Die Rolle von Glutamat bei der Expression von konditioniertem Belohnungsverhalten unter Berücksichtigung des Einflusses der Amygdala

Dissertation

der Fakultät für Biologie
der Eberhard-Karls-Universität Tübingen

zur Erlangung des Grades eines Doktors
der Naturwissenschaften

vorgelegt von
Volker Herzig
aus Leonberg
2004

Tag der mündlichen Prüfung:	6. August 2004
Dekan:	Prof. Dr. H.-U. Schnitzler
1. Berichterstatter:	Prof. Dr. W. J. Schmidt
2. Berichterstatter:	PD Dr. M. Fendt

Danksagung

Zu allererst möchte mich bei Prof. Dr. Werner J. Schmidt für die Überlassung des Themas und für die Doktorandenstelle bedanken. Danke auch für die angenehme Betreuung (einen besseren „Chef" kann ich mir nicht vorstellen) und die vielen anregenden Diskussionen.

Vielen Dank auch an PD Dr. Markus Fendt, der die Aufgabe des Zweitgutachters übernommen hat.

Ferner möchte ich mich bei Dr. Thomas Tzschentke für die Durchsicht einiger meiner Manuskripte und für seine kompetente Hilfe bei Fragen zur Platzpräferenz bedanken.

Des Weiteren bedanke ich mich bei Dr. Andreas Mayerhofer für die wissenschaftlichen und vor allem für die amüsanten Diskussionen.

Ein großer Dank geht außerdem an die gesamte Arbeitsgruppe der Abt. Neuropharmakologie für eine angenehme Arbeitsatmosphäre (mit Humor lassen sich abgelehnte Manuskripte halt doch am besten ertragen !!!) und für die diversen außerdienstlichen Aktivitäten (Videoabende, Grillparties, Squashen gehen, etc.).

Weiterhin danke ich Eleonora M. I. Capuani für die gute Zusammenarbeit während ihrer Lab Rotation.

Natürlich danke ich auch meinen Eltern für ihre Unterstützung während meiner gesamten Studienzeit.

Der größte Dank gebührt allerdings meiner Frau Inge Ruder, die wegen vieler Wochenendversuchstage des Öfteren auf mich verzichten musste.

Inhaltsverzeichnis

1 Einleitung

1.1 Das Belohnungssystem und Sucht 1

1.1.1 Natürliche Belohnung 1

1.1.2 Wirkung von Suchtmitteln 2

1.1.3 Sensitivierung 3

1.1.4 Konditionierte Stimuli („Cues“), Craving und Rückfälle 4

1.1.5 Einfluss von Glutamat 6

1.1.6 Rolle der Amygdala 7

1.2 Tiermodelle für Sucht 8

1.2.1 Modelle für Suchtmittel-induzierte Belohnung („primary reward“) 8

1.2.2 Modelle für konditionierte Belohnung („secondary reward“) 9

1.3 „State-dependency“ 10

1.4 Mikroinfusion 11

1.5 Fragestellung der Arbeit 12

2 Zusammenfassung der Ergebnisse der einzelnen Manuskripte

2.1 Manuskript 1 13

2.2 Manuskript 2 14

2.3 Manuskript 3 15

2.4 Manuskript 4 16

3 Diskussion

3.1 Einfluss von Anti-Craving-Substanzen auf CPP Expression 17

3.1.1 Acamprosat 17

3.1.2 Naloxon 17

3.1.3 Acamprosat + Naloxon (Kombination) 17

3.1.4 MPEP 18

3.2 Rolle der Amygdala bei Kokain-CPP Expression 20

3.3 Rolle von Glutamat bei CPP Expression 21

3.3.1 Amphetamin 21

3.3.2 Kokain 21

3.3.3 MDMA 22

3.3.4 Morphin 22

3.3.5 Natürliche Belohnung 23

4 **Literatur** 23

5 **Abkürzungen und Fachbegriffe** 29

6 **Erklärung zum Eigenanteil an den einzelnen Arbeiten** 30

7 **Lebenslauf** 31

8 **Anhang: einzelne Manuskripte** 33

Manuskript 1 35

eingereicht bei *Behavioural Brain Research*

Volker Herzig and Werner J. Schmidt: "Testing the effectiveness of anti-craving drugs acamprosate and naloxone on expression of morphine or cocaine conditioned place preference by applying a repeated-testing schedule in rats".

Manuskript 2 49

angenommen bei *Neuropharmacology*

Volker Herzig and Werner J. Schmidt: "Effects of MPEP on locomotion, sensitization and conditioned reward induced by cocaine or morphine".

Manuskript 3 65

eingereicht bei *Psychopharmacology*

Volker Herzig, Eleonora M.I. Capuani, Karl-Artur Kovar, Werner J. Schmidt: "Effects of the mGluR5 antagonist MPEP on expression of conditioned place preference to natural (food) or drug (amphetamine, MDMA) reward in rats".

Manuskript 4 79

eingereicht bei *Neuropharmacology*

Volker Herzig and Werner J. Schmidt: "Reduction of cocaine conditioned place preference expression in rats by temporal inactivation of central but not basolateral amygdala".

1 Einleitung

1.1 Das Belohnungssystem und Sucht

1.1.1 Natürliche Belohnung

Das Belohnungssystem im Gehirn von Säugetieren dient dazu, lebensnotwendige (Nahrungs- und Wasseraufnahme) sowie arterhaltende (Fortpflanzung) Vorgänge zu fördern, indem diese mit einer belohnenden Empfindung assoziiert werden. Diese Assoziation mit einem Belohnungswert führt dazu, dass das jeweilige Verhalten wiederholt ausgeführt wird, man spricht von einer positiven Verstärkung („positive reinforcement“). Als „Reinforcer“ wird dabei jedes Ereignis definiert, das die Wahrscheinlichkeit einer Verhaltensantwort verstärkt (Koob & Nestler 1997). Natürliche Belohnungsvorgänge unterliegen ferner Kontrollmechanismen, welche diese Verhaltensweisen beenden, sobald kein Bedürfnis mehr besteht (d.h. ein Individuum nimmt z.B. keine Nahrung mehr auf, wenn es satt ist). Bei Belohnungsprozessen unterscheidet man im Allgemeinen zwei Phasen, an denen unterschiedliche Neurotransmittersysteme beteiligt sind. Die Annäherungsphase (z.B. Futtersuche) führt zu einer Aktivierung des Dopamin- (DA) Systems. In der anschließenden konsumatorischen Phase (z.B. Verzehr der Nahrung) geht die DA-Aktivität wieder zurück und das endogene Opiatsystem wird aktiviert (Schmidt 1997). Für das Belohnungssystem im Gehirn spielen vor allem zwei Strukturen eine wichtige Rolle, der Nucleus accumbens (Nac) und das ventrale tegmentale Areal (VTA). Ein natürlicher Belohnungsreiz führt nun dazu, dass dopaminerge Neurone, die vom VTA zum Nac projizieren, verstärkt DA im Nac freisetzen. Den selben Effekt erzielen endogene Opiate (Endorphine), indem sie an Interneurone im VTA binden, welche den hemmenden Neurotransmitter Gammaaminobuttersäure (GABA) verwenden, und so deren hemmende Wirkung auf die dopaminergen Projektionsneurone reduzieren. Eine verstärkte DA-Ausschüttung im Nac führt schließlich zu einer Hemmung der GABAergen Projektionsneurone des Nac, welche in das ventrale Pallidum projizieren, was als belohnend empfunden wird.

