Meinen Eltern gewidmet

Die Bedeutung des Neurokinin$_3$-Rezeptors für die akuten neurochemischen und Verhaltenseffekte von Kokain

Inauguraldissertation

zur

Erlangung des Doktorgrades der

Mathematisch-Naturwissenschaftlichen Fakultät

der Heinrich-Heine-Universität Düsseldorf

vorgelegt von

Gerhard Jocham

geboren in Wangen im Allgäu

September 2006

Aus dem Institut für Physiologische Psychologie I
der Heinrich-Heine-Universität Düsseldorf

Gedruckt mit Genehmigung der
Mathematisch-Naturwissenschaftlichen Fakultät der
Heinrich-Heine-Universität Düsseldorf

Referent: Prof. Dr. J.P. Huston
Koreferent: PD. Dr. T. Steckler

Tag der mündlichen Prüfung: 15.12.2006

Danksagung

An dieser Stelle möchte ich mich bei allen bedanken, die zum Zustandekommen dieser Arbeit beigetragen haben.

Mein besonderer Dank gilt Herrn Prof. Dr. J.P. Huston für die Möglichkeit, an seinem Institut zum Thema dieser Arbeit forschen zu dürfen, für die Freiheit, eigene Ideen einbringen und verwirklichen zu dürfen, für die Geduld während der Implementierung schwieriger analytischer Methoden und für die Betreuung und Unterstützung in jeder Phase des Projekts.

Dem Graduiertenkolleg 320 „Pathologische Prozesse des Nervensystems: Vom Gen zum Verhalten“ danke ich für die finanzielle und ideelle Unterstützung.

Insbesondere gilt mein Dank auch Frau Dr. Angelica de Souza Silva für die ausgezeichnete und unkomplizierte Betreuung meiner Arbeit sowie für die Freiräume und das unabhängige Arbeiten, die sie mir ermöglicht hat.

Bei Herrn PD. Dr. Thomas Steckler möchte ich mich für die Übernahme des Koreferats bedanken.

Besonders möchte ich mich auch bei unseren Kooperationspartnern Herrn Prof. Dr. C. Tomaz, Frau Prof. Dr. M. Barros und Dr. E.L. Mello Jr. vom Primatenzentrum der Universität Brasília für die hervorragende Zusammenarbeit und die unvergessliche Gastfreundschaft bedanken.

Weiterhin möchte ich mich auch bei Herrn Dr. Christian Müller, Frau Dipl. Psych. Emriye Teke und Frau Dr. Bianca Topic für die zahlreichen Diskussionen, die gute Arbeitsatmosphäre, die fachliche, administrative und moralische Unterstützung in allen Phasen des Projekts besonders bedanken.

Mein Dank gilt auch allen Kolleginnen und Kollegen des Instituts für Physiologische Psychologie I für die stets freundliche, kollegiale und hilfsbereite Arbeitsatmosphäre, sowie Herrn Günther Abel, der mir meine Arbeit in vielen Bereichen erleichterte.

Zusammenfassung

Die Forschung hinsichtlich der neuronalen Grundlagen der suchtinduzierenden Eigenschaften von Kokain fokussierte sich lange Zeit auf den kokaininduzierten Anstieg der extrazellulären Konzentration von Dopamin (DA) im Nucleus accumbens (Nac). Dieser DA-Anstieg ist jedoch lediglich als eine initiale Reaktion zu verstehen, die weitere Folgeerscheinungen nach sich zieht. In den letzten Jahren mehrt sich die Evidenz, dass Kokain mit Neuropeptiden aus der Familie der Tachykinine interagiert, und dass diese ihrerseits die dopaminerge Übertragung beeinflussen. Insbesondere der in diesem Zusammenhang noch völlig unerforschte Neurokinin$_3$- (NK$_3$-) Rezeptor erschien dabei von großem Interesse.

In der vorliegenden Arbeit wurde daher untersucht, wie sich die dopaminergen und Verhaltenseffekte von Kokain ändern, wenn NK$_3$-Rezeptoren zuvor pharmakologisch aktiviert beziehungsweise blockiert wurden.

Zur Untersuchung der belohnenden und hyperlokomotorischen Eigenschaften von Kokain wurde das Paradigma der konditionierten Platzpräferenz (CPP) an Ratten verwendet. Die in vivo Mikrodialyse an der frei beweglichen Ratte wurde genutzt, um die Konzentration von DA sowie seiner Metaboliten in den beiden Subregionen des Nac, „Core“ und „Shell“ zu bestimmen. Aufgrund von Speziesunterschieden wurden zusätzlich vergleichende Untersuchungen in nicht-humanen Primaten (Callithrix penicillata) durchgeführt.

Es zeigte sich, dass in Ratten weder Agonismus noch Antagonismus des NK$_3$-Rezeptors einen Einfluss auf die kokaininduzierte CPP hatte. Auch wenn sie allein verabreicht wurden, hatten weder der Agonist noch der Antagonist einen Einfluß auf die CPP. Die durch Kokain verursachte Hyperlokomotion wurde dagegen durch den Antagonisten abgeschwächt und durch den Agonisten potenziert. Zudem induzierte der Agonist alleine einen unmittelbaren Anstieg der Lokomotion, der von kurzer Dauer war und sich im Zeitraum vor der Kokainverabreichung abspielte.

Kokain führte zu einem Anstieg der DA-Konzentration sowohl in der Core als auch in der Shell des Nac. Dieser Effekt wurde durch den Agonisten in beiden Subregionen potenziert. Der Antagonist dagegen potenzierte den DA-Anstieg

subregionenselektiv in der Core des Nac. Weder der Antagonist noch der Agonist hatten einen Effekt, wenn sie alleine gegeben wurden.

Wie in Ratten konnte die akute Hyperlokomotion durch Kokain auch in Primaten durch Vorbehandlung mit einem NK_3-Rezeptorantagonisten verhindert werden. Die Effekte des Agonisten in Primaten waren dagegen komplexer. Anders als erwartet, wurde die kokaininduzierte Hyperlokomotion durch den Agonisten ebenfalls abgeschwächt.

Diese Arbeit konnte zeigen, dass NK_3-Rezeptoren sowohl an der Generierung kokaininduzierter Hyperaktivität als auch am Dopaminanstieg im Nac beteiligt sind. Im Falle des Antagonisten ist dieser Effekt auf die DA-Konzentration subregionenselektiv und auf die Core des Nac beschränkt. Eine Involvierung von NK_3-Rezeptoren in die belohnenden Eigenschaften von Kokain konnte dagegen in dieser Arbeit nicht nachgewiesen werden. Untersuchungen in Primaten stützten diese Befunde zum Teil, jedoch wiesen sie zumindest im Falle des Agonisten auch erneut auf die Existenz von Speziesunterschieden hin.

Inhaltsverzeichnis

1. Abkürzungsverzeichnis

CPP	- Konditionierte Platzpräferenz
DA	- Dopamin
DOPAC	- L-3,4-Dihydroxyphenylessigsäure
GABA	- γ-Aminobuttersäure
HVA	- Homovanillinsäure
i.c.v.	- intracerebroventricular
i.p.	- intraperitoneal
i.v.	- intravenös
kDa	- kiloDalton
mPFC	- medialer präfrontaler Cortex
Nac	- Nucleus accumbens
NK	- Neurokinin
NKA	- Neurokinin A
NKB	- Neurokinin B
NPK	- Neuropeptid K
NPγ	- Neuropeptid γ
NE	- Noradrenalin
5-HT	- Serotonin
s.c.	- subkutan
SP	- Substanz P
VP	- ventrales Pallidum
VTA	- Area tegmentalis ventralis

2. Einleitung

Es werden zunächst kurz die markantesten neurochemischen und Verhaltenseffekte von Kokain sowie deren Dependenz vom mesolimbischen Dopaminsystem beschrieben. Danach wird die funktionell-anatomische Diversität des Nac und ihre Bedeutung für die Wirkung von Kokain erläutert. Im Anschluss daran erfolgt die Einordnung des NK_3-Rezeptors in die Tachykininfamilie und es wird Evidenz beschrieben, die darauf hindeutet, dass dieser Rezeptor mit den neurochemischen und Verhaltenseffekten von Kokain interagieren könnte. Schließlich werden kritische Punkte bei der Erforschung des NK_3-Rezeptors beleuchtet.

2.1. Kokain

Kokain ist eine Droge aus der Klasse der Psychostimulantien mit hohem Missbrauchspotential. Es entfaltet seine zentralnervösen Effekte durch Wiederaufnahmehemmung freigesetzter Neurotransmitter der Monoaminklasse, d.h. Dopamin (DA), Serotonin (5-HT) und Noradrenalin (NE, Ritz et al., 1990). Dadurch kommt es im Bereich der Projektionsgebiete der jeweiligen Neurone, aber auch in deren Somataregion zu einer Erhöhung der extrazellulären Konzentration der jeweiligen Neurotransmitter (Reith et al., 1997). In hohen Konzentrationen blockiert Kokain zudem spannungsabhängige Na^+-Kanäle (Matthews und Collins, 1983), was seine lokalanästhetische Wirkung begründet. Subjektiv berichten Konsumenten vom Empfinden eines „Euphorie- oder Hochgefühls“. Manche Konsumenten berichten aber auch von Dysphorie oder Angst nach Einnahme der Droge (Resnick et al., 1977; Post et al., 1974). Auch tierexperimentell lässt sich ein potenter verstärkender Effekt der Droge nachweisen: Verhaltensweisen, die zum Erhalt von Kokain führen, werden mit erhöhter Wahrscheinlichkeit wieder gezeigt. So erlernen i.v. katheterisierte Ratten beispielsweise, einen Hebel zu drücken, um eine Kokaininfusion zu erhalten (Roberts et al., 1977). Zudem löst Kokain eine konditionierte Platzpräferenz (CPP) aus: Versuchstiere bevorzugen Orte, an denen

sie zuvor die Wirkung von Kokain erfahren hatten (Tzschentke, 1998). Neben der verstärkenden Wirkung ist die Induktion von Hyperaktivität der am besten beschriebene Effekt der Droge. Während Kokain in mittleren Dosen (bei der Ratte im Bereich von ca. 5-20 mg/kg i.p.) die lokomotorische Aktivität deutlich erhöht (Kelly und Iversen, 1976), wird diese durch höhere Dosen unterdrückt. Anstelle von Hyperlokomotion treten dann Verhaltensstereotypien auf (Fog, 1969).

Sowohl die verstärkenden Eigenschaften von Kokain als auch seine psychomotorisch stimulierende Wirkung hängen in erheblichem Maß von seiner Wirkung auf das mesolimbische DA-System ab. Dieses System hat seinen Ursprung in den dopaminergen Zellen der Area tegmentalis ventralis (VTA), die in zahlreiche Vorderhirngebiete projizieren, darunter der Nucleus accumbens (Nac), die Amygdala, das ventrale Pallidum (VP) und der mediale präfrontale Kortex (mPFC). Insbesondere die gesteigerte dopaminerge Übertragung im Nac scheint hierbei von großer Bedeutung (Koob, 1992; Di Chiara und Imperato, 1988). Sowohl die hyperlokomotorischen als auch die Verstärkereffekte von Kokain, wie sie nach systemischer Verabreichung erzielt werden, können durch eine direkte Injektion in den Nac erreicht werden (Delfs et al., 1990; Rodd-Henricks et al., 2002). Eine Unterbrechung der dopaminergen Übertragung im Nac durch Rezeptorblockade oder durch neurotoxische Läsion mit 6-Hydroxydopamin eliminiert dagegen sowohl die hyperlokomotorischen als auch die Verstärkereffekte systemisch applizierten Kokains (Baker et al., 1996; Baker et al., 1998; Pettit et al., 1984; Ettenberg et al., 1982).

2.2. Funktionelle Unterteilung im Nucleus accumbens

Der Nac ist jedoch keine homogene Struktur. In den letzten Jahren wurde seine Anatomie in beträchtlichem Maß neu konzeptualisiert. Gegenwärtig grenzt man bei Nagern mindestens zwei Subregionen, die „Core“ und die „Shell“ des Nac voneinander ab. Diese unterscheiden sich hinsichtlich immunhistochemischer Marker, ihrer Konnektivität, sowie auch ihrer funktionellen Bedeutung (Zahm und Brog, 1992). So reagieren Core und Shell z.B. unterschiedlich auf diverse

pharmakologische und Umweltreize (Di Chiara, 2002). Vereinfacht kann man sagen, dass die Core-Region in vielerlei Hinsicht eher dem dorsalen Striatum ähnelt, während die Shell als Teil eines „Extended Amygdala"-Systems gesehen wird (de Olmos und Heimer, 1999).

Auch für die Effekte von Kokain scheinen die beiden Subregionen eine unterschiedliche Rolle zu spielen. Lange Zeit erschien es rätselhaft, warum Kokain von Versuchstieren in Selbstverabreichungsstudien nicht in den Nac appliziert wurde (Goeders and Smith, 1983). Diese Studien trugen noch nicht der Core/Shell-Dichotomie Rechnung, und es stellt sich heraus, dass diese Studien für die Mikroinjektion Koordinaten wählten, die vornehmlich auf die Core des Nac abzielten. In neueren Untersuchungen konnte gezeigt werden, dass die Shell des Nac intrakranielle Kokainselbstverabreichung unterstützt, die Core dagegen nicht (Rodd-Henricks et al., 2002). Gleiches gilt für die Induktion von CPP, die sich ebenfalls nur durch Injektion in die Shell auslösen ließ (Liao et al., 2000). Zudem wurde gezeigt, dass Kokain in niedrigen Dosen selektiv die DA-Konzentration in der Shell, nicht aber in der Core erhöht. Bei höheren Dosen tritt dieser Effekt auch in der Core und im dorsalen Striatum auf, ist dort aber weniger ausgeprägt als in der Shell. (Pontieri et al., 1995; Jocham et al., 2006).

Aus diesen Befunden wird ersichtlich, wie wichtig es ist, dieser anatomischen Abgrenzung Rechnung zu tragen, wenn Substanzen in den Nac verabreicht werden oder wenn dort die DA-Konzentration gemessen werden soll. Aus diesem Grund wurden auch in den hier beschriebenen Mikrodialysestudien die DA-Konzentrationen getrennt in der Core und Shell des Nac untersucht.

2.3. Die Tachykinine und der NK_3-Rezeptor

Tachykinine sind Peptide, die durch die gemeinsame carboxyterminale Sequenz Phe-X-Gly-Leu-Met-NH_2 charakterisiert sind. Bei Nicht-Säugern sind zahlreiche Tachykinine bekannt, bei Säugern konnte man dagegen bisher nur fünf Vertreter identifizieren: Substanz P (SP), Neurokinin A (NKA), Neurokinin B (NKB),

Neuropeptid K (NPK) und Neuropeptid γ (NPγ). Die Tachykinine der Säuger werden auch als Neurokinine (NK) bezeichnet. Es wurden bisher drei Rezeptoren charakterisiert, die diese NK binden, die als NK_1-, NK_2- und NK_3-Rezeptoren bezeichnet werden und zur Superfamilie der G-Protein-gekoppelten Rezeptoren mit sieben Transmembrandomänen gehören. SP, NKA und NKB haben die jeweils höchste Affinität zu NK_1-, NK_2- und NK_3-Rezeptoren, jedoch binden alle Neurokinine an alle der drei NK-Rezeptoren (Maggi, 1995; Massi et al., 2000).

Verschiedene Evidenz weist darauf hin, dass NK_3-Rezeptoren sowohl an der Modulation der Aktivität des mesolimbischen DA Systems als auch an Verstärkungsprozessen und der Generierung lokomotorischer Aktivität beteiligt sind. NK_3-Rezeptoren werden in Arealen des Gehirns exprimiert, die in die belohnenden und psychomotorisch stimulierenden Effekte von Kokain involviert sind, wie z.B. das VP, der mPFC, die Amygdala und die Core des Nac (Shughrue et al., 1996). Insbesondere findet man eine hohe Dichte von NK_3-Rezeptoren in der VTA, wo sie auf dopaminergen Zellen lokalisiert sind (Chen et al., 1998). Lokale Verabreichung des NK_3-Rezeptoragonisten Senktide in die VTA führte zu einem Anstieg der Feuerfrequenz der DA-Neurone (Keegan et al., 1992; Overton et al., 1992) sowie zu einer erhöhten Freisetzung von DA in den Zielgebieten (Nac, mPFC; Marco et al., 1998) und zu vermehrter Lokomotion (Elliott et al., 1991; Stoessl et al., 1991). Zudem wirkt die i.c.v. Gabe eines NK_3-Rezeptoragonisten ebenfalls verstärkend (Ciccocioppo et al., 1998). Eine verstärkende Wirkung sowie ein modulatorischer Einfluss auf das mesolimbische DA-System konnte ebenfalls für das Neurokinin SP gezeigt werden. Diese beiden Effekte werden über die carboxyterminale Sequenz des Peptids mediiert, das, anders als SP selbst, eine höhere Affinität für den NK_3-Rezeptor als für die anderen NK-Rezeptoren aufweist (Huston et al., 1993; Regoli et al., 1994).

Aufgrund dieser Befunde wurde postuliert, dass die Verhaltenseffekte von Kokain und seine Wirkung auf das mesolimbische DA-System durch Vorbehandlung mit einem NK_3-Rezeptoragonisten bzw. -antagonisten potenziert respektive abgeschwächt werden sollten.

2.4. Kritische Punkte bei der Untersuchung von NK3-Rezeptoren

Ein Punkt, der bei der Untersuchung von NK-Rezeptoren im Allgemeinen, und auch beim NK3-Rezeptor nicht ausser Acht gelassen werden sollte, ist das Vorhandensein von Speziesunterschieden. Heterologien in der Aminosäuresequenz der NK3-Rezeptoren verschiedener Spezies finden sich vor allem im Bereich der putativen Transmembrandomänen. Während die Bindung von Agonisten vor allem durch die carboxyterminale Sequenz des Rezeptors gewährleistet wird (Zusammen mit weiteren Bindungsepitopen, die über das gesamte Rezeptorprotein verstreut liegen), binden Antagonisten an einer Tasche in den Transmembrandomänen. Infolgedessen ist die Affinität von Agonisten für die NK3-Rezeptoren verschiedener Spezies sehr ähnlich, für Antagonisten dagegen machen sich deutliche Unterschiede bemerkbar. Hinsichtlich der Pharmakologie von Antagonisten lassen sich zwei Speziesgruppen unterscheiden, einerseits Maus und Ratte, andererseits Meerschweinchen und Primaten, die sich jeweils in ihrer Gruppe sehr ähnlich sind, zwischen den Gruppen aber recht deutlich variieren (Maggi, 1995). Aufgrund dieser Gruppierung erschiene es sinnvoll, Meerschweinchen anstelle von Ratten als Versuchstiere zu verwenden, um von den Resultaten bessere Rückschlüsse auf die Situation beim Menschen ziehen zu können. Jedoch sind Meerschweinchen aufgrund ihres geringen Verhaltensrepertoires wenig geeignet für verhaltenspharmakologische Tests, zudem sind die meisten Verhaltensparadigmen für Ratten und Mäuse validiert worden. Da die vorhandenen NK3-Rezeptorantagonisten aber für den potentiellen therapeutischen Einsatz am Menschen konzipiert sind, weisen diese Verbindungen am NK3-Rezeptor von Ratte und Maus eine geringere Potenz und Selektivität auf (Emonds-Alt et al., 1995; Beaujouan et al., 1997; Sarau et al., 2000). Diese Problematik betrifft nicht die Experimente III und IV dieser Arbeit, in denen der Agonist Senktide verwendet wurde. Jedoch bestehen neben Unterschieden in der Rezeptorpharmakologie trotz eines sehr großen Überlappungsbereichs auch einige Unterschiede im zerebralen Expressionsmuster des NK3-Rezeptors zwischen verschiedenen Spezies (Langlois et al., 2001). Um diesem Punkt Rechnung zu tragen,

wurden in Experiment V und VI zusätzlich komparative Studien an nicht-humanen Primaten (Callithrix penicillata) durchgeführt.

3. Verwendete Methoden

In diesem Abschnitt werden die Methoden erläutert, die in den in dieser Arbeit enthaltenen Experimenten verwendet wurden.

3.1. In vivo Mikrodialyse

Die *in vivo* Mikrodialyse ist ein Verfahren zur Bestimmung der extrazellulären Konzentration niedermolekularer Substanzen. Zu diesem Zweck wird eine Sonde ins Gewebe eingeführt. Diese besteht aus einem äusseren und einem inneren Tubus (Abb. 1), wobei der äussere Tubus am unteren Ende aus einer semipermeablen Membran besteht. Durch den äusseren Tubus wird eine isotone Lösung (z.B. Ringerlösung) gepumpt. Diese erreicht dann die Membran und kehrt durch den inneren Tubus wieder nach oben zurück, wo sie in Eppendorfgefäßen gesammelt wird. Da das Perfusionsmedium isoton ist, entsteht kein netto Flüssigkeitsaustausch. Aufgrund der Isotonie des Perfusats existieren weder elektrische Potentialdifferenzen noch ein osmotischer Druck zwischen dem Kompartiment innerhalb und ausserhalb der Sonde. Daher beruht der Transport zwischen den beiden Kompartimenten alleine auf der Diffusion entlang eines Konzentrationsgradienten.

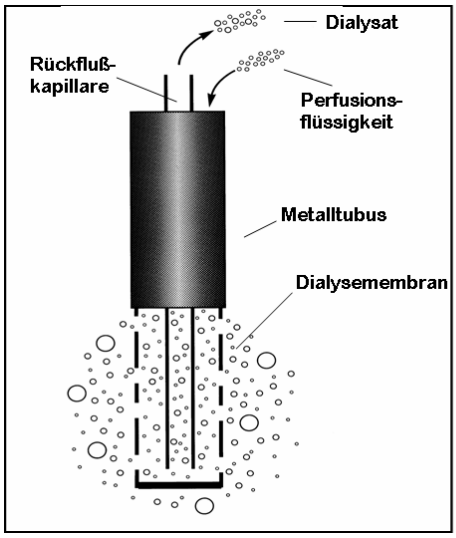

Abb.1: Schematische Darstellung von Aufbau und Funktion einer Mikrodialysesonde

In der aus organischen Polymeren bestehenden Membran befinden sich Poren, die die Passage von Molekülen erlauben, die ein definiertes Molekulargewicht nicht überschreiten. Diese Grenze wird als „Cut-off" bezeichnet, er lag in den hier vorgestellten Studien bei 6 kDa. Durch diesen Cut-off besteht eine mechanische Barriere, die dazu führt, dass degradierende Enzyme nicht in die Sonde eindringen können und die Analyten somit vor enzymatischem Abbau geschützt sind. Ein weiterer Vorteil dieser Barriere besteht darin, dass das Dialysat einen sehr hohen Reinheitsgrad aufweist. Es ist daher keine weitere Aufbereitung der Proben nötig, sie können unmittelbar nach der Gewinnung in ein chromatographisches Analysesystem injiziert werden.

Die in vivo Mikrodialyse kann am anästhesierten oder, wie in den hier vorgestellten Arbeiten, am wachen, frei beweglichen Tier durchgeführt werden (Abb. 2). Die Sonden werden über Schläuche mit einem Drehventil verbunden, das an einem gegenbalancierten Schwenkarm angebracht ist. Über das Drehventil wird mit Mikroinfusionspumpen das Perfusat in die Sonden appliziert, das zurückfließende Dialysat wird in Sammelgefäßen aufgefangen, die am Schwenkarm angebracht sind.

Es ist wichtig, zu beachten, dass die gemessene Transmitterkonzentration im Dialysat nicht der tatsächlichen Konzentration im Extrazellulärraum entspricht. Das Verhältnis von Konzentration des Analyten innerhalb der Sonde zur Konzentration ausserhalb wird als „Wiederfindungsrate" (engl.: recovery) bezeichnet. Die Wiederfindungsrate lässt sich zwar *in vitro* recht einfach bestimmen, indem die

Sonde in ein Bad mit einer Standardlösung (mit definierter Analytenkonzentration) getaucht wird und die Konzentration im Dialysat mit der Konzentration in der Standardlösung verglichen wird. Jedoch ist es nicht möglich, aus der Wiederfindungsrate *in vitro* auf die Wiederfindungsrate *in vivo* zu extrapolieren. Dies liegt zum einen daran, dass der Diffusionskoeffizient eines Analyten (der wiederum die Diffusion über die Dialysemembran beeinflußt) im wässrigen Medium höher ist als in der extrazellulären Matrix, zum Anderen wird *in vivo* die Diffusion in die Sonde durch den Tortuositätsfaktor λ vermindert. Unter Tortuosität versteht man, dass der Pfad des Analyten zur Sonde im Gewebe nicht einer Geraden entspricht, sondern die Moleküle müssen einen „Umweg" um die Zellen herum zurücklegen. Zwar existieren Methoden der quantitativen Mikrodialyse, die eine Bestimmung der tatsächlichen Extrazellulärkonzentration einer Substanz ermöglichen (Parsons und Justice, Jr., 1992), jedoch dauert diese Bestimmung mehrere Stunden und ist daher lediglich für statische Messwerte und nicht für die Abbildung dynamischer Prozesse geeignet. Aus diesen Gründen ist es zu empfehlen, Mikrodialysedaten als Prozentwerte einer Basalkonzentration anzugeben (Westerink, 1995). Durch dieses Vorgehen sind die Daten unabhängig von Schwankungen in der Wiederfindungsrate von Sonde zu Sonde. Dieses Vorgehen entspricht auch der wissenschaftlichen Praxis in der überwiegenden Zahl der Mikrodialysestudien.

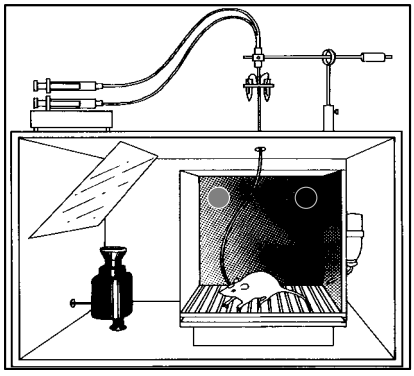

Abb. 2: In vivo Mikrodialyse am wachen, frei beweglichen Tier

Ein weiterer kritischer Punkt bei der in vivo Mikrodialyse ist die geringe zeitliche Auflösung. Da die extrazellulären Substanzkonzentrationen sehr gering sind, muss die Probennahme recht lange andauern, um genügend Dialysat für eine

Analyse zur Verfügung zu haben. Zugleich stellen diese niedrigen Stoffmengen höchste Anforderungen an das analytische System, das Mengen von wenigen fmol reliabel quantifizieren muß. Gängige Probennahmeintervalle liegen zur Zeit im Bereich von 10 bis 30 Minuten, je nach Neurotransmitter und Hirngebiet. Der Autor optimierte das analytische System so, dass die Quantifizierung von DA in Probenintervallen von 10 Minuten reliabel möglich war. Eine weitere Verkürzung der Probenintervalle ist in pharmakologischen Studien nur bedingt sinnvoll, da sich pharmakologisch induzierte Effekte meist nicht in solch kurzen Zeitfenstern abspielen.

Die in vivo Mikrodialyse stellt ein zeitaufwendiges Verfahren dar. Inklusive Vor- und Nachbereitung dauert die Messung eines einzigen Tieres fast einen gesamten Arbeitstag. Weiterer zeitlicher Aufwand ensteht u.a. für die stereotaktische Implantation der Kanülen, die histologische Aufarbeitung der Gehirne sowie die manuelle Konstruktion der Dialysesonden. Gleichzeitig muss gewährleistet sein, dass die chromatographische Analytik stets parallel zum Experiment unter nahezu optimalen Bedingungen operiert. Weiterer zeitlicher Aufwand entsteht durch die hohe Verlustrate, die insbesondere dadurch bedingt ist, dass die fragilen Sonden während des Experiments ihre Funktionalität einbüßen, im Regelfall durch Blockade oder durch das Auftreten von Lecks. Aus diesen Gründen sind die in Mikrodialysestudien verwendeten Tierzahlen eher gering, es werden für gewöhnlich zwischen 4 und 8 Tieren pro Gruppe verwendet.

3.2. Konditionierte Platzpräferenz

Zur Messung der konditionierten Platzpräferenz (CPP) wurde hier ein kreisrundes Offenfeld verwendet, d.h. eine von Wänden umgrenzte, kreisrunde Grundplatte. Diese läßt sich durch eine Plexiglaswand in zwei Hälften unterteilen. Da auf der einen Seite eine glatte, auf der anderen eine geriffelte Matte als Bodenbelag diente, waren die beiden Hälften für die Tiere diskriminierbar.

Abb. 3: Die Platzpräferenzapparatur

Bei der Durchfühung eines CPP-Experiments sind drei Phasen zu unterscheiden: Die Basalmessung, die Konditionierungsphase und die Testmessung. In der Basalmessung erhält das Tier unbeschränkten Zugang zum gesamten Offenfeld, und es wird gemessen, wie lange sich das Tier in den beiden Hälften aufhält. Da die Hälften vorab neutral für die Tiere sind, verbringen sie im Durchschnitt gleich viel Zeit auf beiden Seiten. In der Konditionierungsphase wird die Apparatur mittels der Plexiglaswand abgeteilt. Dem Tier wird die Testsubstanz verabreicht und es wird für eine bestimmte Zeit in eine der beiden Seiten gesetzt. Am nächsten Tag wird dieselbe Prozedur durchgeführt, nur dass dem Tier nun Kochsalzlösung verabreicht wird und es danach in die andere Hälfte der Apparatur gesetzt wird. Dies wird als Pseudokonditionierung bezeichnet. Durch dieses Vorgehen assoziiert das Tier die Wirkung der Droge mit der einen Seite, während es auf der anderen Seite lernt, dass hier kein besonderer Effekt auftritt. Dieses Vorgehen wird mehrfach wiederholt. Während der Testphase wird die Wand dann wieder entfernt, das Tier hat erneut freien Zugang zur gesamten Apparatur und es wird wiederum die Aufenthaltszeit in den beiden Hälften gemessen. Wenn die Droge einen für das Tier angenehmen Zustand herbeigeführt hat, so wird es nun während der Testphase mehr Zeit auf der drogenassoziierten Seite verbringen als während der Basalmessung. Das Gegenteil, d.h. eine verringerte Aufenthaltszeit tritt ein, wenn die Droge einen für das Tier aversiven Zustand induziert, z.B. Übelkeit, Schmerzen oder einen panikartigen Zustand. Im ersten Fall spricht man von einer Platzpräferenz, im zweiten Fall von einer Platzaversion.

Es existieren diverse Variationen des CPP-Paradigmas. Die Apparatur kann in Form, Größe und Gestaltung der Kompartimente variieren. Auch können zur Diskriminierung der Kompartimente Reize einer oder mehrerer Sinnesmodalitäten verwendet werden, z.B. visuelle, taktile oder olfaktorische Reize. Ein wichtiger grundlegender Unterschied besteht zwischen voreingenommenen und unvoreingenommenen Designs (engl.: biased bzw. unbiased design). Man spricht von einem voreingenommenen Design, wenn die Versuchstiere bereits vor der Konditionierung eine deutliche Präferenz für eines der beiden Kompartimente aufweisen. Das klassische Modell der CPP, das auch heute noch oft verwendet wird, ist die Schwarz-Weiss-Box, eine rechteckige Box, bei der eine Seite schwarz und gering ausgeleuchtet, die andere weiss und hell ausgeleuchtet ist. Dies macht die Kompartimente zwar leicht unterscheidbar, jedoch werden Nagetiere aufgrund ihrer natürlichen Verhaltenstendenz die helle Seite meiden. Je nach Helligkeitsbedingungen verbringen die Tiere daher in der Basalmessung nur ca. 30% der Gesamtzeit im hellen Kompartiment. Es besteht dann die Möglichkeit, die Tiere auf die nicht bevorzugte Seite zu konditionieren, was den Nachteil hat, dass man aufgrund des niedrigen Ausgangsniveaus leicht falsch positive Resultate erhält. Werden die Tiere dagegen auf die ohnehin schon hochgradig bevorzugte Seite konditioniert, gestaltet es sich enorm schwierig, überhaupt noch eine weitere Steigerung der Aufenthaltszeit zu erzielen, so dass dieses Vorgehen anfällig für falsch negative Resultate ist (Tzschentke, 1998). In den hier vorgestellten Studien wurde ein unvoreingenommenes Design verwendet. Die Tiere verbrachten auf beiden Seiten nahezu gleichviel Zeit, zudem wurden in jeder Versuchsgruppe jeweils die Hälfte der Tiere auf die glatte und auf die geriffelte Seite konditioniert. In Experiment I wurde initial eine Abwandlung der oben beschriebenen Apparatur verwendet, bei der keine taktilen, sondern visuelle Hinweisreize angebracht waren. Zudem wurde das kreisrunde Feld nicht in zwei Hälften, sondern in vier Quadranten eingeteilt, und es wurden keine Pseudokonditionierungsdurchgänge durchgeführt (modifiziert nach: Hasenöhrl et al., 1989). Da dieses Vorgehen nur eine schwache Präferenz hervorrief, entschieden wir uns, das Experiment mit der oben

beschriebenen Apparatur zu replizieren und diese auch für die folgende CPP-Studie zu verwenden.

3.3. Das Figure-Eight-maze

Für die ethologische Analyse der Effekte von Kokain in Primaten wurde ein „Figure-Eight-maze“ verwendet (Abb. 2). Dieses bestand aus einem rechteckigen Grundfeld, das durch zwei von Barrieren umgrenzte rechteckige Löcher in fünf Arme unterteilt wurde, so dass sich die Form einer kontinuierlich verlaufenden Acht ergab.

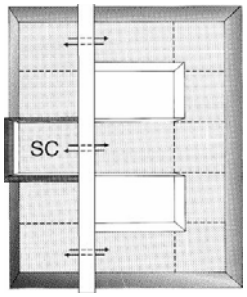

Abb. 2: Schematische Ansicht von oben auf das Figure-Eight-Maze. SC= Startkompartiment

Nicht-humane Primaten verfügen über ein komplexes Verhaltensrepertoire (Stevenson und Poole, 1976). Daher stellen sie eine wichtiges Zwischenstufe für die Übertragbarkeit der Daten von Nagern auf Menschen dar. Da in Primatenstudien aus ethischen und ökonomischen Gründen nur begrenzte Tierzahlen zur Verfügung stehen, ist es meist nicht möglich, mit unabhängigen Gruppen zu arbeiten. Stattdessen werden Experimente an nicht-humanen Primaten oft mittels eines Messwiederholungsdesigns durchgeführt. Ein Vorteil dieser Prozedur besteht darin, dass die Tiere hinsichtlich ihrer Reaktion auf Kokain miteinander verglichen werden können. In früheren Studien mit dem niedrigpotenten Psychostimulans Diethylpropion zeigte sich, dass sich die Tiere aufgrund der lokomotorischen Reaktion auf die Droge in zwei Populationen von „Respondern“ und „Non-Respondern“ einteilen ließ, die sich in ihrem gesamten Reaktionsprofil erheblich unterschieden (Mello, Jr. et al., 2005). Aus diesem Grund wurde auch in den hier vorgestellten Studien der Vergleich zwischen den Behandlungsgruppen nicht nur

über die Gesamtpopulation, sondern auch nach einer Unterteilung in „Responder“ und „Non-Responder“ vorgenommen.

4. Experiment I: NK3-Rezeptorantagonismus und kokaininduzierte CPP und Hyperaktivität

Ausgehend von den Befunden, dass die Aktivierung von NK3-Rezeptoren einerseits selbst verstärkend wirkt (im Sinne einer Ausbildung von CPP), die lokomotorische Aktivität erhöht und das mesolimbische DA-System aktiviert, leiteten wir die Hypothese ab, dass Blockade dieses Rezeptors die Ausbildung einer CPP durch Kokain sowie dessen akute hyperlokomotorische Effekte unterbindet. Zur Überprüfung dieser Hypothesen wurden in einem CPP-Experiment acht Gruppen untersucht, die zur Konditionierung eine kombinierte Behandlung erhielten, die aus einer Vorbehandlung mit dem selektiven NK3-Rezeptorantagonisten SR142801 (0; 0,2; 2 oder 20 mg/kg i.p.) und der eigentlichen Behandlung mit Kokain (0 oder 10 mg/kg i.p.) bestand. Die beiden Injektionen lagen 30 Minuten auseinander.

Kokain führte im Vergleich zur Kontrollgruppe (Vehikel + Vehikel) zu einer signifikanten Erhöhung der Lokomotion und des Aufrichtverhaltens. Die Hyperlokomotion konnte durch Vorbehandlung mit SR142801 in der Dosis von 0.2 mg/kg antagonisiert werden, die höheren Dosen dagegen waren ineffektiv. Im Falle der Erhöhung des Aufrichtverhaltens konnte lediglich eine Tendenz zur Abschwächung durch SR142801 in den Dosen von 0.2 und 2 mg/kg beobachtet werden, die im Falle von 0.2 mg/kg knapp die Signifikanzgrenze verfehlte.

Ein weiterer reliabel zu beobachtender Effekt von Kokain besteht in der Unterdrückung von Putzverhalten (Müller et al., 2002). Eine klare Reduktion des Putzverhaltens im Vergleich zur Kontrollgruppe (Vehikel + Vehikel) konnte auch hier festgestellt werden, jedoch wurde dieser Effekt nicht durch Vorbehandlung mit dem Antagonisten moduliert.

Kokain induzierte eine CPP im Sinne einer signifikant gesteigerten Aufenthaltszeit im Konditionierungskompartiment während des Testdurchganges

(Innergruppenvergleich). Jedoch konnte kein Zwischengruppenunterschied zur Vergleichsgruppe (der mit Vehikel + Vehikel behandelten Gruppe) festgestellt werden. Es zeigte sich aber, dass die Tiere, die vor der Kokainverabreichung mit SR142801 vorbehandelt wurden, im Innergruppenvergleich keinen Anstieg der Aufenthaltszeiten von der Basal- zur Testphase aufwiesen. Am effektivsten zeigten sich dabei die Dosen von 0.2 und 2 mg/kg.

SR142801 alleine hatte keinerlei Effekte, weder auf die Lokomotion, das Aufricht- oder Putzverhalten, noch auf die CPP.

Da die kokaininduzierte CPP in diesem Messdesign nur recht schwach ausgeprägt war, und nur im Sinne eines Innergruppenvergleichs, nicht aber im Zwischengruppenvergleich zur Kontrollgruppe Bestand hatte, wurde eine Teilreplikation dieses Experiments mit einer anderen CPP-Apparatur durchgeführt. Da bereits die niedrigste Dosis von SR142801 die maximale Effektivität in der Unterdrückung der akuten Kokaineffekte zeigte, fügten wir zudem eine weitere Dosis von 0.02 mg/kg hinzu. Kokain induzierte nun eine deutliche CPP, die jedoch durch SR142801 nicht beeinflusst wurde. Die Reduktion der Hyperlokomotion durch SR142801 konnte dagegen bestätigt werden. Die effektive Dosis war dabei erneut 0.2 mg/kg, während 0.02 mg/kg ohne Einfluss blieb. Des Weiteren induzierte Kokain hier einen konditionierten lokomotorischen Effekt, d.h. einen Anstieg der Lokomotion vom Basal- zum Testdurchgang. Auch dieser Effekt wurde jedoch durch Vorbehandlung mit SR142801 nicht moduliert.

Diese Ergebnisse legen nahe, dass NK_3-Rezeptoren an der Generierung eines hyperaktiven Zustandes durch Kokain beteiligt sind. Eine Beteiligung des NK_3-Rezeptors an den belohnenden Eigenschaften von Kokain konnte dagegen nicht nachgewiesen werden. Gleiches gilt für die konditionierten lokomotorischen Effekte von Kokain. Weiterhin zeigte sich, dass Blockade des NK_3-Rezeptors alleine keine Effekte auf die Aktivitätsparameter hatte und auch keine Platzpräferenz oder –aversion auslöste. Dies spricht dafür, dass NK_3-Rezeptoren nicht tonisch aktiviert sind. Möglicherweise induziert Kokain die Freisetzung eines NK_3-Rezeptorliganden, der an der erhöhten Lokomotion beteiligt ist. Auf der transkriptionellen Ebene wurde bereits gezeigt, dass Kokain die Synthese von Preprotachykinin A (dem

Vorläuferpeptid von SP und NKA) im Striatum und im Nac hochreguliert (Mathieu-Kia and Besson, 1998). Ob dies auch mit einer erhöhten Freisetzung dieser Liganden einhergeht, wurde bisher nicht untersucht, ist jedoch nicht unwahrscheinlich. Ein Einfluss von Kokain auf NKB wurde bisher nicht untersucht, jedoch kann endogene Aktivität am NK_3-Rezeptor auch über SP und NKA vermittelt werden, die ebenfalls an diesen Rezeptor binden. Tatsächlich liegt ein hervorstechendes Paradoxon bei den NK-Rezeptoren im sogenannten „Mismatch Problem". Dies besteht entweder im Vorhandensein einer Rezeptorpopulation ohne die Gegenwart des dazugehörigen Transmitters oder vice versa im Vorhandensein einer hohen Konzentration eines Transmitters bei Absenz eines passenden Rezeptors. Am besten wird dieser „Mismatch" wohl am Beispiel der pars reticulata der Substantia nigra illustriert, die eine äusserst hohe Dichte SPerger Nervenendigungen enthält, jedoch nahezu frei von NK_1-Rezeptoren ist. Stattdessen findet man dort eine hohe Dichte von NK_3-Rezeptoren, die möglicherweise die Effekte von SP mediieren (Mussap et al., 1993).

Interessanterweise fand diese Studie eine Dissoziation zwischen den belohnenden und verhaltensaktivierenden Effekten von Kokain. Während letztere durch NK_3-Rezeptorantagonismus blockiert wurden, blieben erstere davon unbeeinflusst. Zwar wurden bereits solche Dissoziationen berichtet (Robledo et al., 1993), dennoch sind sie insofern überraschend, als dass man davon ausgeht, dass beide Effekte über den DA-Anstieg im Nac vermittelt werden. Jedoch ist der Nac als eine Art Relaisstation anzusehen, von der aus das kokaininduzierte DA-Signal in verschiedene Richtungen weitergeleitet wird. Wahrscheinlich vermitteln unterschiedliche Zielstrukturen des Nac die belohnenden und hyperlokomotorischen Effekte von Kokain.

