Die Bedeutung von Serotonin im Kortex für die Effekte von Kokain: neurochemische- und Verhaltensbefunde

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vorgelegt von

Martin Erich Pum

geboren in Wolfsberg, Österreich

Aus dem Institut für Experimentelle Psychologie,
Arbeitsgruppe für Physiologische Psychologie der Heinrich-Heine-Universität Düsseldorf

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Referent: Prof. Dr. J.P. Huston
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Zusammenfassung

Kokain ist eine Droge mit sehr hohem Suchtpotential, deren fortgesetzter Konsum mit negativen Konsequenzen verbunden sein kann. Aus Studien mit bildgebenden Verfahren ist bekannt, dass die Applikation von Kokain sowie die Präsentation von Reizen, die mit Kokain assoziiert sind, zu Veränderungen der neuronalen Aktivität auch außerhalb des, in der Forschung im Fokus stehenden, mesolimbischen Dopaminsystems führt. Besonders von Bedeutung scheinen hier kortikale Hirngebiete zu sein die stark von serotonergen Projektionen innerviert werden. In der hier vorgelegten Arbeit sollte daher die Rolle von Serotonin in Kortizes, die mit Gedächtnisfunktionen in Verbindung gebracht werden, bei der Vermittlung der Wirkung von Kokain untersucht werden. Dazu wurden zunächst in einem in vivo Mikrodialyse-Experiment am freibeweglichen Tier die neurochemischen Effekte von Kokain im medialen präfrontalen Kortex, perirhinalen Kortex und im entorhinalen Kortex charakterisiert, und mit denen von Amphetamin, einem anderen Wirkstoff aus der Gruppe der Psychostimulantien, verglichen. Es zeigte sich, dass Kokain in allen drei Kortizes zu einem dosisabhängigen Anstieg von Dopamin und Serotonin führte, und dass diese neurochemischen Effekte weitestgehend mit denen von Amphetamin vergleichbar waren. In der Folge sollte die funktionelle Bedeutung speziell der serotonergen Effekte geprüft werden. Dazu wurde die serotonerge Innervation des medialen präfrontalen Kortex, des entorhinalen und des occipitalen Kortex durch die Injektion des Neurotoxins 5,7-Dihydroxytryptamin (5,7-DHT) zerstört, und die so behandelten Tiere wurden auf die kokain-evozierten hyperlokomotorischen Effekte und auf die kokain-induzierte konditionierte Platzpräferenz getestet. Es zeigte sich, dass die Läsion des medialen präfrontalen Kortex nach der ersten, jedoch nicht nach den drei darauf folgenden Injektionen, zur Blockade der kokain-evozierten Hyperaktivität führte. Weiterhin kam es nach der serotonergen Läsion dieses Kortex zur Verringerung der kokain-induzierten Platzpräferenz. Die 5,7-DHT-Läsion im entorhinalen

Kortex verringerte die kokain-induzierte Platzpräferenz, hatte jedoch keine Effekte auf die Hyperlokomotion, während die Läsion des occipitalen Kortex keine Effekte auf das hier gemessene Verhalten hatte. Zusammenfassend wurde also gezeigt, dass Kokain profunde neurochemische Effekte im Kortex auslöst, und dass Serotonin besonders im präfrontalen Kortex sowie im entorhinalen Kortex eine Rolle bei der Vermittlung der Verhaltenseffekte von Kokain spielt.

Summary

Cocaine is a highly addictive drug which's continued use can has negative consequences. Neuroimaging studies show, that the application of cocaine as well as the presentation of cocaine-related stimuli lead to wide-spread neuronal activation also outside the mesolimbic dopamine system, which had been in the focus of research. Whereas cortical areas, which are strongly innervated by serotonergic projections, seem to be of special relevance. Thus, the present series of studies investigated the role of serotonin (5-HT) in cortical areas associated with memory-functions in the mediation of the effects of cocaine. To this end, the neurochemical effects of cocaine were assessed in the medial prefrontal cortex (mPFC), the perirhinal cortex (PRC) and the entorhinal cortex (EC) by in vivo microdialysis, and compared to the effects of d-amphetamine another psychostimulant drug. It was shown that cocaine induced a dose-dependent increase of 5-HT and dopamine in all three cortices, and that these effects were largely comparable to the effects of d-amphetamine. Subsequently, the functional relevance of the effects of cocaine on 5-HT in the cortex was investigated. To this end, the serotonergic innervation of the mPFC, the EC, or the occipital cortex (OccC) was lesioned by local infusion of the serotonergic neurotoxin 5,7-dihydroxytryptamine (5,7-DHT), and animals treated in such a way were tested for cocaine-induced hyperactivity and conditioned place preference. The lesion of the mPFC blocked cocaine-evoked hyperactivity

following the first, but not following the three subsequent injections, of cocaine. Furthermore, the serotonergic lesion of the mPFC lead to an attenuation of cocaine-induced conditioned place preference. The 5,7-DHT-lesion of the EC reduced cocaine induced conditioned place preference, but had no effect on hyperlocomotion, while the lesion of the OccC had no effect on the measured behaviours. In summary, it was shown that cocaine had significant neurochemical effects in the cortex, and that 5-HT plays a role in the behavioural effect of cocaine especially in the mPFC and the EC.

Inhaltsverzeichnis

1. Einleitung

Kokain ist eine Suchtdroge deren chronischer Missbrauch zu Beeinträchtigungen führen kann (Nutt et al., 2007). Trotzdem verzeichnet die Europäische Beobachtungsstelle für Drogen und Drogensucht für die letzten Jahre einen bedeutsamen Anstieg des Konsums dieser Droge (Europäische Beobachtungsstelle für Drogen und Drogensucht: EU Drogenreport 2007; verfügbar unter http://www.emcdda.europa.eu/html.cfm /index419EN.html), was der genauen Klärung der Wirkmechanismen von Kokain besondere Relevanz verleiht. Bekannt ist, das Kokain im Gehirn an Monoamintransportern bindet und diese blockiert (Elliott & Beveridge, 2005). In Folge dessen kommt es sowohl in den terminalen- als auch den Zellkerngebieten der Monoamine, Dopamin, Noradrenalin und Serotonin (5-HT), zu einem signifikanten Anstieg der extrazellulären Konzentration dieser Transmitter (Parsons & Justice, 1993; Reith et al., 1997).

Menschen berichten nach der Applikation von Kokain von seiner euphorisierenden Wirkung (Johanson & Fischman, 1989), und auch das im Tiermodell gemessene Verhalten lässt den Schluss zu, dass die Effekte der Droge als positiv empfunden werden, da es zu Selbstverabreichungsverhalten (z. B. Olmstead et al., 2001; Richardson & Roberts, 1996; Wee et al., 2007) und zur Ausbildung einer Präferenz für einen kokain-assoziierten Kontext (Bardo et al., 1995; Tzschentke, 2007) kommt. Vor allem der Dopaminanstieg im Nucleus Accumbens wird mit der Vermittlung der positiv verstärkenden Effekte von Suchtdrogen in Verbindung gebracht, da hier durch alle vom Menschen missbrauchten Substanzen ein Anstieg von Dopamin induziert wird (Di Chiara & Bassareo, 2007; Koob et al., 1998). Der Nucleus Accumbens ist das wichtigste Projektionsareal der im Mittelhirn gelegenen dopaminergen Zellen der Area ventralis tegmentalis (VTA; Oades & Halliday, 1987; Swanson, 1982), und die lokale Blockade der Dopaminrezeptoren in diesem Kern vermindert

die verstärkende Wirkung von Kokain (Bari & Pierce, 2005; Caine et al, 1995; McGregor & Roberts, 1993).

Allerdings zeigen Studien mit genetisch modifizierten Mäusen, dass neben Dopamin auch 5-HT eine bedeutende Rolle bei der Vermittlung der Wirkung von Kokain hat. So konnte gezeigt werden, dass sich in Tieren ohne Dopamintransporter (sog. Dopamintransporter kockout Mäuse) noch eine konditionierte Platzpräferenz für eine mit Kokain assoziierte Umgebung ausbildet (Sora et al., 2001), und dass derartige Tiere auch Kokain Selbstverabreichung zeigen (Rocha et al., 1998). Wenn jedoch sowohl der Dopamin- als auch der Serotonintransporter eliminiert wird, kommt es nicht mehr zur Ausbildung einer Kokain Platzpräferenz (Sora et al., 2001). Diese Befunde stützen eine Reihe von neuropharmakologischen Befunden, die eine wichtige Rolle für das serotonerge System bei der Vermittlung der Wirkung von Kokain implizieren (Higgins & Fletcher, 2003; Müller et al., 2007a; Müller & Huston, 2006).

1.1 Das serotonerge System und die Effekte von Suchtdrogen

Das serotonerge System zeichnet sich im Vergleich zum dopaminergen durch eine ausgedehntere Innervierung des Neokortex aus (Jacobs & Azmitia, 1992; Oades & Halliday, 1987; Swanson, 1982; Vertes, 1991; Vertes et al., 1999). Die Zellkerne der serotonergen Neurone, die aufsteigende Bahnen zum Kortex, dem limibischen System und den Basalganglien schicken, liegen in den Raphé Nuclei im Mesencephalon (Jacobs & Azmitia, 1992; Vertes, 1991; Vertes et al., 1999). Grundsätzlich werden hier der Nucleus Raphé dorsalis (DRN) und der Nucleus Raphé medianus (MRN) unterschieden (Jacobs & Azmitia, 1992). Der DRN innerviert präferentiell den Kortex, das dorsale Striatum, den Nucleus Accumbens, das VTA und die Amygdala (Vertes, 1991), während der MRN der Ursprung der serotonergen Afferenzen zum Hippokampus, zum medialen Septum und zum Hypothalamus

ist (Vertes et al., 1999). Das unterschiedliche Projektionsmuster dieser beiden Zellkerngruppen schlägt sich auch in funktionalen Unterschieden nieder (Lechin et al., 2006). Zum Beispiel wurde demonstriert, dass die Injektion eines Agonisten am Serotonin 1A (5-HT 1A) Rezeptor unterschiedliche Effekte auf die Hyperaktivität nach chronischer systemischer Applikation von Kokain und nach akuter Applikation von Kokain hervorruft (Szumlinksi et al., 2004).

Wie oben beschrieben, ist die Blockade des Serotonintransporters durch Kokain zusätzlich zur Blockade des Dopamintransporters notwendig, um eine kokain-induzierte Platzpräferenz zu entwickeln (Sora et al., 2001). Pharmakologische Studien, in denen die Möglichkeit zu rezeptorspezifischen systemischen und lokalen Manipulationen gegeben ist, zeigen hier jedoch ein differenzierteres Bild. Zum Beispiel wurde gefunden, dass die systemische Behandlung mit einem Agonisten am Serotonin 1B (5-HT 1B) Rezeptor, die im Gehirn zu einer Reduktion der extrazellulären 5-HT Konzentration führt (Adell et al., 2001), vor der Injektion von Kokain die Akquisition einer konditionierten Platzpräferenz förderte und nicht inhibierte (Cervo et al., 2002). Die systemische Erhöhung des 5-HT Spiegels hingegen verringerte die Expression kokain-induzierter Platzpräferenz (Harris et al., 2001). Zum Unterschied hierzu führte die Blockade von Serotonin 3 (5-HT3) oder Serotonin2 (5-HT 2) Rezeptoren zu einer Verringerung der Platzpräferenz für einen mit Kokain assoziierten Kontext (Kankaanpää et al., 2002; Nomikos et al., 1988). In Selbstverabreichungsstudien hat man gefunden, dass sowohl die Erhöhung des 5-HT Spiegels durch Fluoxetin als auch die globale Läsion des serotonergen Systems durch die Applikation von 5,7-DHT in die zerebralen Ventrikel zur Verringerung des Kokain Suchverhaltens führt (Burmeister et al., 2003; Tran-Nguyen et al., 2001). Allerdings zeigt keine der beiden Manipulationen Effekte auf die Wiederaufnahme des Selbstverabreichungsverhaltens nach der nicht-kontingenten Applikation von Kokain (Burmeister et al., 2003; Tran-Nguyen et al., 2001).

Die Widersprüchlichkeit dieser Befunde legt nahe, dass (a) die Rolle von 5-HT bei der Vermittlung von drogen-bezogenem Verhalten abhängig von dem jeweils auszuführenden Verhalten sein könnte, und (b) dass 5-HT in unterschiedlichen Hirngebieten und abhängig davon welche Rezeptortypen stimuliert werden unterschiedliche Funktionen vermitteln könnte. Allerdings besteht zurzeit ein Mangel an Befunden zu den Effekten lokaler Manipulationen des serotonergen Systems auf durch Suchtdrogen vermitteltes Verhalten (Übersicht bei Müller & Huston, 2006).

1.2 Die Rolle von Gedächtnisprozessen im Suchtverhalten

Eine Vielzahl von Studien belegt, dass Suchtdrogen starke Modulatoren neuronaler Plastizität sind (Übersicht z.B. bei Berke & Hyman, 2000; Jones & Bonci, 2005). Vor allem die Ähnlichkeit der molekularen Prozesse die durch Lernen und durch Suchtdrogen in Gang gesetzt werden (Nestler, 2002; Nestler & Aghajanian, 1997), hat dazu geführt, Sucht als, durch wiederholte Drogenapplikation ausgelösten, dysfunktionalen Lernprozess zu betrachten (Hyman, 2005; Kelly, 2004). Die Steigerung der Aktivität der intra-zellulären cAMP (cyklisches Adenosinmonophosphat) Signalkaskade scheint hier eine besondere Rolle zu spielen (Nestler, 2002; Nestler & Aghajanian, 1997). Tatsächlich konnte gezeigt werden, dass die cAMP-PKA (Protein Kinase A) Kaskade sowohl bei der Kontext Furchtkonditionierung als auch bei der Akquisition konditionierter Platzpräferenzen notwendig ist (Bourtchouladze et al., 1998; Cervo et al., 1997).

Die Modulation neuronaler Plastizität durch Psychostimulantien konnte in einer Reihe von Hirngebieten nachgewiesen werden (Wolf et al., 2004). Von besonderer Bedeutung erscheint hier die Beeinflussung von Plastizität in Hirngebieten die mit der Bildung von Gedächtnis in Verbindung gebracht werden, wie dem Hippokampus (Thompson et al., 2002), dem Striatum (Nishioku et al., 1999) oder der Amygdala (Huang et al., 2003). Zudem kommt

es nach Applikation von Kokain oder der Präsentation von kokain-assoziierten Stimuli in einem weit verteilten Netzwerk an Hirnregionen zur erhöhten Expression von Genen und Proteinen die mit Lernen und neuronaler Plastizität assoziiert sind (Thomas & Everitt, 2001; Thomas et al., 2003; Valjent et al., 2004). Im Menschen findet man nach Applikation von Kokain erhöhte Aktivität in einem weitverteilten Netzwerk aus Hirnregionen (Breiter et al., 1997), und ähnlich weit verteilte Aktivität zeigt sich auch bei der Präsentation von kokain-assoziierten Reizen (Grant et al., 1996; Kosten et al., 2006). 5-HT könnte besonders in Bereichen des Gehirns eine wichtige Rolle bei der Vermittlung der Effekte von Kokain spielen, die weniger stark durch dopaminerge Fasern innerviert sind (Jacobs & Azmitia, 1992; Oades & Halliday, 1987; Swanson, 1982; Vertes, 1991; Vertes et al., 1999). Wichtig könnten dabei Hirnregionen sein, die bei der Bildung und Speicherung von Gedächtnisinhalten eine Rolle spielen. Zum Beispiel wurden prominente Effekte von Kokain auf den 5-HT-Spiel im Hippokampus, wo diese Droge auch neuronale Plastizität moduliert (Thompson et al., 2002), nachgewiesen (Müller et al., 2002; 2004). Tatsächlich spielt 5-HT eine Rolle sowohl bei „normalen“ Gedächtnisprozessen als auch bei der Bildung und dem Abruf von mit Suchtdrogen assoziierten Gedächtnisinhalten (Nic Dhonnchadha & Cunningham, in press). Über die Funktion von 5-HT in gedächtnisrelevanten Hirngebieten wie dem Kortex oder dem Hippokampus bei der Vermittlung der neurochemischen- und Verhaltenseffekte von Kokain ist allerdings nur wenig bekannt. Für 5-HT Rezeptoren im medialen präfrontalen Kortex (mPFC) wurde eine Rolle für die Vermittlung der hyperlokomotorischen und subjektiven Effekte von Kokain vorgeschlagen (Filip und Cunningham, 2003). Für weiter posterior gelegene Kortizes konnten profunde Effekte von Kokain auf den extrazellulären 5-HT-Spiegel im temporalen sowie im occipitalen Kortex gefunden werden, welche der neurochemischen Reaktion auf sensorische Stimulation ähneln (Müller et al., 2007b). Zusätzlich konnte gezeigt werden, dass Serotonin 1A (5-HT1A) Rezeptoren im Hippokampus die hyperlokomotorischen Effekte von 5-HT modulieren (Müller et al., 2004). Allerdings ist

noch nichts bekannt über die Effekte von Kokain auf 5-HT in anderen, mit Gedächtnisfunktionen in Verbindung gebrachten, kortikalen Hirngebieten, und über die funktionale Bedeutung eines zu erwartenden 5-HT-Anstiegs in diesen Bereichen in Folge der Applikation von Kokain. Entsprechend hatten die aktuellen Experimente zum Ziel (a) die neurochemischen Effekte von Kokain im mPFC, dem entorhinalen Kortex (EC), und dem perirhinalen Kortex (PRC) zu charakterisieren, und (b) die funktionale Relevanz der serotonergen Veränderungen durch Kokain in diesen gedächtnisrelevanten kortikalen Hirngebieten zu testen. Um dies zu erreichen, wurde die Methode der in vivo Mikrodialyse am freibeweglichen Tier verwendet um die kokain-evozierten Veränderungen der extrazellulären Konzentrationen von 5-HT und Dopamin im Kortex zu messen. Um die Generalisierbarkeit dieser Befunde zu prüfen, wurden die Effekte von Kokain mit jenen eines anderen Wirkstoffes aus der Gruppe der Psychostimulantien – d-Amphetamin – verglichen. Im zweiten Schritt wurde die funktionale Relevanz des kokain-induzierten 5-HT-Anstiegs im mPFC, EC, und im occipitalen Kortex getestet. Dazu wurde die serotonerge Neurotransmission in diesen Arealen, durch die lokale Injektion von 5,7-DHT, eines selektiv gegen serotonerge Nervenendigungen gerichteten Neurotoxins, dauerhaft vermindert, und die Auswirkungen dieser Läsion auf die kokain-induzierte konditionierte Platzpräferenz (CPP) und Hyperaktivität getestet.

2. Methoden

2.1 in vivo Mikrodialyse

Mit der zerebralen in vivo Mikrodialyse steht eine Methode zur Verfügung Veränderungen der extrazellulären Konzentration von Neurotransmittern in umschriebenen Hirngebieten zu messen. Die Kopplung an die Hochdruckflüssigkeitschromatographie

(HPLC) mit elektrochemischer Detektion ermöglicht die neurochemisch selektive Messung von Monoaminen. Zur Extraktion der Bestandteile der extrazellulären Flüssigkeit wird eine Mikrodialyse-Sonde (Abb. 1) ins Gehirn implantiert, die aus einer semi-permeablen Membran besteht, und von einer physiologischen Ringerlösung durchspült wird. Da die Zusammensetzung der Ringerlösung jener der extrazellulären Flüssigkeit entspricht, entsteht zwischen dem Inneren der Sonde und dem umgebenden Hirngewebe ein Konzentrationsgefälle bezüglich der Neurotransmitter, die vom Extrazellulärraum in die Sonde diffundieren, und über die Sonde und eine Silicafaser in ein Probengefäß gepumpt werden. Anschließend erfolgt die Analyse der Monoamin-Konzentrationen in der Probe Mittels HPLC und elektrochemischer Detektion.