1.1.2 Wirkung von Suchtmitteln

Missbrauchsdrogen (fortan als Suchtmittel bezeichnet) binden ebenfalls an verschiedene Strukturen des Belohnungssystems und bewirken, dass die Belohnung von der Ausführung der eigentlich zu belohnenden Vorgänge entkoppelt wird. Mit anderen Worten werden normale Verhaltensweisen sowie motivationelle Prozesse durch Suchtmittel kurzgeschlossen (Robbins & Everitt 1999). Im Gegensatz zu natürlicher Belohnung gibt es ferner bei Suchtmittel-induzierter Belohnung keine Kontrollmechanismen, die zu einer Beendigung der Suchtmitteleinnahme führen würden. Ein weiterer wichtiger Aspekt bei Suchtmitteln ist, dass sie sowohl Toleranz als auch Sensitivierung auslösen können. Dabei kann das gleiche Suchtmittel durch wiederholte Gabe bei einem Verhaltensparameter Toleranz bewirken, während ein anderes Verhalten im gleichen Individuum sensitiviert (Hyman & Malenka 2001). Man nimmt an, dass sich das unwiderstehliche Verlangen nach dem Suchtmittel (Craving) bei wiederholtem Konsum analog zu einem Sensitivierungsprozess (siehe auch 1.1.3) steigert und so zu einem Kontrollverlust führen kann (Wolffgramm & Heyne 1995; Koob & Le Moal 1997). Trotz ernsthafter negativer Konsequenzen der Suchtmitteleinnahme (Hyman & Malenka 2001) entwickelt sich ein zwanghaftes Gewohnheitsverhalten (Everitt *et al.* 2001), welchem als Hauptantrieb die Beschaffung und Einnahme des Suchtmittels zu Grunde liegt (Koob & Nestler 1997). Obwohl verschiedene Suchtmittel an unterschiedlichen Strukturen des Belohnungssystems angreifen, bewirken sie in der Regel ebenfalls eine Hemmung der GABAergen Projektionsneurone des Nac und erzeugen somit eine belohnende Wirkung. Kokain z.B. blockiert die Wiederaufnahme von DA in die Präsynapse, so dass sich die DA-Konzentration im synaptischen Spalt erhöht. Ein erhöhter DA-Spiegel im Nac erklärt somit die belohnende Wirkung von Kokain. Es wurde gezeigt, dass schon eine einmalige Gabe von Kokain synaptische Plastizität in Neuronen des VTA bewirken kann, die über mehrere Tage anhält (Ungless *et al.* 2001). Amphetamin wirkt ähnlich wie Kokain, außer dass nicht nur die DA-Wiederaufnahme blockiert wird, sondern noch zusätzlich der DA-Wiederaufnahme Transporter umgekehrt wird. Die Partydroge Ecstasy (3,4-Methylendioxymethylamphetamin, MDMA) hingegen blockiert zwar ebenfalls die DA-Wiederaufnahme, ihr Hauptwirkungsmechanismus liegt aber in der Blockade der Serotonin-Wiederaufnahme (zur Übersicht siehe Morgan 2000). Opiate (z.B. Heroin oder Morphin) wirken in gleicher Weise wie die Endorphine (siehe 1.1.1) und führen über eine Hemmung GABAerger Interneurone im VTA zu einer verstärkten DA-Freisetzung im Nac und somit zu einer Hemmung GABAerger Projektionsneurone des Nac.

1.1.3 Sensitivierung

Die Sensitivierung ist ein Phänomen, das bei wiederholter Gabe von Suchtmitteln beobachtet werden kann. Unter Sensitivierung versteht man die progressive Verstärkung eines Verhaltens nach wiederholter Applikation einer Substanz, man spricht daher auch von „umgekehrter Toleranz“ (Koob & Nestler 1997). Sensitivierung kann bei unterschiedlichen Verhaltensparametern auftreten. Sowohl die durch ein Suchtmittel induzierte Lokomotion, als auch die durch ein Suchtmittel erzeugte Belohnung unterliegen Sensitivierungsprozessen (Carlezon *et al.* 1997). Aber auch nach wiederholter Gabe von Haloperidol, einem DA-D2-Antagonisten welcher als Tiermodell für die Parkinsonkrankheit eingesetzt wird, kann man eine progressive Verstärkung der Katalepsie beobachten (z.B. Klein & Schmidt 2003; Amtage & Schmidt 2003), d.h. die behandelten Ratten bewegen sich im Gegensatz zur Sensitivierung mit Suchtmitteln immer weniger. Obwohl Sensitivierung bisher hauptsächlich in Tierexperimenten nachgewiesen wurde, gibt es auch Hinweise auf eine psychomotorische Sensitivierung beim Menschen (Strakowski *et al.* 1996). Sensitivierung ist für das Verständnis von Sucht besonders interessant, da es sich bei Sensitivierung (in Analogie zur Sucht) um eine sehr lang anhaltende, wenn nicht sogar permanente Veränderung handelt. Ferner geht man davon aus, dass an der Sensitivierung zumindest teilweise Strukturen des Belohnungssystems beteiligt sind. So zeigte sich, dass die Entwicklung der Sensitivierung vor allem über das VTA gesteuert wird, während der Nac entscheidend für die Expression der Sensitivierung ist (zur Übersicht siehe Tzschentke & Schmidt 2003). In einem sehr einflussreichen Übersichtsartikel stellten Robinson und Berridge (1993) ihre „incentive-sensitization“-Theorie der Sucht vor. Danach entsteht eine Sucht dadurch, dass die motivierenden Anreize („incentive salience“), welche das Suchtmittel selbst und die damit assoziierten Stimuli darstellen, über Sensitivierungsprozesse immer stärker werden und schließlich zu einem unwiderstehlichen Verlangen (Craving) nach der Droge führen.