5. Experiment II: NK_3-Rezeptorantagonismus und kokain-induzierter Dopaminanstieg im Nac

Um mögliche neuronale Mechanismen der in Experiment I gefundenen Effekte zu klären, wurde mittels in vivo Mikrodialyse die Konzentration von DA sowie dessen

Metaboliten DOPAC und HVA im Nac von frei beweglichen Ratten gemessen. Aufgrund der unter 2.2. beschriebenen funktionellen Diversität des Nac wurde bei jedem Tier eine Sonde in die Core und in der kontralateralen Hemisphäre in die Shell des Nac eingebracht. Nach vier Probennahmen zur Etablierung einer Ausgangslage der Neurotransmitterkonzentrationen wurde zunächst der NK_3-Rezeptorantagonist SR142801 (0; 0,2 oder 2 mg/kg i.p.) und nach einer 30 minütigen Absorptionsphase Kokain (0 oder 10 mg/kg i.p.) verabreicht. Es wurde erwartet, dass die Vorbehandlung mit SR142801 den kokaininduzierten DA-Anstieg abschwächen sollte.

Kokain erhöhte die extrazelluläre Konzentration von DA in der Core des Nac auf ein Maximum von 350% der Ausgangslage. In der Shell erreichte dieser Anstieg 450%. Der Anstieg erreichte sein Maximum innerhalb der ersten beiden Probennahmeintervalle (Minuten 0 bis 20) und kehrte dann bis zum Ende der zweistündigen Messung langsam wieder auf Ausgangsniveau zurück. Entgegen den Erwartungen wurde dieser DA-Anstieg durch NK_3-Rezeptorantagonismus nicht abgeschwächt, sondern sogar in einer subregionenspezifischen Weise potenziert: Während der NK_3-Rezeptorantagonist keinen Einfluss auf den DA-Anstieg nach Kokain in der Shell hatte, potenzierte er den DA-Anstieg in der Core bis auf 550% der Ausgangslage. Somit wurde die stärkere DA-Reaktion der Shell des Nac durch Blockade des NK_3-Rezeptors aufgehoben.

Kokain induzierte zudem einen zeitlich verzögerten Abfall der Konzentrationen von DOPAC und HVA. Dieser Effekt wird in Zusammenhang mit der vermehrten Aktivierung somatodendritischer Autorezeptoren und der daraus resultierenden verminderten Feuer- und Syntheserate der dopaminergen Zellen gebracht (Zetterström et al., 1988). Diese Reduktion wurde durch Vorbehandlung mit SR142801 nicht moduliert.

SR142801 alleine hatte keinerlei Einfluss auf die extrazellulären Konzentrationen von DA, DOPAC oder HVA.

Diese Ergebnisse waren in zweierlei Hinsicht erstaunlich: i) Aufgrund der Lokalisation der NK_3-Rezeptoren auf DA-Zellen der VTA und ihrer Physiologie sollte NK_3-Rezeptorantagonismus den DA-Anstieg nach Kokain abschwächen und ii)

würde man als neuronales Korrelat abgeschwächter Verhaltenseffekte von Kokain ebenfalls einen verminderten DA-Anstieg im Nac, nicht aber eine Potenzierung erwarten. Über den Mechanismus für den potenzierten DA-Anstieg in der Core des Nac kann nur spekuliert werden, da der Antagonist systemisch und nicht intrakraniell in diskrete Hirnareale verabreicht wurde. Dennoch können einige Möglichkeiten in Betracht gezogen werden. In der VTA hat SR142801 keinen Einfluss auf die basale Feuerrate der DA-Zellen, und es verändert auch nicht die DA-Konzentration im Nac. Es unterbindet lediglich depolarisierende Einflüsse, wie z.B. die durch Haloperidol verursachte Disinhibition (Gueudet et al., 1999). Da Kokain diese Zellen nicht depolarisiert, sondern im Gegenteil sogar hyperpolarisiert (Einhorn et al., 1988), ist davon auszugehen, dass SR142801 auf Ebene der VTA keinen Einfluss auf die Feuerrate der Zellen hatte. Ein alternativer Angriffspunkt für den NK_3-Rezeptorantagonismus wäre im Nac selbst. Interessanterweise ist die Expression des Rezeptors dort auf die Core beschränkt, was im Einklang mit der in dieser Studie gefundenen Subregionenselektivität steht. Im Striatum konnte zudem gezeigt werden, dass NK_3-Rezeptoren dort die Freisetzung von GABA aus Interneuronen modulieren (Preston et al., 2000). Daher könnten die Verhaltenseffekte des NK_3-Rezeptorantagonismus unabhängig von der potenzierten dopaminergen Reaktion über eine veränderte GABAerge Übertragung im Nac vermittelt worden sein. Zudem ist zu beachten, dass ausser dem Nac weitere Strukturen, die an den Verhaltenseffekten von Kokain beteiligt sind, wie z.B. das VP (Gong et al., 1996; Gong et al., 1997) eine hohe Dichte an NK_3-Rezeptoren aufweisen (Shughrue et al., 1996). Zudem sind nicht nur andere Hirnstrukturen als der Nac, sondern auch andere Überträgersysteme als das dopaminerge an der Wirkungsweise von Kokain beteiligt. Insbesondere dem serotonergen System scheint eine besondere Bedeutung zuzukommen (Sora et al., 2001; Müller und Huston, 2006). Da NK_3-Rezeptoren auf serotonergen Zellen der Raphé Nuclei lokalisiert sind (Stoessl und Hill, 1990) und deren Funktion modulieren (Paris et al., 1989; Mason und Elliott, 1992), erscheint eine Mediation der gefundenen Effekte über das serotonerge System nicht unwahrscheinlich.

6. Experiment III: NK3-Rezeptoragonismus und kokaininduzierte CPP und Hyperaktivität

Ausgehend von den Überlegungen, die auch Experiment I zugrunde lagen, wurde gefolgert, dass eine Aktivierung von NK3-Rezeptoren sowohl die kokaininduzierte CPP als auch die Hyperaktivität potenzieren sollte. Zur Überprüfung dieser Annahme wurden in einem CPP-Experiment acht Gruppen untersucht, die zur Konditionierung eine kombinierte Behandlung erhielten, die aus einer Vorbehandlung mit dem selektiven NK3-Rezeptoragonisten Senktide (0; 0,1; 0,2 oder 0,4 mg/kg s.c.) und der eigentlichen Behandlung mit Kokain (0 oder 10 mg/kg i.p.) bestand. Die beiden Injektionen lagen 30 Minuten auseinander.

Kokain alleine (d.h. Vorbehandlung: Senktide 0 mg/kg) induzierte eine deutliche CPP im Vergleich zur Kontrollgruppe. Jedoch wurde diese CPP nicht durch Vorbehandlung mit Senktide moduliert, die Magnitude der kokaininduzierten CPP war in allen mit Senktide vorbehandelten Gruppen vergleichbar mit der der Kokaingruppe. Auch Senktide alleine hatte keinerlei Einfluss auf die CPP-Scores.

Kokain führte zu einer Erhöhung der Lokomotion und des Aufrichtverhaltens. Dies wurde durch Vorbehandlung mit Senktide (0,2 mg/kg) zusätzlich potenziert. Dagegen wurde der kokaininduzierte Anstieg der Lokomotion und des Aufrichtverhaltens durch die höchste Dosis von Senktide (0,4 mg/kg) sogar abgeschwächt. Senktide alleine hatte keinen Einfluss auf die lokomotorische Aktivität oder das Aufrichtverhalten der Tiere. Zusätzlich wurde über die vier Konditionierungsdurchgänge der Steigungsindex für die Parameter Lokomotion und Aufrichtverhalten berechnet und zwischen den Gruppen verglichen. Während sich in der Saline-Kokain-Gruppe ein negativer Steigungsindex ergab, erzielten die mit Senktide vorbehandelten Gruppen einen positiven Wert, d.h. ihre Aktivität stieg über die vier Durchgänge hinweg an. Dieser Effekt könnte auf eine erleichterte Sensitisierung der hyperaktivierenden Eigenschaften von Kokain hindeuten.

Im Vergleich von Baseline- und Testdurchgang zeigte sich, dass Kokain einen konditionierten lokomotorischen Effekt verursachte, d.h. die Aktivität dieser Gruppe war im Testtrial höher als in der Baselinemessung. Dieser Unterschied konnte

allerdings nur gezeigt werden, wenn jeweils die ersten fünf Minuten der beiden Durchgänge, nicht aber die kompletten Durchgänge miteinander verglichen wurden. Dieser konditionierte lokomotorische Effekt wurde durch Vorbehandlung mit Senktide nicht beeinflusst.

Das Putzverhalten wurde durch Kokain unterdrückt, dies wurde aber durch Vorbehandlung mit Senktide nicht moduliert. In der Dosis von 0,4 mg/kg unterdrückte Senktide alleine das Putzverhalten, allerdings verfehlte dieser Effekt knapp die statistische Signifikanz ($p=0.055$) und blieb auf den ersten Konditionierungstag limitiert.

Die Ergebnisse dieser Studie stehen im Einklang mit den Befunden aus Experiment I. Agonismus des NK_3-Rezeptors führte entsprechend den Erwartungen zu einer Potenzierung der kokaininduzierten Hyperaktivität. Die Annahme einer Beteiligung dieses Rezeptors an den verstärkenden Eigenschaften und an den konditionierten lokomotorischen Effekten von Kokain konnte dagegen erneut nicht untermauert werden. Interessanterweise konnte auch ein verstärkender Effekt des NK_3-Rezeptoragonisten selbst nicht nachgewiesen werden. Dies liegt möglicherweise an dem relativ langen Intervall von 30 Minuten zwischen der Verabreichung der Vorbehandlung und der Platzierung der Tiere in der Apparatur. Dieser Zeitrahmen scheint zwar optimal für eine Beeinflussung der Kokaineffekte, jedoch sind die Effekte des Agonisten selbst nach dieser Phase möglicherweise bereits abgeklungen. Diese Erklärung wird auch durch die Befunde aus Experiment IV gestützt.

7. Experiment IV: NK_3-Rezeptoragonismus und kokaininduzierter Dopaminanstieg im Nac

Um mögliche neuronale Mechanismen der in Experiment III gefundenen Effekte zu klären und um einen Vergleich mit dem in Experiment II gefundenen Wirkungsprofil des Antagonisten SR142801 zu erhalten, wurde mittels in vivo Mikrodialyse die Konzentration von DA sowie dessen Metaboliten DOPAC und HVA in den beiden Subregionen Core und Shell des Nac von frei beweglichen Ratten

gemessen. Das Vorgehen entsprach dem von Experiment II, mit der Ausnahme, dass inzwischen ein Infrarotlichtschrankensystem zur Verfügung stand, das eine simultane Quantifizierung der horizontalen und vertikalen Aktivität der Tiere parallel zur Mikrodialyse ermöglichte. Nach vier Probennahmen zur Etablierung einer Ausgangslage der Neurotransmitterkonzentrationen wurden zunächst der NK_3-Rezeptoragonist Senktide (0; 0,2 oder 0,4 mg/kg s.c.) und nach einer 30 minütigen Absorptionsphase Kokain (0 oder 10 mg/kg i.p.) verabreicht. Es wurde erwartet, dass die Vorbehandlung mit Senktide den kokaininduzierten Anstieg sowohl der DA-Konzentration als auch der horizontalen und vertikalen Aktivität potenzieren sollte.

Wie in Experiment II bewirkte Kokain eine unmittelbare und deutliche Erhöhung der DA-Konzentration in beiden Subarealen des Nac. Der Anstieg erreichte sein Maximum im zweiten Probenintervall (Minuten 10 bis 20 nach Kokain) mit 404% (Core) bzw. 480% (Shell) der Ausgangslage. In Übereinstimmung mit der Hypothese bewirkte Senktide (0,2 mg/kg) in beiden Subregionen eine Potenzierung dieser Reaktion auf 666% (Core) bzw. 869% (Shell) der Ausgangslage.

Auch der in Experiment II beschriebene kokaininduzierte, zeitlich verzögerte Abfall der Konzentrationen der beiden Metaboliten DOPAC und HVA in beiden Subregionen des Nac konnte hier erneut gezeigt werden. Jedoch wurde keiner dieser Effekte durch Aktivierung des NK_3-Rezeptors beinflusst.

Senktide alleine hatte nur marginale Effekte auf die neurochemischen Parameter. Während die DA-Konzentrationen gänzlich unbeeinflusst blieben, zeigte sich in der Shell des Nac in vereinzelten Probenintervallen eine leicht verminderte Konzentration von DOPAC und HVA im Vergleich zur Saline-Saline-Gruppe.

Kokain induzierte auch hier einen Anstieg der horizontalen und vertikalen Aktivität auf 584% bzw. 227% der Ausgangslage. Im Falle der vertikalen Aktivität erreichte dieser Anstieg jedoch keine statistische Signifikanz. Vorbehandlung mit Senktide (0,2 mg/kg) potenzierte diesen Anstieg auf 1685% bzw. 724% der Ausgangslage.

Senktide alleine (0,2 mg/kg) führte zu einem unmittelbaren, kurz andauernden Anstieg der horizontalen und vertikalen Aktivität. Dieser Effekt wurde in den 30 Minuten vor der Kokainverabreichung beobachtet.

Diese Ergebnisse stehen im Einklang mit der in der Literatur beschriebenen Rolle der NK_3-Rezeptoren in der Modulation der Aktivität mesencephaler dopaminerger Zellen (Keegan et al., 1992; Marco et al., 1998), und sie zeigen, dass sich diese Modulation auch auf kokaininduzierte Veränderungen der dopaminergen Übertragung auswirkt. Der kokaininduzierte DA-Anstieg im Nac ist von kritischer Bedeutung für die verstärkenden und gewohnheitsbildenden Eigenschaften von Kokain (Wise, 2004). Insbesondere DA in der Shell des Nac scheint von entscheidender Bedeutung für die Belohnungswirkung von Kokain zu sein (Rodd-Henricks et al., 2002; Bari und Pierce, 2005). Vor diesem Hintergrund erscheint es erstaunlich, dass die Potenzierung des kokaininduzierten DA-Anstiegs im Nac durch den NK_3-Rezeptoragonisten zwar mit einer gesteigerten Hyperlokomotion, nicht aber mit einer erhöhten CPP einherging. Jedoch wurde über die letzten Jahre zunehmend evident, dass DA im Nac nicht, wie ursprünglich angenommen, den belohnenden Effekt appetitiver Stimuli kodiert (Wise et al., 1978), sondern vielmehr für die Induktion neuroplastischer Veränderungen notwendig ist, die Stimulus-Belohnungs- oder Reaktions-Belohnungsassoziationen zugrunde liegen (Salamone et al., 2005; Smith-Roe and Kelley, 2000). Daher scheint der DA-Anstieg im Nac durch Suchtdrogen zwar notwendig für die Bildung solcher Assoziationen, eine weitere Erhöhung des dopaminergen Tonus sollte demzufolge aber weder den Belohnungswert der Droge erhöhen, noch das Lernen der Assoziation weiter intensivieren.

Interessanterweise bewirkte Senktide alleine einen kurz andauernden (10 bis 20 Minuten) Anstieg der horizontalen und vertikalen Aktivität. Dieser spielte sich im Zeitraum vor der Kokainverabreichung ab, also in einem Zeitfenster, in dem sich in den neurochemischen Parametern keinerlei Veränderungen zeigten. Dies deutet darauf hin, dass die motorische Aktivierung über einen DA-unabhängigen Mechanismus mediiert wurde. Wie in Experiment II bereits beschrieben, stellt das serotonerge System einen möglichen Kandidaten hierfür dar. Injektion von Senktide

in die Raphé Nuclei induziert lokomotorische Aktivierung über einen serotonergen Mechanismus (Mason und Elliott, 1992; Paris et al., 1989). Da der Effekt von Senktide nur von kurzer Dauer und zum Zeitpunkt der Kokaininjektion bereits abgeklungen war, handelt es sich bei der Potenzierung der kokaininduzierten Hyperaktivität nicht um einen rein additiven, sondern tatsächlich um einen genuin potenzierenden Effekt. Der Befund, dass sich die Effekte von Senktide in einem kurzen Zeitraum nach der Injektion abspielen, erklärt möglicherweise, warum es in Experiment III keine CPP auslöste. Sollte sich die verstärkende Wirkung von Senktide in einem vergleichbaren Zeitfenster wie die motorischen Effekte ereignen, dann wären diese in Experiment III zum Zeitpunkt der Platzierung der Tiere in die Apparatur bereits abgeklungen. Daher ist das Intervall von 30 Minuten zwischen den beiden Injektionen scheinbar optimal für eine Modulation der Kokaineffekte, zur Untersuchung der Effekte des NK_3-Rezeptoragonismus per se jedoch nicht geeignet.

8. Experiment V: NK_3-Rezeptorantagonismus und kokain-induziertes Verhalten in Primaten

Aufgrund der oben beschriebenen Unterschiede in der Rezeptorpharmakologie für Antagonisten sowie dem unterschiedlichen zerebralen Expressionsmuster zwischen verschiedenen Spezies untersuchten wir die Effekte des NK_3-Rezeptorantagonisten SR142801 auf kokaininduziertes Verhalten in Primaten (Callithrix penicillata) im Figure-Eight-Maze. Der Dosierungsbereich von SR142801 wurde entsprechend der Speziesunterschiede angeglichen. Die Tiere erhielten eine kombinierte Behandlung mit SR142801 (0; 0,02; 0,2 oder 2 mg/kg i.p.) und Kokain (0 oder 10 mg/kg i.p.), wobei die beiden Injektionen 30 Minuten auseinander lagen.

Kokain erhöhte die lokomotorische Aktivität, führte aber zugleich zu einer Reduktion der exploratorischen Aktivität, des Duftmarkierungsverhaltens, der Körperpflegeaktivitäten und des terrestrischen Scannings. Interessanterweise konnte eine erhöhte Lokomotion nach Kokain nur bei fünf der insgesamt zwölf Tiere (42%) gezeigt werden, während die verbleibenden sieben Tiere (58%) nicht mit erhöhter

Lokomotion reagierten. Daher wurden die Tiere aufgrund ihrer lokomotorischen Reaktion auf Kokain, die gewöhnlich als Indikatior für die stimulierenden Eigenschaften von Kokain angesehen wird, in „Responder“ und „Non-Responder“ unterteilt. Die nachfolgende Analyse ergab, dass sich diese beiden Populationen nicht nur hinsichtlich ihrer lokomotorischen Reaktion unterschieden. Die Responder zeigten zusätzlich eine Erhöhung des terrestrischen Scannings und des aufwärtsgerichteten Blickverhaltens, sowie vermindertes aufwärtsgerichtetes Scanning. Als aufwärtsgerichtetes Scanning wurde das Verhalten gewertet, wenn es länger als fünf Sekunden andauerte. Unterschritt es eine Dauer von zwei Sekunden, wurde das Verhalten als aufwärtsgerichtetes Blickverhalten klassifiziert (De Souza Silva et al., 2006). Die Non-Responder zeigten dagegen anstelle von Hyperlokomotion eine Erhöhung des aufwärtsgerichteten Scannings. In beiden Populationen führte Kokain gleichermaßen zu einer Hemmung von exploratorischer Aktivität, Körperpflegeaktivitäten und Duftmarkierungsverhalten. Dieses differentielle Reaktionsprofil steht im Einklang mit dem Muster, das in einer vorherigen Studie mit dem niedrigpotenten Psychostimulans Diethylpropion gefunden wurde (Mello, Jr. et al., 2005).

Der NK_3-Rezeptorantagonist SR142801 alleine beeinflusste keinen der Verhaltensparameter. Auch die Kokaineffekte wurden nicht durch SR142801 moduliert, wenn alle Tiere zusammen analysiert wurden. Wurden die beiden Populationen der Responder und Non-Responder dagegen separat analysiert, ergaben sich beachtliche Effekte. SR142801 reduzierte die kokaininduzierte Hyperlokomotion und den Anstieg des terrestrischen Scannings sowie des aufwärtsgerichteten Blickverhaltens selektiv in der Gruppe der Responder. Bei den Non-Respondern dagegen verminderte SR142801 den Anstieg des aufwärtsgerichteten Scannings. Der kokaininduzierte Rückgang der exploratorischen Aktivität, der Körperpflegeaktivitäten und des Duftmarkierungsverhaltens dagegen wurde durch SR142801 nicht beinflußt.

Bei Callitrichiden dient visuelles Scanning, das terrestrisches und aufwärtsgerichtetes Scanning umfasst, der Entdeckung von Objekten in der Umgebung und hat hohen adaptiven Wert (Caine, 1984). Die Präsentation einer

potentiellen Bedrohung geht einher mit einer Zunahme des visuellen Scannings (Caine, 1998). Die Zunahme des terrestrischen Scannings und des aufwärtsgerichteten Blickverhaltens zusammen mit der reduzierten exploratorischen Aktivität gilt als Indikatior eines anxiogenen Zustands (Barros et al., 2004), der durch anxiolytisch wirkende Substanzen wie Diazepam aufgehoben werden kann (Barros et al., 2000). Während in den Respondern Kokain zwar einen Anstieg des terrestrischen Scannings und des aufwärtsgerichteten Blickverhaltens bewirkte, wurde das vorherrschende aufwärtsgerichtete Scanning vermindert, was darauf hindeutet, dass die anxiogene Komponente der Kokainwirkung in dieser Population nicht dominant war. In der Gruppe der Non-Responder dagegen bestand der markanteste Verhaltenseffekt von Kokain im Anstieg des aufwärtsgerichteten Scannings, was auf eine vorwiegend anxiogene Reaktion auf Kokain hindeutet. Dies gleicht der Situation bei Menschen, die Kokain konsumieren. Auch dort verspürt ein gewisser Anteil der Konsumenten kein Euphoriegefühl, sondern stattdessen Angst oder Dysphorie (Resnick et al., 1977). Da der kokaininduzierte Anstieg des aufwärtsgerichteten Scannings in der Gruppe der Non-Responder durch SR142801 antagonisiert wurde, deutet dies auf eine Inhibition der anxiogenen Eigenschaften von Kokain hin.

Die Beteiligung des NK_3-Rezeptors an den akuten Verhaltenseffekten von Kokain scheint bei Ratten und bei der Gruppe der Responder bei Callithrix penicillata vergleichbar zu sein. Auch bei den Primaten wurde durch SR142801 nur die Hyperlokomotion verhindert, nicht aber die Unterdrückung der Körperpflegeaktivitäten durch Kokain. Wie auch in Ratten hatte SR142801 alleine keinerlei Verhaltenseffekte. Zur Blockade der Kokaineffekte war bei Primaten eine 10-fach niedrigere Dosis nötig, als bei der Ratte (Jocham et al., 2006). Dies steht im Einklang mit den Befunden von Emonds-Alt et al. (1995), die zeigten, dass SR142801 bei Menschen oder Meerschweinchen mit 10- bis 100-fach höherer Affinität an den NK_3-Rezeptor bindet als bei Ratten.

9. Experiment VI: NK3-Rezeptoragonismus und kokaininduziertes Verhalten in Primaten

Obwohl sich die Pharmakologie von NK3-Rezeptoragonisten zwischen verschiedenen Spezies nicht signifikant unterscheidet, bleibt doch das Problem des differentiellen Expressionsmusters des Rezeptors bei Menschen und Ratten bestehen. Daher war es von Interesse, zu überprüfen, ob sich die in Ratten gefundenen Effekte des NK3-Rezeptoragonisten Senktide auch in Callithrix penicillata zeigen lassen. Zu diesem Zweck wurde den Tiere 30 Minuten nach der Vorbehandlung mit Senktide (0; 0,1; 0,2 oder 0,4 mg/kg s.c.) Kokain verabreicht (10 mg/kg i.p.), wonach ihr Verhalten, analog zu Experiment V, im Figure-Eight-Maze analysiert wurde.

Kokain erhöhte die lokomotorische Aktivität, führte aber zugleich zu einer Reduktion der exploratorischen Aktivität, der Körperpflegeaktivitäten, des terrestrischen Blickverhaltens und der Häufigkeit des aufwärtsgerichteten Scannings. Das Duftmarkierungsverhaltens wurde durch Kokain ebenfalls unterdrückt, allerdings verfehlte dieser Unterschied die statistische Signifikanz. Wie in Experiment V zeigte sich auch hier, dass nicht alle Tiere mit einer erhöhten Lokomotion auf Kokain reagierten. Sechs der insgesamt acht Tiere zeigten nach Kokain Hyperlokomotion und wurden demzufolge als Responder klassifiziert. Aufgrund der insgesamt geringen Tierzahl und, daraus folgend, nur zwei Tieren in der Gruppe der Non-Responder, hat die nachfolgende Analyse jedoch nur deskriptiven Charakter. Es zeigte sich, dass die Responder generell in allen Verhaltensparametern höhere Werte aufwiesen als die Non-Responder, mit der Ausnahme der Dauer und Häufigkeit des aufwärtsgerichteten Scannings, bei dem die Non-Responder höhere Werte erzielten. Diese Befunde entsprechen im Wesentlichen dem in Experiment V gefundenen Reaktionsprofil (De Souza Silva et al., 2006). Aufgrund der geringen Tierzahl waren manche Effekte allerdings weniger stark ausgeprägt bzw. verfehlten die statistische Bedeutsamkeit. Dies gilt insbesondere für die Unterteilung der Population in Responder und Non-Responder, die hier nur explorativen Charakter hat und zum Vergleich mit anderen Studien dient.

Der NK_3-Rezeptoragonist Senktide alleine bewirkte keine statistisch bedeutsamen Effekte. Senktide verhinderte jedoch in allen drei Dosen die kokaininduzierte Hyperlokomotion. Im Gegensatz dazu potenzierte Senktide die kokaininduzierte Reduktion der exploratorischen Aktivität sowie der Häufigkeit des aufwärtsgerichteten Scannings und des terrestrischen Blickverhaltens.

Entgegen den Erwartungen und im Widerspruch zu unseren Befunden mit Ratten (Jocham et al., 2006) blockierte Senktide die kokaininduzierte Hyperlokomotion. Eine mögliche Erklärung dafür wäre, dass Senktide die Dosis-Wirkungskurve für Kokain nach links verschoben hatte. Diese Kurve weist eine umgekehrt U-förmige Beziehung auf, die in hohen Dosen das Auftreten von Stereotypien anstelle von Hyperlokomotion beinhaltet (Johanson und Fischman, 1989). Da jedoch keine Stereotypien beobachtet werden konnten, ist diese Erklärung unwahrscheinlich.

Senktide alleine bewirkte eine tendenzielle Reduktion der Dauer des aufwärtsgerichteten Scannings. Dies war besonders ausgeprägt in der Gruppe der Non-Responder. Da anxiolytische Substanzen das aufwärtsgerichtete Scanning vermindern (Barros et al., 2000), deutet dies auf einen anxiolytischen Effekt von Senktide hin. Bei Mäusen wurden bereits anxiolytische Effekte von Senktide beschrieben (Ribeiro and De Lima, 1998; Ribeiro et al., 1999). Weitere Untersuchungen zur potentiellen Rolle von NK_3-Rezeptoren bei angstassoziiertem Verhalten in Primaten mit größeren Tierpopulationen als in der vorliegenden Studie erscheinen daher vielversprechend.

10. Zusammenfassende Diskussion

In dieser Arbeit sollte die Rolle des NK3-Rezeptors bei den neurochemischen und Verhaltenseffekten von Kokain untersucht werden. Eine Beteiligung des NK3-Rezeptors an den aktivitätssteigernden Eigenschaften von Kokain konnte bestätigt werden. Die Blockade des NK3-Rezeptors bewirkte eine Abschwächung der kokaininduzierten Hyperaktivität, ohne dabei selbst das spontane Verhalten zu beeinflussen. Die Unterdrückung des Putzverhaltens wurde durch die Rezeptorblockade nicht beeinflusst. Komplementär dazu führte die Aktivierung des NK3-Rezeptors zu einer weiteren Potenzierung der kokaininduzierten Hyperaktivität. Auch hier ergab sich kein Einfluss auf die Suppression des Putzverhaltens durch Kokain. Jedoch bewirkte der Agonist selbst eine kurz andauernde Erhöhung der horizontalen und vertikalen Aktivität. Diese Befunde deuten darauf hin, dass der NK3-Rezeptor unter basalen Bedingungen nicht an der Generierung spontanen Verhaltens beteiligt ist, aber durch Kokainverabreichung aktiviert wird.

Untersuchungen an Primaten bestätigten im Falle der NK3-Rezeptorblockade die mit Ratten erzielten Befunde. Im Falle der Aktivierung des NK3-Rezeptors dagegen ergaben sich konträre Resultate. Dies verdeutlicht die funktionelle Bedeutung von Speziesunterschieden in der Expression des NK3-Rezeptors und wirft die Frage nach dem bestgeeigneten Tiermodell auf.

Im Gegensatz zu den verhaltensaktivierenden Wirkungen konnte eine Rolle des NK3-Rezeptors an den belohnenden Eigenschaften von Kokain nicht nachgewiesen werden. Weder die Blockade noch die Aktivierung des Rezeptors hatte einen Einfluß auf die kokaininduzierte CPP. Zudem induzierten weder der Agonist noch der Antagonist selbst eine Platzaversion oder eine Platzpräferenz, was darauf hindeutet, dass sie weder einen appetitiven noch einen aversiven Zustand auslösten. Letzteres steht im Widerspruch zu Befunden anderer Autoren, die zeigten, dass sowohl selektive wie nichtselektive NK3-Rezeptoragonisten in der Lage sind, eine CPP auszulösen (Ciccocioppo et al., 1998; Hasenöhrl et al., 1992). Das Ausbleiben einer CPP auf den NK3-Rezeptoragonisten könnte an dem langen

Intervall zwischen den beiden Injektionen liegen. Dieses war unter dem Gesichtspunkt gewählt, eine optimale Modulation der Kokaineffekte zu erzielen. Für eine gezielte Untersuchung der Effekte des NK_3-Rezeptoragonisten selbst wäre eine Verabreichung unmittelbar vor der Testsituation ratsam.

Ein wesentliches Ziel dieser Arbeit bestand darin, mögliche neurochemische Mechanismen für die gefundenen Verhaltenseffekte aufzuklären. Zu diesem Zweck wurden Parameter der dopaminergen Transmission im Nac untersucht. Kokain führte zu einem Anstieg von DA im Nac, der in der Shell stärker ausgeprägt war als in der Core. Dieser Effekt wurde bereits in der Literatur beschrieben (Pontieri et al., 1995). Aktivierung des NK_3-Rezeptors potenzierte diesen Anstieg in beiden Subregionen des Nac. Zudem induzierte Kokain ein zeitlich verzögertes Absinken der Konzentration der beiden DA-Metaboliten DOPAC und HVA. Diese Reduktion wurde durch Aktivierung des NK_3-Rezeptors nicht beeinflusst. Blockade des NK_3-Rezeptors potenzierte den kokaininduzierten DA-Anstieg selektiv in der Core des Nac. Wie beim Agonisten war auch hier kein Einfluss auf die kokaininduzierte Reduktion der Konzentrationen von DOPAC und HVA zu beobachten. Weder Aktivierung noch Blockade des NK_3-Rezeptors alleine hatte einen Einfluss auf die Konzentrationen von DA, DOPAC oder HVA.

Über die Mechanismen der gefundenen neurochemischen Effekte kann nur spekuliert werden, da die Substanzen systemisch verabreicht wurden. Jedoch erscheint es möglich, dass die Effekte des Agonisten über eine direkte Wirkung auf die dopaminergen Zellen der VTA vermittelt wurden. Im Gegensatz dazu könnten NK_3-Rezeptoren in der Core des Nac den Angriffspunkt für die Wirkung des Antagonisten darstellen. Da der NK_3-Rezeptor selektiv in der Core des Nac exprimiert wird, und Befunde existieren, dass der von uns verwendete Antagonist nicht die basale Feuerrate der DA-Zellen der VTA beinflusst (Gueudet et al., 1999), würde dies die Begrenzung seiner Effektivität auf die Core des Nac erklären. Der Agonist Senktide dagegen erhöht die Feuerrate der DA-Zellen der VTA (Keegan et al., 1992; Overton et al., 1992). Da diese Zellen beide Subregionen des Nac innervieren, ist eine Wirkung auf die DA-Transmission in beiden Arealen die logische Konsequenz.

Diese Ergebnisse zeigen, dass NK_3-Rezeptoren an kokaininduzierten Veränderungen sowohl der dopaminergen Übertragung wie auch der lokomotorischen Aktivität beteiligt sind. Dagegen wurden die belohnenden Eigenschaften von Kokain durch Modulation des NK_3-Rezeptors nicht beeinflusst. Die Dissoziation zwischen der Modulation der verstärkenden und der aktivitätssteigernden Wirkungen von Kokain weist darauf hin, dass diese beiden Verhaltenseffekte nicht über identische neurale Mechanismen mediiert werden, wie in der Vergangenheit oft angenommen wurde (Gold et al., 1989). Zudem weisen die Ergebnisse darauf hin, dass die Verhaltenseffekte von Kokain moduliert werden können, ohne den kokaininduzierten DA-Anstieg im Nac zu reduzieren: Die Blockade des NK_3-Rezeptors bewirkte eine abgeschwächte lokomotorische Reaktion auf Kokain, obwohl die DA-Reaktion in der Shell des Nac unverändert, in der Core sogar potenziert war. Im Gegensatz dazu führte eine Potenzierung der DA-Reaktion im Nac durch Aktivierung des NK_3-Rezeptors nicht zu einer weiteren Erhöhung der Belohnungswirkung von Kokain. Dies zeigt auf, dass der kokaininduzierte DA-Anstieg im Nac zwar sowohl für die verhaltensaktivierenden wie auch für die verstärkenden Eigenschaften von Kokain notwendig, aber bei weitem nicht hinreichend ist. Der Nac stellt zwar eine zentrale Schaltstelle dar, die eine Vielzahl von sensomotorischen, emotional-motivationalen und viszeralen Informationen integriert (Mogenson et al., 1980), gleichzeitig ist er aber auch Ausgangspunkt mehrer striatopallidothalamocorticaler Schleifen (Zahm und Brog, 1992; Zahm, 2000). Durch DA im Nac modulierte Signale können daher „stromabwärts“ auf jeder dieser weiteren Stationen ohne jeglichen Einfluss auf die dopaminerge Transmission moduliert werden.

11. Ausblick

Die hier vorgestellten Ergebnisse konnten zeigen, dass der NK_3-Rezeptor an den verhaltensaktivierenden und dopaminergen Wirkungen von Kokain beteiligt ist. Diese Daten geben allerdings keinen Aufschluß darüber, welche

Rezeptorpopulationen im Gehirn diese Effekte mediiert hatten. Zu diesem Zweck müssten in nachfolgenden Untersuchungen Agonisten und Antagonisten des NK_3-Rezeptors lokal in diskrete Hirnareale verabreicht werden. Zudem wurde die DA-Konzentration lediglich im Nac als möglichem Mediator der gefundenen Verhaltenseffekte untersucht. Doch auch andere Strukturen, die an den Verhaltenseffekten von Kokain beteiligt sind, und dopaminerg innerviert werden, wären von Interesse. Dazu zählen die Amygdala, der mPFC, aber auch das dorsale Striatum. Zudem häuft sich in den letzten Jahren die Evidenz, dass andere Neurotransmitter als DA in die Generation der Verhaltenseffekte von Kokain involviert sind. Insbesondere dem serotonergen System scheint dabei eine besondere Rolle zuzukommen (Sora et al., 2001; Müller und Huston, 2006).

Des weiteren stellt sich die Frage, ob der NK_3-Rezeptor an den belohnenden Eigenschaften anderer Suchtdrogen, z.B. Amphetamin, Nikotin, Cannabis, Alkohol oder Morphin beteiligt ist. Für den NK_1-Rezeptor z.B. konnte gezeigt werden, dass er für die belohnenden Eigenschaften von Opiaten, nicht aber von Kokain notwendig ist (Murtra et al., 2000; Ripley et al., 2002). Daher ist eine weitere Untersuchung der Bedeutung des NK_3-Rezeptors für die Wirkung anderer Suchtdrogen durchaus vielversprechend.

12. Zusammenfassende Thesen

- NK_3-Rezeptoren sind an den verhaltensaktivierenden Eigenschaften von Kokain beteiligt. Unter basalen Bedingungen ist der NK_3-Rezeptor dagegen nicht in die Generation lokomotorischer Aktivität involviert, da seine Blockade keinen Einfluss auf dieses Verhalten hat. Dies deutet darauf hin, dass der NK_3-Rezeptor nicht konstitutiv aktiviert ist.

- Aktivierung des NK_3-Rezeptors verursacht eine Erhöhung der lokomotorischen Aktivität. Dies wird nicht über einen dopaminergen

Mechanismus im Nac vermittelt. Stattdessen wird eine Mediierung über das serotonerge System vorgeschlagen.

- Der NK_3-Rezeptor ist nicht in die belohnenden Eigenschaften von Kokain involviert. Eine Beteiligung an den belohnenden Eigenschaften anderer Suchtdrogen ist dagegen weiterhin möglich und sollte Gegenstand zukünftiger Untersuchungen sein.

- Der NK_3-Rezeptor hat unter Basalbedingungen keinen Einfluss auf die dopaminerge Übertragung im Nac. Er moduliert jedoch in in einer komplexen, subregionenspezifischen Weise die Reaktion des dopaminergen Systems im Nac auf Kokain.

- Durch den NK_3-Rezeptor können die dopaminergen und Verhaltenseffekte von Kokain in dissoziierbarer Weise moduliert werden. Während die Aktivierung dieses Rezeptors sowohl den Anstieg von DA im Nac als auch der lokomotorischen Aktivität nach Kokainverabreichung potenzierte, blieben die belohnenden Eigenschaften von Kokain unverändert.

13. Literatur

Baker DA, Fuchs RA, Specio SE, Khroyan TV, Neisewander JL (1998) Effects of intraaccumbens administration of SCH-23390 on cocaine-induced locomotion and conditioned place preference. Synapse 30: 181-193.

Baker DA, Khroyan TV, O'Dell LE, Fuchs RA, Neisewander JL (1996) Differential effects of intra-accumbens sulpiride on cocaine-induced locomotion and conditioned place preference. J Pharmacol Exp Ther 279: 392-401.

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

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

Beaujouan JC, Saffroy M, Torrens Y, Glowinski J (1997) Potency and selectivity of the tachykinin NK3 receptor antagonist SR 142801. Eur J Pharmacol 319: 307-316.

Caine NG (1984) Visual Scanning by Tamarins - A Description of the Behavior and Tests of 2 Derived Hypotheses. Folia Primatologica 43: 59-67.

Caine NG (1998) Cutting costs in response to predatory threat by Geoffroy's marmosets (Callithrix geoffroyi). American Journal of Primatology 46: 187-196.

Chen LW, Guan ZL, Ding YQ (1998) Mesencephalic dopaminergic neurons expressing neuromedin K receptor (NK3): a double immunocytochemical study in the rat. Brain Research 780: 150-154.

Ciccocioppo R, Panocka I, Polidori C, Froldi R, Angeletti S, Massi M (1998) Mechanism of action for reduction of ethanol intake in rats by the tachykinin NK-3 receptor agonist aminosenktide. Pharmacol Biochem Behav 61: 459-464.

De Souza Silva MA, Mello EL, Jr., Müller CP, Jocham G, Maior RS, Huston JP, Tomaz C, Barros M (2006) The tachykinin NK3 receptor antagonist SR142801 blocks the behavioral effects of cocaine in marmoset monkeys. Eur J Pharmacol 536: 269-278.

Delfs JM, Schreiber L, Kelley AE (1990) Microinjection of cocaine into the nucleus accumbens elicits locomotor activation in the rat. J Neurosci 10: 303-310.

Di Chiara G (2002) Nucleus accumbens shell and core dopamine: differential role in behavior and addiction. Behav Brain Res 137: 75-114.

Einhorn LC, Johansen PA, White FJ (1988) Electrophysiological effects of cocaine in the mesoaccumbens dopamine system: studies in the ventral tegmental area. J Neurosci 8: 100-112.

Elliott PJ, Mason GS, Stephens-Smith M, Hagan RM (1991) Behavioural and biochemical responses following activation of midbrain dopamine pathways by receptor selective neurokinin agonists. Neuropeptides 19: 119-126.

Emonds-Alt X, Bichon D, Ducoux JP, 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.

Ettenberg A, Pettit HO, Bloom FE, Koob GF (1982) Heroin and cocaine intravenous self-administration in rats: mediation by separate neural systems. Psychopharmacology (Berl) 78: 204-209.

Fog R (1969) Stereotyped and non-stereotyped behaviour in rats induced by various stimulant drugs. Psychopharmacologia 14: 299-304.

Goeders NE, Smith JE (1983) Cortical dopaminergic involvement in cocaine reinforcement. Science 221: 773-775.

Gold LH, Geyer MA, Koob GF (1989) Neurochemical mechanisms involved in behavioral effects of amphetamines and related designer drugs. NIDA Res Monogr 94: 101-126.

Gong W, Neill D, Justice JB, Jr. (1996) Conditioned place preference and locomotor activation produced by injection of psychostimulants into ventral pallidum. Brain Res 707: 64-74.

Gong W, Neill D, Justice JB, Jr. (1997) 6-Hydroxydopamine lesion of ventral pallidum blocks acquisition of place preference conditioning to cocaine. Brain Res 754: 103-112.

Gueudet C, Santucci V, Soubrie P, Le Fur G (1999) Blockade of neurokinin3 receptors antagonizes drug-induced population response and depolarization block of midbrain dopamine neurons in guinea pigs. Synapse 33: 71-79.

Hasenöhrl RU, Gerhardt P, Huston JP (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 RU, Oitzl MS, Huston JP (1989) Conditioned place preference in the corral: a procedure for measuring reinforcing properties of drugs. J Neurosci Methods 30: 141-146.