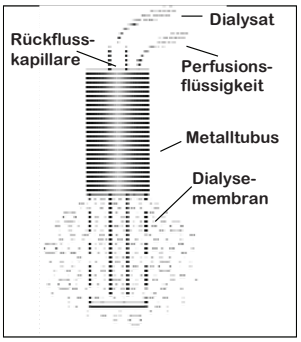

Abb. 1: schematische Darstellung einer Mikrodialyse-Sonde

Die in vivo Mikrodialyse kann sowohl am narkotisierten als auch am wachen, frei beweglichen Tier (Abb. 2) durchgeführt werden. Letztere Herangehensweise hat die Vorteile, dass hier (a) keine Beeinflussung der Hirn-Neurochemie durch ein Anästhetikum vorliegt (De Souza Silva et al., 2007), und dass (b) parallel zu den neurochemischen Daten auch Verhaltensdaten erhoben werden können (Müller et al., 2002; 2004).

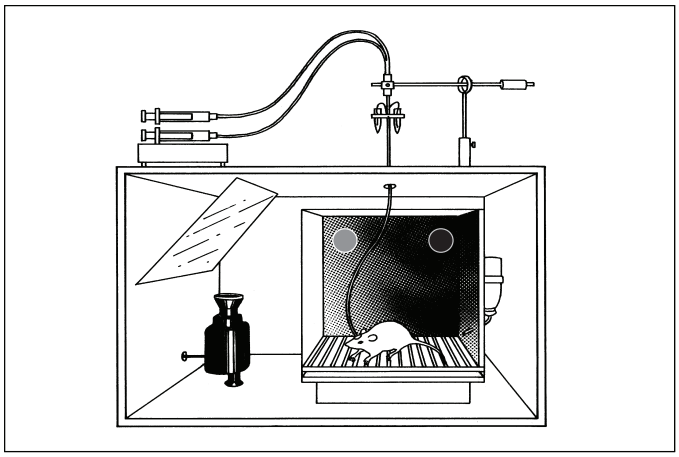

Abb. 2: Exemplarische Testapparatur für ein in vivo Mikrodialyse Experiment am frei beweglichen Tier.

Ein Nachteil der in vivo Mikrodialyse sind die relativ großen Probenintervalle die nötig sind genug Perfusat für die neurochemische Analyse zu sammeln. Dies liegt daran, dass man in Abhängigkeit von der jeweiligen Sonde von einer sog. recovery-rate von etwa 15% ausgeht. Das heißt, dass pro Probe immer nur etwa 15 % des im Extrazellulärraums befindlichen Neurotransmitters im Perfusat extrahiert wird. Dies hat zwar keine Auswirkungen auf die Aussagekraft der erhobenen neurochemischen Daten, da es vor allem um die Erfassung der Veränderungen der Transmitterkonzentrationen in Abhängigkeit einer bestimmten Manipulation geht, und diese im Allgemeinen für jede Sonde relativ zu einer Baseline-Messung ausgedrückt wird (Westerink, 2000), allerdings kommt es dadurch zu den relativ langen Probenintervallen. So sind, bei einer Flussrate der Ringerlösung durch die Mikrodialyse-Sonde von 1 µl/ min, und der intendierten Messung von Monoaminen im Kortex Probenintervalle von 15-20 Minuten notwendig, um im Perfusat reliabel Dopamin und 5-HT messen zu können.

2.2 Die Konditionierte Platzpräferenz

Die konditionierte Platzpräferenz (CPP) ist eine weit verbreitete, reliable Methode zur Messung der verstärkenden Wirkung einer Substanz (Bardo & Bevins, 2000; Tzschenkte, 2007). Die Prozedur wird für gewöhnlich in einer Apparatur mit mindestens zwei unterscheidbaren Kammern durchgeführt. Sie beruht auf dem Prinzip, dass, nach wiederholter Paarung einer Umwelt mit einem bestimmten belohnenden Stimulus (z.B. der Applikation einer Droge), in Tieren eine Präferenz für diese Umgebung herausgebildet wird. Diese Präferenz äußert sich in der erhöhten Aufenthaltszeit in dem gepaarten Kontext im Vergleich zu einem Kontext, der mit einem neutralen Stimulus (z.B. der Injektion einer Kochsalzlösung) assoziiert wird. Im Gegensatz dazu entwickelt sich bei der Paarung eines aversiven Stimulus mit einem Umgebungsreiz eine Aversion vor diesem Kontext. Diese Methode bietet den Vorteil, dass der Test im drogen-freien Zustand durchgeführt werden kann, wobei hier gezeigt werden konnte, dass die Expression der Platzpräferenz nicht zustandsabhängig ist (Bardo & Bevins, 2000). Zusätzlich können hier neben der Platzpräferenz auch die Effekte einer Behandlung auf die Aktivität der Versuchstiere gemessen werden, was besonders bei der Untersuchung von Psychostimulantien von Bedeutung ist (z.B. Baker et al., 1996; Jocham et al., 2007).

Es gibt zwei Herangehensweisen bei der Durchführung von CPP-Experimenten. In einem Design verwendet man eine Apparatur, in der eine *a priori* Präferenz für eine bestimmte Seite besteht, und die Droge wird mit der weniger bevorzugten Kammer gepaart, man spricht hier von einem „biased design“. Alternativ werden Apparaturen verwendet, bei denen keine Präferenz für eine bestimmte Seite der Apparatur besteht, und die Konditionierungskammer wird zufällig zugewiesen, man spricht von einem „unbiased design“ (Bardo & Bevins, 2000). Letzteres Design hat den Vorteil, dass hier weniger Interpretationsprobleme entstehen, da im biased design der Vergleich der Aufenthaltszeiten in

den unterschiedlichen Teilen des Apparates zwischen einem Vortest (Baseline) und dem Test Durchgang gezogen wird. Es wird also eigentlich nicht die Präferenz für die drogen-assoziierte Seite gemessen, sondern die durch die Droge verminderte Aversion für die zuvor gemiedene Seite. Dies könnte jedoch teilweise auch einfach durch die wiederholte Erfahrung mit dieser Seite bewirkt werden (Bardo & Bevins, 2000).

In den aktuellen Experimenten wurde eine modifizierte Platzpräferenzapparatur angewandt (Jocham et al., 2007). Es wurde ein rundes Offenfeld verwendet, das in der Mitte durch eine Plexiglas Wand teilbar war. Die beiden Hälften unterschieden sich an Hand des verwendeten Boden- und Wandbelages, der auf einer Seite aus einer glatten und auf der anderen Seite aus einer rauen, geriffelten Kunststoffmatte bestand. Das Protokoll bestand aus drei Phasen: die Baseline-Messung, die Konditionierungsphase und der Test auf Platzpräferenz. In der Baseline-Messung bekommen die Tiere Gelegenheit für 15 min das gesamte, nicht unterteilte Offenfeld zu explorieren, und es wird geprüft, dass keine a priori Präferenz für eine der beiden Seiten besteht. Dies war in den hier beschriebenen Experimenten der Fall, es handelt sich also um ein unbiased design. In der acht Tage dauernden Konditionierungsphase wird das Offenfeld durch eine Trennwand in zwei Teile geteilt, die Tiere bekommen eine Injektion von Kokain (10 mg/kg, i.p.) oder Saline (1 ml/kg) und werden für 30 min in eine der beiden Hälften gesetzt. Dabei wird für jedes Tier immer dieselbe Seite mit Kokain oder Saline assoziiert, und sie bekommen immer abwechselnd an einem Tag eine Kokaininjektion und am nächsten Tag eine Salineinjektion. Entsprechend wird viermal Kokain und viermal Saline injiziert. In den Kontrollgruppen werden die Tiere an allen Tagen mit Saline behandelt und ebenfalls abwechseln auf die eine und die andere Hälfte gesetzt. Für den Testdurchgang wird die Trennwand entfernt, und die Tiere können sich wieder für 15 min frei im Offenfeld bewegen. Die konditionierte Platzpräferenz äußert sich durch längere Aufenthaltszeiten auf der kokain-assoziierten Seite als auf der saline-gepaarten Seite.

3. Experiment I: Die neurochemischen Effekte von Kokain im medialen präfrontalen, perirhinalen und entorhinalen Kortex: Vergleich mit d-Amphetamin

Als erster Schritt im Projekt sollten die neurochemischen Effekte von Kokain in drei kortikalen Hirngebieten charakterisiert werden. Dazu wurden die kokain-evozierten Änderungen der extrazellulären Konzentrationen von Dopamin und 5-HT im mPFC, EC und PRC gemessen. Es wurden pro Tier drei Mikrodialyse-Sonden verwendet, und zunächst die basalen Konzentrationen der Neurotransmitter gemessen (vier Proben; 20 min Probenintervall). Danach erfolgte die Injektion von Kokain (0, 5, 10, 20 mg/kg; i.p.), und die Messung wurde für weitere drei Stunden fortgesetzt. Während des gesamten Experimentes wurden neben den neurochemischen Daten auch einfache Verhaltensmaße (horizontale und vertikale Aktivität) erhoben. Um zu prüfen in wie fern die neurochemischen Befunde auch auf andere Psychostimulantien generalisiert werden können, erhielten andere Tiere in separaten Gruppen Injektionen von d-Amphetamin (0, 0.5, 1.0, 2.5 mg/kg; i.p.), in einem Dosisbereich der zu einem mit den Kokaindosen vergleichbaren drogen-induzierten Hyperaktivitätsniveau führt.

Kokain und d-Amphetamin erhöhten in Abhängigkeit von der Dosis die extrazellulären Konzentrationen von Dopamin und 5-HT in allen drei Kortizes. Für d-Amphetamin wurde gefunden, dass hier ein besonders starker Effekt auf die Dopamin-Level im mPFC induziert wird, während Kokain in allen drei Hirngebieten die Dopaminkonzentrationen in vergleichbarem Ausmaß erhöhte. Allerdings konnten nach einer Kokaininjektion, verglichen mit den anderen Kortizes, besonders starke Effekte auf 5-HT im EC gefunden werden. Diese Unterschiede im neurochemischen Profil der beiden Drogen,

schlugen sich nicht in Unterschieden bezüglich der drogen-induzierten Hyperaktivität nieder, wo für beide Drogen im gewählten Dosisbereich kein Unterschied gefunden wurde.

Es konnte in diesem Experiment also gezeigt werden, dass Kokain in gedächtnis-relevanten Hirnarealen deutliche Effekte auf die Neurotransmitter Dopamin und 5-HT hat. Zudem ist die neurochemische Reaktion auf Kokain und d-Amphetamin in diesen Hirngebieten weitestgehend vergleichbar.

4. Experiment II: Die Rolle von 5-HT im medialen präfrontalen Kortex für die kokain-induzierte Hyperaktivität und konditionierte Platzpräferenz

Nach der Demonstration serotonerger Effekte im mPFC nach einer systemischen Injektion von Kokain, sollte im nächsten Experiment die funktionale Relevanz des 5-HT-Anstiegs getestet werden. Dazu wurde die serotonerge Neurotransmission im mPFC durch die Läsion serotonerger Synapsen chronisch vermindert, und die Tiere wurden auf kokain-induzierte konditionierte Platzpräferenz und Hyperlokomotion getestet. Die selektive Zerstörung der serotonergen Innervation des mPFC wurde durch die Injektion des serotonergen Neurotoxins 5,7-DHT während einer stereotaktischen Operation durchgeführt. Kontrolltiere wurden der exakt gleichen Prozedur unterzogen, mit dem Unterschied, dass hier lediglich die Vehikellösung ohne 5,7-DHT in den mPFC appliziert wurde (Scheinläsion). Etwa zwei Wochen nach der Operation begann das Experiment, in dem bei jeweils etwa der Hälfte der Läsionstiere und Scheinläsionstiere eine Seite des Offenfeldes mit Kokain (10 mg/kg; i.p.) assoziiert wurde. Die andere Hälfte der Läsions- und Scheinläsionstiere wurde lediglich mit Kochsalzlösung (1 ml/ kg; i.p.) behandelt (zur Prozedur siehe Abschnitt 2.2). Während der Konditionierungsphase wurde die Aktivität der Tiere im Offenfeld gemessen. Während des Vortests und des Platzpräferenztests wurden die Aufenthaltszeiten in den beiden Hälften

erhoben, und es wurde ein Platzpräferenzindex berechnet, indem die Aufenthaltszeit auf der saline-assoziierten Seite von der auf der kokain-assoziierten Seite subtrahiert wurde.

Es zeigte sich, dass durch die Läsion des mPFC die akuten hyerlokomotorischen Effekte von Kokain geblockt wurden. Nach der ersten Kokaininjektion zeigten die lädierten Tiere also keine Hyperaktivität im Vergleich zu den lädierten Tieren die Saline injiziert bekommen hatten. Diese unkonditionierte Reaktion auf Kokain zeigte sich jedoch an den folgenden drei Kokainbehandlungstagen. Konditionierte Platzpräferenz konnte in den Tieren mit einer Scheinläsion, aber nicht in Tieren mit der 5,7-DHT-Läsion der serotonergen Innervation des mPFC, gefunden werden.

In diesem Experiment wurde gezeigt, dass der Anstieg von 5-HT im mPFC nach einer systemischen Injektion von Kokain, der im Experiment I gemessen wurde, funktional relevant ist. Wird diese neurochemische Reaktion blockiert, kommt es bei erstmaliger Gabe nicht zu den hyperlokomotorischen Effekten von Kokain. Der vorübergehende Effekt der Läsion auf die kokain-induzierte Hyperaktivität passt zu vorangehenden Befunden, die nahe legen, dass es einen Unterschied bei der neuronalen Vermittlung der akuten und chronischen hyperlokomotorischen Effekte von Kokain gibt. So zeigten Morrow & Roth (1996), dass, nach globaler Verringerung des 5-HT Gehalts im Gehirn durch intracerebroventrikuläre Injektion von 5,7-DHT, es zu einer Intensivierung der Hyperlokomotion nach der ersten aber nicht mehr nach der vierten Kokainapplikation kommt. Auch Szumlinski et al. (2004) fanden unterschiedliche Effekte einer lokalen Injektion eines 5-HT1A Agonisten in den DRN, die die extrazellulären Level von 5-HT in dessen Projektionsarealen verminderte, auf die akuten und chronischen hyperlokomotorischen Effekte von Kokain. So kam es nach der Injektion des 5-HT1A Agonisten in den DRN zu einer Potenzierung der hyperlokomotorischen Effekte von Kokain, während die durch die chronische Behandlung mit dieser Droge induzierte Sensitivierung der Hyperaktivität durch die selbe intra-zerebrale Behandlung nicht beeinflusst wurde (Szumlinski et al., 2004).

Auch die Ausbildung der Platzpräferenz für einen mit Kokain assoziierten Kontext ist nach der Zerstörung der serotonergen Fasern im mPFC nicht mehr möglich. Eine Rolle des mPFC für die kokain-induzierte Platzpräferenz wurde bereits durch eine Studie, die unspezifische Zellkernläsionen dieses Hirngebietes verwendeten, vorgeschlagen (Tzschentke & Schmidt, 1999). Hier werden diese Resultate erweitert, indem wir zeigen konnten, dass speziell die serotonerge Innervation des mPFC eine wichtige Rolle zu spielen scheint. Der Dopamin-Anstieg im mPFC hingegen, scheint für die Bildung einer konditionierten Platzpräferenz keine Bedeutung zu haben, da gezeigt wurde, dass die chronische Verringerung des Dopamingehalts des mPFC keine Effekte auf die kokain-induzierte Platzpräferenz hat (Hemby et al., 1992). Eine derartige Rolle passt zu den Befunden von Filip und Cunningham (2003), die bezüglich der subjektiven Effekte von Kokain modulatorische Funktionen der 5-HT2 Rezeptoren im mPFC vorschlagen. Zudem konnten auch Studien mit globalen 5-HT Läsionen des Gehirns eine Beeinflussung von Kokain Selbstverabreichungsverhalten durch einen verringerten 5-HT-Spiegel messen (Loh & Roberts, 1990; Tran-Nguyen et al., 2001). Zusammengenommen, sprechen diese Ergebnisse also für eine Rolle von 5-HT bei der Modulation der verstärkenden Wirkung von Kokain, und eröffnen die Möglichkeit, dass es hier eine besondere Rolle für die serotonerge Innervation des mPFC gibt.

5. Experiment III: Die Rolle von 5-HT im entorhinalen Kortex für die kokain-induzierte Hyperaktivität und konditionierte Platzpräferenz

In Experiment III wurde die Rolle des 5-HT-Anstiegs im EC nach Applikation von Kokain untersucht. Äquivalent zu Experiment II wurde dazu eine Läsion der serotonergen

Innervation des EC durchgeführt, und die Tiere wurden anschließend auf die Effekte dieser Läsion auf die kokain-induzierte Hyperaktivität und CPP getestet.

Im Gegensatz zu Experiment II wurden nach der 5,7-DHT Läsion des EC keine Effekte auf die kokain-induzierte Hyperaktivität gefunden. Allerdings zeigte sich in den Läsionstieren eine Verminderung der Platzpräferenz für einen mit Kokain assoziierten Kontext. Die selektive Reduktion der konditionierten Platzpräferenz nach serotonergen Läsionen des EC, spricht, berücksichtigt man die Funktion des EC, dafür, dass hier vor allem die Bildung von Droge-Kontext Assoziationen, also die für die CPP bedeutsame Gedächtniskomponente, gestört ist. Diese Interpretation passt zu den Ergebnissen von Grant et al. (1996), die, nach Präsentation eines kokain-assoziierten Stimulus, erhöhte Aktivität im parahippokampalen Kortex finden. Sie wird auch durch Resultate gestützt, die für den EC sowohl im Menschen als auch in der Ratte eine besondere Rolle für emotionale Gedächtnisinhalte vorschlagen (Dolcos et al., 2004; LaBar & Cabeza, 2006; Roesler et al., 2002).

6. Experiment IV: Die Rolle von 5-HT im occipitalen Kortex für die kokain-induzierte Hyperaktivität und konditionierte Platzpräferenz

In Experiment IV wurde die Rolle des 5-HT-Anstiegs im occipitalen Kortex (OccC; Müller et al., 2007b) nach Applikation von Kokain untersucht. Äquivalent zu Experiment II wurde dazu eine Läsion der serotonergen Innervation des OccC durchgeführt, und die Tiere wurden anschließend auf die Effekte dieser Läsion auf die kokain-induzierte Hyperaktivität und konditionierte Platzpräferenz getestet. Eine mögliche Rolle des OccC für die kokain-induzierte CPP konnte angenommen werden, da in einer vorangehenden Studie gefunden wurde, dass hier die systemische Applikation von Kokain zu einem Anstieg der extrazellulären Konzentration von 5-HT führt, der dem bei einfacher visueller Stimulation ähnelt (Müller et al., 2007b). Zudem konnte in einer Bildgebungsstudie mit kokain-

abhängigen Probanden gezeigt werden, dass unter anderem die neuronale Aktivität im visuellen Kortex bei Präsentation von kokain-assoziierten Stimuli mit der Rückfallswahrscheinlichkeit korrelierte (Kosten et al., 2006).

Im vorliegenden Experiment konnten jedoch keine Effekte der 5,7-DHT-Läsion auf die kokain-induzierte Platzpräferenz oder Hyperaktivität gefunden werden. Der Grund hierfür könnte darin liegen, dass den Tieren in diesem Experiment vor allem taktile Reize zur Unterscheidung der Hälften des Offenfeldes zu Verfügung standen, und entsprechend die visuellen Stimuli im Experimentalraum weniger von Bedeutung waren. Alternativ dazu ist auch möglich, dass die frühe Verarbeitung visueller Stimuli, und deren Modulation durch Kokain (Devonshire et al., 2007), keine wichtige Rolle bei der Vermittlung kokain-induzierter CPP spielt.

