Universidade de Brasília
Instituto de Ciências Biológicas
Programa de Pós-Graduação em Biologia Animal

Efeitos Comportamentais da Dietilpropiona e Cocaína em Primatas Não-Humanos (*Callithrix penicillata*) após Administração Sistêmica de Antagonistas dos Receptores de 5-HT_{1A} e NK_3

Eldon Londe Mello Junior

Brasília – 2006

Universidade de Brasília
Instituto de Ciências Biológicas
Programa de Pós-Graduação em Biologia Animal

Efeitos Comportamentais da Dietilpropiona e Cocaína em Primatas Não-Humanos (*Callithrix penicillata*) após Administração Sistêmica de Antagonistas dos Receptores de 5-HT_{1A} e NK_3

Eldon Londe Mello Junior

Tese de Doutorado apresentada ao Instituto de Ciências Biológicas da Universidade de Brasília, como requisito parcial para obtenção do título de Doutor em Ciências (Área: Biologia Animal)

Prof. Dr. Carlos A. B. Tomaz
Orientador

Brasília – 2006

UNIVERSIDADE DE BRASÍLIA
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA ANIMAL

TESE DE DOUTORADO

Eldon Londe Mello Júnior

Título

"Efeitos Comportamentais da Dietilpropiona e Cocaína em Primatas Não-Humanos (*Callithrix penicillata*) após Administração Sistêmica de Antagonistas dos Receptores de 5-HT_{1A} e NK_3"

Comissão Examinadora:

Prof. Dr. Carlos Alberto Bezerra Tomazz
Universidade de Brasília
Presidente/orientador

Prof. Dr. Carlos Arturo Conde Cotes
Universidad Industrial de Santander
Membro Titular Externo

Prof. Dr. José Gilberto Ortiz
University of Puerto Rico
Membro Titular Externo

Prof. Dr. Valdir Filgueiras Pessoa
Universidade de Brasília
Membro Titular Interno

Profa. Dra. Vânia Maria Moraes Ferreira
Universidade de Brasília
Membro Titular Interno

Brasília, 29 de março de 2006.

Os trabalhos apresentados nesta tese foram realizados no Centro de Primatologia e analisados no Laboratório de Neurociências e Comportamento – Departamento de Ciências Fisiológicas do Instituto de Ciências Biológicas da Universidade de Brasília, sob a orientação do Prof. Dr. Carlos Tomaz e em cooperação com o Prof. Dr. Joseph Huston do Instituto de Psicologia Fisiológica I da Universidade Heinrich-Heine de Düsseldorf na Alemanha.

“... and in the end,
the love you take is equal to
the love you made...”

The Beatles

Dedico este trabalho à minha mãe e à minha irmã

AGRADECIMENTOS

- Ao Prof. Dr. Carlos Tomaz pela valorosa oportunidade dada a mim de experienciar o cume da atividade acadêmico-científica e pela enriquecedora orientação que transcende o cientista e fez do convívio um aprendizado de vida. Grato de coração por tudo!

- À Prof. Dra. Marilia Barros pela cooperação direta e orientação de uma jovem cientista brilhante. Esta é a sua segunda tese, parabéns!

- Ao Prof. Dr. Joseph Huston e ao Dr. Christian Müller pela inestimável parceria em todos os momentos desta ousada empreitada. Impossível também não mencionar minha admiração pelas pessoas fantásticas que são!

- Ao colega de pesquisa Rafael Maior pela valiosa assistência e dedicação. Arigatô!

- Aos estagiários: Ana América, Ana Katarina Santos, Anand Dacier, Carolina Alencar, Danúbia Cristina Reis, Gonçalo Camargo, Naiá Vilas Boas, Pollyana Sousa, Rodrigo Vilhena e Vinicius Ribeiro Covre – que são uma engrenagem essencial no sistema, eu já fui um! Obrigado!

- Ao Dr. Raimundo de Oliveira, Washington Vargas e Geinaldo Vieira da Silva pelo cuidado com os animais e aquela "mãozinha" fundamental na hora certa. Obrigadão!

- A todos do Laboratório de Neurociências e Comportamento pela convivência gratificante e agradável: professores Joaquim, Valdir, João e Clotilde. Muito obrigado! À Marta e a todos os alunos. Valeu!

- À Nara e Dani, do Programa de Pós-Graduação em Biologia Animal e do Departamento de Ciências Fisiológicas, respectivamente. Obrigado mesmo!

- A todos os professores do Programa de Pós-Graduação em Biologia Animal pelo sucesso que o programa já é e pelo o que está por vir. Ricardo, logo chegaremos ao tão sonhado 7!!! Meu agradecimento especial ao ex-coordenador Prof. Dr. Guarino que conduziu o programa magistralmente durante grande parte do meu doutorado, além de eu ter tido a oportunidade de ser seu aluno! Também não posso deixar de mencionar a Prof. Dra. Rosana e a delícia que é pensar a Evolução!

- À minha "enorme" família: mãe e irmã, vocês são as mulheres da minha vida!

- Aos amigos que tornam o fardo mais leve e a vida mais alegre. E o que dizer então do melhor amigo? Grande Flavão!

- À Universidade de Brasília, ao Programa de Pós-Graduação em Biologia Animal, todo o Instituto de Biologia e em especial ao Centro de Primatologia. Este foi o meu mundo por quase dez anos!

- Ao CNPq pela Bolsa de Doutorado, sem a qual eu não teria como ter seguido os estudos.

- À FINATEC e ao programa CAPES/PROBAL pelo apoio financeiro essencial para a viabilização deste projeto.

SUMÁRIO

RESUMO

Há muitos anos vinha sendo atribuído, exclusivamente, ao sistema dopaminérgico o papel de mediar os efeitos comportamentais da cocaína. Isso se deveu à descoberta de que a cocaína se liga ao transportador de dopamina e, assim, bloqueia a recaptação da mesma na fenda sináptica. Conseqüentemente, tem-se um aumento da concentração extracelular de dopamina, o que resulta nos típicos efeitos psicoestimulantes da cocaína. Outros estimulantes, como a anfetamina, atuam do mesmo modo. Entretanto, estudos posteriores demonstraram que o aumento dos níveis extracelulares de dopamina *per se* não explicam todos os efeitos atribuídos aos psicoestimulantes. Também se observou que a cocaína interfere nos níveis extracelulares de outros transmissores como a serotonina. Nesse contexto, vários estudos foram realizados a fim de avaliar as possíveis interações entre a via dopaminérgica e outros transmissores, obtendo resultados interessantes. O presente estudo objetivou contribuir nesse sentido ao avaliar os efeitos comportamentais da cocaína e dietilpropiona (um derivado da anfetamina) em primatas não-humanos (*Callithrix penicillata*) após administração sistêmica de antagonistas dos receptores de 5-HT_{1A} e NK_3 (WAY 100635 e SR142801, respectivamente). Os animais foram devidamente manipulados e habituados ao labirinto em "8" elevado, onde os comportamentos foram registrados em sessões de 20 a 30 minutos, dependendo do tratamento (dietilpropiona ou cocaína). Os antagonistas foram injetados de 20 a 30 minutos antes da administração dos tratamentos. Nem o WAY 100635 nem o SR142801 promoveram efeitos comportamentais isoladamente. A dietilpropiona e a cocaína induziram hiperlocomoção e respostas comportamentais ansiogênicas. O pré-tratamento com os antagonistas bloqueou, com êxito, a hiperlocomoção e os efeitos ansiogênicos induzidos pelos psicoestimulantes, o que reforça a hipótese alternativa de participação de vias não-dopaminérgicas sobre os efeitos indesejados da cocaína e dos anfetamínicos.

ABSTRACT

For many years it was attributed to the dopamine system the sole mediation of cocaine's behavioral effects. That was due to the finding that cocaine binds to the dopamine transporter and, thus, blocks the reuptake of dopamine in the synaptic cleft. This leads to an increase in the extracellular concentration of dopamine, which results in cocaine's well-known psychostimulant effects. Other stimulants, like amphetamine, act the same way. However, further investigations suggested that the extracellular dopamine increase itself does not explain all the observed effects of the referred stimulants. It was also noticed that cocaine interferes in the normal extracellular concentration of other transmitters. In this context, several studies were carried out in order to evaluate possible interactions between the dopaminergic system with other transmitters, such as serotonin, yielding interesting results. The present study tried to contribute in this regard by assessing the behavioral effects of cocaine and diethylproprion (a derivative of amphetamine) in non-human primates (*Callithrix penicillata*) after systemic injection of 5-HT_{1A}- and Nk_3- receptor antagonists WAY 100635 and SR142801, respectively. The animals were properly handled and habituated to an elevated "8"-shaped maze, where the behaviors were recorded during 20 to 30 minutes trials, depending on the treatment (diethylproprion or cocaine). The antagonists were injected 20 to 30 minutes prior to the treatment administration. Neither WAY 100635 nor SR142801 had independent effects upon the animals' behaviors. Diethylproprion and cocaine induced hyperlocomotion and anxiogenic-like behavioral responses. Pretreatment with WAY 100635 and SR142801 successfully blocked the psychostimulants' induced hyperlocomotion and behavioral effects which strengthens the alternative hypothesis of non-dopaminergic neurotransmitter systems' role in the undesirable effects of cocaine and amphetamines.

INTRODUÇÃO

PSICOESTIMULANTES

Anfetaminas

As anfetaminas são compostos sintéticos, fabricados em laboratórios e, devido a propriedades inibidoras de apetite (anorexígenas), foram amplamente empregadas em regimes de emagrecimento. Outra propriedade das anfetaminas explorada clinicamente, no tratamento da depressão, é a de produzir hiperatividade. Entretanto, foi observado que o uso destes compostos levava à dependência física e psicológica, além de apresentar alta toxicidade em super dosagem. Devido ao risco de abuso e toxicidade, muitas anfetaminas passaram a ser consideradas drogas ilícitas enquanto outras são de uso controlado. Por exemplo, nas ruas, a anfetamina é popularmente conhecida como "rebite", “bola”, “bolinha”, “desbutal”, “peruitin” e “speed”. São comuns entre pessoas que viram noites estudando e motoristas que precisam cumprir longas jornadas em um curto período de tempo. Os anfetamínicos são de uso oral ou injetáveis. Nos Estados Unidos (EUA), a metanfetamina é fumada em cachimbos, recebendo o nome de *ice* (gelo). Outra anfetamina, a metilenodioximetanfetamina (MDMA), o *ecstasy*, tem sido uma das drogas com maior aceitação pela juventude inglesa e com um consumo crescente nos EUA e Brasil, especialmente entre os freqüentadores de festas de música eletrônica chamadas de *rave*. (Drummond & Filho, 1998).

Efeitos perceptíveis em curto prazo de consumo contínuo são: diminuição do sono, falta de apetite, pouco cansaço, pupilas dilatadas, aumento da pressão, pouca sede e melhora no desempenho físico de atletas. Por sua vez, os efeitos perceptíveis a médio e longo prazo são: parada cardíaca, hipertensão arterial, febre alta, overdose, alucinações, sensação de energia exagerada, euforia, hiperatividade, convulsões, agressividade, comportamentos estereotipados, dor de cabeça e ranger de dentes. As

crises de abstinência se evidenciam através de uma enorme apatia, medo, angústia, pânico, paranóia profunda, sonolência e depressão grave (Drummond & Filho, 1998; Rang *et al.*, 2001).

A anfetamina e seu dextroisômero ativo, a dextroanfetamina, juntamente com a metanfetamina, MDMA, o metilfenidato e a fenfluramina (embora com efeitos farmacológicos levemente distintos), atuam através da liberação de monoaminas das terminações nervosas cerebrais. A noradrenalina e a dopamina são os mediadores mais importantes mas também ocorre liberação de 5-HT (Rang *et al.*, 2001).

Tendo em vista os efeitos indesejados da anfetamina, seguiu-se a busca por derivados que mantivessem apenas os seus efeitos anorexígenos. Atualmente, anfetamínicos como a dietilpropiona, empregada neste estudo, junto com o metilfenidato são os principais agentes usados no tratamento da obesidade. Contudo, o uso indiscriminado dessas drogas gera um estado de psicose paranóica que se assemelha bastante ao que é observado na esquizofrenia, chegando a ter seus efeitos revertidos com a administração de drogas antipsicóticas como a clorpromazina. Na verdade, este efeito vem sendo explorado em estudos com voluntários como um "modelo de psicose". A administração crônica de anfetamina em alguns ratos em uma colônia levou a uma interação social anormal, incluindo comportamento de isolamento social e agressividade. Além disso, a administração de anfetamina em ratos, que libera tanto dopamina quanto noradrenalina, resulta em comportamentos estereotipados, ou seja, não relacionados a estímulos externos. Esses efeitos são evitados por antagonistas dopaminérgicos ou pela destruição dos corpos celulares que contêm dopamina no mesencéfalo, mas não por drogas que inibem o sistema noradrenérgico. Portanto, acredita-se que os efeitos motores induzidos por anfetamínicos reflitam uma hiperatividade dopaminérgica nigroestriatal. Além disso, a administração de anfetamina promove um aumento geral na atividade locomotora. Tal efeito, ao contrário da

estereotipia, parece estar relacionado com as vias dopaminérgicas mesolímbica e mesocortical (Cooper *et al.*, 1996; Rang *et al.*, 2001).

Dietilpropiona

A fenileltilamina dietilpropiona (1 fenil-2-dietilamina-1-propanona hidrocloreto) é um psicoestimulante de baixa potência (Johanson *et al.*, 1976; Hoekenga *et al.*, 1978). Como a anfetamina e outras feniletilaminas, a dietilpropiona promove um aumento na atividade locomotora, (Tang & Kirch, 1971; Safta *et al.*, 1976; Reimer *et al.*, 1995; Gevaerd *et al.*, 1999; Da Silva & Cordellini, 2003), induz preferência condicionada por lugar em roedores (Reimer *et al.*, 1995; Planeta & DeLucia, 1998), é auto-administrada por macacos (Johanson *et al.*, 1976), e substitui a cocaína em modelos de auto-administração em ratos (Wood & Emmett-Oglesby, 1988) e macacos (Johanson & Schuster, 1976; Griffiths *et al.*, 1976, 1978). Em humanos, a dietilpropiona pode causar "alegria" (Jonsson *et al.*, 1967) e "euforia" (Jasinski *et al.*, 1974), mas também nervosismo, irritabilidade, insônia e hipercinese (*hyperkinesis*) (Khan *et al.*, 1987), e pode induzir uma psicose semelhante a uma esquizofrenia sob altas doses ou uso prolongado (Fookes, 1976; Carney, 1988; Brooke *et al.*, 1988). Entretanto, sua potência é consideravelmente menor que a da anfetamina (Jasinski *et al.*, 1974). Como outros derivados de anfetamina, ela tem propriedades anorexígenas em animais (Tang & Kirch, 1971; Garattini *et al.*, 1978; Foltin, 1989, 2001) e é usada para tratar obesidade em humanos (Bray, 2000; Ryan, 2000). Atualmente, a dietilpropiona é considerada uma das drogas mais seguras no tratamento de curto-prazo da obesidade em pacientes com hipertensão leve ou moderada (Weiser *et al.*, 1997).

Como discutido anteriormente, a anfetamina e outras feniletilaminas têm profundos efeitos na atividade monoaminérgica. Entretanto, o perfil farmacológico

depende, em grande parte, dos principais efeitos de cada droga nos sistemas serotonérgico, noradrenérgico e dopaminérgico. Como a anfetamina, a dietilpropiona tem uma afinidade maior com o sistema noradrenérgico e dopaminérgico do que com o serotonérgico (Garattini *et al.*, 1978). Tem sido demonstrado que os efeitos anorexígenos da dietilpropiona são mediados pelos seus efeitos noradrenérgicos ao invés de suas propriedades dopaminérgicas ou serotonérgicas (Borsini *et al.*, 1979; Samanin & Garattini, 1993). Contudo, a dietilpropiona induz hiperlocomoção e preferência por lugar com base em seus efeitos sobre as vias dopaminérgicas e/ou serotonérgicas (Gevaerd *et al.*, 1999). A mediação de diferentes efeitos comportamentais dos psicoestimulantes por distintos sistemas monoaminérgicos é embasada pelos resultados de Griffiths e colaboradores (1976), que demonstram não haver relação entre a potência de uma droga anorexígena e suas propriedades de induzir auto-administração. Uma vez que os efeitos anorexígenos da dietilpropiona parecem ser dependentes de noradrenalina, outros efeitos comportamentais podem ser inibidos por um mecanismo não noradrenérgico sem afetar suas propriedades anorexígenas, assim, contribuindo para um melhor uso terapêutico da dietilpropiona.

Cocaína

A cocaína pura, hidrocloreto de cocaína, era extraída originalmente a partir da folha do arbusto *Erythroxylon*, a coca, típico do Peru e da Bolívia na metade do século XIX (Leshner, 2004). Essas folhas eram e ainda são utilizadas pelas suas propriedades estimulantes, especialmente úteis para aqueles que vivem em elevadas altitudes e as utilizam para reduzir a fadiga durante o trabalho (Rang *et al.*, 2001).

No início do século XX, a cocaína tornou-se a principal droga estimulante presente na maioria dos tônicos e chás usados para tratar uma grande variedade de doenças. Freud foi um dos grandes responsáveis pela divulgação da droga tendo, inclusive, a receitado a pacientes. Köller, oftalmologista e colega de Freud, descobriu sua ação anestésica local (Rang *et al.*, 2001).

Porém, logo se constatou que seus efeitos variavam consideravelmente entre indivíduos, não eram clinicamente tão satisfatórios, além de sua alta capacidade em levar à dependência, fato que a tornou a principal substância de abuso no ocidente, o que resultou em sua proibição em diversos países (Hooks *et al.*, 1991; Homberg *et al.*, 2002; Deroche-Gamonet *et al.*, 2004). Nos Estados Unidos, a cocaína é uma droga nível 2, o que significa dizer que apresenta um grande potencial de abuso podendo, entretanto, ser empregada legitimamente na medicina como, por exemplo, anestésico local para cirurgias nos olhos, ouvidos ou garganta (Leshner, 2004).

Dentre os principais efeitos observados em animais estão a hiperlocomoção a inibição do apetite e do comportamento de catação (*grooming*). Em humanos, o uso de cocaína produz uma sensação de euforia (Breiter *et al.*, 1997; Volkow *et al.*, 1997) assim como também pode produzir ansiedade (Yang *et al.*, 1992; Rogerio & Takahashi, 1992).

Estudos mais recentes demonstram que após um período de abstinência, memórias de euforia associadas à droga ou a mera exposição a elementos que lembrem o uso de cocaína, podem desencadear uma grande tensão, resultando numa recaída do paciente fazendo buscar novamente a droga. Isto já foi observado mesmo após longos períodos de abstinência. Os efeitos da cocaína são quase que imediatos e desaparecem dentro de alguns minutos ou até horas, dependendo da quantidade empregada e da via de administração. Em doses baixas, até 100 mg, o usuário sente-se eufórico, energético, falante e alerta, especialmente a estímulos visuais, sonoros e táteis. Pode, também, reduzir temporariamente o apetite e a vontade de dormir. A via de administração também influi na intensidade e duração dos efeitos. Se aspirada, por exemplo, o clímax demora um pouco a chegar, porém dura de 15 a 30 min, uma vez que, sendo fumada, pode ir de 5 a 10 min. Os efeitos fisiológicos a curto-prazo da cocaína são: constrição dos vasos sangüíneos, pupilas dilatadas, aumento de temperatura, dos batimentos cardíacos e da pressão sangüínea. Apesar de raro, algumas pessoas podem sofrer de morte súbita em sua primeira experiência com a cocaína ou, inesperadamente, mais tarde, depois de um certo período de uso da droga. As mortes relacionadas ao uso de cocaína são devidas, na maioria dos casos, a paradas cardíacas ou ataques cardíacos seguidos por paradas respiratórias (Drummond & Filho, 1998; Rang *et al.*, 2001; Leshner, 2004).

Existem duas formas básicas de cocaína: o sal hidrocloreto e a base livre. O primeiro se apresenta na forma de pó podendo ser dissolvido em água para injeção intravenosa ou sendo aspirado pelo nariz (intranasal). Na forma de base livre, isto é, o composto não neutralizado por um ácido, o que resultaria no sal hidrocloreto, a cocaína pode ser fumada. A forma mais comum da cocaína vendida nas ruas é, justamente, como um pó fino, branco e cristalino sendo vulgarmente chamado de “coca”, “c”, “neve”, “floco” ou “soco”. A cocaína é vendida, nas ruas, diluída em substâncias inertes como

farinha de milho, alguma espécie de talco/açúcar em pó, com outras drogas ativas como a procaína (um anestésico químico local) e/ou outros estimulantes como as anfetaminas (Drummond & Filho, 1998; Leshner, 2004).

O “crack” é o nome vulgar dado à cocaína na forma de base livre processada com amônia ou bicarbonato de sódio e se apresenta como uma pequena pedra esbranquiçada. Como dito anteriormente, neste estado a cocaína é fumada o que faz com que o usuário sinta seus efeitos em menos de dez segundos. Vale ressaltar que o crack é uma forma mais barata do que a cocaína em pó sendo consumida basicamente por pessoas com menor poder aquisitivo (Drummond & Filho, 1998; Leshner, 2004).

Muitos dos estudos feitos com cocaína buscaram investigar o mecanismo pelo qual a cocaína proporciona uma sensação prazerosa e, também, porque causa tanta dependência. Foi observado que o sistema neural mais afetado pela cocaína está localizado dentro do cérebro, na área tegmentar ventral (ATV). Células nervosas originárias desta região possuem prolongamentos que levam até o núcleo accumbens (Nac), um dos centros-chave de prazer do cérebro. Estudos com animais empregando vários estímulos prazerosos como comida, água, sexo assim como outras drogas que causam dependência, aumentam a atividade neural no Nac. Mais especificamente, sob um estímulo prazeroso, há um grande aumento de DA que é liberada no Nac pelos neurônios da ATV (Cooper *et al.*, 1996; Rang *et al.*, 2001; Leshner, 2004).

Como veremos na seção sobre a Dopamina, a cocaína é um potente inibidor da recaptação desse neurotransmissor, embora também afete a recaptação de outras catecolaminas, como a noradrenalina e a serotonina (Cunningham *et al.*, 1992; Herges & Taylor, 1998). A permanência de DA na fenda sináptica faz com que o estímulo prazeroso seja prolongado, o que pode estar correlacionado com a euforia relatada por usuários da droga. A dependência estaria relacionada com a tolerância desenvolvida pelo organismo devido ao uso prolongado da cocaína. Isto significa que doses cada vez

maiores e mais freqüentes passam a serem necessárias para que se obtenha o mesmo prazer inicial (Cooper *et al.*, 1996; Rang *et al.*, 2001; Leshner, 2004).

VIAS NEURAIS

Dopamina

Até a metade da década de 1950, a dopamina (DA) era considerada exclusivamente como um intermediário na biossíntese das catecolaminas norepinefrina e epinefrina. Contudo, Montagu, Carlsson e colaboradores demonstraram que a DA se encontrava distribuída no cérebro nas mesmas concentrações da norepinefrina. Além disso, a distinta distribuição das duas catecolaminas no sistema nervoso central (SNC) levou os pesquisadores suecos a propor um papel para a DA independente daquele de precursor de norepinefrina (Cooper *et al.*, 1996).

A dopamina é classicamente considerada um inibidor, em função de estudos realizados em suas projeções nigro-estriatais, que concentram cerca de 75% da dopamina presente no cérebro. Pesquisadores levantaram a hipótese de que a DA estaria envolvida no controle motor e que uma redução dos níveis estriatais do neurotransmissor poderiam explicar os sintomas extrapiramidais do mal de Parkinson. Tal hipótese ganhou força com a comprovação de que os pacientes com o mal de Parkinson apresentavam severa depleção de DA no estriado e que seu precursor, L-DOPA, ameniza esses sintomas (Cooper *et al.*, 1996; Rang *et al.*, 2001).

Na figura 1, pode-se observar as três principais vias de atuação dopaminérgica: a via nigroestriatal, envolvida com o controle motor; as vias mesolímbica e mesocortical, envolvida nos efeitos comportamentais e a via túbero-hipofisária, envolvida no controle endócrino.

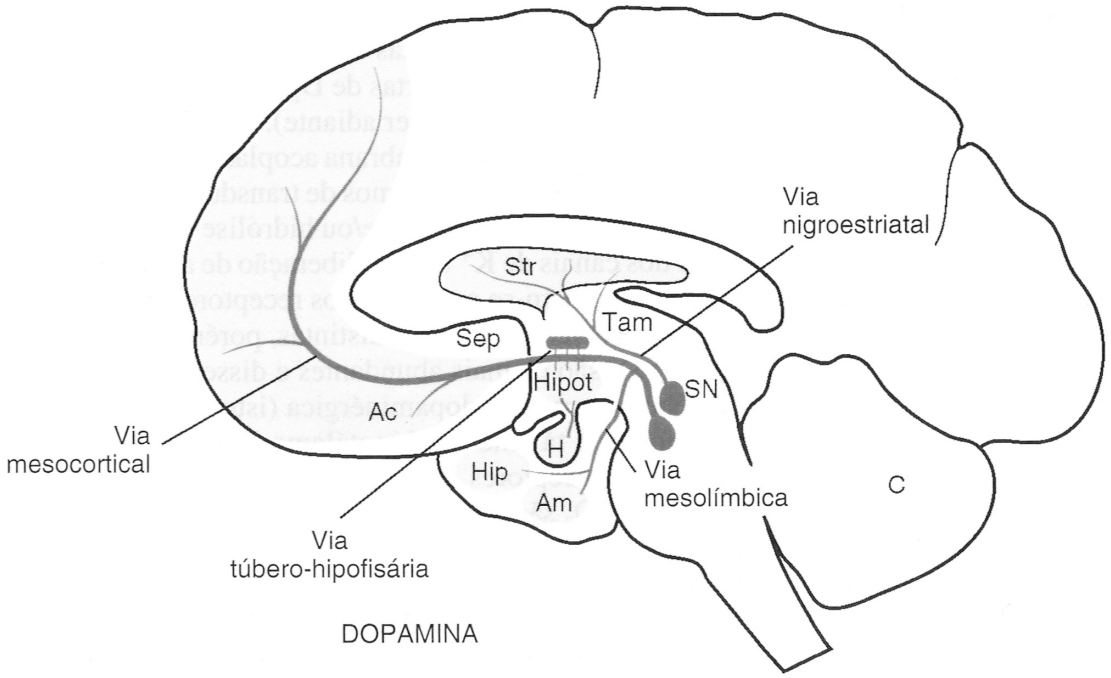


Figura 1. Via da dopamina no cérebro: **Ac** = núcleo accumbens; **Am** = núcleo amigdalóide; **C** = cerebelo; **H** = hipófise; **Hip** = hipocampo; **Hipot** = hipotálamo; **Sep** = septo; **SN** = substância negra; **Str** = corpo estriado; **Tam** = tálamo (Rang *et al.*, 2001).

Receptores de DA

Inicialmente foram caracterizados dois tipos de receptores de dopamina, nomeados D_1 e D_2, os quais são molecularmente distintos, e efeitos bioquímicos igualmente distintos. Porém, em alguns casos, apresentaram efeitos sinergísticos. Nos anos seguintes, estudos de natureza bioquímica, farmacológica e comportamental apontavam para a existência de outros receptores dopaminérgicos além dos já conhecidos D_1 e D_2. A clonagem desses receptores apresentou dois subtipos de D_1 (D_1 e D_5) e três outros de D_2 (D_2, D_3 e D_4). Os receptores D_1 e D_2 são abundantes na região do neo-estriado, isto é, no núcleo caudato, putâmen e núcleo accumbens. Os

receptores do tipo D_1 são os mais abundantes e estão presentes no estriado, no sistema límbico, no tálamo e no hipotálamo (Girault & Greengard, 2004). Os receptores D_2 também estão presentes na hipófise. Os receptores D_3 são encontrados no sistema límbico e, embora desempenhe um papel bastante restrito em circunstâncias normais, se mostrou um alvo potencial no desenvolvimento de fármacos para tratar desordens neurais e psiquiátricas (Luedtke & Mach, 2003). Receptores D_3 pré-sinápticos são encontrados em neurônios dopaminérgicos estão situados no sistema estriado e límbico, onde atuam ao inibir a síntese e a liberação de dopamina. Além disso, antagonistas do receptor D_3, reduzem o efeito recompensador, a auto-administração, da cocaína. Embora seja fracamente expresso no córtex e no sistema límbico, o receptor D_4, tem despertado interesse em virtude de sua possível relação com o mecanismo da esquizofrenia e a dependência de drogas. Por fim, os receptores de dopamina também medeiam efeitos periféricos, nesse caso, devido à presença de receptores D_1 e D_5 no hipotálamo, exercendo funções de controle autônomo e endócrino (Cooper *et al.*, 1996; Rang *et al.*, 2001).

Pscicoestimulantes e a Dopamina (o papel do transportador de DA)

Neurônios dopaminérgicos mesolímbicos estão envolvidos nas propriedades de recompensa de várias drogas de abuso, inclusive psicoestimulantes como a cocaína e a anfetamina. Essas drogas se ligam ao transportador de dopamina (DAT) impedindo a recaptação do neurotransmissor, aumentando sua concentração extracelular (Figura 2). Isto se correlaciona bem com os efeitos reforçadores e estimulantes observados para estas drogas. De fato, os transportadores de dopamina são considerados os principais “receptores de cocaína”. Entretanto, essas drogas não atuam exclusivamente no transportador de dopamina, isto é, elas também apresentam uma considerável afinidade

para sítios de outras catecolaminas como a norepinefrina e a serotonina. Normalmente, o DAT recolhe a dopamina logo após seja liberada na fenda sináptica, de forma a modular sua concentração extracelular e as interações tempo-dependente com receptores pré e pós-sinápticos (Cooper *et al.*, 1996; Rang *et al.*, 2001).

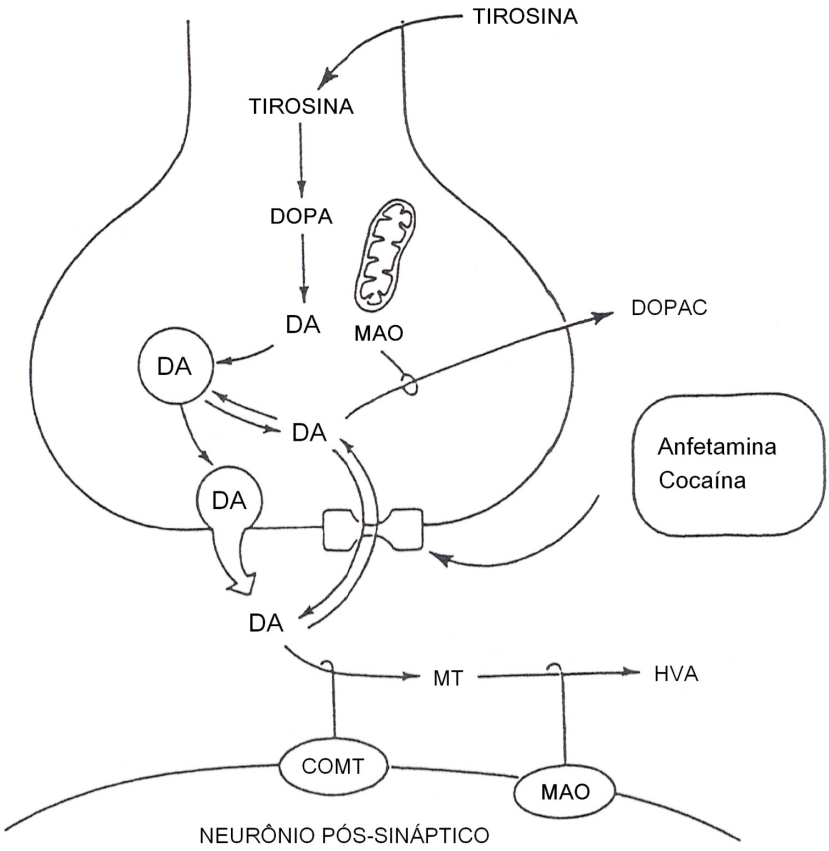


Figura 2. Sítio de ação da anfetamina e da cocaína (adaptado de Cooper *et al.*, 1996).

Serotonina

Desde meados do século XIX, já se sabia que uma substância encontrada no soro sangüíneo (*serum*) causava fortes contrações na musculatura lisa - daí o nome "serotonina". Mais de um século se passou até que a substância foi isolada pela primeira vez – que também seria a causadora da alta pressão sangüínea devido às suas propriedades vasoconstritoras. Enquanto isso, uma substância encontrada em altas concentrações em células enterocromafins da mucosa intestinal estava sendo caracterizada. Enquanto o material extraído da corrente sangüínea, após coagulação do sangue, ficou conhecido como serotonina, o extrato obtido a partir do trato intestinal (células enterocromafins) foi chamado de "enteramina". Em 1948, após purificação e cristalização dos materiais coletados se chegou a 5-hidroxitriptamina (5-HT) e foi demonstrado que era originária das plaquetas. Em seguida, foi detectada no trato gastrintestinal e no sistema vascular periférico. A serotonina também passou a ser sintetizada em laboratório apresentando todas as características da substância natural (Cooper *et al.*, 1996; Rang *et al.*, 2001).

O interesse pela 5-HT como possível transmissor do SNC data de 1953, quando Gaddum descobriu que o ácido lisérgico (LSD), poderoso alucinógeno, atuava como antagonista de 5-HT nos tecidos periféricos. Sugeriu-se, então, que seus efeitos centrais poderiam estar relacionados com essa observação. Contudo, a presença de 5-HT no cérebro só foi demonstrada alguns anos mais tarde (Rang *et al.*, 2001).

Apenas cerca de 2% da serotonina disponível em nosso organismo é encontrada no cérebro. Uma vez que a mesma não consegue cruzar a barreira hematoencefálica, ficou evidente que as células nervosas sintetizavam serotonina por conta própria (Cooper *et al.*, 1996). No cérebro, altas concentrações de 5-HT são encontradas no mesencéfalo (Fig. 3; Rang *et al.*, 2001).

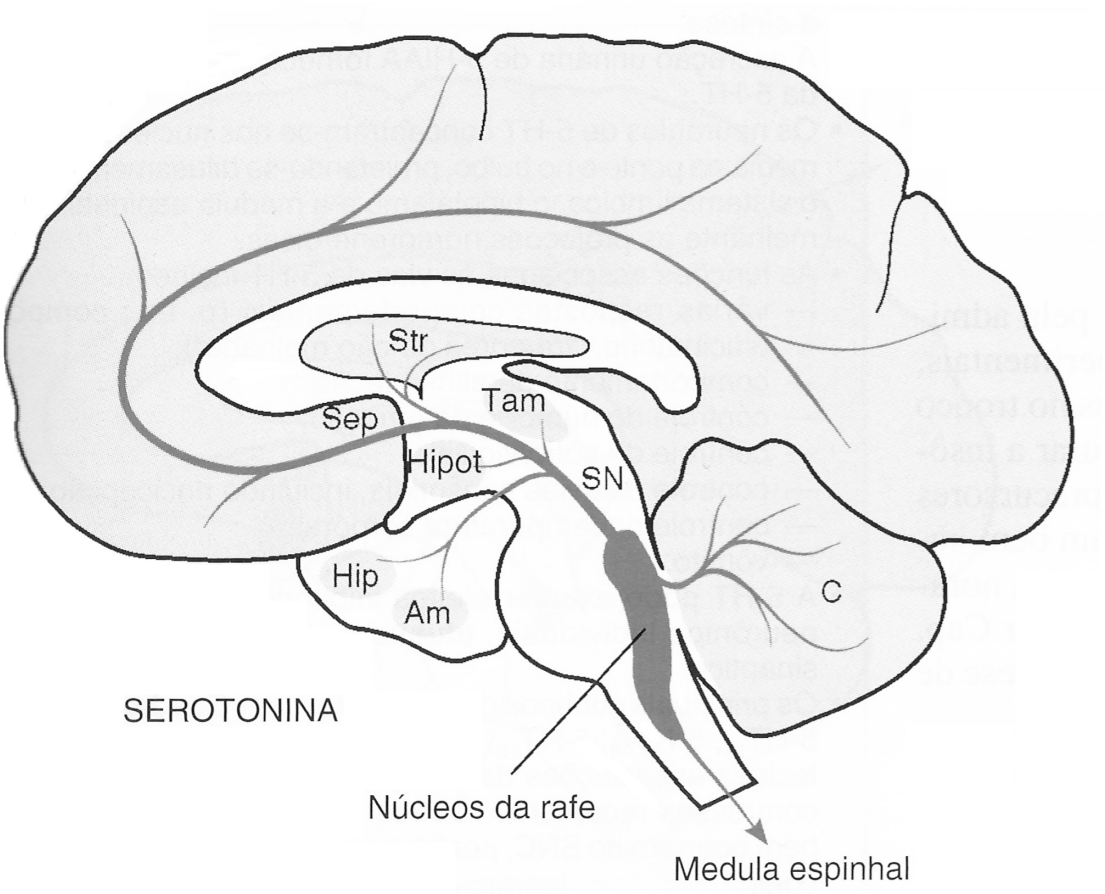


Figura 3. Via da serotonina no cérebro: **Am** = núcleo amigdalóide; **C** = cerebelo; **Hip** = hipocampo; **Hipot** = hipotálamo; **Sep** = septo; **SN** = substância negra; **Str** = corpo estriado; **Tam** = tálamo (Rang *et al*., 2001).

A serotonina e a ativação motora

Estudos eletrofisiológicos com animais não-anestesiados demonstraram uma maior atividade serotonérgica no despertar agitado, verificando o oposto no despertar calmo. Atribui-se tal atividade serotonérgica a um aumento na excitabilidade de neurônios motores, provavelmente preparando o indivíduo para uma resposta mais

eficiente ante um despertar agitado. Outro achado interessante é a ausência de atividade serotonérgica durante o sono REM, estado caracterizado por grande excitação interior, porém com reduzida resposta motora. A não-ativação serotonérgica, portanto, estaria envolvida com a baixa atividade motora observada no sono REM (Cooper *et al.*, 1996). Assim, pode-se estabelecer uma correlação com a atividade serotonérgica e a função motora.

Receptores de 5-HT

Foram identificados pelo menos sete tipos de receptores de 5-HT, numerados de 5-HT_{1-7}, sendo que os receptores dos tipos 1 e 2 apresentam três subtipos designados pelas letras de A-D. Enquanto os receptores dos tipos 2 e 3 ocorrem principalmente no sistema nervoso periférico, os receptores 5-HT_1 ocorrem principalmente no cérebro, sendo os subtipos distinguidos de acordo com sua distribuição e atividade farmacológica. São, em geral, receptores pré-sinápticos inibitórios (Rang *et al.*, 2001).

Os vários tipos de receptores identificados de 5-HT e sua vasta distribuição neural e corporal nos permitem compreender melhor como um único neurotransmissor pode estar envolvido em diferentes padrões comportamentais, clínicos e efeitos de drogas. Como exemplo podemos citar as desordens afetivas, obsessivo-compulsivas, esquizofrenia, estados de ansiedade, fobias, enxaquecas, desordens do sono e de apetite (Cooper *et al.*, 1996). Conseqüentemente, várias drogas psicotrópicas empregadas no tratamento das desordens acima mencionadas apresentam alguma interação com a via serotonérgica.

Pscicoestimulantes e a Serotonina (Receptor 5-HT_{1A})

O subtipo 5-HT_{1A} é particularmente importante no cérebro por sua relação com o humor e vários padrões comportamentais. Estudos eletrofisiológicos demonstraram que os receptores deste tipo mediam a inibição do núcleo da rafe (Cooper *et al.*, 1996).

Uma vez estabelecida a relação entre 5-HT e seu principal receptor, 5-HT_{1A}, começaram a aparecer estudos com ligantes de 5-HT_{1A} com o intuito de estabelecer uma correlação entre a modulação da via serotonérgica com a via dopaminérgica. Recentemente, foi demonstrado que o bloqueio farmacológico do receptor de 5-HT_{1A} com um antagonista seletivo do mesmo, N-{2-[4-(2-metoxifenil)-1-piperazinil]etil}-N-(2-piridinil) ciclohexano-carboxamida trihidrocloreto (WAY 100635; Fletcher *et al.*, 1996), pode inibir a hiperlocomoção induzida por cocaína por um mecanismo serotonérgico (Carey *et al.*, 2001; Müller *et al.*, 2002a). Contrariamente, o agonista 5-HT1-A, 8-hidroxi-2-(di-n-propilamino) tetralina (8-OHDPAT) facilita esses mesmos efeitos induzidos pela cocaína (De La Garza & Cunningham, 2000). Tais evidências sugerem uma participação do receptor de 5-HT_{1A} na hiperatividade induzida por psicoestimulantes. Além disso, Carey e colaboradores (2001) demonstraram o WAY 100635 e 8-OHDPAT, antagonista e agonista de 5-HT_{1A}, respectivamente, produzem seus efeitos sem alterar o metabolismo da DA nem da cocaína no cérebro de ratos. Isto sugere que as duas substâncias afetaram apenas a via serotonérgica e influenciaram os efeitos da cocaína atuando nesta via, deixando clara uma relação entre o sistema dopaminérgico e serotonérgico nos efeitos comportamentais produzidos pela cocaína.

Neuropeptídeos (Taquicininas – receptor NK_3)

Em 1931, von Euler e Gaddum descobriram uma substância com ação farmacológica inesperada, extraída do cérebro e do intestino, a qual foi chamada Substância P (SP, do alemão *pulver*, que significa pó) por ter sido obtida a partir de um extrato de acetona das amostras estudadas (Cooper *et al.*, 1996). Contudo, somente cerca de 40 anos depois, Leeman e colaboradores (1975) purificaram e caracterizaram a natureza peptídica da SP. Desde então o estudo do papel neuromodulador de alguns peptídeos vem se consolidando e hoje já apresenta uma literatura consistente.

Os neuropeptídeos pertencentes à chamada família das taquicininas (de ação rápida, distintos da bradicinina) possuem uma seqüência C-terminal comum (Phe-X-Gly-Leu-Met-NH_2). Os cinco neuropeptídeos identificados em mamíferos até agora são: a SP, Neuroquinina A (NKA), Neuroquinina B (NKB) e os neuropeptídeos K e **γ**. Três receptores são conhecidos: neuroquinina-1 (NK_1), NK_2 e NK_3. Enquanto os receptores NK_1 e NK_3 possuem ampla distribuição no cérebro, os receptores do tipo NK_2 são localizados em áreas mais restritas e em baixas concentrações, a saber, no corpo estriado, substância negra e bulbo olfatório (Helke *et al.*, 1990) e, em altas concentrações, nos tecidos periféricos como o intestino e glândulas adrenais (Otsuka & Yoshioka, 1993). A SP, NKA e NKB possuem maior afinidade pelos receptores NK_1, NK_2 e NK_3, respectivamente, embora todos possam se ligar a qualquer um dos receptores (Cooper *et al.*, 1996; Massi *et al.*, 2000; Hökfelt *et al.*, 2001).