Huston JP, Hasenohrl RU, Boix F, Gerhardt P, Schwarting RK (1993) Sequence-specific effects of neurokinin substance P on memory, reinforcement, and brain dopamine activity. Psychopharmacology (Berl) 112: 147-162.

Jocham G, Lezoch K, Müller CP, Kart-Teke E, Huston JP, de Souza Silva MA (2006) Neurokinin3 receptor antagonism attenuates cocaine's behavioral activating effects yet potentiates its dopamine-enhancing action in the nucleus accumbens core. Eur J Neurosci 24: 1721-1732.

Keegan KD, Woodruff GN, Pinnock RD (1992) The selective NK3 receptor agonist senktide excites a subpopulation of dopamine-sensitive neurones in the rat substantia nigra pars compacta in vitro. Br J Pharmacol 105: 3-5.

Langlois X, Wintmolders C, te Riele P, Leysen JE, Jurzak M (2001) Detailed distribution of neurokinin 3 receptors in the rat, guinea pig and gerbil brain: a comparative autoradiographic study. Neuropharmacology 40: 242-253.

Liao RM, Chang YH, Wang SH, Lan CH (2000) Distinct accumbal subareas are involved in place conditioning of amphetamine and cocaine. Life Sci 67: 2033-2043.

Maggi CA (1995) The mammalian tachykinin receptors. Gen Pharmacol 26: 911-944.

Marco N, Thirion A, Mons G, Bougault I, Le Fur G, Soubrie P, Steinberg R (1998) Activation of dopaminergic and cholinergic neurotransmission by tachykinin NK3 receptor stimulation: an in vivo microdialysis approach in guinea pig. Neuropeptides 32: 481-488.

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

Mathieu-Kia AM, Besson MJ (1998) Repeated administration of cocaine, nicotine and ethanol: effects on preprodynorphin, preprotachykinin A and preproenkephalin mRNA expression in the dorsal and the ventral striatum of the rat. Brain Res Mol Brain Res 54: 141-151.

Mello EL, Jr., Maior RS, Carey RJ, Huston JP, Tomaz C, Müller CP (2005) Serotonin1A-receptor antagonism blocks psychostimulant properties of diethylpropion in marmosets (Callithrix penicillata). Eur J Pharmacol 511: 43-52.

Mogenson GJ, Jones DL, Yim CY (1980) From motivation to action: functional interface between the limbic system and the motor system. Prog Neurobiol 14: 69-97.

Müller CP, Souza Silva MA, DePalma G, Tomaz C, Carey RJ, Huston JP (2002) The selective serotonin(1A)-receptor antagonist WAY 100635 blocks behavioral stimulating effects of cocaine but not ventral striatal dopamine increase. Behav Brain Res 134: 337-346.

Murtra P, Sheasby AM, Hunt SP, De Felipe C (2000) Rewarding effects of opiates are absent in mice lacking the receptor for substance P. Nature 405: 180-183.

Mussap CJ, Geraghty DP, Burcher E (1993) Tachykinin receptors: a radioligand binding perspective. J Neurochem 60: 1987-2009.

Overton P, Elliott PJ, Hagan RM, Clark D (1992) Neurokinin agonists differentially affect A9 and A10 dopamine cells in the rat. Eur J Pharmacol 213: 165-166.

Pettit HO, Ettenberg A, Bloom FE, Koob GF (1984) Destruction of dopamine in the nucleus accumbens selectively attenuates cocaine but not heroin self-administration in rats. Psychopharmacology (Berl) 84: 167-173.

Pontieri FE, Tanda G, Di Chiara G (1995) Intravenous cocaine, morphine, and amphetamine preferentially increase extracellular dopamine in the "shell" as compared with the "core" of the rat nucleus accumbens. Proc Natl Acad Sci U S A 92: 12304-12308.

Post RM, Kotin J, Goodwin FK (1974) Effects of Cocaine on Depressed-Patients. American Journal of Psychiatry 131: 511-517.

Preston Z, Richardson PJ, Pinnock RD, Lee K (2000) NK-3 receptors are expressed on mouse striatal gamma-aminobutyric acid-ergic interneurones and evoke [(3)H] gamma-aminobutyric acid release. Neurosci Lett 284: 89-92.

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

Reith ME, Li MY, Yan QS (1997) Extracellular dopamine, norepinephrine, and serotonin in the ventral tegmental area and nucleus accumbens of freely moving rats during intracerebral dialysis following systemic administration of cocaine and other uptake blockers. Psychopharmacology (Berl) 134: 309-317.

Resnick RB, Kestenbaum RS, Schwartz LK (1977) Acute systemic effects of cocaine in man: a controlled study by intranasal and intravenous routes. Science 195: 696-698.

Ribeiro SJ, De Lima TC (1998) Naloxone-induced changes in tachykinin NK3 receptor modulation of experimental anxiety in mice. Neurosci Lett 258: 155-158.

Ribeiro SJ, Teixeira RM, Calixto JB, De Lima TCM (1999) Tachykinin NK3 receptor involvement in anxiety. Neuropeptides 33: 181-188.

Ripley TL, Gadd CA, De Felipe C, Hunt SP, Stephens DN (2002) Lack of self-administration and behavioural sensitisation to morphine, but not cocaine, in mice lacking NK1 receptors. Neuropharmacology 43: 1258-1268.

Ritz MC, Cone EJ, Kuhar MJ (1990) Cocaine inhibition of ligand binding at dopamine, norepinephrine and serotonin transporters: a structure-activity study. Life Sci 46: 635-645.

Roberts DC, Corcoran ME, Fibiger HC (1977) On the role of ascending catecholaminergic systems in intravenous self-administration of cocaine. Pharmacol Biochem Behav 6: 615-620.

Robledo P, Maldonado R, Koob GF (1993) Neurotensin injected into the nucleus accumbens blocks the psychostimulant effects of cocaine but does not attenuate cocaine self-administration in the rat. Brain Res 622: 105-112.

Rodd-Henricks ZA, McKinzie DL, Li TK, Murphy JM, McBride WJ (2002) Cocaine is self-administered into the shell but not the core of the nucleus accumbens of Wistar rats. J Pharmacol Exp Ther 303: 1216-1226.

Salamone JD, Correa M, Mingote SM, Weber SM (2005) Beyond the reward hypothesis: alternative functions of nucleus accumbens dopamine. Curr Opin Pharmacol 5: 34-41.

Sarau HM, Griswold DE, Bush B, Potts W, Sandhu P, Lundberg D, Foley JJ, Schmidt DB, Webb EF, Martin LD, Legos JJ, Whitmore RG, Barone FC, Medhurst AD, Luttmann MA, Giardina GA, Hay DW (2000) Nonpeptide tachykinin receptor antagonists. II. Pharmacological and pharmacokinetic profile of SB-222200, a central nervous system penetrant, potent and selective NK-3 receptor antagonist. J Pharmacol Exp Ther 295: 373-381.

Shughrue PJ, Lane MV, Merchenthaler I (1996) In situ hybridization analysis of the distribution of neurokinin-3 mRNA in the rat central nervous system. J Comp Neurol 372: 395-414.

Smith-Roe SL, Kelley AE (2000) Coincident activation of NMDA and dopamine D1 receptors within the nucleus accumbens core is required for appetitive instrumental learning. J Neurosci 20: 7737-7742.

Stoessl AJ, Szczutkowski E, Glenn B, Watson I (1991) Behavioural effects of selective tachykinin agonists in midbrain dopamine regions. Brain Res 565: 254-262.

Tzschentke TM (1998) Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. Prog Neurobiol 56: 613-672.

Westerink BH (1995) Brain microdialysis and its application for the study of animal behaviour. Behav Brain Res 70: 103-124.

Wise RA (2004) Dopamine, learning and motivation. Nat Rev Neurosci 5: 483-494.

Wise RA, Spindler J, deWit H, Gerberg GJ (1978) Neuroleptic-induced "anhedonia" in rats: pimozide blocks reward quality of food. Science 201: 262-264.

Zetterström T, Sharp T, Collin AK, Ungerstedt U (1988) In vivo measurement of extracellular dopamine and DOPAC in rat striatum after various dopamine-releasing drugs; implications for the origin of extracellular DOPAC. Eur J Pharmacol 148: 327-334.

14. Einzelarbeiten

Die in dieser Arbeit durchgeführten Versuche an Wirbeltieren wurden in Übereinstimmung mit dem Tierschutzgesetz von 1998 und den „Principles of laboratory animal care“ (NIH Publikation No. 85-23, revidiert von 1996) durchgeführt und durch die Bezirksregierung von Düsseldorf bzw. durch die Tierschutzkommission des Instituts für Biologie der Universität Brasília genehmigt.

Nachfolgend sind die Veröffentlichungen aufgeführt, auf denen diese Arbeit basiert. Die darin zitierte Literatur ist im Anhang der jeweiligen Arbeit aufgeführt.

13.1. Jocham G, Lezoch K, Müller CP, Kart-Teke E, Huston JP, de Souza Silva MA (2006) Neurokinin3 receptor antagonism attenuates cocaine's behavioral activating effects yet potentiates its dopamine-enhancing action in the nucleus accumbens core. Eur J Neurosci 24(6): 1721-1732.

13.2. Jocham G, Lauber AC, Müller CP, Huston JP, de Souza Silva MA (2006) Neurokinin3 receptor activation potentiates the psychomotor and nucleus accumbens dopamine response to cocaine, but not its rewarding effects. Submitted.

13.3. De Souza Silva MA, Mello EL Jr, Müller CP, Jocham G, Maior RS, Huston JP, Tomaz C, Barros M (2006) The tachykinin NK3 receptor antagonist SR142801 blocks the behavioral effects of cocaine in marmoset monkeys. Eur J Pharmacol 536(3): 269-278.

13.4. De Souza Silva MA, Mello EL Jr, Müller CP, Jocham G, Maior RS, Huston JP, Tomaz C, Barros M (2006) Interaction of the tachykinin NK3 receptor agonist senktide with behavioral effects of cocaine in marmosets (Callithrix penicillata). Peptides 27(9): 2214-2223.

European Journal of Neuroscience, Vol. 24, pp. 1721–1732, 2006 doi:10.1111/j.1460-9568.2006.05041.x

Neurokinin$_3$ receptor antagonism attenuates cocaine's behavioural activating effects yet potentiates its dopamine-enhancing action in the nucleus accumbens core

Gerhard Jocham, Katharina Lezoch, Christian P. Müller, Emriye Kart-Teke, Joseph P. Huston and M. Angélica de Souza Silva
Institute of Physiological Psychology and Center for Biological and Medical Research, University of Düsseldorf, Universitätsstr. 1, D-40225, Düsseldorf, Germany

Keywords: cocaine, NK_3 receptors, SR142801, rats, reinforcement

Abstract

Several lines of evidence indicate a role for neurokinin$_3$ receptors (NK_3-Rs) in behavioural activation and mechanisms governing reinforcement processes. In this study we investigated the effect of pretreatment with the NK_3-R antagonist, SR142801, (0.2 and 2.0 mg/kg) on the cocaine-induced (10.0 mg/kg i.p.) increase in extracellular dopaminergic activity in the nucleus accumbens (NAc). *In vivo* microdialysis in the NAc of freely moving rats showed that cocaine increased concentrations of dopamine (DA) to ~350% in the core and ~450% in the shell. Pre-treatment with SR142801 significantly potentiated this effect in the core (to ~550%), whereas this effect was not found in the shell. We also investigated the effects of NK_3-Rs antagonism on cocaine-induced hyperactivity and conditioned place preference. SR142801 blocked the hyperactivity, but neither the conditioned place preference nor the conditioned locomotor activity induced by cocaine, although there was a slight tendency towards a reduced place preference. When given alone, SR142801 had no effects on behaviour or extracellular dopamine concentrations in any of the structures investigated. These data provide evidence for a contribution of NK_3-Rs in the acute behavioural and neurochemical effects of cocaine, involving dopaminergic activity in the core of the nucleus accumbens.

Introduction

The five known mammalian neurokinins are substance P (SP), neurokinin A (NKA), neurokinin B (NKB), neuropeptide K and neuropeptide α. Three G protein-coupled receptors (Rs) have been characterized. SP, NKA and NKB have higher binding affinity to NK_1-, NK_2- and NK_3-Rs, respectively, but all neurokinins bind to all three NK-Rs (Massi *et al*., 2000).

NK_3-Rs have been implicated in reinforcement processes. Conditioned place preference (CPP) has been demonstrated with peripheral and central administration of SP (Hasenöhrl *et al*., 2000) and of the NK_3-R agonist aminosenktide (Ciccocioppo *et al*., 1998). Self-injection of SP was shown in the ventromedial caudate-putamen (Krappmann *et al*., 1994). SP also increased the extracellular concentration of dopamine (DA) in the nucleus accumbens (NAc) (Boix *et al*., 1992b). As both CPP and increase in dopaminergic activity in the NAc, were induced by systemic and central injection of C-terminal fragments of SP, DiMe-C7 (Boix *et al*., 1992a; Hasenöhrl *et al*., 1992; Boix *et al*., 1995), which has a high affinity for the NK_3-R (Regoli *et al*., 1994), it is possible that NK_3-Rs play a major role in the mediation of reinforcement processes. CPP was also induced by injection of a C-terminal fragment of SP into the nucleus basalis magnocellularis (NBM). This effect was not completely blocked by pretreatment with a NK_1-R antagonist, suggesting an involvement of NK_2- or NK_3-Rs (Nikolaus *et al*., 1999). Injection of SP and DiMe-C7 into the ventral tegmental area (VTA) and substantia nigra (SN) enhances locomotor and rearing behaviours (Eison *et al*., 1982; Barnes *et al*., 1990; Elliott *et al*., 1991). These effects are mediated by NK_3-, but not NK_1- and NK_2-Rs (Stoessl *et al*., 1991).

NK_3-Rs are present in high density in the SNc and the VTA, neocortex, striatum and habenular nuclei (Shughrue *et al*., 1996). In the SN and VTA, NK_3-Rs are localized on DAergic cells (Chen *et al*., 1998). Local application of the NK_3-R agonist senktide into the VTA enhanced DA cell firing (Seabrook *et al*., 1995). NK_3-R blockade decreased the release of DA induced by the NK_3-R agonist senktide applied to DAergic cell bodies in the SNc and VTA (Marco *et al*., 1998). These results suggest that NK_3-R play a key role in midbrain dopaminergic functions.

Cocaine increases the activity of the dopaminergic mesocorticolimbic reward system through blockade of the presynaptic DA transporter. Such an increase in the dopaminergic activity is a likely basis for its reinforcing action that also promotes its abuse (Woolverton & Johnson, 1992; Di Chiara *et al*., 2004). Given the localization of NK_3-Rs in crucial parts of the mesolimbic dopaminergic system, their ability to modulate DA activity and their role in reinforcement processes, we hypothesized that NK_3-Rs are also involved in the mediation of the neurochemical and behavioural effects of cocaine. Accordingly, the aim of this study was to investigate the role of NK_3-Rs in (i) cocaine's effects on the activity of the mesolimbic DA system in the core and shell regions of the NAc using *in vivo* microdialysis, and (ii) cocaine's locomotor stimulant action as well as its potency to induce CPP.

Correspondence: Dr M. A. de Souza Silva, as above.
E-mail: desouza@uni-duesseldorf.de

Received 5 February 2006, revised 8 June 2006, accepted 19 July 2006

Materials and methods

Subjects

Eighty-three adult male Wistar rats weighing 338 ± 4.2 g (SEM) at the beginning of the experiment (obtained from the local breeding facility, Tierversuchsanlage, University of Düsseldorf) were used for the microdialysis experiments. For the place preference experiments I and II, 76 and 45 adult male Wistar rats weighing 287 ± 2.5 g (SEM) and 355 ± 6.2 g (SEM), respectively, were used. All animals were kept in macrolon cages in groups of four animals per cage; animals for the microdialysis experiments were housed individually after surgery. They were maintained under a reversed 12-h light : 12-h dark cycle (lights on 07:00 h) with food and water available *ad libitum*. Experiments were performed during the animals' active period between 08:00 h and 15:00 h. All experiments were carried out according to the German Law of Animal Protection of 1998 and approved by the North-Rhine Westphalia State authority.

Drugs

The NK_3-R antagonist, SR142801, ((R)-(N)-[1-[3-[1-benzoyl-3-(3,4-dichlorophenyl) piperidin-3-yl]propyl]-4-phenylpiperidin-4-yl]-*N*-methylacetamide, Sanofi-Aventis, France; Emonds-Alt *et al.*, 1995) and cocaine (Merck, Germany) were used. SR142801 was suspended in 0.01% Tween 80 (Sigma–Aldrich, USA) in distilled water. Cocaine was dissolved in phosphate buffered saline. SR142801 and its vehicle were injected i.p. in a volume of 2.0 mL/kg; cocaine and its vehicle were injected i.p. in a volume of 1.0 mL/kg.

Experiment I – Dopamine and its metabolites in the core and shell regions of the nucleus accumbens

Surgery

Animals were deeply anaesthetized with a mixture of ketaminhydrochloride (90.0 mg/kg, Ketavet, Pharmacia & Upjohn GmbH, Erlangen, Germany) and xylazinhydrochloride (8.0 mg/kg, Rompun, Bayer, Leverkusen, Germany) and fixed in a Kopf stereotactic frame. Two guide-cannulae with a thread on the top (15 mm, 22 G, stainless steel) were aimed at the NAc core (AP +1.2 mm, ML 2.8 mm, DV −5.8 mm, 10° angle tilted laterally) and the nucleus accumbens shell (NAc shell, AP +1.2 mm, ML 0.8 mm, DV −6.2 mm, all coordinates relative to Bregma, according to the atlas of Paxinos & Watson (1986). They were fixed to the skull with two screws (stainless steel, $d = 1.4$ mm) and dental cement. After surgery and again 6 and 12 h later, the animals were administered 100 μL Novalgin orally (containing 500 mg/mL metamizol-sodium, Aventis Pharma, Frankfurt, Germany). They were allowed to recover for at least 6 days preceding microdialysis experimentation. During this time they were handled daily.

Microdialysis procedure

Sixteen to 18 h before experimentation, microdialysis probes (2 mm active membrane length) of a concentric design (for construction details see Boix *et al.*, 1995) were inserted through the guide-cannulae and fixed to the thread. The animal was then placed into a Plexiglas box (29 × 29 × 30 cm) which was located in a sound-attenuating chamber (110 × 70 × 70 cm). The inverted light-dark cycle was maintained in the chamber. To allow videotaping of behaviour, the chamber was illuminated with dim red light (60 W). The probe tubing was connected to a microinfusion pump (CMA 100, Carnegie, Sweden) via a liquid swivel mounted on a balanced arm over the chamber. Probes were perfused with artificial cerebrospinal fluid containing Na^+ 146 mM, K^+ 4 mM, Ca^{2+} 2.2 mM and Cl^- 156 mM. The flow rate was 0.5 μL/min overnight. The next morning at 08:00 h the flow was set to 2.0 μL/min and was left to stabilize for 1 h. Thereafter, samples were collected every 10 min into vials containing 5 μL of 0.05 M $HClO_4$ in which 100 pg of deoxyepinephrine was dissolved, which served as an internal standard for chromatographic analysis. After four baseline samples, SR142801 (0.2 or 2.0 mg/kg) or its vehicle was injected (i.p.). After 30 min, i.e. three samples later, cocaine (10.0 mg/kg) or its vehicle was injected (i.p.). After the second injection sampling was continued for 2 h.

Analysis

Immediately after collection, the samples were analysed for their content of DA and its metabolites 3,4-dihydroxyphenyl acetic acid (DOPAC) and homovanillic acid (HVA) using high performance liquid chromatography. For the separation a 125 × 2 mm reversed-phase column (120–5 C18, Macherey Nagel, Düren, Germany), perfused with a mobile phase composed of 99.5 mM chloroacetic acid, 0.53 mM sodium octyl sulphate, 0.5 mM ethylenediaminetetraacetic acid, 4 mM KCl, 6% v/v acetonitrile and 0.8% v/v tetrahydrofuran, pH adjusted to 3.25 using 6 M NaOH solution, was used. Quantification was performed by amperometric detection (Intro, Antec, Leyden, Netherlands) with the potential set at +530 mV vs. an ISAAC reference electrode (Antec, Leyden, Netherlands) at 28 °C. The limit of detection was 1 fmol per sample for DA at a signal to noise ratio of 2 : 1.

Histology

After completion of the last sampling interval, the animals were administered an overdose of pentobarbital (Nembutal, Sanofi Ceva, Libourne, France) and transcardially perfused with phosphate-buffered saline followed by 10% phosphate-buffered formalin. The brains were removed and stored in formalin (10%) until they were sliced on a cryotome, stained with cresyl-violet and examined under a light microscope. Only animals with probes that were placed within the boundaries of the NAc core or NAc shell were considered for data analysis.

Experiment II – Conditioned place preference

Apparatus

A black corral-maze (modified from Hasenöhrl *et al.*, 1989; see for detail) was used for place preference conditioning. It consisted of a circular open-field (83 cm diameter, walls 43.5 cm height). Two crossed lines marked four quadrants of equal size and floor and wall texture. For the conditioning trials, two Plexiglas barriers were inserted, thus separating the four quadrants. The maze was indirectly lit by four 40 W bulbs placed around the bottom of the maze, resulting in an illumination of the floor of 2.2 lx (walls) to 2.5 lx (centre). Sheets of paper (30 cm width, 8.5 cm height) with different black-white patterns were attached at the inner walls in each quadrant and served as intramaze cues. Except for the intramaze cues, the four quadrants were uniform, providing no bias for any of the quadrants.

Procedure

Each animal was exposed to the corral maze once on each of five consecutive days. The sequence of testing was as follows: on the first day the baseline trial, from the second to the fourth day the conditioning trials 1–3, and on the fifth day the test trial. For the

baseline trial, the animals were placed into the centre of the field and allowed to explore the entire arena for 15 min. Based on the time spent in each quadrant during the baseline trial, the conditioning quadrant was determined to be one of the two quadrants in which the animal neither spent the most nor the least time of the total 15 min. On the conditioning trials, the field was divided into four quadrants by insertion of the separating walls. SR142801 (0.2, 2.0, 20.0 mg/kg) or its vehicle was administered i.p. Thirty minutes after the first injection the animals received a second i.p. injection of either cocaine (10.0 mg/kg) or its vehicle. One minute after the second injection the animal was placed into the conditioning quadrant for 20 min. For the test trial the walls were removed and the animal was placed into the centre of the field and allowed access to the whole arena for 15 min. The animal's sojourn times in each virtual quadrant and the distance moved were tracked automatically using Ethovision Software (Noldus BV, Wageningen, Netherlands). The frequency and duration of rearing (raising both forelimbs off the ground) and the duration of grooming behaviour (self-grooming using paws or snout) were scored semiautomatically by an observer blind to the treatment using Chromotrack software (San Diego Instruments, San Diego, CA) by mouse-clicking the respective fields ('rearing', 'grooming' and 'others') on the computer screen.

Experiment III – Conditioned place preference

In order to investigate further the effects of NK_3-Rs antagonism on cocaine-induced CPP and hyperlocomotion in the corral maze, we performed an additional experiment in a two-compartment-design. As the lowest dose of SR142801 used was the maximally effective one in inhibiting cocaine-induced hyperlocomotion, we tested an additional lower dose of the compound (0.02 mg/kg i.p.).

Apparatus

The same circular open field as described above was used for this experiment. However, this time, a square metal rod (8 × 8 mm) attached to the floor divided the field into two semicircles. In one compartment, the floor was covered with a smooth rubber mat; in the other compartment, both the floor and the wall were covered with a rough black rubber mat. The apparatus was located in a sound-attenuating chamber and was illuminated by a single 100 W light bulb, which resulted in an average illumination of the floor of 1.5 lux (walls) to 2.5 lux (centre).

Procedure

On day 1, the animals were allowed to explore the entire arena for 20 min (baseline trial). On days 2–9, the two compartments of the arena were separated for conditioning by insertion of a transparent perspex wall. On even days, conditioning trials were performed. The animals were injected with SR142801 (0; 0.02 or 0.2 mg/kg i.p.) 30 min before an i.p. injection of cocaine (10 mg/kg). One minute after cocaine injection, each animal was placed into the conditioning compartment for 30 min. On odd days, pseudoconditioning trials were performed. Instead of drug treatments, the animals received only vehicle injections and were placed into the alternate compartment for 30 min. In each group, 50% of the animals were conditioned to the rough compartment and the other 50% were conditioned to the smooth compartment. On day 10, the wall was removed and animals were again allowed to explore the arena for 20 min (test trial). Behavioural scoring was performed as described above, with the exception that this time rearing and grooming were also scored using Ethovision Software (Noldus BV, Wageningen, Netherlands) by manually pressing keys on the keyboard upon occurrence of the specified behaviour.

Statistical analysis

The neurochemical data were expressed as percentage of the mean of the four baseline samples taken as 100%. Two-way ANOVA with repeated measures were calculated with the factors TIME (19 time points) and GROUP (six groups). When appropriate, this was followed by one-way ANOVA and *posthoc* Scheffé test to analyse group differences at individual time points.

For the CPP experiments, the sojourn time in the conditioning quadrant during baseline was analysed by one-way ANOVA with 'group' as factor and 'time in treatment quadrant during the baseline trial' as dependent variable. Differences from chance level (15 min ÷ 4 = 3 min 45 s) were analysed by *t* tests. The analysis of the sojourn times in the treatment quadrant during baseline and test trials was performed using two-way ANOVA with repeated measures and *posthoc* paired *t*-test for within-groups differences when appropriate. Separate two-way ANOVA with repeated measures were calculated for the analysis of the data for locomotor activity, rearing and grooming during the conditioning trials, with *posthoc* Scheffé test when appropriate.

Results

Experiment I – Dopamine and its metabolites in the core and shell of the nucleus accumbens

After the histological screening for correct probe placement, or due to leakage of dialysis probes during the course of experimentation, 57 animals were considered for statistical analysis. In some animals only the data from NAc core or shell were considered. The sample sizes are as follows: NAc core, vehicle–vehicle, $n = 6$; SR142801 0.2 mg/kg–vehicle, $n = 6$; SR142801 2.0 mg/kg–vehicle, $n = 6$; vehicle–cocaine, $n = 8$; SR142801 0.2 mg/kg–cocaine, $n = 7$; SR142801 2.0 mg/kg–cocaine, $n = 7$. NAc shell, vehicle–vehicle, $n = 5$; SR142801 0.2 mg/kg–vehicle, $n = 6$; SR142801 2.0 mg/kg–vehicle, $n = 5$; vehicle–cocaine, $n = 8$; SR142801 0.2 mg/kg–cocaine, $n = 6$; SR142801 2.0 mg/kg–cocaine, $n = 5$.

Nucleus accumbens core

The localization of the dialysis membrane in the NAc core is shown in Fig. 1A and C. The basal levels of DA were 1.27 ± 0.13 pg (mean ± SEM) in the NAc core. Basal values of DA did not differ between groups ($P > 0.05$, one-way ANOVA). Two-way ANOVA yielded an effect of TIME ($F_{18,630} = 69.504$, $P < 0.001$) and GROUP ($F_{5,35} = 22.548$, $P < 0.001$) and a TIME–GROUP interaction ($F_{90,630} = 69.504$, $P < 0.001$). One-way ANOVA followed by *posthoc* tests showed that cocaine increased DA beginning in the first and lasting until the third interval after injection (Ps < 0.015 vs. vehicle–vehicle). This effect was maximal in the second interval after cocaine with a level of 330% of baseline (Fig. 2A). This increase was further potentiated to 592% and 562% of baseline by pretreatment with 0.2 mg/kg and 2.0 mg/kg SR142801, respectively. One-way ANOVA followed by *posthoc* tests showed that DA levels were significantly higher compared to the vehicle–cocaine group in the first to third intervals in the group pretreated with 0.2 mg/kg of SR142801 (Ps < 0.017) and in the second and third intervals in the group pretreated with 2.0 mg/kg of SR142801 ($P = 0.007$ and $P = 0.046$, respectively). When compared to the vehicle–vehicle group, the levels of DA were significantly higher in the first to seventh intervals in the group pretreated with 0.2 mg/kg SR142801 (Ps = 0.001, except for the seventh interval where $P = 0.039$) and from the first to the sixth intervals in the group treated with 2.0 mg/kg SR142801 prior to

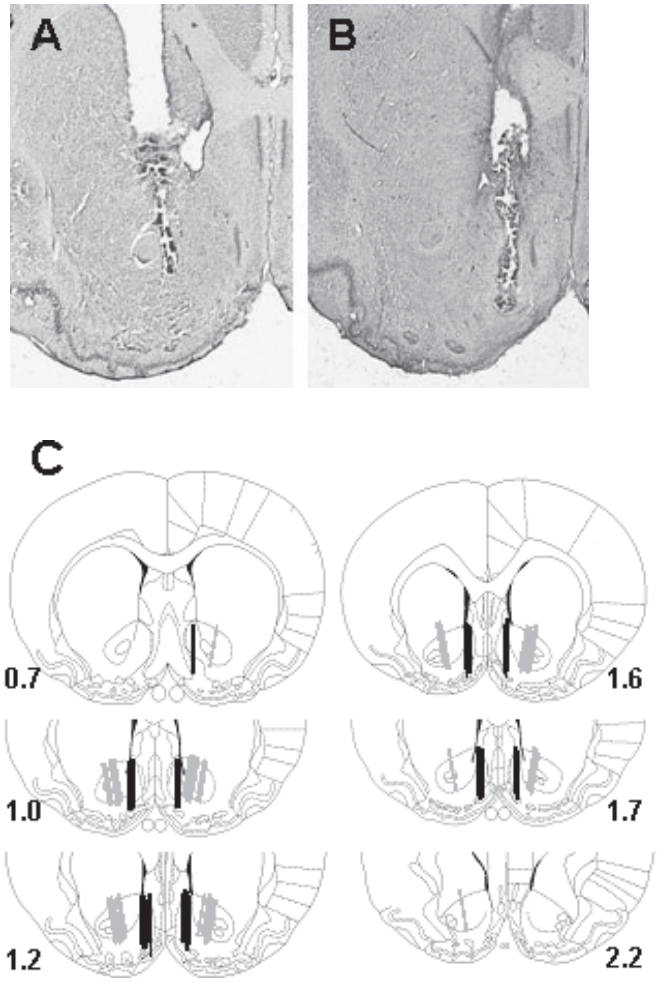

FIG. 1. Photomicrograph of a coronal section through the rat brain depicting the localization of the dialysis probes in the nucleus accumbens shell (A) and core (B). (C) Schematic representation of the placement of the dialysis probes in the core and shell of the nucleus accumbens. The grey bars represent the localization of the dialysis membrane in the nucleus accumbens core, and the black in the nucleus accumbens shell.

cocaine (Ps = 0.004); SR142801 alone had no effect at both doses tested.

Basal levels of DOPAC were 1016.65 ± 58.45 pg (mean ± SEM) in the NAc core. Basal values of DOPAC did not differ between groups ($P > 0.05$). Cocaine decreased DOPAC levels to 69% of baseline in the eleventh interval after cocaine (Fig. 2B). Two-way ANOVA yielded an effect of TIME ($F_{18,630} = 52.466$, $P < 0.001$) and GROUP ($F_{5,35} = 2.691$, $P < 0.04$) and a TIME–GROUP interaction ($F_{90,630} = 3.246$, $P < 0.001$). One-way ANOVA followed by *posthoc* test showed that pretreatment with SR142801 prior to cocaine incurred a decline in DOPAC levels compared to the vehicle–vehicle group in the seventh interval ($P = 0.05$, at the dose of 0.2 mg/kg). The antagonist on its own was without effect.

Basal levels of HVA were 539.58 ± 37.20 pg (mean ± SEM) in the NAc core. Basal values of HVA did not differ between groups ($P > 0.05$). Cocaine incurred a decrease in HVA levels (Fig. 2C). Two-way ANOVA yielded an effect of TIME ($F_{18,630} = 23.638$, $P < 0.001$) and a TIME–GROUP interaction ($F_{90,630} = 1.937$, $P < 0.004$), but no effect of GROUP ($P > 0.05$). One-way ANOVA followed by *posthoc* tests did not reveal any differences between groups at any of the 19 time points ($P > 0.05$).

Nucleus accumbens shell

The localization of the dialysis membrane in the NAc shell is shown in Fig. 1B and C. The basal levels of DA were 0.83 ± 0.12 pg (mean ± SEM) in the NAc shell. Basal values of DA did not differ between groups ($P > 0.05$, one-way ANOVA). Two-way ANOVA yielded an effect of TIME ($F_{18,504} = 40.989$, $P < 0.001$) and GROUP ($F_{5,28} = 10.153$, $P < 0.001$) and a TIME–GROUP interaction ($F_{90,504} = 7.463$, $P < 0.001$). One-way ANOVA followed by *posthoc* tests showed that cocaine led to an increase in DA that was significant in the second, third and sixth intervals after injection (Ps < 0.041). This effect was maximal in the second interval after cocaine injection with a level of 480% of baseline (Fig. 3A). Unlike in the NAc core, this was not influenced by pretreatment with either dose of the antagonist (Ps > 0.05 vs. vehicle–cocaine). When compared against the vehicle–vehicle group, DA levels were significantly increased in the first to third and seventh intervals in the group pretreated with 0.2 mg/kg SR142801 (Ps = 0.044) and from the first to the seventh intervals in the group treated with 2.0 mg/kg SR142801 prior to cocaine (Ps = 0.017, except for the first interval where $P = 0.044$). Again, SR142801 alone was without effect at both doses (Ps > 0.05).

Mean basal level of DOPAC was 593.84 ± 51.87 pg in the NAc shell. Basal values of DOPAC did not differ between groups ($P > 0.05$). In the NAc shell cocaine decreased DOPAC levels to 73% of baseline in the eighth and eleventh sampling intervals after injection (Fig. 3B). Two-way ANOVA yielded an effect of TIME ($F_{18,504} = 59.243$, $P < 0.001$) and GROUP ($F_{5,28} = 4.625$, $P < 0.004$) and a TIME–GROUP interaction ($F_{90,504} = 4.299$, $P < 0.001$). One-way ANOVA followed by *posthoc* tests indicated that 0.2 mg/kg SR142801 followed by cocaine decreased DOPAC levels compared to the vehicle–vehicle group in the fourth to eighth sampling intervals (Ps = 0.043). Similarly, pretreatment with 2.0 mg/kg SR142801 prior to cocaine led to a decline in DOPAC levels compared to the vehicle–vehicle group in the fifth, sixth and eighth intervals (Ps = 0.045). The antagonist on its own was without effect.

Basal levels of HVA were 253.34 ± 24.23 pg (mean ± SEM) in the NAc shell. Basal values of HVA did not differ between groups ($P > 0.05$; Fig. 3C). Two-way ANOVA yielded an effect of TIME ($F_{18,504} = 33.043$, $P < 0.001$) and GROUP ($F_{5,28} = 2.693$, $P < 0.05$) and a TIME–GROUP interaction ($F_{90,504} = 3.524$, $P < 0.001$). One-way ANOVA followed by *posthoc* test showed that pretreatment with 2.0 mg/kg SR142801 prior to cocaine decreased HVA levels compared to the vehicle–vehicle group in the sixth, eight and ninth sampling intervals (Ps = 0.04, Fig. 3E).

Comparison of DA dynamics between nucleus accumbens core and shell

It had been reported previously that the dopamine response to drugs of abuse is greater in the shell compared to the core of the NAc (Pontieri *et al.*, 1995). Therefore, we also compared the DA per cent values after cocaine injection between the two subterritories by two-way ANOVA with repeated measures and *posthoc t*-test when appropriate. A significant effect of TIME ($F_{11,132} = 30.366$, $P < 0.001$) and STRUCTURE ($F_{1,12} = 7.734$, $P = 0.017$) but no TIME–STRUCTURE interaction was obtained. *Posthoc* tests showed that the DA response to cocaine was greater in the NAc shell than in the NAc core from the second to the sixth ($P < 0.03$) and in the ninth and twelfth sampling intervals after cocaine ($P < 0.025$).

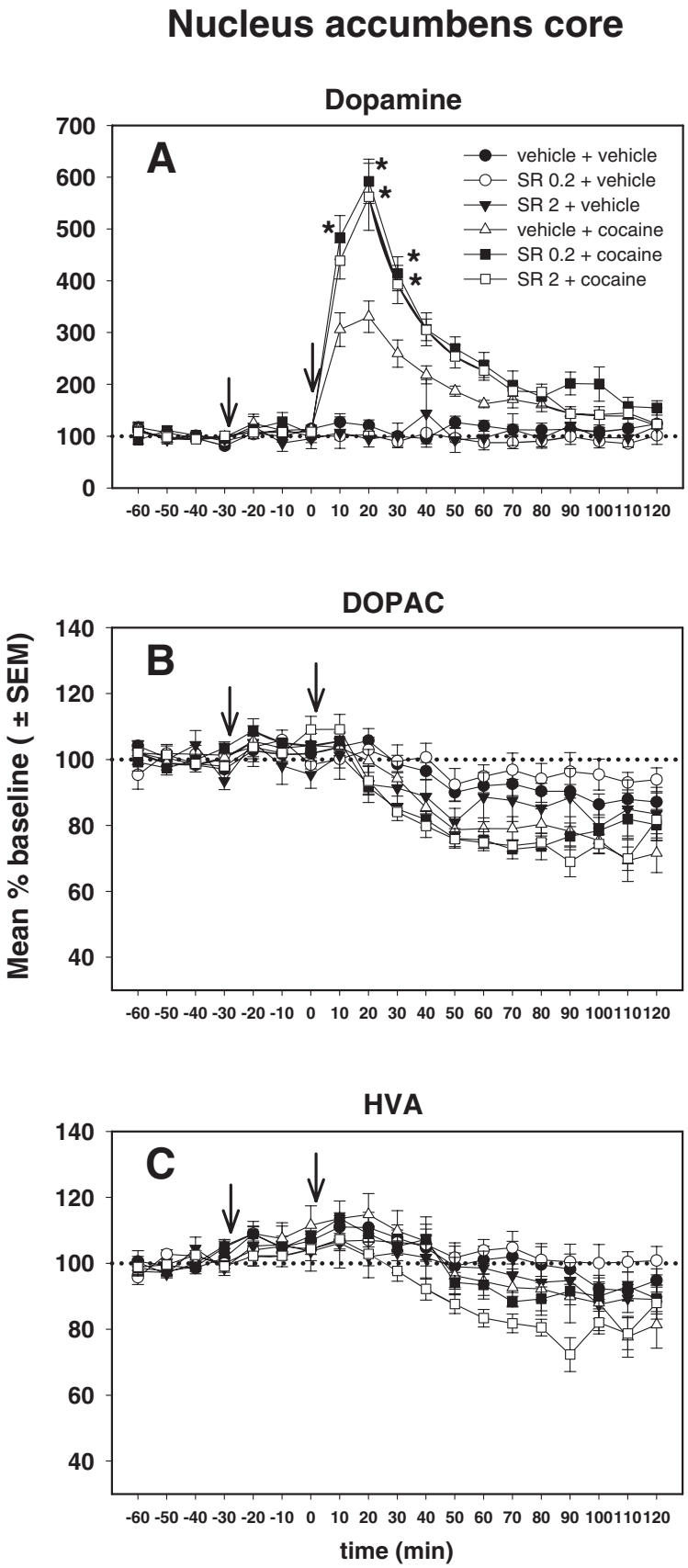

FIG. 2. Effect of the combined treatment with vehicle or SR142801 (0.2 and 2 mg/kg) followed 30 min later by vehicle or cocaine on the time course of extracellular levels of DA (A), DOPAC (B) and HVA (C) in the nucleus accumbens core. Values are expressed as mean (± SEM) percentage of baseline (average of the four baseline samples taken as 100%). Arrows indicate the time-point of the injections. Only differences from the vehicle–cocaine group are represented. *$P < 0.05$ vs. vehicle–cocaine group. ANOVA followed by *posthoc* Scheffé test.

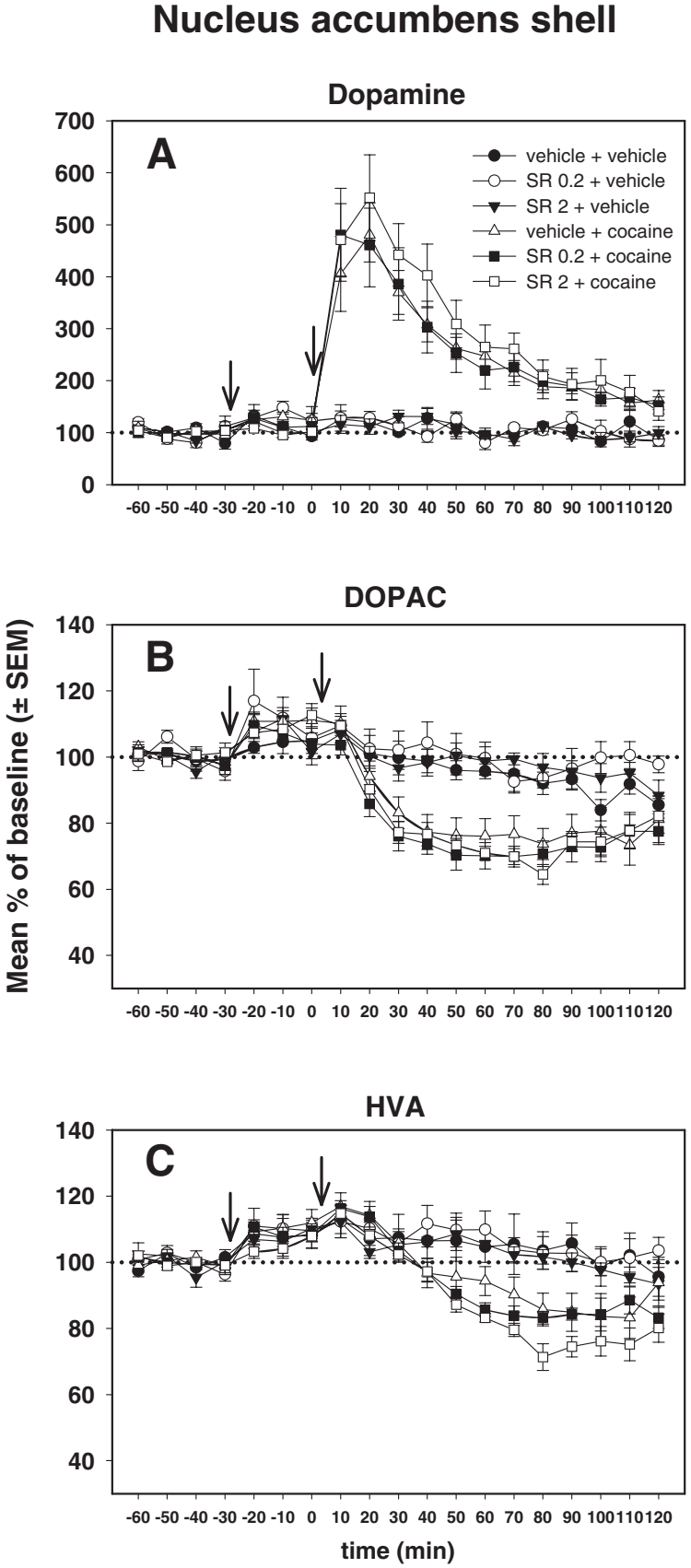

FIG. 3. Effect of the combined treatment with vehicle or SR142801 (0.2 and 2 mg/kg) followed 30 min later by vehicle or cocaine on the time course of extracellular levels of DA (A), DOPAC (B) and HVA (C) in the nucleus accumbens shell. Values are expressed as mean (± SEM) percentage of baseline (average of the four baseline samples taken as 100%). Arrows indicate the time-point of the injections.