7. Zusammenfassende Diskussion

Drogen-assoziierte Reize spielen eine bedeutende Rolle sowohl bei der Aufrechterhaltung von als auch beim Rückfall ins Drogensuch- und Drogenkonsumverhalten (Di Ciano & Everitt, 2004; Gawin, 1991; See, 2005; Zernig et al., 2007). So wurde auch die Reaktivität auf derartige Reize in klinischen Studien als wichtiger Indikator für die Effektivität einer Behandlungsmaßnahme vorgeschlagen (z. B. Berger et al., 1996; Robbins et al., 1992). Entsprechend bedeutsam ist es zu klären welche neurochemischen Systeme bei der Vermittlung der Wirkung von klassisch konditionierten Reizen auf drogen-bezogenes Verhalten beteiligt sind. Berücksichtig man, dass derartiges Verhalten für gewöhnlich in einem drogenfreien Zustand initiiert wird, bekommt besonders die Untersuchung von Gedächtnisprozessen und von Hirngebieten die hier eine Rolle spielen Bedeutung. In der vorliegenden Reihe von Untersuchungen wurde gezeigt, dass Kokain, eine der gefährlichsten und am weitesten verbreiteten Suchdrogen (Nutt et al., 2007), sowohl die extrazelluläre

Konzentration von Dopamin als auch 5-HT im mPFC, EC und PRC erhöht. Der mPFC in Ratten ist für Aufgaben wichtig die Funktionen wie Arbeitsgedächtnis oder Aufmerksamkeit beanspruchen (Dalley et al., 2004), er moduliert jedoch auch, über direkte glutamaterge Projektionen die Funktion des mesolimbischen Dopaminsystems, und spielt deshalb auch eine Rolle im „Belohungssystem“ des Gehirns (Kalivas & Nakamura, 1999; Tzschentke, 2000; Tzschentke & Schmidt, 2000). So konnte in einer Reihe von Studien gezeigt werden, dass die Stimulation von 5-HT Rezeptoren im mPFC zu einer Erhöhung der Dopaminspiegel im VTA sowie zur erhöhten Aktivität dopaminerger Zellen in diesem Kern führt (Bortolozzi et al., 2005; Díaz-Mataix et al., 2005; Pehek et al., 2006). In der aktuellen Studie führte die Läsion der serotonergen Synapsen im mPFC zur Blockade der akuten hyperlokomotorischen Effekte von Kokain sowie zur Verminderung der kokain-induzierten konditionierten Platzpräferenz. Dieser Befund stützt und erweitert vorangehende Befunde die zeigen, dass der mPFC eine Rolle bei der konditionierten Platzpräferenz für Kontexte die mit Psychostimulantien assoziiert werden spielt (Tzschente & Schmidt, 1999). Zudem sind diese Resultate konsistent mit dem Vorschlag, dass 5-HT2 Rezeptoren im mPFC die subjektiven Effekte von Kokain modulieren (Filip & Cunningham, 2003). Wenn man die Rolle präfrontaler 5-HT Rezeptoren bei der Modulation des mesolimbischen Dopaminsystems berücksichtigt (Bortolozzi et al., 2005; Díaz-Mataix et al., 2005; Pehek et al., 2006), liegt die Vermutung nahe, dass der Verlust der modulatorischen Wirkung von 5-HT im mPFC auf dieses „Belohnungssystem“ des Gehirns bei den hier gefundenen Effekten eine Rolle spielt.

Über die Rolle des EC bei der Vermittlung der Effekte von Suchdrogen ist noch sehr wenig bekannt. In den aktuellen Experimenten wurde gezeigt, dass Kokain zu einer dosisabhängigen Erhöhung extrazellulärer Konzentrationen von Dopamin und 5-HT im EC führt, und dass dieser Anstieg wichtig für die Herausbildung einer kokain-induzierten konditionierten Platzpräferenz ist. Studien mit bildgebenden Verfahren zeigen erhöhte Aktivität des EC sowohl nach der Gabe von Kokain (Breiter et al., 1997) als auch bei der

Präsentation von kokain-assoziierten Reizen (Grant et al., 1996). Vor allem der letztere Befund spricht für eine Rolle des EC bei Gedächtnissprozessen die einen signifikanten Faktor bei Drogensucht darstellen (Hyman, 2005; Kelly, 2004). Diese Hypothese wird durch verschiedene Resultate sowohl im Menschen als auch im Tiermodell gestützt, die zeigen, dass der EC speziell bei emotionalen Gedächtnisinhalten involviert ist (Dolcos et al., 2004; LaBar & Cabeza, 2006; Roesler et al., 2002), wobei die vorliegende Studie vermuten lässt, dass die serotonerge Innervation des EC dabei eine Rolle spielt.

Auch im OccC wurden in einer vorangehenden Untersuchung aus unserem Labor dosisabhängige neurochemische Effekte von Kokain berichtet (Müller et al., 2007b). Bezüglich der Funktion des OccC bei der Vermittlung der Wirkung von Kokain ist noch wenig bekannt. Elektrophysiologische Studien zeigen jedoch, dass Kokain profunde Effekte auf die Aktivität der Zellen im OccC hat (Devonshire et al., 2007). Zudem wurde ein Zusammenhang zwischen der neuronalen Aktivität im visuellen Kortex von kokainabhängigen Patienten und deren Rückfallswahrscheinlichkeit postuliert (Kosten et al., 2006). Allerdings konnten im aktuellen Platzpräferenzexperiment keine Effekte serotonerger Läsionen im OccC auf die kokain-induzierte Hyperaktivität oder CPP gefunden werden.

Zusammenfassend lässt sich also sagen, dass 5-HT in unterschiedlichen kortikalen Hirngebieten von einander unterscheidbare Funktionen erfüllt. Im mPFC werden durch 5-HT sowohl unkonditionierte als auch konditionierte Effekte mediiert, während 5-HT im EC lediglich bei den konditionierten Effekten von Kokain eine Rolle spielt. Im OccC hingegen wurde keine funktionale Bedeutung der serotonergen Innervation für kokain-induziertes Verhalten gefunden.

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

9.1 Pum M, Carey RJ, Huston JP, Müller CP (2007) Dissociating effects of cocaine and d-amphetamine on dopamine and serotonin in the perirhinal, entorhinal, and prefrontal cortex of freely moving rats. Psychopharmacology 193, 375-390

9.2 Pum ME, Carey RJ, Huston JP, Müller CP (2008) Role of medial prefrontal, entorhinal and occipital 5-HT in cocaine-induced place preference and hyperlocomotion: Evidence for multiple dissociations. Submitted

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ORIGINAL INVESTIGATION

Dissociating effects of cocaine and *d*-amphetamine on dopamine and serotonin in the perirhinal, entorhinal, and prefrontal cortex of freely moving rats

M. Pum · R. J. Carey · J. P. Huston · C. P. Müller

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Abstract
Rationale Neuroimaging studies with humans showed widespread activation of the cortex in response to psychostimulant drugs. However, the neurochemical nature of these brain activities is not characterized.
Objective The aim of the present study was to investigate the effects of cocaine and *d*-amphetamine on dopamine (DA) and serotonin (5-HT) in cortical areas of the hippocampal network in comparison to the prefrontal cortex (PFC).
Materials and methods We conducted in vivo microdialysis experiments in behaving rats measuring DA and 5-HT in the perirhinal cortex (PRC), entorhinal cortex (EC), and PFC, after application of cocaine (0, 5, 10, 20 mg/kg; i.p.) or *d*-amphetamine (0, 0.5, 1.0, 2.5 mg/kg; i.p.).
Results Cocaine and *d*-amphetamine dose-dependently increased DA and 5-HT levels in the PRC, EC, and PFC. A predominant DA response to *d*-amphetamine was only found in the PFC, but not in the PRC and EC. Cocaine increased DA and 5-HT to an equal extent in the PFC and PRC but induced a predominant 5-HT response in the EC. When comparing the neurochemical responses between the drugs at an equal level of behavioral activation, cocaine was more potent than *d*-amphetamine in increasing 5-HT in the PFC, while no differences were found in the PRC or EC or in the DA responses in all three cortical areas.
Conclusions We conclude that cocaine and *d*-amphetamine increase DA and 5-HT levels in PRC and EC largely to the same extent as in the PFC.

Keywords Cocaine · *d*-amphetamine · Dopamine · Serotonin · Cortex · Behavior

M. Pum · J. P. Huston · C. P. Müller (✉)
Institute of Physiological Psychology and Center for Biological and Medical Research, University of Düsseldorf,
Universitätsstr. 1,
40225 Düsseldorf, Germany
e-mail: muellecr@uni-duesseldorf.de

R. J. Carey
Research and Development (151),
VA Medical Center and SUNY Upstate Medical University,
800 Irving Avenue,
Syracuse, NY 13210, USA

Introduction

Drug addiction is a major neuropsychiatric disease with no effective treatment currently available (Johanson and Fischman 1989; Gorelick et al. 2004). The establishment of the so-called addiction memory (Heyne et al. 2000; Nestler 2002) appears to be an important factor contributing to the persistence of drug-seeking and drug-taking and to the high prevalence of relapse. Modulation of neuronal plasticity in the mesolimbic dopaminergic (DAergic) system, which is thought to be essential in the mediation of the behavioral effects of drugs of abuse (Di Chiara et al. 2004; Koob et al. 1998), has been demonstrated in a number of studies (Jones and Bonci 2005; Kelly 2004; Wolf et al. 2004). However, psychostimulant drugs also influence plasticity in memory-related brain areas such as the hippocampus (Thompson et al. 2002, 2005), the dorsal striatum (Nishioku et al. 1999), and the amygdala (Goussakov et al. 2006; Huang et al. 2003). In addition, drugs of abuse lead to structural plasticity within various neocortical areas (Robinson and Kolb 2004), which is in line with a recent study reporting dose-dependently elevated levels of serotonin (5-HT) and DA in the temporal and occipital cortex after cocaine (Müller et al. 2007; Müller and Huston 2007). It is widely accepted that the hippocampus is crucial for acquiring information, but it is not the locus of memory storage, which

is hypothesized to take place within cortical networks (Eichenbaum 2000; Wiltgen et al. 2004). Cortical areas connected with the hippocampus are the entorhinal (EC) and the perirhinal cortex (PRC), which together with the hippocampus proper, form the hippocampal complex (Witter et al. 2000). The rhinal cortices are the main in-and output stations of the hippocampus (De Curtis and Paré 2004; Sewards and Sewards 2003), receive input from sensory association cortices of all modalities (Naber et al. 1998; Burwell and Amaral 1998), and are, therefore, suggested to be involved in memory storage (Eichenbaum 2000). The prefrontal cortex (PFC), a brain region that was repeatedly implicated in the mediation of the effects of psychostimulant drugs (Carey and Damianopoulos 1994; Jentsch and Taylor 1999; Kalivas and Volkow 2005), is also connected to the hippocampus (Thierry et al. 2000) and the cortices of the hippocampal complex (Delatour and Witter 2002). All three interconnected cortical areas are innervated by the ventral tegmental area (VTA; Oades and Halliday 1987) and by the raphé nuclei (Steinbusch 1981). Accordingly, DA and 5-HT have been found to modulate neuronal activity in these areas (Schmitz et al. 1998, 1999; Stenkamp et al. 1998).

In continuation of previous studies from our lab showing effects of systemic cocaine on the hippocampus proper (Müller et al. 2002, 2004a, b) and on cortical extracellular 5-HT and DA activity (Müller et al. 2007; Müller and Huston 2007), the goal of this study was to characterize psychostimulant effects on DA and 5-HT levels in cortical regions of the hippocampal network. To this end, we used triple-probe microdialysis in freely behaving rats to monitor alterations in extracellular DA and 5-HT in the PRC, EC, and PFC, which was used as a reference area. To compare the neurochemical effects of cocaine and *d*-amphetamine, the doses were selected in a way to induce a comparable amount of behavioral activation. We hypothesized that, in addition to the PFC, also cortical areas of the hippocampal network display a strong dose-dependent DA and 5-HT activation after cocaine and *d*-amphetamine, parallel to a dose-dependent behavioral activation. Furthermore, we expect to isolate substance-specific neurochemical response profiles within these structures.

Materials and methods

Animals and surgery

Male Wistar rats (Tierversuchsanlage, University of Düsseldorf, Germany), weighing between 230 and 320 g before surgery, were used. Until surgery, they were housed, four animals per cage under standard laboratory conditions, with a reversed light–dark rhythm (light on from 1900 to 0700 hours) with food and water provided ad libitum. For surgery, rats were deeply anesthetized with a mixture of 0.9 ml/kg Ketavet (containing 100 mg/ml Ketamine; Pharmacia and Upjohn, Germany) and 0.4 ml/kg Rompun (containing 20 mg/ml Xylazin; Bayer, Germany) and placed in a Kopf stereotaxic frame. Three guide cannulae with a thread on the top (15 mm, 22 gauge, stainless steel, see Boix et al. 1995) were aimed at the PRC (AP −2.3, ML +/−5.0, DV −6.2, angle 12° to midline), the EC (AP −5.3, ML +/−5.0, DV −7.6, angle 13° to midline), and PFC (AP +2.8, ML +/−1.0, DV −2.4, angle 5° from midline; all coordinates are relative to bregma; Paxinos and Watson 1986). The PFC and EC cannulae were implanted unilaterally, and the PRC cannula was placed at the contralateral position. They were fixed to the skull with two screws (stainless steel, d=1.4 mm) and dental cement. To prevent postoperative pain, 100 µl Novaminsulfonratiopharm (containing 500 mg/ml Metamizol sodium) was administered p.o. after rats awoke from anesthesia. After surgery, the animals were housed individually. The animals were handled daily and were allowed to recover for at least 4 days. All rats gained weight during this period.

Microdialysis procedure

On the day of the experiment, microdialysis probes of a concentric design (membrane length 2 mm; 6 kDa molecular cutoff) were inserted into the guide cannulae and fixed to the thread under short (3–5 min) isoflurane anesthesia. The construction of the probes has been described by Boix et al. (1995). After insertion of the probes, the animal was placed into an open field (40×40×39 cm) of a TruScan system (Coulbourn Instruments, Allentown, USA), which was situated in a sound and light isolated chamber (110×70×70 cm). The animals were investigated in the dark phase activity period, thus, kept under red light conditions (luminous density 2.6 lux) with free access to food and water. The temperature in the room/box was between 21 and 23°C. The microdialysis probes were connected to a microinfusion pump (CMA 100, Carnegie, Sweden) via a liquid swivel mounted on a balanced arm on top of the chamber and were perfused with artificial cerebrospinal fluid containing Na^+ 147 mmol, K^+ 4 mmol, Ca^{2+} 2.2 mmol, Cl^- 156 mmol, pH=7.4 at room temperature. The perfusion flow was set at 1.08 µl/min and was allowed to stabilize for at least 2 h. Thereafter, samples were collected every 20 min into vials containing 2 µl of 0.1 M perchloric acid and 500 pg dihydroxybenzylamine (DHBA) or 5 µl of 0.1 M perchloric acid and 400 pg deoxyepinephrine (DA assay) as internal standard.

Analytical procedure

The samples were immediately assayed after collection using high-performance liquid chromatography with

electrochemical detection. The column was an ET 125/2, Nucleosil 120-5, C-18 reversed phase column (Macherey–Nagel, Germany) perfused with a mobile phase composed of 75 mM NaH_2PO_4, 4 mM KCl, 20 μM ethylenediamine tetraacetic acid (EDTA), 1.5 mM sodium dodecyl sulfate, 100 μl/l diethylamine, 12% methanol, and 12% acetonitrile adjusted to pH 6.0 using phosphoric acid (modified from Chen and Reith 1994). The electrochemical detector (Intro, Antec, The Netherlands) was set at 500 mV vs an in situ Ag/AgCl (ISAAC) reference electrode (Antec, Leyden, The Netherlands) at 30°C. This setup allows the measurement of 5-HT and DA in cortical samples. The detection limit of the assay was 0.1 pg for 5-HT and DA with a signal–noise ratio of 2:1. Dialysates from some animals were analyzed for their DA contents by the assay described by Jocham et al. (2006), while all the microdialysis procedures were kept constant. Neurochemical data were not corrected for recovery.

Experimental procedures

The effects of cocaine at doses of 0, 5, 10, or 20 mg/kg and *d*-amphetamine at doses of 0, 0.5, 1.0, or 2.5 mg/kg on extracellular levels of DA and 5-HT in the PRC, EC, and PFC were measured. Four samples were obtained to establish a stable baseline. Subsequently cocaine or *d*-amphetamine was injected, and sampling was continued for another 3 h (nine samples), to cover the whole time span in which behavioral effects of cocaine can be observed (Müller et al. 2004a, 2004b). Drugs were dissolved in 0.9% saline (pH=7.4) and injected i.p. in a volume of 1 ml/kg.

Behavioral analysis

In parallel with microdialysis, behavioral activity was automatically measured by the TruScan light beam system. The following behavioral measures were scored for all treatments. *Horizontal activity*: distance moved in the horizontal plane of the maze. *Vertical activity*: number of light beam interruptions at a height of 12 cm above the floor of the open field. For behavioral analysis, all intervals of 20 min were recorded for the behavioral measures according to the microdialysis sampling intervals.

Histological analysis

After the experiment, the animals were deeply anesthetized with 0.5 ml Nembutal (containing 60 mg/ml Pentobarbital; Sanofi, France) and transcardially perfused with saline followed by 10% phosphate buffered formalin. The brains were removed, sliced on a cryotome, and stained with cresyl violet for analysis of probe placement. Only animals with probe placement within the PRC, EC, or PFC were considered for data analysis.

Statistics

The neurochemical data were transformed to percent baseline values, taking the average of the four baseline values as 100%. Analysis of the neurochemical data was carried out by using repeated measures one-way analyses of variance (ANOVAs) for the factor time, followed by Tukey's post hoc tests vs last baseline sample. For between brain area comparisons, two-way ANOVAs with the factors area and dose were used. For each brain area and drug dose, the area under the curve (AUC) was calculated by summing up the values after injection. Preplanned two-tailed *t*-tests were conducted on the AUCs to compare the PRC and EC with the PFC, respectively. To compare the response of DA vs 5-HT within brain area, preplanned two-tailed *t*-tests were conducted on the respective AUC values. Behavioral data were analyzed by two-way ANOVAs with the factors dose and time, and by one-way ANOVAs with the repeated measures factor time separately for each dose group, which were followed by Tukey's post hoc comparisons vs the last baseline interval (corresponding to microdialysis). For comparison of behavioral data between drugs, a two-way drug × dose (high, medium, low, and control) ANOVA on AUCs was used. For comparisons of the neurochemical response between drugs, three-way ANOVAs (drug × brain area × dose) and preplanned two-tailed *t*-tests within brain areas were used. The software Statistica 5.0 was used for analyses. The *p* value for significant effects was 0.05.

Results

Histological results for cocaine-treated animals

Probe placements for the rats receiving cocaine or vehicle are shown in Fig. 1. Only animals with probes localized in the respective brain areas were considered for analysis. However, probe losses due to leakage or undetectability of one neurochemical occasionally resulted in different sample sizes.