A liberação de SP na periferia quando os nociceptores são ativados contribui para a inflamação neurogênica que, junto com a transmissão nociceptiva, são mediadas principalmente pelos receptores NK_1. O antagonismo dos receptores NK_1 vem sendo estudado para o desenvolvimento de futuros fármacos analgésicos (Rang *et al.*, 2001).

Administração central (Stäubli & Huston, 1985; Holzhäuer-Oitzl *et al.*, 1987, 1988; Hasenöhrl *et al.*, 1990) e sistêmica de SP apresentou características de reforçadoras, no teste de preferência condicionada por lugar (*conditioned place preference* – CPP; Hasenöhrl *et al.*, 1990). Auto-administração de SP foi observada na porção ventro-medial do núcleo caudato-putâmen (Krappmann *et al.*, 1994). SP também apresentou aumento da concentração extracelular de DA no núcleo accumbens (Nac; Boix *et al.*, 1992b). CPP e aumento na atividade dopaminérgica no Nac, foram obtidos por meio da administração de um análogo da porção C-terminal da SP que apresenta maior afinidade pelo receptor NK3 (Regoli *et al.*, 1994; Boix *et al.*, 1992a; Hasenöhrl *et al.*, 1992; Boix *et al.*, 1995). Por sua vez, a porção N-terminal, SP_{1-7}, não apresentou os mesmo resultados (Gerhardt *et al.*, 1992).

Reforçamento (CPP) induzido pela administração da porção C-terminal de SP no núcleo basal magnocelular (NBM) foi parcialmente bloqueado por um antagonista seletivo de receptor NK1, sugerindo o envolvimento de receptores NK2 ou NK3 (Nikolaus *et al.*, 1999). Recentemente foi demonstrado que o receptor NK3 modula tanto os efeitos hiperlocomotores quanto os de recompensa da cocaína (Jocham *et al.*, submetido).

Foram encontradas projeções recíprocas entre neurônios estriatais que produzem SP e neurônios dopaminérgicos da substância negra. Antagonistas de DA administrados na substância negra promoveram uma diminuição na concentração de SP (Cooper *et al.*, 1996).

Além dos referidos efeitos reforçadores, vale destacar o papel da SP em importantes efeitos sobre a memória e a aprendizagem (revisão em Hasenöhrl *et al.*, 2000). Efeitos de longa-duração (Tomaz *et al.*, 1997) foram observados por meio de tarefas com diferentes níveis de complexidade, exigindo, assim, respostas diferenciadas (Tomaz *et al.*, 1990). Além disso, Costa e Tomaz (1998) bloquearam os conhecidos

efeitos amnésicos do diazepam por meio da administração do fragmento N-terminal da SP (SPN). Com base em nesses estudos prévios, realizados em roedores, e em outros que atribuíam um papel ansiolítico à SP (Hasenöhrl *et al.*, 1998), nosso grupo de pesquisa avaliou os possíveis efeitos comportamentais do SPN em calitriquídeos, em nosso modelo de confronto com predador taxidermizado, observando efeitos ansiolíticos por meio da administração sistêmica de SPN (Barros *et al.*, 2002). Em seguida, constatamos um efeito duradouro do SPN na aprendizagem de esquiva em função da posição do predador taxidermizado, sugerindo claros efeitos mnemotrópicos desse neuropeptídeo em sagüis da espécie *Callithrix penicillata* (Maior *et al.*, 2002).

Justificativa e relevância do estudo

O uso, e abuso, de drogas como a cocaína e anfetamínicos é um problema mundial que aflige países ricos e pobres, atinge todas as classes sociais e está diretamente relacionado com a violência urbana e onera o Estado ao ter que considerar recursos adicionais para a Saúde Pública no tratamento de dependentes. Portanto, todos os esforços são necessários para que este problema seja, se não resolvido, ao menos controlado.

Estudos científicos que buscam conhecer os mecanismos fisiológicos e neurológicos de ação de drogas são de extrema importância para que se entenda o comportamento apresentado pelo usuário a curto, médio e longo prazo. Além disso, saber como funciona a dependência química abre perspectivas para o tratamento de dependentes.

Estudos com roedores vêm apontando certas substâncias neuroativas capazes de bloquear os efeitos hiperlocomotores típicos da cocaína e anfetamínicos por meio de vias neurais que não a classicamente reconhecida via dopaminérgica. Em especial, podemos mencionar o papel da via serotonérgica e de neuropeptídeos que interagem com receptores do tipo NK_3.

Resultados advindos de tais estudos poderão contribuir não apenas para o conhecimento sobre a neurobiologia das drogas e vias neurais estudadas, mas também para o desenvolvimento de novas substâncias com valor terapêutico no tratamento da dependência.

Uso de primatas não-humanos

Primatas não-humanos apresentam um repertório comportamental amplo (Stevenson & Poole, 1976; King *et al*., 1988; Barros *et al*., 2004a) que, em particular, permite uma análise acurada dos efeitos de psicoestimulantes como os abordados neste estudo. Além disso, a proximidade filogenética faz do uso desses animais um modelo transitório de extrema importância para estudos pré-clínicos em humanos a partir dos achados provenientes dos tradicionais estudos com ratos. Como exemplo podemos justificar o estudo dos receptores NK_3, cujos resultados obtidos em ratos (Jocham *et al*., submetido) não podem ser generalizados para humanos uma vez que foram observadas diferenças significativas entre os receptores humanos e os de ratos (Emonds-Alt *et al*., 1995; Nguyen-Le *et al*., 1996).

OBJETIVO DO ESTUDO

Objetivos gerais

O objetivo deste trabalho foi investigar os possíveis papéis dos receptores de 5-HT_{1A} e NK_3 sobre os efeitos comportamentais induzidos pela administração do anfetamínico dietilpropiona e da cocaína, respectivamente, por meio do antagonista serotonérgico WAY 100635 e do antagonista do receptor NK_3 SR142801 em primatas da espécie *Callithrix penicillata*.

Objetivos específicos

Como grande parte dos estudos que sustentam teoricamente este trabalho são de dados provenientes de ratos ou de seres humanos, faz-se necessária atenção a possíveis diferenças interespecíficas que, por ventura, podem haver. Naturalmente, a opção pelo emprego de primatas-não humanos se justifica pela grande proximidade filogenética com os seres humanos, fato este que minimiza mas não torna impossível eventuais respostas neurofisiológicas distintas sob a administração de fármacos. Feitas estas considerações, foram nossos objetivos específicos:

Experimento 1: WAY 100635 e dietilpropiona

a) Analisar os efeitos comportamentais da administração sistêmica do WAY 100635;

b) Analisar os efeitos comportamentais da administração sistêmica de dietilpropiona (anfepramona);

c) Analisar os efeitos comportamentais da administração sistêmica de dietilpropiona (anfepramona) e possível bloqueio de seus efeitos locomotores através do pré-tratamento com WAY 100635;

Experimento 2: SR142801 e cocaína

a) Analisar os efeitos comportamentais da administração sistêmica do SR142801;

b) Analisar os efeitos comportamentais da administração sistêmica de cocaína;

c) Analisar os efeitos comportamentais da administração sistêmica de cocaína e possível bloqueio de seus efeitos locomotores através do pré-tratamento com SR12801;

Por fim, espera-se validar este modelo experimental, originalmente desenvolvido para o estudo de fármacos moduladores da ansiedade (Barros & Tomaz, 2002), agora adaptado para o estudo de psicoestimulantes. Esperamos, assim, contribuir com um modelo simples e de baixo-custo, adequado à realidade científica brasileira, empregando animais de nossa fauna, os sagüis, para a realização de pesquisa de ponta envolvendo fármacos com ação psicoestimulante.

EXPERIMENTO 1

Efeitos do Antagonista Seletivo de 5-HT_{1A}, WAY 100635 Sobre os Efeitos Estimulantes do Anfetamínico Dietilpropiona

A dietilpropiona, também conhecida como anfepramona, é um composto com atividade central empregada para tratamento de obesidade que apresenta efeitos colaterais comuns a todas as drogas inibidoras de apetite com ação dependente de catecolaminas (Silverstone, 1992) sendo, portanto, considerada uma droga de abuso (Bray, 2000; Levine *et al*., 2000).

Já foi demonstrado que a dietilpropiona aumenta a atividade locomotora em ratos, além de produzir comportamentos estereotipados e condicionamento de preferência condicionada por lugar (Reimer *et al*., 1995; Planeta & DeLucia, 1998; Da Silva & Cordellini, 2003).

Tais estudos sugerem uma ação dopaminérgica, em receptores D_1, muito semelhante à neuroquímica da cocaína. Tomando por base a ação bloqueadora dos efeitos locomotores (hiperatividade) induzidos por cocaína pelo antagonista serotonérgico WAY 100635 (Carey *et al*., 1999; 2000; 2001; De La Garza & Cunningham, 2000; Müller *et al*., 2002b) há fortes indícios para se acreditar que o pré-tratamento com WAY 100635 poderia, também, bloquear os efeitos locomotores induzidos pela dietilpropiona.

Assim sendo, este experimento foi delineado para investigar a contribuição do receptor de 5-HT_{1A} nos efeitos comportamentais agudos do psicoestimulante dietilpropiona, em primatas não-humanos (*Callithrix penicillata*). Devido às grandes diferenças interindividuais dos efeitos da dietilpropiona em macacos e humanos (Sjöberg & Jonsson, 1967; Johanson *et al*., 1976), os efeitos do WAY 100635 foram analisados com base na sensibilidade à dietilpropiona de cada sujeito experimental.

Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 511 (2005) 43–52

www.elsevier.com/locate/ejphar

Serotonin$_{1A}$-receptor antagonism blocks psychostimulant properties of diethylpropion in marmosets (*Callithrix penicillata*)

Eldon L. Mello Jr.[a], Rafael S. Maior[a], Robert J. Carey[c], Joseph P. Huston[b], Carlos Tomaz[a], Christian P. Müller[b,*]

[a]*Department of Physiological Sciences, Institute of Biology, University of Brasilia, CEP 70910-900 Brasilia, DF, Brazil*
[b]*Institute of Physiological Psychology I and Center for Biological and Medical Research, University of Düsseldorf, Universitätsstr. 1, 40225 Düsseldorf, Germany*
[c]*Research and Development (151), VA Medical Center and SUNY Upstate Medical University, 800 Irving Avenue, Syracuse, NY 13210, USA*

Received 24 January 2005; accepted 28 January 2005

Abstract

Diethylpropion (1-phenyl-2-diethylamine-1-propanone hydrochloride) is a stimulant drug with reinforcing properties that is used to treat obesity in humans. While the anorectic properties of diethylpropion are mediated by a noradrenergic mechanism, stimulant properties depend on its effects on the serotonergic (5-HT) and/or dopaminergic systems. In this study we investigated the role of the 5-HT$_{1A}$-receptor in the acute behavioral effects of diethylpropion in marmosets (*Callithrix penicillata*). Animals were pretreated with the selective 5-HT$_{1A}$-receptor antagonist, *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl) cyclohexane-carboxamide trihydrochloride (WAY 100635; 0.2, 0.4, 0.8 mg/kg, i.p.) or saline (i.p.) and received a treatment with diethylpropion (10 mg/kg, i.p) or saline (i.p.). Diethylpropion induced an increase in locomotor activity in 60% of the monkeys, which were classified as diethylpropion sensitive, but did not affect locomotion in 40% of the monkeys (diethylpropion insensitive). Sensitivity analysis revealed two types of responders to diethylpropion. In the sensitive animals (type A) diethylpropion increased locomotor activity and anxiogenic-like behavior, but decreased bodycare activities. In the insensitive animals (type B) diethylpropion did not affect locomotor and bodycare activity after diethylpropion, but led to a strong increase in anxiogenic-like behavioral responses. Selective 5-HT$_{1A}$-receptor antagonism modulated the acute diethylpropion effects responder type specifically. In the sensitive (type A) monkeys WAY 100635 blocked the diethylpropion-induced increase in locomotor activity, while not affecting anxiogenic-like behavioral responses or the suppression of bodycare activities. In the insensitive monkeys, WAY 100635 had no effect on locomotor activity after diethylpropion, but blocked diethylpropion effects on some anxiogenic-like behavioral responses. In conclusion, these results suggest an essential contribution of the 5-HT$_{1A}$-receptor to the stimulant effects of diethylpropion, which is responder type specific. It also suggests the 5-HT$_{1A}$-receptor to be a source of the interindividual variance in the acute behavioral response to the stimulant diethylpropion in monkeys.

Keywords: Diethylpropion; WAY 100635; Marmoset; Figure-eight maze; Sensitivity

1. Introduction

The phenylethylamine diethylpropion (1 phenyl-2-diethylamine-1-propanone hydrochloride) is a low potency psychostimulant (Johanson et al., 1976; Hoekenga et al., 1978). Like amphetamine and other phenylethylamines diethylpropion typically causes an increase in locomotor activity (Tang and Kirch, 1971; Safta et al., 1976; Reimer et al., 1995; Gevaerd et al., 1999; Da Silva and Cordellini, 2003), induces conditioned place preference in rodents (Reimer et al., 1995; Planeta and DeLucia, 1998), is self-administered by monkeys (Johanson et al., 1976), and substitutes for cocaine in self-administration paradigms in rats (Wood and Emmett-Oglesby, 1988) and monkeys

* Corresponding author. Tel.: +49 211 81 13491; fax: +49 211 81 12024.
E-mail address: muellecr@uni-duesseldorf.de (C.P. Müller).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.01.037

(Johanson and Schuster, 1976; Griffiths et al., 1976, 1978). In humans diethylpropion can cause "happiness" (Jonsson et al., 1967) and "euphoria" (Jasinski et al., 1974), but also nervousness, irritability, insomnia and hyperkinesis (Khan et al., 1987), and may at high doses or after prolonged application induce a schizophrenia-like psychosis (Fookes, 1976; Carney, 1988; Brooke et al., 1988). However, its potency is considerably lower than that of amphetamine (Jasinski et al., 1974). Like other amphetamine derivates it has anorectic properties in animals (Tang and Kirch, 1971; Garattini et al., 1978; Foltin, 1989, 2001) and is used to treat obesity in humans (Bray, 2000; Ryan, 2000). Currently, diethylpropion is considered to be one of the safest short-term anti-obesity drugs in patients with mild to moderate hypertension (Weiser et al., 1997).

Amphetamine and other phenylethylamines have profound effects on monoaminergic activity. However, the pharmacological profile depends to a large extent on the major effects of each drug on either the serotonergic, noradrenergic or dopaminergic system. Like amphetamine, diethylpropion has a stronger affinity to the noradrenergic and dopaminergic than to the serotonergic system (Garattini et al., 1978). It has been reported that the anorectic effects of diethylpropion are mediated by its noradrenergic rather than by its dopaminergic or serotonergic effects (Borsini et al., 1979; Samanin and Garattini, 1993). In contrast, diethylpropion induced hyperlocomotion and place preference depend on dopa-minergic and/or serotonergic effects (Gevaerd et al., 1999). The mediation of different behavioral effects of psychostimulants by different monoaminergic systems is supported by the findings of Griffiths et al. (1976) showing that there is no relationship between the potency as an anorectic drug and the self-administration properties. Since anorectic effects of diethylpropion appear to be noradrenalin dependent, other behavioral effects may be inhibited by a non-noradrenergic mechanism without affecting the anorectic properties, thus, providing a useful approach to improve therapeutic utility of diethylpropion. Recently it was shown that pharmacological blockade of the 5-HT_{1A}-receptor with the selective 5-HT_{1A}-receptor antagonist, *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl) cyclohexane-carboxamide trihydrochloride (WAY 100635; Fletcher et al., 1996), can inhibit cocaine-induced hyperlocomotion by a serotonergic mechanism (Carey et al., 2001; Müller et al., 2002a), suggesting a participation of the 5-HT_{1A}-receptor in psychostimulant induced hyperactivity. This experiment was designed to investigate the contribution of the 5-HT_{1A}-receptor to the acute behavioral effects of the low potency psychostimulant, diethylpropion, in non-human primates (*Callithrix penicillata*). Given the high interindividual differences in the diethylpropion effects in monkeys and humans (Sjöberg and Jonsson, 1967; Johanson et al., 1976), WAY 100635 effects were evaluated with respect to the diethylpropion sensitivity of each animal.

2. Materials and methods

2.1. Subjects

Ten adult marmosets (*Callithrix penicillata*, 2 males and 8 females) were used as subjects. Animals weighed 220–410 g at the beginning of experiments. Before and during the experiment all animals were socially housed in separate male/female groups in indoor/outdoor cages (2×1.3×2 m) of the same colony room (not all members of the housing colony were tested in this experiment). Maintenance and testing of subjects were performed at the Primate Center, University of Brasilia. Except during the 20 min after a pretreatment and the 30 min test periods, food and water were available ad libitum. All procedures were approved by the Animal Ethics Committee of the Institute of Biology, University of Brasilia, Brazil, and followed the 'Principles of Laboratory Animal Care' (NIH publication No. 85-23, revised 1996).

2.2. Drugs

WAY 100635 (*N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl) cyclohexane-carboxamide trihydrochloride; Sigma, USA) was dissolved in 0.9% physiological saline and injected i.p. in the doses of 0.2, 0.4 and 0.8 mg/kg. The dose range was based on previous behavioral experiments investigating the effects of WAY 100635 in non-human primate tests of anxiety (Barros et al., 2003). Diethylpropion (1-phenyl-2-diethylamine-1-propanone hydrochloride; Henrifarma, Brazil) was dissolved in 0.9% physiological saline and injected i.p. in a dose of 10 mg/kg. This dose was shown to induce hyperlocomotion and conditioned place preference in rats (Reimer et al., 1995). The injection volume for WAY 100635, saline and diethylpropion injections was 1 ml/kg.

2.3. Apparatus

Testing was conducted in a figure-eight continuous maze (Barros and Tomaz, 2002). The maze consisted of a rectangular field (125×103×35 cm) suspended 1 m from the floor and divided into five arms by two holes and barriers, forming a continuous figure-eight maze (Fig. 1). The apparatus, made of 4 mm transparent glass on a metal frame support, was divided into two segments (front and back chambers) by a concrete visual barrier (147×8×218 cm). The back chamber consisted of an arm (125×30×35 cm) with a central guillotine-type door. The latter formed the start compartment. The front chamber had three parallel arms (40×25×35 cm), 25 cm apart, ending in a common perpendicular arm (125×25×35 cm). Both chambers were interconnected through holes in the visual barrier at each of the three parallel arms.

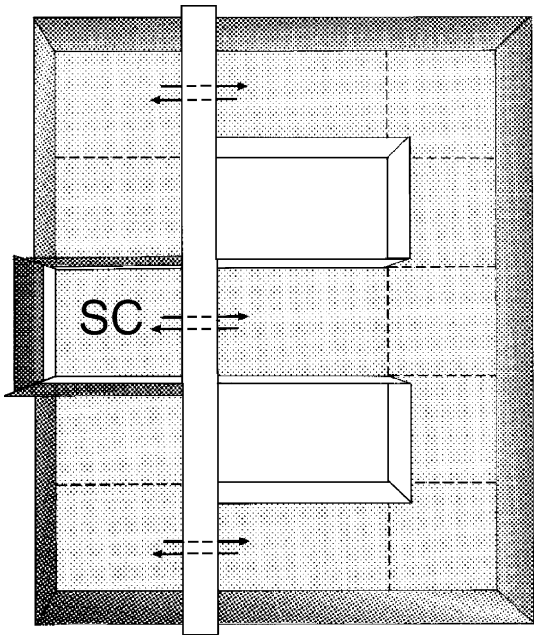


Fig. 1. Top view of the figure-eight continuous maze used for testing (SC indicates the start compartment; for a detailed description: see text).

2.4. Procedure

Before habituation to the test environment took place, the animals were handled and habituated to the transport cage (35×20×23 cm) in four sessions of 5, 10, 15 and 20 min duration, spaced 24 h apart. To avoid confounding effects of exposing the marmosets to a novel environment while measuring their response to the diethylpropion treatment, all subjects were submitted to four 30-min habituation trials to the figure-eight maze, spaced 48 h apart. Previous studies had shown that after four habituation trials activity levels remain constant (Barros et al., 2003). Following the habituation trials, five pseudo-randomly assigned treatment trials were performed with each subject with a wash out period of 72 h between the treatments. As a pretreatment the animals received either an i.p. injection of WAY 100635 (0.2, 0.4 and 0.8 mg/kg) or saline. After the pretreatment the animals were returned to the transport cage for 20 min before they received an i.p. injection of 10 mg/kg diethylpropion or saline. Immediately following the treatment injection the marmoset was released into the maze's back chamber start compartment, thus commencing a 30-min trial. Barriers from this compartment were promptly removed upon the animal's exit, permitting free access to the whole apparatus. After the session, the subject was returned to its home environment in the transport cage. Treatments and order of subjects were pseudo-randomly assigned for each test day. Video cameras were used for online monitoring and all trials were recorded for later behavioral analysis. All test sessions were performed between 8:00 a.m. and 1:00 p.m.

2.5. Behavioral analysis

For behavioral analysis, the maze was divided into 13 sections. The following behavioral parameters were scored for each 30-min trial by an experienced observer blind to the experimental treatment: (1) *Locomotor activity*: the number of maze sections crossed with both forelimbs; (2) *Exploratory activity*: the number of times that the animal spent sniffing and/or licking any part of the apparatus; (3) *Bodycare activities*: number of times the animal spent grooming (slow and precise repetitive movements of the hand through the fur) or scratching (quick repetitive movements of hands or foot through the fur); (4) *Scent marking*: the number of times that the animal rubbed the anogenital region on any substratum; (5) *Aerial scanning*: time the animals spent scanning the environment from the horizontal plane upwards; (6) *Terrestrial scanning*: time the animals spent scanning the environment below the horizontal. Locomotor activity was scored using a semi-automated behavior analysis program (Chromotrack 4.02, San Diego Instruments), whereas the frequency of exploratory activities was measured by focal-all occurrences samplings.

2.6. Statistical analysis

The data were analyzed by means of one-way analysis of variance (ANOVA) for repeated measures on the treatment factor. In order to identify differences versus the saline–saline treatment pre-planned comparisons were calculated using LSD-tests. In order to identify diethylpropion-sensitive animals the locomotor response was used as a criterion. Animals which showed an increase in locomotor activity after the saline–diethylpropion treatment compared to the saline–saline treatment were considered to be "diethylpropion sensitive". All other animals were considered to be "diethylpropion insensitive". All behavioral parameters were further analyzed with respect to the diethylpropion sensitivity of the animals. In order to identify differences in the behavioral response to the treatments between diethylpropion-sensitive and -insensitive animals pre-planned comparisons were calculated using the LSD-test. All statistical results were interpreted as measures of effect with a P-value of 0.05 as a criterion.

3. Results

The injection of saline–diethylpropion caused an increase in the locomotor activity which, however, did not reach P-levels <0.05 when all animals were analyzed together (Fig. 2A). The pretreatment with WAY 100635 did not substantially modify the diethylpropion effects on locomotion. The sensitivity analysis (Fig. 2B) revealed that 6 of the 10 animals tested showed an increase in the locomotor response to diethylpropion (P<0.05). These animals were considered to be diethylpropion sensitive. The other 4

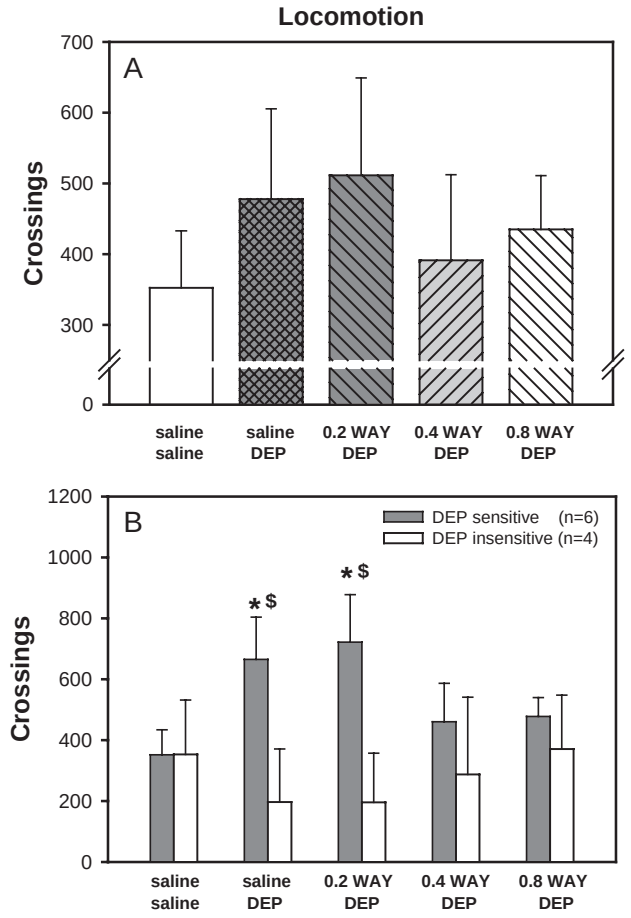


Fig. 2. The effects of diethylpropion (10 mg/kg, i.p.) on locomotor activity (mean±SEM) and its modulation by the 5-HT_{1A}-receptor antagonist, WAY 100635 (0.2–0.8 mg/kg, i.p.), during a 30-min test trial. (A) Effects for all animals tested (n=10). (B) Sensitivity analysis: group splitting according to the animals response to diethylpropion (*sensitive*: increased locomotor activity after saline–diethylpropion vs. saline–saline; *insensitive*: no increase in locomotor activity after saline–diethylpropion vs. saline–saline; *P<0.05 vs. saline–saline; $^{\$}P$<0.01 sensitive vs. insensitive).

animals did not show an increase in locomotor activity as a response to diethylpropion compared to saline–saline (P>0.05). These animals were considered to be diethylpropion insensitive. The WAY 100635 pretreatment did not modify the locomotor activity in the diethylpropion insensitive animals after the diethylpropion treatment. However, it blocked the diethylpropion-induced increase in locomotor activity in the diethylpropion sensitive animals at a dose of 0.4 and 0.8 mg/kg WAY 100635 (P>0.05 vs. saline–saline). The pretreatment with 0.2 mg/kg WAY 100635 did not modify the diethylpropion-induced increase in locomotor activity in the diethylpropion sensitive animals (P<0.05 vs. saline–saline).

The treatments had a profound effect on the exploratory activity ($F_{4,36}$=6.17, P<0.001; Fig. 3A). The injection of diethylpropion decreased exploratory activity (P<0.01) compared to saline–saline treatment. This decrease was not affected by the pretreatment with WAY 100635 (P<0.001, all WAY 100635–diethylpropion groups vs. saline–saline). Sensitivity analysis (Fig. 3B) showed a decreased exploratory activity after the diethylpropion treatment in the diethylpropion sensitive (P<0.01) and as a tendency in the insensitive animals (P<0.052). The WAY 100635 pretreatment did not affect the diethylpropion-induced decrease in exploratory activity in the diethylpropion sensitive group (P<0.01, all WAY 100635–diethylpropion groups vs. saline–saline). In the diethylpropion insensitive group WAY 001635 slightly potentiated the inhibitory effect of diethylpropion (P<0.05, 0.2 mg/kg WAY 100635–diethylpropion; P<0.01, 0.4 and 0.8 mg/kg WAY 100635–diethylpropion vs. saline–saline). However, there was no statistical difference in the effect of WAY 100635 on diethylpropion-induced suppression of exploratory activity between diethylpropion sensitive and insensitive animals (P>0.05).

Bodycare activities comprise grooming and scratching behavior of the animals (Fig. 4A). The treatments did not

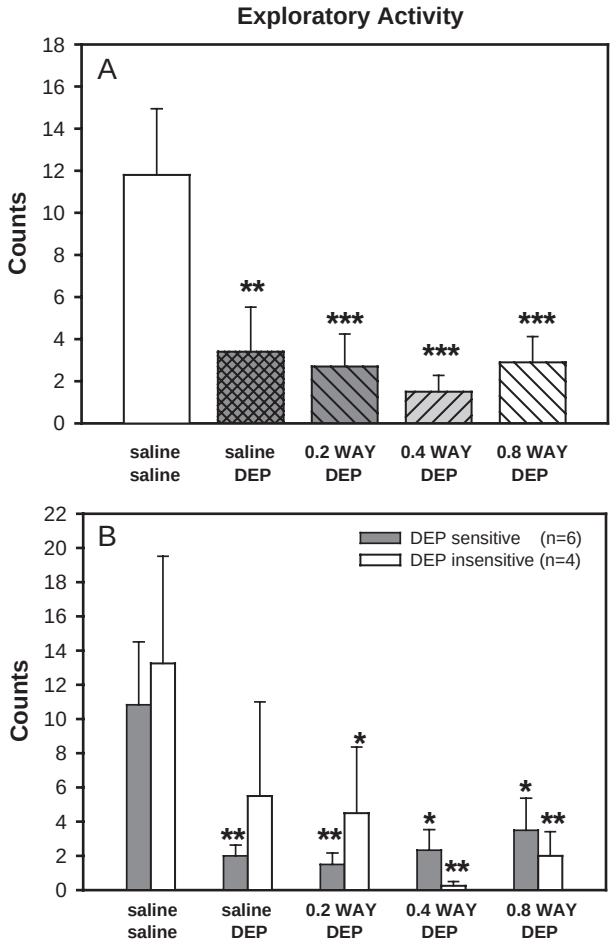


Fig. 3. The effects of diethylpropion (10 mg/kg, i.p.) on exploratory activity (mean ±SEM) and its modulation by the 5-HT_{1A}-receptor antagonist, WAY 100635 (0.2–0.8 mg/kg, i.p.), during a 30-min test trial. (A) Effects for all animals tested (n=10). (B) Sensitivity analysis: group splitting according to the animals response to diethylpropion (*sensitive*: increased locomotor activity after saline–diethylpropion vs. saline–saline; *insensitive*: no increase in locomotor activity after saline–diethylpropion vs. saline–saline; *P<0.05, **P<0.01, ***P<0.001 vs. saline–saline).

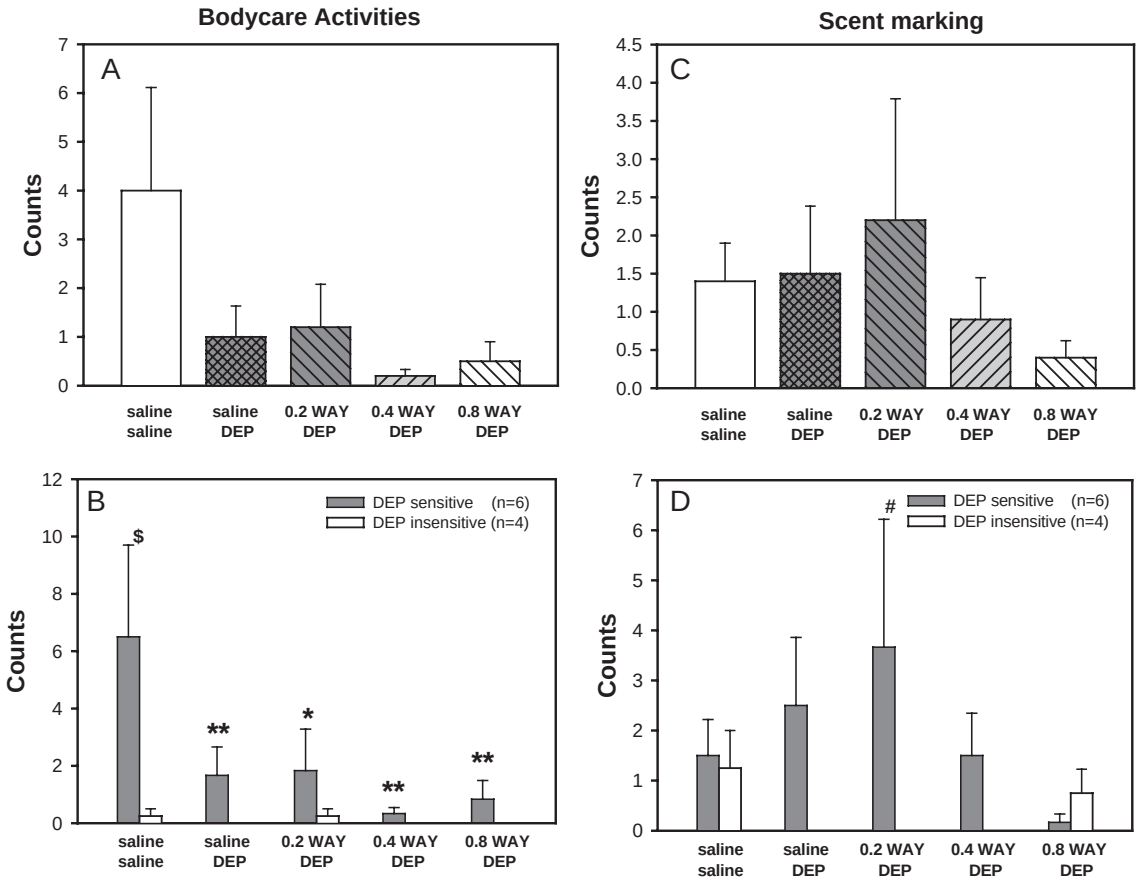


Fig. 4. The effects of diethylpropion (10 mg/kg, i.p.) on bodycare activities (scratching and grooming) and on scent marking (mean ±SEM) and its modulation by the 5-HT_{1A}-receptor antagonist, WAY 100635 (0.2–0.8 mg/kg, i.p.), during a 30 min test trial. (A, C) Effects for all animals tested (n=10). (B, D) Sensitivity analysis: group splitting according to the animals response to diethylpropion (*sensitive*: increased locomotor activity after saline–diethylpropion vs. saline–saline; *insensitive*: no increase in locomotor activity after saline–diethylpropion vs. saline–saline; *P<0.05, **P<0.01 vs. saline–saline; $^{\#}P$<0.05, $^{\$}P$<0.01 sensitive vs. insensitive).

affect this parameter. However, there was a tendency ($F_{4,36}$=2.39, P<0.069) for a decreased response after the diethylpropion treatment which was not affected by the pretreatment with WAY 100635. The sensitivity analysis revealed a strong difference in the bodycare activities between diethylpropion-sensitive and -insensitive animals after the saline–saline treatment (P<0.01). The diethylpropion insensitive animals hardly showed any bodycare activities in the 30 min test trials, which might have masked a possible inhibitory effect of diethylpropion in these animals. In the diethylpropion sensitive animals the diethylpropion treatment attenuated bodycare activities (P<0.01). The pretreatment with WAY 100635 did not modulate this effect (P<0.05, 0.2 mg/kg WAY 100635–diethylpropion vs. saline–saline; P<0.01, 0.4 and 0.8 mg/kg WAY 100635–diethylpropion vs. saline–saline).

The diethylpropion treatment did not affect scent marking behavior (P>0.05; Fig. 4C). Sensitivity analysis showed that scent marking behavior virtually disappeared in the diethylpropion insensitive group after diethylpropion treatments (Fig. 4D). Statistical comparisons showed a difference between diethylpropion sensitive and insensitive animals only after the 0.2 mg/kg WAY 100635–diethylpropion treatment (P<0.05).

The diethylpropion treatment caused a pronounced increase in aerial scanning time ($F_{4,36}$=2.86, P<0.04; saline–diethylpropion vs. saline–saline: P<0.01; Fig. 5A) but not in the frequency of aerial scanning (P>0.05; Fig. 5C). Pretreatment with 0.4 and 0.8 mg/kg WAY 100635 blocked the increase in aerial scanning time (P>0.05 vs. saline–saline). The sensitivity analysis showed profound differences in the levels of aerial scanning time between the diethylpropion sensitive and insensitive animals (Fig. 5B). Interestingly, diethylpropion sensitive animals showed only a small increase after the diethylpropion treatment (P>0.05), while in the insensitive animals diethylpropion strongly increased aerial scanning time (diethylpropion-saline vs. saline–saline: P<0.001). WAY 100635 partially blocked this effect in the diethylpropion insensitive animals dose-dependently with 0.8 mg/kg WAY 100635 as the most effective dose (P>0.05 vs. saline–saline). Sensitive and insensitive animals differed not only in the time of aerial scanning after the diethylpropion treatment (P<0.001) but also after all doses of WAY 100635–diethylpropion

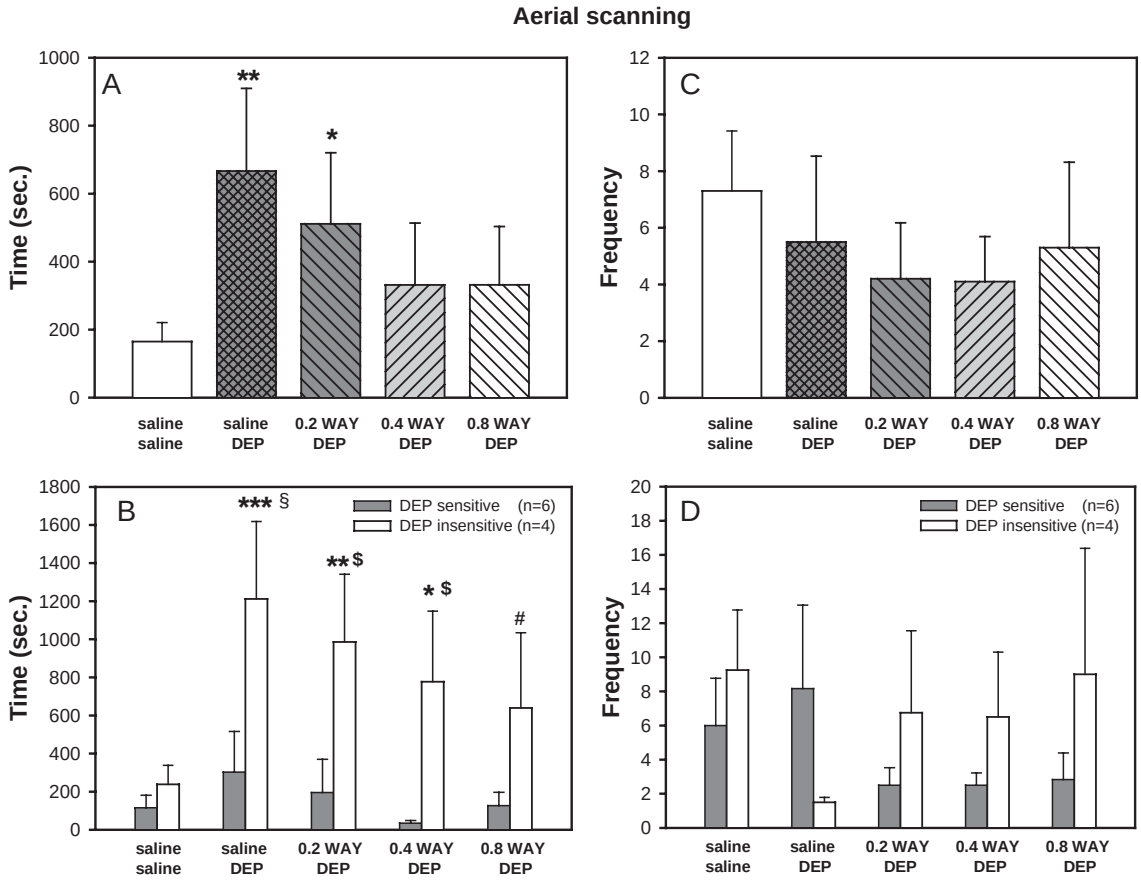


Fig. 5. The effects of diethylpropion (10 mg/kg, i.p.) on aerial scanning (mean±SEM) and its modulation by the 5-HT_{1A}-receptor antagonist, WAY 100635 (0.2–0.8 mg/kg, i.p.), during a 30-min test trial. (A, C) Effects for all animals tested (n=10). (B, D) Sensitivity analysis: group splitting according to the animals response to diethylpropion (*sensitive*: increased locomotor activity after saline–diethylpropion vs. saline–saline; *insensitive*: no increase in locomotor activity after saline–diethylpropion vs. saline–saline; *P<0.05, **P<0.01, ***P<0.001 vs. saline–saline; $^{\#}P$<0.05, $^{\$}P$<0.01, $^{\S}P$<0.001 sensitive vs. insensitive).

(P<0.01, 0.2 and 0.4 mg/kg WAY 100635–diethylpropion, P<0.05, 0.8 mg/kg WAY 100635–diethylpropion). There were no clear effects on the frequency of aerial scanning (Fig. 5D).

In contrast to the increase in aerial scanning time, diethylpropion caused a decrease in terrestrial scanning which reached P-levels <0.05 for the frequency ($F_{4,36}$=3.51, P<0.02; saline–diethylpropion vs. saline–saline: P<0.01; Fig. 6C) but not for the time (Fig. 6A). A dose of 0.4 mg/kg WAY 100635 partially reversed this decrease (P>0.05 vs. saline–saline), while 0.2 and 0.8 mg/kg WAY 100635 did not have any effect (P<0.01 and P<0.05 vs. saline–saline). The sensitivity analysis (Fig. 6D) showed that diethylpropion had the strongest effects in the insensitive animals (diethylpropion-saline vs. saline–saline: P< 0.01). However, in these animals none of these doses of WAY 100635 reversed the inhibitory effect of diethylpropion (P<0.01, 0.2 and 0.4 mg/kg WAY 100635–diethylpropion; P<0.05, 0.8 mg/kg WAY 100635–diethylpropion vs. saline–saline). However, there were no differences between the diethylpropion or WAY 100635 effects between the sensitive and insensitive animals (P>0.05). Sensitivity analysis further revealed a difference between diethylpropion-sensitive and -insensitive animals for the time spent in terrestrial scanning (Fig. 6B) indicating a different effect of 0.4 mg/kg WAY 100635 on the diethylpropion-induced suppression (P<0.05).