Experiment II – Conditioned place preference

The sample sizes are as follows: vehicle–vehicle, $n = 10$; SR142801 0.2 mg/kg–vehicle, $n = 9$; SR142801 2.0 mg/kg–vehicle, $n = 9$; SR142801 20.0 mg/kg–vehicle, $n = 10$; vehicle–cocaine, $n = 9$; SR142801 0.2 mg/kg–cocaine, $n = 9$; SR142801 2.0 mg/kg–cocaine, $n = 10$; SR142801 20.0 mg/kg–cocaine, $n = 10$.

Place preference

There was no difference in the time spent in the conditioning quadrant in the baseline trial between groups ($F_{7,68} = 0.235$, $P = 0.975$). For all the groups, the mean time spent in the conditioning quadrant during baseline trial were not different from chance level (Ps > 0.45). The two-way ANOVA showed a significant TRIAL effect for the sojourn times during baseline and test trials ($F_{1,68} = 4.822$, $P = 0.032$), but no GROUP effect ($F_{7,68} = 0.832$, $P = 0.564$) and no TRIAL–GROUP interaction ($F_{7,68} = 0.472$, $P = 0.851$). Descriptively, there was an increase of time spent in the conditioning quadrant in the test trial compared to baseline for the vehicle–cocaine group. The mean time (± SEM) for the vehicle–cocaine group in the baseline and test trials were 221.42 (± 9.78) and 297.82 (± 34.01), respectively (Fig. 4). When the animals were pretreated with SR142801 at doses of 2.0 mg/kg, the mean time (± SEM) in the baseline and test trials were 213.55 (± 13.66) and 210.90 (± 31.44), respectively, indicating a slight tendency of SR142801 for antagonizing the CPP induced by cocaine.

Activity parameters

Two-way ANOVA yielded a significant GROUP effect ($F_{7,68} = 21.382$, $P < 0.001$) for locomotor activity (Fig. 5A). As the two-way ANOVA did not yield an effect of time over the three conditioning trial, *posthoc* Scheffé tests were calculated on the mean values of the three conditioning trial. They showed that cocaine increased locomotor activity compared to the vehicle–vehicle group ($P < 0.001$). This effect was blocked by pretreatment with 0.2 mg/kg SR142801 ($P = 0.033$ vs. vehicle–cocaine) but not by either 2.0 mg/kg or 20.0 mg/kg ($P > 0.05$). None of the doses of SR142801 had an influence on locomotor activity when followed by vehicle injection ($P > 0.05$ vs. vehicle–vehicle).

Two-way ANOVA also yielded a significant GROUP effect for the frequency of rearing ($F_{7,68} = 14.053$, $P < 0.001$, Fig. 5B). As there was no effect of time, *posthoc* tests were calculated on the mean frequency of rearing over the three conditioning trials. It showed that cocaine led to an increase in the frequency of rearing compared to the vehicle–vehicle group ($P < 0.001$). This effect of cocaine on rearing behaviour was tendencially antagonized by pretreatment with 0.2 mg/kg ($P < 0.069$ vs. vehicle–cocaine), but not 2.0 mg/kg or 20.0 mg/kg SR142801 ($P > 0.05$). While the group treated with 0.2 mg/kg and 2.0 mg/kg SR142801 prior to cocaine did not show a higher frequency of rearing than the vehicle–vehicle group ($P = 0.378$ and $P = 0.143$, respectively) the group treated with 20 mg/kg SR142801 prior to cocaine differed from the vehicle–vehicle group ($P = 0.005$). None of the doses of SR142801 had an influence on the frequency of rearing when given alone ($P > 0.05$ vs. vehicle–vehicle). A significant GROUP effect was also observed for the total duration of rearing ($F_{7,68} = 6.808$, $P < 0.001$, two-way ANOVA, data not shown). The mean of the total duration of rearings for the three conditioning trials was calculated. *Posthoc* test on the mean values showed that cocaine led to an increase in the duration of rearing compared to the vehicle–vehicle group ($P = 0.003$). None of the doses of SR142801 significantly influenced the duration of rearing when given alone ($P > 0.05$ vs. vehicle–vehicle).

The total duration of grooming behaviour was decreased by cocaine (Fig. 5C). Two-way ANOVA yielded a significant GROUP effect for this parameter ($F_{7,68} = 13.607$, $P < 0.001$). Again, *posthoc* tests were calculated on the mean values of the three conditioning trials. They showed that cocaine suppressed grooming compared to the vehicle–

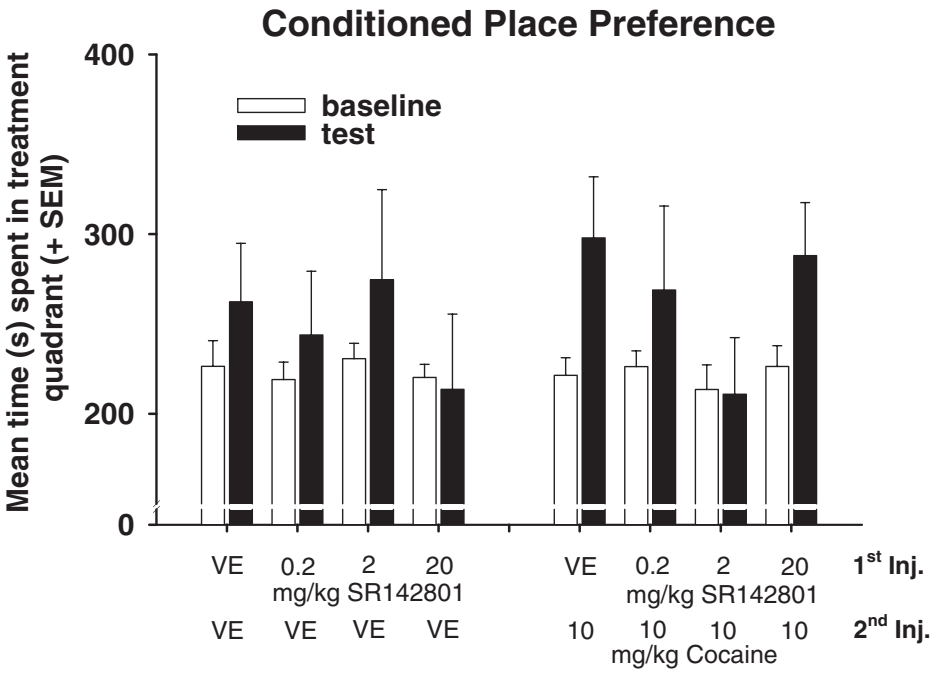

FIG. 4. Conditioned place preference – time spent in the treatment quadrant during baseline (white bars) and test trials (black bars, mean ± SEM). During the conditioning trials, animals received either vehicle (VE) or SR142801 (0.2; 2 or 20 mg/kg i.p.) followed 30 min later by vehicle (VE) or cocaine.

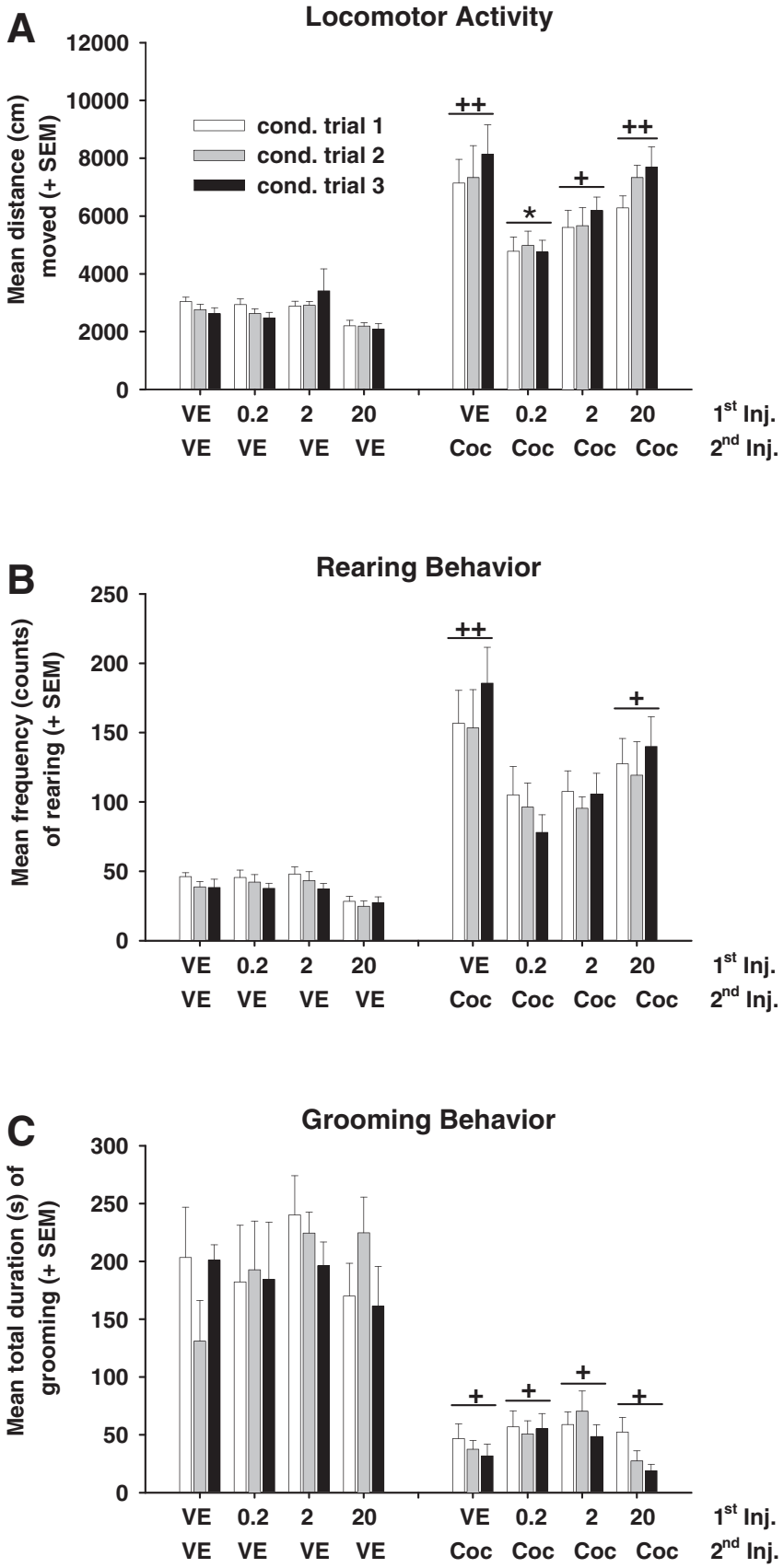

FIG. 5. Behavioural activity during the conditioning trials. Animals received vehicle (VE) or SR142801 (0.2; 2 or 20 mg/kg i.p.) followed 30 min later by vehicle or cocaine (Coc). (A) Lomotor activity: mean distance (cm) + SEM moved. (B) Rearing behaviour, mean frequency of rearings + SEM. (C) Grooming behaviour, mean total duration (seconds) + SEM. *$P < 0.05$, vs. vehicle–cocaine group, +$P < 0.05$, ++$P < 0.01$ vs. vehicle–vehicle group. ANOVA followed by Scheffe test.

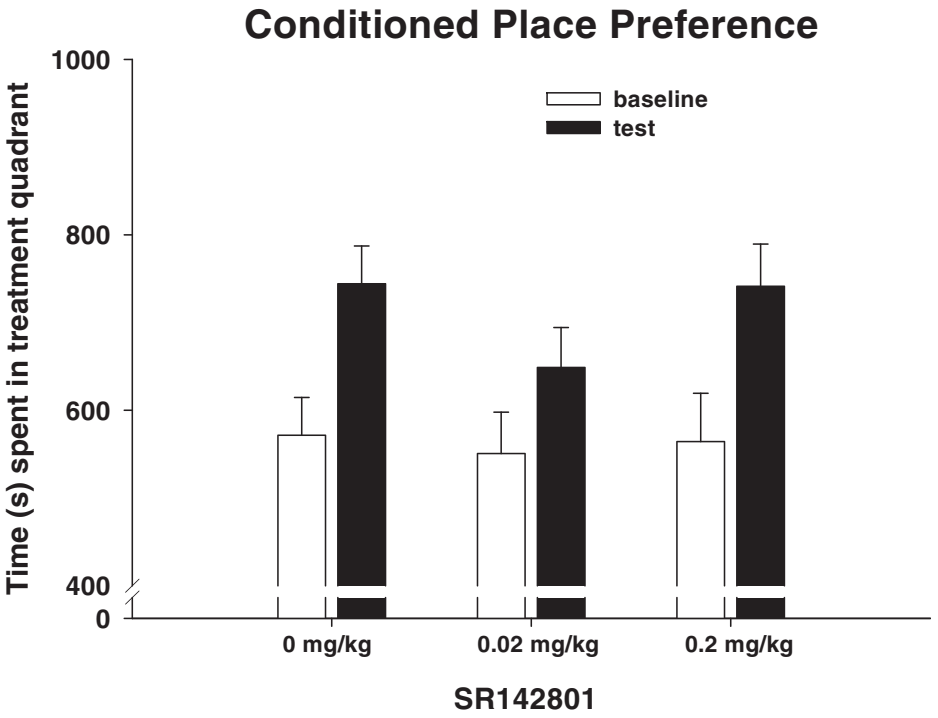

FIG. 6. Conditioned place preference in the two-compartment procedure – time spent in the treatment quadrant during baseline (white bars) and test trials (black bars, mean ± SEM). During the conditioning trials, animals received either vehicle or SR142801 (0.02; or 0.2 mg/kg i.p.) followed 30 min later by cocaine (10 mg/kg).

vehicle group ($P = 0.008$). This effect was not influenced by pretreatment with SR142801 at any of the three doses tested ($P < 0.05$ in all cases). SR142801 on its own had no effect on grooming behaviour at any of the three doses tested ($P > 0.05$ vs. vehicle–vehicle). There was no effect of TRIAL or a TRIAL–GROUP interaction for locomotion, rearing or grooming behaviours.

Experiment III – Conditioned place preference

The sample sizes are as follows: vehicle–cocaine, $n = 15$; SR142801 0.02 mg/kg–cocaine, $n = 15$; and SR142801 0.2 mg/kg–cocaine, $n = 15$.

Place preference

Two-way ANOVA showed a significant TRIAL effect for the sojourn times during baseline and test trials ($F_{1,42} = 33.667$, $P < 0.001$), but no GROUP effect ($F_{2,42} = 0.599$, $P = 0.554$) and no TRIAL–GROUP interaction ($F_{2,42} = 0.987$, $P = 0.381$). Descriptively, there was again an increase of time spent in the conditioning quadrant in the test trial compared to baseline for the vehicle–cocaine group. The mean times (± SEM) for the vehicle–cocaine group in the baseline and test trials were 571.48 (± 43.02) and 744.20 (± 43.09), respectively (Fig. 6). When the animals were pretreated with SR142801 at doses of 0.02 mg/kg, the mean time (± SEM) in the baseline and test trials was 550.60 (± 47.14) and 648.85 (± 45.48), respectively. With SR142801 at doses of 0.2 mg/kg, the mean time (± SEM) in the baseline and test trials was 564.27 (± 55.16) and 741,33 (± 48.16), respectively.

Activity parameters

Like in experiment II, pretreatment with SR142801 reduced cocaine-induced locomotor activation (Fig. 7A). Two-way ANOVA yielded a significant GROUP effect ($F_{2,42} = 3.846$, $P = 0.029$), but no TRIAL effect nor a GROUP–TRIAL interaction. *Posthoc* Scheffé test showed that SR142801 at dose of 0.2 ($P = 0.036$) but not 0.02 mg/kg decreased locomotor activity compared to the vehicle–cocaine group. For a more detailed analysis of locomotor activity during baseline and test trials, we divided the trials into four 5-min bins. When comparing the first 5 min of the baseline with the first 5 min of the test trial, a pronounced conditioned locomotor effect became evident (Fig. 7B). Two-way ANOVA showed an effect of TIME ($F_{1,42} = 62.69$, $P < 0.001$) and an effect of GROUP ($F_{2,42} = 3.324$; $p = 0.046$) but no TIME–GROUP interaction. However, *posthoc* tests revealed no differences between individual groups. Calculation of the slopes from the four bins during baseline and test trial showed that there was a steeper decrease in activity in the test trial compared to the baseline trial (two-way ANOVA, Factor TIME: $F_{1,42} = 78.139$, $P < 0.001$) but this decrease did not differ between groups (Factor GROUP: $F_{2,42} = 1.2$; $P = 0.311$).

Analysis of the parameters rearing frequency, total duration of rearing and grooming did not show any differences between groups (data not shown).

Discussion

The results of the present study demonstrated that NK$_3$-R antagonism potentiated the cocaine-induced increase in DA-levels in the NAc core, but not in the NAc shell. DA levels were increased by cocaine to a larger extent in the shell as compared to the core of the NAc. Cocaine caused a decline in DOPAC and HVA levels in both NAc core and shell; however, this was not influenced by NK$_3$–R antagonism. Neither DA nor DOPAC or HVA concentrations in the NAc core and shell were significantly influenced by SR142801 alone. NK$_3$–Rs antagonism blocked the acute locomotor stimulant effects of cocaine,

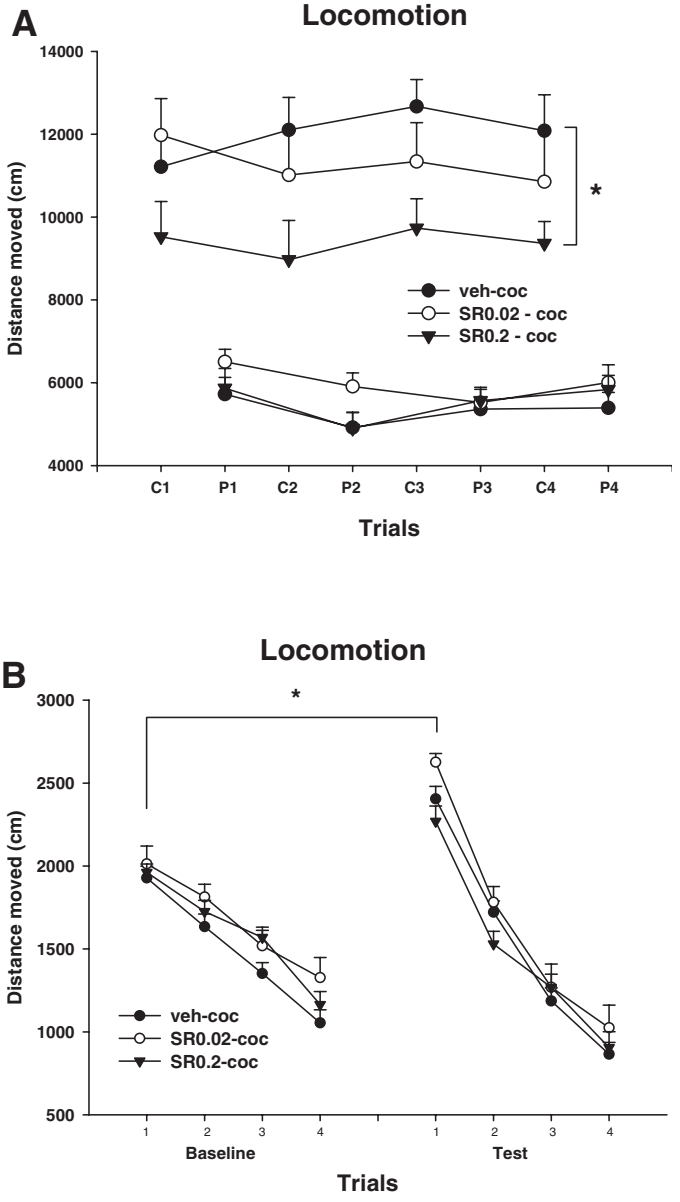

FIG. 7. Behavioural activity during the conditioning trials and during the baseline and test trial. Animals received vehicle (veh) or SR142801 (0.02 or 0.2 mg/kg i.p.) followed 30 min later by cocaine (coc) during the conditioning trials and injections of the corresponding vehicle during the pseudoconditioning trials. Values are mean + SEM. (A) Lomotor activity, distance (cm) moved during the conditioning (C1 to C4) and pseudoconditioning (P1 to P4) trials. $*P < 0.05$, vs. vehicle–cocaine group, two-way ANOVA followed by Scheffe test. (B) Time course of locomotor activity during the baseline and test trials. The time points represent the four five minute intervals of the baseline and test trial. $*P < 0.05$, baseline vs. test (first 5-min intervals), two-way ANOVA followed by paired *t*-test.

but not the suppression of grooming. While in the first CPP experiment there was an indication for an attenuation of cocaine-induced CPP by NK_3–R antagonism at the dose of 2 mg/kg, this effect was no longer found when we employed lower doses of the NK_3–R antagonist in a two-compartment CPP procedure. Repeated cocaine application induced a conditioned locomotor response in the test phase of the CPP experiment, which, however, was unaffected by NK_3–R antagonism. The NK_3–R antagonist by itself had no effect on CPP, locomotion, rearing and grooming behaviour.

An increase in dopaminergic transmission in the NAc is commonly regarded as a critical substrate for the place preference conditioning elicited by drugs of abuse. Cocaine increases DA transmission preferentially in the NAc shell as compared to the NAc core (Pontieri *et al*., 1995; Di Chiara, 2002). While the NAc shell supports intracranial self-administration of cocaine, the NAc core does not (Rodd-Henricks *et al*., 2002). In line with the findings of Pontieri *et al*. (1995), in the present study cocaine increased DA preferentially in the NAc shell to 480% of baseline compared to 330% in the NAc core. Pretreatment with SR142801 (0.2 and 2.0 mg/kg) potentiated the DA response, specifically in the NAc core, but not in the NAc shell. The time course of the potentiated DA response did not differ between the 0.2 and the 2.0 mg/kg SR142801–cocaine groups.

Previous findings suggest a contribution of NK_3-Rs to reinforcement processes. In a CPP paradigm, reinforcing effects of SP and its C-terminal fragment DiMe-C7 were found upon peripheral and central administration (Hasenöhrl *et al*., 1990). Blockade of the NK_1-Rs did not completely antagonize CPP induced by injection of DiMe-C7 into the NBM region (Nikolaus *et al*., 1999). The NK_3-R agonist aminosenktide reduced alcohol consumption in alcohol preferring rats (Ciccocioppo *et al*., 1995), and induced CPP after i.c.v. administration (Massi *et al*., 2000). Thus, while there is evidence that activation of NK_3-Rs, per se, is reinforcing, it is not clear whether these receptors are also necessary for cocaine-mediated reinforcement. In contrast, an absence of a relation between NK_3–Rs antagonism and cocaine-induced CPP is in agreement with the observed lack of effect of NK_3–Rs antagonism on cocaine-evoked increase in DA levels in the shell of the NAc, which is the subregion of the NAc that is involved in the mediation of CPP by cocaine (Rodd-Henricks *et al*., 2002).

Cocaine-induced hyperlocomotion was antagonized by SR142801 at the dose of 0.2 mg/kg, but not by higher or lower doses. The cocaine-induced increase in rearing frequency, although not significantly antagonized, was diminished both by 0.2 mg/kg and 2.0 mg/kg SR142801. Cocaine also caused a conditioned increase in locomotor activity, which, however, was not affected by NK_3–R antagonism. Interesting in this respect is that injections of SP and DiMe-C7 into the VTA and SN enhance locomotor and rearing behaviours (Kelley *et al*., 1979; Eison *et al*., 1982; Barnes *et al*., 1990; Elliott *et al*., 1991). This modulation appears to be mediated by a dopaminergic mechanism, as the locomotor enhancing effects of intra-VTA administration of the NK_3-R agonist DiMe-C7 were blocked by both D_1- or D_2-R antagonists (Placenza *et al*., 2004). These results are in line with our finding of an involvement of the NK_3-R in cocaine-mediated locomotor and rearing behaviour.

The suppression of grooming behaviour induced by cocaine was not antagonized by any of the three doses of SR142801 tested. Indirectly, the lack of antagonism of SR142801 on cocaine-induced inhibition of grooming behaviour supports a selective action of this compound on NK_3-R, as grooming behaviour is elicited by administration of NK_1-R agonists into the SN (Stoessl *et al*., 1991; Stoessl *et al*., 1995). Treatment with the NK_3-R antagonist alone did not significantly influence any of the behavioural measures analysed.

The doses for this study were chosen taking into consideration the known species specificity of the nonpeptide NK_3-R antagonists (Emonds-Alt *et al*., 1995; Jung *et al*., 1996). SR142801 has approximately 35 times higher binding affinity to the gerbil's NK_3-R than to the rat's NK_3-R (Emonds-Alt *et al*., 1995). In the gerbil, 0.3 mg/kg SR142801 i.p. was the smallest dose required to antagonize the contralateral rotations induced by intrastriatal senktide (Emonds-Alt *et al*., 1995). The fact that lower doses of SR142801 were the most effective in antagonizing the hyperlocomotor effects of cocaine in this

study could indicate that in the rat higher amounts of the compound reach the brain after i.p. administration. Unfortunately, data on brain penetrability of SR142801 are available only for the gerbil after subcutaneous administration (Langlois *et al*., 2001a). The effectiveness of lower doses of SR142801 in antagonizing the behavioural effects of cocaine in the rat could also be due to the higher density of NK_3-R in areas of the brain known to mediate such effects of cocaine, such as the VTA, SNc, striatum and NAc, in comparison to the gerbil and guinea pig (Langlois *et al*., 2001b). Indeed, senktide can have markedly different behavioural effects in different species, such as a reduction of locomotor activity in gerbils and cause head twitches in rats and mice (Jung *et al*., 1996; Sarau *et al*., 2000). In the marmoset (*Callithrix penicillata*), pretreatment with 0.02 mg/kg SR142801 antagonized the enhanced locomotor activity induced by systemic cocaine administration (10 mg/kg; de Souza Silva *et al*., 2006). It is also unlikely that the effects of SR142801 are due to a binding to other NK-Rs, as SR142801 exerts inhibitory effects on NK_1- and NK_2-Rs and on the binding of specific ligands to other membrane proteins, such as opioid-Rs and calcium and sodium channels at much higher concentrations (Nguyen *et al*., 1996).

The potentiation of the cocaine-induced DA increase in the NAc core was surprising, given that local application of senktide into the VTA enhanced DA cell firing (Seabrook *et al*., 1995), and NK_3-R blockade decreased the release of dopamine induced by senktide applied into the SNc and VTA (Marco *et al*., 1998). Given that the drugs were administered systemically, the brain mechanisms involved in such findings are difficult to discern. However, a few possibilities can be considered. (i) The NAc core has been proposed to be part of a subcircuit, which is more extensively connected with motor structures, and includes the dorsal PFC, dorsolateral area of the ventral pallidum (VP) and SN, whereas the NAc shell is part of a subcircuit, which is interconnected with limbic structures, and also includes the ventral PFC, medial VP, amygdala and VTA; these are referred to as the 'motor' and 'limbic' subcircuits, respectively (Deutch & Cameron, 1992; Zahm & Brog, 1992; Groenewegen *et al*., 1996). Although NK_3-Rs in the SN and VTA are localized on DAergic cells (Chen *et al*., 1998), there is indication that in the SN the majority of the NK_3-Rs are localized on dopaminergic neurons, while in the VTA two-third of the NK_3-Rs are localized on nondopaminergic neurons (Stoessl, 1994). Therefore, the different effects of NK_3-R antagonism on cocaine-induced increase in levels of DA in the NAc core and shell could have their source at the level of SN/VTA. (ii) It is also possible that the enhanced DA levels in the NAc core are the result of an action of the NK_3 antagonist at the level of the DA terminals within this area, considering that NK_3-Rs are expressed in the NAc core, but not in the NAc shell (Ding *et al*., 1996). (iii) Besides the SN/VTA and NAc, other structures, such as the neocortex and VP, which are also rich in NK_3-R (Ding *et al*., 1996; Shughrue *et al*., 1996), may have influenced the effects of SR142801 on the cocaine-induced behavioural stimulation and increase of DA in the NAc core (Goeders & Smith, 1993). (iv) Finally, it is also possible that the action of the NK_3-R antagonist on the reinforcing and hyperlocomotor effects of cocaine involves monoaminergic systems besides the dopaminergic ones. The serotonergic system, e.g. which has been shown to play a major role in cocaine effects on behaviour (Sora *et al*., 2001; Müller & Huston, 2006), can be modulated by NK_3-Rs (Stoessl *et al*., 1990). While the increase of DA levels in the NAc seems to be necessary for the expression of the behavioural activating and reinforcing effects of cocaine (Di Chiara *et al*., 2004), these effects can be modulated without affecting the dopaminergic activity in these structures (Müller *et al*., 2002).

This study provides the first evidence for an involvement of NK_3-Rs in the acute effects of cocaine. The NK_3-R antagonist SR142801 potentiated cocaine-induced DA activity in the NAc core, whereas no such effect was observed in the NAc shell. These effects were concomitant with a blockade of the cocaine-induced hyperactivity. These results tentatively suggest that the pharmacological manipulation of NK_3-Rs might find application in the treatment of cocaine addiction.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (DFG, DE 792/2–2). G.J. received a stipend from the Graduiertenkolleg 320 'Pathological processes of the nervous system: from genes to behaviour'. We thank Sanofi Recherche for the generous supply of SR142801 and Dr H. Frahm and C. Stolze for the skilfull preparation of the photomicrographs.

Abbreviations

CPP, conditioned place preference; DA, dopamine; DOPAC, 4-dihydroxyphenyl acetic acid; HVA, homovanillic acid; NAC, nucleus accumbens; NK-Rs, neurokinin receptors; SP, substance P; SN, substantia nigra; VP, ventral pallidum; VTA, ventral tegmental area.

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 analogue, DiMe-C7, into the rat ventral tegmental area. *Pharmacol. Biochem. Behav.*, **37**, 839–841.

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.

Chen, L.W., Guan, Z.L. & Ding, Y.Q. (1998) Mesencephalic dopaminergic neurons expressing neuromedin K receptor (NK_3): a double immunocytochemical study in the rat. *Brain Res.*, **780**, 150–154.

Ciccocioppo, R., Panocka, I., Polidori, C., Froldi, R., Angeletti, S. & Massi, M. (1998) Mechanism of action for reduction of ethanol intake in rats by the tachykinin NK-3 receptor agonist aminosenktide. *Pharmacol. Biochem. Behav.*, **61**, 459–464.

Ciccocioppo, R., Panocka, I., Pompei, P., Polidori, C., De Caro, G. & Massi, M. (1995) Subcutaneous injections of the tachykinin senktide reduce alcohol intake in alcohol-preferring rats. *Peptides*, **16**, 533–537.

Deutch, A.Y. & Cameron, D.S. (1992) Pharmacological characterization of dopamine systems in the nucleus accumbens core and shell. *Neuroscience*, **46**, 49–56.

Di Chiara, G. (2002) Nucleus accumbens shell and core dopamine: differential role in behavior and addiction. *Behav. Brain Res.*, **137**, 75–114.

Di Chiara, G., Bassareo, V., Fenu, S., De Luca, M.A., Spina, L., Cadoni, C., Acquas, E., Carboni, E., Valentini, V. & Lecca, D. (2004) Dopamine and drug addiction: the nucleus accumbens shell connection. *Neuropharmacology*, **47**, 227–241.

Ding, Y.Q., Shigemoto, R., Takada, M., Ohishi, H., Nakanishi, S. & Mizuno, N. (1996) Localization of the neuromedin K receptor (NK3) in the central nervous system of the rat. *J. Comp. Neurol.*, **364**, 290–310.

Eison, A.S., Eison, M.S. & Iversen, S.D. (1982) The behavioural effects of a novel substance P analogue following infusion into the ventral tegmental area or substantia nigra of rat brain. *Brain Res.*, **238**, 137–152.

Elliott, P.J., Mason, G.S., Stephens-Smith, M. & Hagan, R.M. (1991) Behavioural and biochemical responses following activation of midbrain dopamine pathways by receptor selective neurokinin agonists. *Neuropeptides*, **19**, 119–126.

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.

Goeders, N.E. & Smith, J.E. (1993) Intracranial cocaine self-administration into the medial prefrontal cortex increases dopamine turnover in the nucleus accumbens. *J. Pharmacol. Exp. Ther.*, **265**, 592–600.

Groenewegen, H.J., Wright, C.I. & Beijer, A.V. (1996) The nucleus accumbens: gateway for limbic structures to reach the motor system? *Prog. Brain Res.*, **107**, 485–511.

Hasenöhrl, R.U., de Souza-Silva, M.A., Nikolaus, S., Tomaz, C., Brandao, M.L., Schwarting, R.K. & Huston, J.P. (2000) Substance P and its role in neural mechanisms governing learning, anxiety and functional recovery. *Neuropeptides*, **34**, 274–280.

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., Oitzl, M.S. & Huston, J.P. (1989) Conditioned place preference in the corral: a procedure for measuring reinforcing properties of drugs. *J. Neurosci. Meth.*, **30**, 141–146.

Jung, M., Michaud, J.C., Steinberg, R., Barnouin, M.C., Hayar, A., Mons, G., Souilhac, J., Emonds-Alt, X., Soubrie, P. & Le Fur, G. (1996) Electrophysiological, behavioural and biochemical evidence for activation of brain noradrenergic systems following neurokinin NK3 receptor stimulation. *Neuroscience*, **74**, 403–414.

Kelley, A.E., Stinus, L. & Iversen, S.D. (1979) Behavioural activation induced in the rat by substance P infusion into ventral tegmental area: implication of dopaminergic A10 neurones. *Neurosci. Lett.*, **11**, 335–339.

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.

Langlois, X., te Riele, P., Wintmolders, C., Leysen, J.E. & Jurzak, M. (2001a) Use of the beta-imager for rapid *ex vivo* autoradiography exemplified with central nervous system penetrating neurokinin 3 antagonists. *J. Pharmacol. Exp. Ther.*, **299**, 712–717.

Langlois, X., Wintmolders, C., te Riele, P., Leysen, J.E. & Jurzak, M. (2001b) Detailed distribution of neurokinin 3 receptors in the rat, guinea pig and gerbil brain: a comparative autoradiographic study. *Neuropharmacology*, **40**, 242–253.

Marco, N., Thirion, A., Mons, G., Bougault, I., Le Fur, G., Soubrie, P. & Steinberg, R. (1998) Activation of dopaminergic and cholinergic neurotransmission by tachykinin NK3 receptor stimulation: an *in vivo* microdialysis approach in guinea pig. *Neuropeptides*, **32**, 481–488.

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., de Souza Silva, M.A., DePalma, G., Tomaz, C., Carey, R.J. & Huston, J.P. (2002) The selective serotonin (1A) -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. & Huston, J.P. (2006) Determining the region-specific contributions of 5-HT receptors to the psychostimulant effects of cocaine. *Trends Pharmacol. Sci.*, **27**, 105–112.

Nguyen, L., 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.

Paxinos, G. & Watson, C. (1986) *The Rat Brain in Stereotactic Coordinates*. Academic Press, New York.

Placenza, F.M., Fletcher, P.J., Rotzinger, S. & Vaccarino, F.J. (2004) Infusion of the substance P analogue, DiMe-C7, into the ventral tegmental area induces reinstatement of cocaine-seeking behaviour in rats. *Psychopharmacology (Berl)*, **177**, 111–120.

Pontieri, F.E., Tanda, G. & Di Chiara, G. (1995) Intravenous cocaine, morphine, and amphetamine preferentially increase extracellular dopamine in the 'shell' as compared with the 'core' of the rat nucleus accumbens. *Proc. Natl Acad. Sci. USA*, **92**, 12304–12308.

Regoli, D., Boudon, A. & Fauchere, J.L. (1994) Receptors and antagonists for substance P and related peptides. *Pharmacol. Rev.*, **46**, 551–599.

Rodd-Henricks, Z.A., McKinzie, D.L., Li, T.K., Murphy, J.M. & McBride, W.J. (2002) Cocaine is self-administered into the shell but not the core of the nucleus accumbens of Wistar rats. *J. Pharmacol. Exp. Ther.*, **303**, 1216–1226.

Sarau, H.M., Griswold, D.E., Bush, B., Potts, W., Sandhu, P., Lundberg, D., Foley, J.J., Schmidt, D.B., Webb, E.F., Martin, L.D., Legos, J.J., Whitmore, R.G., Barone, F.C., Medhurst, A.D., Luttmann, M.A., Giardina, G.A. & Hay, D.W. (2000) Nonpeptide tachykinin receptor antagonists. II. Pharmacological and pharmacokinetic profile of SB-222200, a central nervous system penetrant, potent and selective NK-3 receptor antagonist. *J. Pharmacol. Exp. Ther.*, **295**, 373–381.

Seabrook, G.R., Bowery, B.J. & Hill, R.G. (1995) Pharmacology of tachykinin receptors on neurones in the ventral tegmental area of rat brain slices. *Eur. J. Pharmacol.*, **273**, 113–119.

Shughrue, P.J., Lane, M.V. & Merchenthaler, I. (1996) *In situ* hybridization analysis of the distribution of neurokinin-3 mRNA in the rat central nervous system. *J. Comp. Neurol.*, **372**, 395–414.

Sora, I., Hall, F.S., Andrews, A.M., Itokawa, M., Li, X.F., Wei, H.B., Wichems, C., Lesch, K.P., Murphy, D.L. & Uhl, G.R. (2001) Molecular mechanisms of cocaine reward: combined dopamine and serotonin transporter knockouts eliminate cocaine place preference. *Proc. Natl Acad. Sci. USA*, **98**, 5300–5305.

de Souza Silva, M.A., Mello, E.L., Müller, C.P., Jocham, G., Major, R.S., Huston, J.P., Tomaz, C. & Barros, M. (2006) The tachykinin NK-3 receptor antagonist SR142801 blocks the behavioural effects of cocaine in marmoset monkeys. *Eur. J. Pharmacol.*, **536**, 269–278.

Stoessl, A.J. (1994) Localization of striatal and nigral tachykinin receptors in the rat. *Brain Res.*, **646**, 13–18.

Stoessl, A.J., Brackstone, M., Rajakumar, N. & Gibson, C.J. (1995) Pharmacological characterization of grooming induced by a selective NK-1 tachykinin receptor agonist. *Brain Res.*, **700**, 115–120.

Stoessl, A.J., Dourish, C.T. & Iversen, S.D. (1990) Pharmacological characterization of the behavioural syndrome induced by the NK-3 tachykinin agonist senktide in rodents: evidence for mediation by endogenous 5-HT. *Brain Res.*, **517**, 111–116.

Stoessl, A.J., Szczutkowski, E., Glenn, B. & Watson, I. (1991) Behavioural effects of selective tachykinin agonists in midbrain dopamine regions. *Brain Res.*, **565**, 254–262.

Woolverton, W.L. & Johnson, K.M. (1992) Neurobiology of cocaine abuse. *Trends Pharmacol. Sci.*, **13**, 193–200.

Zahm, D.S. & Brog, J.S. (1992) On the significance of subterritories in the 'accumbens' part of the rat ventral striatum. *Neuroscience*, **50**, 751–767.