1.1.4 Konditionierte Stimuli („Cues“), Craving und Rückfälle

Stimuli, welche mit der Wirkung eines Suchtmittels assoziiert wurden (Cues), erlangen über klassische (Pavlovsche) Konditionierungsmechanismen (siehe 1.2.2) selbst belohnende Eigenschaften. Dabei stellt das Suchtmittel den unkonditionierten Stimulus dar, während die Cues den konditionierten Stimulus darstellen. Cues können sowohl diskrete (z.B. Lichtreiz, Ton, Geruch), als auch kontextuelle (z.B. Umgebung) Stimuli sein. Sowohl Sensitivierungs- (Bell & Kalivas 1996; Amtage & Schmidt 2003), als auch Belohnungsprozesse (Hotsenpiller *et al.* 2001) werden durch Cues beeinflusst. Suchtmittel-Cues können bei entwöhnten Abhängigen das Verlangen nach dem Suchtmittel (Craving) steigern und so einen Rückfall auslösen (Childress *et al.* 1999; Ciccocioppo *et al.* 2001). Weitere Auslöser für Rückfälle sind z.B. das Suchtmittel selbst (Mueller & Stewart 2000; McFarland & Kalivas 2001), Stress (Erb *et al.* 1996; Shaham *et al.* 2000) oder mit Stress assoziierte Stimuli (Sanchez & Sorg 2001) sowie eine elektrische Reizung des Hippocampus (Vorel *et al.* 2001). Häufig wiederkehrende Rückfälle stellen eines der größten Probleme bei der Therapie der Suchtmittelabhängigkeit dar, weshalb Sucht auch als chronische Rückfallskrankheit bezeichnet wurde (Leshner 1997). Die Bedeutung kontextueller Cues für die Rückfälligkeit wird besonders deutlich am Beispiel amerikanischer Vietnamveteranen, die während des Vietnamkrieges opiatabhängig wurden, nach ihrer Rückkehr in die Heimat diese Abhängigkeit aufgrund des anderen Umfeldes aber nicht mehr zeigten (Robins *et al.* 1974). Besonders problematisch bei Cues ist, dass sie nicht immer bewusst wahrgenommen werden (Helmuth 2001) und somit auch nicht aktiv gemieden werden können. Zwar zeigte sich, dass die Präsentation von Suchtmittel-Cues zu einem raschen Anstieg der DA-Freisetzung in Nac führt (Ito *et al.* 2000; Phillips *et al.* 2003). Ein durch die Präsentation Heroin-assoziierter Stimuli ausgelöster Rückfall war jedoch trotz pharmakologischer Blockade von DA-Rezeptoren zu beobachten und somit unabhängig von DA (McFarland & Ettenberg 1997). Daher wird vermutet, dass noch andere Neurotransmitter an Cue-induzierten Rückfällen beteiligt sein müssen, wobei Glutamat (Glu) als ein wichtiger Kandidat gehandelt wird (siehe 1.1.5).

Bezüglich der Entstehung von Craving und seiner Beteiligung an Rückfällen gibt es verschiedene theoretische Modelle. Nach der **„incentive sensitization“-Theorie** von Terry Robinson und Kent Berridge (1993) lässt sich die Zunahme des Cravings wie bereits (in 1.1.3) beschrieben über eine Sensitivierung des Suchtmittelverlangens („drug wanting“) erklären. Eine ähnliche Erklärung für die Zunahme von Craving liefert auch das **„reward-allostasis“-Konzept** aus der Arbeitsgruppe von George Koob (siehe z.B. Ahmed *et al.* (2002)), wobei der hedonische Wert eines Suchtmittel aufgrund einer (vermutlich auf Sensitivierungsmechanismen beruhenden) Abnahme in der Sensitivität des Belohnungssystems verringert wird. Dies führt dann wiederum zu einem hedonischen Defizit, das als Ursache von Craving und gesteigerter Anfälligkeit für Rückfälle angesehen werden kann. Ein Vorteil des „reward-allostasis“-Konzeptes ist, dass es auch das Auftreten von

Toleranz erklären kann: die zunehmende Verringerung der Sensitivität des Belohnungssystems erfordert nämlich eine gesteigerte Dosis des Suchtmittels um die gleichen Effekte zu erreichen (ohne dass sich dabei die Wirkungsweise des Suchtmittels verändert). In den bisher vorgestellten Theorien, die beide auf den Ergebnissen tierexperimenteller Arbeiten beruhen, ist Craving die Ursache für die darauf folgende, erneute Einnahme des Suchtmittels. Es gibt jedoch noch andere theoretische Modelle, in denen kein oder nur ein geringer Zusammenhang zwischen Craving und Rückfällen besteht. Nach dem **„cognitive processing"-Modell** aus der Arbeitsgruppe des Psychologen Steve Tiffany verläuft die Suchtmitteleinnahme unabhängig von dem Prozess, der Craving kontrolliert (Tiffany & Carter 1998; Tiffany & Conklin 2000). In diesem Modell wird die zwanghafte Suchtmitteleinnahme als eine Form von automatisierter Gewohnheit („habit") angesehen, die sich während der wiederholten Suchtmitteleinnahme entwickelt. Craving hingegen ist (im Gegensatz zur Suchtmitteleinnahme) ein nicht-automatischer Prozess, der aktiviert wird um unterbrochenen Suchtmittelkonsum zu vervollständigen oder um automatisierte Suchtmitteleinnahmesequenzen zu blockieren. Damit ist Craving weder zentral noch irrelevant für Suchtmitteleinnahme, sondern fungiert als ein „kognitiver Marker" von Prozessen, die (manchmal) mit der Suchtmittelsuche und- einnahme assoziiert sein können (Tiffany & Conklin 2000). Ein ganz anderer Ansatz aus der Ökonomie zur Erklärung von Sucht und Craving stammt von Gene Heyman, dessen Theorie auf Analogien zwischen Alkoholismus und der ökonomischen Analyse von Konsumverhalten basiert. Diese **„ökonomische"-Theorie** (Heyman 2000) besagt, dass das Suchtmittel bei Abhängigen einer unelastischen Nachfrage („inelastic demand") unterliegt, d.h. eine Steigerung des Preises (also der Anzahl an Hebeldrücken pro Alkoholdosis) steht in keinem linearen Zusammenhang zum Konsumverhalten. Als Grund für Sucht und Rückfälle wird nicht ein unstillbares Verlangen (also Craving), sondern insbesondere das Fehlen einer „substituierbaren Ware" angesehen. Dies bedeutet, dass es möglich sein sollte Bedingungen zu schaffen, die zu einer Beendigung des Suchtmittelmissbrauchs führen können. Als Therapiekonzept wird daher vorgeschlagen Verhaltensweisen zu stärken, die das Suchtmittel substituieren (also z.B. alle Arten von belohnendem Verhalten), und parallel dazu noch die positiven Wirkungen des Suchtmittels pharmakologisch zu blockieren. Welche der vorgestellten Theorien nun am besten die Zusammenhänge zwischen Rückfällen und Craving beschreibt, ist noch nicht abschließend geklärt. Die Beantwortung dieser Frage ist derzeit Gegenstand einer Diskussion auf dem Gebiet der Suchtforschung.