4. Discussion

The effects of the low potency psychostimulant diethylpropion were investigated in a broad range of marmoset behaviors. An increase in locomotor activity could be found only in 60% of the animals after diethylpropion. This increase, which is usually considered as an indicator of the stimulant properties of a drug (Hoekenga et al., 1978), was used to subdivide the population of the animals into diethylpropion-sensitive and diethylpropion-insensitive animals. The behavioral profile of diethylpropion also comprised an inhibitory effect on exploratory activity. Diethylpropion furthermore potentiated the dominant aerial scanning, but inhibited the less pronounced terrestrial scanning. There was also an overall tendency to block bodycare activities, while it had no obvious effect on scent marking behavior. Sensitivity

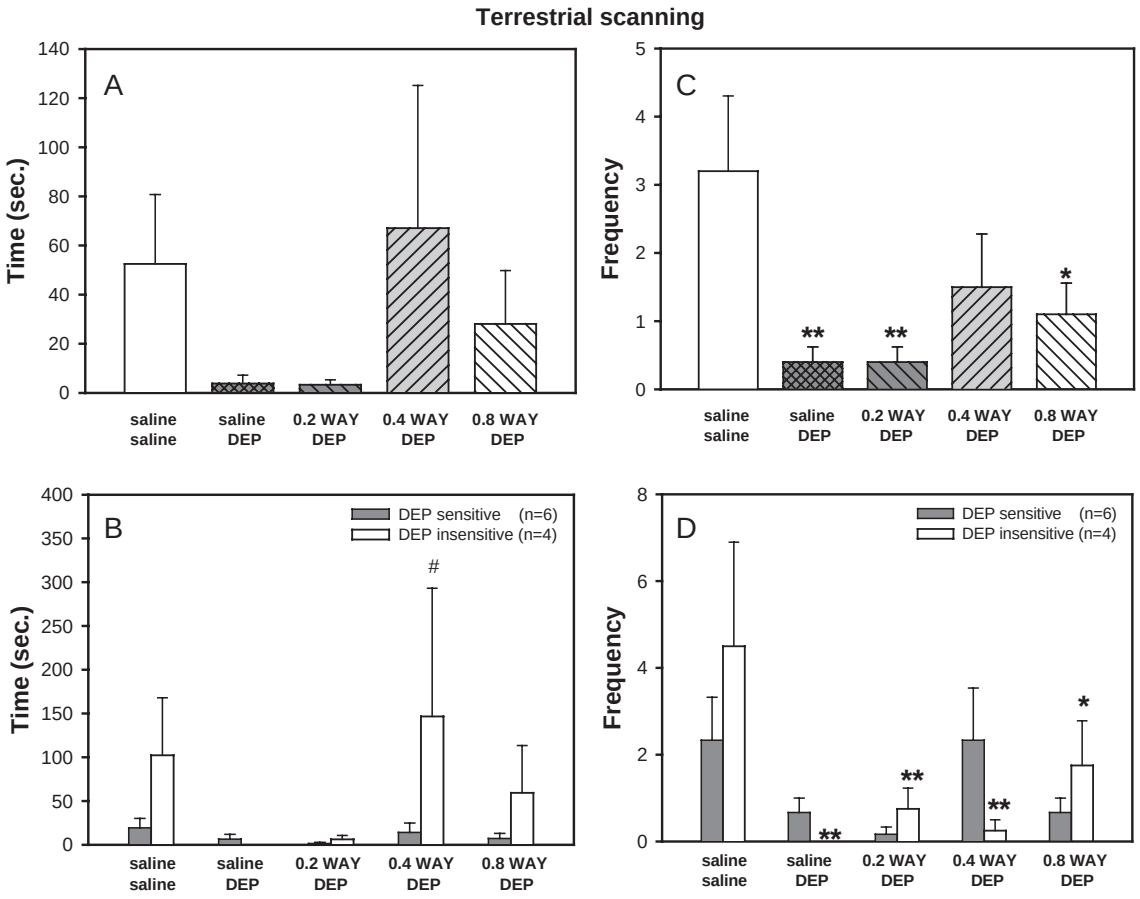


Fig. 6. The effects of diethylpropion (10 mg/kg, i.p.) on terrestrial scanning (mean±SEM) and its modulation by the 5-HT_{1A}-receptor antagonist, WAY 100635 (0.2–0.8 mg/kg, i.p.), during a 30-min test trial. (A, C) Effects for all animals tested (n=10). (B, D) Sensitivity analysis: group splitting according to the animals response to diethylpropion (*sensitive*: increased locomotor activity after saline–diethylpropion vs. saline–saline; *insensitive*: no increase in locomotor activity after saline–diethylpropion vs. saline–saline; *P<0.05, **P<0.01 vs. saline–saline; $^{\#}P$<0.05 sensitive vs. insensitive).

analysis revealed that there are two principle types of responses to diethylpropion in the marmosets. Type A monkeys (sensitive) are characterized in their response to diethylpropion by a profound increase in locomotor activity, a decrease in exploratory activity and a decrease in bodycare activities. Type B monkeys (insensitive) did not show hyperlocomotion and a decrease in bodycare activities, but showed a profound increase in aerial scanning and a decrease in terrestrial scanning following diethylpropion treatment. Exploratory activity was only decreased as a tendency. This study furthermore showed that the 5-HT_{1A}-receptor antagonist, WAY 100635, affects both responder types in different ways. In the sensitive animals (type A) it blocked the diethylpropion-induced hyperlocomotion while not affecting locomotor response in the insensitive (type B) animals. In the insensitive animals WAY 100635 partially reversed the diethylpropion-induced increase in aerial scanning. In contrast, there were no clear effects on the visual scanning response to diethylpropion in the sensitive (type A) animals. These data suggest that the 5-HT_{1A}-receptor does not only play an essential role in the mediation of important behavioral effects of the low potency psychostimulant diethylpropion, but is also the source of some interindividual differences in the response to diethylpropion. Interestingly, WAY 100635 did not have obvious effects on the diethylpropion-induced suppression of the exploratory activity in both types of animals and on the suppression of bodycare activities, which occurred only in the sensitive (type A) animals. Accordingly, the 5-HT_{1A}-receptor does not contribute to the whole spectrum of the acute behavioral effects of diethylpropion.

The observation that diethylpropion did not increase overall locomotor activity was surprising since other groups had shown it (Tang and Kirch, 1971; Safta et al., 1976; Reimer et al., 1995; Gevaerd et al., 1999; Da Silva and Cordellini, 2003). However, highly variant locomotor responses to a stimulant drug in a medium dose range are not unique to diethylpropion but were also reported for other psychostimulants (e.g. Müller et al., 2004). The main reason for these effects are interindividual differences of the animals (e.g. Hooks et al., 1991). The failure to induce hyperlocomotion in all animals tested may be due to the dose choice for diethylpropion, which was in a range that was found by others to have reinforcing properties (Reimer et al., 1995) and to be sufficient to reduce food intake (Garattini et al., 1978). Using the locomotor response as a criterion for

diethylpropion-sensitivity in this study, 60% of the animals increased activity, while 40% did not show an obvious effect. In that, our observation confirms studies in monkeys and humans which showed high interindividual differences in the behavioral effects of diethylpropion (Sjöberg and Jonsson, 1967; Johanson et al., 1976). Further behavioral analysis according to the locomotor response to diethylpropion revealed that these animals, which showed a high locomotor response, also showed a decrease in exploratory activity and a decrease in bodycare activities, but no obvious alterations in the aerial or terrestrial scanning behavior. A decrease in exploratory activity in marmosets is associated with an anxiogenic state (Barros et al., 2004a), which can be reversed by anxiolytic drugs like diazepam (Barros et al., 2000). Thus, the diethylpropion-induced decrease in exploratory activity may be interpreted as an anxiogenic effect. Anxiogenic-like behavioral effects had also been demonstrated for other psychostimulants like cocaine (Yang et al., 1992; Goeders, 1992) and may be considered to be an integral part of the behavioral spectrum of psychostimulants. This counts also for the suppression of bodycare activities, as a decrease in grooming activity was reported in rats after diethylpropion (Da Silva and Cordellini, 2003) and other psychostimulants (e.g. Cooper and Van der Hoek, 1993; Müller et al., 2002b). Interestingly, diethylpropion did not obviously affect aerial or terrestrial scanning behavior in the sensitive (type A) animals. In callitrichids, visual scanning, which includes the predominant aerial and the less frequent terrestrial scanning, facilitates the detection of objects in the environment and has a high adaptive value (Caine, 1984; Hardie and Buchanan-Smith, 1997). The presentation of a potential threat is associated with an increase in visual scanning (Caine, 1984, 1998; Ferrari and Lopes Ferrari, 1990; Hardie and Buchanan-Smith, 1997; Koenig, 1998). In conclusion, the diethylpropion-sensitive marmosets (type A) response to diethylpropion can be characterized as hyperlocomotor, anxiogenic and bodycare suppressive. In contrast to the sensitive (type A) animals, the insensitive animals (type B) did not show hyperlocomotion after the diethylpropion treatment. In addition, there was only a tendency for an inhibitory effect on exploratory activity. Furthermore, the bodycare activities of these animals had been so low that a suppressive action of diethylpropion may have been masked by a floor effect. These animals, however, showed a strong increase in aerial scanning but a suppression of terrestrial scanning after diethylpropion. The magnitude of this effect also argues against the possibility that the lack of a hyperlocomotor response in these animals is due to a lowered bioavailability of diethylpropion. The effect on exploratory activity and the potent effect on aerial scanning suggest a more pronounced anxiogenic effect of diethylpropion in the type B responders (Barros et al., 2004b). In conclusion, the behavioral response to diethylpropion by the insensitive marmosets (type B) is characterized by a lack of hyperlocomotor effects, a high anxiogenic component and no bodycare suppressive effects.

The pretreatment with WAY 100635 can not only provide information about which role the 5-HT_{1A}-receptor plays in the general population in the mediation of the behavioral effects of diethylpropion, but can also provide clues about the role of the 5-HT_{1A}-receptor as a source of the interindividual variance in the response to the low potency psychostimulant diethylpropion. When all animals were pooled WAY 100635 did not modulate the locomotor, exploratory or bodycare activities after a diethylpropion treatment. It only attenuated the effects on aerial and terrestrial scanning dose-dependently. The lack of effect on the diethylpropion-induced suppression of exploratory activity in this experiment is surprising since it was recently shown that WAY 100635 can reverse a decrease in exploratory activity induced by predatory stress in *Callithrix penicillata* (Barros et al., 2003). Accordingly, it may be speculated that the anxiety states induced by predatory stress and that induced by a psychostimulant like diethylpropion, which both lead to a suppression of exploratory activity, may be different and/or differentially involve a 5-HT_{1A}-receptor contribution.

When WAY 100635 effects were analyzed with regard to the diethylpropion sensitivity of the monkeys, profound effects could be detected. In the sensitive (type A) animals WAY 100635 attenuated the diethylpropion-induced increase in locomotor activity, but had no effect on the suppression of exploratory activity and bodycare activities. These results expand the findings in rats, where WAY 100635 reversed the cocaine-induced hyperlocomotion, but had no effect on the suppression of grooming behavior (Carey et al., 2001; Müller et al., 2002b). The lack of an effect on exploratory and bodycare activities after diethylpropion suggests that the 5-HT_{1A}-receptor is neither involved in the mediation of the anxiogenic-like response or the bodycare suppression of diethylpropion in the sensitive animals. Overall, the findings in the diethylpropion sensitive monkeys support the data in rodents that the 5-HT_{1A}-receptor plays mainly a role in the "positive effects" of psychostimulants but not in their "negative effects" (Müller et al., 2004).

In the insensitive (type B) animals WAY 100635 had no effect on locomotion after diethylpropion, but it attenuated diethylpropion effects on aerial and terrestrial scanning, an anxiety related behavioral response. However, also in the insensitive animals (type B) not all anxiety related behavioral effects of diethylpropion were affected by the WAY 100635 pretreatment. As in the sensitive animals WAY 100635 did not reverse the decrease in exploratory activity. When comparing the effects of WAY 100635 on the behavioral profile of diethylpropion between sensitive (type A) and insensitive (type B) monkeys a double dissociation became obvious: WAY 100635 reversed the hyperlocomotor effects in the sensitive animals not affecting the diethylpropion effects on locomotion in the insensitive animals. In contrast, WAY 100635 reversed an anxiogenic-like diethylpropion effect in the insensitive animals, not affect-

ing this behavior in the sensitive animals. From that observations it is suggested that the 5-HT_{1A}-receptor is one source of the interindividual differences in the acute behavioral response to the low potency psychostimulant diethylpropion in monkeys.

Acknowledgement

This work was supported by the Deutsche Forschungsgemeinschaft (HU 306/23-2 and HU 306/23-3 to JPH and CPM), by FINATEC (to CT), by CAPES/DAAD/PROBAL (to CT and JPH), a VA Merit Review grant (to RJC), and a NIDA grant (DAROI 05366 to RJC). ELM was a recipient of a fellowship from CAPES, and CT a recipient of a CNPq researcher fellowship (no. 300364/1986-5).

References

Barros, M., Tomaz, C., 2002. Non-human primate models for investigating fear and anxiety. Neurosci. Biobehav. Rev. 26, 187–201.

Barros, M., Boere, V., Huston, J.P., Tomaz, C., 2000. Measuring fear and anxiety in the marmoset (*Callithrix penicillata*) with a novel predator confrontation model: effects of diazepam. Behav. Brain Res. 108, 205–211.

Barros, M., Mello, E.L., Maior, R.S., Müller, C.P., De Souza-Silva, M.A., Carey, R.J., Huston, J.P., Tomaz, C., 2003. Anxiolytic-like effects of the selective 5-HT1A receptor antagonist WAY 100635 in non-human primates. Eur. J. Pharmacol. 482, 197–203.

Barros, M., De Souza-Silva, M.A., Huston, J.P., Tomaz, C., 2004a. Multibehavioral analysis of fear and anxiety before, during, and after experimentally induced predatory stress in *Callithrix penicillata*. Pharmacol. Biochem. Behav. 78, 357–367.

Barros, M., Alencar, C., Tomaz, C., 2004b. Differences in aerial and terrestrial visual scanning in captive black tufted-ear marmosets (*Callithrix penicillata*) exposed to a novel environment. Folia Primatol. 75, 85–92.

Borsini, F., Bendotti, C., Carli, M., Poggesi, E., Samanin, R., 1979. The roles of brain noradrenaline and dopamine in the anorectic activity of diethylpropion in rats: a comparison with d-amphetamine. Res. Commun. Chem. Pathol. Pharmacol. 26, 3–11.

Bray, G.A., 2000. A concise review on the therapeutics of obesity. Nutrition 16, 953–960.

Brooke, D., Kerwin, R., Lloyd, K., 1988. Diethylpropion hydrochloride-induced psychosis. Br. J. Psychiatry 152, 572–573.

Caine, N.G., 1984. Visual scanning by tamarins: a description of the behavior and tests of two derived hypothesis. Folia Primatol. 43, 59–67.

Caine, N.G., 1998. Cutting costs in response to predatory threat by Geoffroy's marmostes (*Callithrix geoffroyi*). Am. J. Primatol. 46, 187–196.

Carey, R.J., DePalma, G., Damianopoulos, E., 2001. Cocaine and serotonin: a role for the 5-HT(1A) receptor site in the mediation of cocaine stimulant effects. Behav. Brain Res. 126, 127–133.

Carney, M.W., 1988. Diethylpropion and psychosis. Br. J. Psychiatry 152, 146–147.

Cooper, S.J., Van der Hoek, G.A., 1993. Cocaine: a microstructural analysis of its effects on feeding and associated behaviour in the rat. Brain Res. 608, 45–51.

Da Silva, L.B., Cordellini, S., 2003. Effects of diethylpropion treatment and withdrawal on aorta reactivity, edothelial factors and rat behavior. Toxicol. Appl. Pharmacol. 190, 170–176.

Ferrari, S.F., Lopes Ferrari, M.A., 1990. Predator avoidance behavior in the buffy-headed marmoset (*Callithrix flaviceps*). Primates 31, 323–338.

Fletcher, A., Forster, E.A., Bill, D.J., Brown, G., Cliffe, I.A., Hartley, J.E., Jones, D.E., McLenachan, A., Stanhope, K.J., Critchley, D.J., Childs, K.J., Middlefell, V.C., Lanfumey, L., Corradetti, R., Laporte, A.M., Gozlan, H., Hamon, M., Dourish, C.T., 1996. Electrophysiological, biochemical, neurohormonal and behavioural studies with WAY-100635, a potent, selective and silent 5-HT1A receptor antagonist. Behav. Brain Res. 73, 337–353.

Foltin, R.W., 1989. Effects of anorectic drugs on the topography of feeding behavior in baboons. J. Pharmacol. Exp. Ther. 249, 101–109.

Foltin, R.W., 2001. Effects of amphetamine, dexfenfluramine, diazepam, and other pharmacological and dietary manipulations on food "seeking" and "taking" behavior in non-human primates. Psychopharmacology 158, 28–38.

Fookes, B.H., 1976. Schizophrenia-like reaction to diethylpropion. Lancet 2, 1206.

Garattini, S., Borroni, E., Mennini, T., Samanin, R., 1978. Differences and similarities among anorectic agents. In: Garattini, S., Samanin, R. (Eds.), Central Mechanisms of Anorectic Drugs. Raven Press, New York, pp. 127–143.

Gevaerd, M.S., Sultowski, E.T., Takahashi, R.N., 1999. Combined effects of diethylpropion and alcohol on locomotor activity of mice: participation of the dopaminergic and opioid systems. Braz. J. Med. Biol. Res. 32, 1545–1550.

Goeders, N.E., 1992. Potential involvement of anxiety in the neurobiology of cocaine. Ann. N.Y. Acad. Sci. 654, 357–367.

Griffiths, R.R., Winger, G., Brady, J.V., Snell, J.D., 1976. Comparison of behavior maintained by infusions of eight phenylethylamines in baboons. Psychopharmacology 50, 251–258.

Griffiths, R.R., Brady, J.V., Snell, J.D., 1978. Relationship between anorectic and reinforcing properties of appetite suppressant drugs: implications for assessment of abuse liability. Biol. Psychiatry 13, 283–290.

Hardie, S.M., Buchanan-Smith, H.M., 1997. Vigilance in single and mixed-species groups of tamarins (*Saguinus labiatus* and *S. fuscicollis*). Int. J. Primatol. 18, 217–234.

Hoekenga, M.T., Dillon, R.H., Leyland, H.M., 1978. A comprehensive review of diethylpropion hydrochloride. In: Garattini, S., Samanin, R. (Eds.), Central Mechanisms of Anorectic Drugs. Raven Press, New York, pp. 391–404.

Hooks, M.S., Jones, G.H., Smith, A.D., Neill, D.B., Justice, J.B., 1991. Response to novelty predicts the locomotor and nucleus accumbens dopamine response to cocaine. Synapse 9, 121–128.

Jasinski, D.R., Nutt, J.G., Griffith, J.D., 1974. Effects of diethylpropion and d-amphetamine after subcutaneous and oral administration. Clin. Pharmacol. Ther. 16, 645–652.

Johanson, C.E., Schuster, C.R., 1976. A comparison of cocaine and dietylpropion under two different schedules of drug presentation. In: Ellinwood, E.H., Kilbey, M.M. (Eds.), Cocaine and Other Stimulants. Plenum, New York, pp. 545–570.

Johanson, C.E., Balster, R.L., Bonese, K., 1976. Self-administration of psychomotor stimulant drugs: the effects of unlimited access. Pharmacol. Biochem. Behav. 4, 45–51.

Jonsson, C.O., Sjöberg, L., Ek, S., Vallbo, S., 1967. Studies in the psychological effects of a new drug (diethylpropion). Time curves for five subjective variables. Scand. J. Psychol. 8, 39–46.

Khan, S.A., Spiegel, D.A., Jobe, P.C., 1987. Psychotomimetic effects of anorectic drugs. Am. Fam. Phys. 36, 107–112.

Koenig, A., 1998. Visual scanning by common marmostes (*Callithrix jacchus*): functional aspects and the special role of adult males. Primates 39, 85–90.

Müller, C.P., Carey, R.J., De Souza Silva, M.A., Jocham, G., Huston, J.P., 2002a. Cocaine increases serotonergic activity in the hippocampus and nucleus accumbens in vivo: 5-HT1A-receptor antagonism blocks behavioral but potentiates serotonergic activation. Synapse 45, 67–77.

Müller, C.P., De Souza Silva, M.A., DePalma, G., Tomaz, C., Carey, R.J., Huston, J.P., 2002b. The selective serotonin1A-receptor antagonist WAY 100635 blocks behavioral stimulating effects of cocaine but not ventral striatal dopamine increase. Behav. Brain Res. 134, 337–346.

Müller, C.P., Thönnessen, H., Jocham, G., Barros, M., Tomaz, C., Carey, R.J., Huston, J.P., 2004. Cocaine-induced "active immobility" and its modulation by the serotonin$_{1A}$-receptor. Behav. Pharmacol. 15, 481–493.

Planeta, C.S., DeLucia, R., 1998. Involvement of dopamine receptors in diethylpropion-induced conditioning place preference. Braz. J. Med. Biol. Res. 31, 561–564.

Reimer, A.R., Martin-Iverson, M.T., Urichuk, L.J., Coutts, R.T., Byrne, A., 1995. Conditioned place preferences, conditioned locomotion, and behavioral sensitization occur in rats treated with diethylpropion. Pharmacol. Biochem. Behav. 51, 89–96.

Ryan, D.H., 2000. Use of sibutramine and other noradrenergic and serotonergic drugs in the management of obesity. Endocrine 13, 193–199.

Safta, L., Cuparencu, B., Sirbu, A., Secareanu, A., 1976. Experimental observations on the effect of amphepramone on the behavior, locomotion, pentretrazol seizures and electroencephalogram. Psychopharmacology 50, 165–169.

Samanin, R., Garattini, S., 1993. Neurochemical mechanism of action of anorectic drugs. Pharmacol. Toxicol. 73, 63–68.

Sjöberg, L., Jonsson, C.O., 1967. Studies in the psychological effects of a new drug (diethylpropion): individual differences. Scand. J. Psychol. 8, 81–87.

Tang, A.H., Kirch, J.D., 1971. Appetite suppression and central nervous system stimulation in the rhesus monkey. Psychopharmacologia 21, 139–146.

Weiser, M., Frishman, W.H., Michaelson, M.D., Abdeen, M.A., 1997. The pharmacologic approach to the treatment of obesity. J. Clin. Pharmacol. 37, 453–473.

Wood, D.M., Emmett-Oglesby, M.W., 1988. Substitution and cross-tolerance profiles of anorectic drugs in rats trained to detect the discriminative stimulus properties of cocaine. Psychopharmacology 95, 364–368.

Yang, X.M., Gorman, A.L., Dunn, A.J., Goeders, N.E., 1992. Anxiogenic effects of acute and chronic cocaine administration: neurochemical and behavioral studies. Pharmacol. Biochem. Behav. 41, 643–650.

EXPERIMENTO 2

Efeitos do Antagonista do receptor NK_3, SR142801
Sobre os Efeitos Estimulantes da Cocaína

(O presente estudo encontra-se aceito para publicação no *European Journal of Pharmacology*)

A cocaína é um psicoestimulante clássico, de origem vegetal e cuja história se confunde com a da própria humanidade, em especial, com os povos nativos que habitam as grandes altitudes da cordilheira dos Andes, na América do Sul. Teve seu uso difundido na Europa por Freud mas logo seus efeitos aditivos sobrepuseram eventuais benefícios. Tornou-se então uma das drogas de abuso mais usadas no meio artístico e na alta sociedade. Seus derivados, mais baratos, são uma opção acessível também às classes mais baixas da sociedade. Caracterizou-se, na década de 80 do século passado, como um dos maiores problemas de saúde em países ricos como os Estados Unidos, por exemplo. Desde então, uma série de pesquisas vêm sendo conduzidas a fim de conhecer melhor os mecanismos de ação da cocaína; seus efeitos a curto, médio e longo prazo; o mecanismo da dependência; métodos de desintoxicação e reabilitação de dependentes.

Inserido neste contexto, o presente trabalho, consoante com o trabalho previamente apresentado, é mais um esforço na compreensão dos mecanismos pelos quais a cocaína produz seus efeitos indesejáveis. Com base em estudos recentes com neuropeptídeos que relacionam estes transmissores com comportamentos de auto-administração (Krappmann *et al.*, 1994) e, mais especificamente, com o receptor NK_3 (Jocham *et al.*, submetido), sugerem esta via como mais uma porta para acesso e possível controle indireto dos efeitos estimulantes da cocaína.

Este experimento foi delineado para investigar a contribuição do receptor de NK_3 nos efeitos comportamentais agudos da cocaína, em primatas não-humanos (*Callithrix penicillata*). Assim como no estudo anterior, diferenças interindividuais na resposta à cocaína são melhores avaliadas com base na sensibilidade à droga de cada sujeito experimental. Além disso, a manutenção da metodologia anteriormente empregada permite um intercâmbio maior e melhor entre os dois trabalhos, permitindo chegar a conclusões mais significativas.

The neurokinin-3 receptor antagonist SR142801 blocks the behavioral effects of cocaine in marmoset monkeys

Maria A. De Souza Silva[a], Eldon L. Mello Jr.[b], Christian P. Müller[a], Gerhard Jocham[a], Rafael S. Maior[b], Joseph P. Huston[a], Carlos Tomaz[b], and Marilia Barros[c]

[a] Institute of Physiological Psychology and Center for Biological and Medical Research, University of Düsseldorf, Universitätsstr. 1, 40225 Düsseldorf, Germany

[b] Department of Physiological Sciences, Institute of Biology, University of Brasilia, CEP 70910-900 Brasilia, DF, Brazil

[c] Department of Pharmaceutical Sciences, School of Health Sciences, University of Brasilia, CEP 70910-900 Brasilia, DF, Brazil

Corresponding author: Dr. Christian P. Müller
Institute of Physiological Psychology
University of Düsseldorf, Universitätsstr. 1
40025 Düsseldorf, Germany
Tel.: +49-211/81-13491
Fax.: +49-211/81-12024
Email.: muellecr@uni-duesseldorf.de

Abstract

Brain neuropeptide transmitters of the tachykinin family are involved in the organization of many behaviors. However, little is known about their contribution to the behavioral effects of drugs of abuse. Recently, the neurokinin3 (NK_3)-receptor, one of the three tachykinin receptors in the brain, was shown to attenuate the acute and chronic behavioral effects of cocaine in rats. In order to test if these findings can be generalized to primates we investigated the role of the NK_3-receptor in the acute behavioral effects of cocaine in marmoset monkeys (*Callithrix penicillata*) using a figure-eight maze procedure. Animals were pretreated with the NK_3-receptor antagonist, (R)-(N)-[1-[3-[1-benzoyl-3-(3,4-dichlorophenyl) piperidin-3-yl]propyl]-4-phenylpiperidin-4-yl]-N-methylacetamide (SR142801; 0, 0.02, 0.2, 2.0 mg/kg, i.p.), and received either a treatment with cocaine (10 mg/kg, i.p) or saline (i.p.). Cocaine increased locomotor activity and aerial glance behavior, but reduced exploratory and bodycare activities, scent marking and terrestrial scanning behavior. A sensitivity analysis revealed that two responder types can be differentiated in relation to the occurrence of a hyperlocomotor response to cocaine. SR142801 blocked the actions of cocaine on several behaviors dose-dependently for each responder type respectively. There was no effect of SR142801 alone on any behavior measured. These data suggest, that the NK_3-receptor contributes to the individual behavioral response to cocaine in marmoset monkeys. Having no behavioral effects on its own, but blocking the cocaine effects, might suggest the NK_3-receptor antagonist, SR142801, as a potential treatment of cocaine-addiction in humans.

Keywords: cocaine, NK_3-receptor, SR 142801, marmoset, behavior, sensitivity

1. Introduction

Neuropeptides belonging to the tachykinin family are characterized by having the common C-terminal sequence Phe-X-Gly-Leu-Met-NH_2. Five mammalian tachykinins have so far been identified, namely substance P (SP), neurokinin A (NKA), neurokinin B (NKB), neuropeptide K and neuropeptide γ. Three distinct G protein-coupled receptors, neurokinin1 (NK_1), NK_2 and NK_3, have been characterized. NK_1- and NK_3-receptors are widely distributed in the brain, while the NK_2-receptors are found in restricted areas. SP, NKA and NKB have higher binding affinity to NK_1-, NK_2- and NK_3-receptors, respectively, but all the neurokinins bind to all three NK-receptors (Regioli et al., 1994; Massi et al., 2000; Hökfelt et al., 2001). Compelling evidence suggests that NK_3-receptors are involved in memory-, anxiety- and reinforcement related processes (Hasenöhrl et al., 1990, 1992; Huston et al., 1993; Krappmann et al., 1994). Recently it was shown in rats that the NK_3-receptor also mediates the acute as well as the chronic behavioral effects of cocaine (Jocham et al., submitted). However, the findings in rats may not automatically generalize to humans due to the considerable species differences in NK_3-receptors between humans and rats (Emonds-Alt et al., 1995; Nguyen-Le et al., 1996).

Cocaine is a potent pharmacological reinforcer and drug of abuse (Vanderschuren and Everitt, 2004). Already the acute application of cocaine causes complex behavioral patterns in humans and animals, including hyperlocomotion, and the suppression of grooming and eating behavior (Müller et al., 2003). Cocaine can induce euphoria in humans (Breiter et al., 1997; Volkow et al., 1997) but also anxiety, as shown in rodent studies (Yang et al., 1992; Rogerio and Takahashi, 1992). However, the acute effects of cocaine as well as the liability to develop cocaine addiction differ considerably between individuals (Hooks et al., 1991; Homberg et al., 2002; Deroche-Gamonet et al. 2004). Non-human primates with their complex general behavioral repertoire

(Stevenson and Poole, 1976; King et al., 1988; Barros et al., 2004a) and distinguished response profiles to psychostimulants provide a valuable model in the transition from rodents to humans. Even small effects of psychostimulants can, thus, be dissected, identifying high and low hyperlocomotor responding animals, and revealing complex differences in the whole response pattern (Mello et al., 2005).

In this study we investigate the role of the NK_3-receptor in the behavioral effects of cocaine in non-human primates (*Callithrix penicillata*) using a figure-eight maze procedure. In line with a previous study on the acute behavioral effects of a low potency psychostimulant (Mello et al., 2005), we asked whether there are also different responder types for cocaine in non-human primates, and how NK_3-receptor antagonism affects them. According to our findings in rats we hypothesized that pharmacological antagonism of the NK_3-receptor with the non-peptide NK_3-receptor antagonist, (R)-(N)-[1-[3-[1-benzoyl-3-(3,4-dichlorophenyl) piperidin-3-yl]propyl]-4-phenylpiperidin-4-yl]-N-methylacetamide (SR142801), will not have behavioral effects on its own, but should attenuate the acute behavioral effects of cocaine. Furthermore, we expected responder type differences also after cocaine treatment in monkeys, and a differential influence of NK_3-receptor antagonism.

2. Materials and methods

2.1. Subjects

Twelve adult black tufted-ear marmosets (*Callithrix penicillata*, 5 males and 7 females) were used as subjects. Animals weighed 280-405 g at the beginning of experiments. Before and during the experiment all animals were socially housed in separate male/female groups in indoor/outdoor cages (2 x 1.3 x 2 m) of the same colony room (not all members of the housing colony were tested in this experiment). Maintenance and testing of subjects were performed at the Primate Center, University of Brasilia. Except during the 20 min test periods, food and water were available *ad libitum*. All procedures were approved by the Animal Ethics Committee of the Institute of Biology, University of Brasilia, and followed the 'Principles of Laboratory Animal Care' (NIH publication No. 85-23, revised 1996).

2.2. Drugs

The NK_3-receptor antagonist SR142801 ((R)-(N)-[1-[3-[1-benzoyl-3-(3,4-dichlorophenyl) piperidin-3-yl]propyl]-4-phenylpiperidin-4-yl]-N-methylacetamide, Sanofi-Synthelabo, Montpellier, France) was suspended in 0.01 % Tween 80 (Sigma-Aldrich, USA) in distilled water and injected i.p. in the doses of 0, 0.02, 0.2, and 2 mg/kg. The dose range was based on previous behavioral experiments investigating the effects of SR142801 in rats (Jocham et al., submitted) with regards to the species differences between rats and primates (Emonds-Alt et al., 1995; Nguyen-Le et al., 1996). Cocaine (Sigma, USA) was dissolved in 0.9% physiological saline and injected i.p. in a dose of 0 and 10 mg/kg. The injection volume was 2 ml/kg for SR142801 and 1 ml/kg for cocaine.

2.3. Apparatus

Testing was conducted in a figure-eight continuous maze (Barros and Tomaz, 2002). The maze consisted of a rectangular field (125 x 103 x 35 cm) suspended 1 m from the floor and divided into five arms by two holes and barriers, forming a continuous figure-eight maze (Fig. 1). The apparatus, made of 4 mm transparent glass on a metal frame support, was divided into two segments (front and back chambers) by a concrete visual barrier (147 x 8 x 218 cm). The back chamber consisted of an arm (125 x 30 x 35 cm) with a central guillotine-type door. The latter formed the start compartment. The front chamber had three parallel arms (40 x 25 x 35 cm), 25 cm apart, ending in a common perpendicular arm (125 x 25 x 35 cm). Both chambers were interconnected through holes in the visual barrier at each of the three parallel arms.

2.4. Procedure

All animals were habituated to the maze and the transport cage (35 x 20 x 23 cm) prior to the beginning of the experiment. All subjects were submitted to one more 20 min habituation trial in the figure-eight maze, which showed stable and, thus, a well habituated activity compared to the last maze exposure. Following the habituation trial, two test sessions were spaced four weeks apart. In the first session the effects of SR142801 plus saline were tested, while in the second session the effects of SR142801 in combination with cocaine were evaluated.

In each session four pseudo-randomly assigned treatment trials were performed with each subject, with a wash out period of 72 h between the treatments. As a pretreatment the animals received an i.p. injection of SR142801 (0, 0.02, 0.2 and 2 mg/kg). After the pretreatment the animals were returned to the home cage for 30 min before they received an i.p. injection of 10 mg/kg cocaine or saline. Immediately following the treatment the animal was released into the maze's start compartment, thus commencing a 20 min trial. Barriers from this compartment were

promptly removed upon the animal's exit, permitting free access to the whole apparatus. After the session, the subject was returned to its home environment in the transport cage. Treatments and order of subjects were pseudo-randomly assigned for each test day. Video cameras were used for online monitoring, and all trials were recorded for later behavioral analysis. All test sessions were performed between 8:00 am and 1:00 p.m.

2.5. Behavioral analysis

For behavioral analysis, the maze was divided into 13 sections. The following behavioral parameters based on the ethograms of marmoset behavior (Stevenson and Poole, 1976; Stevenson and Rylands, 1988; Barros et al., 2002a, 2003, 2004a, 2004b) were scored for each 20 min trial by experienced observers (inter-rater reliability: ≥95%) blind to the experimental treatment: (1) *Locomotor activity*: the number of maze sections crossed with both forelimbs; (2) *Exploratory activity*: the number of times that the animal spent sniffing and/or licking any part of the apparatus or standing on the hind legs; (3) *Bodycare activities*: number of times the animal spent grooming (slow and precise repetitive movements of the hand through the fur) or scratching (quick repetitive movements of hand or foot through the fur); (4) *Scent marking*: the number of times that the animal rubbed the anogenital region on any substratum; (5) *Aerial scanning*: time and frequency the animals spent scanning the environment from the horizontal plane upwards, persisting ≥ 5 seconds while the animal remained stationary; (6) *Terrestrial scanning*: time and frequency the animals spent scanning the environment below the horizontal plane, persisting ≥ 5 seconds while the animal remained stationary; (7) *Aerial glance*: frequency of rapid upward sweeping movements of the head lasting ≤ 2 seconds while stationary and (8) *Terrestrial glance*: frequency of rapid downward movements of the head lasting ≤ 2 seconds while stationary. For

semi-automated behavioral analysis, the program PROSTCOM 3.20 (Conde et al., 2000) was used.

2.6. Statistical analysis

The data were analyzed by means of a two-way analysis of variance (ANOVA) with pretreatment (4) and treatment (2) as factors. In order to differentiate between cocaine-sensitive and -insensitive animals, the locomotor response was used as a criterion. Animals which showed an increase in locomotor activity after the vehicle-cocaine treatment compared to the vehicle-saline treatment were considered to be “cocaine sensitive”. All other animals were considered to be “cocaine insensitive”. All behavioral parameters were further analyzed with respect to the cocaine sensitivity of the animals. In order to identify differences in the behavioral response to the treatments between cocaine-sensitive and -insensitive animals pre-planned comparisons were calculated using the LSD-test. All statistical results were interpreted as measures of effect with a P-value of .05 as a criterion.

3. Results

The injection of cocaine led to an increase in the locomotor activity when all animals were considered together (Fig. 2A; two-way ANOVA, treatment: $F_{1,88} = 15.12$, $p < 0.0002$). Neither spontaneous nor cocaine-induced locomotor activity was affected by pretreatment with SR142801 when all animals were analyzed together (pretreatment and interaction: $p > 0.05$). Sensitivity analysis (Fig. 2B), however, revealed that only 5 of the 12 animals tested (42 %) showed increased locomotor activity after vehicle-cocaine treatment compared to vehicle-saline, and were, thus, considered to be cocaine sensitive (high responders, HR). Seven of the 12 animals tested (58 %) showed less activity after vehicle-cocaine compared to vehicle-saline treatment, and were considered to be cocaine insensitive (low responders, LR). The cocaine but not the saline effect on locomotor activity differed considerably between the two responder types (HR vs. LR, vehicle-cocaine: $p < 0.003$; vehicle-saline: $p > 0.05$). While pretreatment with SR142801 did not have an effect when all animals were pooled, sensitivity analysis revealed striking responder type differences. The pretreatment reduced the hyperlocomotor effects of cocaine in the HR animals with an inverted U-shaped dose-response curve. The HR vs. LR difference in the locomotor response to cocaine was attenuated by pretreatment with 0.02 and 0.2 mg/kg SR142801 ($p > 0.05$) but not after pretreatment with 2 mg/kg SR142801 ($p < 0.004$).

The cocaine treatment caused a decrease in exploratory activity when all animals were considered together (Fig. 3A; two-way ANOVA, treatment: $F_{1,88} = 3.8$, $p = 0.05$). Neither spontaneous nor cocaine-induced decrease in exploratory activity was affected by pretreatment with SR142801 when all animals were analyzed together (pretreatment and interaction: $p > 0.05$). Sensitivity analysis (Fig. 3B) did not reveal differences between the HR and LR animals (all treatments: $p > 0.05$).

Bodycare activity and scent marking behavior were also decreased after cocaine treatment (Fig. 4A and 4C; two-way ANOVA, treatment, bodycare activity: $F_{1,88} = 10.56$, $p < 0.002$; scent marking: $F_{1,88} = 4.97$, $p < 0.03$). Both behaviors were virtually eliminated by the cocaine treatment. Neither spontaneous nor the cocaine-induced decrease in both behaviors was affected by pretreatment with SR142801 when all animals were analyzed together (pretreatment and interaction: $p > 0.05$). Sensitivity analysis (Fig. 4B and 4D) showed that there was no obvious difference in bodycare activity and scent marking behavior after cocaine between HR and LR animals ($p > 0.05$). Neither spontaneous nor the cocaine-induced decline in these behaviors was affected by SR142801 in either responder group ($p > 0.05$).

Cocaine neither affected the time nor the frequency of aerial scanning behavior when all animals were considered together (Fig. 5A and 5C; two-way ANOVA, treatment: $p > 0.05$). Neither spontaneous aerial scanning nor the aerial scanning after cocaine was affected by pretreatment with SR142801 when all animals were analyzed together (pretreatment and interaction: $p > 0.05$). Sensitivity analysis (Fig. 5B and 5D), however, showed a dissociating effect of cocaine on the time of aerial scanning between the HR and LR animals (HR vs. LR, vehicle-cocaine: $p < 0.007$, vehicle-saline: $p > 0.05$). While cocaine increased the time of aerial scanning in the LR animals, it decreased aerial scanning time in the HR animals. This HR vs. LR difference in the response to cocaine was eliminated by pretreatment with 0.02 and 0.2 mg/kg SR142801 ($p > 0.05$), but not after pretreatment with 2 mg/kg SR142801 ($p < 0.05$). No such effect was observed for the frequency of aerial scanning (HR vs. LR, all treatments: $p > 0.05$).

The time (Fig. 6A; two-way ANOVA, treatment, $F_{1,88} = 4.98$, $p < 0.03$) as well as frequency of terrestrial scanning (Fig. 6C; two-way ANOVA, treatment, $F_{1,88} = 4.93$, $p < 0.03$) were decreased after cocaine when all animals were considered together. SR142801 pretreatment completely eliminated terrestrial scanning after cocaine, however, statistical analysis yielded neither a

pretreatment effect nor a pretreatment x treatment interaction ($p > 0.05$). Sensitivity analysis (Fig. 6B and 6D), on the other hand, showed a dissociating cocaine effect. Cocaine alone increased terrestrial scanning in the HR animals, while it eliminated the behavior in the LR animals (HR vs. LR, vehicle-cocaine, time: $p < 0.03$; frequency: $p < 0.008$, vehicle-saline, time and frequency: $p > 0.05$). The difference between HR and LR animals in their cocaine response was no longer observed after pretreatment with SR142801 (HR vs. LR, all doses: $p > 0.05$).

Aerial glance was increased after cocaine treatment when all animals were considered together (Fig. 7A; two-way ANOVA, treatment: $F_{1,88} = 4.86$, $p < 0.03$). Spontaneous and the cocaine-induced increase in aerial glance was reduced by pretreatment with SR142801 as a tendency when all animals were analyzed together, although statistical analysis did not yield a pretreatment effect or a pretreatment x treatment interaction ($p > 0.05$). Sensitivity analysis (Fig. 7B) showed that the increase in aerial glance after cocaine only occurred in the HR animals but not in the LR animals (HR vs. LR, vehicle-cocaine: $p < 0.03$, vehicle-saline: $p > 0.05$). Pretreatment with SR142801 attenuated the HR vs. LR difference by reducing the increase in aerial glance in the HR animals at doses of 0.2 and 2 mg/kg (HR vs. LR, $p > 0.05$), but not at a dose of 0.02 mg/kg (HR vs. LR, $p < 0.009$).

There was no effect of cocaine on terrestrial glance when all animals were considered together (Fig. 7C; two-way ANOVA; treatment: $p > 0.05$). Spontaneous terrestrial glance and terrestrial glance after cocaine were not affected by pretreatment with SR142801 (pretreatment and interaction: $p > 0.05$). Sensitivity analysis (Fig. 7D) showed a tendency for more terrestrial glance behavior in the HR animals, although statistical analysis did not yield a HR vs. LR difference at any treatment combination ($p > 0.05$).

4. Discussion

The effects of cocaine were investigated on a broad range of marmoset behaviors. Cocaine increased locomotor activity and aerial glance behavior. At the same time exploratory activity, bodycare activities, scent marking and terrestrial scanning behavior were decreased. There was no overall cocaine effect on aerial scanning and terrestrial glance. Interestingly, an increase in locomotor activity after cocaine could be found only in 5 of the 12 animals (42%) tested. Seven of the 12 animals (58%) did not respond with an increased locomotor activity. The analysis of the individual variability indicated a bimodal distribution of effects, very similar to the one found recently in a study investigating the effects of the low potency stimulant, diethylpropion, in marmoset monkeys (Mello et al., 2005). Thereby, the increase in behavioral activity, which is usually considered as an indicator of the stimulant properties of cocaine, was used to subdivide the population of the animals into cocaine sensitive (high responders, HR) and cocaine insensitive (low responders, LR) animals. The subsequent sensitivity analysis revealed that there are two principle types of responses to cocaine in marmoset monkeys. The HR animals were characterized in their response to cocaine by a profound increase in locomotor activity. But HR vs. LR differences after acute cocaine also occurred in aerial and terrestrial scanning and aerial glance behavior. In the HR animals cocaine increased terrestrial scanning and aerial glance, but decreased aerial scanning. Exploratory activity, bodycare activities, and scent marking were also decreased, but did not differ from the LR animal's response. The LR animals did not show hyperlocomotion after cocaine, but instead, responded with an increase in aerial scanning.