Behavioral effects of cocaine

Cocaine dose-dependently increased locomotor activity. The two-way ANOVA on locomotion yielded significant main effects of time [$F_{(12,708)}$=5.4; p<0.000001] and dose [$F_{(3,59)}$=7.2; p=0.0003] as well as a significant interaction [$F_{(36,708)}$=3.3; p<0.000001] (Fig. 2). Post hoc tests revealed that locomotion was significantly enhanced in the 20 mg/kg group (n=15) compared to the 0 mg/kg (n=17; p=0.0006),

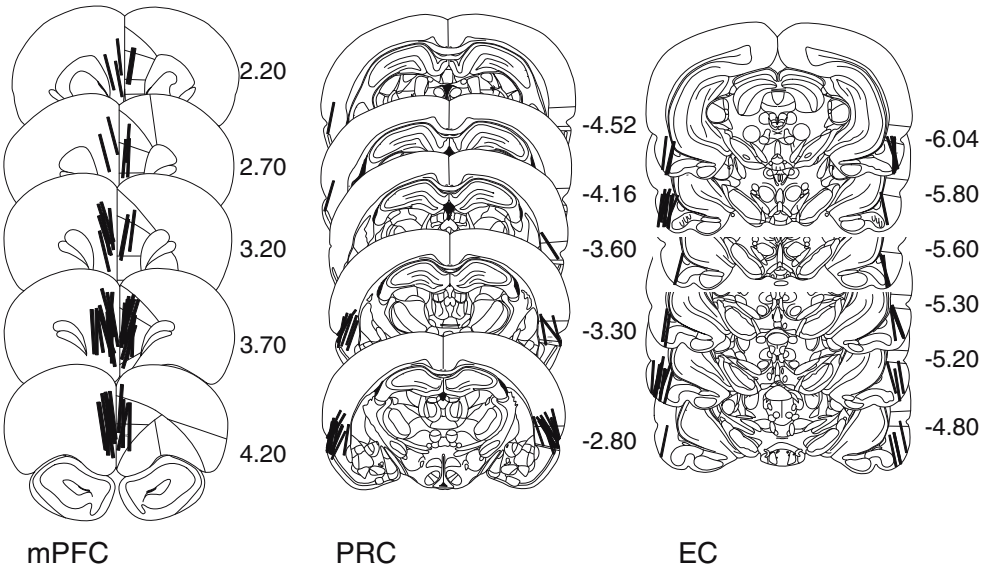

Fig. 1 Localization of microdialysis probes within the prefrontal cortex (*PFC*), perirhinal cortex (*PRC*), and entorhinal cortex (*EC*) in animals treated with cocaine, based on the stereotaxic atlas of the rat brain by Paxinos and Watson (1986). *Caption* refers to the anterior–posterior coordinates relative to bregma

5 mg/kg (n=16; p=0.02), and 10 mg/kg (n=15; p=0.002) groups. No differences between the other groups were found (p>0.05). One-way ANOVAs conducted separately for each group on the factor time found significant effects of time for the 0 mg/kg group [$F_{(12,192)}$=2.9; p=0.001], the 10 mg/kg group [$F_{(12,168)}$=3.6; p=0.0001], and the 20 mg/kg group [$F_{(12,168)}$=6.6; p<0.000001], but not for the 5 mg/kg group (p>0.05). Post hoc tests vs last baseline interval indicated significantly enhanced locomotion during the first 20-min interval after 10 mg/kg cocaine (p=0.009) and during the first (p=0.00002) and second (p=0.0002) interval after the 20 mg/kg treatment. In the saline group, locomotion was significantly enhanced 160 min after injection (p=0.04), but not during any other interval. Tukey's test on the AUC values yielded higher values for the 20 mg/kg group as compared to the 0 mg/kg group (p=0.007), the 5 mg/kg group (p=0.01), and the 10 mg/kg group (p=0.02). No other significant differences were found in the AUC analyses.

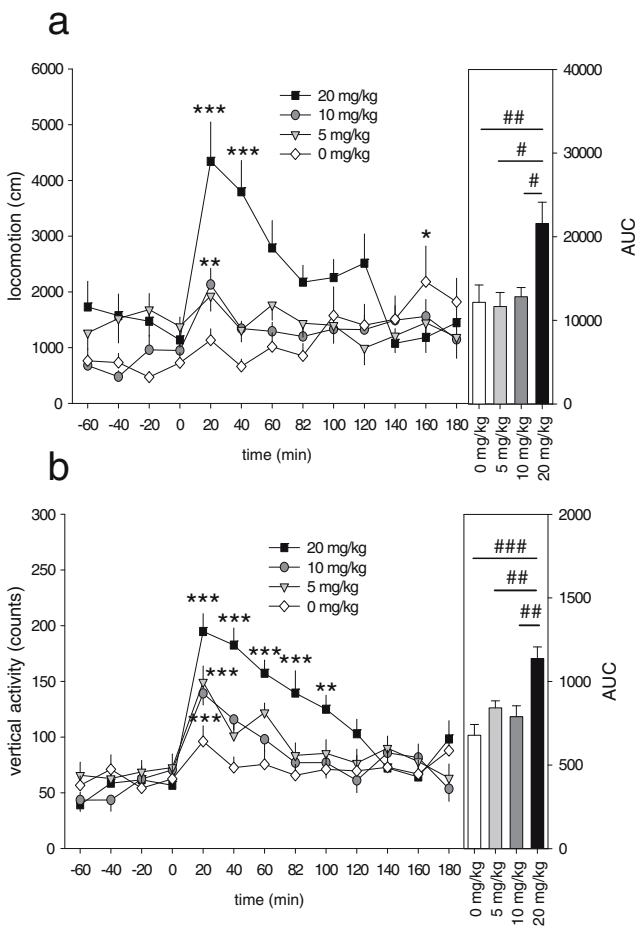

Fig. 2 The effects (mean±SEM) of cocaine (i.p.) on locomotion (**a**) and vertical activity (**b**) (*p<0.05, **p<0.01, ***p<0.001, Tukey's test vs last baseline; $^{\#}p$<0.05, $^{\#\#}p$<0.01, $^{\#\#\#}p$<0.001, Tukey's test)

Analysis of vertical activity showed a similar picture. Thus, the two-way ANOVA revealed significant main effects of dose [$F_{(3,59)}$=6.4; p=0.0008] and time [$F_{(12,708)}$=22.5; p<0.000001] and a significant interaction [$F_{(36,708)}$=3.5; p<0.000001] (Fig. 2). Post hoc tests showed that vertical activity was significantly higher in the 20 mg/kg group compared to the 0 mg/kg (p=0.0006), 5 mg/kg (p=0.02), and 10 mg/kg (p=0.002) dose. No further differences between the other groups were found. One-way ANOVAs conducted separately for each dose on the factor time found significant effects of time for the 5 mg/kg group [$F_{(12,180)}$=4.6; p=0.000002], the 10 mg/kg group [$F_{(12,168)}$=7.6; p<0.000001], and the 20 mg/kg group [$F_{(12,168)}$=19.7; p<0.000001], but not for the 0 mg/kg group (p>0.05). Post hoc tests vs the last baseline interval indicated significantly enhanced vertical activity during the first 20-min interval after injection for animals treated with 5 mg/kg cocaine (p=0.0003) and also during the first interval after the 10 mg/kg treatment (p=0.0001). After 20 mg/kg cocaine, vertical activity was enhanced 20 min (p=0.00002), 40 min

(p=0.00002), 60 min (p=0.00002), 80 min (p=0.00005), and 100 min (p=0.002) after injection. Tukey's test on the AUC values yielded higher values for the 20 mg/kg group as compared to the 0 mg/kg group (p=0.0002), the 5 mg/kg group (p=0.008), and the 10 mg/kg group (p=0.001). No other significant differences were found in the AUC analyses.

Dopamine responses to cocaine

Basal values of DA and 5-HT are shown in Table 1. They did not differ between treatment groups in the PRC, EC, or PFC (p>0.05). There was also no significant difference in baseline values of DA and 5-HT between brain areas (p>0.05).

Although cocaine enhanced DA values in the PRC (Fig. 3), this increase failed to reach statistical significance at all doses. There was only a significant effect of time for the 0 mg/kg group [$F_{(12,48)}$=2.5; p=0.01; n=5], suggesting a continuous decrease in DA during the course of the experiment. However, post hoc tests vs last baseline did not yield any significant differences. There was a trend towards an effect of time in the 10 mg/kg group [$F_{(12,48)}$=1.8; p=0.067; n=5], which was due to a nearly significant increase in DA during the first 20-min sample after injection (223%; p=0.051). There was no significant effect of time for the 5 mg/kg group (n=5) or the 20 mg/kg group (n=6; p>0.05).

Cocaine increased DA levels in the EC (Fig. 3). There was an effect of time on DA values in all cocaine treatment groups [5 mg/kg: $F_{(12,48)}$=3.4, p=0.001, n=5; 10 mg/kg: $F_{(12,48)}$=1.99, p=0.046, n=5; 20 mg/kg: $F_{(12,60)}$=2.1, p=0.03, n=6], but not in animals receiving saline (p>0.05; n=5). Post hoc comparisons vs last baseline sample failed to reach significance (p>0.05).

In the PFC, cocaine increased DA levels dose-dependently (Fig. 3). A significant effect of time was found for all groups receiving a cocaine treatment [5 mg/kg: $F_{(12,72)}$=1.6, p=0.03, n=8; 10 mg/kg: $F_{(12,72)}$=5.1, p=0.000004, n=7; 20 mg/kg: $F_{(12,108)}$=5.6, p<0.000001, n=10], but not for the 0 mg/kg group (p>0.05; n=7). This was supported by the results of the post hoc comparisons showing a significant increase in DA following the 5 mg/kg treatment 20 min after injection (195%; p=0.04). Accordingly, there was a significant increase in DA 20 min (328%; p=0.001) and 40 min (308%; p=0.004) after 10 mg/kg cocaine, and for the 20 mg/kg group, a significant DA response was evident at the 20 min (278%; p=0.02), 40 min (322%; p=0.001), and 60 min (321%; p=0.001) intervals after injection.

A comparison of the effects of cocaine on DA levels between brain areas revealed only a trend for differences between the PRC, EC, and PFC. The area × dose ANOVA on DA AUC values did not yield significant effects (p>0.05), but the preplanned comparisons conducted on the AUCs for the 20 mg/kg dose showed a trend towards a larger response in the PFC as compared to the EC (t=1.9; p=0.09), but not between PFC and PRC (p>0.05).

Table 1 Mean basal values±SEM of serotonin (5-HT) and dopamine (DA) in the perirhinal (PRC), entorhinal (EC), and prefrontal cortex (PFC) of animals receiving cocaine or vehicle (in pg/20 μl)

Area	5-HT	DA
PFC	1.10 ± 0.19 (n = 37)	1.10 ± 0.25 (n = 32)
PRC	3.04 ± 0.93 (n = 30)	1.40 ± 0.36 (n = 21)
EC	7.02 ± 4.14 (n = 29)	0.75 ± 0.16 (n = 21)
	$F_{(2,95)}$ = 1.8; p = 0.2	$F_{(2,73)}$ = 1.5; p = 0.2

Serotonin responses to cocaine

In the PRC, cocaine provoked a dose-dependent increase in 5-HT levels (Fig. 4). There was an effect of time in the groups receiving 10 mg/kg [$F_{(12,72)}$=2.47; p=0.009; n=7] or 20 mg/kg [$F_{(12,60)}$=2.96; p=0.0027; n=6] cocaine, but not for the 5 mg/kg dose (p>0.05; n=11). These results were supported by post hoc tests showing a significant increase as compared to the last baseline sample during the first 20-min interval in animals treated with the 20 mg/kg dose (202%; p=0.002), but no effect was found for the other groups (p>0.05). In the animals treated with saline, 5-HT levels decreased over time, resulting in a significant effect of time [$F_{(12,72)}$=2.25; p=0.017; n=6]. However, post hoc tests vs the last baseline sample failed to reach significance (p>0.05).

A dose-dependent increase in 5-HT was found in the EC (Fig. 4). One-way ANOVAs showed significant effects of time for 5-HT in the EC for all treatment groups [0 mg/kg: $F_{(12,60)}$=9.08, p<0.000001, n=9; 5 mg/kg: $F_{(12,72)}$=2.66, p=0.005, n=7; 10 mg/kg: $F_{(12,72)}$=6.63, p<0.000001, n=7; 20 mg/kg: $F_{(12,60)}$=9.08, p<0.000001, n=6]. After application of 10 mg/kg cocaine, 5-HT levels were significantly elevated to 180% of baseline in the 20-min interval (p=0.002) after injection. For the 20 mg/kg group, the 5-HT response was markedly enhanced and prolonged, which was reflected by a significant 5-HT increase to 342% within the first 20 min (p=0.0001) and to 309% (p=0.0003), 293% (p=0.0008), and 307% (p=0.0003) in the following three intervals.

Cocaine increased 5-HT levels in the PFC (Fig. 4). This was shown by a significant effect of time for all three cocaine treatment groups [5 mg/kg: $F_{(12,96)}$=1.87, p=0.047, n=9; 10 mg/kg: $F_{(12,108)}$=4.9, p=0.000002, n=10; 20 mg/kg: $F_{(12,96)}$=5.8, p<0.000001, n=9], but not for the saline group (p>0.05; n=9). Post hoc tests vs the last baseline sample revealed significantly increased levels of extracellular 5-HT for the 20 mg/kg group 20 min (412%; p=0.0002) and 40 min (304%; p=0.03) after cocaine injection.

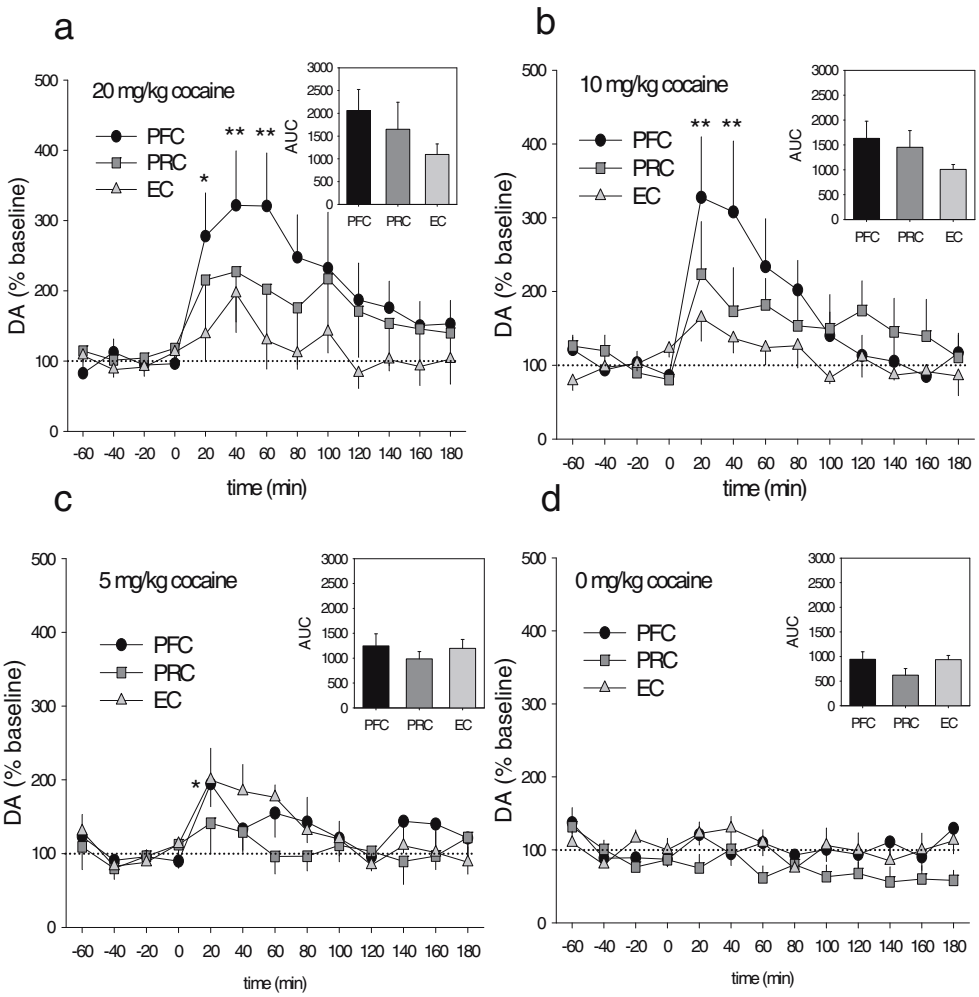

Fig. 3 The effects (mean±SEM) of cocaine (i.p.) on extracellular dopamine activity in the perirhinal cortex (*PRC*), entorhinal cortex (*EC*), and prefrontal cortex (*PFC*). The values are presented as percent baseline, taking the average of the four baseline samples as 100% (*$p<0.05$, **$p<0.01$, Tukey's test vs last baseline sample)

Cocaine had different effects on 5-HT levels in the PRC, EC, and PFC. A significant area effect [$F_{(2,84)}=4$; $p=0.02$] was revealed by the area×dose ANOVA on the 5-HT AUC values. Further, there was a significant main effect of dose [$F_{(3,84)}=15.3$; $p<0.001$], but no significant interaction of area and dose [$F_{(6,84)}=1.88$; $p=0.09$]. Preplanned comparisons conducted separately for each dose showed a more pronounced 5-HT response in the EC as compared to the PRC after 20 mg/kg cocaine ($t=3.4$; $p=0.006$; Fig. 4a). Despite the visual impression, the difference between PFC and PRC in the 20 mg/kg group did not reach significance ($p>0.05$).

Dopamine vs serotonin response to cocaine

To evaluate the relative impact of cocaine on monoamines, the response of DA and 5-HT was compared within brain areas. In the PRC, no difference between the extent of DA and 5-HT increase was found ($p>0.05$). However, comparisons showed a significant difference between the DA and 5-HT response to cocaine in the EC after application of 20 mg/kg cocaine ($t=3.9$; $p=0.004$), indicating a predominant 5-HT response. In the PFC, no differences between the DA and 5-HT response to cocaine became evident ($p>0.05$).

Histological results for *d*-amphetamine-treated animals

Probe placements for the rats receiving *d*-amphetamine or vehicle are shown in Fig. 5. Only animals with probes localized in the respective brain areas were considered for analysis. However, probe losses due to leakage or undetectability of one neurochemical occasionally resulted in different sample sizes.