1.1.5 Einfluss von Glutamat

Erst im letzten Jahrzehnt wurde allmählich klar, dass DA alleine nicht alle Effekte erklären kann, die man nach wiederholter Suchtmittelgabe beobachtet. Es stellte sich heraus, dass Glu eine ebenso wichtige Rolle bei Sensitivierungs- und Belohnungsprozessen spielt wie DA (zur Übersicht siehe Tzschentke & Schmidt 2003). Glu scheint dabei insbesondere für die lang andauernden Effekte von Suchtmitteln von entscheidender Bedeutung zu sein (Kalivas 2004). In einer Übersichtsarbeit verdeutlichten Tzschentke und Schmidt (2003), dass der Einfluss von Glu zu einem großen Teil auf die Modifikation dopaminerger Neurotransmission zurückzuführen ist. Einerseits kann Glu die DA-Transmission modulieren, andererseits kann aber auch DA die Glu-Transmission beeinflussen. So führt eine erhöhte Glu-Freisetzung im Nac oder VTA zu einer gesteigerten DA-Ausschüttung im Nac. Eine erhöhte DA-Freisetzung in den kortikalen Ursprungsgebieten der glutamatergen Neurone (präfrontaler Kortex (PFC), Amygdala oder Hippocampus) führt wiederum zu einer gesteigerten Aktivität der glutamatergen Projektionsneurone und somit zu verstärkter Glu-Freisetzung im Nac und VTA. Allerdings zeigen aktuelle Ergebnisse, dass der über metabotrope Glu-Rezeptoren des Subtyps 5 (mGluR5) gesteuerte Einfluss von Glu auf Kokain-induziertes Verhalten unabhängig von dopaminerger Transmission ist (Chiamulera *et al.* 2001), wobei der genaue Wirkungsmechanismus bisher noch nicht bekannt ist.

Bezüglich der Beteiligung von Glu an Sensitivierungsprozessen konnte bisher gezeigt werden, dass zwischen der Glu-Freisetzung im Nac und der Kokain-induzierten Sensitivierung ein Zusammenhang besteht (Pierce *et al.* 1996; Reid & Berger 1996; Shippenberg *et al.* 2000). Außerdem führt eine durch Kokain induzierte Sensitivierung zu Veränderungen in der Dichte glutamaterger Synapsen in verschiedenen Arealen des Belohnungssystems (Kozell & Meshul 2001). Ferner induziert die wiederholte Gabe von Kokain ein verändertes Expressionsmuster von N-Methyl-D-Aspartat (NMDA)- und Alpha-Amino-3-Hydroxy-5-Methyl-4-Isoxazol-Propionsäure (AMPA)-Subtypen ionotroper Glu-Rezeptoren (Ghasemzadeh *et al.* 1999). Wie bereits erwähnt (siehe 1.1.4) sind auch kontextabhängige Mechanismen an Kokain-induzierter Sensitivierung beteiligt, wobei AMPA-Glu-Rezeptoren eine wichtige Rolle spielen (Bell & Kalivas 1996; Carlezon *et al.* 1997; Carlezon & Nestler 2002).

Die Beteiligung von Glu bei konditionierten Belohnungsprozessen wurde sowohl für deren Entwicklung als auch für deren Expression gezeigt (Slusher *et al.* 2001). Beim Erlernen einer Assoziation zwischen einer belohnenden Kokainwirkung und kontextuellen Stimuli spielt Glu im VTA eine entscheidende Rolle (Harris & Aston-Jones 2003), wobei bereits eine einzige Gabe von Kokain die Glu-Transmission im VTA fünf Tage lang erhöht (Ungless *et al.* 2001). Insbesondere NMDA-Rezeptoren scheinen an der Entwicklung von Suchtmittel-konditioniertem Verhalten beteiligt zu sein (Tzschentke & Schmidt 1997; Di Ciano *et al.* 2001), während bei der Expression vermutlich AMPA-Rezeptoren eine wichtigere Rolle

spielen (Cornish & Kalivas 2000; Di Ciano *et al.* 2001; Hotsenpiller *et al.* 2001). Erst neuere Studien konnten nachweisen, dass auch mGluR5 an suchtrelevanten Prozessen beteiligt sind (Chiamulera *et al.* 2001). Des Weiteren wurde gezeigt, dass Kokain-assoziierte Stimuli einen direkten Einfluss auf die Glu-Freisetzung im Nac haben (Hotsenpiller *et al.* 2001), was von Bedeutung ist, da die Präsentation solcher Stimuli schon ausreicht um Rückfälle auszulösen (siehe 1.1.4). Zusätzlich zu seiner Beteiligung an konditionierten Belohnungsprozessen spielt Glu aber auch eine Rolle bei Kokain-induziertem Reinstatement, wobei selektiv die Glu-Freisetzung im Nac core erhöht wird (McFarland *et al.* 2003). Dieser Effekt scheint jedoch spezifisch für Suchtmittel-induzierte Rückfälle zu sein, da in der gleichen Studie keine Änderung der Glu-Freisetzung bei Futter-induziertem Reinstatement beobachtet wurde.