NK_3-receptor antagonism with SR142801 alone did not affect any of the behaviors measured in marmosets. The cocaine effects on the marmosets behavior did not appear to be modulated by the NK_3-receptor antagonism when all animals were pooled. However, sensitivity analysis revealed

that SR142801 had striking effects when responder types were evaluated separately. SR142801 selectively attenuated the cocaine-induced hyperlocomotion and the increase in terrestrial scanning and aerial glance in the HR animals, while it reduced the increase in aerial scanning in the LR animals. In all these behaviors NK_3-receptor antagonism also attenuated the HR vs. LR differences in the acute behavioral response to cocaine. But also after sensitivity analysis, the NK_3-receptor antagonist did not appear to affect all cocaine-induced changes in behavior. The cocaine-induced decreases in exploratory activity, bodycare activities and scent marking, which did not differ between the HR and LR animals, was not affected by SR142801.

This study revealed a complex behavioral response to cocaine in marmoset monkeys. Within this pattern two principal response types could be distinguished, that were clearly segregated from one another, reflecting strong interindividual differences in the acute behavioral response to cocaine in non-human primates. In that, the present study confirms principle responder type differences in marmosets as they were found in a recent study with the low potency psychostimulant, diethylpropion (Mello et al., 2005). In the present study HR animals not only showed an increase in locomotor response but also an increase in terrestrial scanning and aerial glance. The increase in terrestrial scanning and aerial glance, together with the tendential decrease in exploratory activity, is associated with an anxiogenic state (Barros et al., 2004a, 2004b), which can be reversed by anxiolytic drugs like diazepam (Barros et al., 2000). At the same time the predominant aerial scanning behavior was decreased in the HR animals, which may indicate that the anxiogenic component was not dominant in the HR animals. In callitrichids, visual scanning, which includes the predominant aerial and the less frequent terrestrial scanning, facilitates the detection of objects in the environment and has a high adaptive value (Caine, 1984; Hardie and Buchanan-Smith, 1997). In general, the presentation of a potential threat is associated with an increase in visual scanning (Caine, 1984, 1998; Ferrari and Ferrari, 1990; Hardie and

Buchanan-Smith, 1997; Koenig, 1998). In the LR animals the increase in the aerial scanning is the most pronounced behavioral effect of cocaine, which may reflect a predominant anxiogenic response. Both responder types share the almost complete suppression of bodycare activities and scent marking behavior. Interestingly, both responder types match closely to the responder types to the low potency psychostimulant, diethylpropion (Mello et al., 2005). The most important difference in the behavioral response to the two psychostimulants may be the additional increase in the terrestrial scanning after cocaine in the HR animals. This might reflect a more pronounced anxiogenic component in the HR animals to cocaine compared to diethylpropion.

The hyperlocomotor effects of cocaine as well as the increase in terrestrial scanning and aerial glance were attenuated in the HR animals by NK_3-receptor antagonism. The suppressory effects of cocaine on bodycare activity and scent marking, however, were not reversed by SR142801. Thus, in the HR marmoset monkeys the contribution of the NK_3-receptor to the acute behavioral effects of cocaine appears to be comparable with that in rats. In rats SR142801 blocked the hyperlocomotor effects of cocaine without affecting the suppression of grooming behavior (Jocham et al., submitted). Since SR142801 alone did not significantly affect locomotor activity in primates and rats, but blocked cocaine-induced locomotor activity, it is suggested that a tonic stimulation of the NK_3-receptor is not required for the generation of spontaneous behavior, but rather, that NK_3-receptors contribute to an induced increase in locomotor activity. This view is also supported by the findings that the local injection of SP or its C-terminal analogue, DiMe-C7, into the ventral tegmental area (VTA) and the substantia nigra (SN) is well known to enhance locomotor activity in rats (Kelley et al., 1979; Eison et al., 1982; Barnes et al., 1990). Also the local application of the NK_3-receptor agonist senktide, but not of NK_1- or NK_2-receptor agonists, into the SN and VTA induced locomotor activity and rearing behavior in rats (Stoessl et al., 1988). The present study also showed that the anxiety-related effects of cocaine can be blocked

by NK_3-receptor antagonism. In the LR animals NK_3-receptor antagonism reduced the cocaine-induced increase in aerial scanning, and thus, the predominant anxiogenic response. The attenuation of the cocaine-induced anxiety-related behavior by the NK_3-receptor antagonist was rather surprising, since the NK_3-receptor agonist, senktide (Ribeiro and De Lima, 1998; Ribeiro et al., 1999), SP (Echeverry et al., 2001), and the SP N-terminal fragment, SP_{1-7} (Barros et al., 2002b), were found to be anxiolytic in mice, rats, and monkeys respectively. Also the local application of SP, and both C- and N-terminal fragments, SP_{7-11} and SP_{1-7}, into the ventral pallidum of rats had anxiolytic effects (Nikolaus et al., 2000). However, SP as well as its C-terminal fragment, SP_{7-11}, can also have anxiogenic effects when injected into the dorsal periaqueductal gray of rats (De Araujo et al., 1999; Hasenöhrl et al., 2000). The NK_3-receptor antagonist, SR142801, had either an anxiogenic or no effects in mice (Ribeiro and De Lima, 1998; Ribeiro et al., 1999), and no effect on panic symptoms was found in humans (Kronenberg et al., 2005). In this study no behavior was affected by SR142801 alone in HR and LR animals. Altogether, the NK_3-recptor antagonism attenuated the acute cocaine effects in HR and LR marmoset monkeys respectively. The most effective doses for antagonizing the behavioral effects of cocaine in monkeys were 0.02 and 0.2 mg/kg SR142801. Blocking the acute cocaine effects in rats required a 10 fold higher dose of SR142801 (Jocham et al., submitted). These findings are in line with the report by Emonds-Alt et al. (1995), which showed a 10-100 fold higher binding of SR142801 in guinea-pigs, gerbils and humans compared to rats. At the highest dose tested in the marmoset monkeys (2.0 mg/kg) no inhibition of the cocaine-induce hyperlocomotion in the HR animals and of the increase in aerial scanning in the LR animals was observed, indicating an inverted U-shaped dose-response curve for the effects of SR142801. Such a dose-response curve is described in many neuropeptide studies (Huston et al., 1993; Hasenöhrl et al., 2000), and was also observed in rats blocking the acute hyperlocomotor and the reinforcing effects of cocaine

(Jocham et al., submitted). At the highest dose tested, the low affinity of SR142801 to calcium and sodium channels (Emonds-Alt et al., 1995) may have counteracted the NK_3-receptor effects.

In summary, the present study showed that cocaine has a wide range of different acute effects on behavior in marmoset monkeys. However, the behavioral response is not uniform. Two responder types could be differentiated, which showed a similar response profile as it was previously described for the low potency psychostimulant, diethylpropione (Mello et al., 2005). NK_3-receptor antagonism blocks the acute cocaine effects on behavior in each responder type, respectively. Having no behavioral effects on its own, but blocking individual cocaine effects, suggests the NK_3-receptor antagonist SR142801 as a potential treatment of cocaine-addiction in humans.

References

Barnes, J.M., Barnes, N.M., Costall, B., Cox, A.J., Domeney, A.M., Kelly, M.E., Naylor, R.J., 1990. Neurochemical consequences following injection of the substance-P analog, Dime-C7, into the rat ventral tegmental area. Pharmacol. Biochem. Behav. 37, 839-841.

Barros, M., Boere, V., Huston, J.P., Tomaz, C., 2000. Measuring fear and anxiety in the marmoset (Callithrix penicillata) with a novel predator confrontation model: effects of diazepam. Behav. Brain Res. 108, 205-211.

Barros, M., Tomaz, C., 2002. Non-human primate models for investigating fear and anxiety. Neurosci. Biobehav. Rev. 26, 187-201.

Barros, M., Boere, V., Mello, E.L., Tomaz, C., 2002a. Reactions to potential predators in captive-born marmosets (Callithrix penicillata). Int. J. Primatol. 23, 443-454.

Barros, M., De Souza Silva, M.A., Huston, J.P., Tomaz, C., 2002b. Anxiolytic-like effects of substance P fragment (SP(1-7)) in non-human primates (Callithrix penicillata). Peptides 23, 967-973.

Barros, M., Mello, E.L., Maior, R.S., Müller, C.P., De Souza Silva, M.A., Carey, R.J., Huston, J.P., Tomaz, C., 2003. Anxiolytic-like effects of the selective 5-HT1A receptor antagonist WAY 100635 in non-human primates. Eur. J. Pharmacol. 482, 197-203.

Barros, M., De Souza Silva, M.A., Huston, J.P., Tomaz, C., 2004a. Multibehavioral analysis of fear and anxiety before, during, and after experimentally induced predatory stress in Callithrix penicillata. Pharmacol. Biochem. Behav. 78, 357-367.

Barros, M., Alencar, C., Tomaz, C., 2004b. Differences in aerial and terrestrial visual scanning in captive black tufted-ear marmosets (Callithrix penicillata) exposed to a novel environment. Folia Primatol. 75, 85-92.

Breiter, H.C., Gollub, R.L., Weisskoff, R.M., Kennedy, D.N., Makris, N., Berke, J.D., Goodman, J.M., Kantor, H.L., Gastfriend, D.R., Riorden, J.P., Mathew, R.T., Rosen, B.R., Hyman, S.E., 1997. Acute effects of cocaine on human brain activity and emotion. Neuron 19, 591-611.

Caine, N.G., 1984. Visual scanning by tamarins - a description of the behavior and tests of 2 derived hypotheses. Folia Primatol. 43, 59-67.

Caine, N.G., 1998. Cutting costs in response to predatory threat by Geoffroy's marmosets (Callithrix geoffroyi). Am. J. Primatol. 46, 187-196.

Conde, C.A., Costa, V., Tomaz, C., 2000. PROSTCOM: Un conjunto de programas para registro y procesamiento de datos comportamentales en investigaciones de fisiologia y farmacologia. Biotemas 13, 357-367.

De Araujo, J.E., Silva, R.C.B., Huston, J.P., Brandao, M.L., 1999. Anxiogenic effects of substance P and its 7-11 C terminal, but not the 1-7 N terminal, injected into the dorsal periaqueductal gray. Peptides 20, 1437-1443.

Deroche-Gamonet, V., Belin, D., Piazza, P.V., 2004. Evidence for addiction-like behavior in the rat. Science 305, 1014-1017.

Echeverry, M., Hasenöhrl, R.U., Huston, J.P., Tomaz, C., 2001. Comparison of neurokinin SP with diazepam in effects on memory and fear parameters in the elevated T-maze free exploration paradigm. Peptides 22, 1031-1036.

Eison, A.S., Eison, M.S., Iversen, S.D., 1982. The behavioral-effects of a novel substance-P analog following infusion into the ventral tegmental area or substantia nigra of rat-brain. Brain Res. 238, 137-152.

Emonds-Alt, X., Bichon, D., Ducoux, J.P., Heaulme, M., Miloux, B., Poncelet, M., Proietto, V., Van Broeck, D., Vilain, P., Neliat, G., 1995. SR 142801, the first potent non-peptide antagonist of the tachykinin NK3 receptor. Life Sci. 56, L27-L32.

Ferrari, S.F., Ferrari, M.A.L., 1990. Predator avoidance-behavior in the buffy-headed marmoset, Callithrix-Flaviceps. Primates 31, 323-338.

Hardie, S.M., Buchanan-Smith, H.M., 1997. Vigilance in single- and mixed-species groups of tamarins (Saguinus labiatus and Saguinus fuscicollis). Int. J. Primatol. 18, 217-234.

Hasenöhrl, R.U., Gerhardt, P., Huston, J.P., 1990. Evidence for dose-dependent positively and negatively reinforcing effects of the substance P C-terminal analog DIME-C7. Neuropeptides 17, 205-211.

Hasenöhrl, R.U., Gerhardt, P., Huston, J.P., 1992. Positively reinforcing effects of the neurokinin substance P in the basal forebrain: mediation by its C-terminal sequence. Exp. Neurol. 115, 282-291.

Hasenöhrl, R.U., De Souza Silva, M.A., Nikolaus, S., Tomaz, C., Brandao, M.L., Schwarting, R.K.W., Huston, J.P., 2000. Substance P and its role in neural mechanisms governing learning, anxiety and functional recovery. Neuropeptides 34, 272-280.

Hökfelt, T., Pernow, B., Wahren, J., 2001. Substance P: a pioneer amongst neuropeptides. J. Intern. Med. 249, 27-40.

Homberg, J.R., van den Akker, M., Raaso, H.S., Wardeh, G., Binnekade, R., Schoffelmeer, A.N.M., De Vries, T.J., 2002. Enhanced motivation to self-administer cocaine is predicted by self-grooming behaviour and relates to dopamine release in the rat medial prefrontal cortex and amygdala. Eur. J. Neurosci. 15, 1542-1550.

Hooks, M.S., Jones, G.H., Smith, A.D., Neill, D.B., Justice, J.B., 1991. Response to novelty predicts the locomotor and nucleus accumbens dopamine response to cocaine. Synapse 9, 121-128.

Huston, J.P., Hasenöhrl, R.U., Boix, F., Gerhardt, P., Schwarting, R.K.W., 1993. Sequence-specific effects of neurokinin substance-P on memory, reinforcement, and brain dopamine activity. Psychopharmacology 112, 147-162.

Jocham, G., Lezoch, K., Müller, C.P., Kart-Teke, E., Huston, J.P., De Souza Silva, M.A., Neurokinin3 receptor antagonism attenuates cocaine's rewarding and hyperlocomotor effects yet potentiates its dopamine-enhancing action in the nucleus accumbens core. submitted.

Kelley, A.E., Stinus, L., Iversen, S.D., 1979. Behavioral activation induced in the rat by substance-P infusion into ventral tegmental area - implication of dopaminergic A10 neurons. Neurosci. Lett. 11, 335-339.

King, F.A., Yarbrough, C.J., Anderson, D.C., Gordon, T.P., Gould, K.G., 1988. Primates. Science 240, 1475-1482.

Koenig, A., 1998. Visual scanning by common marmosets (Callithrix jacchus): functional aspects and the special role of adult males. Primates 39, 85-90.

Krappmann, P., Hasenöhrl, R.U., Frisch, C., Huston, J.P., 1994. Self-administration of neurokinin substance P into the ventromedial caudate-putamen in rats. Neuroscience 62, 1093-1101.

Kronenberg, G., Berger, P., Tauber, R.F., Bandelow, B., Henkel, V., Heuser, I., 2005. Randomized, double-blind study of SR142801 (Osanetant). A novel neurokinin-3 (NK3) receptor antagonist in panic disorder with pre- and posttreatment cholecystokinin tetrapeptide (CCK-4) challenges. Pharmacopsychiatry 38, 24-29.

Massi, M., Panocka, I., de Caro, G., 2000. The psychopharmacology of tachykinin NK-3 receptors in laboratory animals. Peptides 21, 1597-1609.

Mello, E.L., Maior, R.S., Carey, R.J., Huston, J.P., Tomaz, C., Müller, C.P., 2005. Serotonin(1A)-receptor antagonism blocks psychostimulant properties of diethylpropion in marmosets (Callithrix penicillata). Eur. J. Pharmacol. 511, 43-52.

Müller, C.P., Carey, R.J., Huston, J.P., 2003. Serotonin as an important mediator of cocaine's behavioral effects. Drugs Today 39, 497-511.

Nguyen-Le, X.K., Nguyen, Q.T., Gobeil, F., Pheng, L.H., Emonds-Alt, X., Breliere, J.C., Regoli, D., 1996. Pharmacological characterization of SR 142801: A new non-peptide antagonist of the neurokinin NK-3 receptor. Pharmacology 52, 283-291.

Nikolaus, S., Huston, J.P., Hasenöhrl, R.U., 2000. Anxiolytic-like effects in rats produced by ventral pallidal injection of both N- and C-terminal fragments of substance P. Neurosci. Lett. 283, 37-40.

Regoli, D., Boudon, A., Fauchere, J.L., 1994. Receptors and antagonists for substance P and related peptides. Pharmacol. Rev. 46, 551-599.

Ribeiro, S.J., De Lima, T.C.M., 1998. Naloxone-induced changes in tachykinin NK3 receptor modulation of experimental anxiety in mice. Neurosci. Lett. 258, 155-158.

Ribeiro, S.J., Teixeira, R.M., Calixto, J.B., De Lima, T.C.M., 1999. Tachykinin NK3 receptor involvement in anxiety. Neuropeptides 33, 181-188.

Rogerio, R., Takahashi, R.N., 1992. Anxiogenic Properties of Cocaine in the Rat Evaluated with the Elevated Plus-Maze. Pharmacol. Biochem. Behav. 43, 631-633.

Stevenson, M.F., Poole, T.B., 1976. Ethnogram of common marmoset (Calithrix-Jacchus-Jacchus) - general behavioral repertoire. Animal Behav. 24, 428-451.

Stevenson, M.F., Rylands, A.B., 1988. The marmosets, genus Callithrix. In: Mittermeier, R.A., Rylands, A.B., Coimbra-Filho, A., Fonseca, G.A.B. (Eds.), Ecology and Behavior of Neotropical Primates, pp. 131-222.

Stoessl, A.J., Dourish, C.T., Iversen, S.D., 1988. The NK-3 tachykinin receptor agonist senktide elicits 5-HT-mediated behavior following central or peripheral administration in mice and rats. Br. J. Pharmacol. 94, 285-287.

Vanderschuren, L.J.M.J., Everitt, B.J., 2004. Drug seeking becomes compulsive after prolonged cocaine self-administration. Science 305, 1017-1019.

Volkow, N.D., Wang, G.J., Fischman, M.W., Foltin, R.W., Fowler, J.S., Abumrad, N.N., Vitkun, S., Logan, J., Gatley, S.J., Pappas, N., Hitzemann, R., Shea, C.E., 1997. Relationship between subjective effects of cocaine and dopamine transporter occupancy. Nature 386, 827-830.

Yang, X.M., Gorman, A.L., Dunn, A.J., Goeders, N.E., 1992. Anxiogenic effects of acute and chronic cocaine administration: neurochemical and behavioral studies. Pharmacol. Biochem. Behav. 41, 643-650.

Acknowledgement

This work was supported by the Deutsche Forschungsgemeinschaft, by FINATEC (to C.T.), by CAPES/DAAD/PROBAL (to C.T. and J.P.H.). G.J. was supported by the Graduiertenkolleg 320 “Pathological processes of the nervous system: from genes to behavior”. E.L.M. was a recipient of a fellowship from CAPES, and C.T. and M.B. recipients of CNPq researcher fellowships (no. 300364/1986-5 and no. 412542/2003). We thank Sanofi Recherche for the generous supply of SR142801, and Mrs. Anna A. Vieira Souto and Mrs. Naia Vilas Boas for assistance in the data collection.

Figure Legends

Fig. 1. Top view of the figure-eight continuous maze used for testing (SC indicates the start compartment; for a detailed description: see text).

Fig. 2. The effects of cocaine (10 mg/kg, i.p.) on locomotor activity (mean ±SEM) and its modulation by the NK_3-receptor antagonist, SR142801 (0.02-2.0 mg/kg, i.p.), during a 20 min test trial. **A**.: effects for all animals tested (n=12). **B**.: sensitivity analysis: group split according to the animals response to cocaine (*high responder*: increased locomotor activity after vehicle-cocaine vs. vehicle-saline (n=5); *low responder*: no increase in locomotor activity after vehicle-cocaine vs. vehicle-saline (n=7); *** $p < 0.001$, two-way ANOVA, factor treatment; $^{\#\#}$ $p < 0.01$, high responders vs. low responders).

Fig. 3. The effects of cocaine (10 mg/kg, i.p.) on exploratory activity (mean ±SEM) and its modulation by the NK_3-receptor antagonist, SR142801 (0.02-2.0 mg/kg, i.p.), during a 20 min test trial. **A**.: effects for all animals tested (n=12). **B**.: sensitivity analysis: group split according to the animals response to cocaine (*high responder*: increased locomotor activity after vehicle-cocaine vs. vehicle-saline (n=5); *low responder*: no increase in locomotor activity after vehicle-cocaine vs. vehicle-saline (n=7); two-way ANOVA, factor treatment).

Fig. 4. The effects of cocaine (10 mg/kg, i.p.) on bodycare activities and scent marking behavior (mean ±SEM) and its modulation by the NK_3-receptor antagonist, SR142801 (0.02-2.0 mg/kg, i.p.), during a 20 min test trial. **A./C**.: effects for all animals tested (n=12). **B./D**.: sensitivity

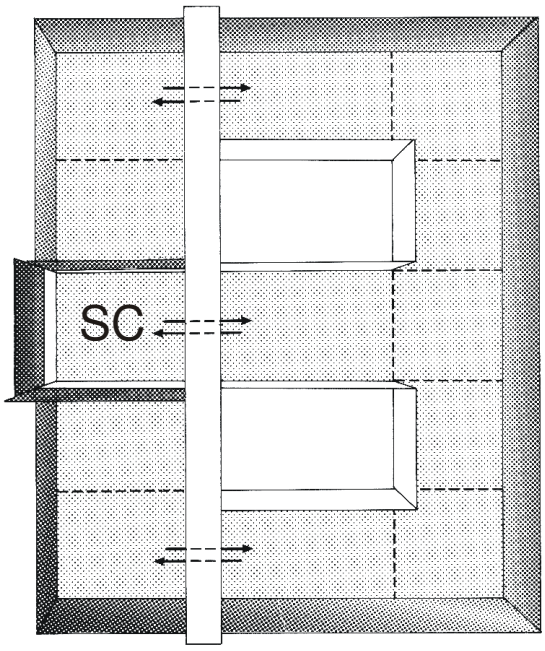
analysis: group split according to the animals response to cocaine (*high responder*: increased locomotor activity after vehicle-cocaine vs. vehicle-saline (n=5); *low responder*: no increase in locomotor activity after vehicle-cocaine vs. vehicle-saline (n=7); * $p < 0.05$, ** $p < 0.01$, two-way ANOVA, factor treatment).

Fig. 5. The effects of cocaine (10 mg/kg, i.p.) on aerial scanning time and frequency (mean ±SEM) and its modulation by the NK_3-receptor antagonist, SR142801 (0.02-2.0 mg/kg, i.p.), during a 20 min test trial. **A./C.**.: effects for all animals tested (n=12). **B./D.**.: sensitivity analysis: group split according to the animals response to cocaine (*high responder*: increased locomotor activity after vehicle-cocaine vs. vehicle-saline (n=5); *low responder*: no increase in locomotor activity after vehicle-cocaine vs. vehicle-saline (n=7); $^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.01$, high responders vs. low responders).

Fig. 6. The effects of cocaine (10 mg/kg, i.p.) on terrestrial scanning time and frequency (mean ±SEM) and its modulation by the NK_3-receptor antagonist, SR142801 (0.02-2.0 mg/kg, i.p.), during a 20 min test trial. **A./C.**.: effects for all animals tested (n=12). **B./D.**.: sensitivity analysis: group split according to the animals response to cocaine (*high responder*: increased locomotor activity after vehicle-cocaine vs. vehicle-saline (n=5); *low responder*: no increase in locomotor activity after vehicle-cocaine vs. vehicle-saline (n=7); * $p < 0.05$, two-way ANOVA, factor treatment; $^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.01$, high responders vs. low responders).

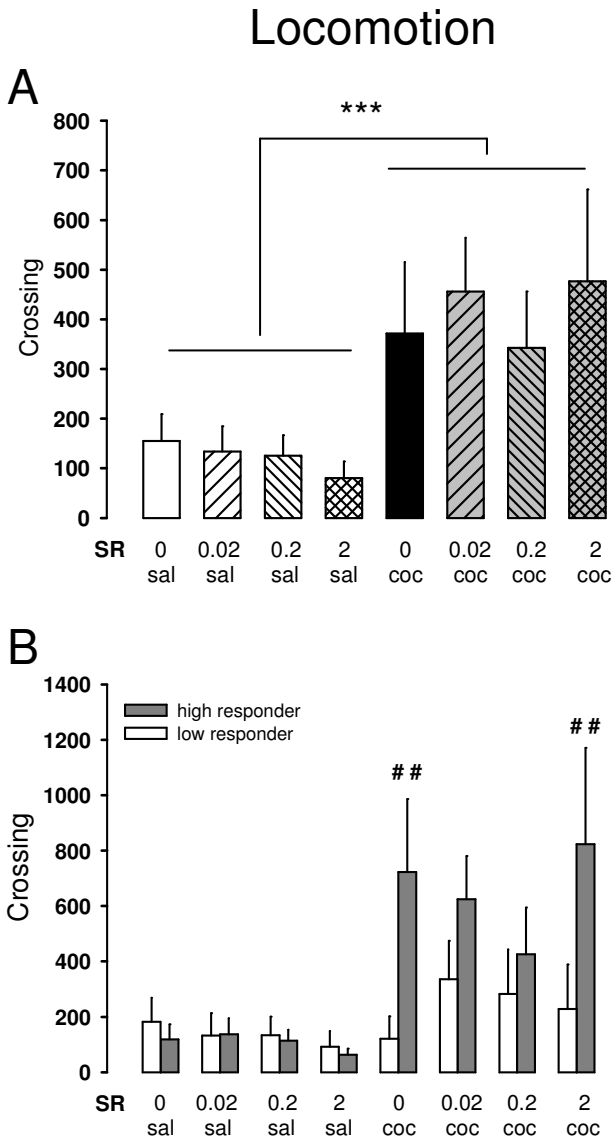
Fig. 7. The effects of cocaine (10 mg/kg, i.p.) on aerial and terrestrial glance (mean ±SEM) and its modulation by the NK_3-receptor antagonist, SR142801 (0.02-2.0 mg/kg, i.p.), during a 20 min

test trial. **A./C.**.: effects for all animals tested (n=12). **B./D.**.: sensitivity analysis: group split according to the animals response to cocaine (*high responder*: increased locomotor activity after vehicle-cocaine vs. vehicle-saline (n=5); *low responder*: no increase in locomotor activity after vehicle-cocaine vs. vehicle-saline (n=7); * $p < 0.05$, two-way ANOVA, factor treatment; $^{\#}$ $p < 0.05$, high responders vs. low responders).



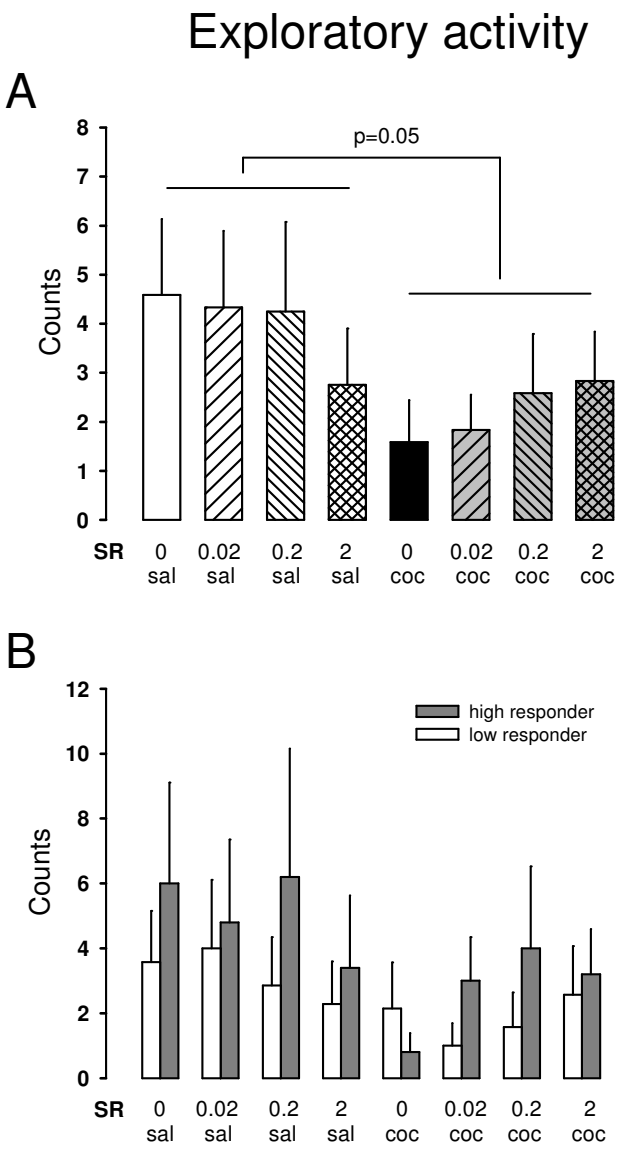
De Souza et al.

Fig. 1



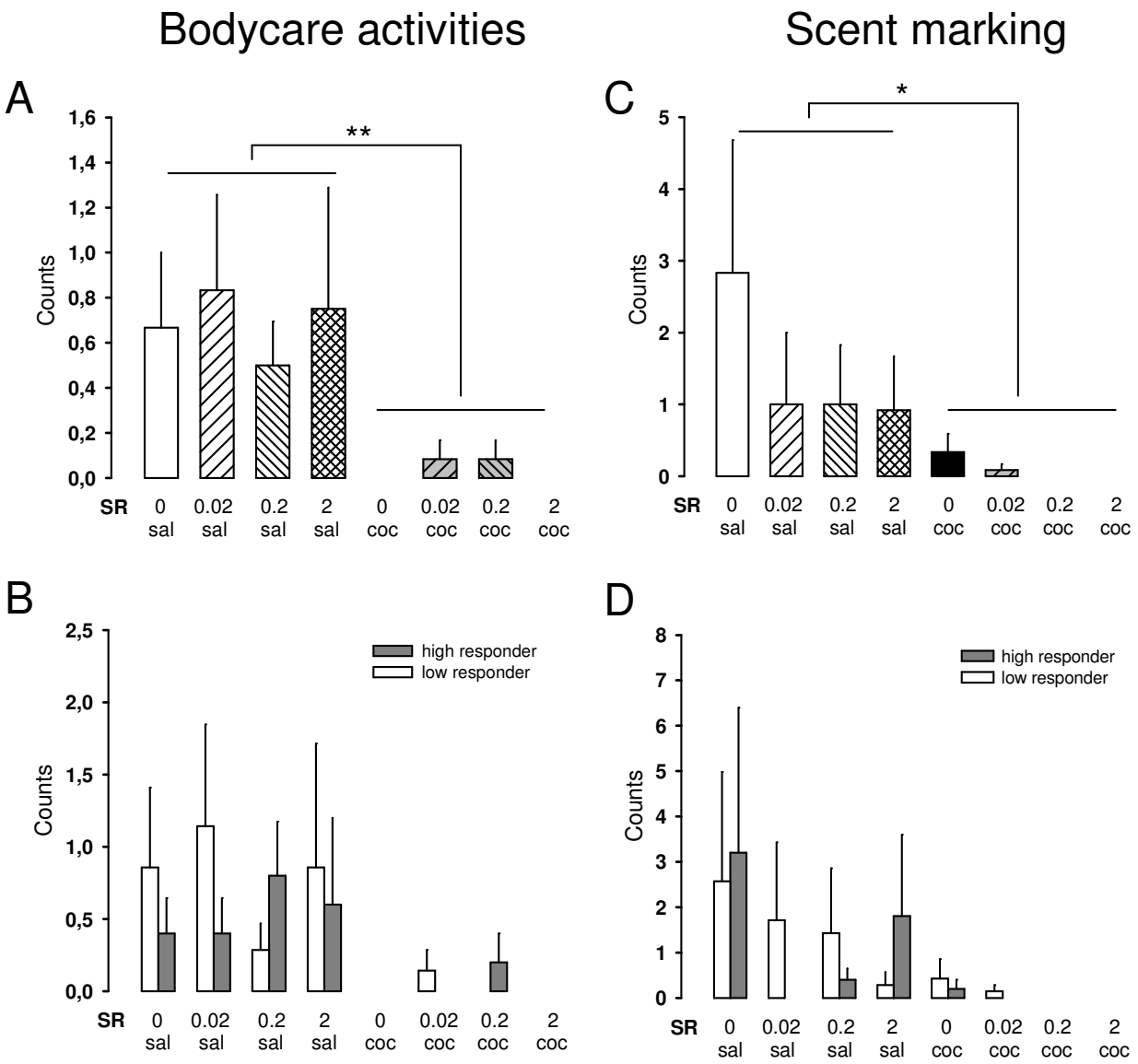
De Souza et al.

Fig. 2



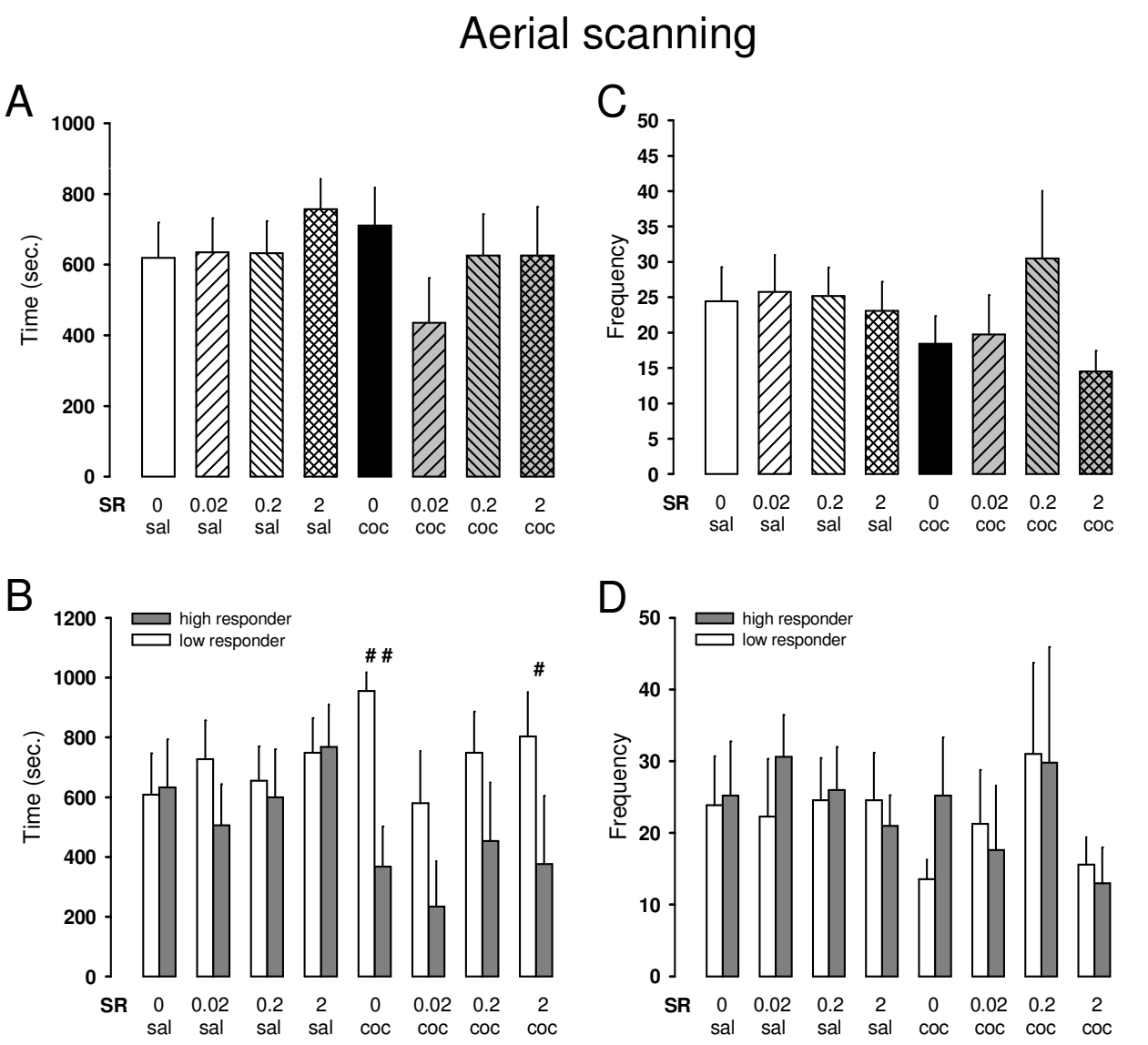
De Souza et al.

Fig. 3



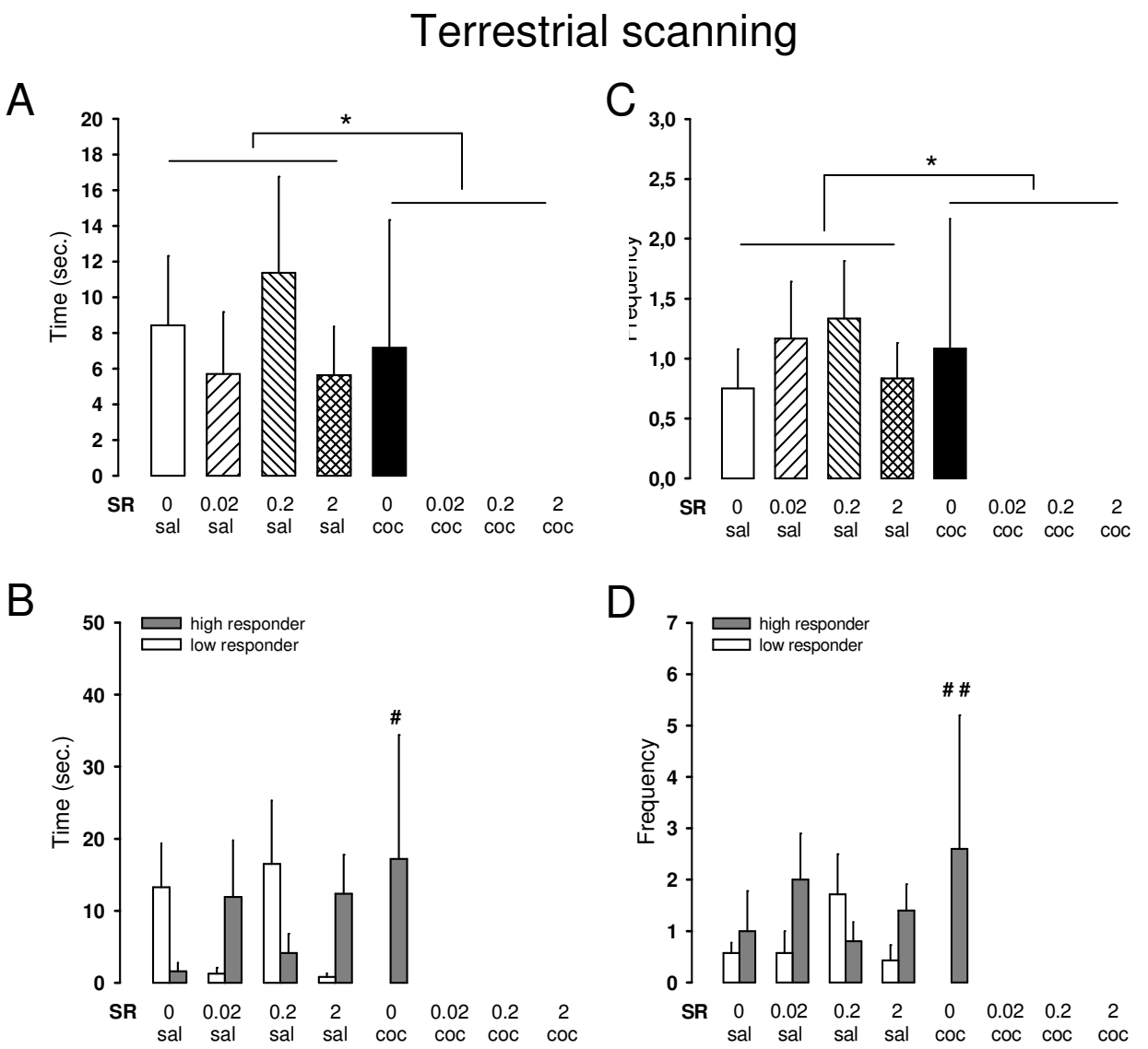
De Souza et al.

Fig. 4



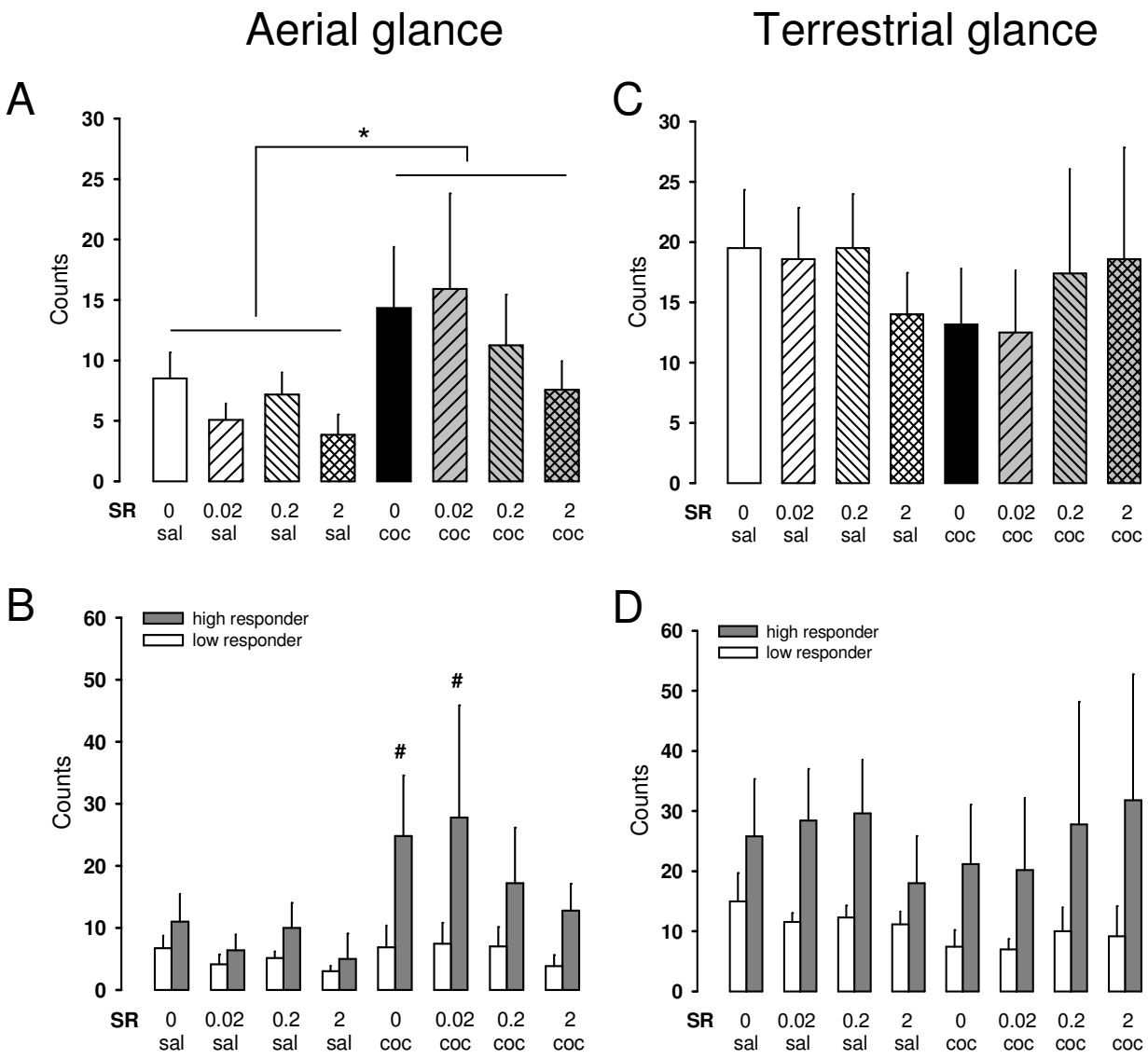
De Souza et al.

