=6)***	***+8-OH-DPAT (n=7)***	***+Vehicle (n=7)***	***+CP-93,129 (n=6)***
Attack bites	10.0±2.3	13.6±6.2	14.4±4.3	17.4±5.6	20.5±3.4
Sideways threat	13.8±3.8	8.1±4.9	7.4±2.7	24.29±7.7	27.0±3.9
Sniff	9.8±2.1	15.8±4.3	13.4±3.3	4.8±2.3	5.5±1.3
Tail rattle	18.1±2.5	8.5±4.3	10.0±3.4	20.5±3.3	26.8±5.6
Duration					
Groom (s)	7.2±2.0	4.5±1.3	3.3±1.2	15.3±6.0	15.7±2.8
Rear (s)	20.1±7.8	13.12±3.8	14.7±5.0	20.2±9.5	21.1±7.9
Walk (s)	46.4±5.6	58.9±9.4	58.6±10.8	37.8±4.6	50.1±4.7

5 CONCLUSÕES E PERSPECTIVAS

Este estudo confirma o envolvimento dos receptores 5-HT_{1A} e 5-HT_{1B} presentes na região ventro orbital do córtex pré-frontal sobre a modulação da agressividade. Efeitos anti-agressivos foram observados com a microinjeção de 8-OH-DPAT e CP-93,129 em camundongos apresentando níveis intensos de agressão devido à provocação social. Em especial, o tratamento com a menor dose de CP-93,129 reduziu de maneira significativa o comportamento agressivo desses animais, sugerindo uma maior especificidade dos receptores 5-HT_{1B} em comparação aos receptores 5-HT_{1A}. A participação seletiva desses receptores foi verificada pela reversão dos efeitos anti-agressivos com a utilização dos antagonistas WAY-100,635 e SB-224,289.

Demais atividades motoras não foram significativamente alteradas com nenhum dos tratamentos analisados, diferentemente do que observa-se na clínica com os agentes farmacológicos empregados atualmente para o manejo de formas violentas de agressividade.

A redução no comportamento agressivo com a utilização desses agonistas serotonérgicos pode estar associada com um aumento nos níveis de serotonina na região ventro orbital do córtex pré-frontal devido à ativação de sítios pós-sinápticos. Assim, a hipótese original da deficiência de serotonina poderia ser relevante no que diz respeito às formas violentas de agressão. Todavia, estudos futuros com técnicas de microdiálise e microinjeção de drogas e/ou neurotoxinas em outras regiões encefálicas relacionadas com a agressão são necessários para elucidar o mecanismo de ação desses receptores e a sua localização precisa.

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