Behavioral and Cognitive Neurosciences /Neuropsychopharmacology
Dr. M. Sarter
Department of Physiology, University of Michigan,
4032 East Hall, 525 E. University Avenue
Ann Arbor, MI 48109-1109, USA

Neurokinin$_3$ receptor activation potentiates the psychomotor and nucleus accumbens dopamine response to cocaine, but not its rewarding effects

Gerhard Jocham, Andrea C. Lauber, Christian P. Müller, Joseph P. Huston and M. Angélica de Souza Silva*

Institute of Physiological Psychology and Center for Biological and Medical Research, University of Düsseldorf, Universitätsstr. 1, D-40225, Düsseldorf, Germany

Running title: Neurokinin$_3$ receptor and cocaine

Number of
Text pages: 24
Figures: 11
Tables: 1
Words: Manuscript: 8.334; Abstract: 252 (max. 250); Introduction: 527 (max. 500)

Keywords: Senktide, NK$_3$ receptor, reinforcement, cocaine, rats, microdialysis, dopamine

*Corresponding author:
M.A. de Souza Silva
Institute of Physiological Psychology
University of Düsseldorf
Universitätsstr. 1
D-40225 Düsseldorf, Germany
Phone: +49- 211-8114297
Fax: +49- 211-8112024
E-mail: desouza@uni-duesseldorf.de

Abstract

Neurokinin$_3$ receptors (NK_3-Rs) have been implicated in psychomotor activity and reinforcement mechanisms. Recently, we showed that NK_3-R antagonism blocked the psychostimulant properties of cocaine both in rats and in primates. Here, using in vivo microdialysis in the nucleus accumbens (Nac) of freely moving rats, we investigated the effect of the NK_3-R agonist senktide (0.2 and 0.4 mg/kg s.c.) on the cocaine-evoked increase in extracellular dopamine. Cocaine (10 mg/kg i.p.) increased dopamine levels to 404% and 480% of baseline in the core and shell of the Nac, respectively. Pretreatment with senktide at the dose of 0.2 mg/kg potentiated this effect to 666% (core) and 869% (shell) of baseline, without having any effect on dopamine when given alone. Parallel behavioral measurements revealed that this was accompanied by an increase in horizontal and vertical activity. This enhanced activity was also markedly potentiated by senktide at the dose of 0.2 mg/kg. Senktide alone induced a short-lasting increase in activity that was not accompanied by any alterations of the neurochemical parameters. In conditioned place preference (CPP) experiments, senktide pretreatment did not alter cocaine-induced CPP, and had no effect when given alone. Likewise, cocaine-conditioned locomotor activity was not affected by the NK_3-R agonist. However, like in the microdialysis studies, cocaine-induced hyperactivity was potentiated by senktide, and there was evidence for a facilitation of sensitization to the hyperlocomotor effects of cocaine by senktide. These data provide evidence that NK_3-R are involved in the control of the hyperlocomotor and Nac DA response to cocaine, but not in its rewarding effects.

Abbreviatons:

CPP – Conditioned place preference

DA – Dopamine

DOPAC –3,4-dihydroxyphenyl acetic acid

HVA- Homovanillic acid

Nac – Nucleus accumbens

NKA – Neurokinin A

NKB – Neurokinin B

NK-R – Neurokinin receptors

NK_1-R – Neurokinin-1 receptors

NK_2-R – Neurokinin-2 receptors

NK_3-R – Neurokinin-3 receptors

SN_C – pars compacta of the substantia nigra

SP – Substance P

VP – Ventral pallidum

VTA – Ventral tegmental area

1. Introduction

For the five known mammalian tachykinins substance P (SP), neurokinin A (NKA), neurokinin B (NKB), neuropeptide K and neuropeptide γ, three receptors have to date been characterized, designated NK_1-, NK_2- and NK_3-Rs. SP, NKA and NKB have higher affinity to NK_1-, NK_2- and NK_3-Rs, respectively, but all neurokinins bind to all three NK-Rs (Massi et al., 2000)

Both the reinforcing and the psychomotor stimulant properties of cocaine depend to a large extent on its capacity to increase extracellular dopamine (DA) levels in terminal areas of the mesolimbic DA system, which originates in the ventral tegmental area (VTA) and projects to several forebrain structures, including the nucleus accumbens (Nac, Wise, 1996). Several lines of evidence implicate NK-Rs both in the activity of the mesolimbic DA system and in processes related to reinforcement and behavioral activity.

NK_3-Rs are expressed in regions of the brain that are crucial for cocaine reward, such as the medial prefrontal cortex, amygdala and ventral pallidum (VP, Shughrue et al., 1996). Of particular interest is that NK_3-Rs are abundantly expressed by DA neurons of the VTA and pars compacta of the subtantia nigra (SN_C, Chen et al., 1998). Application of the selective NK_3-R agonist senktide to the VTA or SN_C enhances firing of DA neurons (Overton et al., 1992; Keegan et al., 1992) and the release of DA in terminal areas (Marco et al., 1998). Furthermore, SP enhances release of DA in the Nac after systemic administration (Boix et al., 1992b) and this effect is mediated by the C-terminal sequence of SP (Boix et al., 1992a). Since the C-terminal fragment of SP has a higher affinity for NK_3-Rs than NK_1-Rs (Regoli et al., 1994), it appears likely that this effect is at least in part mediated by NK_3-R.

Conditioned place preference (CPP) can be induced by systemic and central administration of SP (Hasenohrl et al., 2000) and the NK_3-R selective agonist aminosenktide (Ciccocioppo et al., 1998). Like the effects on the mesolimbic DA system, this effect of SP is also mediated by the C-terminal fragment (Hasenohrl et al., 1992), suggesting that NK_3-Rs contribute to the reinforcing effects of SP. This conclusion is supported by the finding that an NK_1-R antagonist failed to completely prevent the CPP induced by injection of the C-terminal fragment of SP into the nucleus basalis magnocellularis (Nikolaus et al., 1999). Injection of SP or DiMe-C7 into the VTA or SN_C also enhances locomotor and rearing behaviors (Eison et al., 1982; Barnes et al., 1990; Elliott et al., 1991), and these effects are mediated by NK_3-Rs (Stoessl et al., 1991).

Recently, we showed that pretreatment with an NK_3-R antagonist blocked the behavioral effects of cocaine both in rats and in non-human primates (Jocham et al., 2006; De Souza Silva et al., 2006b).

Based on these data, we hypothesized that pretreatment with the NK_3-R agonist senktide would potentiate both the psychomotor and the DA response to cocaine, and thereby increase its rewarding effects. Accordingly, we investigated the effects of senktide on cocaine-induced CPP, hyperactivity and behavioral sensitization. Using in vivo microdialysis, we measured the effects of senktide on the cocaine-induced increase in DA transmission in the core and shell of the Nac.

2. Materials and Methods

2.1. Subjects

Adult male Wistar rats (obtained from the local breeding facility, Tierversuchsanlage, University of Düsseldorf) were used for the microdialysis (weight: 312 ± 4.6 g, mean ± SEM) and place preference experiments (weight: 364 ± 4.7 g, mean ± SEM). All animals were kept in macrolon cages in groups of five animals per cage. Animals for the microdialysis experiments were housed individually after surgery. They were maintained under a reversed 12 h light-dark-cycle (lights on: *7:00 am*) with food and water available *ad libitum*. Experiments were performed during the animals' active period between *8:00 am* and *5:00 pm*. All experiments were carried out according to the German Law of Animal Protection of 1998.

2.2. Drugs

The NK_3-R agonist Senktide ([succinyl-Asp^6, Me-Phe^8]SP_{6-11}, Bachem, Bubendorf, Switzerland) and cocaine (Merck, Germany) were used. Both drugs were dissolved in phosphate buffered saline and injected in a volume of 1 ml/kg. Senktide was injected s.c., cocaine was injected i.p.

2.3. Experiment I: Dopamine and its metabolites in the core and shell regions of the nucleus accumbens

2.3.1. Surgery

Animals were deeply anaesthetised with a mixture of ketaminhydrochloride (90.0 mg/kg, Ketavet, Pharmacia & Upjohn GmbH, Erlangen, Germany) and xylazinhydrochloride (8.0 mg/kg, Rompun, Bayer, Leverkusen, Germany) and fixed in a Kopf stereotactic frame. Two guide-cannulae with a thread on the top (15 mm, 22 G, stainless steel) were aimed at the Nac core (AP: +1.2 mm, ML: 2.8 mm, DV: -5.8 mm, 10° angle tilted laterally) and the Nac shell (AP: +1.2 mm, ML: 0.8 mm, DV: -6.2 mm, all coordinates relative to Bregma, according to the atlas of Paxinos and Watson (1986). They were fixed to the skull with two screws (stainless steel, d=1.4 mm) and dental cement. After surgery and again 6 and 12 h later, the animals were administered 100 µl Novalgin orally (containing 500 mg/ml metamizol-sodium, Aventis Pharma, Frankfurt, Germany). They were allowed to recover for at least 7 days preceding microdialysis experimentation. During this time they were handled daily.

2.3.2. Microdialysis procedure

Sixteen to 18 h before experimentation, microdialysis probes (2 mm active membrane length) of a concentric design (for construction details see: Boix et al., 1995) were inserted through the guide-cannulae and fixed to the thread. The animal was then placed into a Truscan® photobeam activity cage (40 x 40 x 40 cm, Coulbourn instruments, Allentown, USA) which was located in a sound-attenuating chamber (110 x 70 x 70 cm). The inverted light-dark cycle was maintained in the chamber. To allow videotaping of behavior, the chamber was illuminated with dim red light (60 W). The probe tubing was connected to a microinfusion pump (CMA 100, Carnegie, Sweden) via a liquid swivel mounted on a balanced arm on top of the chamber. Probes were perfused with artificial cerebrospinal fluid containing Na^+ 146 mM, K^+ 4 mM, Ca^{2+} 2.2 mM and Cl^- 156 mM. The flow rate was 0.5µl/min overnight. The next morning at 8:00 am the flow was set to 2.0µl/min and was left to stabilize for 1 h. Thereafter, samples were collected every 10 min into vials containing 5 µl of 0.05M $HClO_4$ in which 100 pg of deoxyepinephrine was dissolved, which served as an internal standard for chromatographic analysis. After four baseline samples, senktide (0.2 or 0.4 mg/kg) or its vehicle was injected (s.c.). After 30 min, i.e. three samples later, cocaine (0 or 10 mg/kg) was injected (i.p.). After the second injection sampling was continued for 2 h.

2.3.3. Analysis

Immediately after collection, the samples were analyzed for the content of DA and its metabolites 3,4-dihydroxyphenyl acetic acid (DOPAC) and homovanillic acid (HVA) using high performance liquid chromatography. For the separation a 125 x 2 mm reversed-phase column (120-5 C18, Macherey Nagel, Düren, Germany), perfused with a mobile phase composed of 99.5 mM chloroacetic acid, 0.53 mM sodium octyl sulphate, 0.5 mM ethylenediaminetetraacetic acid, 4 mM KCl, 6% v/v acetonitrile and 0.8% v/v tetrahydrofuran, pH adjusted to 3.25 using 6 M NaOH solution, was used. Quantification was performed by amperometric detection (Intro, Antec, Leyden, Netherlands) with the potential set at +530 mV vs. an ISAAC reference electrode (Antec, Leyden, Netherlands) at 28 °C. The limit of detection was 1 fmol per sample for DA at a signal to noise ratio of 2:1.

2.3.4. Histology

After completion of the last sampling interval, the animals were administered an overdose of pentobarbital (Nembutal, Sanofi Ceva, Libourne, France) and transcardially perfused with

phosphate-buffered saline followed by 10% phosphate-buffered formalin. The brains were removed and stored in formalin (10%) until they were sliced on a cryotome, stained with cresyl-violet and examined under a light microscope. Only animals with probes that were placed within the boundaries of the NAc core or NAc shell were considered for data analysis.

2.4. Experiment II: Conditioned place preference

Apparatus

A circular open field (83 cm diameter, walls 43 cm height) made of black steel was used for place preference conditioning. A square metal rod (8x8 mm) attached to the floor divided the field into two semicircles. In one compartment, the floor was covered with a smooth black rubber mat; in the other compartment, both the floor and the wall were covered with a rough black rubber mat. The apparatus was located in a sound-attenuating chamber and was illuminated by a single 100 W light bulb, which resulted in an average illumination of the floor of 1.5 lux (walls) to 2.5 lux (center). A video camera installed below the ceiling of the room was connected to a TV screen and a computer located outside of the experimental chamber.

2.4.2. Procedure

On day 1, the animals were allowed to explore the entire arena for 20 min (baseline trial). On days 2-9, the two compartments of the arena were separated for conditioning by insertion of a transparent perspex wall. On even days, conditioning trials were performed. The animals were injected with senktide (0; 0.1; 0.2 or 0.4 mg/kg s.c.) 30 minutes before an i.p. injection of cocaine (0 or 10 mg/kg). One minute after cocaine injection, each animal was placed into the conditioning compartment for 30 minutes. On odd days, pseudoconditioning trials were performed. Instead of drug treatments, the animals received vehicle injections and were placed into the alternate compartment for 30 minutes. In each group, 50% of the animals were conditioned to the rough compartment and the other 50% were conditioned to the smooth compartment. On day 10, the wall was removed and animals were again allowed to explore the arena for 20 minutes (test trial). The animals' sojourn times in each compartment and the distance moved were tracked automatically using Ethovision Software (Noldus BV, Wageningen, Netherlands). The frequency and duration of rearing (raising both forelimbs off the ground) and the duration of grooming behavior (self-grooming using paws or snout) were scored using Ethovision Software by an observer blind to the treatment by manually pressing keys on the keyboard upon occurrence of the specified behaviour.

2.5. Statistical analysis

For the behavioral experiment, place preference scores were calculated by subtracting the time spent in the treatment compartment during the baseline trial from the time spent in the treatment compartment during the test trial. CPP scores and basal sojourn times were analyzed using one-way ANOVA with GROUP (8 groups) as factor, followed by post hoc Dunnett test to compare the groups against the saline-saline- and saline-cocaine-groups. For the analysis of locomotor activity, rearing and grooming, two-way ANOVA with repeated measures were calculated with TIME (4 time points during the conditioning trials, 2 time points for comparison of baseline vs. test trial) and GROUP (8 groups) as factors. When appropriate, this was followed by one-way ANOVA and post hoc Dunnett test to analyze group differences from the saline-saline- and saline-cocaine-groups at individual time points.

The neurochemical data were expressed as percentage of the mean of the four baseline samples taken as 100%. Two-way ANOVA with repeated measures were calculated with the factors TIME (19 time points) and GROUP (6 groups). When appropriate, this was followed by one-way ANOVA and post hoc Dunnett test to analyze group differences from the saline-saline- and saline-cocaine-groups at individual time points. For graphical purposes, area under the curve (AUC) scores for the behavioral data were calculated by summing the values from the four conditioning trials.

3. Results

3.1. Experiment I: Dopamine and its metabolites in the core and shell of the Nac

3.1.1. Nac core

The localization of the dialysis membrane in the Nac core is shown in Fig. 1. The mean basal levels of DA were 2.33 ± 0.35 pg (mean ± SEM) in the Nac core. Basal values of DA did not differ between groups ($p>0.05$). Cocaine increased DA levels to a maximum of 404% of baseline in the second sample after injection (Fig. 2 A). Two-way ANOVA yielded an effect of TIME ($F_{18,558}=48.063$, $p<0.001$) and GROUP ($F_{5,31}=14.997$, $p<0.001$) and a TIME x GROUP interaction ($F_{90,558}=10.93$, $p<0.001$). One-way ANOVA followed by post hoc tests showed that cocaine increased dialysate DA levels in the first 40 minutes after injection ($p<0.02$ compared to saline-saline). This increase was further potentiated to a maximum of 666% of baseline in the second interval after cocaine injection by pretreatment with senktide at the dose of 0.2 but not 0.4 mg/kg. Compared to the saline-cocaine group, DA levels were higher in the second to fifth sampling interval after cocaine injection in the group pretreated

with 0.2 mg/kg senktide ($p<0.004$). Senktide alone did not influence dialysate DA levels at either dose tested ($p>0.05$).

Basal levels of DOPAC were 2284.86 ± 181.83 pg (mean ± SEM) in the Nac core. Basal values of DOPAC did not differ between groups ($p>0.05$). Cocaine led to a decline in DOPAC levels to a minimum of 67% of baseline in the tenth interval after injection (Fig. 3 A). Two-way ANOVA yielded an effect of TIME ($F_{18,558}=31.92$, $p<0.001$) and GROUP ($F_{5,31}=3.849$, $p=0.01$) and a TIME x GROUP interaction ($F_{90,558}=2.282$, $p=0.001$). One-way ANOVA followed by post hoc tests showed that cocaine decreased dialysate DOPAC levels from the sixth to the tenth interval after injection ($p<0.03$ compared to saline-saline). However, this decline was not influenced by pretreatment with senktide at either of the two doses tested ($p>0.05$). Senktide alone was without effect ($p>0.05$).

Basal levels of HVA were 698.06 ± 54.95 pg (mean ± SEM) in the Nac core. Basal values of HVA did not differ between groups ($p>0.05$). Cocaine caused a decline in HVA levels to a minimum of 71% of baseline in the tenth interval after injection (Fig. 4 A). Two-way ANOVA yielded an effect of TIME ($F_{18,558}=23.787$, $p<0.001$) and a TIME x GROUP interaction ($F_{90,558}=1.816$, $p=0.012$) but no effect of GROUP ($p>0.05$). One-way ANOVA followed by post hoc tests showed no differences between groups at any of the time points ($p>0.05$).

3.1.2. Nac shell

The localization of the dialysis membrane in the Nac shell is shown in Fig. 1. The mean basal levels of DA were 1.92 ± 0.31 pg (mean ± SEM) in the Nac shell. Basal values of DA did not differ between groups ($p>0.05$). Cocaine increased DA levels to a maximum of 480% of baseline in the second sample after injection (Fig. 2 B). Two-way ANOVA yielded an effect of TIME ($F_{18,522}=21.627$, $p<0.001$) and GROUP ($F_{5,29}=6.35$, $p<0.001$) and a TIME x GROUP interaction ($F_{90,522}=6.577$, $p<0.001$). One-way ANOVA followed by post hoc tests showed that cocaine increased dialysate DA levels in the first 20 minutes after injection ($p<0.05$ compared to saline-saline). This increase was further potentiated to a maximum of 869% of baseline in the second interval after cocaine injection by pretreatment with senktide at the dose of 0.2 but not 0.4 mg/kg. Compared to the saline-cocaine group, DA levels were higher in the second to seventh sampling interval after cocaine injection in the group pretreated with 0.2 mg/kg senktide ($p<0.035$). Senktide alone did not affect dialysate DA levels at either dose tested ($p>0.05$).

Basal levels of DOPAC were 1342.05 ± 157.89 pg (mean ± SEM) in the Nac shell. Basal values of DOPAC did not differ between groups ($p>0.05$). Cocaine led to a decline in DOPAC levels to a minimum of 59% of baseline in the sixth interval after injection (Fig. 3 B). Two-way ANOVA yielded an effect of TIME ($F_{18,522}=61.123$, $p<0.001$) and GROUP ($F_{5,29}=10.461$, $p<0.001$) and a TIME x GROUP interaction ($F_{90,522}=6.62$, $p<0.001$). One-way ANOVA followed by post hoc tests showed that cocaine decreased dialysate DOPAC levels beginning in the third interval after injection and lasting until the end of the 2 h post-injection sampling period ($p<0.01$ compared to saline-saline). However, this decline was not affected by pretreatment with senktide at either of the two doses tested. From the tenth to the eleventh interval after cocaine injection, senktide alone, at both doses, and in the ninth interval at the dose of 0.2 mg/kg, caused a slight but significant decrease in DOPAC levels compared to the saline-saline group ($p<0.04$).

Basal levels of HVA were 317.44 ± 33.29 pg (mean ± SEM) in the Nac shell. Basal values of HVA did not differ between groups ($p>0.05$). Cocaine led to a decline in HVA levels to a minimum of 60% of baseline in the eleventh interval after injection (Fig. 4 B). Two-way ANOVA yielded an effect of TIME ($F_{18,522}=34.717$, $p<0.001$) and GROUP ($F_{5,29}=7.751$, $p<0.001$) and a TIME x GROUP interaction ($F_{90,522}=6.49$, $p<0.001$). One-way ANOVA followed by post hoc tests showed that cocaine decreased dialysate HVA levels beginning in the fifth interval after injection and lasting until the end of the 2 h post-injection sampling period ($p<0.02$ compared to saline-saline). However, this decline was not affected by pretreatment with senktide at either of the two doses tested. In the eleventh interval after cocaine injection, senktide alone at both doses incurred a decrease of HVA levels ($p<0.02$ compared to saline-saline).

3.1.3. Horizontal and vertical activity

Cocaine increased horizontal activity to a maximum of 584% of baseline in the first interval after injection (Fig. 5 A). Two-way ANOVA yielded an effect of TIME ($F_{18,1008}=15.914$, $p<0.001$) and GROUP ($F_{5,56}=5.723$, $p<0.001$) and a TIME x GROUP interaction ($F_{90,1008}=4.487$, $p<0.001$). One-way ANOVA followed by post hoc tests showed that cocaine increased horizontal activity compared to the saline-saline-group in the seventh interval after injection ($p<0.05$). This increase was further potentiated to a maximum of 1685% of baseline in the second interval after cocaine injection by pretreatment with senktide at the dose of 0.2 but not 0.4 mg/kg. Compared to the saline-cocaine group, levels of horizontal activity were higher in the first 70 minutes after cocaine injection in the group pretreated with 0.2 mg/kg

senktide ($p<0.04$). Senktide alone at the dose of 0.2 mg/kg increased horizontal activity compared to the vehicle-cocaine group in the first 20 minutes after senktide administration ($p<0.002$).

Cocaine increased the frequency of vertical activity to a maximum of 227% of baseline in the first interval after injection (Fig. 5 B). Two-way ANOVA yielded an effect of TIME ($F_{18,1008}=10.834$, $p<0.001$) and GROUP ($F_{5,56}=5.35$, $p<0.001$) and a TIME x GROUP interaction ($F_{90,1008}=3.249$, $p<0.001$). However, one-way ANOVA followed by post hoc tests showed that this increase by cocaine did not reach statistical significance when compared to the saline-saline-group ($p>0.05$). However, pretreatment with senktide at the dose of 0.2 but not 0.4 mg/kg induced a pronounced potentiation of this effect to a maximum of 724% of baseline in the second interval after cocaine injection. Compared to the saline-cocaine group, levels of vertical activity were higher throughout the entire 2 h post-cocaine period, except for the last interval, in the group pretreated with 0.2 mg/kg senktide ($p<0.025$).

3.1.4. Effects of senktide alone on Nac DA and DOPAC and behavioral activity

To obtain more detailed information on the effects of senktide alone, we pooled the respective senktide-pretreated groups. Then, we analyzed behavior and DA and DOPAC in the 30 minutes immediately after the senktide injection.

Senktide at both doses had no effect on extracellular levels of DA or DOPAC in either core or shell of the Nac in the 30 minutes after injection ($p<0.05$, one-way ANOVA followed by post hoc test, data not shown).

However, senktide increased both horizontal and vertical activity (Fig 6 A to D). One-way ANOVA yielded an effect of GROUP for the parameter horizontal activity ($F_{2,61}=4.255$ and 4.696, $p<0.02$, for the first and second interval after injection, respectively). Post hoc tests showed that senktide at the dose of 0.2 mg/kg increased horizontal activity in the two intervals following injection ($p<0.015$ compared to saline-saline). One-way ANOVA yielded an effect of GROUP for the parameter vertical activity ($F_{2,61}=4.872$, $p<0.012$ for the first interval after injection). Post hoc test showed that senktide at the dose of 0.2 mg/kg increased vertical activity in the first interval following injection ($p<0.035$ compared to saline-saline).

3.2. Experiment II: Conditioned place preference and psychomotor activity

3.2.1. Place preference

One-way ANOVA revealed that basal sojourn times in the treatment compartment did not differ between groups ($p>0.99$), nor did they differ from chance level (600 s, $p>0.82$), thus confirming the use of an unbiased CPP procedure.

Cocaine led to a significant preference for the treatment compartment (Fig. 7). One-way ANOVA revealed an effect of GROUP for the CPP score ($F_{7,111}=3.053$, $p<0.007$). Post hoc tests showed that the CPP scores were significantly higher in all of the cocaine groups compared to the saline-saline group ($p<0.05$). Cocaine-induced CPP was not modulated in any direction by pretreatment with senktide at any of the doses tested ($p>0.05$ compared to saline-cocaine). Senktide alone also was without influence on CPP scores ($p>0.05$ compared to saline-saline), indicating that it had neither appetitive nor aversive properties.

3.2.2. Activity parameters

Locomotor activity was increased by cocaine (Fig. 8 A, B). Two-way ANOVA yielded a significant effect of GROUP ($F_{7,111}=39.504$, $p<0.001$) and a TIME x GROUP interaction ($F_{21,333}=1.831$, $p=0.017$) but no effect of TIME ($p>0.05$). Post hoc test showed that locomotor activity was higher in the saline-cocaine group compared to the saline-saline group ($p<0.001$). This increase was further potentiated by senktide at the dose of 0.2 mg/kg ($p=0.002$) but not 0.1 or 0.4 mg/kg. Subsequent one-way ANOVA additionally revealed that during the first conditioning trial, senktide at the dose of 0.4 mg/kg attenuated cocaine-induced hyperlocomotion ($p=0.001$). Calculation of the slopes across the four conditioning trials showed that, while in the saline-cocaine-group the slope was negative, i.e. there was a slight decrease of activity over trials, the senktide-cocaine groups increased their activity over trials (as evidenced by a positive slope, Table 1). One-way ANOVA revealed an effect of GROUP ($F_{7,111}=3.428$, $p=0.002$) on the slope. Post hoc tests showed that the slopes were significantly higher in the cocaine-groups that were pretreated with senktide at all of the three doses ($p<0.03$ compared to saline-cocaine).

During the pseudoconditioning trials, locomotor activity declined over trials (data not shown). Two-way ANOVA yielded a significant effect of TIME ($F_{3,333}=39.504$, $p<0.001$) but no effect of GROUP nor a TIME x GROUP interaction.

Locomotor activity decreased from the baseline to the test trial (Fig. 10 A). Two-way ANOVA yielded a significant effect of TIME ($F_{1,111}=23.011$, $p<0.001$) but no effect of GROUP nor a TIME x GROUP interaction. For a more detailed analysis of locomotor activity during baseline and test trials, we divided the trials into four 5 minute bins (Fig. 11 A). When comparing the first five minutes of the baseline with the first five minutes of the test trial, a

pronounced conditioned locomotor effect became evident. Two-way ANOVA showed an effect of TIME ($F_{7,777}$=368.212, p<0.001) but no effect of GROUP nor a TIME x GROUP interaction.

The frequency of rearing was increased by cocaine (Fig. 9 A, B). Two-way ANOVA yielded a significant effect of GROUP ($F_{7,111}$=22.317, p<0.001) and TIME ($F_{3,333}$=6.66, p<0.001) and a TIME x GROUP interaction ($F_{21,333}$=2.668, p<0.001). Post hoc tests showed that rearing frequency was higher in all of the cocaine groups compared to the saline-saline group (p<0.013). This increase was attenuated by senktide at the dose of 0.4 mg/kg (p<0.05 compared to saline-cocaine) but not 0.1 or 0.2 mg/kg. Subsequent one-way ANOVA additionally revealed that, while senktide at the dose of 0.2 mg/kg initially slightly decreased cocaine-induced rearing frequency (p=0.025 compared to saline-cocaine, conditioning trial 1), this relationship reversed over trials, such that on conditioning trial 3, the same dose of senktide potentiated the cocaine-induced increase in rearing frequency (p=0.025). Calculation of the slopes across the four conditioning trials showed that, while in the saline-cocaine-group the slope was negative (i.e. there was a slight decrease of activity over trials), the senktide-cocaine groups increased their activity over trials (as evidenced by a positive slope, Table 1). One-way ANOVA revealed an effect of GROUP ($F_{7,111}$=5.237, p<0.001) on the slope. Post hoc tests showed that the slopes were significantly higher in the cocaine-groups that were pretreated with senktide at all of the three doses (p≤0.001 compared to saline-cocaine).

The frequency of rearing either increased or decreased from the baseline to the test trial, depending on the group (Fig. 10 B). Two-way ANOVA yielded no effect of TIME or GROUP, but a significant TIME x GROUP interaction ($F_{7,111}$=2.154, p=0.044). Subsequent one-way ANOVA followed by post hoc tests revealed that during the test trial, the senktide 0.4 mg/kg-saline-group performed fewer rearings than the saline-cocaine group (p=0.01). When comparing the first five minutes of the baseline with the first five minutes of the test trial (Fig. 11 B), a conditioned increase in rearing frequency became evident. Two-way ANOVA yielded an effect of TIME ($F_{7,777}$=58.875, p<0.001), but no effect of GROUP nor a TIME x GROUP interaction. Subsequent one-way ANOVA showed that in the senktide 0.4 mg/kg-saline-group the frequency of rearing was reduced compared to the saline-saline-group in the third (p=0.04) and tendentially during the fourth interval (p=0.103) of the test trial.

The duration of rearing was increased by cocaine (Fig. 9 C, D). Two-way ANOVA yielded a significant effect of GROUP ($F_{7,111}$=5.89, p<0.001) and TIME ($F_{3,333}$=9.227, p<0.001) and a TIME x GROUP interaction ($F_{21,333}$=2.058, p=0.005). Post hoc tests showed that the duration of rearing in the saline-cocaine-group was higher compared to the saline-

saline group ($p<0.05$). This increase was attenuated by senktide at the dose of 0.4 mg/kg ($p=0.004$ compared to saline-cocaine) but not 0.1 or 0.2 mg/kg. Calculation of the slopes across the four conditioning trials showed that, while in the saline-cocaine-group the slope was negative (i.e. there was a slight decrease of activity over trials), the senktide-cocaine groups increased their activity over trials (as evidenced by a positive slope, Table 1). One-way ANOVA revealed an effect of GROUP ($F_{7,111}=3.256$, $p=0.004$) on the slope. Post hoc tests showed that the slopes were significantly higher in the cocaine-groups that were pretreated with senktide at all of the three doses ($p<0.025$ compared to saline-cocaine).

For the comparison of the duration of rearing during the baseline and test trials (Fig. 10 C), two-way ANOVA yielded no effect of TIME or GROUP, nor a significant TIME x GROUP interaction. When comparing the first five minutes of the baseline with the first five minutes of the test trial (Fig. 11 C), two-way ANOVA yielded an effect of TIME ($F_{7,777}=14.956$, $p<0.001$), but no effect of GROUP nor a TIME x GROUP interaction. Subsequent one-way ANOVA showed that in the senktide 0.4 mg/kg-saline-group the duration of rearing was reduced compared to the saline-saline-group in the third 5-min bin of the test trial ($p=0.03$).

Grooming was decreased by cocaine (Fig. 8 C, D). Two-way ANOVA yielded a significant effect of GROUP ($F_{7,111}=29.015$, $p<0.001$) and TIME ($F_{3,333}=3.231$, $p=0.023$), but no TIME x GROUP interaction ($p>0.05$). Post hoc tests showed that there was significantly less grooming in all of the cocaine groups compared to the saline-saline group ($p<0.001$). This suppression of grooming was not modulated in any direction by pretreatment with senktide. Subsequent one-way ANOVA additionally revealed that during the first conditioning trial, senktide at the dose of 0.4 mg/kg tended to decrease grooming behaviour ($p=0.055$ compared to saline-saline).

Grooming behavior increased from the baseline to the test trial (Fig. 10 D). Two-way ANOVA yielded a significant effect of TIME ($F_{1,111}=45.247$, $p<0.001$) but no effect of GROUP nor a TIME x GROUP interaction. When comparing the first five minutes of the baseline with the first five minutes of the test trial (Fig. 11 D), two-way ANOVA across the eight bins yielded an effect of TIME ($F_{7,777}=24.422$, $p<0.001$) but no effect of GROUP nor a TIME x GROUP interaction.

4. Discussion

The present results demonstrate that NK_3-R agonism potentiated the cocaine-induced increase in extracellular levels of DA in both the core and shell subregions of the Nac. Cocaine decreased DOPAC levels in the core and shell, and HVA levels in the shell of the Nac. These decreases were not altered by pretreatment with the NK_3-R agonist senktide. The cocaine-induced neurochemical alterations were accompanied by an increase in horizontal and vertical activity, which was potentiated by pretreatment with senktide. Senktide alone increased horizontal and vertical activity immediately after injection, a time at which no neurochemical alterations were observed. In a separate CPP study, neither cocaine-induced CPP nor cocaine-conditioned locomotor activity were influenced by senktide. However, pretreatment with senktide dose-dependently modulated cocaine-induced hyperactivity and enhanced sensitization to the locomotor effects of cocaine. The suppression of grooming by cocaine was not affected by senktide. Senktide alone was without effect on CPP and locomotion, rearing and grooming behavior.

Cocaine increased levels of DA in the core and shell of the Nac. This effect tended to be more pronounced in the shell compared to the core, which is in line with previous reports (Pontieri et al., 1995; Jocham et al., 2006). This increase was further potentiated by senktide at the dose of 0.2 but not 0.4 mg/kg, which is in agreement with the finding that senktide enhances firing of midbrain DA neurons (Overton et al., 1992; Keegan et al., 1992). Cocaine inhibits firing of midbrain DA neurons (Einhorn et al., 1988), thereby self-limiting the DA increase in terminal areas. It may be speculated that senktide counteracted this effect, thereby potentiating the DA response to cocaine. Our finding that senktide alone did not increase DA levels is interesting in comparision with the finding by Marco et al. (1998) of an increase in Nac DA levels after administration of senktide into the VTA of guinea pigs. However, Floresco et al. (2003) showed that only treatments that increase the population response of DA neurons cause an increase in Nac DA as detectable by microdialysis. Such an increase by induction of burst firing could only be observed in Nac dialysate when reuptake was inhibited. Possibly, the different route of administration used in our study did not enhance the population response of DA neurons.

The neurochemical response was accompanied by an increase in horizontal and vertical activity. This response was also potentiated by senktide at the dose of 0.2 but not 0.4 mg/kg. The time course of this potentiation paralleled the neurochemical response. These findings are consistent with the role of Nac DA in the generation of cocaine-induced

hyperactivity (Delfs et al., 1990). Interestingly, senktide alone induced an immediate but short-lasting (20 minutes) increase in horizontal and vertical activity, without a concomitant change in DA transmission. This effect could be mediated by an effect on serotonergic systems, since it has been shown that NK_3-Rs are expressed by serotonergic neurons (Stoessl and Hill, 1990) and that infusion of senktide into the median raphé nucleus increases locomotor activity in a serotonin-dependent fashion (Paris et al., 1989; Mason and Elliott, 1992).

Cocaine injection led to conditioned place preference in an unbiased CPP paradigm. This CPP was not affected by pretreatment with senktide at any of the doses tested. Senktide alone did not influence CPP scores either. CPP has been demonstrated after i.c.v. administration of the NK_3-R agonist aminosenktide (Ciccocioppo et al., 1998). In that study, there was no delay between the i.c.v. injection and the placement into the conditioning compartment, whereas we used a 30-minute delay prior to the cocaine (or saline) injection. In the microdialysis studies, we could also observe that the effects of senktide alone on locomotor activity took place within the 30 minutes preceding the administration of cocaine. Therefore it is possible that our inter-injection interval was optimal for investigating the effects of senktide on cocaine's actions, given that senktide was administered s.c., but too long for detecting possible reinforcing effects of senktide alone.

Concordant with the neurochemical effects, we observed a potentiation of cocaine-induced increases in locomotion and rearing behavior by the dose of 0.2, but not 0.1 or 0.4 mg/kg. Unlike in the first experiment however, this potentiation was not evident during the initial exposure (first conditioning trial), but gradually developed over trials. The dose of 0.4 mg/kg initially even suppressed the cocaine-induced increase in locomotion and rearing, which may indicate an inverted U-shaped dose-response relationship. These differences from our first experiment may be due to the differential degree of habituation of the animals to the test situation. In the microdialysis experiments, the animals were habituated to the experimental chamber overnight and therefore the basal levels of spontaneous activity were extremely low, whereas in the CPP experiments even saline-treated animals displayed rather high levels of activity. Calculation of the slopes of activity over the four conditioning trials also revealed a steeper slope in the cocaine-animals that were pretreated with senktide at all of the doses used. This may indicate an enhanced sensitziation to the psychostimulant effects of cocaine. Microanalysis of locomotor activity during the baseline and test trials revealed a conditioned increase in locomotor activity in the first 5-minute bin of the test trial, which however, was not influenced by pretreatment with senktide. In addition, senktide alone at the

dose of 0.4 mg/kg decreased rearing behavior during the third interval of the test trial. The reasons for this remain elusive, but it might reflect a facilitation of habituation learning.

The suppression of grooming behavior was not modulated by senktide at any of the doses tested. However, senktide alone at the dose of 0.4 mg/kg tended to suppress grooming during the first conditioning trial.

Our findings are consistent with the described role of NK_3-R in the modulation of the activity of midbrain DA neurons and they show that this modulation also extends to cocaine-induced alterations in DA transmission. The increase in DA levels in the Nac is considered to be crucial for the reinforcing and habit-forming actions of cocaine (Di Chiara, 2002; Wise, 2004). In particular, DA in the shell is of relevance to the rewarding effects of cocaine (Rodd-Henricks et al., 2002; Bari and Pierce, 2005). However, it is becoming more and more evident that Nac DA does not encode the primary hedonic impact of appetitive stimuli, but rather is necessary for induction of neuroplastic changes that underly stimulus-reward- or response-reward-associations (Smith-Roe and Kelley, 2000; Wise, 2004; Salamone et al., 2005). Therefore, while the increase in DA transmission by drugs of abuse seems to be necessary for the formation of such associations, further increasing of DA tone will neither enhance the hedonic ("rewarding") value of the drug stimulus, nor will it be able to further intensify the learning of such associations. Indeed, there are other reports showing that pharmacological treatments can modulate the effects of cocaine on reinforcement and Nac DA in opposite directions (Pierce et al., 1997).

The doses of senktide used in the present study were chosen on the basis of evidence from other studies (Stoessl et al., 1988a; Stoessl et al., 1988b) showing behavioral activity of the compound in the range of 0.1 to 1.0 mg/kg after s.c. administration. Senktide is a ligand with both high selectivity and equally high affinity for NK_3-Rs of different species (Laufer et al., 1986). Nevertheless, it should be kept in mind that there are considerable inter-species differences in terms of the cerebral expression pattern of NK_3-Rs (Langlois et al., 2001), which might account for the discrepant results that we obtained when studying the effects of senktide on cocaine-induced behaviors in primates (De Souza Silva et al., 2006a). In that study, senktide, unlike in rats, blocked the cocaine-induced hyperlocomotion.

Taken together, the results of this study show that NK_3-Rs are involved in the generation of behavioral hyperactivity and the modulation of mesolimbic DA transmission by cocaine. However, an involvement of NK_3-Rs in cocaine reward could not be demonstrated.

Acknowledgements:

This study was supported by the Deutsche Forschungsgemeinschaft through grant De-792/2-2 to M.A. de Souza Silva. G.J. received a stipend from the Graduiertenkolleg 320 “Pathological processes of the nervous system: From genes to behavior”.

5. References

Bari, A.A. & Pierce, R.C. (2005) D1-like and D2 dopamine receptor antagonists administered into the shell subregion of the rat nucleus accumbens decrease cocaine, but not food, reinforcement. *Neuroscience*, **135**, 959-968.

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 analogue, DiMe-C7, into the rat ventral tegmental area. *Pharmacol.Biochem.Behav.*, **37**, 839-841.

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.

Chen, L.W., Guan, Z.L. & Ding, Y.Q. (1998) Mesencephalic dopaminergic neurons expressing neuromedin K receptor (NK3): a double immunocytochemical study in the rat. *Brain Research*, **780**, 150-154.

Ciccocioppo, R., Panocka, I., Polidori, C., Froldi, R., Angeletti, S. & Massi, M. (1998) Mechanism of action for reduction of ethanol intake in rats by the tachykinin NK-3 receptor agonist aminosenktide. *Pharmacol.Biochem.Behav.*, **61**, 459-464.

De Souza Silva, M.A., Mello, E.L., Jr., Müller, C.P., Jocham, G., Maior, R.S., Huston, J.P., Tomaz, C. & Barros, M. (2006a) Interaction of the tachykinin NK(3) receptor agonist senktide with behavioral effects of cocaine in marmosets (Callithrix penicillata). *Peptides*.

De Souza Silva, M.A., Mello, E.L. Jr., Müller, C.P., Jocham, G., Maior, R.S., Huston, J.P., Tomaz, C. & Barros, M. (2006b) The tachykinin NK3 receptor antagonist SR142801 blocks the behavioral effects of cocaine in marmoset monkeys. *Eur.J.Pharmacol.*, **536**, 269-278.

Delfs, J.M., Schreiber, L. & Kelley, A.E. (1990) Microinjection of cocaine into the nucleus accumbens elicits locomotor activation in the rat. *J.Neurosci.*, **10**, 303-310.

Di Chiara, G. (2002) Nucleus accumbens shell and core dopamine: differential role in behavior and addiction. *Behav.Brain Res.*, **137**, 75-114.

Einhorn, L.C., Johansen, P.A. & White, F.J. (1988) Electrophysiological effects of cocaine in the mesoaccumbens dopamine system: studies in the ventral tegmental area. *J.Neurosci.*, **8**, 100-112.

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

Elliott, P.J., Mason, G.S., Stephens-Smith, M. & Hagan, R.M. (1991) Behavioural and biochemical responses following activation of midbrain dopamine pathways by receptor selective neurokinin agonists. *Neuropeptides*, **19**, 119-126.

Hasenohrl, 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.

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

Jocham, G., Lezoch, K., Müller, C.P., Kart-Teke, E., Huston, J.P. & de Souza Silva, M.A. (2006) Neurokinin3 receptor antagonism attenuates cocaine's behavioral activating effects yet potentiates its dopamine-enhancing action in the nucleus accumbens core. *Eur.J.Neurosci.*, **24**, 1721-1732.

Keegan, K.D., Woodruff, G.N. & Pinnock, R.D. (1992) The selective NK3 receptor agonist senktide excites a subpopulation of dopamine-sensitive neurones in the rat substantia nigra pars compacta in vitro. *Br.J.Pharmacol.*, **105**, 3-5.

Langlois, X., Wintmolders, C., te Riele, P., Leysen, J.E. & Jurzak, M. (2001) Detailed distribution of neurokinin 3 receptors in the rat, guinea pig and gerbil brain: a comparative autoradiographic study. *Neuropharmacology*, **40**, 242-253.

Laufer, R., Gilon, C., Chorev, M. & Selinger, Z. (1986) Characterization of a neurokinin B receptor site in rat brain using a highly selective radioligand. *J.Biol.Chem.*, **261**, 10257-10263.

Marco, N., Thirion, A., Mons, G., Bougault, I., Le Fur, G., Soubrie, P. & Steinberg, R. (1998) Activation of dopaminergic and cholinergic neurotransmission by tachykinin NK3 receptor stimulation: an in vivo microdialysis approach in guinea pig. *Neuropeptides*, **32**, 481-488.