Fig. 5



De Souza et al.

Fig. 6



De Souza et al.

Fig. 7

DISCUSSÃO GERAL

Diferenças comportamentais interindividuais

Um dos aspectos mais importantes a serem considerados em estudos dessa natureza é a resposta diferenciada, interindividual, a um mesmo tratamento farmacológico (e.g. Hooks *et al.*, 1991). Primeiramente, deve-se considerar a natureza essencialmente comportamental deste estudo neurofarmacológico. À medida que o repertório comportamental dos animais estudados é mais amplo, mais variada e diversa pode ser a reação ante um mesmo estímulo, como foi observado, neste caso, por meio da administração de fármacos.

Em especial, com relação à atividade locomotora, indicador-chave das propriedades estimulantes de uma droga (Hoekenga *et al.*, 1978), para uma análise mais acurada dos efeitos obtidos pela administração dos psicoestimulantes empregados nesse estudo, o anfetamínico dietilpropiona e a cocaína, agrupamos os animais em função da ocorrência, ou não, de hiperlocomoção. Dessa forma, nos dois experimentos, havia um grupo dos animais "sensíveis" ao psicoestimulante administrado, ou seja, aqueles que apresentaram um aumento na atividade locomotora após a administração do mesmo, se comparado ao controle salina. Por outro lado, os animais que não apresentaram aumento na atividade locomotora após a administração do psicoestimulante, formaram o grupo de animais "insensíveis" ao psicoestimulante.

Vale ressaltar que a referida variação na resposta locomotora já foi observada em outros estudos empregando cocaína (e.g. Müller *et al.*, 2004); enquanto os estudos clássicos com a dietilpropiona já apontavam para diferentes respostas comportamentais (Sjöberg & Jonsson, 1967; Johanson *et al.*, 1976).

No experimento da dietilpropiona, 60% dos animais (n=10), apresentaram hiperlocomoção sendo, portanto, considerados "sensíveis" à droga. Por sua vez, no

experimento com cocaína, 42% dos animais (5 em 12), apresentaram aumento da atividade locomotora após administração da droga. Por conseguinte, os animais restantes, os que não apresentaram hiperlocomoção, formaram o grupo dos sujeitos "insensíveis" ao tratamento.

A relevância desta subdivisão se torna clara ao compararmos os efeitos dos pré-tratamentos, para os dois experimentos, usando os antagonistas serotonérgico e do receptor NK_3, WAY 100635 e SR142801, respectivamente. Tomados em conjunto, ou seja, como um único grupo, resultados significativos eram mascarados. Desta forma, a divisão em subgrupos de animais sensíveis ou não ao psicoestimulante administrado, nos permitiu identificar respostas diferenciadas, considerando os diversos parâmetros comportamentais analisados, com relação ao respectivo antagonista empregado em cada experimento.

Outro aspecto importante a ser considerado é que embora tenhamos adotado a hiperlocomoção como critério para a caracterização dos grupos ditos "sensíveis" ou "insensíveis" ao psicoestimulante administrado, temos consciência de que mesmo na ausência de hiperlocomoção, os animais classificados como "insensíveis" ao tratamento podem, por outros meios, terem sido afetados pelos pscicoestimulantes estudados. De fato, como pode ser visto, alguns efeitos dose-dependente envolvendo o pré-tratamento com WAY 100635 e o SR142801, sobre os sujeitos ditos insensíveis, não descartam a possibilidade de um efeito sinérgico dos respectivos psicoestimulantes administrados.

Ademais, as respostas diferenciadas observadas após pré-tratamento com WAY 100635 e SR142801, entre os sujeitos sensíveis e insensíveis ao respectivo psicoestimulante, dietilpropiona ou cocaína, sugerem que os receptores de $5\text{-}HT_{1A}$ e NK_3 estejam envolvidos nas diferenças comportamentais interindividuais em primatas não-humanos em resposta a psicoestimulantes.

Efeitos isolados dos antagonistas (WAY 100635 & SR142801)

O WAY 100635 foi escolhido para o presente estudo por ser um antagonista seletivo de 5-HT_{1A} que não interfere nos níveis de dopamina (Di Chiara & Imperato, 1988; Müller *et al.*, 2002b), além de termos observados em um estudo prévio não afetar a atividade locomotora *per se* (Barros *et al.*, 2003). Portanto, nos permitiu avaliar o papel deste receptor sobre os efeitos estimulantes do anfetamínico dietilpropiona e, devido à grande semelhança no modo de ação dos anfetamínicos com a cocaína, estabelecer uma ponte entre seus efeitos.

Por sua vez, o SR142801, por se tratar de um fármaco com efeitos desconhecidos em nosso modelo animal, foi realizada uma primeira sessão experimental para observar eventuais efeitos comportamentais e locomotores que a droga, por ventura, pudesse produzir em sagüis da espécie *Callithrix penicillata*. Para tanto, foram empregadas as mesmas doses que viriam a ser usadas no teste em conjunto com a cocaína. Contudo, o SR142801 não apresentou nenhuma alteração comportamental ou na atividade locomotora em nenhuma dose testada.

Desta forma, constatou-se que os dois antagonistas empregados como pré-tratamento, a fim de bloquear os efeitos dos psicoestimulantes testados, não produzem nenhum tipo de efeito comportamental ou locomotor isoladamente.

Efeitos dos psicoestimulantes (dietilpropiona e cocaína)

Enquanto a cocaína duplicou a atividade locomotora, a dietilpropiona não produziu um aumento significativo para este mesmo parâmetro, tomando os animais como um único grupo. Entretanto, considerando os animais em função de sua resposta à droga, houve um aumento significativo na atividade locomotora dos animais sensíveis à dietilpropiona se comparados ao controle e aos animais "insensíveis" à droga. Para a cocaína, por sua vez, foi observada uma atividade

locomotora significativamente maior dos animais “sensíveis” comparados aos “insensíveis” ao psicoestimulante. Entretanto, a hiperlocomoção não passou de uma tendência se comparada ao controle salina. Como discutido anteriormente, o aumento na atividade locomotora é um efeito clássico dos psicoestimulantes e foi tomado como critério para a subdivisão dos sujeitos em grupos de acordo com a sensibilidade aos efeitos hiperlocomotores dos psicoestimulantes testados.

Tanto a cocaína quanto a dietilpropiona levaram a uma diminuição significativa dos comportamentos exploratórios. Analisando os grupos, contudo, a dietilpropiona a atividade exploratória no grupo de animais sensíveis enquanto que, para a cocaína, tal efeito nos comportamentos exploratórios dos sujeitos sensíveis à droga não passou de uma mera tendência. A redução observada nos comportamentos exploratórios pode ser considerada um efeito ansiogênico (Barros *et al*., 2004a).

Com relação aos comportamentos de cuidado corporal e marcação de cheiro, a cocaína reduziu de forma significativa esses comportamentos enquanto a dietilpropiona não interferiu na marcação de cheiro e apresentou apenas uma tendência de redução dos comportamentos de cuidado corporal. Seguindo a análise de grupos, a baixa freqüência destes comportamentos, tanto no estudo com a cocaína quanto no estudo com a dietilpropiona, pode ter mascarado um possível efeito inibitório tal qual o observado considerando-se todos os sujeitos. Ainda assim, foi observada uma redução significativa na ocorrência de comportamentos de cuidado corporal para os sujeitos sensíveis à dietilpropiona. Assim, como para os comportamentos exploratórios, a redução observada para esses comportamentos, pode, também, estar relacionada a um estado de ansiedade.

A vigilância (*scanning*) é um comportamento de rastreamento visual, anti-predatório e, portanto, de alto valor adaptativo para os calitriquídeos (Caine, 1984; Hardie & Buchanan-Smith, 1997). A vigilância pode ser classificada como aérea ou terrestre, em função da característica arborícola destes animais que possuem

predadores terrestres e aéreos (Barros *et al*., 2004b). Tomando os animais como um todo, a dietilpropiona potenciou o tempo de vigilância aérea (*aerial scanning*), enquanto a cocaína não apresentou nenhum efeito neste sentido, a não ser por uma diferença entre os grupos "sensível" e "insensível", onde os animais insensíveis apresentaram tempo de vigilância significativamente maior que os animais sensíveis à cocaína. Tal efeito também foi observado para a dietilpropiona que, além disso, promoveu um aumento acentuado no tempo de vigilância aérea dos sujeitos insensíveis à droga. A freqüência da vigilância terrestre (*terrestrial scanning*) foi significativamente reduzida após administração da dietilpropiona enquanto observou-se uma tendência de redução no tempo do referido comportamento. A cocaína, por sua vez, não produziu efeitos claros sobre a vigilância terrestre considerando os sujeitos como um todo. Observando os grupos em função de sua sensibilidade às drogas, constatou-se que os animais "insensíveis" à dietilpropiona foram responsáveis pelos efeitos significativos na redução da freqüência da vigilância terrestre, assim como pela tendência na diminuição do tempo da mesma. Para a cocaína, observou-se efeitos claramente antagônicos: uma supressão completa da vigilância terrestre entre os sujeitos "insensíveis" à droga enquanto os sujeitos "sensíveis", tanto na duração quanto na freqüência do comportamento, foram diferentes.

Por fim, um novo parâmetro comportamental foi adotado para o experimento com a cocaína, a análise da varredura rápida (*glance*), que assim como a vigilância, pode ser aérea (*aerial glance*) ou terrestre (*terrestrial glance*). Trata-se, portanto, de uma rápida varredura visual. Observou-se, assim, um aumento significativo da varredura aérea rápida considerando todos os animais como um único grupo, enquanto este aumento não passou de uma tendência entre os sujeitos sensíveis à cocaína que, no entanto, apresentaram uma freqüência três vezes maior do que os animais insensíveis ao psicoestimulante. Não houve nenhuma alteração no padrão da varredura terrestre rápida.

O aumento observado na vigilância como um todo, tanto para a cocaína quanto para a dietilpropiona, levando em consideração os efeitos observados em todos os animais agrupados ou de acordo com a sensibilidade às drogas, está relacionado a um contexto ansiogênico (Caine, 1998; Ferrari & Lopes Ferrari, 1990).

Efeitos dos pré-tratamentos (WAY 100635 e SR142801)

Os efeitos observados da cocaína sobre os comportamentos dos calitriquídeos não parecem ter sido modulados pelo pré-tratamento com o antagonista do receptor tipo NK_3, SR142801, quando considerando todos os animais como um grupo homogêneo. O mesmo não pode ser dito com relação aos efeitos do pré-tratamento com o antagonista serotonérgico do receptor de 5-HT_{1A}, WAY 100635. As doses de 0,4 e 0,8 mg/kg do antagonista reverteram o aumento induzido pela dietilpropiona sobre o tempo de vigilância aérea. Além disso, a dose de 0,4 mg/kg de WAY 100635 reverteu a redução induzida pelo anfetamínico sobre a freqüência de vigilância terrestre. Esses efeitos sugerem uma ação ansiolítica parcial sobre os efeitos ansiogênicos induzidos pela dietilpropiona.

Entretanto, é na análise dos animais segundo sua sensibilidade às drogas que se observam efeitos significativos dos pré-tratamentos. A hiperlocomoção induzida pela dietilpropiona sobre os animais sensíveis à droga foi revertida sob as doses de 0,4 e 0,8 mg/kg de WAY 100635. De forma análoga, a hiperlocomoção induzida pela cocaína sobre os animais sensíveis ao psicoestimulante apresentou uma redução parcial na dose de 0,2 mg/kg de SR142801.

Não foram observados efeitos claros do antagonista serotonérgico sob a redução da atividade exploratória induzida pela dietilpropiona nos sujeitos sensíveis à droga. Entretanto, o WAY 100635 potenciou a redução dos referidos comportamentos nos sujeitos insensíveis à droga para todas as doses, em

destaque, a de 0,4 mg/kg. Já o antagonista de NK_3, SR142801, parece não ter tido nenhum efeito sobre os comportamentos exploratórios.

Ambos pré-tratamentos não apresentaram nenhum efeito evidente sobre os comportamentos de cuidado corporal nem na marcação de cheiro.

O WAY 100635 reverteu parcialmente, na dose de 0,8 mg/kg, o aumento induzido pela dietilpropiona no tempo de vigilância aérea dos sujeitos insensíveis à droga. O pré-tratamento com SR142801, por sua vez, apresentou apenas uma leve tendência de redução no tempo de vigilância aérea induzida pela cocaína.

Com respeito à vigilância terrestre, observou-se um efeito dose-dependente, não significativo, por parte do antagonista serotonérgico, que reverteu a tendência de redução do tempo de vigilância, induzida pela dietilpropiona, entre os animais insensíveis ao psicoestimulante. Já a tendência de aumento no tempo e na freqüência da vigilância terrestre entre os animais insensíveis à cocaína, foi suprimida para todos as doses de SR142801. O mesmo efeito foi observado para o parâmetro varredura aérea rápida, analisado no experimento com a cocaína.

CONCLUSÃO

A análise dos animais em função de sua sensibilidade à droga se revelou extremamente relevante, considerando as já mencionadas diferenças interindividuais em resposta a administração de fármacos psicoestimulantes. Por sua vez, o agrupamento dos resultados, considerando todos os animais em um único grupo, embora necessário e relevante para se obter uma visão geral dos efeitos dos tratamentos e pré-tratamentos sobre o conjunto dos indivíduos, quando não foi pouco informativo, mascarou diferenças importantes e até mesmo antagônicas entre os grupos caracterizados de acordo com a sensibilidade aos psicoestimulantes empregados neste trabalho. Este aspecto é de grande relevância para futuros estudos.

Tal subdivisão, sugere a ocorrência de dois tipos, perfis distintos de calitriquídeos, que apresentam uma resposta diferenciada a psicoestimulantes como a dietilpropiona e a cocaína. Os animais do primeiro tipo apresentam hiperlocomoção típica sob efeito de tais estimulantes, além de apresentarem alterações comportamentais que sugerem, também, um estado de ansiedade induzido pelas drogas. Por sua vez, os animais do segundo tipo, seriam aqueles que não apresentam hiperlocomoção, mas apresentam alterações comportamentais distintas dos animais do primeiro tipo que sugerem um estado de ansiedade induzido por psicoestimulantes.

Mais especificamente, os animais do primeiro tipo se caracterizaram, além da hiperlocomoção, por um aumento marcante na atividade locomotora em detrimento da atividade exploratória e dos comportamentos de cuidado corporal. Os animais do segundo tipo que, embora não apresentem hiperlocomoção nem redução dos comportamentos de cuidado corporal, se caracterizam por um aumento na vigilância como um todo.

De um modo geral, os pré-tratamentos com os antagonistas serotonérgico (5-HT_{1A}) e do receptor neuropeptídico NK_3, WAY 100635 e SR142801 bloquearam os efeitos comportamentais de seus respectivos psicoestimulantes, dietilpropiona e cocaína. Além de um bloqueio da atividade locomotora, também pôde ser observado um bloqueio dos efeitos ansiogênicos intrínsecos aos estimulantes empregados. Nesse sentido, em um estudo prévio, já havíamos observado a propriedade ansiolítica do WAY 100635 (Barros *et al*., 2003). Contudo, tal efeito por parte do SR142801 foi inesperado, uma vez que estudos com agonistas do receptor NK_3 (Echeverry *et al*., 2001) e, inclusive, um estudo nosso prévio com o fragmento N-terminal da substância-P (Barros *et al*., 2002), resultaram em respostas ansiolíticas. Além disso, outros estudos realizados com o próprio SR142801, relatam efeitos ansiogênicos ou nenhum efeito nesse sentido (Ribeiro & DeLima, 1998; Ribeiro *et al*., 1999).

A diferença mais marcante entre as respostas comportamentais observadas entre os psicoestimulantes testados, dietilpropiona e cocaína, seria o aumento da vigilância terrestre nos animais sensíveis à cocaína. Tal efeito sugere que a cocaína elicie efeitos ansiogênicos mais pronunciados, entre os animais sensíveis, do que a dietilpropiona. Ademais, os efeitos comportamentais de ambas as drogas foram bastante similares, guardadas as devidas proporções, considerando efeitos mais ou menos acentuados em função do comportamento observado. Esta similaridade se mostra bastante útil ao, pelo menos, propiciar um "intercâmbio" dos resultados obtidos para os diferentes psicoestimulantes, considerando, também, a semelhança de seus mecanismos neurofisiológicos, a fim de sugerir mecanismos semelhantes de bloqueio de seus efeitos indesejados, propiciando um leque de opções de intervenção farmacológica por vias neurais distintas no tratamento da dependência.

Por fim, de acordo com os objetivos propostos para este estudo, podemos concluir:

- A administração sistêmica dos antagonistas, WAY 100635 e SR142801, não produziram efeitos comportamentais *per se*;

- A dietilpropiona e a cocaína apresentaram efeitos similares, promovendo hiperlocomoção em parte dos animais e efeitos ansiogênicos;

- Os pré-tratamentos com WAY 100635 e SR142801 foram capazes de bloquear os efeitos hiperlocomotores e ansiogênicos da dietilpropiona e cocaína, respectivamente;

- Os resultados obtidos reforçam a hipótese de que os efeitos de estimulantes como a cocaína e anfetamínicos, como a dietilpropiona, não estão unicamente vinculados à modulação da via dopaminérgica mas que as vias serotonérgica e peptidérgica desempenham um papel fundamental na ação neural desses psicoestimulantes;

- O modelo experimental empregado, incluindo sua metodologia, se mostrou uma ferramenta eficaz para estudos de interação entre fármacos moduladores da ansiedade e psicoestimulantes.

REFERÊNCIAS BIBLIOGRÁFICAS

Barros, M., Boere, V., Huston, J.P., Tomaz, C., 2000. Measuring fear and anxiety in the marmoset (*Callithrix penicillata*) with a novel predator confrontation model: effects of diazepam. Behav. Brain Res. 108, 205-211.

Barros, M., Tomaz, C., 2002. Non-human primate models for investigating fear and anxiety. Neurosci. Biobehav. Rev. 26, 187-201.

Barros, M., De Souza Silva, M.A., Huston, J.P., Tomaz, C., 2002. Anxiolytic-like effects of substance P fragment (SP(1-7)) in non-human primates (Callithrix penicillata). Peptides 23, 967-973.

Barros, M., Mello, E.L., Maior, R.S., Müller, C.P., De Souza-Silva, M.A., Carey, R.J., Huston, J.P., Tomaz, C., 2003. Anxiolytic-like effects of the selective 5-HT_{1A} receptor antagonist WAY 100635 in non-human primates. Eur. J. Pharmacol. 482, 197-203.

Barros, M., De Souza-Silva, M.A., Huston, J.P., Tomaz, C., 2004a. Multibehavioral analysis of fear and anxiety before, during, and after experimentally induced predatory stress in *Callithrix penicillata*. Pharmacol. Biochem. Behav. 78, 357-367.

Barros, M., Alencar, C., Tomaz, C., 2004b. Differences in aerial and terrestrial visual scanning in captive black tufted-ear marmosets (*Callithrix penicillata*) exposed to a novel environment. Folia Primatol. 75, 85-92.

Boix, F., Huston, J.P., Schwarting, R.K., 1992a. The C-terminal fragment of substance P enhances dopamine release in nucleus accumbens but not in neostriatum in freely moving rats. Brain Res 592:181-186.

Boix, F., Mattioli, R., Adams, F., Huston, J.P., Schwarting, R.K., 1992b. Effects of substance P on extracellular dopamine in neostriatum and nucleus accumbens. Eur J Pharmacol 216:103-107.

Boix, F., Sandor, P., Nogueira, P.J., Huston, J.P., Schwarting, R.K., 1995. Relationship between dopamine release in nucleus accumbens and place preference induced by substance P injected into the nucleus basalis magnocellularis region. Neuroscience 64:1045-1055.

Borsini, F., Bendotti, C., Carli, M., Poggesi, E., Samantin, R., 1979. The roles of brain noradrenaline and dopamine in the anorectic activity of diethylpropion in rats: a comparison with d-amphetamine. Res. Commun. Chem. Pathol. Pharmacol. 26, 3-11.

Bray, G.A., 2000, A concise review on the therapeutics of obesity. Nutrition 16, 953-960.

Breiter, H.C., Gollub, R.L., Weisskoff, R.M., Kennedy, D.N., Makris, N., Berke, J.D., Goodman, J.M., Kantor, H.L., Gastfriend, D.R., Riorden, J.P., Mathew, R.T., Rosen, B.R., Hyman, S.E., 1997. Acute effects of cocaine on human brain activity and emotion. Neuron 19:591-611.

Brooke, D., Kerwin, R., Lloyd, K., 1988. Diethylpropion hydrochloride-induced psychosis. Br. J. Psychiatry 152, 572-573.

Caine, N.G., 1984. Visual scanning by tamarins: a description of the behavior and tests of two derived hypothesis. Folia Primatol. 43, 59-67.

Caine, N.G., 1998. Cutting costs in response to predatory threat by Geoffroy's marmosets (*Callithrix geoffroyi*). Am. J. Primatol. 46, 187-196.

Carey, R.J., Damianopoulos, E.N., DePalma, G., 1999. Interoceptive drug cue conditioning of cocaine stimulant effects. Soc Neurosci Abstr. v. 25, p. 308.

Carey, R.J., Damianopoulos, E.N., DePalma, G., 2000. The 5-HT_{1A} antagonist WAY 100635 can block the low dose locomotor stimulant effects of cocaine. Brain Res. v. 862, p. 242-246.

Carey, R.J., DePalma, G., Damianopoulos, E., 2001. Cocaine and serotonin: a role for the 5-HT_{1A} receptor site in the mediation of cocine stimulant effects. Behav. Brain Res. 126, 127-133.

Carney, M.W., 1988. Diethylpropion and psychosis. Br. J. Psychiatry 152, 146-147.

Carraway, R. e Leeman, S.E., 1975. The amino acid sequence of a hypothalamic peptide, neurotensin. F. Neurosci. 9, 4430-4438.

Cooper, S.J., Van der Hoek, G.A., 1993. Cocaine: a microstructural analysis of its effects on feeding and associated behaviour in the rat. Brain Res. 608, 45-51.

Cooper, J.R., Bloom, F.E., Roth, R.H. The Biochemical Basis of Neuropharmacology, 7 ed. New York: Oxford University Press, 1996.

Costa, J.C., Tomaz, C., 1998. Posttraining administration of Substance P and its N-terminal fragment block the amnestic effects of Diazepam. Neurobiol. Learn. Mem. 69:65-70.

Cunningham, K., A., Paris, J., M., Goeders, N., E., 1992. Chronic cocaine enhances serotonin autoregulation and serotinin uptake binding. Synapse. v. 11, p. 112-123.

Da Silva, L.B., Cordellini, S., 2003. Effects of diethylpropion treatment and withdrawal on aorta recativity, endothelial factors and rat behavior. Toxicol. Appl. Pharmacol. 190, 170-176.

De La Garza, R. II, Cunningham, K.A., 2000. The effects of the 5-hydroxytrypamine 1A agonist 8-hydroxy-2-(di-n-propylamino)tetralin on spontaneous activity, cocaine-induced hyperactivity and behavioral sensitization: a microanalysis of locomotor activity. J. Pharmacol Exp Ther. V. 292, p. 610-617.

Deroche-Gamonet, V., Belin, D., and Piazza, P.V., 2004. Evidence for addiction-like behavior in the rat. Science 305:1014-1017.

Di Chiara, G., Imperato, A., 1988. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc. Natl. Acad. Sci. U.S.A. 85, 5274–5278.

Drummond, M.C.C.; Filho, H.C.D. Drogas, a busca de respostas. Edições Loyola, São Paulo; 170 p. 1998.

Echeverry, M., Hasenöhrl, R.U., Huston, J.P., Tomaz, C., 2001. Comparison of neurokinin SP with diazepam in effects on memory and fear parameters in the elevated T-maze free exploration paradigm. Peptides 22, 1031-1036.

Emonds-Alt, X., Bichon, D., Ducoux, J.P., Heaulme, M., Miloux, B., Poncelet, M., Proietto, V., Van Broeck, D., Vilain, P., Neliat, G., 1995. SR 142801, the first potent non-peptide antagonist of the tachykinin NK3 receptor. Life Sci 56:L27-L32.

Ferrari, S.F., Lopes Ferrari, M.A., 1990. Predator avoidance behavior in the buffy-headed marmoset (*Callithrix flaviceps*). Primates 31, 323-338.

Fletcher, A., Forster, E.A., Bill, D.J., Brown, G., Cliffe, I.A., Hartley, J.E., Jones, D.E., McLenachan, A., Stanphope, K.J., Critchley, D.J., Childs, K.J., Middlefell, V.C., Lanfumey, L., Corradetti, R., Laporte, A.M., Gozlan, H., Hamon, M., Dourish, C.T., 1996. Electrophysiological, biochemical, neurohormonal and behavioural studies with WAY-100635, a potent, selective and silent 5-HT_{1A} receptor antagonist. Behav. Brain Res. 73, 337-353.

Foltin, R.W., 1989. Effects of anorectic drugs on the topography of feeding behavior in baboons. J. Pharmacol. Exp. Ther. 249, 101-109.

Foltin, R.W., 2001. Effects of amphetamine, dexfenfluramine, diazepam and other pharmacological and dietary manipulations on food “seeking” and “taking” behavior in non-human primates. Psychopharmacology 158, 28-38.

Fookes, B.H., 1976. Schizophrenia-like reaction to diethylpropion. Lancet 2, 1206.

Garattini, S., Borroni, E., Mennini, T., Samarin, R., 1978. Differences and similarities among anorectic agents. In: Garattini, S., Samanin, R. (Eds.), Central Mechanisms of Anorectic Drugs. Raven Press, New York, pp. 127-143.

Gerhardt, P., Hasenöhrl, R.U., Huston, J.P., 1992. Enhanced learning produced by injection of neurokinin substance P into the region of the nucleus basalis magnocellularis: mediation by the N-terminal sequence. Exp Neurol 118:302-308.

Gevaerd, M.S., Sultowski, E.T., Takahashi, R.N., 1999. Combined effects of diethylpropion and alcohol on locomotor activity of mice: participation of the dopaminergic and opioid systems. Braz. J. Med. Biol. Res. 32, 1545-1550.

Girault, J.A., Greengard, P., 2004. The neurobiology of dopamine signaling. Arch. Neurol. 61, 641-644.

Goeders, N.E., 1992. Potential involvement of anxiety in the neurobiology of cocaine. Ann. N.Y. Acad. Sci. 654, 357-367.

Griffiths, R.R., Winger, G., Brady, J.V., Snell, J.D., 1976. Comparison of behavior maintained by infusions of eight phenylethylamines in baboons. Psychopharmacology 50, 251-258.

Griffiths, R.R., Brady, J.V., Snell, J.D., 1978. Relationship between anorectic and reinforcing properties of appetite suppressant drugs: implications for assessment of abuse liability. Biol. Psychiatry 13, 283-290.

Hardie, S.M., Buchanan-Smith, H.M., 1997. Vigilance in single and mixed-species groups of tamarins (*Saguinus labiatus* and *S. fuscicollis*). Int. J. Primatol. 18, 217-234.

Hasenöhrl, R.U., Gerhardt, P., Huston, J.P., 1990. Evidence for dose-dependent positively and negatively reinforcing effects of the substance P C-terminal analog DIME-C7. Neuropeptides 17:205-211.

Hasenöhrl, R.U., Gerhardt, P., Huston, J.P., 1992. Positively reinforcing effects of the neurokinin substance P in the basal forebrain: mediation by its C-terminal sequence. Exp Neurol 115:282-291.

Hasenöhrl, R.U., Jentjens, O., De Souza-Silva, M.A., Tomaz, C., Huston, J.P., 1998. Anxiolytic-like action of neurokinin substance P administered systemically or into the nucleus basalis magnocellularis region. Eur. J. Pharmacol. 354:123-133.

Hasenöhrl, R.U., De Souza-Silva, M.A., Nikolaus, S., Tomaz, C., Brandão, M.L., Schwarting, R.K.W., Huston, J.P., 2000. Substance P and its role in neural mechanisms governing learning, anxiety and functional recovery. Neuropeptides 34:272-280

Helke, C., Krause, J.E., Mantyh, P.W., Couture, R., Bannon, M.J., 1990. Diversity in mammalian tackykinin peptidergic neurons: multiple peptides, receptors, and regulatory mechanisms. FASEB Journal 4:1606-1615.

Herges, S., Taylor, D.A., 1998. Involvement of serotonin in the modulation of cocaine-induced locomotor activity in the rat. Pharmacol Biochem Behav. 59:595-611.

Hoekenga, M.T., Dillon, R.H., Leyland, H.M., 1978. A comprehensive review of diethylpropion hydrochloride. In: Garattini, S., Samanin, R. (Eds.), Central Mechanisms of Anorectic Drugs. Raven Press, New York, pp. 391-404.

Hökfelt, T., Pernow, B., Wahren, J., 2001. Substance P: a pioneer amongst neuropeptides. J Intern Med 249:27-40.

Holzhäuer-Oitzl, M.S., Boucke, K., Huston, J.P., 1987. Reinforcing properties of substance P in the lateral hypothalamus revealed by conditioned place preference. Pharmacol Biochem Behav 28:511-515.

Holzhäuer-Oitzl, M.S., Hasenöhrl, R., Huston, J.P., 1988. Reinforcing properties of substance P in the region of the nucleus basalis magnocellularis in rats. Neuropharmacology 27:749-756.

Homberg, J.R., van den Akker, M., Raaso, H.S., Wardeh, G., Binnekade, R., Schoffelmeer, A.N.M., De Vries, T.J., 2002. Enhanced motivation to self-administer cocaine is predicted by self-grooming behaviour and relates to dopamine release in the rat medial prefrontal cortex and amygdala. Eur J Neurosci 15:1542-1550.

Hooks, M.S., Jones, G.H., Smith, A.D., Neill, D.B., Justice, J.B., 1991. Response to novelty predicts the locomotor and nucleus accumbens dopamine response to cocaine. Synapse 9, 121-128.

Jasinski, D.R., Nutt, J.G., Griffith, J.D., 1974. Effects of diethylpropion and d-amphetamine after subcutaneous and oral administration. Clin. Pharmacol. Ther. 16, 645-652.

Jocham, G., Lezoch, K., Muller, C.P., Kart-Teke, E., Huston, J.P., De Souza Silva, M.A., Neurokinin3 receptor antagonism attenuates cocaine's rewarding and hyperlocomotor effects yet potentiates its dopamine-enhancing action in the nucleus accumbens core. Submetido para publicação.

Johanson, C.E., Balster, R.L., Bonese, K., 1976. Self-administration of psychomotor stimulant drugs: the effects of unlimited access. Pharmacol. Biochem. Behav. 4, 45-51.

Johanson, C.E., Schuster, C.R., 1976. A comparison of cocaine and diethylpropion under two different schedules of drug presentation. In: Ellinwood, E.H., Kilbey, M.M. (Eds.), Cocaine and Other Stimulants. Plenum, New York, pp. 545-570.

Jonsson, C.O., Sjöberg, L., Ek, S., Vallbo, S., 1967. Studies in the psychological effects of a new drug (diethylpropion). Time curves for five subjective variables. Scand. J. Psychol. 8, 39-46.
Khan, S.A., Spiegel, D.A., Jobe, P.C., 1987. Psychotomimetic effects of anorectic drugs. Am. Fam. Phys. 36, 107-112.

King, F.A., Yarbrough, C.J., Anderson, D.C., Gordon, T.P., Gould, K.G., 1988. Primates. Science. 240:1475-1482.

Koenig, A., 1998. Visual scanning by common marmosets (*Callithrix jacchus*): functional aspects and the special role of adult males. Primates 39, 85-90.

Krappmann, P., Hasenöhrl, R.U., Frisch, C., Huston, J.P., 1994. Self-administration of neurokinin substance P into the ventromedial caudate-putamen in rats. Neuroscience 62:1093-1101.

Leshner, A. I., 2004. Cocaine abuse and addiction Research Report Series NIH Pub. No. 99-4342.

Levine, R.R., Walsh, C.T., Schawartz-Bloom, R.D., 2000. The pharmacologic aspects of drug abuse and drug dependence, in: Levine, R.R.: Walsh, C.T.; Schawartz-Bloom, R.D. (Eds.), Pharmachology: Drug actions and reactions. Parthenon Press, New York, pp. 407-410.

Luedtke, R.R., Mach, R.H., 2003. Progress in developing D_3 dopamine receptor ligands as potential therapeutic agents for neurological and neuropsychiatric disorders. Curr Pharm Des 9:643-671.

Maior, R.S., Mello Jr., E.L., Barros, M., Tomaz, C., 2002. Efeitos mnemotrópicos duradouros do fragmento n-terminal da substância-p (spn) sobre a aprendizagem de esquiva em primatas não-humanos (Callithrix penicillata). Neurobiologia 65:59-62

Massi, M., Panocka, I., de Caro, G., 2000. The psychopharmacology of tachykinin NK-3 receptors in laboratory animals. Peptides 21:1597-1609.

Müller, C.P., Carey, R.J., De Souza Silva, M.A., Jocham, G., Huston, J.P., 2002a. Cocaine increases serotonergic activity in the hippocampus and nucleus accumbens in vivo: 5-HT_{1A}-receptor antagonism blocks behavioral but potentiates serotonergic activation. Synapse 45, 67-77.

Müller, C.P., De Souza Silva, M.A., DePalma, G., Tomaz, C., Carey, R.J., Huston, J.P., 2002b. The selective serotonin 1A-receptor antagonist WAY 100635 blocks behavioral stimulating effects of cocaine but not ventral striatal dopamine increase. Behav. Brai Res. 134, 337-346.

Müller, C.P., Thönnessen, H., Jocham, G., Barros, M., Tomaz, C., Carey, R.J., Huston, J.P., 2004. Cocaine-induced “active immobility” and its modulation by the serotonin$_{1A}$-receptor. Behav. Pharmacol. 15, 481-493.

Nguyen-Le, X.K., Nguyen, Q.T., Gobeil, F., Pheng, L.H., Emonds-Alt, X., Breliere, J.C., Regoli, D., 1996. Pharmacological characterization of SR 142801: A new non-peptide antagonist of the neurokinin NK-3 receptor. Pharmacology 52:283-291.

Nikolaus, S., Huston, J.P., Hasenöhrl, R.U., 1999. Reinforcing effects of neurokinin substance P in the ventral pallidum: mediation by the tachykinin NK1 receptor. Eur J Pharmacol 370:93-99.

Otsuka, M., Yoshioka, K., 1993. Neurotransmitter functions of mammalian tachykinins. Physiol. Rev. 73:229-308.

Planeta, C.S., DeLucia, R., 1998. Involvement of dopamine receptors in diethylpropion-induced conditioning place preference. Braz. J. Med. Biol. Res. 31, 561-564.

Rang, H.P., Dale, M.M., Ritter, J.M. Farmacologia, 4ª ed. Rio de Janeiro: Guanabara Koogan, 2001.

Regoli, D., Boudon, A., Fauchere J.L., 1994. Receptors and antagonists for substance P and related peptides. Pharmacol Rev 46:551-599.

Reimer, A.R., Martin-Iverson, M.T., Urichuk, L.J., Coutts, R.T., Byrne, A., 1995. Conditioned place preferences, conditioned locomotion, and behavioral sensitization occur in rats treated with diethylpropion. Pharmacol. Biochem. Behav. 51, 89-96.

Ribeiro, S.J., De Lima, T.C.M., 1998. Naloxone-induced changes in tachykinin NK3 receptor modulation of experimental anxiety in mice. Neurosci. Lett. 258, 155-158.

Ribeiro, S.J., Teixeira, R.M., Calixto, J.B., De Lima, T.C.M., 1999. Tachykinin NK3 receptor involvement in anxiety. Neuropeptides 33, 181-188.

Rogério, R., Takahashi, R.N., 1992. Anxiogenic Properties of Cocaine in the Rat Evaluated with the Elevated Plus-Maze. Pharmacol Biochem Behav 43:631-633.

Ryan, D.H., 2000. Use of sibutramine and other noradrenergic and serotonergic drugs in the management of obesity. Endocrine 13, 193-199.

Safta, L., Cuparencu, B., Sirbu, A., Secareanu, A., 1976. Experimental observations on the effect of amphepramone on the behavior, locomotion, pentretrazol seizures and electroencephalogram. Psychopharmacology 50, 165-169.

Samanin, R., Garattini, S., 1993. Neurochemical mechanism of action of anorectic drugs. Pharmacol. Toxicol. 73, 63-68.

Silverstone, T., 1992. Appetite suppressants. Drugs v. 43 p. 820-836.

Sjöberg, L., Jonsson, C.O., 1967. Studies in the psychological effects of a new drug (diethylpropion): individual differences. Scand. J. Psychol. 8, 81-87.

Stäubli, U., Huston, J.P., 1985. Central action of substance P: possible role in reward. Behav Neural Biol. 43:100-108.

Stevenson, M.F., Poole, T.B., 1976. Ethnogram of Common Marmoset (Calithrix-Jacchus-Jacchus) - General Behavioral Repertoire. Animal Behav. 24:428-451.

Tang, A.H., Kirch, J.D., 1971. Appetite suppression and central nervous system stimulation in the rhesus monkey. Psychopharmacologia 21, 139-146.

Tomaz, C., Aguiar, M.S., Nogueira, P.J.C., 1990. Facilitation of memory by peripheral administration of substance P using avoidance by post-trial peripheral injection of substance P. Neurosci Biobehav Rev. 14:447-453.

Tomaz, C., Silva, A.C.F., Nogueira, P.J.C., 1997. Long-lasting mnemotropic effect of substance P and its N-terminal fragment (SP1-7) on avoidance learning. Braz J Med Biol Res. 30:231-233.

Volkow, N.D., Wang, G.J., Fischman, M.W., Foltin, R.W., Fowler, J.S., Abumrad, N.N., Vitkun, S., Logan, J., Gatley, S.J., Pappas, N., Hitzemann, R., Shea, C.E., 1997 Relationship between subjective effects of cocaine and dopamine transporter occupancy. Nature 386:827-830.

Weiser, M., Frishman, W.H., Michaelson, M.D., Abdeen, M.A., 1997. The pharmacologic approach to the treatment of obesity. J. Clin. Pharmacol. 37, 453-473.

Wood, D.M., Emmett-Oglesby, M.W., 1988. Substitution and cross-tolerance profiles of anorectic drugs in rats trained to detect the discriminative stimulus properties of cocaine. Psychopharmacology 95, 364-368.

Yang, X.M., Gorman, A.L., Dunn, A.J., Goeders, N.E., 1992. Anxiogenic effects of acute and chronic cocaine administration: neurochemical and behavioral studies. Pharmacol. Biochem. Behav. 41, 643-650.

APÊNDICE 1

Parecer do Comitê de Ética

UNIVERSIDADE DE BRASÍLIA – UnB
INSTITUTO DE CIÊNCIAS BIOLÓGICA - IBD
COMITÊ DE ÉTICA NO USO ANIMAL - CEUA

11 de junho de 2002.

A QUEM POSSA INTERESSAR

Declaramos que o projeto intitulado ***"Possíveis interações entre os sistemas serotonérgico e dopaminérgico no controle dos efeitos comportamentais e neuroquímicos induzidos pela administração de cocaína em primatas não-humanos (Callithriz penicillata)."***, foi avaliado e aprovado pelo Comitê de Ética no Uso Animal (CEUA) do Instituto de ciências Biológicas da Universidade de Brasília.

Cesar Koppe Grisolia
Comitê de Ética do Uso Animal
Presidente

APÊNDICE 2

Trabalhos publicados no período

ELSEVIER

Pharmacology, Biochemistry and Behavior 68 (2001) 255–262

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Behavioral effects of buspirone in the marmoset employing a predator confrontation test of fear and anxiety

Marilia Barros[a], Eldon L. Mello[a], Joseph P. Huston[b], Carlos Tomaz[a,*]

[a]*Primate Center and Department of Physiological Sciences, Institute of Biology, University of Bras lia, Brazil, CEP 70910-900 Bras lia, DF, Brazil*
[b]*Institute of Physiological Psychology I and Center for Biological and Medical Research, Heinrich-Heine-University of Düsseldorf, 40225 Düsseldorf, Germany*

Received 20 June 2000; accepted 26 September 2000

Abstract

In order to further validate the recently developed marmoset (*Callithrix penicillata*) predator confrontation model of fear and anxiety, we investigated the behavioral effects of buspirone with this method. The apparatus consisted of three parallel arms connected at each end to a perpendicular arm, forming a figure-eight continuous maze. A taxidermized wild oncilla cat (*Felis tigrina*) was positioned facing a corner of the parallel arms, alternating between the left or right side of the maze among animals tested. All subjects were first submitted to seven 30-min maze habituation trials (HTs) in the absence of the predator, and then to five randomly assigned treatment trials (TTs) in the presence of the predator: three buspirone sessions (0.1, 0.5 and 1.0 mg/kg), saline and sham injection controls. Twenty minutes after treatment administration, the animal was released into the maze and had free access to the apparatus for 30 min. All trials were taped for later behavioral analysis. Buspirone significantly decreased the frequency of scent marking, while increasing the time spent in proximity to the 'predator' stimulus, indicating an anxiolytic effect. Neither locomotor activity, exposure to a novel environment, stimulus location and habituation, nor gender influenced the effects of the drug treatments. These results further validate this method and demonstrate the potential usefulness of this ethologically based paradigm to test anxiety and fear-induced avoidance in nonhuman primates and its susceptibility to anxiolytic pharmacological manipulations.

Keywords: Marmoset; Anxiety; Fear; Maze; Taxidermized predator; Confrontation; Serotonin; Buspirone

1. Introduction

Ethologically based models of anxiety attempt to approximate natural conditions under which such emotional states are elicited and thus hope to provide comparable results to human anxiety (Blanchard et al., 1998; File, 1980). In fact, various naturalistic models have been developed to test anxiety in rodents, including the social interaction tests, predator confrontations (odor, sound or presence), elevated plus- and T-maze, open field and conspecific confrontations (for reviews, see Blanchard et al., 1998; Griebel, 1995). In nonhuman primates, models like the human threat (Barnes et al., 1991; Carey et al., 1992; Costall et al., 1992; Jones et al., 1988; Newman and Farley, 1995; Walsh et al., 1995), social isolation (Newman and Farley, 1995; Smith and French, 1997; Smith et al., 1998), conspecific confrontation (Cilia and Piper, 1991; French and Inglett, 1991), and social interaction (Palit et al., 1998) have also been employed. Since nonhuman primates exhibit similar physiological and behavioral responses to anxiety-inducing situations as humans (Newman and Farley, 1995; Vellucci, 1990), they can provide important data of relevance to humans (Carey et al., 1992; Newman and Farley, 1995).