1.1.6 Rolle der Amygdala

Verschiedene Hirnareale senden glutamaterge Projektionen in den Nac und das VTA und können dadurch die Funktion des Belohnungssystems regulieren. Umgekehrt erhalten diese Areale wiederum DA-Projektionen aus dem VTA, was eine gegenseitige Modulation ermöglicht (siehe 1.1.5 und Tzschentke & Schmidt 2003). Glutamaterge Eingänge kommen z.B. vom PFC (Carr & Sesack 2000), dem Hippocampus (Vorel *et al.* 2001) oder der Amygdala (Winnicka & Wisniewski 1999). Die Amygdala spielt eine bedeutende Rolle bei emotionalem Verhalten sowie bei der Bewertung („reward evaluation/devaluation“) von Belohnungsvorgängen (zur Übersicht siehe Holland & Gallagher 1999; See 2002; Everitt *et al.* 2003; See *et al.* 2003). Sie stellt ferner einen Schlüsselregulator für die Assoziation zwischen einem Stimulus und einem belohnenden Reiz dar (See 2002). Nach Präsentation von Kokain-assoziierten Cues zeigten Drogenkonsumenten eine erhöhte Aktivität der Amygdala (Grant *et al.* 1996; Childress *et al.* 1999). Die Amygdala spielt also insbesondere bei sekundären (konditionierten) Belohnungsmechanismen eine wichtige Rolle (Grimm & See 2000). Bestimmte Subareale innerhalb der Amygdala, wie z.B. die zentrale (CeA) oder die basolaterale (BLA) Amygdala, weisen unterschiedliche Funktionen bei Suchtmittel-konditioniertem Verhalten auf (Parkinson *et al.* 2000; Kruzich & See 2001; Fuchs *et al.* 2002). Es wird diskutiert, dass die BLA eher für die Verarbeitung einzelner („discrete“) Cues zuständig ist, wohingegen die CeA an der Verarbeitung kontextueller („predictive“) Cues beteiligt ist (See 2002).

1.2 Tiermodelle für Sucht

Die Tiermodelle für Sucht unterscheidet man in Modelle zur Bestimmung der direkten Suchtmittelwirkung sowie in Modelle zur Bestimmung der Wirkung von Stimuli, welche mit der Suchtmittelwirkung assoziiert wurden und somit selbst belohnende Eigenschaften erlangen.

1.2.1 Modelle für Suchtmittel-induzierte Belohnung („primary reward“)

Diese Modelle werden hauptsächlich eingesetzt um festzustellen, ob eine Substanz eine belohnende Wirkung, und damit vermutlich auch ein Suchtpotential, aufweist.

Intrakranielle Selbststimulation (ICSS)

Dieses Suchtmodell geht zurück auf die Studien von Olds und Milner (1954), welche zur Entdeckung des Belohnungssystems führten. In diesem Versuch wurden Ratten Elektroden implantiert und anschließend gab man ihnen die Möglichkeit, sich in den betreffenden Hirnarealen über Hebeldrücke selbst elektrisch zu reizen. Die Bereiche, welche dann dem Belohnungssystem zugeordnet wurden (u.a. der laterale Hypothalamus und der Nac), führten zu einer exzessiven Reizung unter Vernachlässigung aller anderen Bedürfnisse. Die ICSS beruht auf den Prinzipien der operanten (oder auch instrumentellen, Skinnerschen) Konditionierung, d.h. die Ratte lernt ihre Aktion (hier: Hebeldruck) mit der Reaktion (hier: elektrische Reizung, die als belohnend wahrgenommen wird) zu assoziieren und führt die Aktion dann wiederholt aus. Als Suchtmodell ist die ICSS interessant, weil Suchtmittel die belohnende Wirkung der ICSS noch verstärken (zur Übersicht, siehe Wise & Kelsey 1994; Wise 1996). Aus einer Verstärkung der ICSS kann man also auf eine belohnende Wirkung der getesteten Substanz schließen.

Suchtmittel-Selbstadministration (SA)

Auch die Suchtmittel-SA beruht hauptsächlich auf operanter Konditionierung, im Gegensatz zur ICSS erhält das Versuchstier aber keine elektrischen Reizungen, sondern es kann sich durch Hebeldruckverhalten ein Suchtmittel selbst zuführen (meist über einen implantierten Katheter direkt in den Blutkreislauf). Eine Substanz, welche aktivierend auf das Belohnungssystem wirkt, führt dann zu einer Wiederholung des Hebeldruckverhaltens („positive reinforcement“), ganz analog zur elektrischen Reizung von Arealen des Belohnungssystems bei der ICSS. Umgekehrt kann man also daraus schließen, dass eine Substanz vermutlich belohnend wirkt (und somit auch ein Missbrauchspotential aufweist), wenn sie SA-Verhalten auslöst.

1.2.2 Modelle für konditionierte Belohnung („secondary reward“)

Diese Modelle werden hauptsächlich eingesetzt, um den Einfluss konditionierter Stimuli auf Suchtmittelsuchverhalten („drug-seeking“) zu untersuchen.

Suchtmittel-SA

Mit dem Modell der Suchtmittel-SA lässt sich, außer der direkten Suchtmittelwirkung („primary reward“, siehe 1.2.1), auch konditionierte Belohnung („secondary reward“) untersuchen (zur Übersicht siehe Everitt & Robbins 2000). Bei konditionierter Belohnung wird ein bestimmter Stimulus (z.B. Licht) mit der Suchtmittelgabe assoziiert. Es handelt sich dabei also um eine klassische (oder Pavlovsche) Konditionierung. Nach erfolgter Assoziation reicht schon die alleinige Präsentation dieses Stimulus, um Suchtmittelsuchverhalten oder Hebeldruckverhalten auszulösen. An der Suchtmittel-SA sind somit sowohl operante (Hebeldruckverhalten) als auch klassische (Assoziation zu einem Stimulus) Konditionierungsmechanismen beteiligt. Dies könnte auch eine Erklärung dafür sein, weshalb trotz vieler Übereinstimmungen von Ergebnissen aus SA- und Platzpräferenz- (siehe unten) Studien geschlussfolgert wurde, dass beide Modelle grundsätzlich unterschiedliche Lernprozesse messen (Bardo & Bevins 2000).

Konditionierte Platzpräferenz (CPP)

Die CPP beruht vor allem auf dem Prinzip der klassischen Konditionierung, d.h. sie dient insbesondere der Untersuchung sekundärer (konditionierter) Belohnungsmechanismen. Dazu wird in einer (meist) zweigeteilten Platzpräferenz-Box ein Kompartiment mit der Gabe des Suchtmittels gepaart, während das andere Kompartiment z.B. mit der Gabe von physiologischer Kochsalzlösung (Saline) gepaart wird. Lässt man den Tieren nun in einem abschließenden Test die freie Wahl zwischen beiden Kompartimenten, so halten sie sich längere Zeit in dem Kompartiment auf, das mit dem Suchtmittel assoziiert wurde (für eine genauere Beschreibung dieses Modells siehe Tzschentke 1998): Man sagt, die Tiere haben eine konditionierte Platzpräferenz („conditioned place preference“ = CPP) entwickelt. Von den meisten Suchtmitteln (z.B. Heroin, Morphin, Kokain, Amphetamin, MDMA) ist bekannt, dass sie eine CPP induzieren können (zur Übersicht siehe Tzschentke 1998). Im Umkehrschluss (analog zur SA) kann man davon ausgehen, dass eine Substanz vermutlich belohnend wirkt (und somit auch ein Missbrauchspotential aufweist), wenn sie eine CPP erzeugt. Ein Vorteil dieser Methode ist, dass auch Substanzen untersucht werden können, welche aversive Eigenschaften aufweisen. Nach einer Konditionierung mit solch einer Substanz wird sich das Tier länger in dem Kompartiment aufhalten, welches nicht mit der aversiven Substanz gepaart wurde, es zeigt sich eine so genannte konditionierte Platzaversion („conditioned place aversion“ = CPA).