Behavioral effects of *d*-amphetamine

d-Amphetamine dose dependently increased locomotor activity (Fig. 6). The two-way ANOVA yielded significant main effects of time [$F_{(12,696)}=7.2$; $p<0.000001$] and dose [$F_{(3,58)}=9.3$; $p=0.00004$] as well as a significant interaction of those factors [$F_{(36,696)}=4.2$; $p<0.000001$]. Post hoc tests showed that locomotion was significantly higher in the 2.5 mg/kg group ($n=17$) compared to the 0 mg/kg ($p=0.0002$;

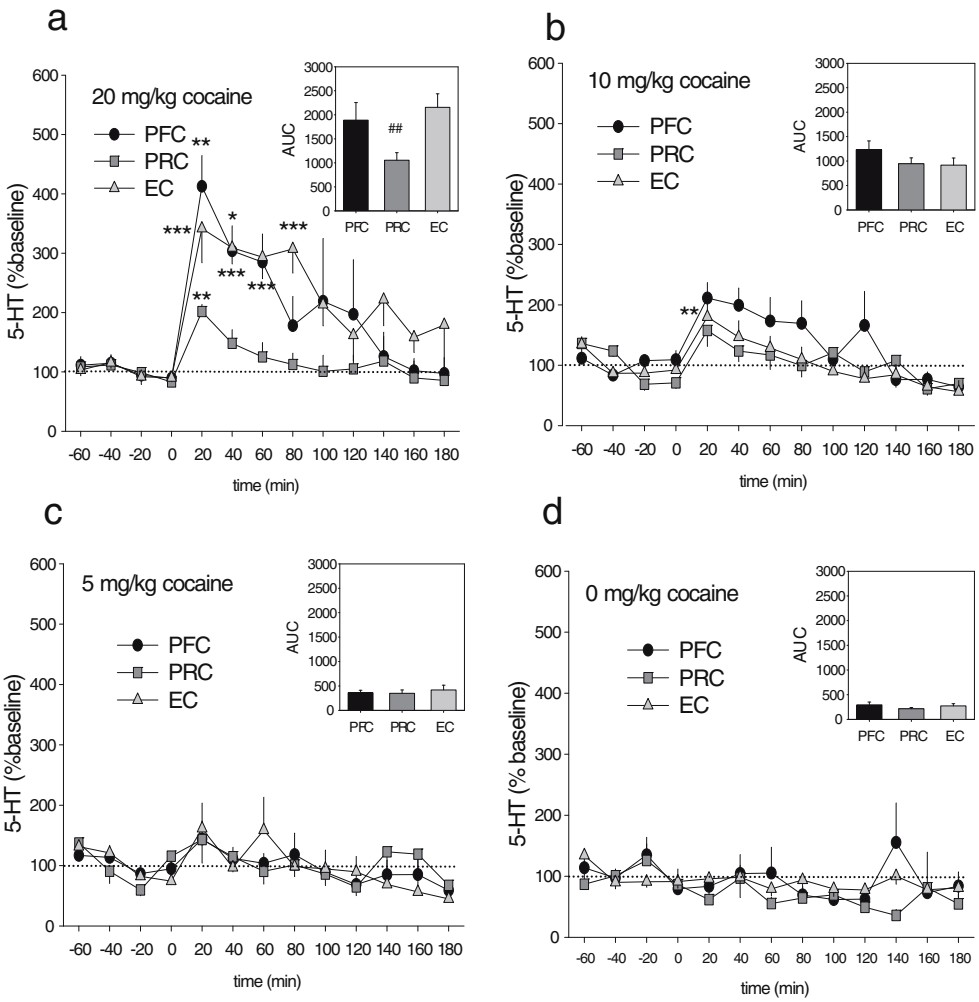

Fig. 4 The effects (mean±SEM) of cocaine on extracellular serotonin activity in the perirhinal cortex (*PRC*), entorhinal cortex (*EC*), and prefrontal cortex (*PFC*). The values are presented as percent baseline, taking the average of the four baseline samples as 100% (*$p<0.05$, **$p<0.01$, ***$p<0.001$, Tukey's test vs last baseline sample; $^{\#\#}p<0.01$ compared to EC, two-tailed *t*-test)

$n=17$), 0.5 mg/kg ($p=0.005$; $n=14$), and 1.0 mg/kg ($p=0.01$; $n=14$) group. No differences between the other groups were found ($p>0.05$). One-way ANOVAs conducted separately for each group on the factor time found significant effects of time for the 1.0 mg/kg group [$F_{(12,156)}=3.6$; $p=0.00008$] and the 2.5 mg/kg group [$F_{(12,156)}=9.3$; $p<0.000001$], but not for the 0.5 mg/kg and the 0 mg/kg groups ($p>0.05$). Post hoc tests vs last baseline interval

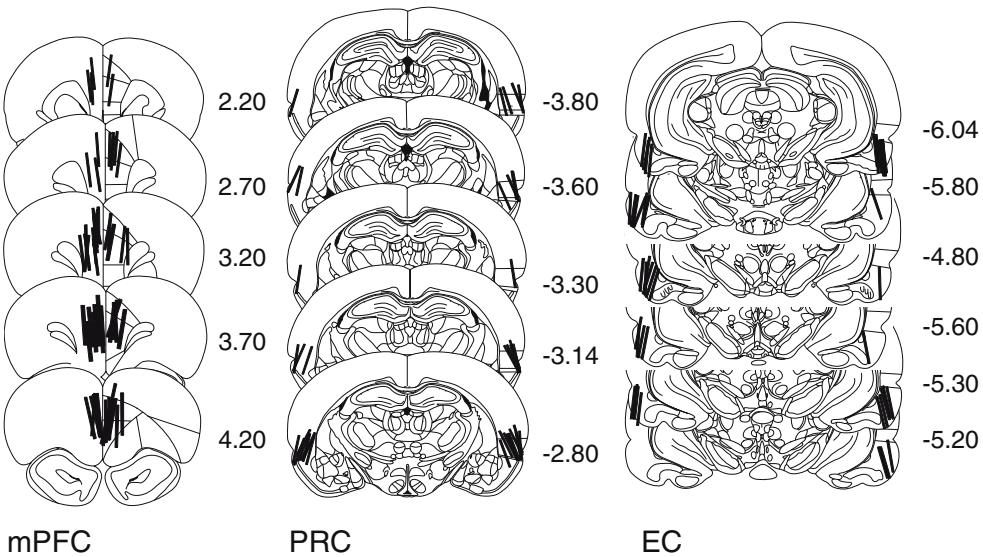

Fig. 5 Localization of microdialysis probes within the prefrontal cortex (*PFC*), perirhinal cortex (*PRC*), and entorhinal cortex (*EC*) in animals treated with *d*-amphetamine, based on the stereotaxic atlas of the rat brain by Paxinos and Watson (1986). *Caption* refers to the anterior–posterior coordinates relative to bregma

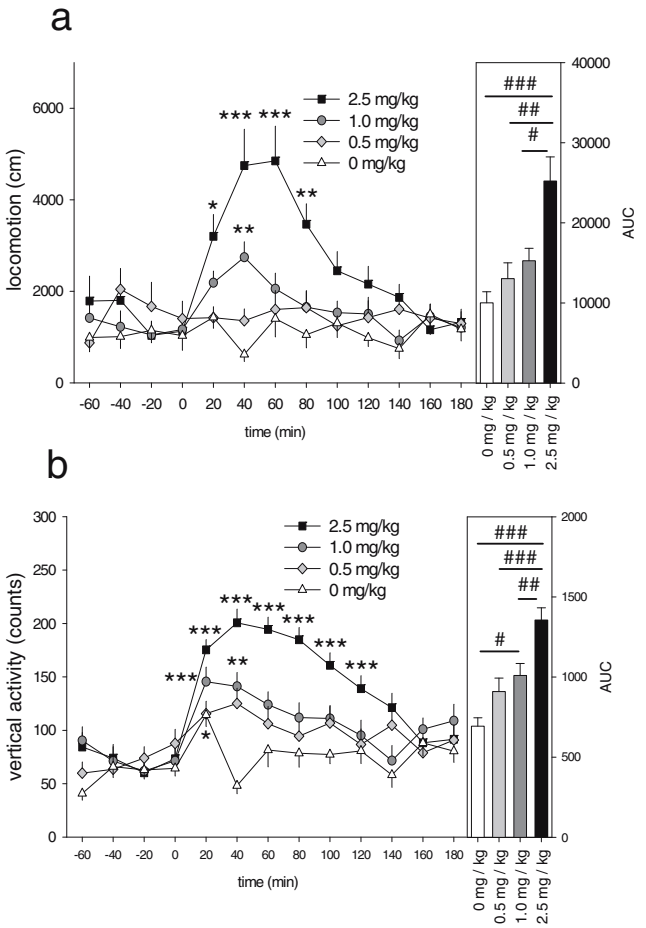

Fig. 6 The effects (mean±SEM) of *d*-amphetamine (i.p.) on locomotion (**a**) and vertical activity (**b**) (*$p<0.05$, **$p<0.01$, ***$p<0.001$, Tukey's test vs last baseline interval; $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$, Tukey's test)

indicated significantly enhanced locomotion during the second 20-min interval after 1.0 mg/kg *d*-amphetamine ($p=0.002$) and during the first ($p=0.04$), second ($p=0.00002$), third ($p=0.00002$), and fourth ($p=0.008$) interval after the 2.5 mg/kg treatment. Tukey's test on the AUC values yielded higher values for the 2.5 mg/kg group as compared to the 0 mg/kg group ($p=0.0002$), the 0.5 mg/kg group ($p=0.001$), and the 1.0 mg/kg group ($p=0.01$).

Analysis of vertical activity showed a similar picture. The two-way ANOVA revealed significant main effects of dose [$F_{(3,58)}=12.7$; $p=0.000002$] and time [$F_{(12,696)}=21.1$; $p<0.000001$] and a significant interaction [$F_{(36,696)}=4.8$; $p<0.000001$] (Fig. 6b). Post hoc tests revealed that vertical activity was enhanced in the 2.5 mg/kg group compared to the 0 mg/kg ($p=0.0002$), 0.5 mg/kg ($p=0.003$), and 1.0 mg/kg ($p=0.03$) group. Further, vertical activity was higher in the 1.0 mg/kg group that in the saline control group ($p=0.02$). One-way ANOVAs conducted separately for each group on the factor time found significant effects of time for all groups [0 mg/kg: $F_{(12,192)}=3.9$, $p=0.00003$; 0.5 mg/kg: $F_{(12,156)}=2.7$, $p=0.002$; 1.0 mg/kg: $F_{(12,156)}=5.4$, $p<0.000001$; 2.5 mg/kg: $F_{(12,192)}=23.3$, $p<0.000001$]. Post hoc tests vs last baseline interval indicated significantly enhanced vertical activity during the first 20-min interval after injection for control animals ($p=0.01$). For animals treated with 1.0 mg/kg *d*-amphetamine, vertical activity was significantly enhanced during the first ($p=0.0005$) and second ($p=0.002$) interval after treatment. Finally, the 2.5 mg/kg treatment induced an increase in vertical activity 20 min ($p=0.00002$), 40 min ($p=0.00002$), 60 min ($p=0.00002$), 80 min ($p=0.00002$), 100 min ($p=0.00002$), and 120 min ($p=0.0009$) after injection. Tukey's test on the AUC values yielded higher values for the 2.5 mg/kg group as compared to the 0 mg/kg group ($p=0.0002$), the 0.5 mg/kg group ($p=0.0005$), and the 1.0 mg/kg group ($p=0.007$). Finally, the AUC value for the 1.0 mg/kg group was significantly higher than the value for the 0 mg/kg group ($p=0.02$).

Dopamine responses to *d*-amphetamine

Basal values of DA and 5-HT are shown in Table 2. They did not differ between treatment groups in the PRC, EC, or PFC ($p>0.05$). There was also no significant difference in baseline values of DA and 5-HT between brain areas ($p>0.05$).

In the PRC, DA was dose dependently increased by *d*-amphetamine application (Fig. 7). Statistical analysis yielded a significant effect of time for the 0.5 mg/kg [$F_{(12,48)}=3.21$; $p=0.002$; $n=5$] and 2.5 mg/kg groups [$F_{(12,48)}=2.7$; $p=0.006$; $n=5$], but not for the 1.0 mg/kg ($n=5$) and 0 mg/kg groups ($p>0.05$; $n=5$). These effects were supported by a trend towards an increase in extracellular DA values 40 min after injection for the 0.5 mg/kg *d*-amphetamine group (210%; $p=0.06$) and by significantly increased DA values for the 2.5 mg/kg dose during the same interval (364%; $p=0.006$).

In the EC, DA levels were dose-dependently increased by *d*-amphetamine (Fig. 7). There was a significant main effect of time for the 1.0 mg/kg [$F_{(12,48)}=4.1$; $p=0.0002$; $n=5$] and

Table 2 Mean basal values±SEM of serotonin (5-HT) and dopamine (DA) in the perirhinal (PRC), entorhinal (EC), and prefrontal cortex (PFC) of animals receiving *d*-amphetamine or vehicle (in pg/20 μl)

Area	5-HT	DA
PFC	2.40 ± 0.51 (n = 37)	1.10 ± 0.35 (n = 28)
PRC	2.00 ± 0.49 (n = 27)	0.62 ± 0.12 (n = 20)
EC	2.40 ± 0.71 (n = 28)	0.69 ± 0.18 (n = 23)
	$F_{(2,92)}$ = 0.2; p = 0.8	$F_{(2,69)}$ = 0.87; p = 0.4

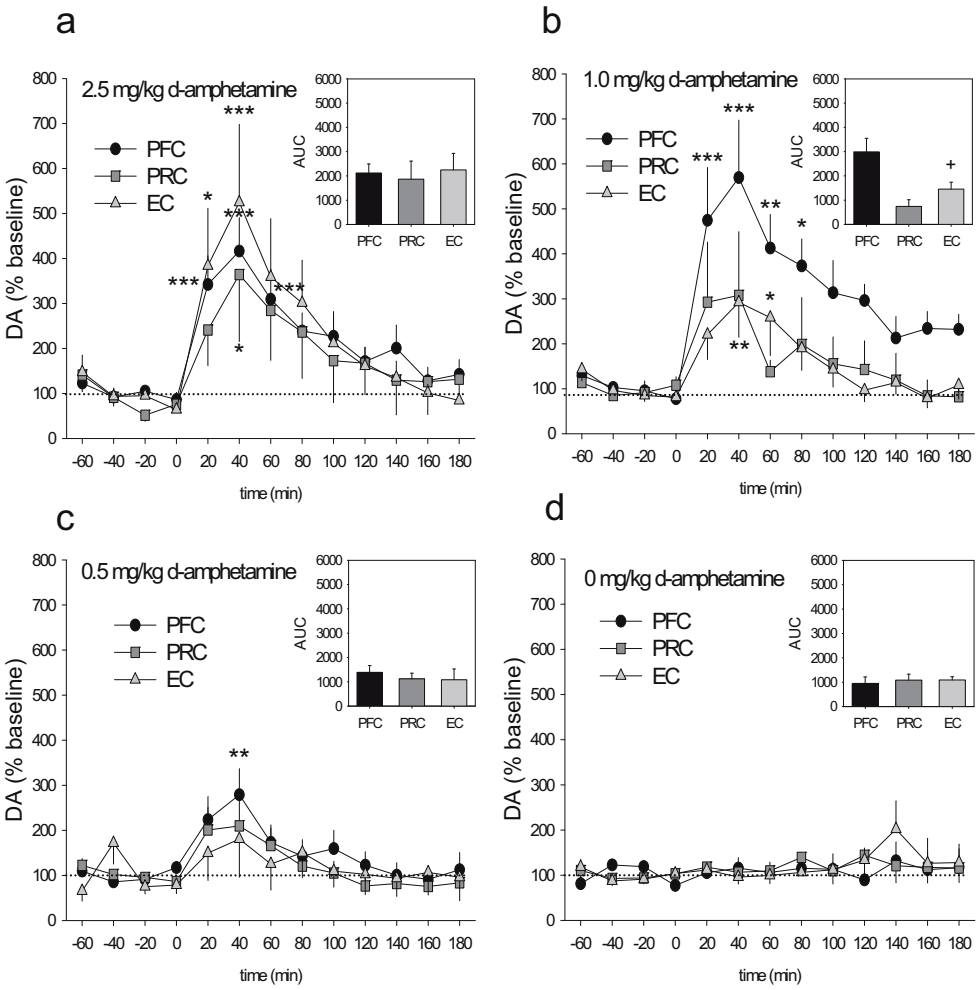

Fig. 7 The effects (mean±SEM) of *d*-amphetamine (i.p.) on extracellular dopamine activity in the perirhinal cortex (*PRC*), entorhinal cortex (*EC*), and prefrontal cortex (*PFC*). The values are presented as percent baseline, taking the average of the four baseline samples as 100% (*$p<0.05$, **$p<0.01$, ***$p<0.001$, Tukey's test vs last baseline sample; $^{+}p<0.05$ compared to PFC, two-tailed *t*-test)

2.5 mg/kg groups [$F_{(12,60)}=5.3$; $p=0.000005$; $n=7$], but not for the 0 mg/kg ($n=5$) and 0.5 mg/kg groups ($p>0.05$; $n=6$). Those results were supported by post hoc tests, yielding a significant increase in DA 40 min (292%; $p=0.007$) and 60 min (258%; $p=0.04$) after 1.0 mg/kg *d*-amphetamine and significantly elevated DA levels 20 min (384%; $p=0.03$) and 40 min (525%; $p=0.0003$) after injection in the 2.5 mg/kg group.

In the PFC, *d*-amphetamine increased DA levels dose-dependently (Fig. 7). Significant effects of time were found for all groups receiving *d*-amphetamine [0.5 mg/kg: $F_{(12,72)}=4.84$, $p=0.000009$, $n=7$; 1.0 mg/kg: $F_{(12,60)}=8.72$, $p<0.000001$, $n=6$; 2.5 mg/kg: $F_{(12,60)}=10.22$, $p<0.000001$, $n=6$], but not for the 0 mg/kg group ($n=9$). Post hoc tests vs the last baseline sample revealed a significant increase in extracellular DA 40 min after 0.5 mg/kg *d*-amphetamine (279%; $p=0.002$). For the 1 mg/kg group, significantly enhanced extracellular DA levels were found at 20 min (475%; $p=0.0002$), 40 min (569%; $p=0.0001$), 60 min (413%; $p=0.002$), and 80 min (373%; $p=0.01$) after drug application. For the 2.5 mg/kg group, significantly enhanced DA levels were found 20 min (342%; $p=0.0002$), 40 min (417%; $p=0.0001$), and 60 min (309%; $p=0.0008$) after injection.

Little differences in the DA response to *d*-amphetamine between PRC, EC, and PFC were found. The brain area × dose ANOVA on DA AUC values did not yield any significant effects ($p>0.05$). Preplanned comparisons, however, revealed a trend towards a more pronounced response in the PFC as compared to the PRC ($p=0.06$) and a significantly more pronounced response in the PFC as compared to the EC ($p=0.02$) in the group treated with 1.0 mg/kg *d*-amphetamine (Fig. 7b).

Serotonin responses to *d*-amphetamine

In the PRC, 5-HT levels increased dose-dependently in response to *d*-amphetamine (Fig. 8). A significant effect of time on 5-HT values was found for the 1.0 mg/kg [$F_{(12,84)}=2.21$, $p=0.018$; $n=8$] and 2.5 mg/kg groups [$F_{(12,72)}=1.91$, $p=0.047$; $n=7$], but not for the 0 mg/kg ($n=7$) and 0.5 mg/kg groups ($p>0.05$; $n=5$). Post hoc

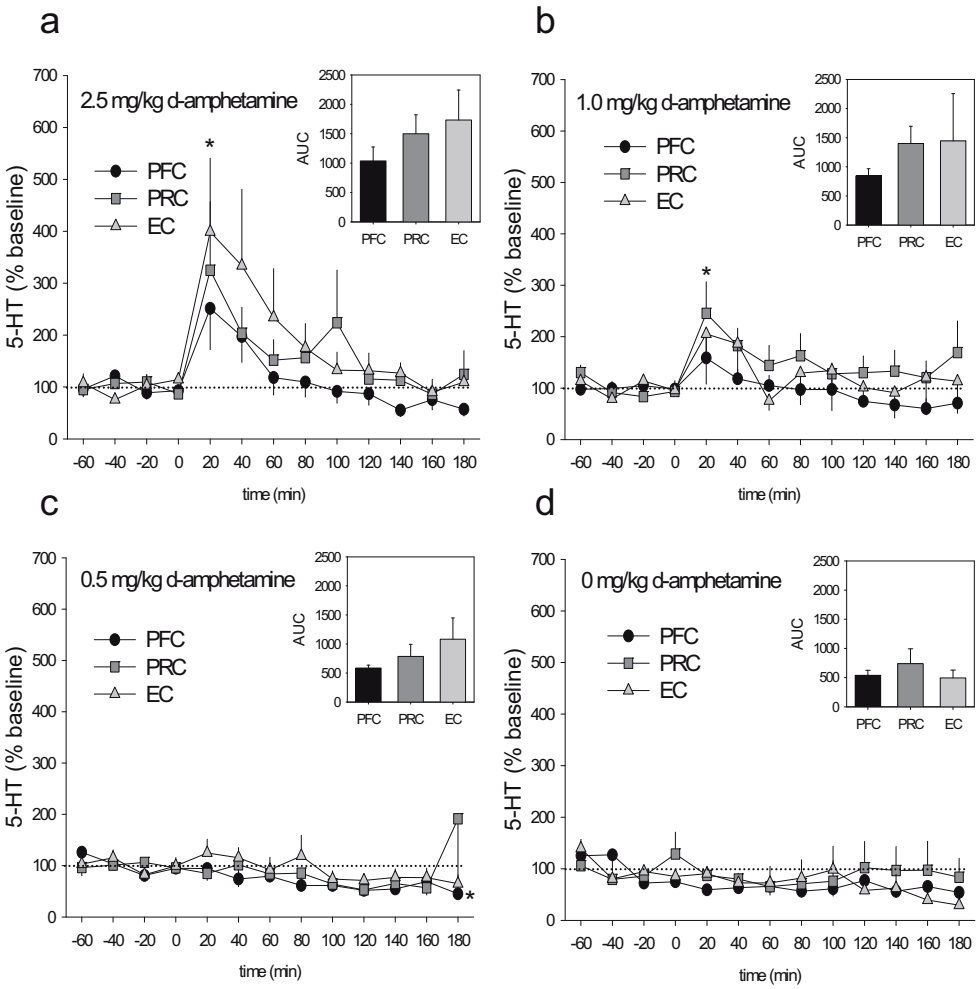

Fig. 8 The effects (mean±SEM) of *d*-amphetamine (i.p.) on extracellular serotonin activity in the perirhinal cortex (*PRC*), entorhinal cortex (*EC*), and prefrontal cortex (*PFC*). The values are presented as percent baseline, taking the average of the four baseline samples as 100% (*$p<0.05$, **$p<0.01$, ***$p<0.001$, Tukey's test vs last baseline sample)

tests vs the last baseline sample indicated that there was a significant increase 20 min after injection for the 1.0 mg/kg group (245%; $p=0.02$) and a trend towards such an increase for the 2.5 mg/kg group (325%; $p=0.055$).