Recently, we have developed a new ethologically based method to study fear and anxiety in *Cerrado* marmosets (*Callithrix penicillata*) (Barros et al., 2000). The strategy employed was to expose these animals to a taxidermized predator (the wild oncilla cat *Felis tigrina*), known to elicit fear and anxiety responses in callitrichids (Barros et al., 2000; Emmons, 1987; Passamani, 1995). This predator confrontation model was shown to be sensitive to diazepam, indicating this method as a potentially useful experimental paradigm for studying anxiety and fear-induced avoidance

* Corresponding author. Tel.: +55-61-274-12-51; fax: +55-61-274-12-51.

E-mail address: ctomaz@unb.br (C. Tomaz).

0091-3057/01/$ – see front matter
PII: S0091-3057(00)00447-0

in marmosets. Administration of diazepam significantly reduced scratching, while increasing the frequency of exploratory behaviors and the time spent near the location of the 'predator' (Barros et al., 2000).

One of the major drawbacks in many existing models of anxiety is that their validation is based essentially on their sensitivity to benzodiazepines (BZDs) (File, 1987; Griebel, 1995; Rodgers, 1997; Rodgers et al., 1997). Preclinical and clinical studies employing non-BZD drugs, like buspirone, have sometimes failed to demonstrate conclusive effects of these novel compounds using various methods (for review, see Griebel, 1995). Serotonin (5-hydroxytryptamine, 5-HT) has been repeatedly demonstrated as an essential component of the central network mediating fear and anxiety-induced behaviors in animals (e.g. Barrett and Vanover, 1993; Graeff et al., 1997), and in human pathological states of anxiety (Graeff et al., 1996). In fact, buspirone, a 5-HT_{1A} ligand, has become the most commonly employed alternative drug to classical BZDs in clinical treatments of anxiety (Lader, 1995).

Therefore, going further in the validation of the marmoset predator confrontation model as a new method to study anxiety and fear-induced avoidance, the aim of the present study was to test the effects of buspirone on the behavior of marmosets using this paradigm.

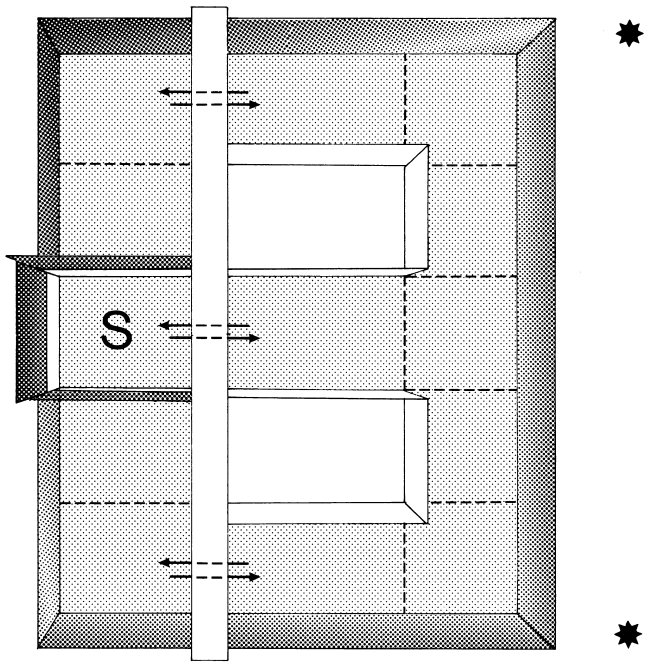


Fig. 1. Topview of the experimental apparatus (figure-eight continuous maze) employed in the marmoset predator confrontation model of fear/anxiety. (S) Start compartment; (✸) locations where the taxidermized wildcat could be positioned.

2. Materials and method

2.1. Subjects

Seven captive born and experimentally naive adult *Cerrado* marmosets (*Ca. penicillata*: four males and three females) were used as subjects. Animals weighed 300–400 g at the beginning of experiments and were housed in male/female pairs in cages ($2 \times 1.3 \times 2$ m). Maintenance and testing of subjects were done at the Primate Center, University of Bras lia. Except during the 30-min experimental sessions, food and water were available ad libitum. The study was approved by the Animals Ethics Committee of the Institute of Biology, University of Bras lia, Brazil.

2.2. Drugs

Buspirone (Bristol-Meyers) was dissolved in physiological saline and injected subcutaneously (sc) in 0.1, 0.5 and 1.0 mg/kg doses, in a volume of 1 ml/kg. Doses of buspirone are expressed as their base and saline was used as vehicle. All treatments were administered in each animal's home cage.

2.3. Apparatus

The experimental apparatus has been described in detail elsewhere (Barros et al., 2000). Briefly, it consists of a rectangular field (125×103 cm) divided into five arms by two holes and barriers, forming a figure-eight continuous maze (see Fig. 1). The apparatus, suspended 1 m from the floor, was divided into two parts (front and back chambers) by a concrete visual barrier (147 cm long, 8 cm wide, 218 cm high). The removable wire mesh start compartment, consisted of a rectangular arm (30 cm long, 25 cm wide, 35 cm high) with a central guillotine-type door. The front chamber, made of 4 mm transparent glass supported by a metal frame, had three parallel arms (40 cm long, 25 cm wide, 35 cm high), 25 cm apart, ending in a common perpendicular arm (125 cm long, 25 cm wide, 35 cm high). The two chambers were connected through holes in the visual barrier at each parallel arm.

Video cameras for monitoring and recording the experimental sessions were used, and a small taxidermized wild oncilla cat (*F. tigrina*) was placed outside the maze facing one corner of the parallel arms. The concrete barrier prevents view of the taxidermized cat as the subject enters the maze (see Barros et al., 2000), enabling a casual encounter through spontaneous exploration of the maze.

2.4. Habituation to the maze

To avoid confounding effects of exposing the marmosets to a novel environment (maze) while measuring their response to a taxidermized predator, seven 30-min habituation trials (HTs) were given in the absence of the 'predator', with 48-h intervals between sessions. These trials are essential to reliably measure the marmoset's fear/anxiety

behaviors in response to the 'predator' stimulus, as these animals can predominantly engage in highly erratic locomotor patterns when first exposed to novel environments. Such behavior tends to decline to a baseline level by the seventh trial (Barros et al., 2000).

2.5. Experimental procedure

After HT, five treatment trials (TTs) were performed on each subject, including three doses of buspirone, saline and a sham injection trial. For HT, each marmoset was quickly captured in its own home cage, handled for 1 min, and then placed in a transportation cage (35 cm long, 20 cm wide, 23 cm high). For TT, after being captured, each animal was administered a treatment, and thereafter placed into the cage. After 20 min, for both HT and TT, the subject was released into the start compartment of the maze, thus commencing a 30 min trial. Barriers from this compartment were promptly removed upon the marmoset's exit. After the test session, the subject was returned to its home cage in the transportation cage.

The 'predator' was presented on either the left or right side of the maze among subjects. Sessions were observed through a closed-circuit television and taped for later analysis. Treatment and order of the subjects were pseudo-randomly assigned for each test day. Sessions were performed between 07:30 and 13:30 hours, with a 72-h interval between test days.

2.6. Behavioral and statistical analysis

The choice of the behaviors analyzed was based on information from the literature, pilot work testing various taxidermized predators as stimuli, and on a previous study of the effects of diazepam using this model (Barros et al., 2000). The figure-eight maze was divided into 13 sections. Locomotor activity (frequency and time spent in each section) was measured using the behavior analysis software CHROMOTRACK 4.02, and the frequency and duration of other behaviors were analyzed by the focal-all occurrences sampling method (Altman, 1974). The following behaviors were measured by an observer blind to the experimental treatment: (1) exploratory behavior: to smell and/or lick any part of the apparatus; (2) locomotor activity; (3) scent marking: to rub the anogenital region to any substratum; and (4) time spent in the vicinity of the 'predator'.

Statistical analysis was carried out using Friedman's test for repeated measures followed by Dunnett's or Tukey's test for pairwise comparisons. Level of significance was set at $P < .05$ and analysis are based on one-tailed levels of significance, except for the different time intervals on the locomotor activity and proximity to the 'predator'. Based on previous studies with buspirone (Costall et al., 1992), one-tailed probabilities were employed since an anxiolytic effect was expected after treatments.

3. Results

For each of the behavioral categories analyzed data were pooled into one group, as no significant differences in gender were observed. The results for scent marking and exploratory behaviors are presented as the averaged frequencies obtained over each 30-min treatment session. Furthermore, we analyzed the number of maze section crossings (locomotor activity) and the time spent in the section closest to the stimulus (proximity to 'predator'), right or left side, over the 30-min testing period for each habituation and treatment session. Analysis of the latter behaviors, divided into three 10-min time intervals, are also presented.

The administration of buspirone in doses of 0.5 and 1.0 mg/kg significantly decreased the frequency of scent marking as compared to saline control ($\chi^2 = 9.415$, $P < .05$; Fig. 2). A relative increase in the frequency of exploratory behaviors (to smell and/or lick the apparatus) was observed for the dose of 0.5 mg/kg, but failed to attain significance level ($\chi^2 = 1.586$, $P = .406$; Fig. 2).

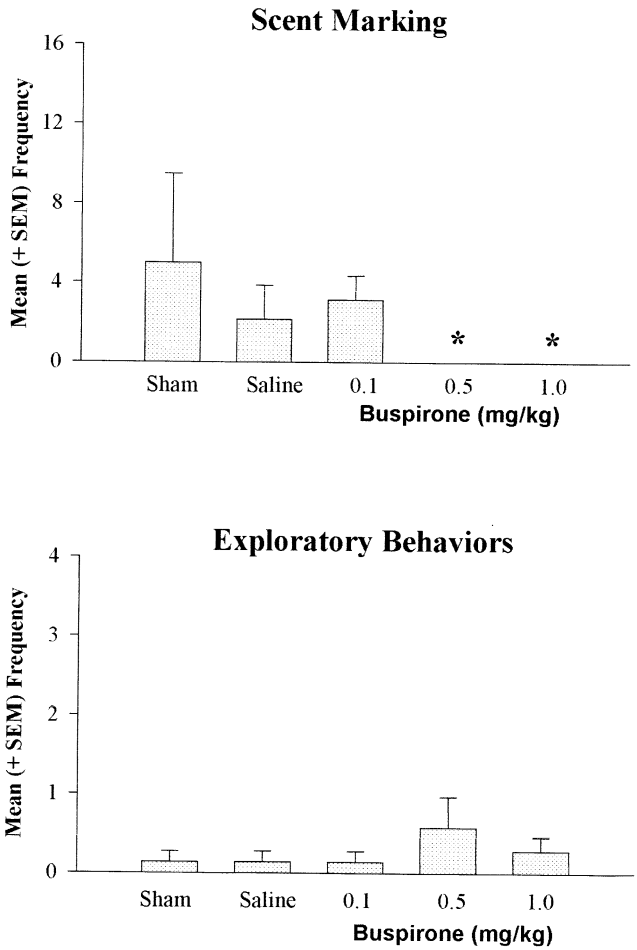


Fig. 2. Effects of treatments on the mean (+S.E.M.) frequency of scent marking (top) and exploratory behaviors (bottom) during 30-min sessions. (Friedman's test followed by Dunnett's one-tailed test. * $P < .05$ compared to saline control, $n = 7$).

Analysis of the time spent in the maze section closest to the taxidermized predator location indicated a significant increase at 0.1- and 0.5-mg/kg doses, compared to saline control ($\chi^2 = 10.185$, $P < .05$; Fig. 3a). Significant differences in this parameter were not observed during the HTs ($\chi^2 = 3.453$, P .1; Fig. 3a), when the 'predator' stimulus was absent. In turn, analysis of the different time intervals (Fig. 3b) did not reveal significant differences between the three intervals of the HTs ($\chi^2 = 2.000$, $P = .486$), while indicating a tendency to increase the time spent in this section during the last 10 min of the buspirone sessions, although not significantly (control: $\chi^2 = 1.130$, $P = .620$; 0.1 mg/kg: $\chi^2 = 1.826$, $P = .486$; 0.5 mg/kg: $\chi^2 = 3.217$, $P = .237$; and 1.0 mg/kg: $\chi^2 = 0.778$, $P = .768$).

A significant decrease in locomotor activity was observed during the course of the HTs when compared to Trial 1 ($\chi^2 = 25.592$, $P < .05$; Fig. 4a). Furthermore, the number of maze section crossings tended to decrease after 10 min of exposure in each HT, except for HT1 (Fig. 4b). Buspirone treatment significantly decreased the level of locomotion

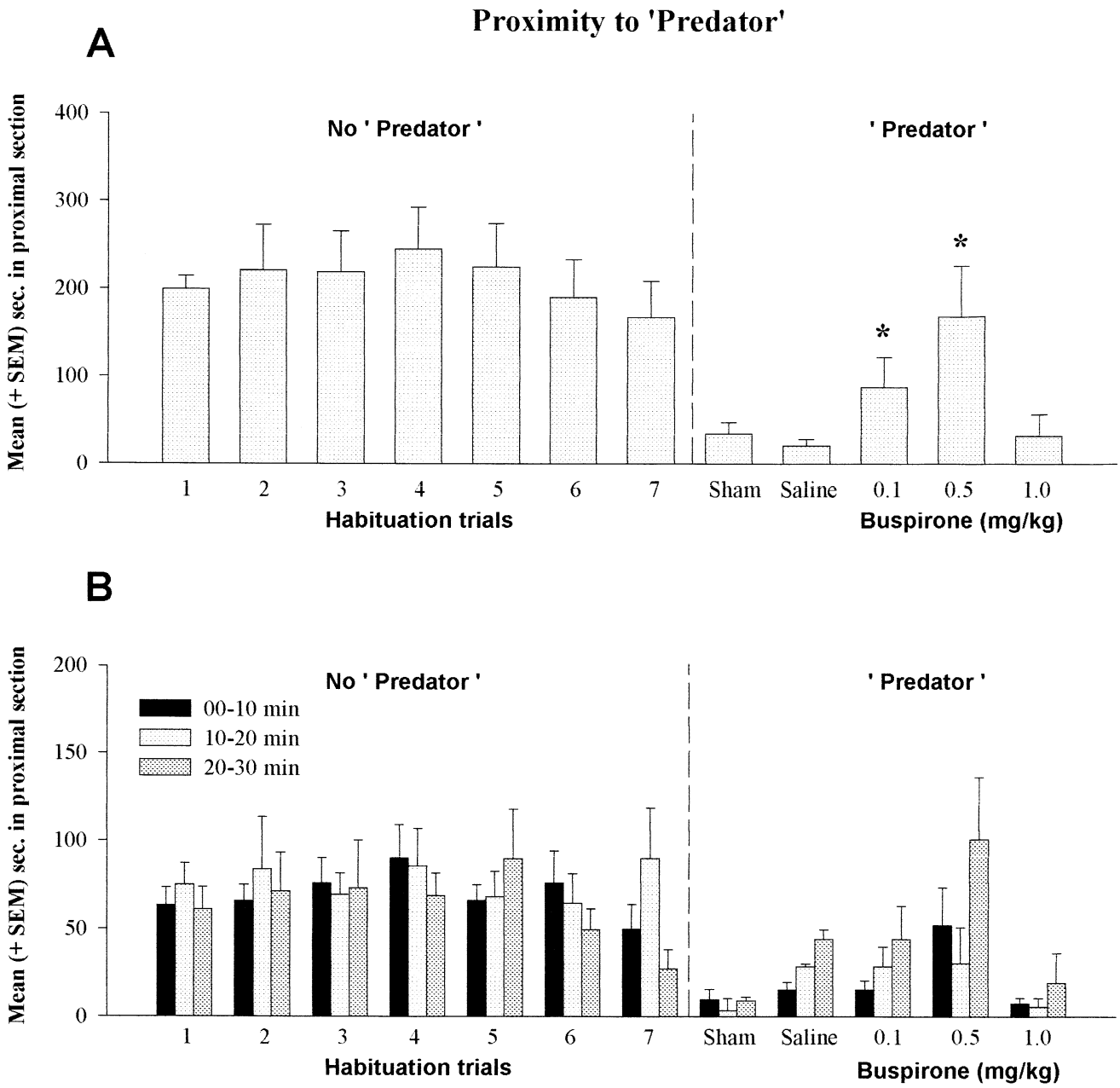


Fig. 3. Effect of habituation trials (left) and control and buspirone treatment sessions (right) on the mean (+S.E.M.) seconds spent in the maze section closest to the 'predator' stimulus during 30 min (A) or three 10-min time intervals (B). (Friedman's test followed by Dunnett's one-tailed or Tukey's two-tailed test. * $P < .05$ compared to saline control, $n = 7$).

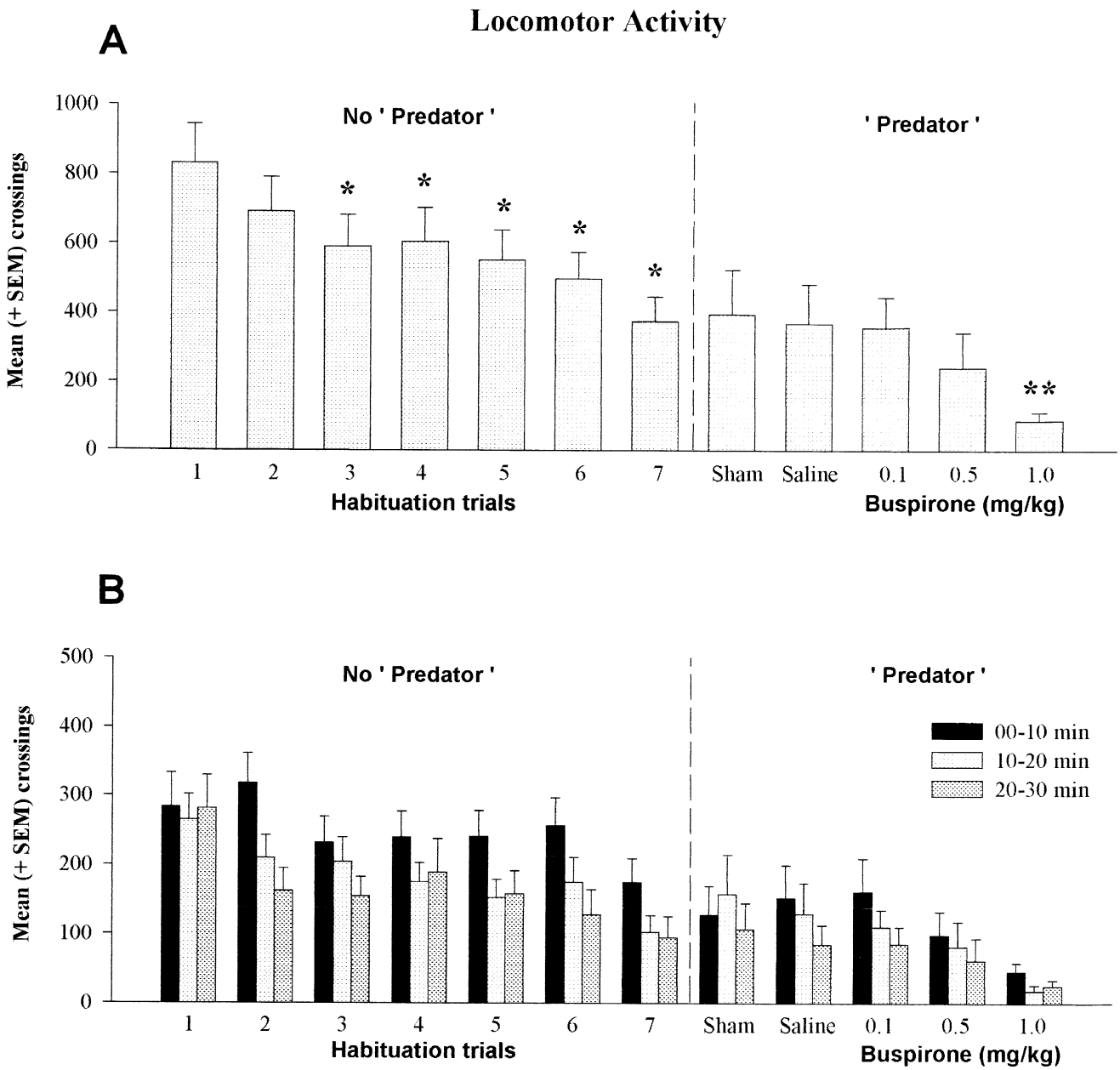


Fig. 4. Mean (+S.E.M.) locomotor activity as defined by the number of the 13 maze sections that were crossed over 30-min periods (A) or during three 10-min time intervals (B). (Left) The seven habituation trials; (right) control and buspirone treatment sessions. (Friedman's test followed by Dunnett's one-tailed or Tukey's two-tailed test. $*P<.05$ compared to habituation trial 1, $**P<.05$ compared to saline control, $n=7$).

only at the 1.0-mg/kg dose, compared to saline control ($\chi^2=9.663$, $P<.05$; Fig. 4a), and no significant decrease during the time course of each trial was observed (control: $\chi^2=5.407$, $P=.085$; 0.1 mg/kg: $\chi^2=4.667$, $P=.112$; 0.5 mg/kg: $\chi^2=3.714$, $P=.192$; and 1.0 mg/kg: $\chi^2=5.556$, $P=.085$; Fig. 4b).

4. Discussion

The present study indicates that the new test of fear and anxiety, the marmoset predator confrontation in the figure-eight maze, is sensitive to serotonergic pharmacological manipulations, inducing significant dose-dependent changes in the behavioral repertoire of the animals tested.

Scent marking, a common behavior in marmosets, disappeared after the administration of buspirone (0.5 and 1.0 mg/kg). In the first validating study of this model, scent marking was also reduced by diazepam treatments, although not statistically significant (Barros et al., 2000). This anxiety-related behavior in marmosets has been shown to increase under various stressful conditions (Epple et al., 1993; Smith et al., 1998). Furthermore, scent marking in marmosets has been shown to be

sensitive not only to BZDs, but also to serotonergic drug manipulations (Barnes et al., 1991; Cilia and Piper, 1991; Costall et al., 1992).

Buspirone at 0.5 mg/kg also induced an increase in exploratory behaviors (to lick and/or smell the apparatus), although not significantly (possibly due to the small sample size). This behavioral pattern is considered an indicator of anxiety levels in rhesus monkeys (Suomi et al., 1981), and has also been demonstrated to increase in marmosets after the administration of diazepam employing our paradigm (Barros et al., 2000). A decrease in exploration under stressful situations has been an indicator of anxiety in many rodents models, in which anxiogenic and anxiolytic compounds have effectively altered the frequency of this behavior (e.g. Bättig, 1969).

The lack of a baseline frequency of scratching observed in our study (data not shown), contrasts with the dose-dependent effect initially obtained for this behavior when first validating the method with diazepam (Barros et al., 2000). The present experiment was conducted during a different period of the year than the previous study, corresponding precisely with the rainy and dry seasons, respectively. These very distinct and opposite seasons are typical for the *Cerrado* region in which *C. penicillata* naturally occur, and are known to significantly influence a wide range of behavioral parameters in callitrichids (Ferrari and Diego, 1992). As the animals used in this study are maintained in indoor–outdoor housing facilities, they are also susceptible to such climatic variations, possibly influencing the baseline levels of scratching observed for this behavior. Previous studies where significant effects on scratching were demonstrated, have in general been conducted under controlled environmental conditions (Cilia and Piper, 1991; Diezinger and Anderson, 1986; Schino et al., 1991; Troisi et al., 1991) where temperature and humidity were not considered influencing factors. Further research, considering such variables, would be necessary for a more reliable evaluation of the effect of climatic conditions on the behavioral parameters observed in this model.

A significant increase in the time spent in the vicinity of the 'predator' after 0.1 and 0.5 mg/kg administration of buspirone indicates that this drug had an effective anxiolytic action on the subjects tested. The same effect was observed in our first study employing diazepam (Barros et al., 2000). The observed tendency to increase the time spent near the stimulus during the last 10 min of each session may relate to the pharmacokinetics of buspirone. Alternatively, possible effects of habituation may have induced the enhanced time in proximity to the 'predator' within each treatment session. However, other reports have also revealed anxiolytic effects for subcutaneously administrated buspirone in marmosets after 47 min (Barnes et al., 1991; Costall et al., 1992), corresponding to the 40–50-min post-administration time interval found in this study, suggesting an anxiolytic rather than a habituation effect.

Place preference (right or left side of the maze) does not confound the results obtained for proximity to the taxidermized predator, as the location of the stimulus was alternated between these two sides among subjects. Therefore, proximity may be a measure of anxiety in this model, in which an increase in the time spent close to the 'predator' indicates an anxiolytic effect.

The behavioral changes observed after the administration of buspirone are also not due to effects of the drug on locomotor activities. This behavior was only significantly altered at the highest dose (1.0 mg/kg), which had a sedative effect. Such an effect has also been observed for the same dose of this drug in other marmosets studies (Barnes et al., 1991; Costall et al., 1992). Changes in the behavioral repertoire are then primarily due to anxiolytic effects of drug administrations, and the results obtained for all behaviors analyzed indicate 0.5 mg/kg as the most effective dose in this new method.

Possible confounding effects of exposing these animals to a novel environment were minimized by prior exposure to the apparatus, in the absence of the 'predator' (HTs). Novel environments can be a potent source of stress among marmosets, where increased locomotor activity is a predominant feature of their behavioral repertoire (Smith et al., 1998). This parameter decreased not only between HTs, but also within each trial, reaching stable baseline levels after the seventh trial and 10 min after initial exposure, respectively. Habituation to the maze environment was also observed in the first validating study of this method (Barros et al., 2000), which may in fact be employed as a useful experimental method for investigating different aspects of habituation learning to a novel environment. In addition, male and female marmosets did not differ significantly on any of the behavioral categories observed, consistent with previous reports employing the same method (Barros et al., 2000), and other experimental models (Barnes et al., 1991; Carey et al., 1992; Cilia and Piper, 1991; Costall et al., 1992; Jones et al., 1988; Smith et al., 1998).

The value of studying serotonin's influence in animal models is greatly supported by the fact that the 5-HT system retains various primitive aspects across species, suggesting similar physiological and behavioral roles among vertebrates (Jacobs and Fornal, 1999; Jacobs and Azmitia, 1992), particularly mammals. However, various studies carried out in rodents, pigeons and nonhuman primates employing different, and even the same paradigms, have often led to conflicting and paradoxical results, suggesting that the role of 5-HT in anxiety is more complex than that initially envisioned (for reviews, see Blanchard et al., 1998; Graeff et al., 1997; Griebel, 1995). Discrepancies in data concerning the effects of 5-HT on anxiety may actually be due to the fact that different models, and sometimes the same model, may be measuring different types of anxiety (Barrett and Vanover, 1993; File, 1995; Handley and McBlane, 1993; Handley et al., 1993; Rodgers, 1997) and therefore are not readily comparable.

Models based on ethologically elicited anxiety (e.g. conspecific confrontations and antipredator responses)

allow differentiation between the various types of anxiety, eliciting relevant defensive behaviors, each differentially sensitive to specific drug manipulations (Blanchard et al., 1998). When trying to evaluate anxiety such an array of responses is more informative than a single parameter, since, more often than not, different aspects of the same pathological state may respond differently to distinct pharmacological manipulations (Blanchard et al., 1993). Furthermore, mammals are highly dependent on behavioral adaptations for self-protection (Blanchard et al., 1993). Defense behaviors and their neural substrates are highly conserved among mammals (Davis, 1992; LeDoux, 1995), susceptible to selective pressures (Blanchard et al., 1990; Nesse, 1999), and are thought by some authors to be the 'primitive' basis of anxiety disorders (Darwin, 1872; Deakin and Graeff, 1991; Nesse, 1999). In regard to callitrichids, these animals are susceptible to a broad range of potential predators due to their small size, and predation has therefore had an important influence in the evolution of their defensive responses, among other aspects (Caine, 1990; Ferrari and Lopez Ferrari, 1990). Thus, such features make defense behaviors a prime target for the development of new animal models of anxiety and for investigative studies about its etiology and possible future treatments (Rodgers, 1997).

At the same time, antipredator models tend to give more consistent results when compared to conspecific confrontations (Blanchard et al., 1998). The use of stimuli that one would normally encounter in the environment of the studied species, such as taxidermized predators, approximate normal situations in which such defensive behaviors are elicited, and therefore can provide more valid data (Blanchard et al., 1998; File, 1980). The predator confrontation model used here can be regarded as a useful method for studying anxiety in marmosets. It provides a substantial behavioral repertoire, which has been associated with fear/anxiety situations in captive, as well as wild marmosets (Epple, 1975; Stevenson and Poole, 1976; Stevenson and Rylands, 1988), and has now been shown to be sensitive to pharmacological manipulations of the serotonergic and BZD systems.

Acknowledgments

This research was supported by CAPES/DAAD/PROBAL (058/98 to C.T. and J.P.H.), and by a grant from the German National Science Foundation to J.P.H. M.B. was a recipient of a doctoral fellowship from CAPES. We are thankful to Dr. R. de Oliveira and W. Vargas for animal care and maintenance, to G. Abel for apparatus schemata, and to J.-S. Li for the behavioral analysis software program.

References

Altmann J. Observational study of behavior: sampling methods. Behaviour 1974;49:227–67.

Barnes NM, Costall B, Domeney AM, Gerrard PA, Kelly ME, Krahling H, Naylor RJ, Tomkins DM, Williams TJ. The effects of umespirone as a potential anxiolytic and antipsychotic agent. Pharmacol, Biochem Behav 1991;40:89–96.

Barrett JE, Vanover KE. 5-HT receptors as targets for the development of novel anxiolytic drugs: models, mechanisms and future directions. Psychopharmacology 1993;112:1–12.

Barros M, Boere V, Huston JP, Tomaz C. Measuring fear and anxiety in the marmoset (*Callithrix penicillata*) with a novel predator confrontation model: effects of diazepam. Behav Brain Res 2000;108:205–11.

Bättig K. Drug effects of a combined maze and open-field system by rats. Ann N Y Acad Sci 1969;159:880–97.

Blanchard DC, Griebel G, Rodgers RJ, Blanchard RJ. Benzodiazepine and serotonergic modulation of antipredator and conspecific defense. Neurosci Biobehav Rev 1998;22:597–612.

Blanchard RJ, Blanchard DC, Rodgers J, Weiss SM. The characterization and modelling of antipredator defensive behavior. Neurosci Biobehav Rev 1990;14:463–72.

Blanchard RJ, Yudko EB, Rodgers RJ, Blanchard CD. Defense system psychopharmacology: an ethological approach to pharmacology of fear and anxiety. Behav Brain Res 1993;58:155–65.

Caine NG. Unrecognized anti-predator behavior can bias observation data. Anim Behav 1990;39:195–7.

Carey GJ, Costall B, Domeney AM, Jones DN, Naylor RJ. Behavioural effects of anxiogenic agents in the commom marmoset. Pharmacol, Biochem Behav 1992;42:143–53.

Cilia J, Piper DC. Marmosets conspecific confrontation: an ethologically-based model of anxiety. Pharmacol, Biochem Behav 1991;58:85–91.

Costall B, Domeney AM, Farre AJ, Kelly ME, Martiney L, Naylor RJ. Profile of action of a novel 5-hydroxytryptamine$_{1A}$ receptor ligand E-4424 to inhibit aversive behavior in the mouse, rat and marmoset. J Pharmacol Exp Ther 1992;262:90–8.

Darwin CR. The expression of the emotions in man and animals. London: John Murray 1872.

Davis M. The role of the amygdala in fear and anxiety. Annu Rev Psychol 1992;15:353–75.

Deakin JFW, Graeff FG. J Psychopharmacol 1991;5:305–15.

Diezinger F, Anderson JR. Starting from scratch: a first look at a 'displacement activity' in group-living rhesus monkeys. Am J Primatol 1986;11:117–24.

Emmons LH. Comparative feeding ecology of felids in a neotropical rainforest. Behav Ecol Sociobiol 1987;20:271–83.

Epple G. The behavior of marmoset monkeys (Callithricidae). In: Rosenblum LA, editor. Primate behavior: developments in field and laboratory research vol. 4. New York: Academic Press, 1975. pp. 195–239.

Epple G, Belcher AM, Kuderling I, Zeller U, Scolnock L, Greenfield KL, Smith AB. Making sense out of scents: species differences in scent glands, scent-marking behavior, and scent mark composition in the Callitrichidae. In: Rylands AB, editor. Marmosets and tamarins: systematics, behaviour and ecology. Oxford, UK: Oxford Univ. Press, 1993. pp. 123–51.

Ferrari SF, Diego VH. Long-term changes in a wild marmoset group. Folia Primatol 1992;58:215–8.

Ferrari SF, Lopes Ferrari MA. Predator avoidance behaviour in the buffy-headed marmoset (*Callithrix flaviceps*). Primates 1990;31:323–38.

File SE. The use of social interactions as a method for detecting anxiolytic activity of chlordiazepoxide-like drugs. J Neurosci Methods 1980; 2:219–38.

File SE. The search for novel anxiolytics. Trends Neurosci 1987;10:461–3.

File SE. Animal models of different anxiety states. Adv Biochem Psychopharmacol 1995;48:93–113.

French JA, Inglett BJ. Responses to novel social stimuli in Callitrichid monkeys: a comparative perspective. In: Box HO, editor. Primate responses to environmental change. Cambridge, UK: Chapman Hall, 1991. pp. 275–94.

Graeff FG, Guimaraes FS, de Andrade TGCS, Deakin JFW. Role of 5-HT in stress, anxiety, and depression. Pharmacol, Biochem Behav 1996;54: 129–41.

Graeff FG, Viana MB, Mora PO. Dual role of 5-HT in defense and anxiety. Neurosci Biobehav Rev 1997;21:791–9.

Griebel G. 5-Hydroxytyptamine-interacting drugs in animal models of anxiety disorders: more than 30 years of research. Pharmacol Ther 1995; 65:319–95.

Handley SL, McBlane JW. 5-HT drugs in animal models of anxiety. Psychopharmacology 1993;112:13–20.

Handley SL, McBlane JW, Critchley MAE, Njung'e K. Multiple serotonin mechanisms in animal models of anxiety: environmental, emotional and cognitive factors. Behav Brain Res 1993;58:203–10.

Jacobs BL, Azmitia EC. Structure and function of the brain serotonin system. Physiol Rev 1992;72:165–229.

Jacobs BL, Fornal CA. Activity of serotonergic neurons in behaving animals. Neuropsychopharmacology 1999;21:9S–15S.

Jones BJ, Costall B, Domeney AM, Kelly ME, Naylor RJ, Oakely NR, Tyers MB. The potential anxiolytic activity of GR38032F, a 5-HT_3-receptor antagonist. Br J Pharmacol 1988;93:985–93.

Lader M. Clinical pharmacology of anxiolytic drugs: past, present and future. Adv Biochem Psycho Pharmacol 1995;48:135–152.

LeDoux JE. Emotion: clues from the brain. Annu Rev Psychol 1995;46: 209–35.

Nesse RM. Proximate and evolutionary studies of anxiety, stress and depression: synergy at the interface. Neurosci Biobehav Rev 1999; 23:895–903.

Newman JD, Farley MJ. An ethologically based, stimulus and gender-sensitive nonhuman primate model for anxiety. Prog Neuropsychopharmacol Biol Psychiatry 1995;19:677–85.

Palit G, Kumar R, Chowdhury SR, Gupta MB, Saxena RC, Srimal RC, Dhawan BN. A primate model of anxiety. Eur Neuropsychopharmacol 1998;8:195–201.

Passamani M. Field observation of a group of Geoffroy's marmosets mobbing a margay cat. Folia Primatol 1995;64:163–6.

Rodgers RJ. Animal models of 'anxiety': where next Behav Pharmacol 1997;8:477–96.

Rodgers RJ, Cao BJ, Dalvi A, Holmes A. Animal models of anxiety: an ethological perspective. Braz J Med Biol Res 1997;30:289–304.

Schino G, Troisi A, Perretta G, Monaco V. Measuring anxiety in nonhuman primates: effects of lorazepam on macaque scratching. Pharmacol, Biochem Behav 1991;38:889–91.

Smith TE, French JA. Psychosocial stress and urinary cortisol excretion in marmoset monkeys (*Callithrix kuhli*). Physiol Behav 1997;62:225–32.

Smith TE, Whitworth-McGreer B, French JA. Close proximity of the heterosexual partner reduces the physiological and behavioral consequences of novel-cage housing in black tufted-ear marmosets (*Callithrix kuhli*). Horm Behav 1998;34:211–22.

Stevenson MF, Poole TB. An ethogram of the commom marmoset (*Callithrix jacchus jacchus*): general behavioural repertoire. Anim Behav 1976;24:428–51.

Stevenson MF, Rylands AB. The marmosets, genus *Callithrix*. In: Mittermeier RA, Rylands AB, Coimbra-Filho A, Fonseca GAB, editors. Ecology and behavior of neotropical primates vol. 2. Contagem: Littera Maciel/WWF, 1988. pp. 131–222.

Suomi SJ, Kraemer GW, Baysinger CM, DeLizio RD. Inherited and experiential factors associated with individual differences in anxious behavior displayed by rhesus monkeys. In: Klein DF, Rabkin J, editors. Anxiety: new research and changing concepts. New York: Raven Press, 1981. pp. 179–99.

Troisi A, Schino G, D'Antoni M, Pandolfi N, Aureli F, D'Amato FR. Scratching as a behavioral index of anxiety in macaque mothers. Behav Neural Biol 1991;56:307–13.

Vellucci SV. Primate social behaviour anxiety or depression. Pharmacol Ther 1990;47:167–80.

Walsh DM, Stratton SC, Harvey FJ, Beresford IJM, Hagan RM. The anxiolytic-like activity of GR159897, a non-peptide NK_2 receptor antagonist, in rodent and primate models of anxiety. Psychopharmacology 1995;121:186–91.

International Journal of Primatology, Vol. 23, No. 2, April 2002 (© 2002)

Reactions to Potential Predators in Captive-born Marmosets (*Callithrix penicillata*)

Marilia Barros, Vanner Boere, Eldon L. Mello, Jr., and Carlos Tomaz[1]

Received October 11, 2000; revised December 5, 2000; accepted April 10, 2001

*We describe the behavioral repertoire of captive-born black tufted-eared marmosets (*Callithrix penicillata*) elicited by brief exposures to three potential mounted taxidermized predators (caracara hawk,* Polyborus plancus*; rattlesnake,* Crotalus durissus*; oncilla,* Leopardus tigrina*), and a stuffed toy. For each of the four stimuli, we submitted the subjects to a 9-min trial divided into three consecutive intervals: a 4-min pre-exposure baseline observation, a 1-min stimulus exposure, and a 4-min postexposure observation period. We positioned stimuli in front of each subject's home cage, and video-taped trials for behavioral analysis. During exposures to the potential taxidermized predators, we heard* tsik-tsik *vocalization and alarm behavior. After exposures, only the cat induced these reactions. All stimuli elicited observational reaction, albeit only during exposure intervals. Further comparisons between the three trial intervals indicated a decrease in the time spent in proximity to the cat during exposures, while an increase in proximity occurred when subjects were exposed to either the hawk or snake for the same period. Taken together, the behavioral responses during and after exposures to the taxidermized oncilla suggest that this stimulus is capable of inducing strong and persistent emotional reactions in* Callithrix penicillata.

KEY WORDS: *Callithrix penicillata*; taxidermized predators; antipredator reactions, fear.

INTRODUCTION

The threat of predation exerts a fundamental selective pressure, which influences numerous features of the behavioral ecology of primates (Caine,

[1]To whom correspondence should be addressed at Primate Center and Department of Physiological Sciences, Institute of Biology, University of Brasília, CEP 70910-900, Brasília, DF, Brazil; e-mail: ctomaz@unb.br.

0164-0291/02/0400-0443/0

1993; van Schaik, 1983). Callitrichids, a family of cryptic diurnal New World primates, have small body sizes, and thus, are susceptible to a diverse range of potential predators (Peres, 1993; Sussman and Kinzey, 1984). Investigations of them have demonstrated that distinct behavioral patterns are elicited towards either terrestrial or arial predators (Bartecki and Heymann, 1987; Ferrari and Lopes-Ferrari, 1990; Peres, 1993).

Raptors (Heymann, 1990), owls (Stafford and Ferreira, 1995), felids (Emmons, 1987), snakes (Bartecki and Heymann, 1987; Corrêa and Coutinho, 1997; Heymann, 1987), and tayras (e.g. Rylands, 1981) prey on callitrichids. They also exhibit defense or avoidance behavioral patterns during encounters with other animals, such as tufted capuchins (Peres, 1993), coatimundis (Rylands, 1981), vultures, toucans and parrots, as well as human observers (Heymann, 1990; Peres, 1993; Rylands, 1981).

Although much is known concerning the common marmoset (*Callithrix jacchus*; Stevenson and Rylands, 1988), information regarding the closely related black tufted-eared marmoset (*Callithrix penicillata*) is scarce. Predation by the ornate hawk-eagle (*Spizaetus ornatus*) is currently the only confirmed event for them (Andrade-Greco and Andrade, 1999). Nonetheless, other hawks (*Elanus leucurus*: Faria, 1984; *Polyborus plancus*: Miranda, 1997) may elicit antipredator behaviors.

As an event rarely observed in the wild (Cheney and Wrangham, 1987), predation and the antipredator behavioral repertoire of *Callithrix penicillata* are poorly established towards specific groups of potential predators; felids, raptors and snakes. Studies in captivity may elucidate specific features of antipredator behaviors by way of experimentally controlled exposures to a variety of potentially threatening stimuli. Accordingly, we investigated, in laboratory settings, the behavioral repertoire of captive-born black tufted-eared marmosets elicited by brief exposures to potential taxidermized predators.

METHODS

Subjects and Maintenance

The subjects are 4 male and 3 female, experimentally naive, captive-born adult *Callithrix penicillata*. They lived in 3 groups—two-male/female groups and a group with one male and two females—in indoor/outdoor enclosures of $2.0 \times 1.3 \times 2.0$ m. The home cages were furnished with a suspended nest box, perches and natural tree trunks. Food and water were available *ad libitum*. Maintenance and testing occurred at the Primate Center, University of Brasília, Brazil, conforming to the regulations of the Brazilian Institute of the Environment and Renewable Natural Resources—IBAMA.