Bei der Untersuchung von Platzpräferenz unterscheidet man zwischen Entwicklung („development“, „acquisition“) und Expression („expression“, „performance“) einer CPP. Zur Untersuchung der Entwicklung einer CPP wird ein Suchtmittel während der Konditionierung immer zusammen mit einer Testsubstanz verabreicht. Falls sich im anschließenden drogenfreien („undrugged“) Test keine CPP zeigt, hat die Testsubstanz die Entwicklung einer CPP verhindert (sofern keine „State-dependency“ vorliegt, siehe 1.3). Um die Expression einer CPP zu untersuchen, testet man nach erfolgter Konditionierung mit dem Suchtmittel unter dem Einfluss einer Testsubstanz, ob das Abrufen des konditionierten Verhaltens verändert ist. Um Substanzen (z.B. Anti-Craving-Substanzen) zu testen, die später bei Suchtmittelabhängigen (die also bereits eine Abhängigkeit entwickelt haben) wirken sollen, untersucht man folglich ihre Wirkung auf die Expression einer Suchtmittel-CPP.

Ein weiterer Vorteil der Platzpräferenz-Methode ist die gleichtzeitige Messung von Platzpräferenz und Lokomotion. Dadurch können bestimmte Effekte, welche bei der CPP beobachtet werden, eventuell auf Änderungen im Lokomotionsverhalten zurückgeführt werden (z.B. bei Substanzen mit sedierender Wirkung). Somit werden auch Nebenwirkungen der Testsubstanzen erfasst, die ihre mögliche Verwendung einschränken könnten. Ferner zeigt sich während der Konditionierungphase auch, ob eine Substanz die Entwicklung einer sensitivierten Lokomotion bewirkt (siehe 1.1.3).

1.3 „State-dependency“

Die „State-dependency“ ist ein Phänomen, welches bei Lernvorgängen z.B. mit NMDA-Antagonisten beobachtet werden kann (Jackson *et al.* 1992). Man versteht darunter, dass ein bestimmtes Verhalten, wenn es unter dem Einfluss einer bestimmten Substanz erlernt wurde, auch nur in demselben pharmakologischen Zustand wieder abgerufen werden kann. „State-dependency“-Effekte werden z.B. bei Sensitivierungsprozessen (siehe 1.1.3) beobachtet (Carlezon *et al.* 1995; Lanis & Schmidt 2001, Tzschentke & Schmidt 1998a). In Platzpräferenz-Studien zeigt sich eine „State-dependency“, wenn man im drogenfreien Test keine CPP-Expression beobachtet, bei einem Test unter dem Einfluss der Konditionierungssubstanz aber eine CPP Expression gefunden wird. Bei der Untersuchung, ob die Gabe einer Substanz die Entwicklung einer CPP (CPP development) beeinflusst, sollte daher zusätzlich zu dem drogenfreien Test immer ein Test unter dem Einfluss der Testsubstanz („drugged“) erfolgen, um eventuelle „State-dependency“-Effekte auszuschließen.

1.4 Mikroinfusion

Um eine gezielte Behandlung von Sucht zu erreichen, ist es unerlässlich, die unterschiedlichen Funktionen der daran beteiligten Gehirnareale genauer zu verstehen. Eine Methode (neben Mikrodialyse- oder Läsionsstudien), die dies ermöglicht, ist die Mikroinfusion. Dabei werden den Versuchstieren anhand vorgegebener Koordinaten gezielt Infusionskanülen in bestimmte Gehirnareale implantiert. Durch Infusion eines Agonisten oder Antagonisten kann dann im frei beweglichen, nicht narkotisierten Tier die Rolle verschiedener Neurotransmitter in dem jeweiligen Gehirnareal bei unterschiedlichen Verhaltensweisen untersucht werden. Durch die Verwendung mehrerer Infusionskanülen in verschiedenen Zielgebieten kann mit dieser Methode z.B. auch geklärt werden, ob bestimmte Gehirnareale mit anderen interagieren und welche Neurotransmitter dabei eine Rolle spielen. Aufgrund der Wirkung von GABA als wichtigstem inhibitorischen Transmitter des Gehirns kann man ferner über die Infusion eines Gemisches der GABA-A- und GABA-B-Rezeptor Agonisten Muscimol und Baclofen gezielt bestimmte Gehirnareale inaktivieren (siehe McFarland & Kalivas 2001; McFarland *et al.* 2003). Vorteilhaft gegenüber permanenten Läsionen ist, dass diese Inaktivierung nur temporär ist, d.h. sie verschwindet nach dem Ende der Substanzwirkungen der GABA-Agonisten wieder. Daher eignet sich diese Mikroinfusionsmethode besonders gut zur gezielten Untersuchung der Expression eines Verhaltens (z.B. der Platzpräferenz, siehe 1.2.2), ohne dessen Entwicklung zu beeinflussen.

1.5 Fragestellung der Arbeit

Ziel der vorliegenden Arbeit war es, die Rolle von Glu im Belohnungssystem der Ratte genauer zu untersuchen. Ein Schwerpunkt lag dabei auf der Suche nach Substanzen, welche durch ihre Wirkung auf das glutamaterge System die Expression von Suchtmittel-konditioniertem Belohnungsverhalten beeinflussen. Insbesondere die durch eine Testsubstanz verursachte Reduktion der Expression einer Suchtmittel-CPP könnte dabei auf eine mögliche therapeutische Verwendung als Anti-Craving-Substanz hinweisen.

In Manuskript 1 (MS1) sollte geklärt werden, ob die Anti-Craving-Substanzen Acamprosat, Naloxon oder eine Kombination aus beiden die Expression einer Kokain- oder Morphin-CPP reduzieren können. Acamprosat (ein funktioneller NMDA-Antagonist) wurde bisher in der Klinik zur Rückfallprophylaxe bei entwöhnten Alkoholikern eingesetzt, während Naloxon (ein μ-Opiatrezeptor-Antagonist) einen positiven Einfluss auf die Wirkung von Acamprosat bei Alkoholikern zeigte. Des Weiteren sollte (in MS1) die Methode des wiederholten Testens („repeated-testing“) der CPP Expression evaluiert werden, um eine sinnvolle Möglichkeit zur Reduzierung der benötigten Versuchstiere zu finden.