In the EC, 5-HT values increased dose dependently in response to *d*-amphetamine (Fig. 8). Significant effects of time were shown for the 2.5 mg/kg [$F_{(12,84)}=3.25$, $p=0.0007$; $n=9$] and 0 mg/kg groups [$F_{(12,48)}=2.96$, $p=0.004$; $n=5$], thus, reflecting a continuous decrease in 5-HT, but not for the 0.5 mg/kg ($n=9$) and 1.0 mg/kg groups ($n=5$; $p>0.05$). Post hoc tests revealed a significant increase in 5-HT 20 min after 2.5 mg/kg *d*-amphetamine (399%; $p=0.03$), but no other significant effects were found ($p>0.05$).

A dose-dependent response of 5-HT was shown in the PFC (Fig. 8). Repeated measures one-way ANOVAs revealed significant effects of time for all treatment groups [0 mg/kg: $F_{(12,84)}=2.95$, $p=0.002$, $n=8$; 0.5 mg/kg: $F_{(12,108)}=5.38$, $p<0.000001$, $n=10$; 1.0 mg/kg: $F_{(12,96)}=2.18$, $p=0.02$, $n=9$; and 2.5 mg/kg: $F_{(12,96)}=4.17$, $p=0.00003$, $n=10$]. Nevertheless, in post hoc tests, a trend to a significant increase in 5-HT values over baseline was only evident for the 2.5 mg/kg dose 40 min after injection (197%; $p=0.09$).

Analyses conducted on the 5-HT AUC values to compare the response between brain areas did not find any significant differences ($p>0.05$).

Dopamine vs serotonin response to *d*-amphetamine

To evaluate the relative impact of *d*-amphetamine on monoamines, the response of DA and 5-HT were compared within brain areas. A more pronounced DA as compared to 5-HT response was found in the PFC in all groups treated with *d*-amphetamine. Thus, in the 2.5 mg/kg *d*-amphetamine group, the DA response was more pro-

nounced in the PFC (t=2.7; p=0.02), but DA and 5-HT responded equally in the PRC and EC (p>0.05). The DA response in the PFC was stronger than the 5-HT response in the 1.0 mg/kg (t=5.7; p<0.001) and 0.5 mg/kg groups (t=2.8; p=0.03), while there were no differences between DA and 5-HT in the PRC or EC (p>0.05). In the saline group, there was a stronger DA than 5-HT response in the EC (t=2.9; p=0.02), whereas there were no differences between DA and 5-HT in the PFC or the PRC (p>0.05).

Comparisons between cocaine and *d*-amphetamine

The doses of cocaine and *d*-amphetamine were selected in a way to yield similar behavioral activation, which allowed an inter-drug comparison of the neurochemical responses.

Behavioral responses

Behavioral data were compared between cocaine and *d*-amphetamine by drug × dose ANOVAs on AUC values. Analysis of horizontal locomotion showed a significant effect of dose [$F_{(3,117)}$=14.4; p<0.001], but not for drug (p>0.05) nor a dose × drug interaction (p>0.05). Also the two-tailed t-test did not reach significance (p>0.05). The two-way ANOVA for vertical activity found significant main effects of dose [$F_{(3,117)}$=25; p<0.001] and drug [$F_{(1,117)}$=7.8; p=0.006], but no significant dose × drug interaction [$F_{(3,117)}$=1.1; p=0.3]. A two-tailed t-test was used to compare cocaine with *d*-amphetamine and revealed significantly higher vertical activity counts for the animals treated with *d*-amphetamine (p=0.02).

Neurochemical responses

A three-way ANOVA on DA values yielded significant main effects of area [$F_{(2,120)}$=3.4; p=0.036], drug [$F_{(1,120)}$=5.4; p=0.021], and dose [$F_{(3,120)}$=7.3; p<0.001], but none of the interactions were significant (p>0.05). Preplanned comparisons failed to show significant differences in the extent of the DA response after cocaine and *d*-amphetamine for the PRC, EC, or PFC (p>0.05). However, for 5-HT, there was a significant main effect of dose [$F_{(3,165)}$=17.2; p<0.001] and, notably, a significant brain area × drug interaction [$F_{(2,165)}$=4.5; p=0.01]. No other main effect or interaction reached significance (p>0.05). To further characterize the area×drug interaction, two-tailed t-tests comparing drugs within each brain area were used. Those results indicated a stronger 5-HT response to cocaine as compared to *d*-amphetamine in the PFC (p=0.009), but no significant effect of drug within the other brain areas, despite a slight trend towards a stronger reactivity to *d*-amphetamine than cocaine within the PRC (p=0.08).

Discussion

The PFC is the cortical area receiving the most attention in rodent studies on the neuronal effects of psychostimulant drugs of abuse. However, brain imaging studies in humans revealed that many more cortical structures are functionally affected (Breiter et al. 1997; Volkow et al. 2002). These studies only showed alterations in glucose or oxygen utilization and do not provide direct evidence for the transmitter systems involved. In vivo microdialysis studies, in particular in rodents, may bridge this gap. The present report characterized the effects of two widely abused psychostimulant drugs, cocaine and *d*-amphetamine, on DA and 5-HT activity in cortical structures of the hippocampal complex and compared them with the effect in the PFC. The main finding of this study is that cocaine and *d*-amphetamine dose-dependently increase both DA and 5-HT in the PRC, EC, and PFC. The DA and 5-HT responses in these brain areas show not only many commonalities but also some differences. The DA response after cocaine tended to be more pronounced in the PFC than in the EC, but not compared to the PRC. The 5-HT response after cocaine was stronger in the EC as compared to the PRC. No differences for the DA or the 5-HT responses to *d*-amphetamine were found between the PRC or EC and the PFC, except for DA after the 1.0 mg/kg dose. After cocaine, a more pronounced response of 5-HT as compared to DA was found in the EC, but not in the PRC or PFC. *d*-Amphetamine, in contrast, preferentially increased DA as compared to 5-HT in the PFC, but not in the PRC or EC.

In the present study, cocaine and *d*-amphetamine doses were selected to induce a comparable activation after both drugs, thus, providing a behavioral reference for neurochemical comparisons. While the DAergic response did not differ between *d*-amphetamine and cocaine at high, medium, low, and control doses, extracellular 5-HT levels in the PFC showed a more pronounced reaction to cocaine than to *d*-amphetamine. Those differences in the neurochemical response profiles were evident despite the use of dose-ranges for cocaine and *d*-amphetamine that induced a comparable amount of horizontal locomotor activity. Notably, the findings of the present study do not suggest a causal link between the monoaminergic response in the investigated cortices and hyperactivity provoked by cocaine and *d*-amphetamine. However, they may be the source of reported differences in the subjective effects between

cocaine and *d*-amphetamine (Gawin and Ellinwood 1989; Kramer et al. 1967).

Given the high expression of 5-HT transporters (SERT) in the cortex (Sur et al. 1996), it was expected that both cocaine and *d*-amphetamine application may lead to elevated levels of extracellular 5-HT. Indeed, a comparable 5-HT response could be shown for cocaine and *d*-amphetamine in the PRC and EC. Only in the PFC, a more pronounced 5-HTergic response was seen after cocaine as compared to *d*-amphetamine. Levels of DA did not differ significantly between brain areas, which conforms to neuroanatomical studies finding relatively dense innervation of the PFC, PRC, and EC by the DAergic cells of the VTA (Berger et al. 1991; Oades and Halliday 1987). The seemingly large difference between basal 5-HT levels in the PFC and the EC were due to a few animals distributed over the treatment groups with particularly high 5-HT levels in the EC. However, no abnormalities in the vascularization or local bleeding was observed in these animals in the histology. And because the percent increase in 5-HT was in no respect different from the other animals in these groups, i.e., from the animals with lower basal values, we did not see a reason to exclude these animals from analysis. The variation in basal values, however, can most likely be explained by variation in the recovery rates of the used probes (Benveniste and Hansen 1991).

Elevated levels of DA could be shown after both psychostimulant drugs in the PRC and EC, which were largely comparable to the DAergic response in the PFC. Given the low expression of dopamine transporters (DAT) especially in caudal cortical areas (Freed et al. 1995), it can be speculated that the DA response found in the present study may be mediated by blockade of the norepinephrine transporter (NET) by cocaine or the release via the NET by *d*-amphetamine, as both drugs bind to the NET (Ritz and Kuhar 1989). Indeed, elevation of extracellular levels of DA in the PFC was found after local application of the selective NET blocker desmethylimipramine (Yamamoto and Novotney 1998) or the systemic application of reboxetine or desipramine (Valentini et al. 2004). Mazei et al. (2002) reported that the combined DAT/NET blocker nomifensine or the selective NET blocker desmethylimipramine was more effective in elevating DA levels in the PFC than the selective DAT blocker GBR 12909. Furthermore, Carboni et al. (2006) demonstrated that combined application of the selective NET blocker reboxetine and the selective DAT blocker GBR 12909 induced a more pronounced increase in prefrontal DA than application of each drug alone. For caudal cortical areas, Valentini et al. (2004) reported that DA levels in the parietal and occipital cortex were not modulated by desipramine. Therefore, it may be argued that the NET is the main target in the mediation of cocaine's and *d*-amphetamine's neurochemical effects in the PFC (Tanda et al. 1997), PRC, and EC.

The stronger 5-HT response in the PFC after cocaine as compared to *d*-amphetamine, reported in the present study, may be explained by the different affinities of the two compounds to the SERT, with cocaine binding more effectively to the SERT than to the DAT or NET and *d*-amphetamine having higher affinity to the NET and DAT as compared to the SERT (Ritz and Kuhar 1989). The same explanation may account for the more pronounced response of DA as compared to 5-HT in the PFC after application of *d*-amphetamine.

Given the limited evidence about the role of monoamines in the rhinal cortices, it can only be speculated on the functional implications of the present results. Breiter et al. (1997) investigated the acute response to cocaine in humans by means of functional magnetic resonance imaging and reported, among others, an activation of the parahippocampal gyrus, the primate equivalent of the rhinal cortices of rodents (Eichenbaum 2000). Neuronal activity in this area was correlated with subjective ratings of drug-craving (Breiter et al. 1997). The PRC and EC are critical brain structures involved in learning and memory formation (Galani et al. 1998; Good and Honey 1997; Hannesson et al. 2004; Izquierdo et al. 2002; Parron and Save 2004; Phillips and LeDoux 1995). Furthermore, in vitro studies found substantial modulation of neuronal activity in the EC by DA (Stenkamp et al. 1998) and 5-HT (Schmitz et al. 1998, 1999). Notably, a role for DA in the EC in reward learning is suggested by Liu et al. (2004), who found in monkeys that dopamine D2 receptors in the EC are involved in learning reward-predicting cues. Posttrial application of cocaine has been found to enhance inhibitory avoidance learning (Cestari et al. 1996; Puglisi-Allegra et al. 1994), an effect that was blocked by pretreatment with a dopamine D1 or D2 receptor antagonist (Puglisi-Allegra et al. 1994). Therefore, elevated DA in the rhinal cortices may have a memory-enhancing effect. There is also evidence suggesting a role for 5-HT in the EC in memory processes. Impaired long-term but enhanced short-term inhibitory avoidance memory was found after stimulation of EC 5-HT_{1A} receptors (Izquierdo et al. 2002). However, the lack of evidence concerning the role of other 5-HT receptor subtypes present in the EC (like 5-HT_{2A} or 5-HT_4 receptors; Barnes and Sharp 1999) limits hypotheses about the direction in which elevated 5-HT modulates memory storage in the EC. Considering recent evidence showing a clear contribution of memory processes in conditioned place preference (Cervo et al. 1997; Hsu et al. 2002; Miller and Marshall 2005) and drug-seeking (Lee et al. 2005), it may be speculated that the monoaminergic response to cocaine and *d*-amphetamine could modulate the way in which signals from sensory and association cortices are

processed by the hippocampus (De Curtis and Paré 2004), and therefore, influence storage of environmental configurations related to the drug experience.

Besides the interaction of the rhinal cortices with the hippocampus, there are also direct (Krayniak et al. 1981) as well as indirect (via the hippocampus; Lisman and Grace 2005) connections between the EC and the NAc. A modulatory role of the EC on the mesolimbic DA system is suggested by Todd and Grace (1999), who demonstrated that chemical stimulation of the EC increased the number of active VTA neurons, an effect that was blocked by infusion of a glutamate receptor antagonist into the NAc. Those results are corroborated by neurochemical findings showing an increase in NAc DA after chemical stimulation of the EC/subiculum (Mitchell et al. 2000). Given the close connection between NAc DA and locomotor behavior (Ikemoto 2002; Mogenson et al. 1980), a modulatory role of the EC on locomotion under certain conditions seems likely. Indeed, Sumiyoshi et al. (2004) found enhanced methamphetamine-induced locomotion after unilateral lesions of the left EC without a parallel enhancement of NAc DA elevation ipsilateral to the EC lesion. In contrast, unilateral DA depletion of the EC did induce an increase in contralateral DA turnover in the NAc but without altering amphetamine-induced locomotion (Louilot and Choulli 1997). These results suggest a functional interaction between EC and NAc, either via direct EC–NAc projections (Krayniak et al. 1981) or mediated by a EC–hippocampal–NAc pathway (Mitchell et al. 2000). Interestingly, a recent hypothesis by Lisman and Grace (2005) suggests that the neuronal circuits between the hippocampal formation and the mesolimbic DA system are involved in controlling the processes leading to the establishment of long-term memory.

The PFC is thought to be involved in various aspects of psychostimulant effects (Jentsch and Taylor 1999; Steketee 2003). In a neuroimaging study with humans, acute infusion of cocaine activated the PFC, which was correlated with reports of a subjective "rush" (Breiter et al. 1997). Prefrontal activation is also associated with drug craving in humans (Volkow et al. 2002) and is suggested to play a role in reduced impulse control and perseverative responding associated with addictive behavior (Hyman 2005; Jentsch and Taylor 1999; Volkow et al. 2002). In the present study, we found a parallel increase in DA and 5-HT in the PFC after cocaine and *d*-amphetamine, with a more pronounced 5-HT response after cocaine. Given the diverse functions of the PFC, it is likely that the relative contribution of different monoamines modulates different aspects of drug-related behaviors. DA in the PFC is associated with learning and plasticity in the cortex (Volkow et al. 2002; Wolf et al. 2004) as well as with the modulation of the mesolimbic DAergic system (Karreman and Moghaddam 1996). Thus, it is likely that increased DA levels after cocaine or *d*-amphetamine modulate the working memory function of the PFC (Durstewitz et al. 1999; Goldman-Rakic et al. 2000). Everitt and Robbins (2005) suggested that the PFC is related to the control of behavior, which is disrupted in the course of the development of drug addiction. In this respect, it is notable that elevated 5-HT in the PFC has been implicated in impulsive choice (Winstanley et al. 2006) and impulsive behavior (Dalley et al. 2002) by recent microdialysis studies. In the present study, we found elevated levels of 5-HT in the PFC in response to both *d*-amphetamine (see also Hedou et al. 2000; Kuroki et al. 1996; Millan et al. 1999) and cocaine. Accordingly, it may be speculated that the elevated levels of 5-HT after application of psychostimulants relates to behavioral disinhibition induced by these drugs (Dalley et al. 2002). Carey and Damianopoulos (1994) reported elevated 5-HT levels in postmortem samples of the PFC of rats, which had been conditioned to a specific environment by cocaine reinforcement. Importantly, in the same study, no effect was found for prefrontal DA or its metabolites (Carey and Damianopoulos 1994), which corroborates the results by Hemby et al. (1992), who found preserved conditioned place preference after cocaine in rats with selective DAergic lesions of the PFC. This suggests that 5-HT in the PFC may be essentially involved in the mediation of addiction-related behaviors of cocaine (Filip and Cunningham 2003; Müller and Huston 2006).

In conclusion, we found increased levels of DA and 5-HT in the PRC, EC, and PFC in response to systemic administration of cocaine and *d*-amphetamine. In large parts, the neurochemical responses were comparable between the brain areas after cocaine and *d*-amphetamine. However, there was a more pronounced reaction of 5-HT in the PFC, but not in the PRC or EC after cocaine as compared to *d*-amphetamine. *d*-Amphetamine, on the other hand, predominantly increased DA as compared to 5-HT in the PFC, but not in the PRC and EC. The neurochemical responses to cocaine and *d*-amphetamine in cortical areas involved in memory storage may contribute to the dysfunctional modulation of memory processes by drugs of abuse as well as to the establishment of an addiction memory.

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Role of medial prefrontal, entorhinal and occipital 5-HT in cocaine-induced place preference and hyperlocomotion: Evidence for multiple dissociations

M.E. Pum[1], R.J. Carey[2], J.P. Huston[1], and C.P. Müller[3]

[1] Institute of Physiological Psychology and Center for Biological and Medical Research, University of Düsseldorf, Universitätsstr. 1, 40225 Düsseldorf, Germany
[2] Research and Development (151), VA Medical Center and SUNY Upstate Medical University, 800 Irving Avenue, Syracuse, NY 13210, USA
[3] Social, Genetic and Developmental Psychiatry Center, Institute of Psychiatry, King's College London, De Crespigny Park, London

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Corresponding author: Christian P. Müller, PhD
MRC-SGDP-Center
Institute of Psychiatry
King's College London
De Crespigny Park
London SE5 8AF
UK

Email: Christian.Muller@iop.kcl.ac.uk
Phone: +44 (0) 20 7848 5679
Fax: +44 (0)20 7 848 0866

Abstract

Application of cocaine or exposure to cocaine-related stimuli induces widespread activation of the cortex in neuroimaging studies with human subjects. In accordance to these findings it was reported in previous microdialysis experiments, that cocaine increased serotonin (5-HT) and dopamine in various cortical brain areas. The present series of studies set out to investigate the functional role of the observed increases in 5-HT in the medial prefrontal cortex (mPFC), the entorhinal cortex (EC), and the occipital cortex (OccC) in the mediation of cocaine-induced conditioned place preference (CPP) and hyperactivity. To reduce 5-HTergic neurotransmission in circumscribed brain areas, bilateral local infusions of the serotonergic neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT), were made into the mPFC, EC, or OccC. Two weeks following surgery, cocaine- (10 mg/kg; i.p.) induced CPP was measured in an unbiased design. The 90 % depletion of 5-HT in the mPFC significantly attenuated the preference for the cocaine-associated environment and the hyperlocomotor response to cocaine. A 61 % depletion of 5-HT in the EC reduced conditioned place preference without modulation of hyperactivity, while a 78 % 5-HT depletion of the OccC cortex had no effect on cocaine-induced CPP and hyperactivity. These results indicate an important role of cortical 5-HT in the mediation of cocaine-induced CPP, and specify the region-dependent contribution of a neurochemical response to cocaine-mediated behaviour.