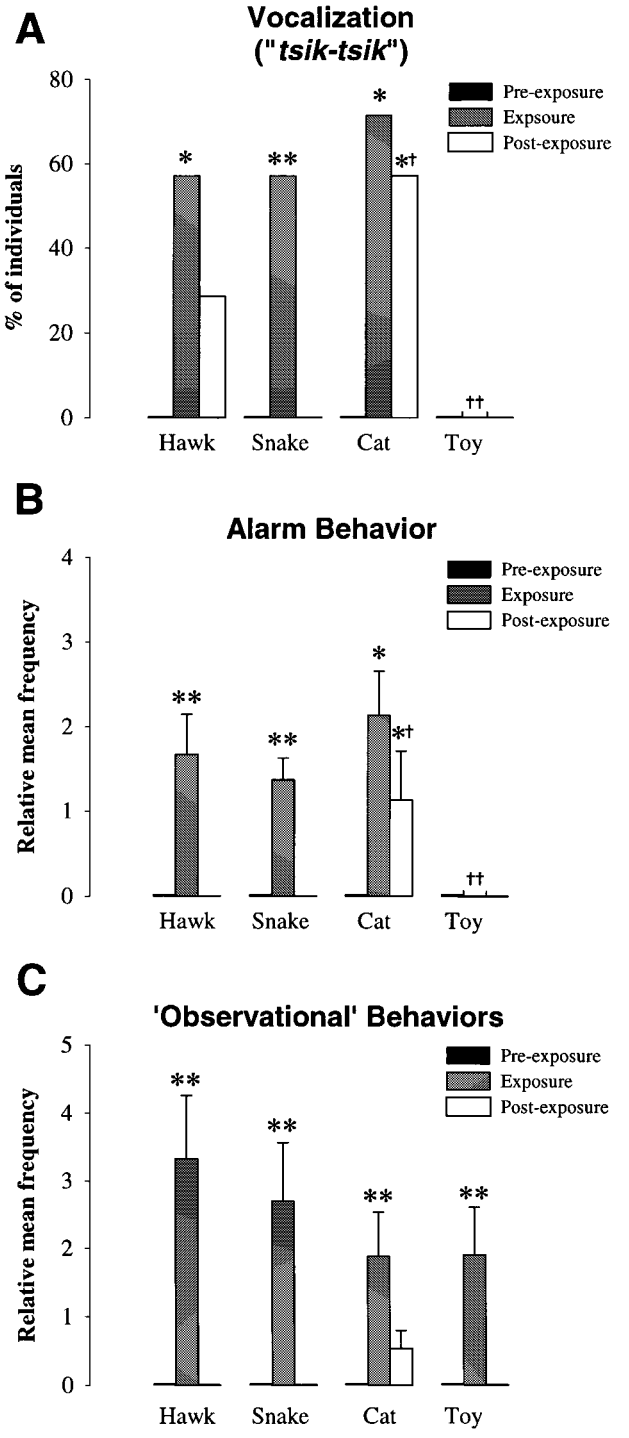
Experimental Procedure

The four stimuli are: 3 mounted taxidermized potential predators—a caracara hawk (*Polyborus plancus*), a rattlesnake (*Crotalus durissus*), and an oncilla (*Leopardus tigrina*)—and a 15 cm purple bear-like stuffed toy. The animals had been taxidermized for >6 years. The toy served as a control stimulus since it does not resemble a potential predator. The stimuli were 60 cm above floor level and 160 cm away from the home cage's front wire mesh. We presented each object tested simultaneously to all members of a group. For each of the 4 stimuli, we submitted the groups to a 9-min trial divided into 3 consecutive time intervals: a 4-min preexposure baseline observation period; a 1-min stimulus exposure interval, where the stimulus was placed in front of the home cage and subsequently removed; and a 4-min postexposure observation period. Each stimulus was initially covered by a cloth and placed on a fixed platform, before the exposure interval, by a caretaker very familiar to the monkeys. Once in place, he removed the cloth. Exposure intervals began immediately after the caretaker's exit. At the end of the exposure, the caretaker reentered the room, covered the object and removed it, and the postexposure interval began. Presentations of stimuli to each group were not visible or audible to other test groups.

Each group was exposed to all four stimuli, one on each test day and with a 48-h interval between trials. Stimuli and group order were pseudorandomly assigned on each test day. Trials were performed between 08:00 and 10:00 h. Before the first test trial we held a sham session in which each group was exposed to the experimental set-up and procedure, but in the absence of any experimental stimuli. We observed behaviors through closed-circuit television and recorded them for later analysis.

Behavioral and Statistical Analyses

We divided the home cage into 4-quadrants (I–IV) of $1 \times 1.3 \times 1$ m. Quadrant I corresponded to the lower rear section, quadrant II the lower front, quadrant III the upper rear, and quadrant IV the upper front. The stimulus was placed in front of section II. We analyzed locomotor activity (time spent in locomotion), use of space (time spent in each quadrant), and proximity to stimulus (time spent in contact with the home cage's front wire mesh—in quadrants II and IV) via the behavioral analysis software PROSTCOM 1.04 (Conde *et al*., 2000). We measured the following behaviors via focal-all occurrences sampling (Altmann, 1974): 1) alarm behavior: to sway—to move the whole body from side to side in a pendular-like fashion while


A
Vocalization
("tsik-tsik")
% of individuals
80
60
40
20
0
Pre-exposure
Expsoure
Post-exposure
*
**
*
*†
††
Hawk
Snake
Cat
Toy
B
Alarm Behavior
Relative mean frequency
4
3
2
1
0
Pre-exposure
Exposure
Post-exposure
**
**
*
*†
††
Hawk
Snake
Cat
Toy
C
'Observational' Behaviors
Relative mean frequency
5
4
3
2
1
0
Pre-exposure
Exposure
Post-exposure
**
**
**
**
Hawk
Snake
Cat
Toy

quadrupedal; 2) observational behaviors: head cock—to move the head from side to side—and leg stand- to raise the body into a bipedal position; 3) vocalizations: presence or absence of *tsik-tsik* calls. We measured alarm and observational behaviors in terms of frequency, while proximity to the stimulus, locomotion and use of space in terms of duration (in sec). We observed and scored each subject individually.

We compared all behavioral responses for variations between trial intervals and between the 4 stimuli. We analyzed data for vocalization via the Cochran test, and further compared them via the McNemar test. We analyzed the remaining behavioral categories via the Friedman test for repeated measures, followed by the Wilcoxon test for correlated samples, when appropriate. Differences are significant when $p \leq 0.05$. Analysis are based on two-tailed levels of significance, except for vocalization and alarm behavior. We expected the latter parameters to increase, as this profile has been extensively reported in different primate species when exposed to potential predatory threat (Caine and Marra, 1988; Vitale *et al*., 1991).

RESULTS

As there was no significant difference between males and females, we pooled each behavioral parameter into single groups. For each behavioral category, data are expressed as the mean frequency or duration observed for all subjects, per stimulus tested, relative to the duration of its respective interval. That is, one min for the exposure period, and 4 min for either pre- or postexposure intervals. We saw no incidence of direct defensive attack or freezing in response to any of the stimuli.

The three taxidermized animals induced a significant number of subjects to emit *tsik-tsik* vocalizations during the exposure intervals (hawk: $Q = 6.000$, $P < 0.05$; snake: $Q = 8.000$, $P < 0.05$—compared to the respective pre- and postexposure intervals; oncilla: $Q = 8.400$, $P < 0.05$—compared to preexposure interval; Fig. 1A). The toy stimulus induced no *tsik-tsik* call during the 3 trial intervals ($Q = 7.080$, $P < 0.05$; Fig. 1A). Furthermore,

Fig. 1. Percentage of individuals that emitted *tsik-tsik* vocalizations (A) during the 3 trial intervals for each stimuli tested. (Cochran test followed by the McNemar test; $n = 7$). Alarm (B) and observational behaviors (C), expressed as the mean (+SEM) frequency observed for all subjects, per each stimulus, relative to the duration of its respective interval (one min for exposure interval and 4 min for either pre- or postexposures). (Friedman test followed by the Wilcoxon test; $n = 7$). [$^{*}P < 0.05$ exposure vs. pre-exposure; $^{**}P < 0.05$ exposure vs. pre- and postexposure; $^{\dagger}P < 0.05$ oncilla vs. snake and toy (vocalization) or oncilla vs. hawk, snake and toy (alarm behavior); $^{\dagger\dagger}P < 0.05$ toy vs. hawk, snake and oncilla].

the oncilla elicited the call in a significant number of subjects after the exposure interval ($Q = 8.400$, $P < 0.05$—compared to pre-exposure interval; Fig. 1A), contrarily to the remaining stimuli ($Q = 9.429$, $P < 0.05$).

The taxidermized animals also elicited an alarm behavior: swaying when the stimuli were present (hawk: $X^2 = 12.000$, $P < 0.05$; snake: $X^2 = 12.000$, $P < 0.05$—compared to the respective pre- and postexposure intervals; oncilla: $X^2 = 8.696$, $P < 0.05$—compared only to the pre-exposure interval; Fig. 1B). The oncilla was the only stimulus that induced an alarm behavior in the postexposure period ($X^2 = 8.696$, $P < 0.05$—relative to the pre-exposure interval; $X^2 = 12.000$, $P < 0.05$—compared to the other objects; Fig. 1B). Alarm behavior was not apparent before, during or after exposure to the toy.

Observational behaviors (head cock and/or leg stand) occurred with all stimuli (Fig. 1C). However, they were only significant during stimuli exposure intervals (hawk: $X^2 = 12.000$, $P < 0.05$; snake: $X^2 = 12.000$, $P < 0.05$; wild cat: $X^2 = 8.957$, $P < 0.05$; toy: $X^2 = 12.000$, $P < 0.05$—relative to the respective pre- and postexposure interval); there is no significant difference among the objects ($X^2 = 0.826$, $P = 0.843$).

A significant increase in locomotor activity during exposures to the hawk and snake occurred (hawk: $X^2 = 7.714$, $P < 0.05$; snake: $X^2 = 8.857$, $P < 0.05$—compared to the respective pre- and postexposure intervals; Fig. 2A). Comparisons of the two stimuli with the oncilla and toy during the exposure intervals demonstrated a significant increase in the time spent in locomotion (hawk: $X^2 = 13.261$, $P < 0.05$; snake: $X^2 = 12.092$; $P < 0.05$; Fig. 2A). Conversely, the locomotor activity observed across the 3 trial intervals for the oncilla and toy did not alter significantly (cat: $X^2 = 0.583$, $P = 0.768$; toy: $X^2 = 1.462$, $P = 0.486$; Fig. 2A).

Comparisons between the four stimuli indicated distinct patterns of spatial occupation of the home cage for each trial interval (Table I). Before exposures, time spent in each quadrant (I-IV) was similar (I: $X^2 = 4.130$, $P = 0.248$; II: $X^2 = 0.778$, $P = 0.855$; III: $X^2 = 1.609$, $P = 0.657$; IV: $X^2 = 0.771$, $P = 0.856$). However, during exposures, the hawk stimulus induced a significant decrease in the amount of time spent in the lower and upper rear quadrants (I and III respectively; I: $X^2 = 7.692$, $P < 0.05$ versus the pre-exposure interval; III: $X^2 = 7.143$, $P < 0.05$ versus the pre- and postexposure intervals), as well as a significant increase in the upper front quadrant (IV: $X^2 = 8.857$, $P < 0.01$, versus the pre- and postexposure intervals). Time spent in each quadrant during presentations to the hawk stimulus is lower for quadrant III and higher for quadrant IV, when compared to the remaining objects tested (III: $X^2 = 12.771$, $P < 0.01$; IV: $X^2 = 14.130$, $P < 0.01$). In addition, the time spent in the lower rear quadrant (I) after exposures to the hawk is also higher versus the snake and oncilla (I: $X^2 = 9.441$, $P < 0.05$). The amount of time spent in the upper quadrants of the home cage during

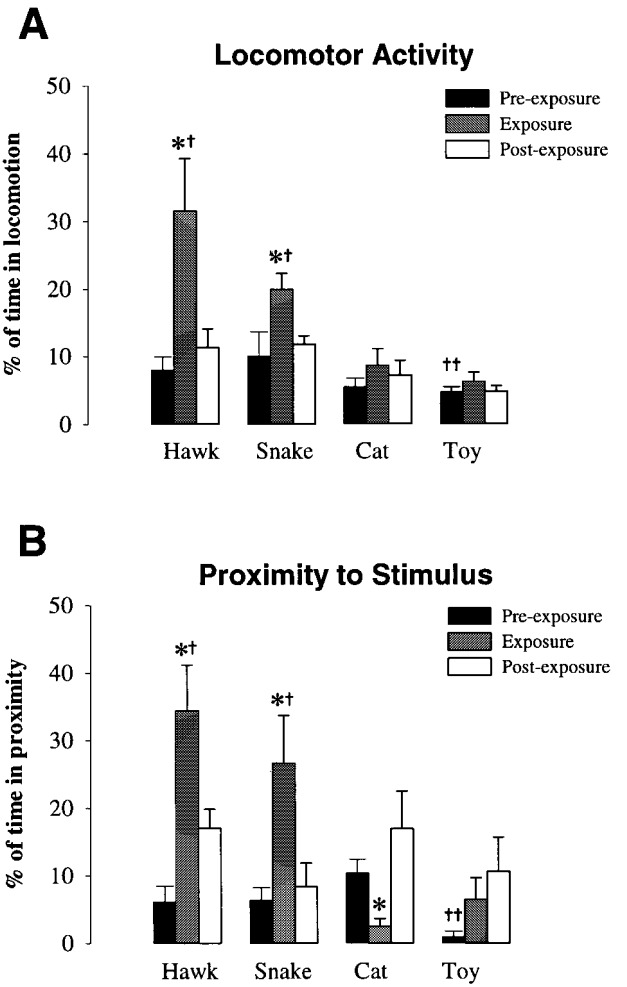


Fig. 2. Mean (+SEM) locomotor activity defined as the time (s) spent in locomotion within the home cage (A) and mean time (s) (+SEM) spent in contact with the wire mesh of the home cage (B) closest to the stimulus, for each stimulus during the 3-trial interval, relative to the total duration of its respective interval (one min for exposure interval and 4 min for either pre- or postexposures). (Friedman test followed by the Wilcoxon test; $n = 7$). [$^{*}P < 0.05$ exposure vs. preexposure;$^{\dagger}P < 0.05$ hawk or snake vs. oncilla and toy;$^{\dagger\dagger}P < 0.05$ toy vs. hawk and snake (locomotion) or toy vs. hawk, snake and oncilla (proximity)].

Table I. Percentage of time spent in each of the 4 quadrants of the home cage during the 3 trial intervals for each stimuli tested

Stimulus	Quadrant	Trial interval		
		Pre[a]	Exposure[a]	Post[a]
Hawk	I	41.0 ± 7.8	6.6 ± 4.3[b]	18.2 ± 3.2[f]
	II	2.5 ± 2.3	0.0 ± 0.0	4.8 ± 3.4
	III	30.7 ± 7.3	11.2 ± 5.2[c,e]	39.7 ± 6.3
	IV	25.8 ± 10.8	82.2 ± 7.4[d,e]	37.3 ± 5.3
Snake	I	16.5 ± 6.6	1.2 ± 1.2	7.7 ± 3.7
	II	14.3 ± 9.3	9.6 ± 9.5	1.1 ± 0.7
	III	28.4 ± 7.8	47.0 ± 15.1	61.2 ± 7.4
	IV	40.8 ± 11.3	42.2 ± 13.2	30.0 ± 5.6
Cat	I	19.4 ± 8.6	9.9 ± 7.4	4.0 ± 2.2
	II	5.2 ± 5.1	0.0 ± 0.0	3.3 ± 2.2
	III	33.9 ± 12.4	45.3 ± 11.7	59.6 ± 14.0
	IV	41.5 ± 15.0	44.8 ± 15.1	33.1 ± 12.7
Toy	I	9.2 ± 4.7	11.0 ± 7.5	13.9 ± 5.9
	II	3.4 ± 3.4	10.9 ± 10.8	0.2 ± 0.3
	III	49.5 ± 13.8	48.0 ± 9.8	47.8 ± 9.9
	IV	37.9 ± 16.1	30.1 ± 11.0	38.1 ± 12.0

[a] Data are expressed as the mean (±SEM) percentage of time (in sec) spent in the 4 quadrants of the home cage (I-IV) for each stimuli tested, relative to the total duration of its respective trial interval (one min for exposure interval and 4 min for either pre- or postexposures). Quadrant I: lower rear section, II: lower front section, III: upper rear section, IV: upper front section. Pre = preexposure; Post = postexposure intervals. Friedman test followed by the Wilcoxon test; $n = 7$.
[b] $P < 0.05$ exposure vs. preexposure.
[c] $P < 0.05$ exposure vs. postexposure.
[d] $P < 0.01$ exposure vs. pre- and postexposure.
[e] $P < 0.01$ hawk vs. snake, oncilla and toy.
[f] $P < 0.05$ hawk vs. snake and oncilla.

and after exposures to the snake and oncilla tended to increase, albeit not significantly when compared to the pre-exposure intervals (snake: I: $X^2 = 5.846$, $P = 0.085$; II: $X^2 = 0.154$, $P = 0.964$; III: $X^2 = 4.963$, $P = 0.085$; IV: $X^2 = 1.143$, $P = 0.620$; wild cat: I: $X^2 = 1.130$, $P = 0.620$; II: $X^2 = 2.000$, $P = 0.768$; III: $X^2 = 3.429$, $P = 0.237$; IV: $X^2 = 0.519$, $P = 0.768$). The toy did not induce significant changes in the home cage occupational pattern during or after exposures (I: $X^2 = 0.080$, $P = 0.964$; II: $X^2 = 0.286$, $P = 0.964$; III: $X^2 = 0.222$, $P = 0.964$; IV: $X^2 = 1.000$, $P = 0.768$ relative to the pre-exposure interval).

Analysis of the time spent in contact with the front wire mesh of the home cage (proximity to the stimuli; Fig. 2B) during exposure indicated a decrease when the oncilla was present ($X^2 = 10.571$, $P < 0.05$), while an increase occurred when the hawk and snake were present (hawk: $X^2 = 8.857$, $P < 0.05$; snake: $X^2 = 8.074$; $P < 0.05$, versus the respective pre- and

postexposure intervals). In addition, subjects spent more time in proximity to the hawk and snake versus the oncilla and toy ($X^2 = 12.739$, $P < 0.05$). The toy failed to alter this behavioral parameter significantly ($X^2 = 4.353$, $P = 0.237$).

DISCUSSION

The marmosets' brief exposure to the 3 potential taxidermized predators significantly elicited *tsik-tsik* vocalization and alarm behavior, responses commonly observed during dangerous or alarming encounters (Stevenson and Rylands, 1988). However, only presentations of the oncilla induced the behaviors in the postexposure interval, indicating its ablity to elicit strong emotional reactions in *Callithrix penicillata*. Conversely, the stuffed toy failed to elicit *tsik-tsik* calls and swaying, during or after exposures, which is consistent with the assumption that callitrichids are able to distinguish potentially dangerous stimuli from harmless objects (Buchanan-Smith *et al*., 1993; Caine and Weldon, 1989).

Conversely, observational behaviors—head cock and/or leg stand—occurred significantly, with all stimuli during the presentation interval. They often evidence an interest of the subject towards a specific and novel stimulus (Stevenson and Rylands, 1988) and may aid to visualize objects (Hampton *et al*., 1966). Hence, it is possible that our subjects may have a natural tendency to observe new and unfamiliar objects in their environment, regardless of its nature or potential threat, as reported for other callitrichid species (Caine, 1984; Cameron and Rodgers, 1999; Forster, 1995).

Increased locomotor activity during the hawk and snake presentations was concentrated in the two upper quadrants of the cage (quadrants III and IV). Increased use of quadrants III and IV may be associated with the presence of a nest box in quadrant IV, a possible concealing behavior common to the antipredator repertoire in marmosets (Ferrari and Lopes-Ferrari, 1990). Alternatively, the increased time in the two quadrants may also be related to the area occupied by the subjects when an alarm-inducing stimulus is encountered. Similar occupational patterns characterize vigilance behavior in tamarins (Caine, 1984). The toy failed to alter the locomotor activity or use of space, further demonstrating its ineffectiveness to alter the behavioral repertoire of the subjects.

Together, the increased locomotor activity and the enhanced use of both upper sections of the home cage, suggest a flee-approach pattern during exposure to the hawk and snake. Our results are in accordance with other reports of snake and cat mobbing in feral (Bartecki and Heymann, 1987; Passamani, 1995) and captive callitrichids (e.g. Buchanan-Smith *et al*., 1993;

Epple, 1968). Mobbing is assumed to result from a conflicting tendency both to approach and to flee/avoid contact with a specific stimulus (Epple, 1968). At the same time, it may act as a learning opportunity for young monkeys to readily identify potential predators in the environment (Bartecki and Heymann, 1987; Passamani, 1995). However, during the exposure intervals it decreased in the postexposure period. Marmosets may reduce the costs of antipredator behaviors by quickly resuming previous activities once the degree of threat has been accurately evaluated (Caine, 1998). The short-lived emotional reactions to the hawk and snake accord with this assumption.

The oncilla, a presumed predator of marmosets (Passamani, 1995), induced strong and persistent emotional reactions in our subjects, which agrees with previous laboratory investigations showing that cats induce strong emotional reactions in marmosets, tamarins and squirrel monkeys, (Caine, 1984; Caine and Marra, 1988; Epple, 1968). Furthermore, the time spent in the vicinity of the stimulus during exposures decreased when the oncilla was present, which resembles a classical fear and anxiety response. We reported a similar behavioral pattern toward the taxidermized oncilla employing a novel predator confrontation model of fear and anxiety in *Callithrix penicillata*. It was reversed when we treated the subjects with different anxiolytic drugs (Barros *et al*., 2000; Barros *et al*., 2001). However, any precise correlation between the type of predator—felids, hawks or snakes—and the behavioral changes observed are still tentative. Long-term field observations and additional laboratory studies, employing distinct potential predators, can elucidate the proximate and ultimate effects of predation on the behavioral ecology of callitrichids.

Cook and Mineka (1990) suggested that primates learn the threatening stimuli in their environment mainly from other members of the group. However, prior contact with a specific threatening object or situation is not always necessary to elicit significant emotional responses. For instance, naive crab-eating macaques and tufted capuchins readily respond to a snake model (Vitale *et al*., 1991), while various marmoset species easily distinguish predatory fecal scents (Caine and Weldon, 1989), and react to models of predators (Caine, 1998; Epple, 1968).

The oncilla elicited a fear-induced avoidance behavior, suggesting that in our naive captive-born subjects such reactions could be related to phylogentic encoding, as has been extensively demonstrated in laboratory-raised rats (Blanchard *et al*., 1998). The persistence of antipredatory responses in naive captive-born marmosets may indicate the importance of feline predation as a selective pressure shaping the behavior of marmosets. Lack of prior experience and consistency in which the oncilla influenced the marmosets' behavior, indicate the potential use of this stimulus to further investigate the impact of predation on callitrichid behavior. In addition, taxidermized felids

may be employed as a quasi-naturalistic aversive stimulus in ethologically-based models of fear/anxiety.

We are first to report the behavioral repertoire of *Callithrix penicillata* when exposed to potential taxidermized predators. We conclude that the oncilla is a powerful threatening stimulus.

ACKNOWLEDGMENTS

Our research was funded by CAPES/DAAD/PROBAL (058/98 to Tomaz). Barros and Boere received doctoral fellowships from CAPES. We thank Dr. R. de Oliveira and W. Vargas for excellent animal care and maintenance.

REFERENCES

Altmann, J. (1974). Observational study of behavior: Sampling methods. *Behaviour* 49: 227–267.

Andrade-Greco, M. V., and Andrade, M. A. (1999). *Callithrix penicillata* na dieta de *Spizaetus ornatus* (Aves: Accipitridae) em área de cerrado no estado de Minas Gerais. Abstract. Annals of the IX Brazilian Congress of Primatology, Brazilian Primatological Society, p. 65.

Barros, M., Boere, V., Huston, J. P., and Tomaz, C. (2000). Measuring fear and anxiety in the marmoset (*Callithrix penicillata*) with a novel predator confrontation model: Effects of diazepam. *Behav. Brain Res.* 108: 205–211.

Barros, M., Mello Jr., E. L., Huston, J. P., and Tomaz, C. (2001). Behavioral effects of buspirone in the marmoset employing a predator confrontation test of fear and anxiety. *Pharmacol. Biochem. Behav*. 68: 255–262.

Bartecki, U., and Heymann, E. W. (1987). Field observation of snake-mobbing in a group of saddle-back tamarins, *Saguinus fuscicollis nigrifrons. Folia Primatol*. 48: 199–202.

Blanchard, D. C., Griebel, G., Rodgers, R. J., and Blanchard, R. J. (1998). Benzodiazepine and serotonergic modulation of antipredator and conspecific defense. *Neurosci. Biobehav. Rev.* 22: 597–612.

Buchanan-Smith, H. M., Anderson, D. A., and Ryan, C. W. (1993). Responses of cotton-top tamarins (*Saguinus oedipus*) to fecal scents of predators and non-predators. *Anim. Welfare* 2: 17–32.

Caine, N. G. (1998). Cutting costs in response to predatory threat by Geoffroy's marmoset (*Callithrix geoffroyi*). *Am. J. Primatol.* 46: 187–196.

Caine, N. G. (1993). Flexibility and co-operation as unifying themes in *Saguinus* social organization and behaviour: Role of predation pressures. In Rylands, A. B. (ed.), *Marmosets and Tamarins: Systematics, Behaviour, and Ecology*, Oxford University Press, New York, pp. 200–219.

Caine, N. G. (1984). Visual scanning by tamarins: A description of the behavior and tests of two derived hypothesis. *Folia Primatol*. 43: 59–67.

Caine, N. G., and Weldon, P. J. (1989). Responses by red-bellied tamarins (*Saguinus labiatus*) to fecal scents of predatory and non-predatory neotropical mammals. *Biotropica* 21: 186–189.

Caine, N. G., and Marra, S. L. (1988). Vigilance and social organization in two species of primates. *Anim. Behav.* 36: 897–904.

Cameron, R., and Rodgers, L. J. (1999). Hand preference of the common marmoset (*Callithrix jacchus*): Problem solving and responses in a novel setting. *J. Comp. Psychology* 113: 149–157.

Cheney, D. L., and Wrangham, R. W. (1987). Predation. In Smuts, B. B., Cheney, D. L., Seyfarth, R. M., Wrangham, R. W., and Struhsaker, T. T. (eds.), *Primate Societies*, University of Chicago Press, Chicago, pp. 227–239.

Conde, C. A., Costa, V., and Tomaz, C. (2000). PROSTCOM, Un conjunto de programas para registro y procesamiento de datos comportamentales en investigaciones de fisiologia y farmacologia. *Biotemas* 13: 145–159.

Cook, M., and Mineka, S. (1990). Selective associations in the observational conditioning of fear in rhesus monkeys. *J. Exp. Psychol. Anim. Behav. Process* 16: 372–389.

Corrêa, H. K. M., and Coutinho, P. E. G. (1997). Fatal attack of a pit viper, *Bothrops jararaca*, on an infant buffy-tufted ear marmoset. *Primates* 38: 215–217.

Emmons, L. H. (1987). Comparative feeding ecology of felids in a neotropical rainforest. *Behav. Ecol. Sociobiol.* 20: 271–283.

Epple, G. (1968). Comparative studies on vocalization in marmoset monkeys (Hapalidae). *Folia Primatol.* 8: 1–40.

Faria, D. S. (1984). Aspectos gerais do comportamento de *Callithrix jacchus penicillata* em mata ciliar do Cerrado. In Mello, M. T. de (ed.), *A Primatologia no Brasil*, Vol. 1, Brazilian Primatological Society, Belo Horizonte, Brazil, pp. 55–65.

Ferrari, S. F., and Lopes-Ferrari, M. A. (1990). Predator avoidance behavior in the buffy-headed marmoset, *Callithrix flaviceps. Primates* 31: 323–338.

Forster, F. C. (1995). Exploratory behavior and learning in laboratory marmosets (*Callithrix jacchus jacchus*): Comparison between experimental-cage and home-cage activity. *Primates* 36: 501–514.

Hampton Jr., J. K., Hampton, S. H., and Landwehr, B. T. (1966). Observations on a successful breeding colony of the marmoset *Oedipomidas oedipus. Folia Primatol*. 4: 265–287.

Heymann, E. W. (1987). A field observation of predation on a moustached tamarin (*Saguinus mystax*) by an anaconda. *Int. J. Primatol.* 8: 193–195.

Heymann, E. W. (1990). Reactions of wild tamarins, *Saguinus mystax* and *Saguinus fuscicollis* to avian predators. *Int. J. Primatol.* 11: 327–337.

Miranda, G. H. B. (1997). Aspectos da ecologia e comportamento do mico-estrela (*Callithrix penicillata*) no cerradão e cerrado denso da Área de Proteção Ambiental (APA) do Gama e Cabeça-de-Veado/DF. Unpubl. Masters thesis, University of Brasília, Brasília, Brazil.

Passamani, M. (1995). Field observation of a group of Geoffroy's marmosets mobbing a margay cat. *Folia Primatol.* 64: 163–166.

Peres, C. A. (1993). Anti-predation benefits in a mixed-species group of Amazonian tamarins. *Folia Primatol*. 61: 61–76.

Rylands, A. B. (1981). Preliminary field observation on the marmoset, *Callithrix humeralifer intermedius* (Hershkovitz, 1977) at Dardanelos, Rio Aripuanã, Mato Grosso. *Primates* 22: 46–59.

Stafford, B. J., and Ferreira, F. M. (1995). Predation attempts on callitrichids in the Atlantic Coastal Rain Forest of Brazil. *Folia Primatol*. 65: 229–233.

Stevenson, M. F., and Rylands, A. B. (1988). The marmosets, genus *Callithrix*. In Mittermeier, R. A., Rylands, A. B., Coimbra-Filho, A. F., and da Fonseca, G. A. B. (eds.), *Ecology and Behavior of Neotropical Primates*, Vol. 2. World Wildlife Fund, Washington DC, pp. 131–222.

Sussman, R. W., and Kinzey, W. G. (1984). The ecological role of the Callitrichidae: A review. *Amer. J. Phys. Anthropol.* 64: 419–449.

van Schaik, C. P. (1983). Why are diurnal primates living in groups? *Behaviour* 87: 120–144.

Vitale, A. F., Visalberghi, E., and de Lillo, C. (1991). Responses to a snake model in captive crab-eating macaques (*Macaca fascicularis*) and captive tufted capuchins (*Cebus apella*). *Int. J. Primatol*. 12: 277–286.

Efeitos mnetrópicos duradouros do fragmento N-terminal da substância P (SPN) sobre a aprendizagem de esquiva em primatas não-humanos (*Callithrix penicillata*)

Long-lasting mnemotropic effects of substance P N-terminal fragment (SPN) on avoidance learning in non-human primates (Callithrix penicillata)

Carlos Tomaz[a,1]
Rafael S. Maior[a,2]
Eldon Mello Jr.[a,3]
Marilia Barros[a,b,4]

Summary

*The effects of substance P N-terminal fragment (SPN) on memory and anxiety were investigated in the marmoset predator confrontation test. The animals were tested in a figure-eight maze with a taxidermized wild cat placed outside facing one of its corners. The study was divided in two parts: [1] each marmoset (*Callithrix penicillata*) was submitted to seven 30 min maze habituation trials (HTs), 48 h apart and in the absence of the "predador", and then to six pseudo-randomly assigned 30 min treatment trials (TTs), in the presence of the "predador", namely four doses of SPN (5, 50, 250 and 500 µg/kg; ip), saline and a* sham *injection, with a 72 h interval between trials; [2] three months later the animals were re-exposed to the maze for a 30 min trial, in the absence of the "predador" stimulus. During the re-exposure, the time spent in the maze section closest to where the "predador" had previously been presented was significantly lower than that for HTs, although higher than saline injection. These results suggest a long-lasting SPN effect on avoidance learning of the taxidermized predator's position, indicating mnemotropic effects for this neuropeptide in marmosets.*

Uniterms: avoidance learning; anxiety; marmoset; predator confrontation test

Recebido em 10/10/2002
Aprovado em 20/10/2002

[a] Primate Center and Department of Physiological Sciences, Institute of Biology, University of Brasilia, CEP 70910-900, Brasilia, DF, Brazil
[b] Institute of Physiological Psychology I and Center for Biological and Medical Research, University of Düsseldorf, Universitätsstr. 1, 40225 Düsseldorf, Germany
[1] Professor Titular
[2] Aluno de iniciação científica
[3] Doutorando em Biologia Animal
[4] Pesquisadora Associada

Resumo

*Os efeitos do fragmento N-terminal da substância P (SPN) sobre memória e ansiedade foram investigados no teste experimental de confronto com predador. Os animais foram testados no labirinto em 8, com um gato selvagem taxidermizado posicionado de frente a um dos seus cantos. O estudo foi dividido em duas partes: [1] cada sagüi (*Callithrix penicillata*) foi submetido a sete sessões de habituação ao labirinto de 30 minutos (HTs), com intervalos de 48 horas e na ausência do "predador", e depois submetidos pseudo-aleatoriamente a seis sessões de tratamento de 30 minutos cada (TTs), na presença do "predador": quatro doses de SPN (5, 50, 250 e 500 µg/kg; ip), uma sessão salina e um controle de manipulação* sham, *em intervalos de 72 horas; [2] três meses depois cada sujeito foi reexposto ao labirinto por 30 minutos, na ausência do "predador". Durante a reexposição, o tempo gasto na seção do labirinto mais próxima de onde o "predador" havia sido exposto anteriormente foi significativamente menor do que nas HTs, apesar de maior que na sessão salina. Esses resultados sugerem um efeito duradouro do SPN na aprendizagem de esquiva à posição do "predador", indicando efeitos mnetrópicos para esse neuropeptídeo em calitriquídeos.*

Unitermos: aprendizagem de esquiva; ansiedade; sagüi; teste de confronto com predador

Introduction

Substance P (SP), a neurokinin widely distributed in the central and peripheral nervous system, plays a vital neuromodulatory role in various neural processes, such as reinforcement, learning and memory [8, 10, 16]. More specifically, systemic post-training administration of this neuropeptide in rats has consistently been shown to enhance memory in a dose- and time-dependent way[14, 17]. These effects were long-lasting[21], observed across tasks with different response requirements[20], and in the absence of explicit punishment[7]. In addition, the observed mnemotropic effects have been shown to be encoded by the SP N-terminal fragment (SPN; 9, 19).

Interest in SP-mediated processes has recently increased, however, due to their possible modulating role in depression and anxiety[4, 15, 18]. SP effects on anxiety seem dependent on the terminal fragment, as well as on the brain site injected and the amount administered. Accordingly, intracerebral injection of SP C-terminal fragment (SPC) into the periaqueductal gray matter, but not SPN, yielded anxiogenic effects in the rat elevated plus-maze test[5]. In the ventral pallidal area, on the other hand, both SPC and SPN treatments induced an anxiolytic-like profile[13]. Following peripheral SP administrations in rats an anxiolytic action has been observed at lower doses, while an anxiogenic one was reported for higher concentrations (for review see 10). Additionally, when systemically administered in rats, the classic benzodiazepine diazepam induced not only strong anxiolytic effects, but anterograde amnesia as well when animals were tested three days later[7]. SP treatment, however, induced an anxiolytic-like profile without negatively influencing retention of learned fear-avoidance, hence indicating a dissociation between anxiolytic and memory-disrupting effects[7].

The behavioral effects of SPN in non-human primates were recently investigated using the marmoset predator confrontation test of fear/anxiety (MPCT; 3). This experimental approach attempted to bridge the gap between rodent and human research on fear/anxiety, given that monkeys and humans exhibit similar physiological and behavioral responses to anxiety-inducing situations (e.g. 12). Systemic administrations of SPN in the marmosets induced anxiolytic-like effects on behavioral indicators of fear/anxiety. Going one step further, the present study aimed to evaluated the long-term mnemonic effects of SPN on fear/anxiety parameters previously observed in the MPCT[1].

Methods

The study was divided in two parts, of which data regarding the first part has been published elsewhere[3]. In the first part, five experimentally naïve marmosets (*Callithrix penicillata; 2 males and 3 females*) were initially submitted to seven 30 min habituation trials (HTs) in the figure-eight continuous maze, with a 48 h interval between trials and in the absence of the "predador" stimulus. This apparatus has been described in detail elsewhere[1]. Briefly, it consists of a rectangular field (125 x 103 cm) elevated to a height of 1 m, divided by two holes into five interconnecting arms. Following the HTs, each subject was submitted to six pseudo-randomly assigned 30 min treatments trials (TTs) in the maze, namely: a drug administration session for each dose of SPN tested (5, 50, 250 and 500 g/kg), a saline control, and a sham manipulation control. Trials were held 5 min following treatment administration, 72 h apart, and in the presence of the "predador" stimulus. The latter consisted of a taxidermized oncilla cat (*Felis tigrina*) placed outside the maze facing one of its corners. Position of the "predador" varied (left or right side) among subjects, yet remained unaltered for each subject during the six TTs. In the second part of the study, subjects were re-exposed (RE) three months later to the maze for a 30 min trial, in the absence of the "predador" stimulus, as previously described for the HTs. Video cameras were used for online monitoring and all trials recorded for later analysis.

For behavioral analyses, the figure-eight maze was divided into 13 imaginary sections. The following parameters were assessed for each 30 min trial via a semi-automated behavior analysis program (Chromotrack 4.02, San Diego Instruments)

by an experienced observer blind to the experimental treatment: [1] *Proximity to "predador"*, the time spent in the maze section closest to the stimulus; and [2] *Locomotor activity*, the number of imaginary maze sections crossed with both forelimbs. Data were analyzed by means of the Friedman's test (non-parametric analysis of variance for repeated measures), followed by post hoc pairwise comparisons using SNK's test whenever significant values were obtained. Significance level was set at $p < 0.05$.

Results

As the data from the first part of the study have been published elsewhere[3], only its most significant aspects will be presented again; namely: the SPN dose of 50 µg/kg (induced the most significant anxiolytic effects), the seven HTs pooled together for proximity to "predador" (no significant difference), and the locomotor activity observed during HT1 and HT7 (significant habituation effect observed).

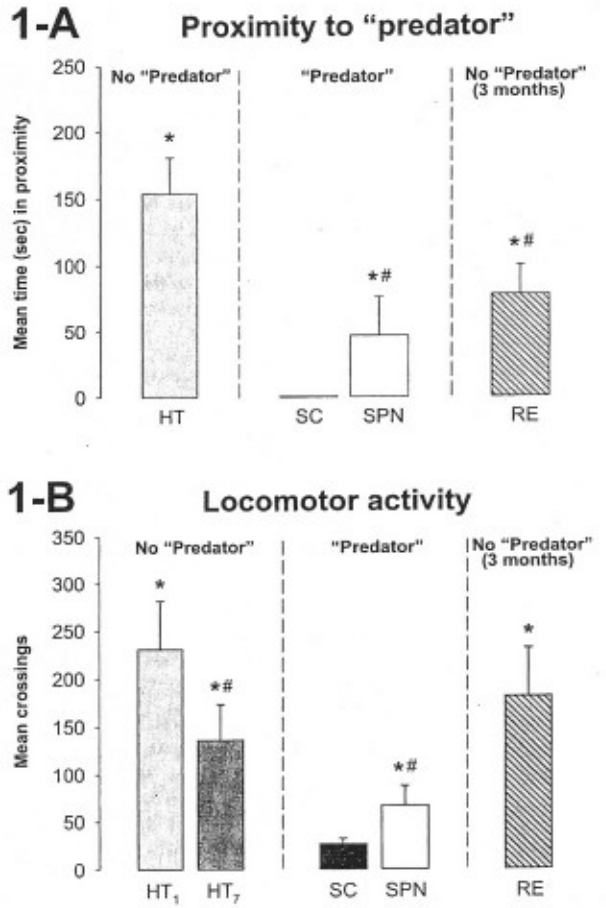


Figure 1 – Mean (+SEM) seconds spent in the maze section closest to the "predador" stimulus location [A] and mean number of maze sections crossed [B] during each 30 min trial. HT = habituation trials 1-7; HT_1 and HT_7 = first and seventh habituation trial, respectively; SC = saline control; SPN = substance P N-terminal fragment (50 µg/kg); and RE = re-exposure trial held 3 months after initial "predador" confrontation (#$p < 0.05$ vs. HT or HT_1; *$p < 0.05$ vs. SC).

The proximity to "predador" differed significantly among trials ($X_2 = 11.125$; $df = 3$; $p = 0.011$; Fig 1A). Post hoc analysis revealed that the presence of the 'predator' induced a significant decrease ($p < 0.05$) in the time spent in the maze section closest to the stimulus (saline control vs. HT). This fear-induced avoidance of the stimulus was significantly reversed by SPN treatment and during the re-exposure trial (RE), compared to saline control ($p < 0.05$). However, the time spent in proximity during the RE was significantly lower than that for the HTs ($p < 0.05$), higher than saline control ($p < 0.05$), and it failed to differ significantly from the SPN treatment ($p > 0.05$).

In addition, analysis of the marmosets' locomotor activity also revealed that this behavior differed significantly among trials ($X_2 = 16.480$; $df = 4$; $p = 0.002$; Fig. 1B). Post hoc comparisons indicated that locomotion decreased significantly ($p < 0.05$) during confrontation with the "predador" (saline control), compared to HT_1 and HT_7. However, SPN treatment significantly increased marmoset locomotor activity ($p < 0.05$), compared to saline control, although remaining significantly lower than HT1 ($p < 0.05$). Re-exposure to the maze significantly increased locomotion only in relation to saline control ($p < 0.05$), not differing significantly from the remaining trials (i.e. HT_1, HT_7 and SPN; $p > 0.05$).

Discussion

The present results confirm in marmosets, a previous report in rodents which suggested that the administration of substance P N-terminal fragment (SPN) induces long-term mnemotropic effects on avoidance learning[21]. The presence of the "predador" elicited in the marmosets a fear-induced avoidance of the maze section closest to where this stimulus was presented (i.e. saline control). Systemically administered SPN significantly reversed this avoidance behavior, indicating an anxiolytic-like effect for this compound. This parameter has been previously used in the MPCT as a behavioral indicator of the anxiolytic actions of acute injections of drugs such as diazepam[1] and buspirone[2]. Interestingly, during re-exposure of the marmosets to the maze, three months following initial "predador" confrontations and in the absence of the stimulus, similar values were not obtained for the initial time spent in the maze section closest to where the "predador" would be presented (HTs); i.e. re-exposure failed to restore the initial levels of "proximity" observed during the HTs. This result suggests that the marmosets may have acquired an avoidance learning towards the location of the "predador" stimulus. Thus, the present SPN profile in the MPCT is similar to that reported in rats for SP[7] and BRL 46470A, a 5-HT_3 antagonist[6], where an anxiolytic-like action was observed without the well known anterograde amnesic effect of diazepam. Importantly, the present results were not influenced by changes in the pattern of locomotor activity.