Mason, G.S. & Elliott, P.J. (1992) Behavioral consequences following infusion of selective neurokinin agonists into the median raphe nucleus of the rat. *Neuropharmacology*, **31**, 757-760.

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

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.

Overton, P., Elliott, P.J., Hagan, R.M. & Clark, D. (1992) Neurokinin agonists differentially affect A9 and A10 dopamine cells in the rat. *Eur.J.Pharmacol.*, **213**, 165-166.

Paris, J.M., Mitsushio, H. & Lorens, S.A. (1989) Intra-raphe neurokinin-induced hyperactivity: effects of 5,7-dihydroxytryptamine lesions. *Brain Res.*, **476**, 183-188.

Pierce, R.C., Meil, W.M. & Kalivas, P.W. (1997) The NMDA antagonist, dizocilpine, enhances cocaine reinforcement without influencing mesoaccumbens dopamine transmission. *Psychopharmacology (Berl)*, **133**, 188-195.

Pontieri, F.E., Tanda, G. & Di Chiara, G. (1995) Intravenous cocaine, morphine, and amphetamine preferentially increase extracellular dopamine in the "shell" as compared with the "core" of the rat nucleus accumbens. *Proc.Natl.Acad.Sci.U.S.A*, **92**, 12304-12308.

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

Rodd-Henricks, Z.A., McKinzie, D.L., Li, T.K., Murphy, J.M. & McBride, W.J. (2002) Cocaine is self-administered into the shell but not the core of the nucleus accumbens of Wistar rats. *J.Pharmacol.Exp.Ther.*, **303**, 1216-1226.

Salamone, J.D., Correa, M., Mingote, S.M. & Weber, S.M. (2005) Beyond the reward hypothesis: alternative functions of nucleus accumbens dopamine. *Curr.Opin.Pharmacol.*, **5**, 34-41.

Shughrue, P.J., Lane, M.V. & Merchenthaler, I. (1996) In situ hybridization analysis of the distribution of neurokinin-3 mRNA in the rat central nervous system. *J.Comp Neurol.*, **372**, 395-414.

Smith-Roe, S.L. & Kelley, A.E. (2000) Coincident activation of NMDA and dopamine D1 receptors within the nucleus accumbens core is required for appetitive instrumental learning. *J.Neurosci.*, **20**, 7737-7742.

Stoessl, A.J., Dourish, C.T. & Iversen, S.D. (1988a) The NK-3 tachykinin agonist senktide elicits yawning and chewing mouth movements following subcutaneous administration in the rat. Evidence for cholinergic mediation. *Psychopharmacology (Berl)*, **95**, 502-506.

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

Stoessl, A.J. & Hill, D.R. (1990) Autoradiographic visualization of NK-3 tachykinin binding sites in the rat brain, utilizing [3H]senktide. *Brain Res.*, **534**, 1-7.

Stoessl, A.J., Szczutkowski, E., Glenn, B. & Watson, I. (1991) Behavioural effects of selective tachykinin agonists in midbrain dopamine regions. *Brain Res.*, **565**, 254-262.

Wise, R.A. (1996) Neurobiology of addiction. *Curr.Opin.Neurobiol.*, **6**, 243-251.

Wise, R.A. (2004) Dopamine, learning and motivation. *Nat.Rev.Neurosci.*, **5**, 483-494.

Figure legends:

Table 1: Slopes values of activity over the four conditioning trials. Values represent group means.

Fig. 1: Schematic representation of the placement of the dialysis probes in the core and shell of the nucleus accumbens. The gray bars represent the localization of the dialysis membranes in the nucleus accumbens core, and the black in the nucleus accumbens shell.

Fig. 2: Effect of the treatment with senktide (senk; 0; 0.2 or 0.4 mg/kg s.c.) followed 30 minutes later by cocaine (coc; 0 or 10 mg/kg i.p.) on extracellular levels of dopamine in the core (A) and shell (B) of the nucleus accumbens. Values are expressed as mean (± SEM) percent of baseline, with the mean of the four baseline samples taken as 100%. Arrows indicate the time points of the injections. Sample sizes are n= 5 to 7. * $p<0.05$ vs. saline-cocaine group, # $p<0.05$ vs. saline-saline group.

Fig. 3: Effect of the treatment with senktide (senk; 0; 0.2 or 0.4 mg/kg s.c.) followed 30 minutes later by cocaine (coc; 0 or 10 mg/kg i.p.) on extracellular levels of DOPAC in the core (A) and shell (B) of the nucleus accumbens. Values are expressed as mean (± SEM) percent of baseline, with the mean of the four baseline samples taken as 100%. Arrows indicate the time points of the injections. Sample sizes are n= 5 to 7. * $p<0.05$ vs. saline-cocaine group, # $p<0.05$ vs. saline-saline group.

Fig. 4: Effect of the treatment with senktide (senk; 0; 0.2 or 0.4 mg/kg s.c.) followed 30 minutes later by cocaine (coc; 0 or 10 mg/kg i.p.) on extracellular levels of HVA in the core (A) and shell (B) of the nucleus accumbens. Values are expressed as mean (± SEM) percent of baseline, with the mean of the four baseline samples taken as 100%. Arrows indicate the time points of the injections. Sample sizes are n= 5 to 7. * $p<0.05$ vs. saline-cocaine group, # $p<0.05$ vs. saline-saline group.

Fig. 5: Effect of the treatment with senktide (senk; 0; 0.2 or 0.4 mg/kg s.c.) followed 30 minutes later by cocaine (coc; 0 or 10 mg/kg i.p.) on horizontal activity (A) and the frequency of vertical activity (B). Values are expressed as mean (± SEM) percent of baseline, with the mean of the four baseline samples taken as 100%. Arrows indicate the time points of the

injections. Sample sizes are n= 8 to 13. * $p<0.05$ vs. saline-cocaine group, # $p<0.05$ vs. saline-saline group.

Fig. 6: Effect of senktide (senk) alone on horizontal (A, C) and vertical activity (B, D) in the first 20 minutes after injection. The groups that received saline and cocaine as their second injections were pooled together. The top row shows the first, the bottom row the second sampling interval after injection. Sample sizes are saline: n=23; senktide 0.2 mg/kg: n=20; senktide 0.4 mg/kg: n=19. * $p<0.05$.

Fig. 7: Effect of the treatment with senktide (0; 0.1; 0.2 or 0.4 mg/kg s.c.) followed 30 minutes later by cocaine (coc; 0 or 10 mg/kg i.p.) on conditioned place preference. CPP scores represent time in the conditioning compartment in the test trial minus time in the treated side in the baseline trial. Sample sizes are n=14-15. * $p<0.05$ vs. saline-saline group.

Fig. 8: Effect of the treatment with senktide (senk; 0; 0.1; 0.2 or 0.4 mg/kg s.c.) followed 30 minutes later by cocaine (coc; 0 or 10 mg/kg i.p.) on locomotor activity (A, B) and grooming (C, D) during the conditioning trials. Sample sizes are n=14-15. * $p<0.05$ vs. saline-cocaine group, # $p<0.05$ vs. saline-saline group.

Fig. 9: Effect of the treatment with senktide (senk; 0; 0.1; 0.2 or 0.4 mg/kg s.c.) followed 30 minutes later by cocaine (coc; 0 or 10 mg/kg i.p.) on rearing frequency (A, B) and rearing duration (C, D) during the conditioning trials. Sample sizes are n=14-15. * $p<0.05$ vs. saline-cocaine group, # $p<0.05$ vs. saline-saline group.

Fig. 10: Effect of the treatment with senktide (senk; 0; 0.1; 0.2 or 0.4 mg/kg s.c.) followed 30 minutes later by cocaine (coc; 0 or 10 mg/kg i.p.) on locomotor activity (A), the frequency (B) and duration (C) of rearing and the duration of grooming (D) during the baseline and test trials. Sample sizes are n=14-15. # $p<0.05$ vs. saline-saline group.

Fig. 11: Effect of the treatment with senktide (senk; 0; 0.1; 0.2 or 0.4 mg/kg s.c.) followed 30 minutes later by cocaine (coc; 0 or 10 mg/kg i.p.) on locomotor activity (A), the frequency (B) and duration (C) of rearing and the duration of grooming (D) during the four 5 minute intervals of baseline and test trials. Sample sizes are n=14-15. # $p<0.05$ vs. saline-saline group, * $p<0.05$ between baseline and test trial.

		Senktide			
		0	**0.1**	**0.2**	**0.4**
Locomotion	**Cocaine 0 mg/kg**	-131,47	-112,31	-221,32	-1,94
	Cocaine 10 mg/kg	-842,21	502,64	319,77	919,69
Rearing Frequency	**Cocaine 0 mg/kg**	3,88	4,58	1,45	5,60
	Cocaine 10 mg/kg	-15,54	23,79	24,95	32,21
Rearing Duration	**Cocaine 0 mg/kg**	4,93	5,63	-2,35	13,81
	Cocaine 10 mg/kg	-26,94	16,27	27,80	32,66

Table 1

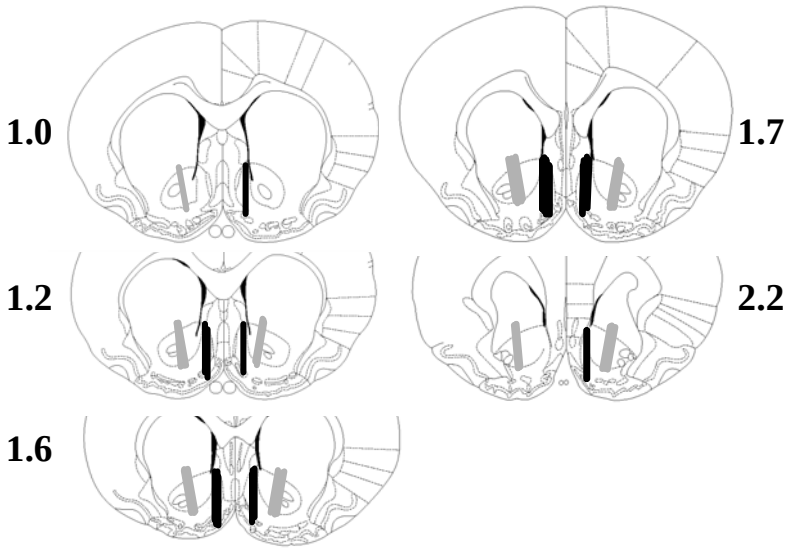

Fig. 1

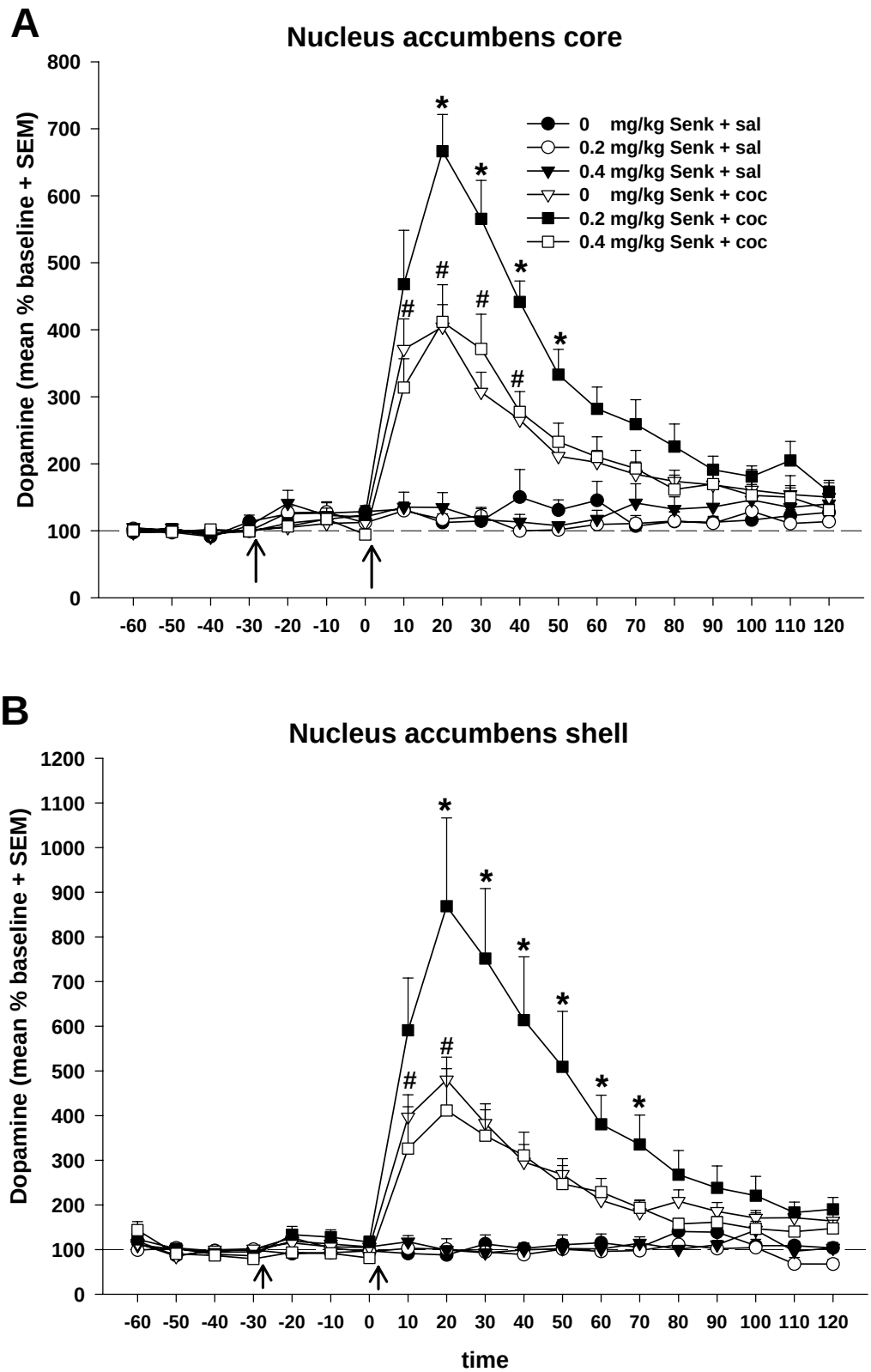
A
Nucleus accumbens core
Dopamine (mean % baseline + SEM)
0 mg/kg Senk + sal
0.2 mg/kg Senk + sal
0.4 mg/kg Senk + sal
0 mg/kg Senk + coc
0.2 mg/kg Senk + coc
0.4 mg/kg Senk + coc
B
Nucleus accumbens shell
Dopamine (mean % baseline + SEM)
time

Fig. 2

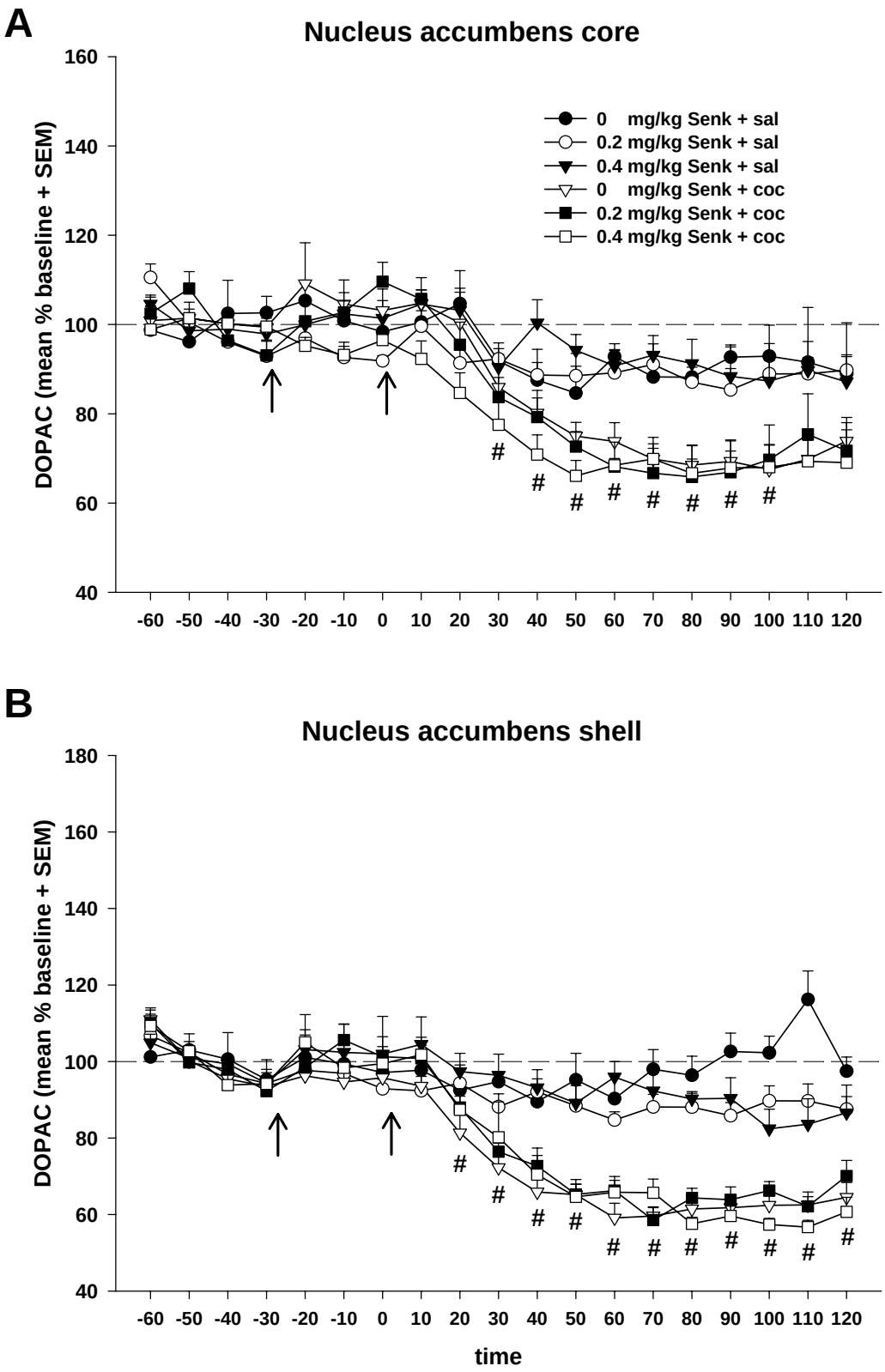
A
Nucleus accumbens core
DOPAC (mean % baseline + SEM)
160
140
120
100
80
60
40
0 mg/kg Senk + sal
0.2 mg/kg Senk + sal
0.4 mg/kg Senk + sal
0 mg/kg Senk + coc
0.2 mg/kg Senk + coc
0.4 mg/kg Senk + coc
-60 -50 -40 -30 -20 -10 0 10 20 30 40 50 60 70 80 90 100 110 120
B
Nucleus accumbens shell
DOPAC (mean % baseline + SEM)
180
160
140
120
100
80
60
40
-60 -50 -40 -30 -20 -10 0 10 20 30 40 50 60 70 80 90 100 110 120
time

Fig. 3

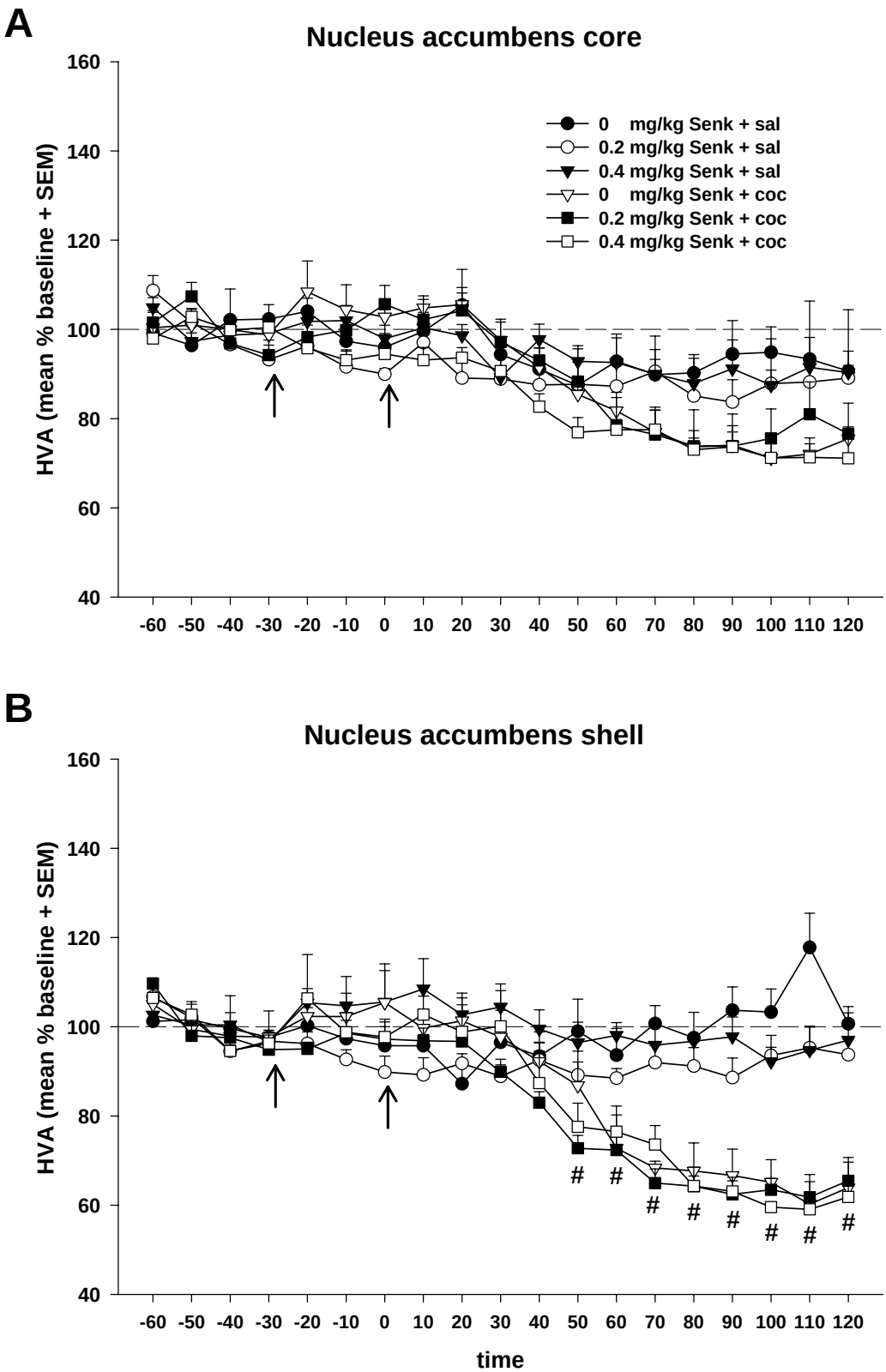
A
Nucleus accumbens core
HVA (mean % baseline + SEM)
160
140
120
100
80
60
40
0 mg/kg Senk + sal
0.2 mg/kg Senk + sal
0.4 mg/kg Senk + sal
0 mg/kg Senk + coc
0.2 mg/kg Senk + coc
0.4 mg/kg Senk + coc
-60 -50 -40 -30 -20 -10 0 10 20 30 40 50 60 70 80 90 100 110 120
B
Nucleus accumbens shell
HVA (mean % baseline + SEM)
160
140
120
100
80
60
40
#
-60 -50 -40 -30 -20 -10 0 10 20 30 40 50 60 70 80 90 100 110 120
time

Fig. 4

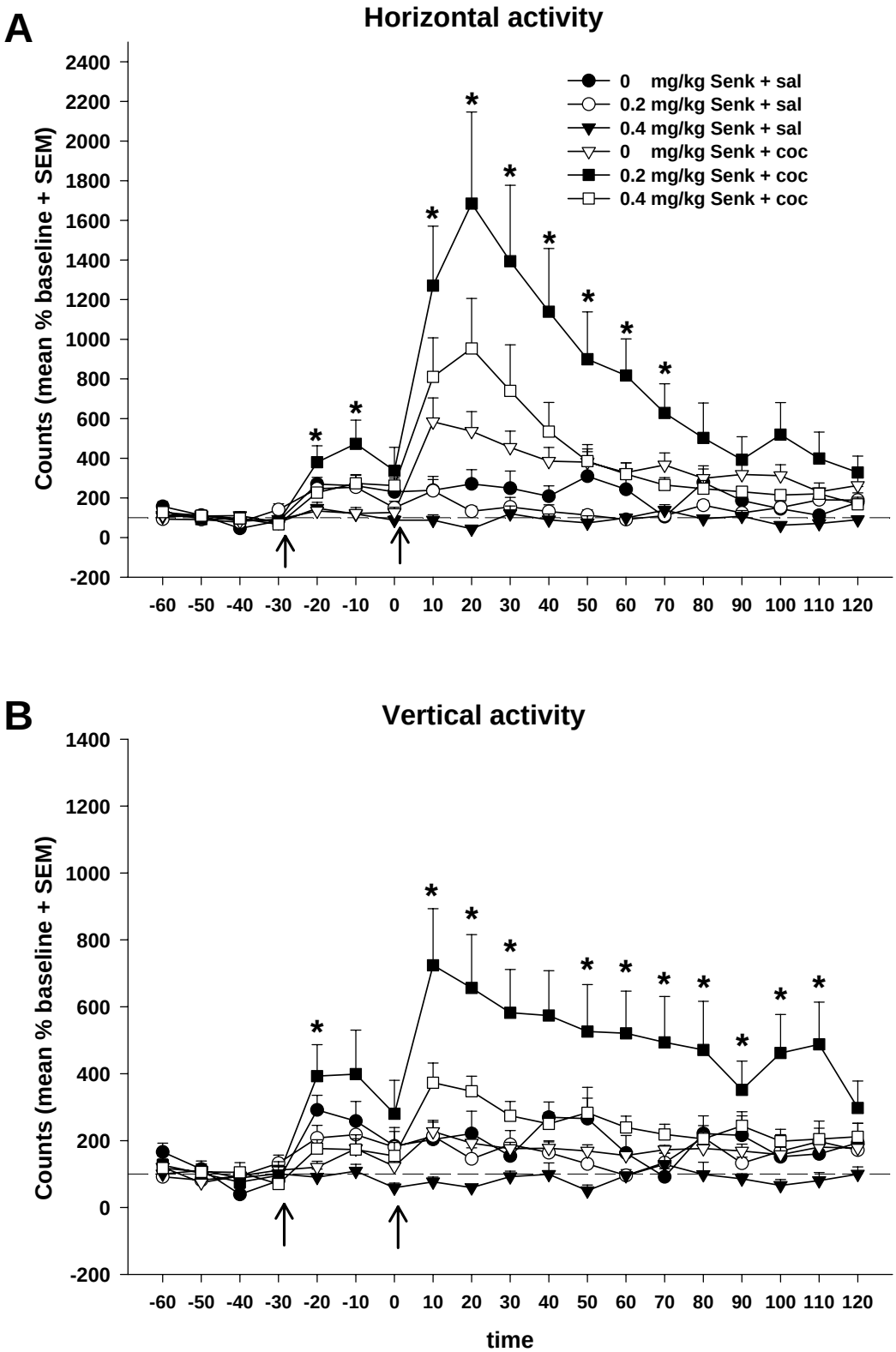
A
Horizontal activity
Counts (mean % baseline + SEM)
0 mg/kg Senk + sal
0.2 mg/kg Senk + sal
0.4 mg/kg Senk + sal
0 mg/kg Senk + coc
0.2 mg/kg Senk + coc
0.4 mg/kg Senk + coc
B
Vertical activity
Counts (mean % baseline + SEM)
time

Fig. 5

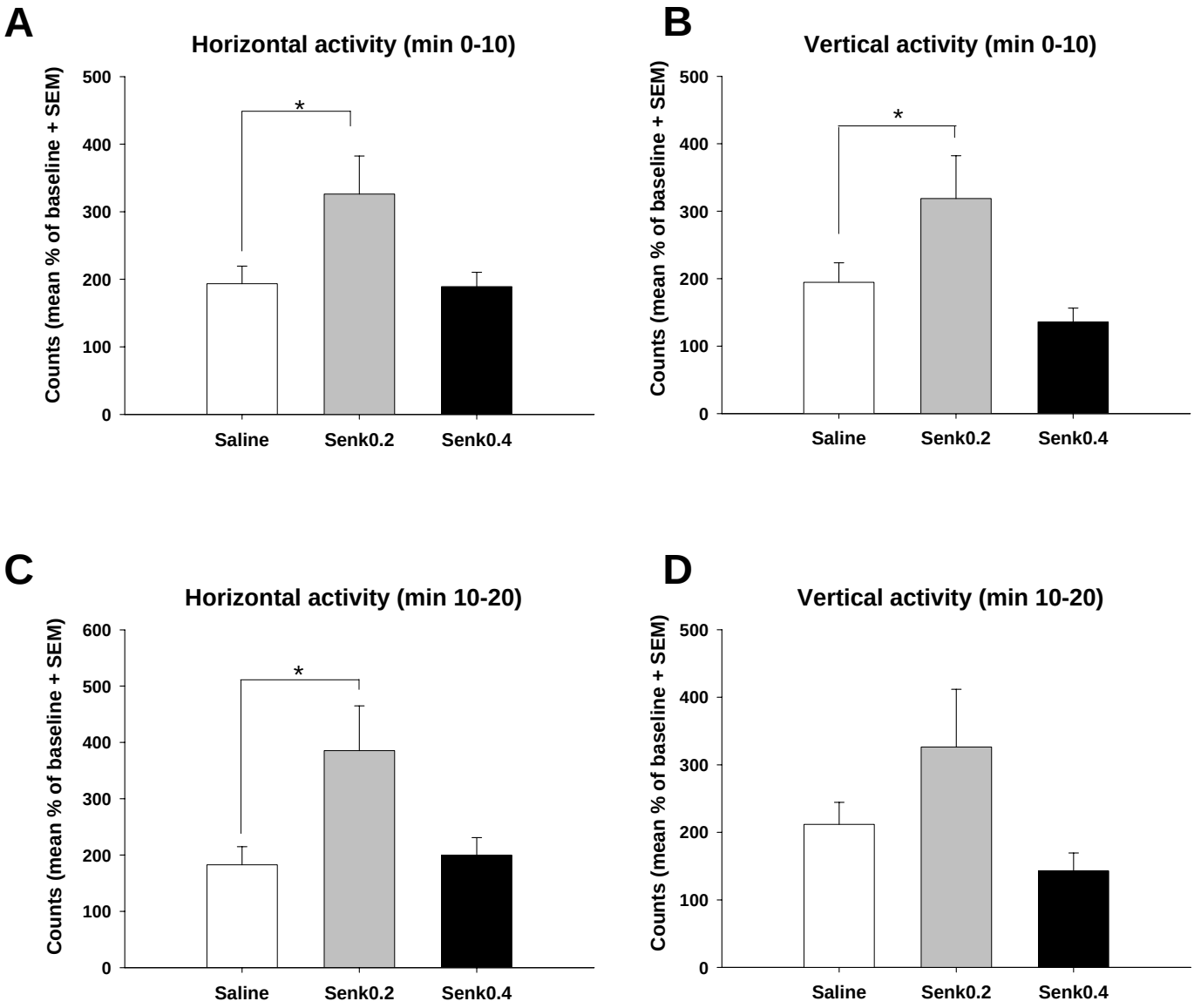
A
Horizontal activity (min 0-10)
Counts (mean % of baseline + SEM)
500
400
300
200
100
0
*
Saline
Senk0.2
Senk0.4
B
Vertical activity (min 0-10)
Counts (mean % of baseline + SEM)
500
400
300
200
100
0
*
Saline
Senk0.2
Senk0.4
C
Horizontal activity (min 10-20)
Counts (mean % of baseline + SEM)
600
500
400
300
200
100
0
*
Saline
Senk0.2
Senk0.4
D
Vertical activity (min 10-20)
Counts (mean % of baseline + SEM)
500
400
300
200
100
0
Saline
Senk0.2
Senk0.4

Fig. 6

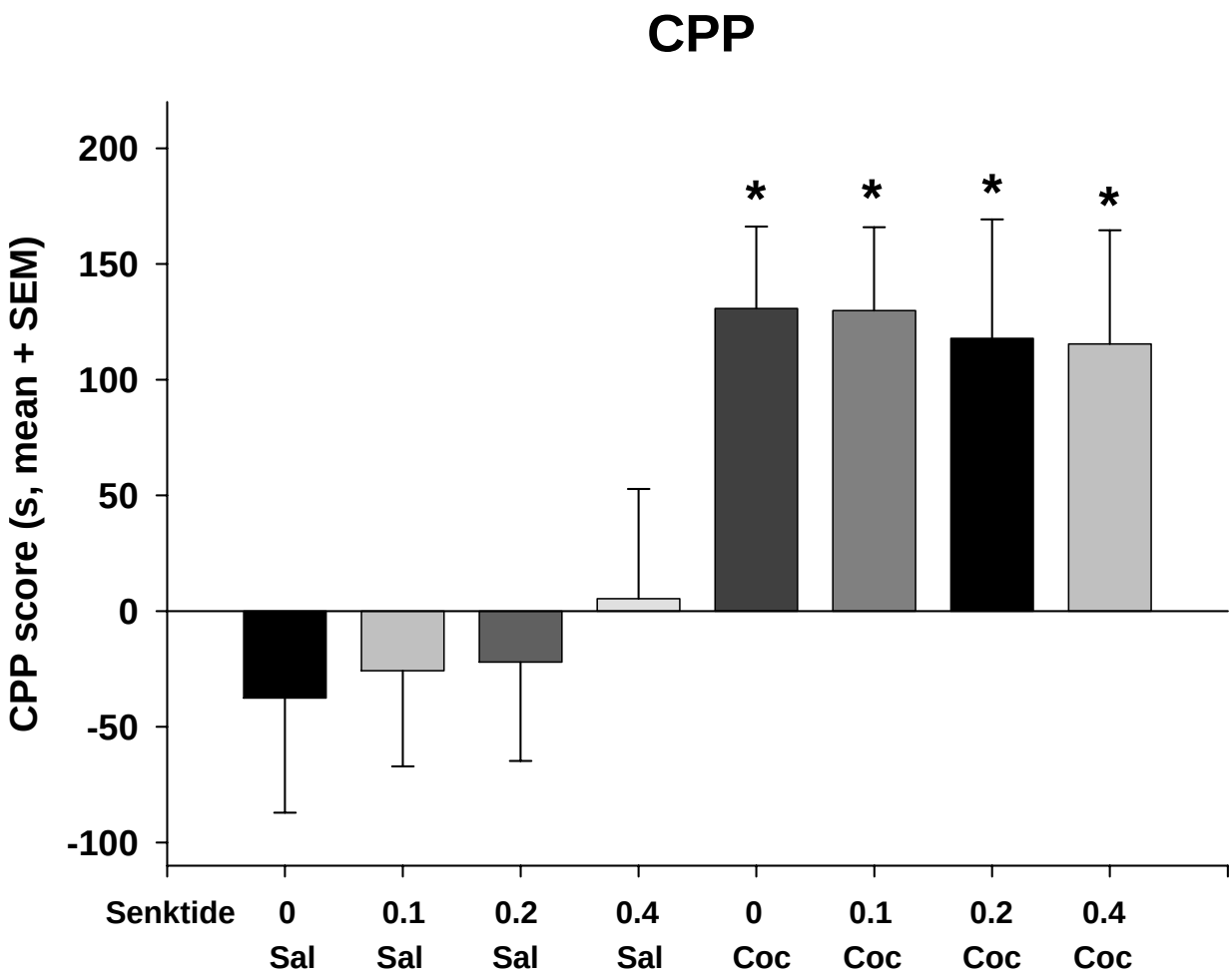
CPP
CPP score (s, mean + SEM)
200
150
100
50
0
-50
-100
*
*
*
*
Senktide
0 Sal
0.1 Sal
0.2 Sal
0.4 Sal
0 Coc
0.1 Coc
0.2 Coc
0.4 Coc

Fig. 7

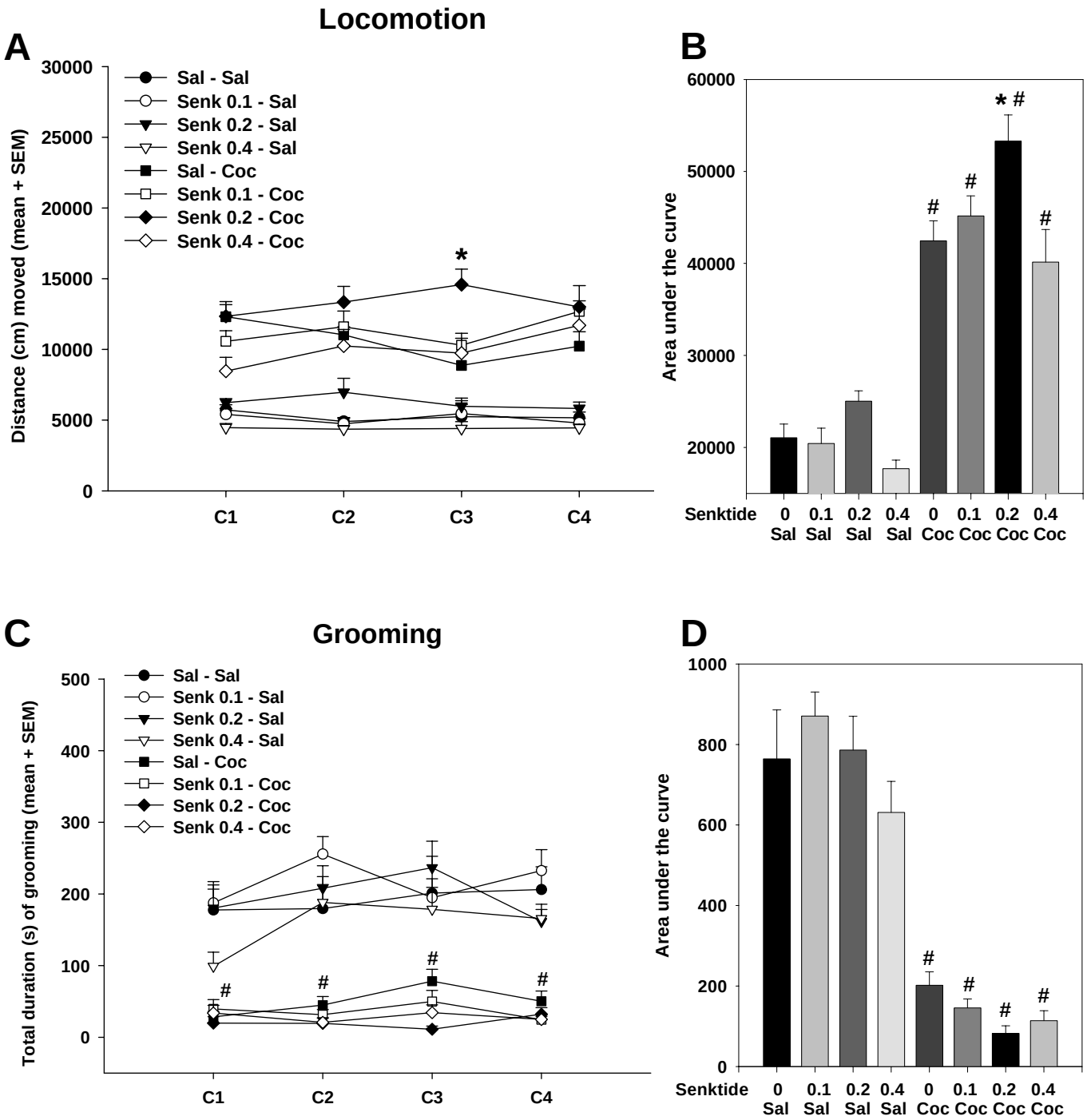
Locomotion
A
Distance (cm) moved (mean + SEM)
30000
25000
20000
15000
10000
5000
0
Sal - Sal
Senk 0.1 - Sal
Senk 0.2 - Sal
Senk 0.4 - Sal
Sal - Coc
Senk 0.1 - Coc
Senk 0.2 - Coc
Senk 0.4 - Coc
*
C1
C2
C3
C4
B
Area under the curve
60000
50000
40000
30000
20000
* #
#
#
#
Senktide
0 Sal
0.1 Sal
0.2 Sal
0.4 Sal
0 Coc
0.1 Coc
0.2 Coc
0.4 Coc
C
Grooming
Total duration (s) of grooming (mean + SEM)
500
400
300
200
100
0
Sal - Sal
Senk 0.1 - Sal
Senk 0.2 - Sal
Senk 0.4 - Sal
Sal - Coc
Senk 0.1 - Coc
Senk 0.2 - Coc
Senk 0.4 - Coc
#
#
#
#
C1
C2
C3
C4
D
Area under the curve
1000
800
600
400
200
0
#
#
#
#
Senktide
0 Sal
0.1 Sal
0.2 Sal
0.4 Sal
0 Coc
0.1 Coc
0.2 Coc
0.4 Coc