In den Manuskripten 2 und 3 (MS2 und MS3) sollte getestet werden, ob 2-Methyl-6-(Phenylethynyl)-Pyridin (MPEP), ein selektiver mGluR5-Antagonist, die CPP Expression reduzieren kann, die durch verschiedene Suchtmittel (Kokain, Morphin, Amphetamin, MDMA) oder natürliche Belohnung (Futter) ausgelöst wurde. In verschiedenen Suchtmodellen zeigte sich bisher, dass MPEP die Entwicklung von Kokain- und Morphin-CPP, die Expression von Morphin-CPP sowie Kokain-SA reduzieren kann.

Als zweiter Schwerpunkt der vorliegenden Arbeit wurde die Rolle der Amygdala bei der Expression von Suchtmittel-konditioniertem Verhalten untersucht. Für das Verständnis der Rolle von Glu bei Belohnungsprozessen spielt die Amygdala eine bedeutende Rolle, da sie als eine wichtige Quelle glutamaterger Afferenzen des Belohnungssystems beschrieben wurde. Ferner zeigten sich teilweise unterschiedliche Funktionen von Unterarealen der Amygdala bei Suchtmittel-induziertem Verhalten. In Manuskript 4 (MS4) sollte daher geklärt werden, ob die Amygdala an der Kokain-CPP Expression beteiligt ist und ob verschiedene Unterareale der Amygdala (BLA, CeA) dabei eventuell unterschiedliche Rollen spielen.

2 Zusammenfassung der Ergebnisse der einzelnen Manuskripte

2.1 Manuskript 1

Volker Herzig and Werner J. Schmidt: "Testing the effectiveness of anti-craving drugs acamprosate and naloxone on expression of morphine or cocaine conditioned place preference by applying a repeated-testing schedule in rats"
eingereicht bei *Behavioural Brain Research.*

Experiment 1:
Es ist bekannt, dass der soziale Kontext einen starken Einfluss auf das Verhalten ausüben kann. Hiermit wollten wir klären, ob er auch Suchtmittel-Suchverhalten („drug-seeking") beeinflussen kann. Des Weiteren wird diskutiert, dass Acamprosat und Naloxon bei unterschiedlichen Arten von Craving wirksam sind: Acamprosat soll Entzugs-Craving verhindern, wohingegen Naloxon eher das Belohnungs-Craving beeinflusst. Als Modell für konditioniertes Suchtmittel-Suchverhalten wurde die CPP eingesetzt. Sozial isolierte und in Gruppe gehaltene Ratten wurden mittels Morphin konditioniert, im Anschluss wurde die CPP Expression nach Behandlung mit Acamprosat oder Naloxon gemessen. Als Ergebnis zeigte sich kein Unterschied in der Morphin-CPP Expression zwischen den unterschiedlich gehaltenen Ratten. Außerdem unterschied sich die CPP Expression nach Behandlung mit Acamprosat oder Naloxon nicht zwischen beiden Gruppen. Als Schlussfolgerung dieses Versuches lässt sich sagen, dass die zweiwöchige Isolationshaltung vor Versuchsbeginn nicht ausreichend war, um die belohnenden Eigenschaften von Morphin oder die Effekte einer Behandlung mit Acamprosat oder Naloxon in Ratten zu beeinflussen.

Experiment 2:
Die kombinierte Gabe von Acamprosat und Naloxon zeigte (im Vergleich zur getrennten Gabe) in klinischen Studien eine bessere Wirksamkeit in der Rückfallsvermeidung bei entwöhnten Alkoholikern. Zusätzlich wurde kürzlich in Tierexperimenten ein Effekt von Acamprosat auf Kokain-konditioniertes Verhalten festgestellt. Basierend auf den Ergebnissen von Experiment 1 wurden nur in Gruppe gehaltene Ratten eingesetzt und entweder auf Morphin oder auf Kokain konditioniert. Danach wurde in beiden Suchtmittel-Gruppen wiederholt die CPP Expression nach Behandlung mit Acamprosat, Naloxon oder einer kombinierten Gabe getestet. Zusätzlich sollte die Nützlichkeit und die Eignung der Methode des wiederholten Testens aufgeklärt werden. Es zeigte sich, dass lediglich Acamprosat die Expression einer Kokain-CPP hemmen kann. Methodisch gesehen ergab das wiederholte Testen sinnvolle Ergebnisse, weshalb es als eine nützliche Methode für zukünftige Studien vorgeschlagen wird, um die Wirkungen von Anti-Craving-Substanzen auf die CPP Expression zu untersuchen, wodurch insbesondere die benötigte Zahl von Versuchstieren verringert werden kann.

2.2 Manuskript 2

Volker Herzig and Werner J. Schmidt: “Effects of MPEP on locomotion, sensitization and conditioned reward induced by cocaine or morphine”
angenommen bei *Neuropharmacology*.

Die Präsentation von Umgebungsreizen wird als ein wichtiger Faktor angesehen, welcher sogar noch nach Jahren der Abstinenz bei entgifteten Drogenabhängigen zu Rückfällen führen kann. Neuere Erkenntnisse konnten eine Beteiligung von Glu bei Cue-induzierten Rückfällen zeigen und experimentelle Evidenzen deuteten darauf hin, dass mGluR5 an konditionierter Suchtmittelbelohnung beteiligt sind. Die vorliegende Studie verwendete das Modell der konditionierten Platzpräferenz, um die Beteiligung von mGluR5 an Kokain- und Morphin-induziertem Verhalten zu bestimmen. Durch Verwendung des selektiven mGluR5-Antagonisten MPEP wurden die bisherigen, mittels Mäusen gewonnenen Erkenntnisse, auf Ratten ausgeweitet. Als Ergebnis zeigte sich, dass die untersuchten Verhaltensparameter dosisabhängig von MPEP beeinflusst wurden. Die niedrige MPEP-Dosis (10 mg/kg, i.p.) hatte keinen Einfluss auf spontane Lokomotion, reduzierte die Kokain-induzierte Hyperlokomotion und erzeugte eine sensitivierte Lokomotion, während sie keine Wirkung auf die durch Kokain oder Morphin induzierte Sensitivierung hatte. Die niedrige MPEP-Dosis reduzierte zwar nicht die Entwicklung einer Kokain- oder Morphin-CPP, machte aber deren Expression „state-dependent“. Die mittlere MPEP-Dosis (30 mg/kg) zeigte ihre stärkste Wirkung in der Reduktion der Spontanlokomotion. Die hohe MPEP-Dosis (50 mg/kg) war hingegen am effektivsten in der Reduktion des Körpergewichts und der Morphin-CPP Expression. Keine der drei MPEP-Dosierungen beeinflusste jedoch die Kokain-CPP Expression signifikant. Daraus lässt sich schlussfolgern, dass mGluR5 an der Modulation von spontaner und Kokain-induzierter Lokomotion, beim „State-dependent“-Lernen und bei der Expression einer Morphin-CPP beteiligt sind. Folglich könnte sich MPEP zur Rückfallsverhinderung bei Morphinabhängigen eignen.