The present study demonstrated that peripheral administrations of the neurokinin substance P N-terminal fragment induce an anxiolytic-like effect, as well as a fear-avoidance learning lasting at least 3 months following the initial training. Such a long-term effect, as originally proposed by McGaugh[11], indicates that memory processes *per se* are affected by the treatment.

Furthermore, the long-lasting SP fear-avoidance learning may be viewed as an advantage over benzodiazepines' amnestic effects, increasing the likelihood that in the future the former may be employed in the treatment of human anxiety disorders. Taken together, these studies corroborate the notion that substance P alters the physiological state of neuronal systems involved in learning, memory and anxiety. However, no functional role for endogenous SP in such processes has yet been reported and the precise mechanisms by which SP acts are presently unknown. Pharmacological and neurochemical experiments using high-affinity receptor agonists and antagonists in non-human primates could provide important insights.

Acknowledgements

This work was supported by CAPES/DAAD/PROBAL (137/02) and FINATEC (to CT). MB was a recipient of a post-doctoral fellowship from CAPES and CT a recipient of a CNPq research fellowship (no. 300364/1986-5). We are thankful to Dr. R. de Oliveira for animal care.

References

1. Barros M, Boere V, Huston JP, Tomaz C. Measuring fear and anxiety in the marmoset (Callithrix penicillata) with a novel predator confrontation model: effects of diazepam. Behav Brain Res 2000; 108: 205-11.
2. Barros M, Mello EL, Huston JP, Tomaz C. Behavioral effects of buspirone in the marmoset employing a predator confrontation test of fear and anxiety. Pharmacol Biochem Behav 2001; 68: 255-62.
3. Barros M, De Souza Silva MA, Huston JP, Tomaz C. Anxiolytic-like effects of substance P fragment (SP1-7) in non-humans primates (Callithrix penicillata). Peptides 2002; 23: 967-73.
4. Brodin E, Rosén A, Schött E, Brodin K. Effects of sequential removal of rats from a group cage, and of individual housing of rats, on substance P, cholecystokinin and somatostatin levels in the periaqueductal grey and limbic regions. Neuropeptides 1994; 26: 253-60.
5. De Araújo JE, Silva RCB, Huston JP, Brandão ML. Anxiogenic effects of substance P and its 7-11 terminal, but not the 1-7 N-terminal, injected into the dorsal periaqueductal gray. Peptides 1999; 20: 1437-43.
6. De Souza Silva MA, Guimarães FS, Graeff FG, Tomaz C. Absence of amnestic effect of an anxiolytic 5-HT3 antagonist (BRL 46470A) injected into the basolateral amygdala, as opposed to diazepam. Behav Brain Res 1993; 59: 141-45.
7. Echeverry MB, Hasenöhrl RU, Huston JP, Tomaz C. Comparisons of neurokinin SP with diazepam in effects on memory and fear parameters in the elevated T-maze free exploration paradigm. Peptides 2001; 22: 1031-36.
8. Hasenöhrl RU, De Souza-Silva MA, Nikolaus S, Tomaz C, Brandão ML, Schwarting RKW, Huston JP. Substance P and its role in neural mechanisms governing learning, anxiety and functional recovery. Neuropeptides 2000; 34: 272-80.
9. Huston JP, Hasenöhrl RU, Boix F, Gerhardt P, Schwarting RKW. Sequence-specific effects of neurokinin substance P on memory, reinforcement, and brain dopamine activity. Psychopharmacology 1993; 112: 147-62.
10. Huston JP, Hasenöhrl RU. The role of neuropeptides in learning: focus on the neurokinin substance P. Behav Brain Res 1995; 66: 117-27.
11. McGaugh JL. Drug facilitation of learning and memory. Annu Rev Pharmacol 1973; 13: 229-41.
12. Newman JD, Farley MJ. An ethologically based, stimulus and gender-sensitive non-human primate model of anxiety. Prog Neuropsychopharmacol Biol Psychiatr 1995; 19: 677-85.
13. Nikolaus S, Huston JP, Hasenöhrl RU. Anxiolytic-like effects in rats produced by ventral pallidal injection of both N- and C-terminal fragments of substance P. Neurosci Lett 2000; 283: 37-40.
14. Nogueira PJC, Tomaz C. Substance P and naloxone enhancement of avoidance conditioning in rats. Braz J Med Biol Res 1990; 23: 163-67.
15. Rupniak NMJ, Kramer MS. Discovery of the anti-depressant and anti-emetic efficacy of substance P receptor (NK1) antagonists. Trends Pharmacol Sci 1999; 20: 485-90.
16. Saria A. The Tachykinin NK1 receptor in the brain: pharmacology and putative functions. Eur J Pharmacol 1999; 375: 51-60.
17. Schlesinger K, Pelleymounter MA, Van de Kamp J, Bader DL, Stewart JM, Chase TN. Substance P facilitation of memory: Effects in appetitive motivated learning task. Behav Neural Biol 1986; 45: 230-9.
18. Shirayama Y, Mitsushio H, Takashima M, Ichiwaka H, Takahashi K. Reduction of substane P after chronic anti-depressant treatments in the striatum, substantia nigra and amygdala of the rat. Brain Res 1996; 739: 70-8.
19. Stewart JM, Hall ME, Harkins J, Friederickson RCA, Terenius L, Hokfelt T, Krivoy WA. A fragment of substance P with specific central activity: SP (1-7). Peptides 1982; 3: 851-3.
20. Tomaz C, Aguiar MS, Nogueira PJC. Facilitation of memory by peripheral administration of substance P using avoidance by post-trial peripheral injection of substance P. Neurosci Biobehav Rev 1990; 14: 447-53.
21. Tomaz C, Silva ACF, Nogueira PJC. Long-lasting mnemotropic effect of substance P and its N-terminal fragment (SP1-7) on avoidance learning. Braz J Med Biol Res 1997; 30: 231-33.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 482 (2003) 197–203
www.elsevier.com/locate/ejphar

Anxiolytic-like effects of the selective 5-HT$_{1A}$ receptor antagonist WAY 100635 in non-human primates

Marilia Barros[a,b], Eldon L. Mello Jr.[a], Rafael S. Maior[a], Christian P. Müller[b], Maria A. de Souza Silva[b], Robert J. Carey[c], Joseph P. Huston[b,*], Carlos Tomaz[a]

[a] *Department of Physiological Sciences, Institute of Biology, University of Brasilia, CEP 70910-900 Brasilia, DF, Brazil*
[b] *Institute of Physiological Psychology I and Center for Biological and Medical Research, University of Düsseldorf, Universitätsstr. 1, D-40225 Düsseldorf, Germany*
[c] *Research and Development (151), VA Medical Center and SUNY Upstate Medical University, 800 Irving Avenue, Syracuse, NY 13210, USA*

Received 1 July 2003; received in revised form 8 September 2003; accepted 30 September 2003

Abstract

Non-human primates provide important insights into the potential use of 5-HT$_{1A}$ receptor antagonists in treating human anxiety disorders and as research tools, given the existent inconsistencies in rodent tests. This study investigated the effects of the selective silent 5-HT$_{1A}$ receptor antagonist *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexane-carboxamide trihydrochloride (WAY 100635), administered systemically, in an ethologically based fear/anxiety test in marmoset monkeys (*Callithrix penicillata*). Subjects were tested using a figure-eight maze and a taxidermized wild cat as 'predator' stimulus. After seven 30-min maze habituations in the absence of the 'predator', each animal was submitted to four pseudo-randomly assigned 30-min treatment trials in the presence of the 'predator': three WAY 100635 (0.2, 0.4 and 0.8 mg/kg, i.p.) sessions and a saline control trial. The 'predator' stimulus caused a significant fear-induced avoidance of the maze sections closest to where it was presented, indicating an anxiogenic effect. However, WAY 100635 treatment reversed, significantly and dose-dependently, this fear-induced avoidance behavior, while increasing maze exploration. Sedation was not observed. This is the first study to suggest an anxiolytic-like effect of the selective silent 5-HT$_{1A}$ receptor antagonist WAY 100635 in non-human primates, indicating its potential use as a therapeutic agent.

Keywords: WAY 100635; Marmoset, monkey; Figure-eight maze; Taxidermized predator

1. Introduction

The 5-HT$_{1A}$ receptor has been extensively investigated regarding its role in fear and anxiety (De Vry, 1995). In general, 5-HT ligands that stimulate postsynaptic 5-HT$_{1A}$ receptors in terminal areas of serotonergic projections have an anxiogenic profile (e.g., File et al., 1996). Compounds that stimulate inhibitory somatodendritic 5-HT$_{1A}$ autoreceptors in the raphe nuclei, on the other hand, decrease the firing frequency of 5-HT neurons and hence reduce 5-HT release, inducing anxiolytic effects (e.g., File et al., 1996).

Numerous investigations employing 5-HT$_{1A}$ receptor agonists (e.g., 8-hydroxy-2-(di-*N*-propylamino)tetralin(8-OH-DPAT), however, yielded highly variable results in different anxiety tests, particularly for systemically administered compounds (for a review, see Griebel, 1995). Controversial effects have also been reported for several 5-HT$_{1A}$ receptor antagonists in rodent tests of anxiety, as for instance 1-(2-methoxyphenyl)-4-(4-(2-phthalimido)butyl)-piperazine (NAN-190), 5-fluoro-8-hydroxy-2-(dipropylamino)tetralin ((*S*)-UH-301) and *N*-*tert*-butyl-3-(4-(2-methoxyphenyl)piperzin-1-yl)-2-phenylpropanamide (WAY 100135) (Moreau et al., 1992; Charrier et al., 1994; Rodgers and Cole, 1994; Griebel et al., 1999). Various 5-HT$_{1A}$ receptor antagonists were later shown to be non-selective and/or possess mixed agonist/antagonist activity (Arborelius et al., 1993; Assie and Koek, 1996; Routledge, 1996), which may partially account for some of these inconsistencies. Thus, the development of selective and silent 5-HT$_{1A}$ receptor antagonists, such as *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexane-carboxa-

* Corresponding author. Tel.: +49-211-81-14296; fax: +49-211-81-12024.
E-mail address: huston@uni-duesseldorf.de (J.P. Huston).

0014-2999/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2003.09.064

mide trihydrochloride (WAY 100635) (Forster et al., 1995; Fletcher et al., 1996), proved essential for more conclusive investigations. In fact, WAY 100635 has been found to decrease terminal 5-HT concentrations after systemic administrations in rats (Hjorth et al., 1997; Müller et al., 2002a), suggestive of an anxiolytic potential for this compound. Surprisingly, investigations on the anxioselective profile of systemically administered WAY 100635 in different rodent tests of anxiety ranged from anxiolysis (Fletcher et al., 1996; Cao and Rodgers, 1997, 1998; Griebel et al., 1999, 2000) and no effect (Stanhope and Dourish, 1996; Bell et al., 1999), to anxiogenesis (Groenink et al., 1995).

Given the controversial findings in rodent tests of anxiety, studies with non-human primates may provide important insights into the potential use of selective 5-HT_{1A} receptor antagonists as therapeutic agents for human affective disorders (King et al., 1988). To our knowledge, the only previous primate study investigating the effects of a 5-HT_{1A} receptor antagonist reported in squirrel monkeys an anxiolytic-like action for (*S*)-UH-301, a non-specific 5-HT_{1A} receptor antagonist (Moreau et al., 1992). The aim of the present study, therefore, was to investigate the effects of the selective silent 5-HT_{1A} receptor antagonist WAY 100635 in an ethologically based fear/anxiety test in non-human primates. Based on previous neurochemical findings showing a 5-HT decrease in terminal areas after WAY 100635 treatment (Hjorth et al., 1997; Müller et al., 2002a), and the overall results of behavioral studies in different rodent tests of anxiety (e.g., Cao and Rodgers, 1997; Griebel et al., 2000), an anxiolytic effect was expected for this drug in non-human primates.

2. Materials and methods

2.1. Subjects

Five experimentally naive adult marmosets (*Callithrix penicillata*, two males and three females) were used as subjects. Animals weighed 300–400 g at the beginning of experiments, and all were socially housed in three separate male/female groups in indoor/outdoor cages (2 × 1.3 × 2 m) of the same colony room. In one group, only the female was used in this study. Maintenance and testing of subjects were performed at the Primate Center, University of Brasilia. Except during the brief 30-min test periods, food and water were available ad libitum. All procedures were approved by the Animal Ethics Committee of the Institute of Biology, University of Brasilia, Brazil, and followed the 'Principles of Laboratory Animal Care' (NIH Publication No. 85-23, revised 1996).

2.2. Drugs

WAY 100635 (Sigma, USA) was dissolved in 0.9% physiological saline and injected i.p. in the doses of 0.2, 0.4 and 0.8 mg/kg. The injection volume for WAY 100635 and saline injections (vehicle control) was 1 ml/kg. All treatments were administered in the animals' home cages. Dose range was based on previous behavioral experiments investigating the effects of WAY 100635 in rodent tests of anxiety (Cao and Rodgers, 1997, 1998; Griebel et al., 1999, 2000).

2.3. Apparatus

Testing was conducted in a figure-eight continuous maze, recently validated as an ethologically based apparatus to measure fear/anxiety in marmosets (for a review, see Barros and Tomaz, 2002). The maze consisted of a rectangular field (125 × 103 × 35 cm) suspended 1 m from the floor and divided into five arms by two holes and barriers, forming a continuous figure-eight maze (Fig. 1). The apparatus, made of 4 mm transparent glass on a metal frame support, was divided into two segments (front and back chambers) by a concrete visual barrier (147 × 8 × 218 cm). The back chamber consisted of an arm (125 × 30 × 35 cm) with a central guillotine-type door and removable barriers. The latter formed the start compartment. The front chamber had three parallel arms (40 × 25 × 35 cm), 25 cm apart, ending in a common perpendicular arm (125 × 25 × 35 cm). Both chambers were interconnected through holes in the visual barrier at each of the three parallel arms. A taxidermized wild oncilla cat (*Felis tigrina*), which is a potential natural predator of marmosets, was placed outside the maze facing one corner of the parallel arms. The concrete barrier prevented subjects from viewing the taxidermized cat as they entered the maze, enabling a casual encounter via

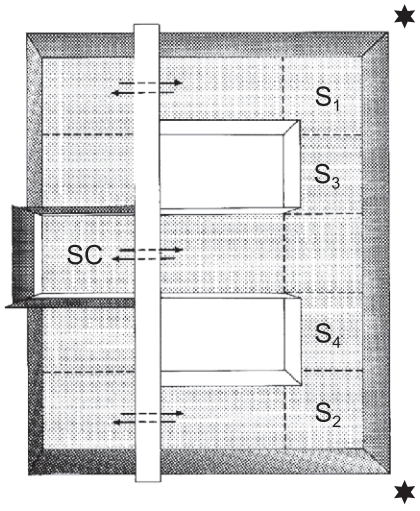


Fig. 1. Topview of the figure-eight maze used in the Marmoset Predator Confrontation Test of fear/anxiety. (SC) indicates the start compartment, the stars show the two locations where the taxidermized predator could be positioned, the dotted lines indicate the divisions of the maze into 13 sections, S_1 and S_2 correspond to the maze sections closest to the 'predator' location, and S_3 and S_4 are the maze sections immediately adjacent to the 'predator' position.

spontaneous exploration of the maze by the subject (for details, see Barros and Tomaz, 2002).

2.4. Procedure

2.4.1. Habituation trials

To avoid confounding effects of exposing the marmosets to a novel environment (i.e., maze) while measuring their response to a taxidermized predator, all subjects were first submitted to seven 30-min habituation trials, 48 h apart and in the absence of the 'predator'. These trials are essential to reliably measure the marmosets' fear/anxiety behavior in response to the 'predator' stimulus, as they predominantly display a highly erratic locomotor activity when first exposed to novel environments. This behavior declines to a baseline level prior to the seventh trial (Barros et al., 2000, 2001, 2002a). The procedure employed for the habituation trials consisted of the same protocol described below for subsequent trials, however, animals were not submitted to any treatment. Instead, subjects were only handled for 1 min and then placed in a transport cage (35 × 20 × 23 cm).

2.4.2. Treatment trials

Following the habituation trials, four pseudo-randomly assigned treatment trials were performed with each subject: three i.p. injections of WAY 100635 (0.2, 0.4 and 0.8 mg/kg) and a saline control. For each trial, the subject was quickly captured in its home cage, administered a treatment and placed thereafter into the transport cage. Following a 10-min interval, the marmoset was released into the maze's back chamber start compartment, thus commencing a 30-min trial. Barriers from this compartment were promptly removed upon the animal's exit, permitting free access to the whole apparatus. After the session, the subject was returned to its home environment in the transport cage. Overall, each marmoset received four i.p. injections (i.e., saline, 0.2, 0.4 and 0.8 mg/kg WAY 100635) spaced 72 h apart. During treatment trials, the 'predator' was present on either the left or right corner of the maze's back chamber (Fig. 1), and its position pseudo-randomly assigned to each subject, remaining constant throughout these trials. Treatments and order of subjects were pseudo-randomly assigned for each test day. Video cameras were used for online monitoring and all trials were recorded for later behavioral analysis. All test sessions were performed between 07:30 and 10:00 a.m.

2.5. Behavioral analysis

For behavioral analysis, the maze was divided into 13 sections (Fig. 1). The following behavioral parameters were scored for each 30-min trial by an experienced observer blind to the experimental treatment (intra-rater reliability ≥ 95%): (1) *exploratory activity*, the frequency of sniffing and/or licking any part of the apparatus, and/or leg stand (to raise the body into a bipedal position); (2) *proximity to 'predator'*, the frequency and time spent in the maze sections closest to (S_1 and S_2) and immediately adjacent to (S_3 and S_4) the 'predator' location (only the adjacent sections equal in size to S_1 and S_2 were analyzed; Fig. 1); and (3) *locomotor activity*, the number of maze sections crossed with both forelimbs. Locomotor activity and proximity to 'predator' were scored using a semi-automated behavior analysis program (Chromotrack 4.02, San Diego Instruments), whereas the frequency of exploratory activities was measured by focal-all occurrences samplings. Exploratory activity and proximity to 'predator' have been consistently shown as fear/anxiety measures in marmosets (e.g., Carey et al., 1992; Barros et al., 2002b), influenced by diazepam, buspirone and substance P in the same fear/anxiety test presently employed (Barros et al., 2000, 2001, 2002a). Locomotor activity was used as a measure of habituation to the maze, as well as to detect possible sedating or activating effects of WAY 100635.

2.6. Statistical analysis

Non-normally distributed data were log transformed. Exploratory and locomotor activity were analyzed by means of one-way analysis of variance (ANOVA) with repeated measures on the time (habituation trials) or treatment factor (treatment trials). Frequency and duration of proximity to

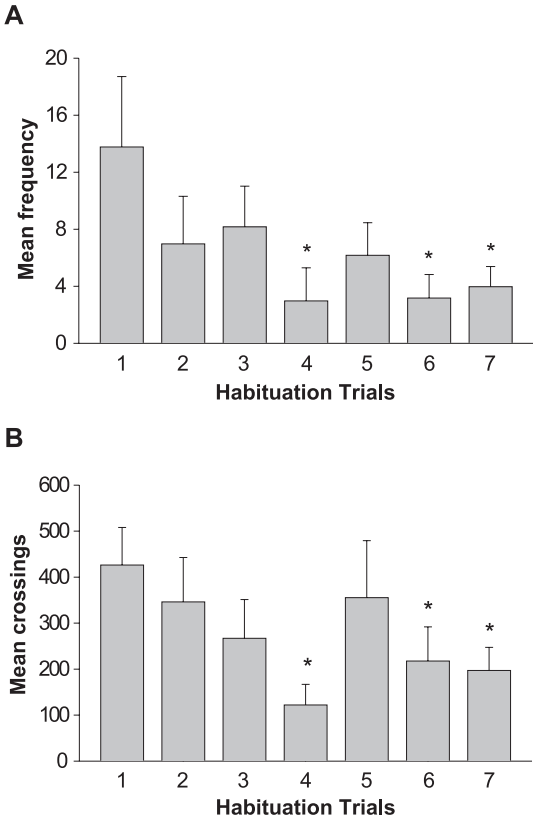


Fig. 2. Effect of each 30-min maze habituation trial, in the absence of the 'predator', on the mean (± S.E.M.) exploratory activity (A) and locomotor activity (B). (*$P < 0.05$ vs. trial 1.)

'predator' were analyzed with two-way ANOVAs for repeated measures (factors: maze section and treatment). Subsequent between- and within-groups analyses were performed using the appropriate error variance terms from the ANOVA summary tables with Duncan's test (habituation trials: trial 1 vs. remaining trials; treatment trials: saline vs. habituation trial 7 and each drug treatment trial). A P value of 0.05 was used for statistical significance.

3. Results

For each behavioral category, the analyzed data were pooled into one group, as no significant gender differences were observed. During the course of the seven maze habituation trials, in the absence of the 'predator', marmosets were found to habituate to the maze environment (Fig. 2). A significant decrease in exploratory [$F(6,28)=3.901$, $P<0.01$] and locomotor activity [$F(6,28)=7.401$, $P<0.001$] were observed during the consecutive seven habituation trials. Post hoc analyses revealed that exploratory and locomotor activity decreased significantly ($P<0.05$) during trials 4, 6 and 7, compared to trial 1. These results indicate that marmosets were fully habituated to the maze environment prior to subsequent trials.

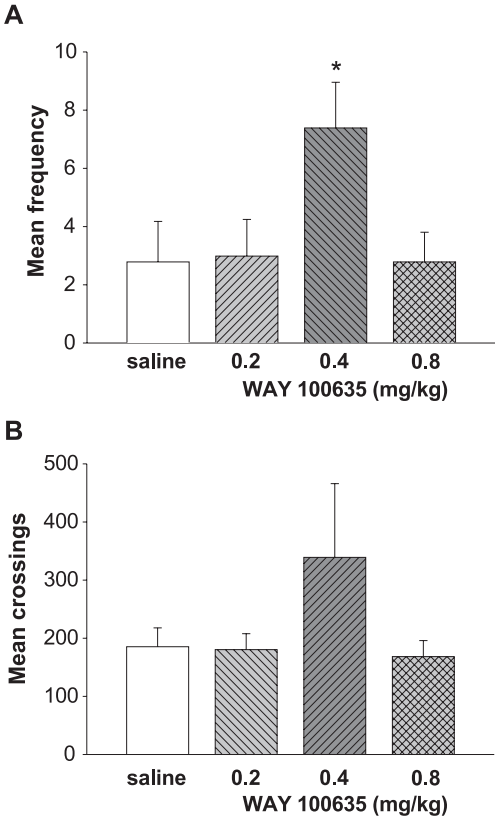


Fig. 3. Effects of WAY 100635 (i.p.) administrations on the mean (± S.E.M.) exploratory (A) and locomotor activity (B) during the 30 min trials in the presence of the 'predator'. (*$P<0.05$ vs. saline.)

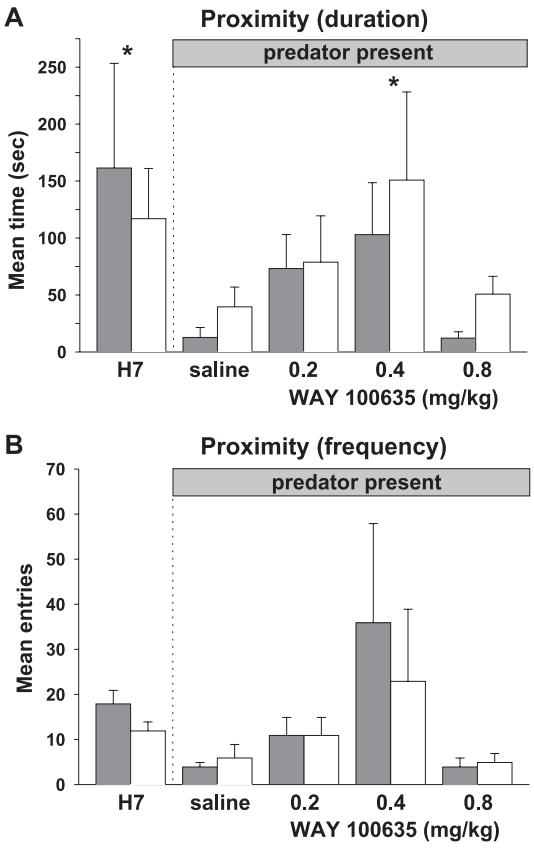


Fig. 4. Effects of WAY 100635 (i.p.) administrations on the mean (± S.E.M.) time spent (A) and frequency (B) in the maze sections closest to (gray bars) and immediately adjacent to (white bars) the 'predator' stimulus location during the 30 min trials. (H7 = habituation trial 7; *$P<0.05$ vs. saline.)

During the treatment trials, when the 'predator' was present, WAY 100635 administration was found to significantly alter exploratory activity [$F(6,28)=4.047$, $P<0.05$; Fig. 3A]. Further analysis indicated that only the dose of 0.4 mg/kg significantly increased this parameter, compared to saline control ($P<0.05$). Notably, analysis of the time spent in the maze sections closest to (S_1/S_2) and immediately adjacent to (S_3/S_4) the 'predator' location (i.e., proximity to 'predator'; Fig. 4A) revealed that the stimulus and treatments significantly influenced this parameter [treatment: $F(4,32)=2.800$, $P<0.05$]. The presence of the 'predator' induced a significant decrease in the duration of proximity (habituation trials 7 vs. saline; $P<0.05$), indicating a fear-induced avoidance of the stimulus. WAY 100635 treatment of 0.4 mg/kg, on the other hand, significantly reversed this fear-induced avoidance behavior ($P<0.05$), relative to saline control. Similarly, analyses of frequency (Fig. 4B) revealed a decrease in proximity to 'predator' (habituation trial 7 vs. saline), whereas 0.4 mg/kg WAY 100635 also reversed the marmosets' fear-induced avoidance, although not significantly [treatment: $F(4,32)=2.430$, $P=0.067$]. Furthermore, marmosets on average spent more time and went more frequently to the adjacent maze section (S_3/S_4)

when the predator was present, compared to the proximal one (S_1/S_2), however, this difference was not found to be significant [duration: $F(1,8)=0.170$, $P=0.680$; frequency: $F(1,8)=0.390$, $P=0.550$]. Maze section vs. treatment interactions were not statistically significant [duration: $F(4,32)=0.330$, $P=0.850$; frequency: $F(4,32)_{4,32}=0.210$, $P=0.920$], and sedation as manifested in decreased locomotion was not observed at any dose of WAY 100635 [$F(3,16)=1.435$, $P=0.281$; Fig. 3B].

4. Discussion

In the Marmoset Predator Confrontation Test–an ethologically based fear/anxiety test in non-human primates (Barros and Tomaz, 2002)–the selective silent 5-HT_{1A} receptor antagonist WAY 100635 altered the animals' behavioral repertoire suggestive of an anxiolytic profile. Consistent with previously findings in this test (Barros and Tomaz, 2002; Barros et al., 2002a), WAY 100635 treatment (0.4 mg/kg) was found to significantly reverse fear-induced avoidance of the maze sections closest to the 'predator', and increase the frequency of maze exploration. Importantly, these results were not influenced by motor impairment. At a similar dose range, WAY 100635 has also failed to modify locomotor activity in rodents (Cao and Rodgers, 1997, 1998; Griebel et al., 1999, 2000). An apparent bell-shaped dose–response curve for the anxiolytic effects was observed, similar to results and dose range found in the different rodent tests of anxiety (Cao and Rodgers, 1997, 1998; Griebel et al., 1999, 2000). Accordingly, WAY 100635 systemically administered at low doses (≤ 0.2 mg/kg) may not yet have attained anxiolytic properties in marmosets. On the other hand, at a higher dose (0.8 mg/kg), an antagonistic action of WAY 100635 and its metabolite, WAY 100634, at α_1-adrenoceptor sites could induce an opposing anxiogenic-like response, counteracting the WAY 100635 anxiolytic effects at postsynaptic 5-HT_{1A} receptors (Cao and Rodgers, 1997). In fact, WAY 100634 demonstrates a high affinity for α_1-adrenoceptors, particularly at high doses, while that of WAY 100635 has been shown to be only moderate to low (Fletcher et al., 1996; Pike et al., 1996). Such a relatively narrow dose–response curve of the observed anxiolytic-like effect is consonant with previous findings in the Marmoset Predator Confrontation Test (Barros et al., 2000, 2001, 2002a, 2002b), albeit not with other primate models (e.g., Kalin et al., 1987; Costall et al., 1992; Cilia and Piper, 1997). This disparity, as also observed among rodent tests of fear/anxiety, may be due to the nature of the response being investigated (i.e., conspecific confrontation vs. social isolation vs. predator stress; e.g., Blanchard et al., 1998). Predatory stress most likely involves different aspects of anxiety, as, for example, conspecific confrontation paradigms do. As such, it may provide a complementary way to assess anxiety-related behaviors, which, however, may have a narrower sensitivity to pharmacological manipulations. To our knowledge, this paradigm is the first attempt to investigate acute predatory stress and its involvement in fear/anxiety responses in primates.

Previous studies in the Marmoset Predator Confrontation Test with the 5-HT_{1A} receptor partial agonist buspirone (Barros et al., 2001) resulted in more significant effects on a wider range of behavioral indicators of anxiety than that with WAY 100635. Both contrary (e.g., Griebel et al., 2000) and similar findings (Cao and Rodgers, 1997; Bell et al., 1999) with rodents, however, indicate that factors aside from inter-species differences should be accountable for this discrepancy. In fact, significant differences between the nature of the response being induced and studied are known to exist, which in turn are thought to be mediated by distinct 5-HT_{1A} receptor mechanisms (Griebel et al., 2000). As a result, differences in the roles of pre- and postsynaptic 5-HT_{1A} receptors in anxiety may account for the WAY 100635 vs. buspirone profiles observed in this test. Accordingly, buspirone derives its anxiolytic properties from an agonistic action at inhibitory somatodendritic 5-HT_{1A} autoreceptors and an antagonistic one at postsynaptic 5-HT_{1A} sites (e.g., Dourish, 1987), of which either or both properties yield a decline in 5-HT neurotransmission. The 5-HT_{1A} receptor antagonist WAY 100635, on the other hand, has a potent and selective antagonistic action at both pre- and postsynaptic 5-HT_{1A} receptor sites (e.g., Fletcher et al., 1996). Therefore, although WAY 100635 can inhibit hippocampal cell firing (Fletcher et al., 1996) and decrease 5-HT concentrations in the hippocampus and nucleus accumbens (Hjorth et al., 1997; Müller et al., 2002a), it can also prevent 5-HT_{1A} receptor-mediated auto-inhibition of the firing frequency of 5-HT neurons in the raphe nuclei (Forster et al., 1995; Fletcher et al., 1996; Fornal et al., 1996; Mundey et al., 1996). These former effects may, in fact, be mediated by suppression of noradrenergic neuron activity in the locus coeruleus (Blier et al., 2001; Szabo and Blier, 2001).

In addition, as both serotonergic compounds studied to date (i.e., buspirone and WAY 100635) are metabolized into compounds known to act on α-adrenoceptors (Caccia et al., 1986; Fletcher et al., 1996; Pike et al., 1996), some caution should be taken when interpreting the involvement of 5-HT_{1A} receptors in the Marmoset Predator Confrontation Test. However, activation of this receptor is expected to induce an anxiogenic-like behavioral response, and the patterns observed with buspirone (Barros et al., 2001) and here, WAY 100635, indicate an anxiolytic-like effect. Accordingly, 5-HT_{1A} receptors are likely to be involved as well, mediating the anxiolytic action of buspirone and WAY 100635. Studies employing more selective 5-HT_{1A} receptor agonist could provide important insights in the involvement of 5-HT_{1A} receptors in this test, as well as the specific role of pre- vs. postsynaptic serotonergic mechanisms.

Nonetheless, direct behavioral testing of the intrinsic pharmacological potential of WAY 100635 in different rodent tests of anxiety (e.g., Cao and Rodgers, 1997;

Griebel et al., 1999, 2000), and now, in the present test with marmosets, has indicated an anxiolytic potential for this antagonist. To our knowledge, this is the first study to report anxiolytic-like effects of the selective 5-HT_{1A} receptor antagonist WAY 100635 in non-human primates. With respect to its potential use as a therapeutic agent for human anxiety disorder, it is interesting that WAY 100635 seems to lack addictive properties, given that it does not affect dopamine levels in the nucleus accumbens (Di Chiara and Imperato, 1988; Müller et al., 2002b).

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (HU 306/23-1 to JPH), by FINATEC (to CT), a NIDA grant R01 DA 05366-14 (to RJC) and by CAPES/DAAD/PROBRAL (137/02 to CT and JPH). MB was a recipient of a post-doctoral fellowship from CAPES, and CT a recipient of a CNPq researcher fellowship (no. 300364/1986-5).

References

Arborelius, L., Chergui, K., Murase, S., Nomikos, G.G., Hook, B.B., Chouvert, G., Hacksell, U., Svensson, T.H., 1993. The 5-HT_{1A} receptor selective ligands, (*R*)-8-OH-DPAT and (*S*)-UH-301, differentially affect the activity of midbrain dopamine neurons. Naunyn-Schmiedeberg's Arch. Pharmacol. 347, 353–362.

Assie, M.B., Koek, W., 1996. Effects of 5-HT_{1A} receptor antagonists on hippocampal 5-hydroxytryptamine levels: (*S*)-WAY 100135, but not WAY 100635, has partial agonist properties. Eur. J. Pharmacol. 304, 15–21.

Barros, M., Tomaz, C., 2002. Non-human primate models for investigating fear and anxiety. Neurosci. Biobehav. Rev. 26, 187–201.

Barros, M., Boere, V., Huston, J.P., Tomaz, C., 2000. Measuring fear and anxiety in the marmoset (*Callithrix penicillata*) with a novel predator confrontation model: effects of diazepam. Behav. Brain Res. 108, 205–211.

Barros, M., Mello Jr., E.L., Huston, J.P., Tomaz, C., 2001. Behavioral effects of buspirone in the marmoset employing a predator confrontation test of fear and anxiety. Pharmacol. Biochem. Behav. 68, 255–262.

Barros, M., De Souza Silva, M.A., Huston, J.P., Tomaz, C., 2002a. Anxiolytic-like effects of substance P fragment (SP_{1-7}) in non-human primates (*Callithrix penicillata*). Peptides 23, 967–973.

Barros, M., Boere, V., Mello Jr., E.L., Tomaz, C., 2002b. Reactions to potential predators in captive-born marmosets (*Callithrix penicillata*). Int. J. Primatol. 23, 443–454.

Bell, R., Lynch, K., Mitchell, P., 1999. Lack of effect of the 5-HT_{1A} receptor antagonist WAY-100635 on murine agonistic behaviour. Pharmacol. Biochem. Behav. 64, 549–554.

Blanchard, D.C., Griebel, G., Rodgers, R.J., Blanchard, R.J., 1998. Benzodiazepine and serotonergic modulation of antipredator and conspecific defense. Neurosci. Biobehav. Rev. 22, 597–612.

Blier, P., Lavoie, N., Haddjeri, N., 2001. Electrophysiological evidence for the tonic activation of the 5-HT_{1A} autoreceptor in vivo in the rat. Abstr.-Soc. Neurosci. 27, 124.9.

Caccia, S., Conti, I., Vigano, G., Garattini, S., 1986. 1-(2-Pyrimidinyl)-piperazine as active metabolite of buspirone in man and rat. Pharmacology 33, 46–51.

Cao, B.J., Rodgers, R.J., 1997. Influence of 5-HT_{1A} receptor antagonism on plus-maze behaviour in mice: II. WAY 100635, SDZ 216-525 and NAN-190. Pharmacol. Biochem. Behav. 58, 593–603.

Cao, B.J., Rodgers, R.J., 1998. Tolerance to acute anxiolysis but no withdrawl anxiogenesis in mice treated chronically with 5-HT_{1A} receptor antagonist, WAY 100635. Neurosci. Biobehav. Rev. 23, 247–257.

Carey, G.J., Costall, B., Domeney, A.M., Jones, D.N.C., Naylor, R.J., 1992. Behavioural effects of anxiogenic agents in the common marmoset. Pharmacol. Biochem. Behav. 42, 142–153.

Charrier, D., Dangoumau, L., Hamon, M., Puech, A.J., Thiebot, M.H., 1994. Effects of 5-HT_{1A} receptor ligands on a safety signal withdrawal procedure of conflict in the rat. Pharmacol. Biochem. Behav. 48, 281–289.

Cilia, J., Piper, D.C., 1997. Marmoset conspecific confrontation: an ethologically-based model of anxiety. Pharmacol. Biochem. Behav. 58, 85–91.

Costall, B., Domeney, A.M., Farre, A.J., Kelly, M.E., Martinez, L., Naylor, R.J., 1992. Profile of action of a novel 5-hydroxytryptamine1A receptor ligand E-4424 to inhibit aversive behavior in the mouse, rat and marmoset. J. Pharmacol. Exp. Ther. 262, 90–98.

De Vry, J., 1995. 5-HT_{1A} receptor agonists: recent developments and controversial issues. Psychopharmacology 121, 1–26.

Di Chiara, G., Imperato, A., 1988. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc. Natl. Acad. Sci. U. S. A. 85, 5274–5278.

Dourish, C.T., 1987. 5-HT_{1A} receptors and anxiety. In: Dourish, C.T., Ahlenius, S., Hutson, P.H. (Eds.), Brain 5-HT_{1A} Receptors. Ellis Horwood, Chichester, pp. 261–277.

File, S.E., Gonzalez, L.E., Andrews, N., 1996. Comparative study of pre- and post-synaptic 5-HT_{1A} receptor modulation of anxiety in two ethological animal tests. J. Neurosci. 16, 4810–4815.

Fletcher, A., Forster, E.A., Bill, D.J., Brown, G., Cliffe, I.A., Hartley, J.E., Jones, D.E., McLenachan, A., Stanhope, K.J., Critchley, D.J., Childs, K.J., Middlefell, V.C., Lanfumey, L., Corradetti, R., Laporte, A.M., Gozlan, H., Hamon, M., Dourish, C.T., 1996. Electrophysiological, biochemical, neurohormonal and behavioural studies with WAY-100635, a potent, selective and silent 5-HT_{1A} receptor antagonist. Behav. Brain Res. 73, 337–353.

Fornal, C.A., Metzler, C.W., Gallegos, R.A., Veasez, S.C., McCreary, A.C., Jacobs, B.L., 1996. WAY-100635, a potent and selective 5-hydroxytryptamine$_{1A}$ antagonist, increases serotonergic neuronal activity in behaving cats: comparison with (*S*)-WAY-100135. J. Pharmacol. Exp. Ther. 278, 752–762.

Forster, E.A., Cliffe, I.A., Bill, D.J., Dover, G.M., Jones, D., Reilly, Y., Fletcher, A., 1995. A pharmacological profile of the selective silent 5-HT_{1A} receptor antagonist, WAY 100635. Eur. J. Pharmacol. 281, 81–88.

Griebel, G., 1995. 5-Hydroxytryptamine-interacting drugs in animal models of anxiety disorders: more than 30 years of research. Pharmacol. Ther. 65, 319–395.

Griebel, G., Rodgers, R.J., Perrault, G., Sanger, D.J., 1999. Behavioural profiles in the mouse defence test battery suggest anxiolytic potential of 5-HT_{1A} receptor antagonists. Psychopharmacology 144, 121–130.

Griebel, G., Rodgers, R.J., Perrault, G., Sanger, D.J., 2000. The effects of compounds varying in selectivity as 5-HT(1A) receptor antagonists in three rat models of anxiety. Neuropharmacology 39, 1848–1857.

Groenink, L., Mos, J., van der Gugten, J., Schipper, J., Olivier, B., 1995. WAY 100635, a silent 5-HT_{1A} receptor antagonist, has anxiogenic effects in rats and does not block stress-induced rises in stress hormones. Abstr.-Soc. Neurosci. 21, 1366.

Hjorth, S., Westlin, D., Bengtsson, H.J., 1997. WAY100635-induced augmentation of the 5-HT-elevating action of citalopram: relative importance of the dose of the 5-HT_{1A} (auto)receptor blocker versus that of the 5-HT reuptake inhibitor. Neuropharmacology 36, 461–465.

Kalin, N.H., Shelton, S.E., Barksdale, C.M., 1987. Separation distress in infant rhesus monkeys: effects of diazepam and Ro 15-1788. Brain Res. 408, 192–198.

King, F.A., Yarbrough, C.J., Anderson, D.C., Gordon, T.P., Gould, K.G., 1988. Primates. Science 240, 75–82.

Moreau, J.L., Griebel, G., Jenck, F., Martin, J.R., Widmer, U., Haefely, W.E., 1992. Behavioral profile of the $5HT_{1A}$ receptor antagonist (*S*)-UH-301 in rodents and monkeys. Brain Res. Bull. 29, 901–904.

Müller, C.P., Carey, R.J., De Souza Silva, M.A., Jocham, G., Huston, J.P., 2002a. Cocaine increases serotonergic activity in the hippocampus and nucleus accumbens in vivo: $5\text{-}HT_{1A}$-receptor antagonism blocks behavioural but potentiates serotonergic activation. Synapse 45, 67–77.

Müller, C.P., De Souza Silva, M.A., De Palma, G., Tomaz, C., Carey, R.J., Huston, J.P., 2002b. The selective serotonin1A-receptor antagonist WAY 100635 blocks behavioural stimulating effects of cocaine but not ventral striatal dopamine release. Behav. Brain Res. 134, 337–346.

Mundey, M.K., Fletcher, A., Marsden, C.A., 1996. Effects of 8-OH-DPAT and $5\text{-}HT_{1A}$ antagonists WAY 100135 and WAY 100635, on guinea-pig behaviour and dorsal raphe 5-HT neuron firing. Br. J. Pharmacol. 117, 750–756.

Pike, V.M., McCarron, J.A., Lammertsma, A.A., Osman, S., Hume, S.P., Sargent, P.A., Bench, C.J., Cliffe, I.A., Fletcher, A., Grasby, P.M., 1996. Exquisite delineation of $5\text{-}HT_{1A}$ receptors in human brain with PET and [carbonyl-^{11}C]WAY-100635. Eur. J. Pharmacol. 301, R5–R7.

Rodgers, R.J., Cole, J.C., 1994. Anxiolytic-like effect of (*S*)-WAY 100135, a $5\text{-}HT_{1A}$ receptor antagonist in the murine elevated plus-maze test. Eur. J. Pharmacol. 261, 321–325.

Routledge, C., 1996. Development of $5\text{-}HT_{1A}$ receptor antagonists. Behav. Brain Res. 73, 153–156.

Stanhope, K.J., Dourish, C.T., 1996. Effects of $5\text{-}HT_{1A}$ receptor agonists, partial agonists and a silent antagonist on the performance of the conditioned emotional response test in the rat. Psychopharmacology 128, 293–303.

Szabo, S.T., Blier, P., 2001. Serotonin1A receptor ligands act on norepinephrine neuro firing through excitatory amino acid and $GABA_A$ receptors: a microiontophoretic study in the rat locus coeruleus. Synapse 42, 203–212.