Fig. 8

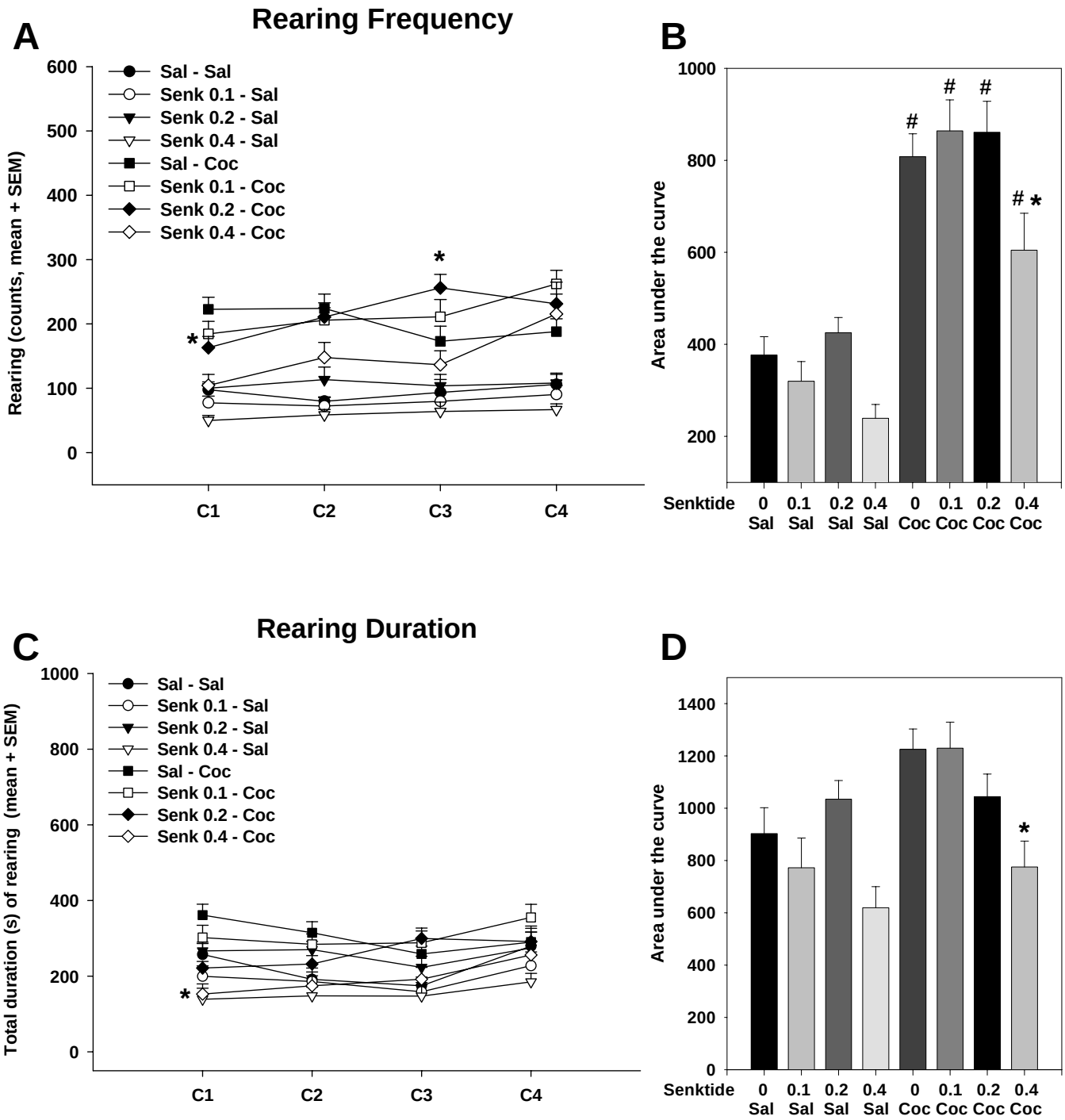
A
Rearing Frequency
Rearing (counts, mean + SEM)
600
500
400
300
200
100
0
Sal - Sal
Senk 0.1 - Sal
Senk 0.2 - Sal
Senk 0.4 - Sal
Sal - Coc
Senk 0.1 - Coc
Senk 0.2 - Coc
Senk 0.4 - Coc
*
*
C1
C2
C3
C4
B
Area under the curve
1000
800
600
400
200
#
#
#
*
Senktide
0 Sal
0.1 Sal
0.2 Sal
0.4 Sal
0 Coc
0.1 Coc
0.2 Coc
0.4 Coc
C
Rearing Duration
Total duration (s) of rearing (mean + SEM)
1000
800
600
400
200
0
Sal - Sal
Senk 0.1 - Sal
Senk 0.2 - Sal
Senk 0.4 - Sal
Sal - Coc
Senk 0.1 - Coc
Senk 0.2 - Coc
Senk 0.4 - Coc
*
C1
C2
C3
C4
D
Area under the curve
1400
1200
1000
800
600
400
200
0
*
Senktide
0 Sal
0.1 Sal
0.2 Sal
0.4 Sal
0 Coc
0.1 Coc
0.2 Coc
0.4 Coc

Fig. 9

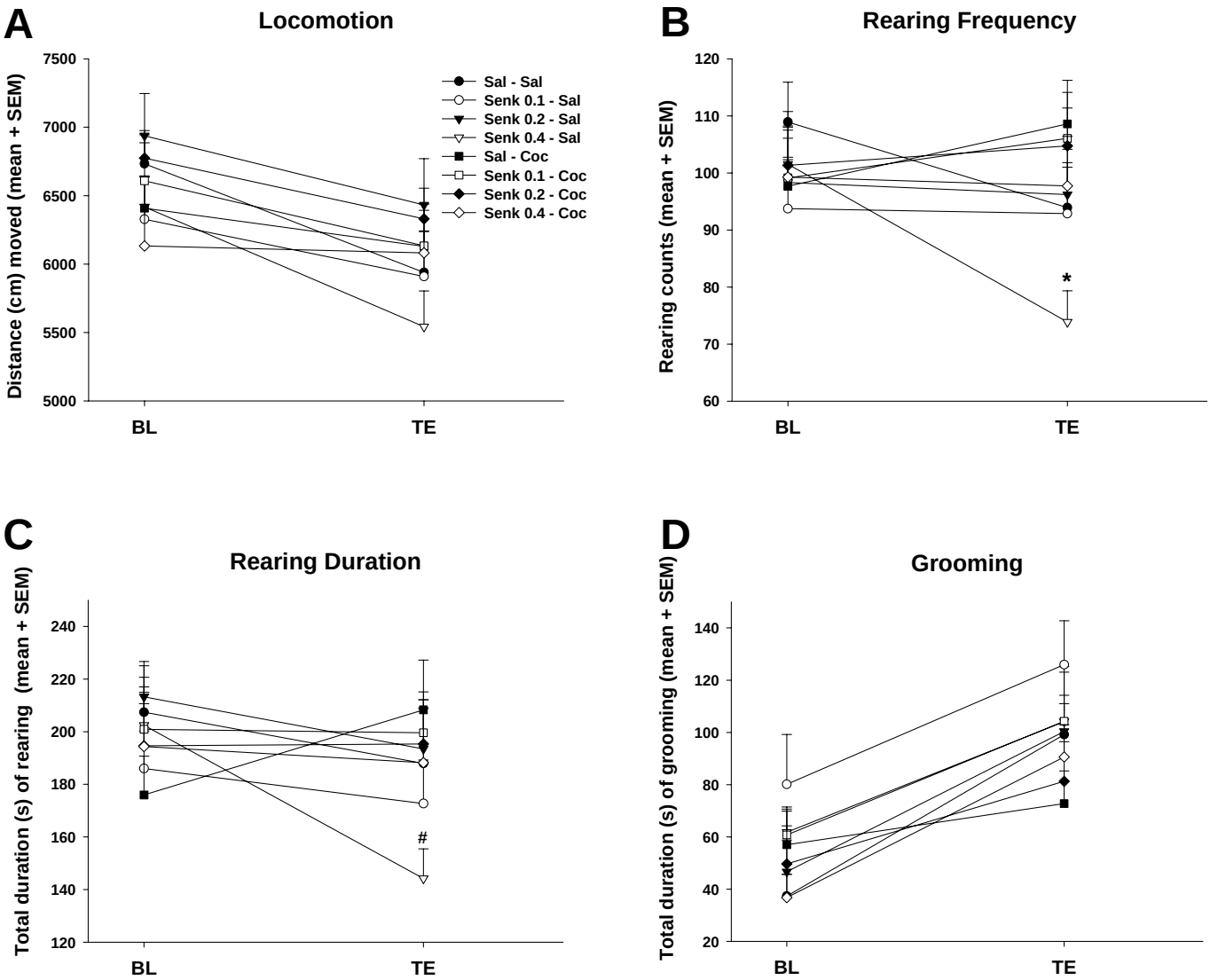
A
Locomotion
Distance (cm) moved (mean + SEM)
7500
7000
6500
6000
5500
5000
BL
TE
Sal - Sal
Senk 0.1 - Sal
Senk 0.2 - Sal
Senk 0.4 - Sal
Sal - Coc
Senk 0.1 - Coc
Senk 0.2 - Coc
Senk 0.4 - Coc
B
Rearing Frequency
Rearing counts (mean + SEM)
120
110
100
90
80
70
60
*
BL
TE
C
Rearing Duration
Total duration (s) of rearing (mean + SEM)
240
220
200
180
160
140
120
#
BL
TE
D
Grooming
Total duration (s) of grooming (mean + SEM)
140
120
100
80
60
40
20
BL
TE

Fig. 10

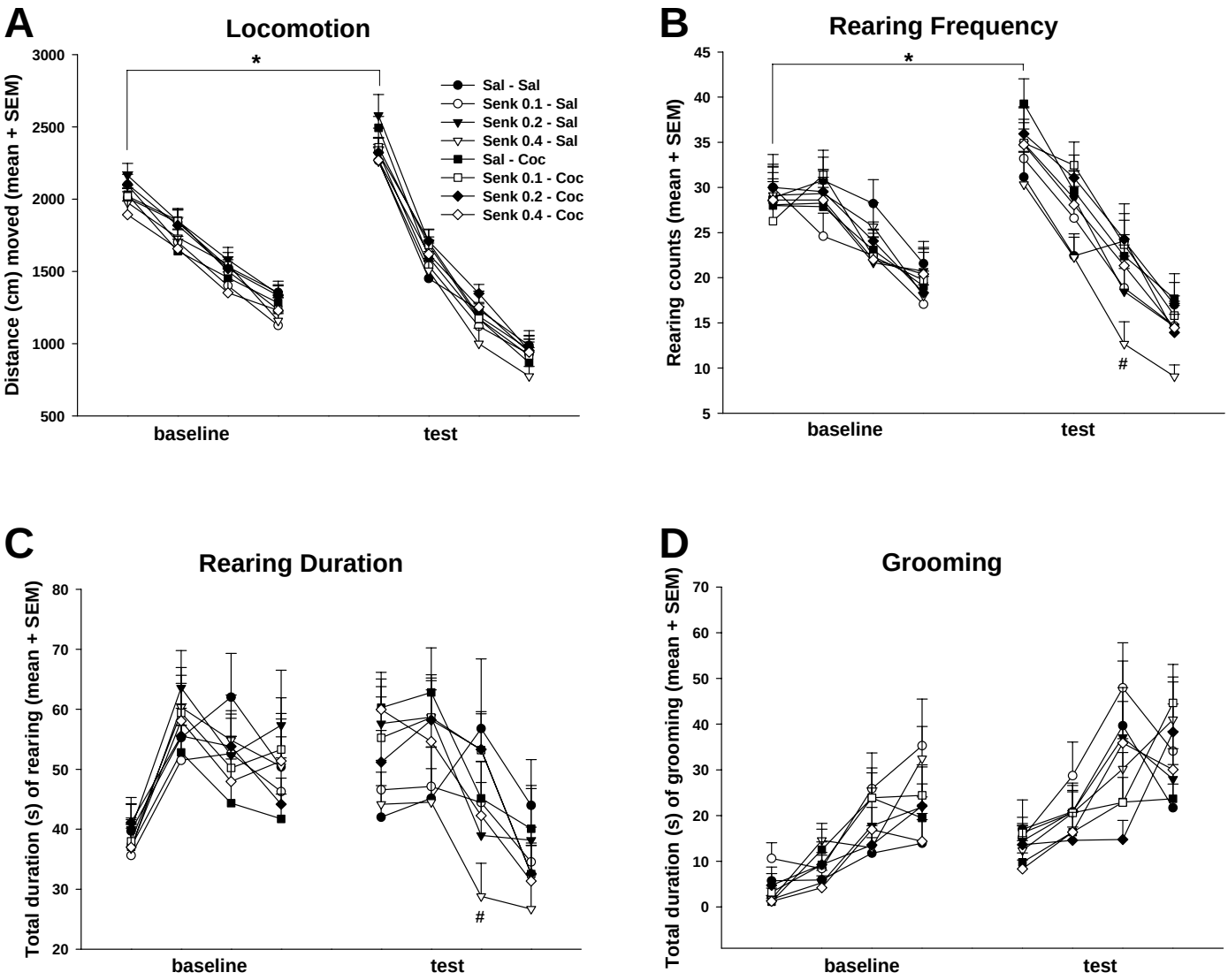
A
Locomotion
Distance (cm) moved (mean + SEM)
3000
2500
2000
1500
1000
500
*
Sal - Sal
Senk 0.1 - Sal
Senk 0.2 - Sal
Senk 0.4 - Sal
Sal - Coc
Senk 0.1 - Coc
Senk 0.2 - Coc
Senk 0.4 - Coc
baseline
test
B
Rearing Frequency
Rearing counts (mean + SEM)
45
40
35
30
25
20
15
10
5
*
#
baseline
test
C
Rearing Duration
Total duration (s) of rearing (mean + SEM)
80
70
60
50
40
30
20
#
baseline
test
D
Grooming
Total duration (s) of grooming (mean + SEM)
70
60
50
40
30
20
10
0
baseline
test

Fig. 11

Available online at www.sciencedirect.com
SCIENCE DIRECT
European Journal of Pharmacology 536 (2006) 269–278
www.elsevier.com/locate/ejphar

The tachykinin NK_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], 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*

Received 13 January 2006; received in revised form 21 February 2006; accepted 6 March 2006
Available online 10 March 2006

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 tachykinin 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 tachykinin 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 tachykinin 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 tachykinin 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 tachykinin NK_3 receptor antagonist, SR142801, as a potential treatment of cocaine addiction in humans.

Keywords: Cocaine; Tachykinin 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, neurokinin A, neurokinin B, neuropeptide K and neuropeptide γ. Three distinct G protein-coupled receptors, tachykinin neurokinin1 (NK_1), NK_2 and NK_3, have been characterized. Tachykinin NK_1 and NK_3 receptors are widely distributed in the brain, while the tachykinin NK_2 receptors are found in restricted areas. Substance P, neurokinin A and neurokinin B have higher binding affinity to tachykinin NK_1, NK_2 and NK_3 receptors, respectively, but all the neurokinins bind to all three tachykinin NK receptors (Regoli et al., 1994; Massi et al., 2000; Hökfelt et al., 2001). Compelling evidence suggests that tachykinin 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 tachykinin NK_3 receptor also mediates the acute as well as the chronic behavioral effects of cocaine (Jocham et al.,

* 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 © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2006.03.010

submitted for publication). However, the findings in rats may not automatically generalize to humans due to the considerable species differences in tachykinin 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 not only 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 tachykinin 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 tachykinin NK_3 receptor antagonism affects them. According to our findings in rats we hypothesized that pharmacological antagonism of the tachykinin NK_3 receptor with the non-peptide tachykinin 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 tachykinin NK_3 receptor antagonism.

2. Materials and methods

2.1. Subjects

Twelve adult black tufted-ear marmosets (*C. penicillata*, five males and seven 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 × 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 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 tachykinin 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 for publication) 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 × 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.

2.4. Procedure

All animals were habituated to the maze and the transport cage (35 × 20 × 23 cm) prior to the beginning of the experiment. All subjects were submitted to one more 20-min habituation trial

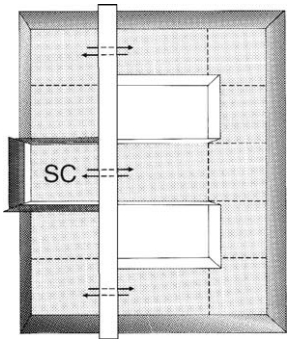

Fig. 1. Top view of the figure-eight continuous maze used for testing (SC indicates the start compartment; for a detailed description, see text).

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 4 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 pm.

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,b) 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 s while the animal remained stationary; (6) *Terrestrial scanning*: time and frequency the animals spent scanning the environment below the horizontal plane, persisting >5 s while the animal remained stationary; (7) *Aerial glance*: frequency of rapid upward sweeping movements of the head lasting <2 s while stationary and (8) *Terrestrial glance*: frequency of rapid downward movements of the head lasting <2 s 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 0.05 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

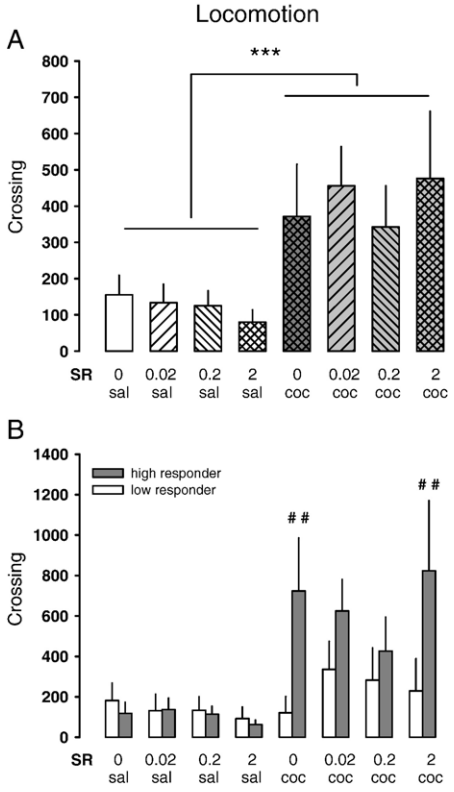

Fig. 2. The effects of cocaine (10 mg/kg, i.p.) on locomotor activity (mean±S.E.M.) and its modulation by the tachykinin NK_3 receptor antagonist, SR142801 (0.02–2.0 mg/kg, i.p.), during a 20-min test trial. (A) Effects for all animals tested ($n=12$). (B) Sensitivity analysis: group split according 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).

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). 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). There was no effect of the treatment day on which an animal received the vehicle-cocaine treatment regarding its locomotor response, and, thus, the high vs. low responder classification. The cocaine but not the saline effect on locomotor activity differed considerably between the two responder types (high vs. low responders, 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 high responder animals with an inverted U-shaped dose–response curve. The high vs. low responder 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 ($P=0.0036$).

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 high and low responder animals (all treatments: $P>0.05$).

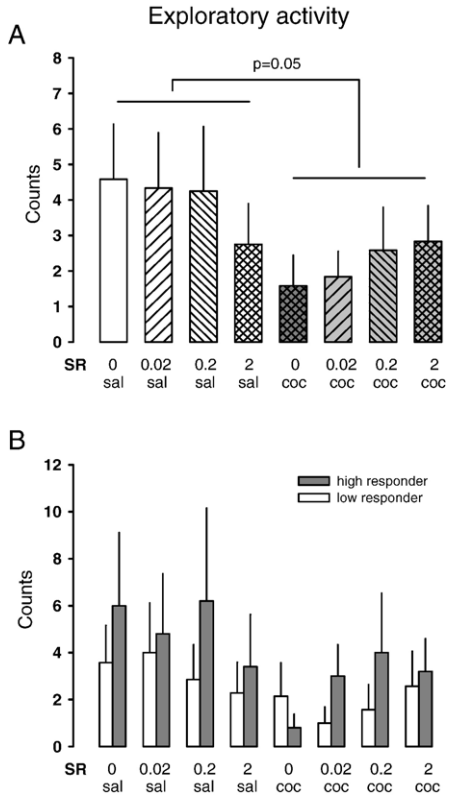

Fig. 3. The effects of cocaine (10 mg/kg, i.p.) on exploratory activity (mean ± S.E.M.) and its modulation by the tachykinin NK_3 receptor antagonist, SR142801 (0.02–2.0 mg/kg, i.p.), during a 20-min test trial. (A) Effects for all animals tested ($n=12$). (B) Sensitivity analysis: group split according 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 ($n=7$); $P=0.05$, two-way ANOVA, factor treatment).

Bodycare activity and scent marking behavior were also decreased after cocaine treatment (Fig. 4A and C; two-way ANOVA, treatment, bodycare activity: $F_{1,88}=10.56$, $P=0.0016$; scent marking: $F_{1,88}=4.97$, $P=0.028$). 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 D) showed that there was no obvious difference in bodycare activity and scent marking behavior after cocaine between high and low responder 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 C; 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 D), however, showed a dissociating effect of cocaine on the time of aerial scanning between the high and low responder animals (high vs. low responders, vehicle-cocaine: $P=0.0063$, vehicle-saline: $P>0.05$). While cocaine increased the time of aerial scanning in the low responder animals, it decreased aerial scanning time in the high responder animals. This high vs. low responder 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 ($P=0.045$). No such effect was observed for the frequency of aerial scanning (high vs. low responders, all treatments: $P>0.05$).

The time (Fig. 6A; two-way ANOVA, treatment, $F_{1,88}=4.98$, $P=0.028$) as well as frequency of terrestrial scanning (Fig. 6C; two-way ANOVA, treatment, $F_{1,88}=4.93$, $P=0.029$) 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 × treatment interaction ($P>0.05$). Sensitivity analysis (Fig. 6B and D), on the other hand, showed a dissociating cocaine effect. Cocaine alone increased terrestrial scanning in the high responder animals, while it eliminated the behavior in the low responder animals (high vs. low responders, vehicle-cocaine, time: $P=0.023$; frequency: $P=0.0075$, vehicle-saline, time and frequency: $P>0.05$). The difference between high and low responder animals in their cocaine response was no longer observed after pretreatment with SR142801 (high vs. low responders, all doses: $P>0.05$).

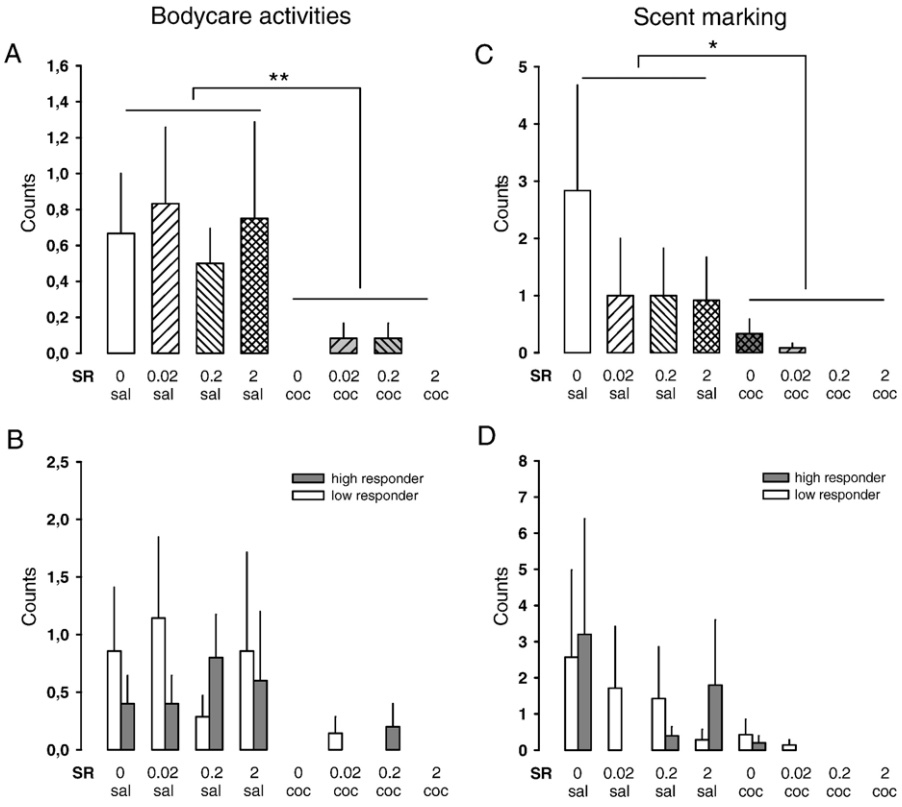

Fig. 4. The effects of cocaine (10 mg/kg, i.p.) on bodycare activities and scent marking behavior (mean ± S.E.M.) and its modulation by the tachykinin NK_3 receptor antagonist, SR142801 (0.02–2.0 mg/kg, i.p.), during a 20 min test trial. (A/C) Effects for all animals tested ($n=12$). (B/D) Sensitivity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

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 × treatment interaction ($P>0.05$). Sensitivity analysis (Fig. 7B) showed that the increase in aerial glance after cocaine only occurred in the high responder animals but not in the low responder animals (high vs. low responders, vehicle-cocaine: $P=0.021$, vehicle-saline: $P>0.05$). Pretreatment with SR142801 attenuated the high vs. low responder difference by reducing the increase in aerial glance in the high responder animals at doses of 0.2 and 2 mg/kg (high vs. low responders, $P>0.05$), but not at a dose of 0.02 mg/kg (high vs. low responders, $P=0.0089$).

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 high responder animals, although statistical analysis did not yield a high vs. low responder 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 responder) and cocaine insensitive (low responder) animals. The subsequent sensitivity analysis revealed that there are two principle types of responses to cocaine in marmoset monkeys. The high responder animals were characterized in their response to cocaine by a profound increase in locomotor activity. But high vs. low responder differences after acute cocaine also occurred in aerial and terrestrial scanning and aerial glance behavior. In the high responder animals, cocaine

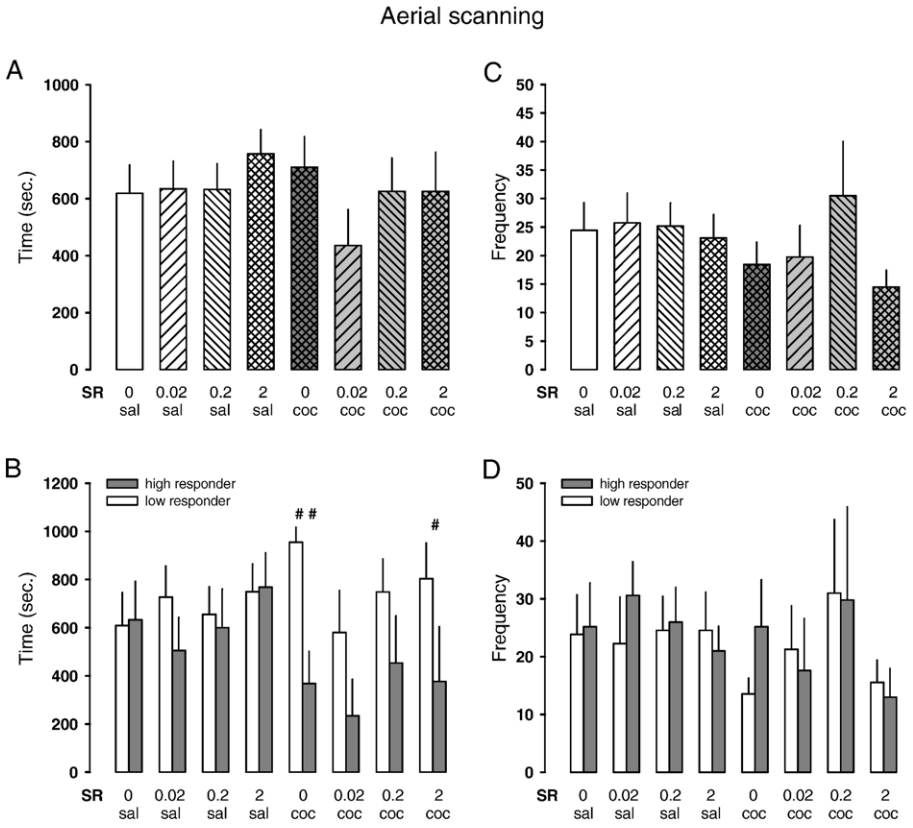

Fig. 5. The effects of cocaine (10 mg/kg, i.p.) on aerial scanning time and frequency (mean ± S.E.M.) and its modulation by the tachykinin NK_3 receptor antagonist, SR142801 (0.02–2.0 mg/kg, i.p.), during a 20-min test trial. (A/C) Effects for all animals tested ($n=12$). (B/D) Sensitivity analysis: 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).

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 low responder animal's response. The low responder animals did not show hyperlocomotion after cocaine, but instead, responded with an increase in aerial scanning.

Tachykinin NK_3 receptor antagonism with SR142801 alone did not affect any of the behaviors measured in marmosets. The cocaine effects on the marmoset behavior did not appear to be modulated by the tachykinin 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 high responder animals, while it reduced the increase in aerial scanning in the low responder animals. In all these behaviors tachykinin NK_3 receptor antagonism also attenuated the high vs. low responder differences in the acute behavioral response to cocaine. But also after sensitivity analysis, the tachykinin 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 high and low responder 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, high responder 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,b), 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 high responder animals, which may indicate that the anxiogenic component was not dominant in the high responder 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; Ferrari and Ferrari, 1990; Hardie and Buchanan-Smith, 1997; Caine, 1998; Koenig, 1998). In the low responder

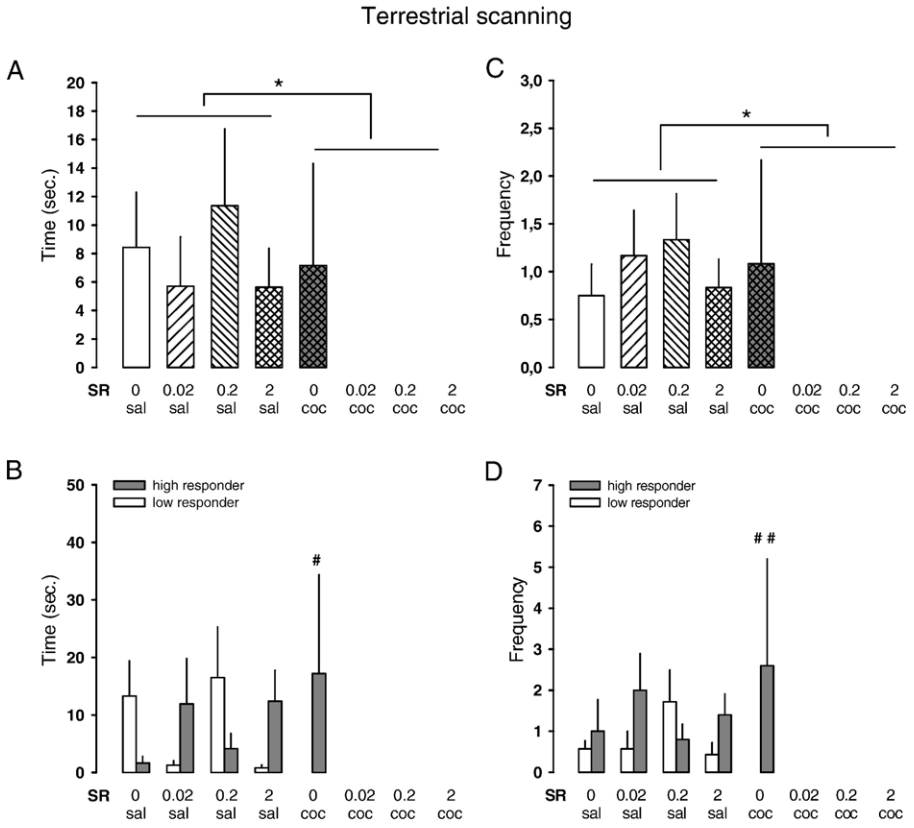

Fig. 6. The effects of cocaine (10 mg/kg, i.p.) on terrestrial scanning time and frequency (mean±S.E.M.) and its modulation by the tachykinin NK_3 receptor antagonist, SR142801 (0.02–2.0 mg/kg, i.p.), during a 20-min test trial. (A/C) Effects for all animals tested ($n=12$). (B/D) Sensitivity analysis: 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).

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 high responder animals. This might reflect a more pronounced anxiogenic component in the high responder 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 high responder animals by tachykinin NK_3 receptor antagonism. The suppressory effects of cocaine on bodycare activity and scent marking, however, were not reversed by SR142801. Thus, in the high responder marmoset monkeys the contribution of the tachykinin 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 for publication). 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 tachykinin NK_3 receptor is not required for the generation of spontaneous behavior, but rather, that tachykinin NK_3 receptors contribute to an induced increase in locomotor activity. This view is also supported by the findings that the local injection of substance P or its C-terminal analogue, DiMe-C7, into the ventral tegmental area and the substantia nigra 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 tachykinin NK_3-receptor agonist senktide, but not of tachykinin NK_1 or NK_2 receptor agonists, into the substantia nigra and ventral tegmental area 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 tachykinin NK_3 receptor antagonism. In the low responder animals tachykinin 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 tachykinin NK_3 receptor antagonist was rather surprising, since the tachykinin NK_3 receptor agonist, senktide (Ribeiro and De Lima, 1998; Ribeiro et al., 1999), substance P (Echeverry et al., 2001), and the substance P N-terminal fragment, SP_{1-7} (Barros et al., 2002b), were found to be anxiolytic in mice, rats, and

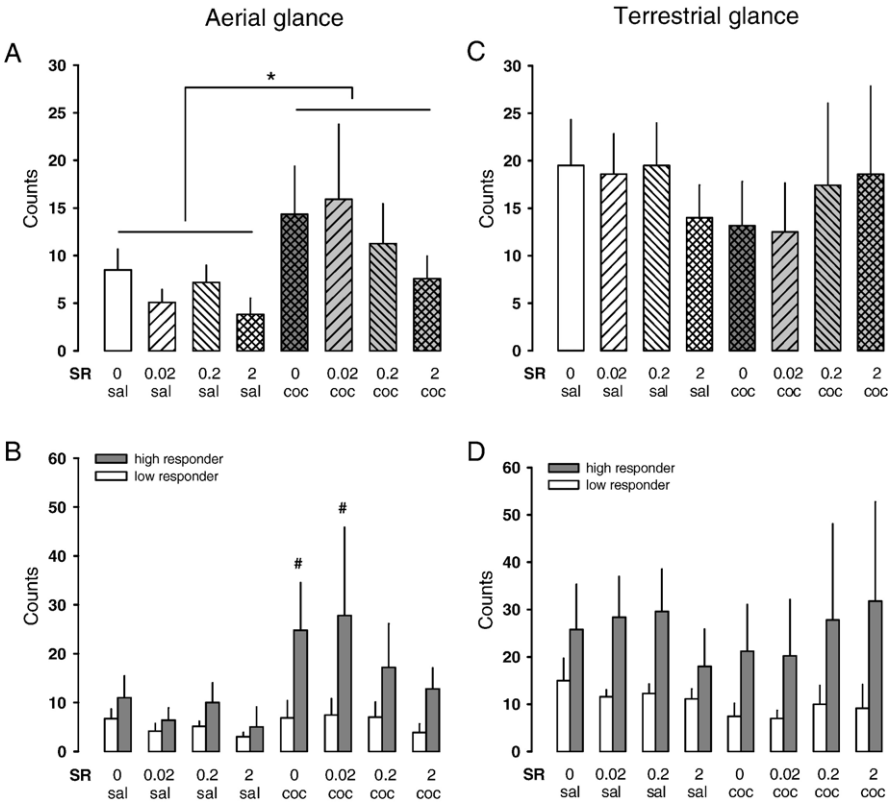

Fig. 7. The effects of cocaine (10 mg/kg, i.p.) on aerial and terrestrial glance (mean ± S.E.M.) and its modulation by the tachykinin NK_3 receptor antagonist, SR142801 (0.02–2.0 mg/kg, i.p.), during a 20-min test trial. (A/C) Effects for all animals tested ($n=12$). (B/D) Sensitivity analysis: group 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).

monkeys respectively. Also the local application of substance P, 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, substance P 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 tachykinin 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 high and low responder animals.

Altogether, the tachykinin NK_3 receptor antagonism attenuated the acute cocaine effects in high and low responder 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 for publication). 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 high responder animals and of the increase in aerial scanning in the low responder 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 for publication). At the highest dose tested, the low affinity of SR142801 to calcium and sodium channels (Emonds-Alt et al., 1995) may have counteracted the tachykinin NK_3 receptor effects. It should be noted that the high vs. low responder subdivision of the animals was made post hoc based on the effects of the vehicle-cocaine treatment administered in the course of the testing. An important drawback, which might also limit the interpretation, is that by the subdivision of the animals group size was reduced, which results in a loss of statistical power when comparing the treatment effects. Results based on the high vs. low responder behavioral profiles have, therefore, an exploratory character and warrant further investigation.

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, diethylpropion (Mello et al., 2005). Tachykinin 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 tachykinin NK_3 receptor antagonist SR142801 as a potential treatment of cocaine-addiction in humans.

Acknowledgement

This work was supported by the Deutsche Forschungsgemeinschaft, by FINATEC (to C.T.), by CAPES/DAAD/PROBRAL (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. were 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.

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., 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., 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., submitted for publication. Neurokinin3 receptor antagonism attenuates cocaine's behavioral activating effects yet potentiates its dopamine-enhancing action in the nucleus accumbens core.

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. Anim. 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.

PEPTIDES 27 (2006) 2214–2223

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/peptides

Interaction of the tachykinin NK_3 receptor agonist senktide with behavioral effects of cocaine in marmosets (*Callithrix penicillata*)

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], *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*

ARTICLE INFO

Article history:
Received 30 January 2006
Received in revised form
9 March 2006
Accepted 9 March 2006
Published on line 18 April 2006

Keywords:
Cocaine
Tachykinin NK_3-receptor
Senktide
Marmoset
Behavior
Sensitivity

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, antagonism of the tachykinin NK_3-receptor (NK_3-R), one of the three tachykinin receptors in the brain, was shown to attenuate the acute and chronic behavioral effects of cocaine in rats and the acute effects in non-human primates. In order to expand these findings we investigated the effects of the NK_3-R agonist, succinyl-[Asp^6, Me-Phe^8]SP_{6-11} (senktide), on the acute behavioral effects of cocaine in marmoset monkeys (*Callithrix penicillata*) using a figure-eight maze procedure. Animals were pretreated with senktide (0, 0.1, 0.2, 0.4 mg/kg, s.c.), and received either a treatment with cocaine (10 mg/kg) or saline (i.p.). Cocaine increased locomotor activity and the duration of aerial scanning behavior, but reduced exploratory activity, bodycare activity, the frequency of aerial scanning, and terrestrial glance behavior. Senktide blocked the effects of cocaine on locomotor activity, but enhanced the cocaine effects on exploratory activity, aerial scanning frequency, and terrestrial glance behavior. Senktide alone did not significantly influence monkey behavior in this study. These data expand previous findings suggesting a complex role of the NK_3-R in the acute behavioral effects of cocaine in non-human primates.

1. Introduction

Neuropeptides belonging to the tachykinin family are characterized by 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 (NPK), and neuropeptide γ (NPγ). Three distinct G protein-coupled receptors, tachykinin NK_1-receptor (NK_1-R), NK_2-R and NK_3-R, have been characterized. NK_1-Rs and NK_3-Rs are widely distributed in the brain, while the NK_2-Rs are found only in restricted areas. SP, NKA and NKB have a high binding affinity to the NK_1-R, NK_2-R and NK_3-R, respectively, but all the tachykinins bind to all three NK-Rs [26,44,46].

Several studies suggest a tachykinin involvement in cocaine effects. Thus, cocaine activated *c*-fos in SP-positive

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

0196-9781/$ – see front matter
doi:10.1016/j.peptides.2006.03.005

cells of the striatum already 30 min after application [31], and preprotachykinin-A (PPT-A) messenger RNA (mRNA) after 3 h in the striatum of rats [1]. Repeated cocaine administration increased PPT-A mRNA in the dorsal and ventral striatum [37], and SP immunoreactivity in the striatum, substantia nigra and frontal cortex of rats [2]. In addition, prolonged cocaine self-administration increased SP mRNA in the striatum [27] and nucleus accumbens (Nac)/shell [3]. However, SP applied i.c.v. did not substitute for cocaine in rats using a drug-discrimination paradigm [51]. Pharmacological NK_1-R antagonism blocked the acute hyperlocomotor effects of cocaine [34], reverses cocaine sensitization [18], and reduces the cocaine-induced dopamine increase in the striatum of rats [35]. Pharmacological activation of NK_1-Rs can reinstate cocaine seeking behavior ([43]; but see also Ref. [45]). In contrast to the NK_1-R, little is known about the role of the NK_3-R in cocaine effects. Massi and co-workers [13–16,36,41] demonstrated an inhibitory role of the NK_3-R in alcohol self-administration in rats, using the NK_3-R agonists, succinyl-[Asp^6, Me-Phe^8]SP_{6-11} (senktide) and [$Asp^{5,6}$, Me-Phe^8]SP_{5-11} (aminosenktide). In contrast, a study by Placenza et al. [42] reported reinstatement of cocaine self-administration after application of the NK_3-R preferring SP analog DiMe-C7 into the ventral tegmental area of rats. In line with these findings is a recent study, which showed that the NK_3-R antagonist, SR142801, attenuates the acute and chronic behavioral effects of cocaine in rats, possibly by modulating the cocaine-induced dopamine (DA) increase in the Nac/core, but not Nac/shell [29]. Despite considerable species differences in NK_3-R antagonist binding between humans and rats [21,40], a study in marmoset monkeys (*Callithrix penicillata*) provided evidence for an inhibitory effect of NK_3-R antagonism on the cocaine-induced hyperlocomotion in a non-rodent species [19]. NK_3-R antagonism not only blocked the cocaine-induced hyperlocomotion in responsive monkeys, but also affected other cocaine-induced behaviors. In order to further characterize the role of the NK_3-R in the acute behavioral effects of cocaine, we investigated the effects of the NK_3-R agonist, senktide, alone, and in combination with cocaine in non-human primates (*C. penicillata*).

2. Materials and methods

2.1. Subjects

Eight adult black tufted-ear marmosets (*C. penicillata*, five males and three females) were used as subjects. Animals weighed 285–370 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 m × 1.3 m × 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-R agonist senktide (Bachem, Bubendorf, Switzerland) was dissolved in 0.9% physiological saline and injected s.c. in the doses of 0, 0.1, 0.2 and 0.4 mg/kg. 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 1 ml/kg for senktide and cocaine.