Introduction

It is established that the mesolimbic dopaminergic system is crucial for the reinforcing and hyperlocomotor effects of psychostimulant drugs (Koob et al., 1998). However, neuroimaging studies in humans have found cocaine-induced activation of cortical areas which are not target of the mesolimbic DA projections (Breiter et al., 1997). Given the substantial role of the cortex in memory and cognition, these results are in accordance with an important role of cognitive and memory-related processes in the development and maintenance of addictive behaviour (Everitt & Robbins, 2005; Hyman, 2005; Kalivas and Volkow, 2005; Kelly, 2004; Zernig et al., 2007).

The prefrontal cortex (PFC) is activated following acute application of cocaine (Breiter et al., 1997) or when drug-experienced individuals were exposed to stimuli associated with the drug (Grant et al., 1996). In rats, excitotoxic lesion of the mPFC affects various components of cocaine-induced conditioned place preference (CPP; Pierce et al., 1998, Tzschentke and Schmidt 1999; 2000; Zalava et al., 2003). However, the neurochemical mechanisms mediating the effects of mPFC lesions on cocaine-induced CPP are not well understood. Hemby et al. (1992) did not find effects of mPFC dopamine (DA) depletion in the mPFC on cocaine-induced CPP, while a recent study suggested an involvement of mPFC norepinephrine in place preference induced by cocaine (Ventura et al., 2007). Studies using mice, with specific and combined deletion of monoamine transporters, provided evidence for a role of the interaction of dopamine and serotonin transporters in the mediation of the rewarding effects of cocaine (Rocha, 2003). Indeed, a significant role of serotonin (5-HT) in the behavioural effects of cocaine is well established (Higgins and Fletcher, 2003; Müller and Huston, 2006; Müller et al., 2007a). Notably, cocaine not only increases DA-activity in the mPFC but also raises extracellular 5-HT levels (Pum et al., 2007). In particular, mPFC 5-HT2C receptors seem to play a role in mediating the hyperlocomotor and discriminative

stimulus effects of cocaine (Filip and Cunningham, 2003). Therefore, a role of 5-HT in the mPFC in cocaine-induced CPP can be expected.

The acute application of psychostimulants results in an increase of DA and 5-HT levels not only in the mPFC, but also in the rhinal and in secondary sensory cortices (Müller et al., 2007b; 2007c; Pum et al., 2007). The rhinal cortices are involved in memory-functions (Eichenbaum, 2000). Cocaine-induced neurochemical alterations in these areas may also contribute to the formation of memories related to drugs of abuse (Pum et al., 2007). In human subjects activation of the entorhinal cortex (EC) was evident following application of cocaine (Breiter et al., 1997), as well as following presentation of cocaine-associated visual stimuli (Grant et al., 1996), which supports the hypothesis that neurochemical alterations in the EC may play a role in drug-related memories. Also, the neurochemical effects of cocaine in the visual cortex (Müller et al., 2007b; 2007c) may be of importance. Especially, given the substantial role of cocaine-associated stimuli in the maintenance and reinstatement of addictive behaviour (Di Ciano and Everitt, 2004; See, 2005). The modulation of early sensory processing by cocaine (Devonshire et al., 2007) might play a role in the motivational strength exercised by these stimuli. This assumption is also corroborated by neuroimaging results, which associate visual cortex activation in cocaine-dependent subjects during exposure to drug-related stimuli with an increased risk for relapse (Kosten et al., 2006). To investigate the functional role of the cocaine-induced 5-HT increase in 5-HT in various cortices (Müller et al., 2007b; Pum et al., 2007), we depleted 5-HT in the mPFC, EC, and occipital cortex (OccC), by local injections of the serotonergic neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT; Baumgarten and Lachenmayer, 2004), and tested the effects of these lesions on the acute behavioural effects of cocaine and on CPP. It was hypothesized that 5-HT in all three cortical areas contributes to cocaine-induced CPP and hyperlocomotion.

Materials and Methods

All experiments were conducted in conformity with the Animal Protection Law of the Federal Republic of Germany and with the guidelines established by the National Institutes of Health Guide for the Care and Use of Animals. All efforts were made to minimize the number of animals used and to minimize any discomfort.

Animals and Surgery

Male Wistar rats (Tierversuchsanlage University of Düsseldorf, Germany) weighting 274.8 (± 2.5) g before surgery were used. They were housed in groups of 4-5 animals under a reversed 12:12h light-dark cycle (lights on at 19:00 h), with food and water provided ad libitum. Following surgery they were temporarily housed in single cages for one or two days, and then, again, kept in groups of 4-5 animals. On the evening before surgery, the animals were treated with 25 mg/kg desipramine-hydrochloride (dissolved in distilled water; injection volume 1 ml/kg, i.p.; Sigma, Steinheim, Germany). On the next day, they were anesthetized with a mixture of 0.9 ml/kg Ketavet (containing 100 mg/ml Ketamine; Pharmacia and Upjohn, Germany) and 0.1 ml/kg Rompun (containing 20 mg/ml Xylazin; Bayer, Germany) and 1 ml/kg Rimadyl (containing 5 mg/kg carprofen; Pfizer, Karlsruhe, Germany) was injected s.c. to prevent post-operative pain. The rats were placed in a Kopf stereotaxic frame, the scalp was cut and retracted to expose the skull. Holes were drilled bilaterally above the targeted cortices and 1µl 5,7-dihydroxytryptamine (10 µg/µl in saline with 0.1% ascorbic acid) or vehicle was infused (mPFC: AP +2.7, ML ± 0.5, DV -3.4; EC: AP -5.3, ML ± 5.0, DV -6.6, angled 13° towards midline; OccC: AP -6.8, ML ± 4.5, DV -2.8, angled 14° towards midline; coordinates relative to bregma or skull surface; Paxinos and Watson, 1986) via a

silica cannula (other diameter: 150 µm) by using a microinfusion pump (flow-rate 0.5 µl/min). Following the infusion, the injection cannula was left in place for 2-min to allow for diffusion. Finally, the wound was sutured and disinfected with a 70 % ethanol solution. Animals were allowed to recover for one week before being tested in the open field and elevated plus maze (data reported elsewhere). Two weeks after surgery, the place preference experiment started.

Apparatus

Place preference conditioning was performed in a round open field (diameter 83 cm, walls height 43 cm), which was divided into two halves, that could be distinguished by the texture of the floor and walls, by a square metal rod (8 x 8 mm). On one side the floor and wall was covered by a rough black rubber mat, and on the other side floor and wall were covered by a smooth black rubber mat. Illumination was provided indirectly by a 100 W light bulb in one of the upper corners of the light- and sound-attenuating room, in which the apparatus was set up (resulting in an illumination level of 1.5 lux near the walls, 2.5 lux in the middle of the apparatus). A camera, connected to a VCR and a PC, was installed above the open field (Jocham et al., 2006; 2007).

Experimental Procedures

On the first day, a 15-min baseline session was conducted, during which both sides of the open field were accessible. Here, previous results were replicated, showing that there was no *a priori* preference for a particular side of the apparatus (Jocham et al., 2007). On days 2-9 the open field was divided into halves by a transparent Plexiglas wall. On each day the animals received an injection and were immediately place on the assigned side for a 30-min conditioning session. Cocaine (10 mg/kg, i.p., Merck, Germany) was administered to the animals in the cocaine-treatment groups (sham–cocaine, lesion–cocaine) on odd days, and to

the saline-vehicle animals on even days, while animals in the saline-groups (sham–saline, lesion–saline) received a saline injection each day. The conditioning phase took 8 days. On the day following the last treatment (day 10), the Plexiglas-wall was removed, and animals could explore the whole open field for a 15-min test of place-preference. Horizontal locomotion and time spent in the halves of the apparatus were automatically recorded by the EthoVision (Noldus, Wageningen, Netherlands) videotracking system (Jocham et al., 2006; 2007).

Post mortem neurochemistry

After the experiment, the animals were deeply anesthetized by CO_2 and killed by decapitation and the brain was excised and placed into cold 0.5 M perchloric acid. The ventral striatum, dorsal striatum, medial prefrontal cortex, hippocampus, entorhinal cortex, and occipital cortex were dissected, homogenised, centrifuged, filtered, and stored at -80°C until analysis for dopamine- and serotonin-content (De Souza Silva et al., 1997). Samples, containing 400 pg deoxyepinephrine as an internal standard, were analysed by HPLC with electrochemical detection. The column was an ET 125/2, Nucleosil 120-5, C-18 reversed phase column (Macherey & Nagel, Düren, Germany). The mobile phase consisted of 0.15 M chloroacetic acid, 0.12 M NaOH, 0.67 mM EDTA, 0.86 M sodium octylsulfate, 3.5 % acetonitrile, and 1.8 % tetrahydrofuran, and was adjusted to pH 3.0. The electrochemical detector (Intro, Antec, Netherlands) was set at 500 mV vs. an ISAAC reference electrode (Antec, Leyden, Netherlands) at 25°C. There was marked variation in the neurotransmitter-content of different control brain-areas between the experiments. As the presented experiments were performed over the time-course of a year, these variations may be explained by seasonal variations in the brain's monoamine-contents (Kempf et al., 1978; Valzelli et al., 1977).

Statistics

For the analysis of conditioned place preference, a place-preference score was calculated by subtracting the time spent on the saline-paired side from the time spent on the cocaine-paired side. The place-preference scores and the area under curve (AUC) for the cocaine-treatment days were analysed by a one-way ANOVA. To compare the effects of single treatments, pre-planned comparisons with Fisher's LSD-tests were used (Ramsey, 1993). Levels of DA and 5-HT were compared by t-tests for independent samples for each brain area. The software SPSS was used for analyses. The p-value for significant effects was 0.05.

Results

Medial prefrontal cortex 5-HT is critical for cocaine-induced hyperlocomotion and CPP

The local injection of 5,7-DHT into the mPFC significantly reduced 5-HT by 90% [$t(49)=4.754$; $p<0.001$; Tab. 1]. There was also a 70 % reduction of 5-HT levels in the hippocampus [$t(49)=2.658$; $p=0.011$]. There was no effect of the lesion on DA levels in the PFC and hippocampus, or on 5-HT and DA levels in the ventral striatum, dorsal striatum, EC, and OccC ($p>0.05$; Tab. 1).

No bias for a particular side of the apparatus was found during the baseline-test ($p>0.05$). Figure 1A shows the place-preference score following conditioning. It can be seen, that sham-animals conditioned with cocaine exhibited a preference for the cocaine-paired side, while the animals with 5,7-DHT lesions of the mPFC did not show conditioned place preference. This was supported by a significant effect of group [$F(3, 50)=3.314$; $p=0.028$]. Pre-planned comparisons showed that the place-preference score differed significantly between saline- and cocaine-treated sham-lesioned animals ($p=0.008$), but not between the 5,7-DHT-lesioned groups ($p>0.05$). Conditioned locomotion was not observed in any of the cocaine-treatment groups.

The hyperactivity-response to cocaine was blocked on the first day of cocaine treatment in animals with 5,7-DHT lesions of the mPFC, but was evident thereafter (Fig. 1B). The ANOVA on the first cocaine treatment day found a significant effect of group [$F(3, 50)=9.066$; $p<0.001$], which was due to a significant difference between the sham-cocaine and the sham-saline groups ($p<0.001$), while no difference was evident between the lesion-groups ($p>0.05$). The effect of group on the second cocaine-treatment day [$F(3, 50)=15.320$; $p<0.001$] could be ascribed to the significant difference between the sham-saline and sham-cocaine groups ($p<0.001$), and here there was also a difference between the lesion-saline and lesion-cocaine groups ($p=0.003$). Also, on the third day of cocaine treatment there were significant differences between the groups [$F(3, 50)=12.167$; $p<0.001$], and a hyperactivity response was evident in the comparisons of the sham ($p<0.001$) as well as the lesioned groups ($p<0.001$). On the last cocaine treatment day there was, again, an effect of group [$F(3, 50)=11.780$; $p<0.001$], and significantly enhanced locomotion could be confirmed in the comparisons of the sham ($p<0.001$) and the lesioned ($p=0.042$) groups.

Entorhinal cortex 5-HT is essential for cocaine-induced CPP, but not hyperlocomotion

In the experiment testing the effect of 5,7-DHT lesions of the EC, analyses of DA and 5-HT levels in the mPFC, ventral striatum, dorsal striatum, hippocampus, entorhinal cortex, and occipital cortex revealed a significant effect of group for 5-HT in the EC [$t(39)=4.387$; $p<0.001$] and in the OccC [$t(39)=2.077$; $p=0.044$], where a reduction of 5-HT levels of respectively, 61% and 40% was found. Furthermore, a 22% reduction of DA in the hippocampus of the lesion group was found to be significant [$t(39)=2.491$; $p=0.017$]. All other comparisons did not reach significance ($p>0.05$; Tab. 2).

There was, again, no *a priori* preference for a particular side of the apparatus during the baseline-trial ($p>0.05$). Figure 2A shows the place-preference score following conditioning. It can be seen that sham-lesioned animals exhibit a preference for the drug-paired side, which

was attenuated in the cocaine-treated animals with 5,7-DHT lesions of the EC. The one-way ANOVA did not show a significant effect of group ($p>0.05$). However, pre-planned comparisons found a significant difference between the place-preference scores of sham-lesioned cocaine- and saline-treated animals ($p=0.03$), while no further comparisons reached significance. Conditioned locomotion was not observed in any of the cocaine-treatment groups.

The hyperactivity-response to cocaine was not influenced by the 5-HT-depletion of the EC (Fig. 2B). The one-way ANOVA on the first cocaine treatment day revealed a significant effect of group [$F(3, 37)=6.36$; $p=0.001$], and subsequent comparisons confirmed cocaine-induced hyperactivity in the sham ($p=0.006$) as well as in the lesion group ($p=0.004$) as compared to the respective saline control groups. On the second cocaine treatment day there was an effect of group [$F(3, 37)=5.41$; $p=0.003$], however, post hoc tests only found a significant difference between the sham-cocaine and sham-saline groups ($p=0.005$), but not between the lesion-cocaine and lesion-saline groups ($p>0.05$). On the third cocaine treatment day, there was, again, a significant effect of group [$F(3, 37)=11.234$; $p<0.001$], and cocaine-induced hyperactivity was evident in both the sham-cocaine ($p=0.001$) and lesion-cocaine groups ($p<0.001$) as compared to their respective saline control groups. Finally, there was an effect of group on the fourth cocaine treatment day [$F(3, 37)=8.332$; $p<0.001$], which was due to significant differences between the sham-cocaine and sham-saline ($p<0.001$) and between the lesion-cocaine and lesion-saline groups ($p=0.007$). On the second saline treatment day (day4 of the conditioning phase) the one-way ANOVA revealed a significant effect of group [$F(3, 37)=7.019$; $p<0.001$], which was due to increased locomotion in the sham-cocaine as compared to the sham-saline group ($p=0.05$), and a trend towards increased locomotion in the lesion-cocaine group as compared to its saline-control group ($p=0.06$). Further group differences were evident on saline treatment day three [$F(3, 37)=2.789$; $p=0.05$], however, there was only a slight trend towards a difference between the sham-cocaine and sham-saline

groups (p=0.09). Locomotor differences were also found on the fourth saline treatment day [F(3, 37)=4.363; p=0.01], where a trend towards enhanced locomotion was seen in the comparison between the sham-cocaine and sham-saline groups (p=0.06), while the difference between the lesion-cocaine and lesion-saline groups reached significance (p=0.04).

No relevance of occipital cortex 5-HT for the behavioural effects of cocaine

The 5,7-DHT lesions of the OccC led to a significant 78% reduction of 5-HT in the OccC (t(42)=3.242; p=0.002). No other differences in DA or 5-HT levels were found in the other brain areas analysed (p>0.05; Tab. 3).

There was no *a priori* preference for either side of the place preference apparatus (p>0.05). The lesion had no effect on cocaine-CPP (Fig. 3A). There was a significant effect of group [F(3, 40)=4.3; p=0.01], showing that place preference was evident in the comparisons between the sham lesioned saline-treated and cocaine-treated groups (p=0.02) as well as between the 5,7-DHT-lesioned saline-treated and cocaine-treated groups (p=0.01). No differences were found between the cocaine treated groups (p>0.05). Conditioned locomotion was not observed in any of the cocaine-treatment groups.

The hyperactivity-response to cocaine was not influenced by the 5-HT-depletion of the OccC (Fig. 3B). On the first cocaine treatment day there was a significant effect of group [F(3, 40)=7.124; p<0.001], and a hyperactivity response was evident in the sham-cocaine (p=0.001) and in the lesion-cocaine (p=0.006) groups as compared to their respective saline-control groups. There was an effect of group on the second cocaine treatment day [F(3, 40)=8.480; p<0.001], and post hoc comparisons confirmed significant differences between the sham-cocaine and sham-saline (p<0.001), the lesion-cocaine and lesion-saline (p=0.002), as well as between the sham-saline and lesion-saline groups (p=0.002). On the third cocaine treatment day the effect of group [F(3, 40)=9.134; p<0.001] can be ascribed to the differences between the sham-cocaine and sham-saline (p<0.001) and the lesion-cocaine and lesion-saline groups

(p=0.008). A similar result was obtained by the ANOVA on the fourth cocaine treatment day [F(3, 40)=8.000; p<0.001], were hyperactivity was again seen in both cocaine groups relative to their saline control groups (sham: p<0.001; lesion p=0.003). A significant effect of group was also seen on the second saline treatment day [F(3, 40)=4.106; p=0.012], which was due to differences between the sham-cocaine and sham-saline groups (p=0.003). Also, on the third saline treatment day, an effect of group was revealed by the one-way ANOVA [F(3, 40)=4.390: p=0.009], which was due to increased activity in the sham-cocaine group as compared to the sham-saline group (p=0.001). No further significant effects were obtained.

Discussion

Previously it was reported that cocaine induced an increase of 5-HT and DA in the temporal and occipital cortex, as well as in the mPFC, EC, and perirhinal cortex (Müller et al., 2007b; 2007c; Pum et al., 2007). Such wide-spread neurochemical effects are consistent with the activation of multiple cortical brain areas in functional neuroimaging studies in humans (Breiter et al., 1997). Given that these are correlative observations, it is necessary to demonstrate a truly functional role of a certain metabolic or neurochemical alteration induced by a specific drug to ascribe to it any functional relevance (Kalivas, 2005). Considering the crucial role of 5-HT in the mediation of the behavioural effects of cocaine (Higgins and Fletcher, 2003; Müller and Huston, 2006; Müller et al., 2007a), we used local infusions of the serotonergic neurotoxin, 5,7-DHT, to deplete circumscribed cortical brain areas of 5-HT, thus, selectively reducing 5-HTergic neurotransmission in these structures. Depletion of 5-HT in the mPFC by 90% and in EC by 61% significantly attenuated cocaine-induced CPP, while a 78% depletion in the OccC had no effect. The serotonergic lesion of the mPFC also attenuated the hyperlocomotor effects of cocaine. In contrast, 5-HT depletion in neither EC nor OccC influenced the effects of cocaine on locomotor activity. This suggests multiple dissociations in the functional role of 5-HT in the cortex in cocaine-induced CPP and hyperlocomotor effects.