2.3. Apparatus

Testing was conducted in a figure-eight continuous maze [5]. The maze consisted of a rectangular field (125 cm × 103 cm × 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 cm × 8 cm × 218 cm). The back chamber consisted of an arm (125 cm × 30 cm × 35 cm) with a central guillotine-type door. The latter formed the start compartment. The front chamber had three parallel arms (40 cm × 25 cm × 35 cm), 25 cm apart, ending in a common perpendicular arm (125 cm × 25 cm × 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 cm × 20 cm × 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. After the habituation trial two test sessions followed,

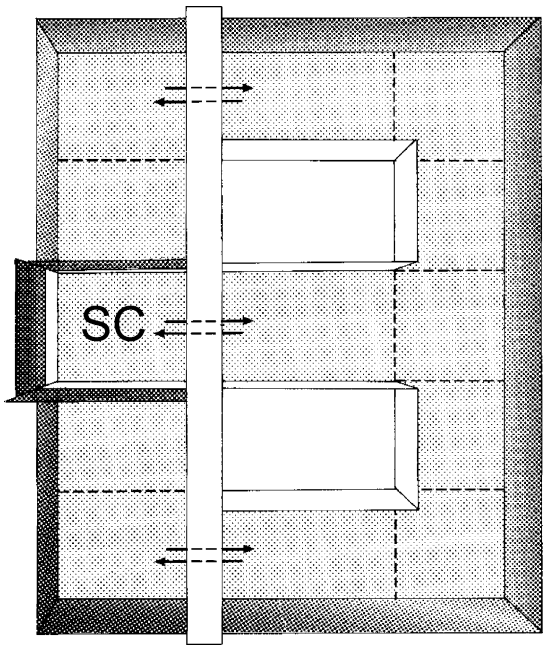

Fig. 1 – Top view of the figure-eight continuous maze used for testing (SC indicates the start compartment; for a detailed description: see text).

spaced 1 week apart. In the first session the effects of senktide plus saline were tested, while in the second session the effects of senktide 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 s.c. injection of senktide (0, 0.1, 0.2 and 0.4 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 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 based on the ethograms of marmoset behavior [5–10,47,48] 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 s while the animal remained stationary; (6) *terrestrial scanning*: time and frequency the animals spent scanning the environment below the horizontal plane, persisting ≥5 s while the animal remained stationary; (7) *aerial glance*: frequency of rapid upward sweeping movements of the head lasting ≤2 s while stationary; (8) *terrestrial glance*: frequency of rapid downward movements of the head lasting ≤2 s while stationary. For semi-automated behavioral analysis, the program PROSTCOM 3.20 [17] 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 determine differences between the treatment groups pre-planned comparisons using the LSD-test were calculated. 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". Behavioral parameters were further analyzed with respect to the cocaine sensitivity of the animals. All statistical results were interpreted as measures of effect with a p-value of <0.05 as a criterion.

3. Results

The injection of cocaine led to an increase in locomotor activity (Fig. 2A). Although a two-way ANOVA failed to show effects for the factor treatment ($p > 0.05$), pre-planed comparisons indicated a statistical difference between the saline–cocaine and the saline–saline group ($p = 0.03$). The cocaine-induced increase in locomotor activity was blocked by the

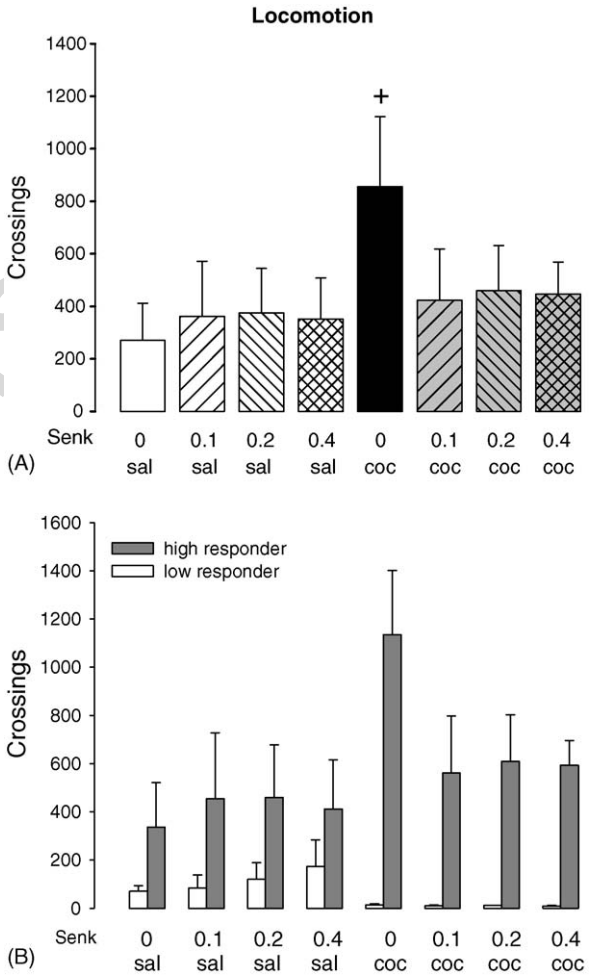

Fig. 2 – The effects of cocaine (10 mg/kg, i.p.) on locomotor activity (mean ± S.E.M.) and its modulation by the NK_3-receptor agonist, senktide (0.1–0.4 mg/kg, s.c.), during a 20 min test trial. (A) Effects for all animals tested ($n = 8$). (B) Sensitivity analysis: group split according to the animals response to cocaine (*high responder*: increased locomotor activity after vehicle-cocaine vs. vehicle-saline ($n = 6$); *low responder*: no increase in locomotor activity after vehicle-cocaine vs. vehicle-saline ($n = 2$); $^{+}p < 0.05$ vs. saline–saline).

pretreatment with 0.1, 0.2, and 0.4 mg/kg senktide in an unspecific way (saline–saline versus all senktide–cocaine groups: $p > 0.05$). Senktide alone did at no dose affect locomotor activity (saline–saline versus all senktide–saline groups: $p > 0.05$). Sensitivity analysis (Fig. 2B) revealed that only six of the eight animals tested (75%) showed an increase in locomotor activity after saline–cocaine compared to saline–saline treatment, and were, thus, considered to be cocaine sensitive (high responders, HR). Two of the eight animals tested (25%) showed less activity after saline–cocaine compared to saline–saline treatment, and were considered to be cocaine insensitive (low responders, LR). Sensitivity analysis showed that the hyperlocomotor effects of cocaine were primarily observed in the HR animals, while LR animals showed a generally lower locomotor activity in all treatment combinations. However, due to the small number of LR animals in this study, sensitivity analysis of the HR and LR animal may primarily serve for comparative reasons with other studies in monkeys investigating psychostimulant effects [29,38].

Cocaine decreased exploratory activity (Fig. 3A and B; two-way ANOVA, factor treatment: $F_{1,56} = 20.37$, $p < 0.0001$). The cocaine-induced decrease in exploratory activity was potentiated by pretreatment with 0.1 mg/kg, and as a tendency with 0.4 mg/kg, but not with 0.2 mg/kg senktide (saline–saline versus 0.1 mg/kg senktide–cocaine: $p = 0.03$; versus 0.4 mg/kg

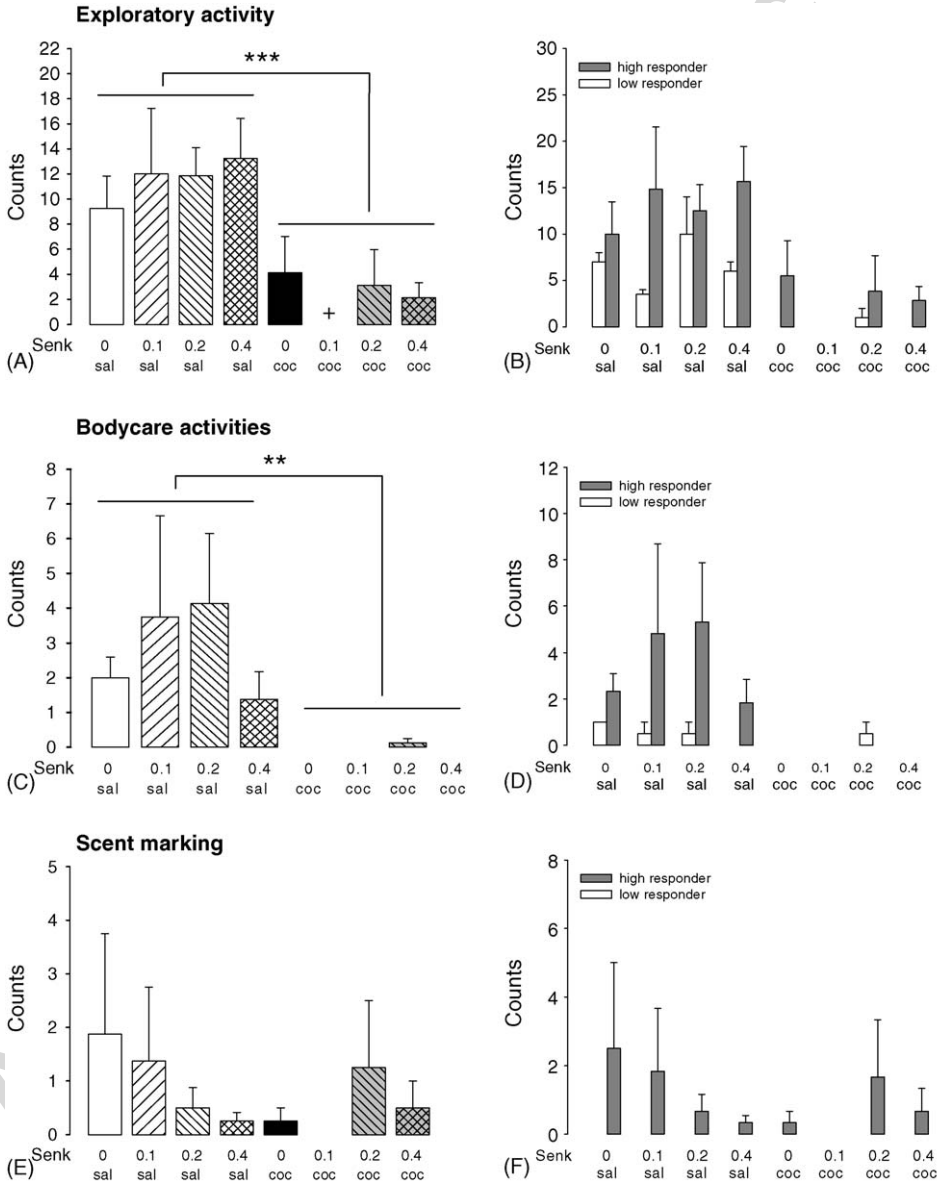

Fig. 3 – The effects of cocaine (10 mg/kg, i.p.) on exploratory activity (A and B), bodycare activities (C and D), and scent marking (E and F; mean ± S.E.M.) and its modulation by the NK_3-receptor agonist, senktide (0.1–0.4 mg/kg, s.c.), during a 20 min test trial. (A, C and E) Effects for all animals tested ($n = 8$). (B, D and F) Sensitivity analysis: group split according to the animals response to cocaine (*high responder*: increased locomotor activity after vehicle-cocaine vs. vehicle-saline ($n = 6$); *low responder*: no increase in locomotor activity after vehicle-cocaine vs. vehicle-saline ($n = 2$); $^{**}p < 0.01$, $^{***}p < 0.001$, two-way ANOVA, factor treatment; $^{+}p < 0.05$ vs. saline–saline).

senktide–cocaine: $p = 0.09$; versus 0.2 mg/kg senktide–cocaine: $p > 0.05$). Senktide alone did at no tested dose affect exploratory activity (saline–saline versus all senktide–saline groups: $p > 0.05$). Sensitivity analysis indicated a floor-effect of cocaine on this behavior in the LR, but not HR animals. The potentiation of the cocaine-induced decrease in exploratory activity was only observed in the HR animals. Cocaine virtually eliminated bodycare activities (Fig. 3C and D; two-way ANOVA, factor treatment: $F_{1,56} = 9.13$, $p = 0.004$). This effect was evident in the HR and LR animals. The cocaine-induced decrease in bodycare activities was not affected by senktide pretreatment (saline–saline versus all senktide–cocaine groups: $p > 0.05$). Although senktide alone slightly increased bodycare activites, this effect did not reach statistical importance (saline–saline versus all senktide–saline groups: $p > 0.05$). Scent marking behavior was only as a tendency decreased by cocaine and senktide (Fig. 3E and F), although, neither the treatement nor pretreatment effect reached statistical significance in a two-way ANOVA ($p > 0.05$). Scent marking behavior was exclusively shown by the HR animals under all treatment conditions in this study.

Cocaine increased the duration of aerial scanning (Fig. 4A and B; two-way ANOVA, factor treatment: $F_{1,56} = 4.33$, $p = 0.04$), but decreased its frequency (Fig. 4C and D; two-way ANOVA, factor treatment: $F_{1,56} = 15.84$, $p = 0.0002$). Interestingly, there was a tendency for a decrease of aerial scanning duration for senktide alone, and an tendency for an increase in combination with cocaine, which, however, failed to reach statistical

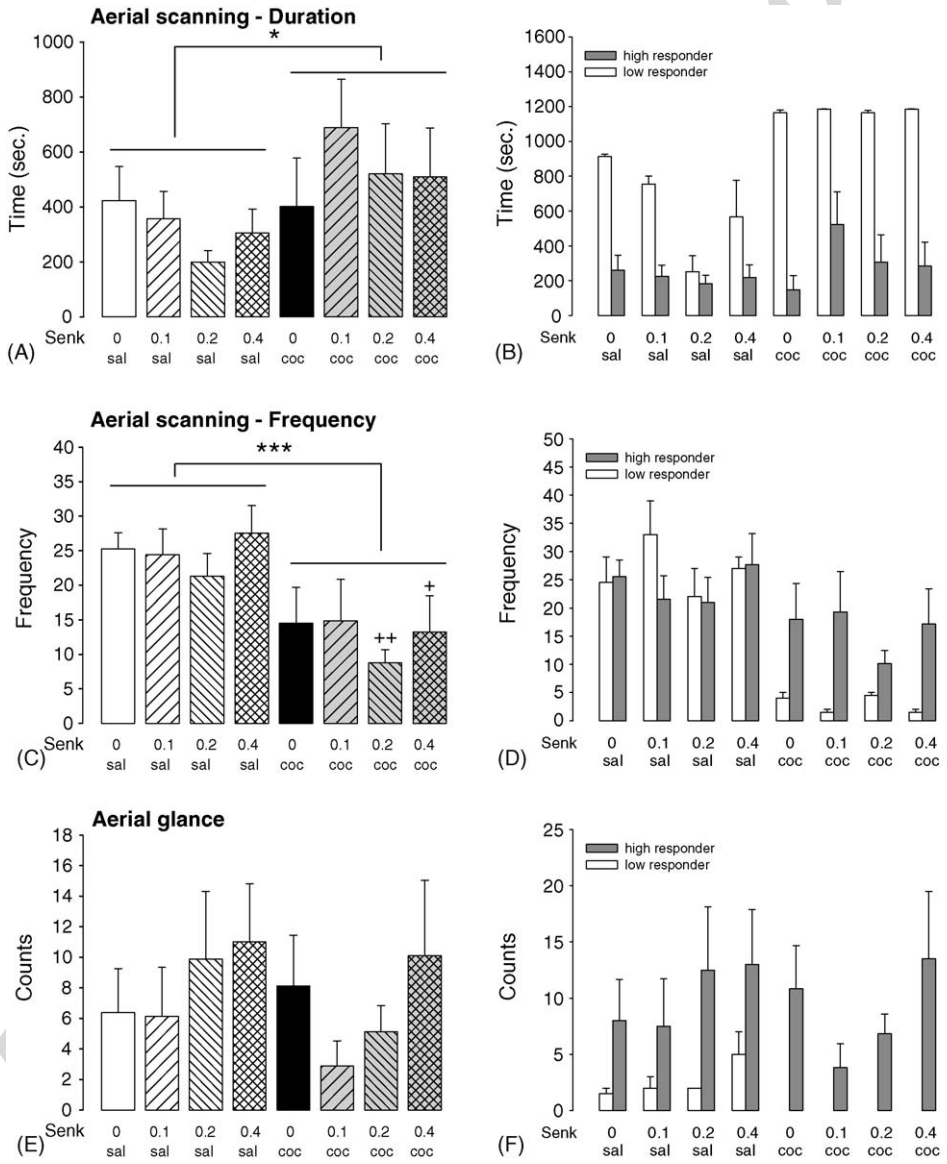

Fig. 4 – The effects of cocaine (10 mg/kg, i.p.) on aerial scanning (A–D) and aerial glance (E and F; mean ± S.E.M.) and its modulation by the NK_3-receptor agonist, senktide (0.1–0.4 mg/kg, s.c.), during a 20 min test trial. (A, C and E) Effects for all animals tested ($n = 8$). (B, D and F) Sensitivity analysis: group split according to the animals response to cocaine (*high responder*: increased locomotor activity after vehicle-cocaine vs. vehicle-saline ($n = 6$); *low responder*: no increase in locomotor activity after vehicle-cocaine vs. vehicle-saline ($n = 2$); $^{*}p < 0.05$, $^{**}p < 0.01$, two-way ANOVA, factor treatment; $^{+}p < 0.05$, $^{++}p < 0.01$ vs. saline–saline).

importance in pre-planed comparisons ($p > 0.05$). The cocaine-induced decline in aerial scanning frequency was potentiated by pretreatment with 0.2 and 0.4 mg senktide (saline–saline versus saline–cocaine: $p = 0.07$; versus 0.1 mg/kg senktide–cocaine: $p = 0.08$; versus 0.2 mg/kg senktide–cocaine: $p = 0.008$; versus 0.4 mg/kg senktide–cocaine: $p = 0.047$). Senktide had no effect on the frequency of aerial scanning by itself ($p > 0.05$). LR animals showed as a tendency an increase in aerial scanning duration and a decrease in the aerial scanning frequency, while HR animals, did not show a cocaine effects on aerial scanning. Aerial glance behavior was neither affected by cocaine nor by senktide (Fig. 4E and F; $p > 0.05$). However, cocaine eliminated aerial glance behavior completely in the LR animals, while it increased it in the HR animals. An attenuating effect of senktide, was, accordingly, only observed in the HR animals.

Terrestrial scanning occurred only for short periods of time and in only a small frequency (Fig. 5A–D). Two-way ANOVA did neither indicate an effect of cocaine nor of senktide on the duration or the frequency of this behavior ($p > 0.05$). However, pre-planed comparisons revealed a tendency for an increase in terrestrial scanning duration after 0.1 mg/kg senktide–saline (versus saline–saline: $p = 0.054$). The were no clear responder type differences observed between HR and LR animals in terrestrial scanning. Terrestrial glance behavior, which occurred at a higher frequency, was reduced by cocaine

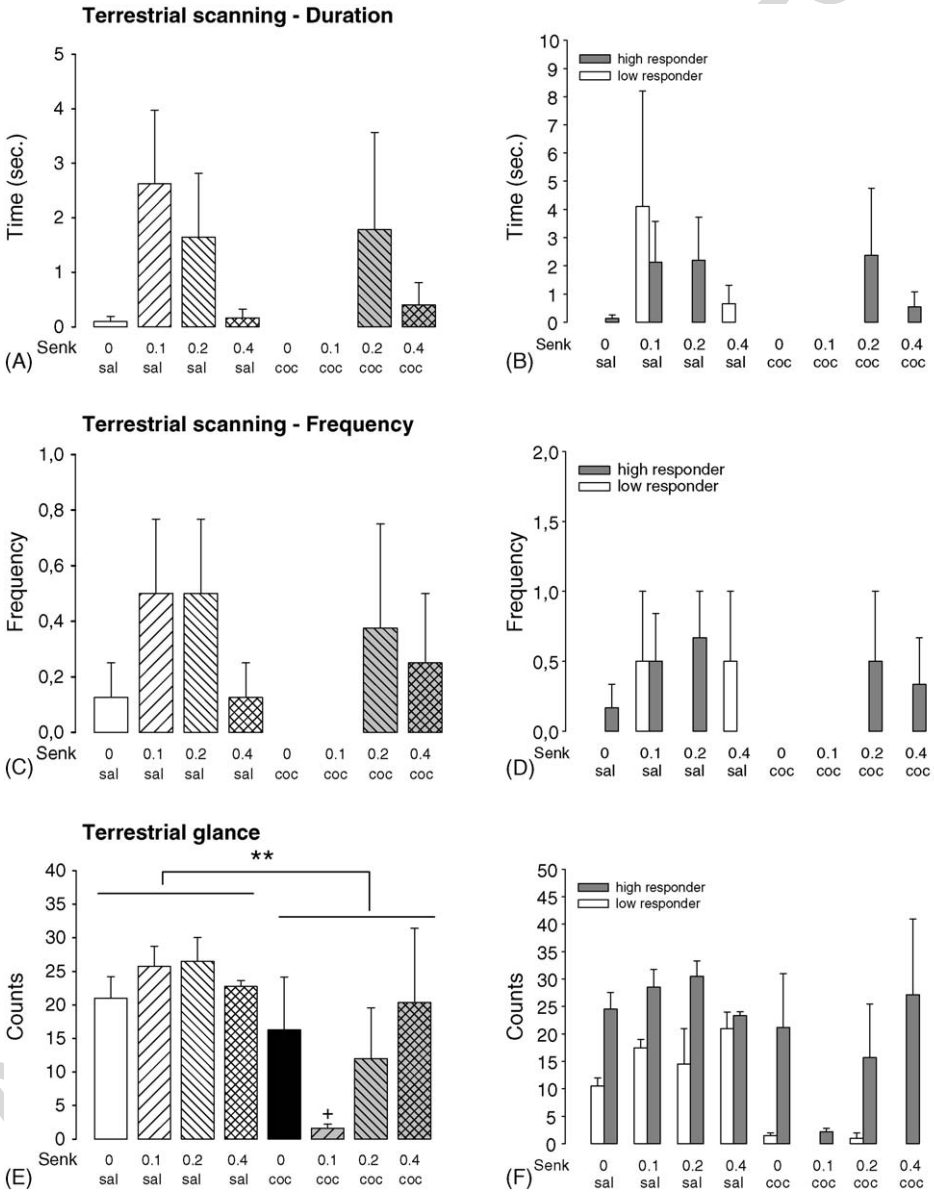

Fig. 5 – The effects of cocaine (10 mg/kg, i.p.) on terrestrial scanning (A–D) and terrestrial glance (E and F; mean ± S.E.M.) and its modulation by the NK_3-receptor agonist, senktide (0.1–0.4 mg/kg, s.c.), during a 20 min test trial. (A, C and E) Effects for all animals tested ($n = 8$). (B, D and F) Sensitivity analysis: group split according to the animals response to cocaine (*high responder*: increased locomotor activity after vehicle-cocaine vs. vehicle-saline ($n = 6$); *low responder*: no increase in locomotor activity after vehicle-cocaine vs. vehicle-saline ($n = 2$); $^{**}p < 0.01$, two-way ANOVA, factor treatment; $^{+}p < 0.05$, vs. saline–saline).

(Fig. 5E and F; two-way ANOVA, factor treatment: $F_{1,56} = 7.66$, $p = 0.008$). The cocaine-induced decline in terrestrial glance was further potentiated by pre-treatment with 0.1 mg/kg senktide (saline–saline versus saline–cocaine: $p > 0.05$; versus 0.1 mg/kg senktide–cocaine: $p = 0.023$). The cocaine effect on terrestrial glance was most prominent in the LR animals than in the HR animals. However, the senktide potentiation of the cocaine effect was more evident in the HR animals, given a floor effect in the LR animals.

4. Discussion

Cocaine increased locomotor activity and the duration of aerial scanning behavior in the marmoset monkeys. At the same time exploratory activity, bodycare activities, the frequency of aerial scanning, and terrestrial glance behavior were decreased. There was no overall cocaine effect on scent marking behavior, aerial glance, and the duration or frequency of terrestrial scanning behavior. Senktide blocked the cocaine-induced increase in locomotor activity. In contrast, senktide potentiated the cocaine-induced decrease in exploratory activity and in the frequency of aerial scanning and terrestrial glance behavior. NK_3-R agonism alone did not have any statistically meaningful effect on marmoset behavior in this study. Sensitivity analysis showed that an increase in locomotor activity after cocaine could be found in six of the eight animals (75%) tested, two of the eight animals (25%) did not respond with an increased locomotor activity. The HR animals generally showed a higher activity in all behavioral parameters, with the only exception of aerial scanning duration and frequency, where LR animals spent more time with.

In this study cocaine profoundly increased locomotor activity in the marmoset monkeys, which confirmed previous findings [19]. Senktide attenuated the cocaine effect on locomotor activity at all doses in an equally effective way. The direction of the senktide effect was rather unexpected, since previous studies in rats and monkeys showed an inhibitiory effect on cocaine-induced hyperlocomotion by NK_3-R antagonist pretreatment [19,29]. Other studies found that the local injection of SP, its C-terminal analogue, DiMe-C7, or of senktide into the ventral tegmental area and substantia nigra enhanced locomotor activity in rats and mice [4,20,32,49]. Considering the equal effectivity of all tested does of senktide to block cocaine-induced hyperlocomotion, it may be argued that senktide shifted the dose–response curve of the locomotor effects of cocaine to the left. It is well known that this curve has an inverted U-shaped form, including the occurrence of behavioral stereotypies at high doses of cocaine [30]. However, since no stereotyped behavior was observed in this study, this explanation appears unlikely. In a study by Ciccocioppo et al. [14] a linear dose–response curve for the inhibitory effects of senktide on alcohol consumption was found for doses of 0.125 and 0.25 mg/kg senktide (s.c.) in rats. It is unclear, why the attenuating effects of senktide did not show a dose–response function in this dose range in monkeys. Since little is known about the behavioral effects of NK_3-R agonists in non-human primates, it cannot be excluded that at a dose of 0.1 mg/kg already a floor effect for the attenuation of this cocaine effect occurred, so that at higher doses no further inhibition was possible. Alternatively, it could be argued that senktide may have unspecifically reduced the availability of cocaine in the brain. However, these views are challenged by the other behavioral effects of cocaine, which were potentiated by senktide in this study, namely the inhibition of exploratory activity, aerial scanning frequency, and terrestrial glance. The senktide modulation of the cocaine effects on these behaviors was maximal at doses of 0.1 and 0.2 mg/kg senktide, indicating at least an inverted U-shaped dose response curve for senktide. Senktide alone did not affect locomotor activity in monkeys in this study using a dose of 0.1–0.4 mg/kg, which is in line with rodent studies that reported gross behavioral effects of senktide similar to the serotonin syndrome only after higher doses of $\geq$0.5 mg/kg senktide [28,49,50].

Exploratory behavior was decreased by cocaine in this study, which is in line with previous findings in marmosets [19]. Senktide further potentiated this effect with the most effective dose of 0.1 mg/kg senktide. The NK_3-R antagonist, SR142801, attenuated the cocaine effect on exploratory behavior in primates [19]. Both findings suggest a facilitory role of the NK_3-R in the cocaine-induced attenuation of exploratory activity. This view is further supported by findings in mice showing an attenuating effect of the SP C-terminal fragment, $pGlu^6$-SP(6-11), on rearing behavior [24]. Cocaine eliminated bodycare activities in monkeys, which supports the findings of a previous study in primates [19], and the effects on grooming behavior in rodents [29,39]. Senktide pretreatment did not affect the cocaine-induced decrease in bodycare activities in this study. Since also pretreatment with a NK_3-R antagonist had no effect on the cocaine-induced suppression of grooming in primates and rats either [19,29], it may be concluded, that the NK_3-receptor is not involved in this acute behavioral effect of cocaine. Senktide alone did not influence bodycare activities to a considerable extent. Findings in mice, in contrast, indicated a facilitatory effect of NK_3-R activation on grooming behavior [24]. In the present study scent marking behavior was not significantly affected by cocaine. A previous study in marmosets, however, found a decrease of scent marking after cocaine [19]. In the present study basal scent marking activity was considerably lower compared to the previous study, which might explain, why the cocaine-induced attenuation failed to reach statistical importance. Neither senktide alone, nor in combination with cocaine affected scent marking behavior to a considerable extent in this study. Nevertheless, an involvement of the NK_3-R in the cocaine-effects on scent marking in marmosets was supported by findings with the NK_3-R antagonist that further potentiated the cocaine-induced decrease [19].

In marmosets, 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 [11,25]. In general, the presentation of a potential threat is associated with an increase in visual scanning [11,12,23,25,33]. In the present study the predominant aerial scanning was increased by cocaine as revealed by ANOVA. Although the pretreatment $\times$ treatment interaction

did not reach statistical importance, visual inspection of the data suggests that this cocaine effect was due to the low levels after the senktide–saline and the high levels after the senktide–cocaine treatments. This view is supported by previous findings, which failed to show a cocaine effect on the duration of aerial scanning in marmosets [19]. However, in this predominant marmoset behavior differences between responder types to psychostimulant treatment become obvious, possibly by amplifying the negative correlation between locomotor activity and aerial scanning duration in experimental environments [8]. Accordingly, LR animals, that do not show a hyperlocomotor response to psychostimulant treatment, show an increase in aerial scanning duration, while HR animals, which responded with hyperlocomotion to psychostimulants, show no change in aerial scanning duration [19,38]. The present study supports this principal responder type difference in marmosets, showing a tendency for an increase in the duration of aerial scanning in the LR animals, but a decrease in the HR animals. Cocaine reduced the frequency of aerial scanning behavior. This effect was observed in all cocaine treated groups and suggests in combination with the elevated duration of aerial scanning longer scanning intervals after cocaine. Also, this cocaine effect was predominantly observed in the LR animals in this and an other study in marmosets [19,38]. Senktide alone tended to reduce the duration, but not the frequency of aerial scanning in this study, with predominant effects in the LR animals. NK_3-R antagonism, in contrast, slightly increased this parameter [19]. These findings might suggest that the NK_3-R suppresses this important behavior in marmoset monkeys. The effects of cocaine on aerial glance did not reach statistical importance in this study, which is in contrast to a previous report [19]. However, the lack of a cocaine treatment effect may be explained by the attenuating effects of senktide–cocaine, indicating that senktide reversed the small cocaine effects on this behavior. Under experimental conditions *Callithrix* monkeys generally emit less terrestrial- than aerial scanning [8–10]. Basal levels in this study appeared to be particularly low. This might explain, why the attenuating effects of psychostimulants on this behavior [19,38] could not be seen here. Neither cocaine nor senktide had a statistically meaningful effect on terrestrial scanning. However, cocaine decreased terrestrial glance behavior. This effect was potentiated by senktide pretreatment with 0.1 mg/kg as the most effective dose. Complementarily, NK_3-R antagonism was found to reverse this cocaine effect [19], which might suggest a facilitatory role of the NK_3-R in this cocaine-induced behavior.

In summary, the present study confirmed previous findings showing that cocaine has a wide range of acute effects on behavior in marmoset monkeys. Cocaine increased locomotor activity and the duration of aerial scanning behavior. In contrast, exploratory activity, bodycare activities, the frequency of aerial scanning, and terrestrial glance behavior were decreased. Several behaviors were not affected by cocaine in this study, including scent marking, aerial glance, and the duration or frequency of terrestrial scanning. NK_3-R agonism blocked the cocaine effect on locomotor activity, but at the same time potentiated other effects, such as the decrease in exploratory activity, aerial scanning frequency, and terrestrial glance behavior. Senktide alone did not affect any behavior tested to a considerable extent. Overall, these data support a complex involvement of the NK_3-R in the acute behavioral effects of cocaine in non-human primates.

Acknowledgements

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 (nos. 300364/1986-5 and 412542/2003). We thank Mrs. Anna A. Vieira Souto and Mrs. Naia Vilas Boas for assistance in the data collection.

REFERENCES

[1] Adams DH, Hanson GR, Keefe KA. Differential effects of cocaine and methamphetamine on neurotensin/neuromedin N and preprotachykinin messenger RNA expression in unique regions of the striatum. Neuroscience 2001;102:843–51.
[2] Alburges ME, Ramos BP, Bush L, Hanson GR. Responses of the extrapyramidal and limbic substance P systems to ibogaine and cocaine treatments. Eur J Pharmacol 2000;390:119–26.
[3] Arroyo M, Baker WA, Everitt BJ. Cocaine self-administration in rats differentially alters mRNA levels of the monoamine transporters and striatal neuropeptides. Mol Brain Res 2000;83:107–20.
[4] Barnes JM, Barnes NM, Costall B, Cox AJ, Domeney AM, Kelly ME, et al. Neurochemical consequences following injection of the substance-P analog, DiMe-C7, into the rat ventral tegmental area. Pharmacol Biochem Behav 1990;37:839–41.
[5] Barros M, Tomaz C. Non-human primate models for investigating fear and anxiety. Neurosci Biobehav Rev 2002;26:187–201.
[6] Barros M, Boere V, Mello EL, Tomaz C. Reactions to potential predators in captive-born marmosets (*Callithrix penicillata*). Int J Primatol 2002;23:443–54.
[7] Barros M, de Souza Silva MA, Huston JP, Tomaz C. Anxiolytic-like effects of substance P fragment (SP(1-7)) in non-human primates (*Callithrix penicillata*). Peptides 2002;23:967–73.
[8] Barros M, Mello EL, Maior RS, Müller CP, de Souza Silva MA, Carey RJ, et al. Anxiolytic-like effects of the selective 5-HT1A receptor antagonist WAY 100635 in non-human primates. Eur J Pharmacol 2003;482:197–203.
[9] Barros M, Alencar C, Tomaz C. Differences in aerial and terrestrial visual scanning in captive black tufted-ear marmosets (*Callithrix penicillata*) exposed to a novel environment. Folia Primatol 2004;75:85–92.
[10] Barros M, de Souza Silva MA, Huston JP, Tomaz C. Multibehavioral analysis of fear and anxiety before, during, and after experimentally induced predatory stress in *Callithrix penicillata*. Pharmacol Biochem Behav 2004;78:357–67.

[11] Caine NG. Visual scanning by tamarins—a description of the behavior and tests of 2 derived hypotheses. Folia Primatol 1984;43:59–67.
[12] Caine NG. Cutting costs in response to predatory threat by Geoffroy's marmosets (*Callithrix geoffroyi*). Am J Primatol 1998;46:187–96.
[13] Ciccocioppo R, Panocka I, Pompei P, DeCaro G, Massi M. Selective agonists at NK3 tachykinin receptors inhibit alcohol intake in Sardinian alcohol-preferring rats. Brain Res Bull 1994;33:71–7.
[14] Ciccocioppo R, Panocka I, Pompei P, Polidori C, DeCaro G, Massi M. Subcutaneous injections of the tachykinin senktide reduce alcohol intake in alcohol-preferring rats. Peptides 1995;16:533–7.
[15] Ciccocioppo R, Panocka I, Polidori C, DeCaro G, Regoli D, Massi M. Stimulation of tachykinin NK-3 receptors in the nucleus basalis magnocellularis reduces alcohol intake in rats. Peptides 1997;18:1349–55.
[16] Ciccocioppo R, Panocka I, Polidori C, Froldi R, Angeletti S, Massi M. Mechanism of action for reduction of ethanol intake in rats by the tachykinin NK-3 receptor agonist aminosenktide. Pharmacol Biochem Behav 1998;61:459–64.
[17] Conde CA, Costa V, Tomaz C. PROSTCOM: Un conjunto de programas para registro y procesamiento de datos comportamentales en investigaciones de fisiologia y farmacologia. Biotemas 2000;13:357–67.
[18] Davidson C, Lee TH, Ellinwood EH. NKI receptor antagonist WIN51708 reduces sensitization after chronic cocaine. Eur J Pharmacol 2004;499:355–6.
[19] De Souza Silva MA, Mello Jr. EL, Müller CP, Jocham G, Souto Maior R, Huston JP, Tomaz C, Barros M. The tachykinin NK3 receptor antagonist SR142801 blocks the behavioral effects of cocaine in marmoset monkeys. Eur J Pharmacol, in press.
[20] Eison AS, Eison MS, Iversen SD. The behavioral-effects of a novel substance-P analog following infusion into the ventral tegmental area or substantia nigra of rat-brain. Brain Res 1982;238:137–52.
[21] Emonds Alt X, Bichon D, Ducoux JP, Heaulme M, Miloux B, Poncelet M, et al. SR 142801, the first potent non-peptide antagonist of the tachykinin NK3 receptor. Life Sci 1995;56:L27–32.
[23] Ferrari SF, Ferrari MAL. Predator avoidance-behavior in the buffy-headed marmoset, Callithrix-Flaviceps. Primates 1990;31:323–38.
[24] Hall ME, Grantham P, Limoli J, Stewart JM. Effects of substance-P and neurokinin-A (substance-K) on motor behavior—unique effect of substance-P attributable to its amino-terminal sequence. Brain Res 1987;420:82–94.
[25] Hardie SM, BuchananSmith HM. Vigilance in single- and mixed-species groups of tamarins (*Saguinus labiatus* and *Saguinus fuscicollis*). Int J Primatol 1997;18:217–34.
[26] Hökfelt T, Pernow B, Wahren J. Substance P: a pioneer amongst neuropeptides. J Intern Med 2001;249:27–40.
[27] Hurd YL, Brown EE, Finlay JM, Fibiger HC, Gerfen CR. Cocaine self-administration differentially alters messenger-RNA expression of striatal peptides. Mol Brain Res 1992;13:165–70.
[28] 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.
[29] Jocham G, Lezoch K, Müller CP, Kart-Teke E, Huston JP, de Souza Silva MA. Neurokinin3 receptor antagonism attenuates cocaine's behavioral activating effects yet potentiates its dopamine-enhancing action in the nucleus accumbens core. submitted for publication.
[30] Johanson CE, Fischman MW. The pharmacology of cocaine related to its abuse. Pharmacol Rev 1989;41:3–52.
[31] Johansson B, Lindstrom K, Fredholm BB. Differences in the regional and cellular-localization of c-Fos messenger-RNA induced by amphetamine, cocaine and caffeine in the rat. Neuroscience 1994;59:837–49.
[32] Kelley AE, Stinus L, Iversen SD. Behavioral activation induced in the rat by substance-P infusion into ventral tegmental area—implication of dopaminergic A10 neurons. Neurosci Lett 1979;11:335–9.
[33] Koenig A. Visual scanning by common marmosets (*Callithrix jacchus*): functional aspects and the special role of adult males. Primates 1998;39:85–90.
[34] Kraft M, Ahluwahlia S, Angulo JA. Neurokinin-1 receptor antagonists block acute cocaine-induced horizontal locomotion. Ann NY Acad Sci 2001;937: 132–9.
[35] Loonam TM, Noailles PAH, Yu J, Zhu JPQ, Angulo JA. Substance P and cholecystokinin regulate neurochemical responses to cocaine and methamphetamine in the striatum. Life Sci 2003;73:727–39.
[36] Massi M, Panocka I, de Caro G. The psychopharmacology of tachykinin NK-3 receptors in laboratory animals. Peptides 2000;21:1597–609.
[37] Mathieu-Kia AM, Besson MJ. Repeated administration of cocaine, nicotine and ethanol: effects on preprodynorphin, preprotachykinin A and preproenkephalin mRNA expression in the dorsal and the ventral striatum of the rat. Mol Brain Res 1998;54:141–51.
[38] Mello EL, Maior RS, Carey RJ, Huston JP, Tomaz C, Müller CP. Serotonin(1A)-receptor antagonism blocks psychostimulant properties of diethylpropion in marmosets (*Callithrix peniccillata*). Eur J Pharmacol 2005;511:43–52.
[39] Müller CP, de Souza-Silva MA, DePalma G, Tomaz C, Carey RJ, Huston JP. The selective serotonin1A-receptor antagonist WAY 100635 blocks behavioral stimulating effects of cocaine but not ventral striatal dopamine increase. Behav Brain Res 2002;134:337–46.
[40] Nguyen-Le XK, Nguyen QT, Gobeil F, Pheng LH, Emonds-Alt X, Breliere JC, et al. Pharmacological characterization of SR 142801: a new non-peptide antagonist of the neurokinin NK-3 receptor. Pharmacology 1996;52:283–91.
[41] Perfumi M, Polidori C, Pompei PL, DeCaro G, Massi M. The tachykinin NH2-senktide inhibits alcohol intake in alcohol-preferring rats. Pharmacol Biochem Behav 1991;38:881–7.
[42] Placenza FM, Fletcher PJ, Rotzinger S, Vaccarino FJ. Infusion of the substance P analogue, DiMe-C7, into the ventral tegmental area induces reinstatement of cocaine-seeking behaviour in rats. Psychopharmacology 2004;177:111–20.
[43] Placenza FM, Vaccarino FJ, Fletcher PJ, Erb S. Activation of central neurokinin-1 receptors induces reinstatement of cocaine-seeking behavior. Neurosci Lett 2005;390:42–7.
[44] Regoli D, Boudon A, Fauchere JL. Receptors and antagonists for substance P and related peptides. Pharmacol Rev 1994;46:551–99.
[45] Ripley TL, Gadd CA, De Felipe C, Hunt SP, Stephens DN. Lack of self-administration and behavioural sensitisation to morphine, but not cocaine, in mice lacking NK1 receptors. Neuropharmacology 2002;43:1258–68.
[46] Severini C, Improta G, Falconieri-Erspamer G, Salvadori S, Erspamer V. The tachykinin peptide family. Pharmacol Rev 2002;54:285–322.

[47] Stevenson MF, Poole TB. Ethnogram of common marmoset (Calithrix-Jacchus-Jacchus)—general behavioral repertoire. Animal Behav 1976;24:428–51.
[48] 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. 1988. p. 131–222.
[49] Stoessl AJ, Dourish CT, Iversen SD. The NK-3 tachykinin receptor agonist senktide elicits 5-HT-mediated behavior following central or peripheral administration in mice and rats. Br J Pharmacol 1988;94:285–7.
[50] Stoessl AJ, Dourish CT, Iversen SD. Pharmacological characterization of the behavioral syndrome induced by the NK-3 tachykinin agonist senktide in rodents—evidence for mediation by endogenous 5-HT. Brain Res 1990;517:111–6.
[51] Ukai M, Mori E, Kameyama T. Effects of centrally administered neuropeptides on discriminative stimulus properties of cocaine in the rat. Pharmacol Biochem Behav 1995;51:705–8.

Die hier vorgelegte Dissertation habe ich eigenständig und ohne unerlaubte Hilfsmittel angefertigt. Die Dissertation wurde in der vorliegenden oder in ähnlicher Form noch bei keiner anderen Institution eingereicht. Ich habe bisher keine erfolglosen Promotionsversuche unternommen.

Düsseldorf, den 24.09.2006

(Gerhard Jocham)