A role of the mPFC in the mediation of the conditioned preference for environments associated with psychostimulant drugs is well established (Tzschentke, 2007). Here, we extended previous findings that reported a blockade of the acquisition of CPP following excitotoxic lesions of the mPFC (Tzschentke and Schmidt, 1999), by showing that this function may be mediated by the 5-HTergic innervation of the mPFC. The observation that cocaine-induced hyperactivity was also attenuated, might suggest that the reduction of CPP may be due to a reduced effectiveness of cocaine as an unconditioned stimulus. Also, in human subjects, depletion of tryptophan, the precursor of 5-HT, reduced subjective ratings of "high" following intra nasal cocaine (Aronson et al., 1995), as well as cue-induced cocaine

craving (Satel et al., 1995). These results underscore a role of 5-HT in the unconditioned stimulus properties of cocaine. However, studies using self-administration techniques in 5,7-DHT-lesioned animals suggest an alternative interpretation. For example, Tran-Nguyen et al. (2001) found an increase in the effectiveness of a cocaine priming-injection in reinstating drug-seeking behaviour, and Roberts et al. (1994) found increased breakpoints for cocaine in a progressive ratio schedule of reinforcement, following global depletion of 5-HT in the brain (see also: Loh and Roberts, 1990). Both findings implicate an increase of the incentive value of cocaine, and, therefore, would suggest that 5-HT has an inhibitory role in cocaine-mediated reinforcement. Such an interpretation is also consistent with the reduction of cocaine-seeking under extinction conditions, and the increased latency to respond for cocaine following global 5,7-DHT lesions (Tran-Nguyen et al., 2001). The observation that cocaine-induced hyperactivity was also reduced in lesioned animals does not necessarily contradict the conclusion of reduced incentive value of cocaine induced by mPFC 5-HT depletion, because there appears to be a dissociation of cocaine's hyperlocomotor and rewarding effects under certain conditions (e.g. Jocham et al., 2006; Szumlinski et al., 2002). However, it could be that reduced locomotion during the conditioning trials with cocaine, contributed to a reduced CPP, because the lesioned animals showed less exploration, and, to that effect, reduced the association of environmental features to the effects of the drug. A serotonergic component for the hyperlocomotor response to cocaine is well established (Müller and Huston, 2006). The main source of 5-HT in the mPFC is the dorsal raphé nucleus (Vertes et al., 1999). Therefore, a role for the mPFC in cocaine-induced hyperactivity is consistent with the finding that intra-dorsal raphé injections of a 5-HT1A antagonist or agonist modulate this behaviour (Herges and Taylor, 1999; Szumlinski et al., 2004). Furthermore, a modulatory effect of prefrontal 5-HT receptors was suggested by Filip and Cunningham (2003), who found reduced cocaine-stimulated locomotion following infusion of a 5-HT2C agonist into the mPFC. The finding, that there was only a transient effect of mPFC 5-HT depletion on the hyperactivity response to

cocaine, suggests, that there may be differences in the mediation of acute versus chronic hyperlocomotor effects of this drug. This is supported by findings with global 5-HT depletions (Morrow and Roth, 1996) and local injection of a 5-HT1A agonist into the dorsal or medial raphé nuclei (Szumlinski et al., 2004), where differential effect of the treatments were evident following an acute or chronic injection of cocaine.

The role of prefrontal 5-HT may derive from the important interaction of local 5-HT receptors with the mesolimbic dopaminergic system. For example, a stimulatory role of 5-HT1A and 5-HT2A receptors in the mPFC on the ventral tegmental area (VTA) was reported in a series of recent studies combining in vivo microdialysis and electrophysiological techniques (Bortolozzi et al., 2005; Díaz-Mataix et al., 2005; Pehek et al., 2006). Reduced stimulation of prefrontal 5-HT receptors in animals with 5,7-DHT lesions following application of cocaine may result in modulated glutamatergic output from the mPFC to the VTA as compared to non-lesioned controls. A reduction of glutamatergic neurotransmission by intra-VTA injections of an AMPA and a NMDA antagonist was found to impair place conditioning for cocaine (Harris and Aston-Jones, 2003). Thus, the applied lesions might interfere with an important circuit within the brain's reward and motivation system (Wise, 2002), and, thus, reduce the incentive value of cocaine and/ or stimuli associated with the drug.

While the effects of 5-HT depletion of the mPFC is most likely due to the attenuation of the cocaine-induced attribution of incentive value to an environment, the reduction of CPP following depletion of the EC might be attributed to an impairment of drug-environment associations. This explanation is consistent with the role of the EC in learning and memory (Eichenbaum, 2000). Notably, the interaction between the medial temporal lobe and the amygdala was found to be essential for encoding of emotional memories in fMRI studies in humans (Dolcos et al., 2004; LaBar and Cabeza, 2006). These findings corroborated animal studies, which also argued for a role of the interaction between EC and amygdala in acquiring emotional memories (Roesler et al., 2002). More specifically, activation of the EC was also

evident during the presentation of cocaine-associated stimuli (Grant et al., 1996), which further supports the notion, that the attenuated CPP induced by 5-HT depletion of the EC was due to an impairment of drug-environment associations. It should be noted that the lesion procedure in this experiment also had some neurochemical effects in the OccC and hippocampus. While the negative findings from the OccC lesion experiment rule out that the attenuation of CPP following EC 5-HT depletion is due to the unintended reduction of 5-HT in the OccC, the reduced levels of DA in the hippocampus might contribute to impaired drug-context associations. Given the neurochemical results reported for the mPFC experiment, were no reduction of DA-levels in the lesioned cortex was evident, the reduction of DA in the hippocampus is unlikely to be due to diffusion of 5,7-DHT from the EC. Thus, this neurochemical effect was putatively caused by changed input from the EC, induced by depletion of 5-HT in this brain structure.

Previous in vivo microdialysis studies found that simple visual stimulation induced an increase of 5-HT in the OccC, that partly mimics the one following administration of cocaine (Müller et al., 2007b; Pum et al., 2008). Indeed, there is a significant role of visual stimuli in maintaining drug-related behaviour (Di Ciano and Everitt, 2004) and in relapse to drug-seeking (See, 2005). In human cocaine-dependent subjects, it was found that the degree of activation within the OccC during exposure to drug-related cues was one factor predicting treatment outcome (Kosten et al., 2006). Therefore, it was hypothesised, that a blockade of the cocaine-induced increase of 5-HT in the OccC would attenuate cocaine-induced CPP. However, no effects of 5,7-DHT lesions of the OccC on cocaine CPP were evident in the present study. The most likely explanation for this lack of effect is that in our apparatus, where animals are provided predominantly with proximal tactile cues, visual stimuli may have been less relevant for the distinction of the drug-paired and vehicle-paired side of the apparatus. Further studies with systematic variation of environmental features and lesioned brain area are needed to resolve this issue.

In summary, we found that 5,7-DHT lesions of the mPFC reduced CPP to a cocaine-associated environment as well as the unconditioned hyperactivity-provoking effects of cocaine. 5-HT depletion of the EC selectively reduced CPP, but not hyperactivity. Neither cocaine-induced CPP nor hyperactivity was affected by 5-HT lesions of the OccC. These findings suggest multiple dissociations in the role of cortical 5-HT in cocaine-induced CPP and hyperlocomotion.

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Figure and table legends

Figure 1: **A** Place-preference score (mean ± SEM) of rats with sham-lesions or 5,7-DHT-lesions (n=10-12/ group) of the medial prefrontal cortex. **B** Horizontal locomotion (cm; mean ± SEM) on the cocaine-treatment days during the conditioning phase. **C** Horizontal locomotion (cm; mean ± SEM) on the saline-treatment days during the conditioning phase. (* $p<0.05$ vs. saline-control).

Figure 2: **A** Place-preference score (mean ± SEM) of rats with sham-lesions or 5,7-DHT-lesions of the entorhinal cortex (n=9-11/ group). **B** Horizontal locomotion (cm; mean ± SEM) on the cocaine-treatment days during the conditioning phase. **C** Horizontal locomotion (cm; mean ± SEM) on the saline-treatment days during the conditioning phase. (* $p<0.05$ vs. saline-control).

Figure 3: **A** Place-preference score (mean ± SEM) of rats with sham-lesions or 5,7-DHT-lesions of the occipital cortex (n=10-12/ group). **B** Horizontal locomotion (cm; mean ± SEM) on the cocaine-treatment days during the conditioning phase. **C** Horizontal locomotion (cm; mean ± SEM) on the saline-treatment days during the conditioning phase. (* $p<0.05$ vs. saline-control).

Table 1: Concentration of dopamine (DA) and serotonin (5-HT) expressed as pg/ mg tissue (± SEM) in post-mortem samples of various brain areas of animals with sham lesions (n=21) or 5,7-DHT lesions (n=23) of the medial prefrontal cortex. PFC - medial prefrontal cortex, NAC - Nucleus Accumbens, EC - entorhinal cortex, OccC - occipital cortex (* $p<0.05$; *** $p<0.001$ vs. sham).

Table 2: Concentration of dopamine (DA) and serotonin (5-HT) expressed as pg/ mg tissue (± SEM) in post-mortem samples of various brain areas of animals with sham lesions (n=21) or 5,7-DHT lesions (n=20) of the entorhinal cortex. PFC - medial prefrontal cortex, NAC - Nucleus Accumbens, EC - entorhinal cortex, OccC - occipital cortex (* $p<0.05$; *** $p<0.001$ vs. sham).

Table 3: Concentration of dopamine (DA) and serotonin (5-HT) expressed as pg/ mg tissue (± SEM) in post-mortem samples of various brain areas of animals with sham lesions (n=21) of 5,7-DHT lesions (n=23) of the occipital cortex. PFC - medial prefrontal cortex, NAC - Nucleus Accumbens, EC - entorhinal cortex, OccC - occipital cortex (** $p<0.01$ vs. sham).

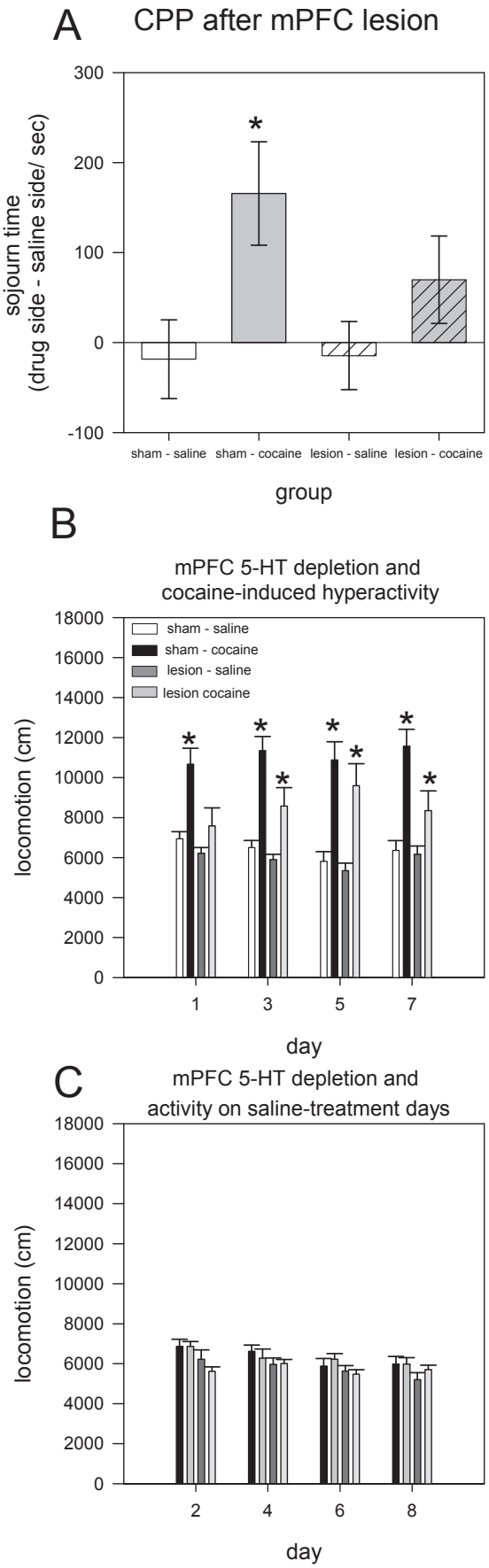

Figure 1

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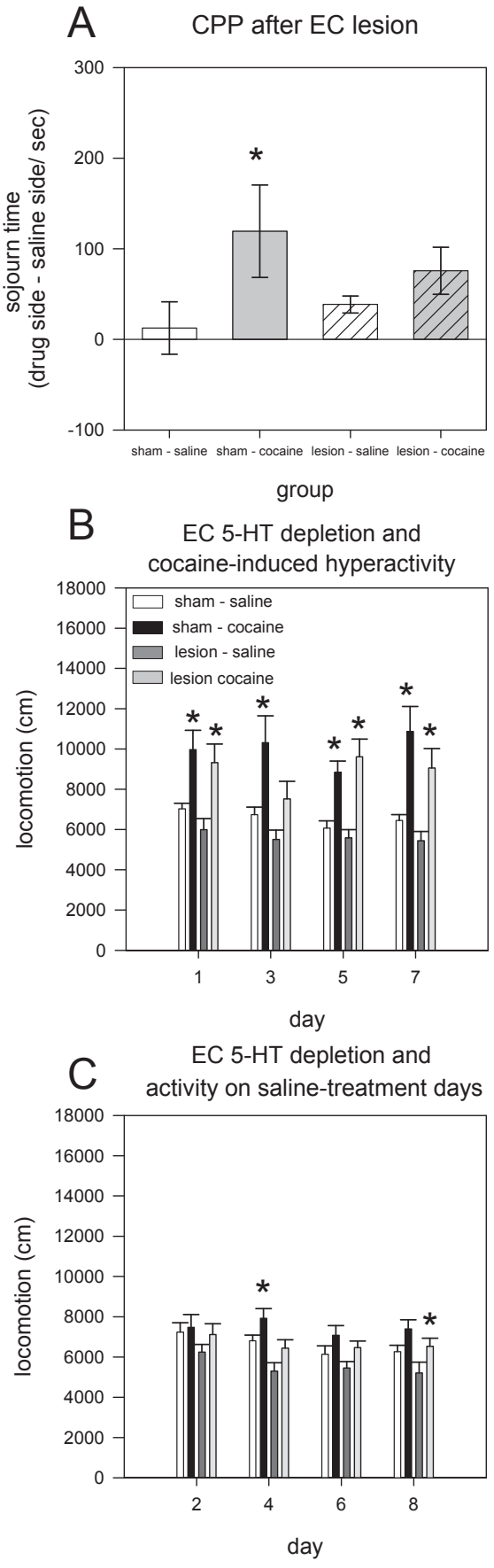

Figure 2

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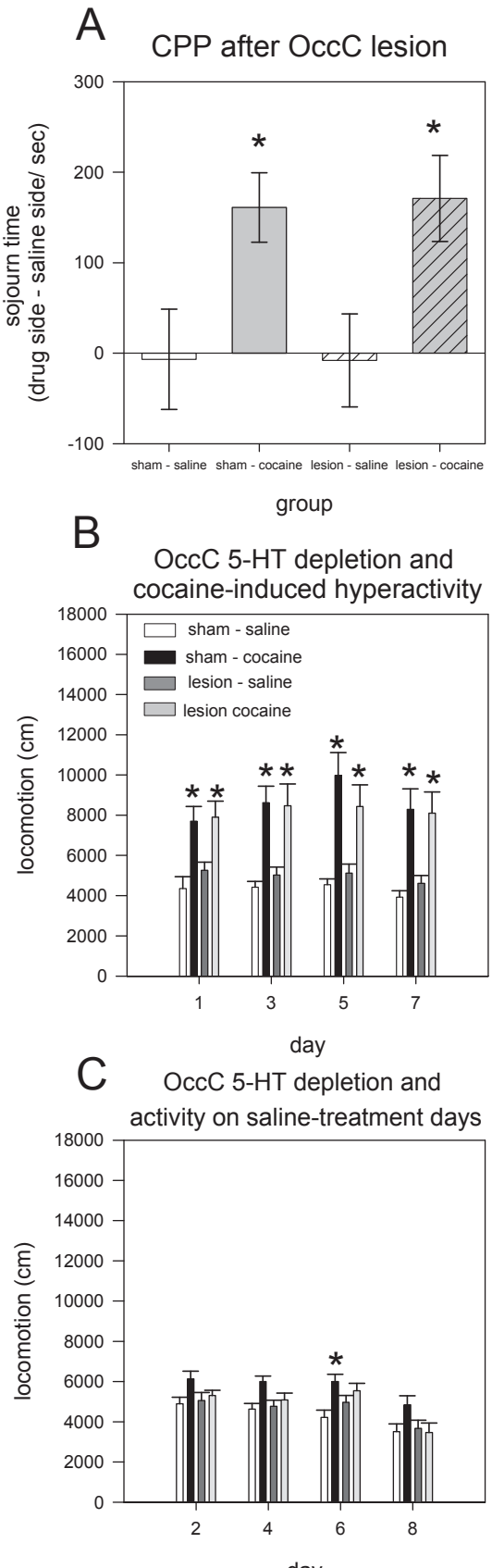

Figure 3

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		sham	lesion
PFC	DA	34.98 (± 18.64)	37.95 (± 18.25)
	5-HT	27.40 (± 5.01)	2.93 (± 0.58)***
NAC	DA	1461.44 (± 480.76)	602.84 (± 135.70)
	5-HT	177.07 (± 91.43)	83.99 (± 14.75)
Striatum	DA	688.76 (± 199.42)	569.68 (± 60.69)
	5-HT	30.74 (± 13.28)	43.78 (±13.82)
Hippocampus	DA	26.17 (± 8.58)	27.40 (± 9.02)
	5-HT	38.17 (± 7.00)	18.36 (± 2.17)*
EC	DA	64.37 (± 22.89)	42.47 (±12.76)
	5-HT	34.15 (± 8.27)	26.63 (± 4.64)
OccC	DA	95.16 (± 97.30)	85.66 (± 36.57)
	5-HT	15.69 (± 5.79)	11.66 (± 6.85)

		sham	lesion
PFC	DA	69.45 (±21.00)	46.60 (±21.20)
	5-HT	20.89 (±1.30)	28.80 (±5.00)
NAC	DA	674.30 (±92.80)	981.60 (±276.30)
	5-HT	157.60 (±31.80)	234.10 (±59.80)
Striatum	DA	1042.10 (±201.80)	1095.30 (±241.00)
	5-HT	97.40 (±19.20)	86.90 (±19.00)
Hippocampus	DA	111.19 (±30.88)	22.98 (±13.02)*
	5-HT	31.90 (±12.80)	14.70 (±3.00)
EC	DA	111.19 (±307.88)	10.60 (±3.00)
	5-HT	37.50 (±4.70)	14.20 (±1.80)***
OccC	DA	8.60 (±5.30)	9.60 (±5.80)
	5-HT	12.60 (±2.00)	7.70 (±1.00)*

		sham	lesion
PFC	DA	15.71 (± 1.90)	48.45 (± 26.31)
	5-HT	37.70 (± 3.04)	4865.18 (± 4803.95)
NAC	DA	1388.48 (± 216.70)	1058.21 (± 159.34)
	5-HT	245.78 (± 44.56)	259.64 (± 33.79)
Striatum	DA	871.50 (± 80.55)	862.59 (± 106.92)
	5-HT	101.58 (± 15.84)	110.88 (± 19.00)
Hippocampus	DA	8.92 (± 2.43)	27.14 (± 10.22)
	5-HT	70.94 (± 13.83)	147.22 (± 44.24)
EC	DA	81.40 (± 18.84)	80.46 (± 21.41)
	5-HT	322.10 (± 64.56)	254.73 (± 54.60)
OccC	DA	36.48 (± 15.63)	9.78 (± 4.11)
	5-HT	148.93 (± 39.08)	32.10 (± 5.15)**

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 02.07.2008

(Martin Pum)