2.3 Manuskript 3

Volker Herzig, Eleonora M.I. Capuani, Karl-Artur Kovar, Werner J. Schmidt: "Effects of the mGluR5 antagonist MPEP on expression of conditioned place preference to natural (food) or drug (amphetamine, MDMA) reward"
eingereicht bei *Psychopharmacology*.

Neuere Studien konnten eine Beteiligung von mGluR5 in verschiedenen Tiermodellen der Sucht nachweisen. Es wurde gezeigt, dass eine mGluR5-Blockade die Expression von Kontext-konditionierter Morphinbelohnung blockiert. Die vorliegende Studie untersucht nun, ob mGluR5 auch an der Expression von Kontext-konditionierter Belohnung beteiligt sind, die durch andere Suchtmittel (Amphetamin oder MDMA) oder durch natürliche (Futter) Belohnung induziert wurde. Dazu wurden drei Gruppen von Ratten in einem Platzpräferenzversuch mit Amphetamin, MDMA oder Futter konditioniert. Nach erfolgter Konditionierung wurde die CPP Expression sowohl im drogenfreien („undrugged"), als auch im belohnten Zustand („drugged") sowie nach Behandlung mit 50 mg/kg (i.p.) MPEP, einem hoch-selektiven mGluR5-Antagonisten, getestet. Als Ergebnis zeigte sich in allen Gruppen eine Reduktion der Lokomotion durch MPEP. Allerdings wurde nur die Expression der Amphetamin-CPP durch MPEP reduziert, während die Expression der Futter- und MDMA-CPP unbeeinflusst blieb. Daraus lässt sich schlussfolgern, dass mGluR5 sowohl an der Modulation von spontaner Lokomotion, als auch an der Expression von Kontext-konditionierter Amphetaminbelohnung beteiligt sind. Ferner scheinen mGluR5 keinen Einfluss auf Kontext-konditionierte natürliche oder MDMA-Belohnung zu haben. Zusammenfassend zeigt sich, dass die Blockade von mGluR5 eine nützliche Behandlungsmethode zur Rückfallsverhinderung bei Kontext-konditioniertem Amphetamin-Craving sein könnte.

2.4 Manuskript 4

Volker Herzig and Werner J. Schmidt: “Reduction of cocaine conditioned place preference expression in rats by temporal inactivation of central but not basolateral amygdala” eingereicht bei *Neuropharmacology*.

Kontextuelle Stimuli sind ein wichtiger Faktor, der bei entwöhnten Drogenabhängigen Rückfälle induzieren kann. Die Amygdala spielt dabei eine wichtige Rolle, da sie an der Assoziation dieser Stimuli mit Belohnungsreizen beteiligt ist. Eine genauere Untersuchung zeigte Unterschiede in der Funktion von Subarealen der Amygdala, d.h. der CeA und der BLA während der Entwicklung einer Pavlovschen Kontext-Konditionierung durch Suchtmittel. Die Rolle von CeA und BLA bei der Expression einer Kontext-Konditionierung ist hingegen weiter unklar. Aus diesem Grund verwendeten wir die CPP, ein Modell das auf Pavlovscher Konditionierung basiert, um die Funktionen beider Amygdala-Kerne bei der Expression einer Kontext-Konditionierung zu untersuchen. Dazu wurden Mikroinfusionskanülen entweder in die CeA oder in die BLA von Ratten, welche später mittels Kokain konditioniert wurden, implantiert. Nur die Ratten, die eine Kokain-Platzpräferenz entwickelten wurden auf deren Expression getestet, indem zuvor entweder Saline oder aber eine Kombination von Baclofen und Muscimol (um eine temporäre Inaktivierung der betreffenden Hirnareale zu bewirken) infundiert wurde. Unsere Ergebnisse zeigen, dass eine temporäre Inaktivierung der CeA, nicht jedoch der BLA die Expression einer Kokain-CPP reduziert, wohingegen die Lokomotion (als Gradmesser der allgemeinen Aktivität) unverändert bleibt. Zusammenfassend lässt sich sagen, dass die CeA an der Expression einer Kontext-Konditionierung durch Kokain beteiligt ist.

3 Diskussion

3.1 Einfluss von Anti-Craving-Substanzen auf CPP Expression

3.1.1 Acamprosat

Ergänzend zu bisherigen Studien, die einen Effekt von Acamprosat auf die Entwicklung einer Kokain-CPP zeigen konnten (McGeehan & Olive 2003a), reduzierte Acamprosat in der vorliegenden Arbeit (MS1) die Expression einer Kokain-CPP. Die Morphin-CPP Expression hingegen blieb nach Acamprosatbehandlung unbeeinflusst. Dies lässt darauf schließen, dass unterschiedliche Mechanismen an der Expression von Kokain- oder Morphin-konditioniertem Verhalten beteiligt sind. Somit könnte Acamprosat, zusätzlich zu dem bisherigen Einsatz in der Rückfallprophylaxe bei Alkoholikern (zur Übersicht siehe Spanagel & Zieglgänsberger 1997), auch bei Kokain- nicht jedoch bei Opiatabhängigen (siehe MS1 und Spanagel *et al.* 1998) eingesetzt werden, um die rückfallsauslösende Wirkung konditionierter Umweltstimuli abzuschwächen. Das geringe Suchtpotential von Acamprosat (McGeehan & Olive 2003a; Kratzer & Schmidt 2003) erweist sich bei einer möglichen Verwendung als Anti-Craving-Substanz ebenfalls als vorteilhaft.

3.1.2 Naloxon

Für Naloxon konnte keine Wirkung auf die Expression einer Kokain- oder Morphin-CPP beobachtet werden (MS1). Im Falle von Morphin wird die CPP Expression durch Naloxon sogar eher noch verstärkt (Neisewander *et al.* 1990; Noble *et al.* 1993). Dies weist auf eine Unabhängigkeit der CPP Expression von μ-Opiatrezeptoren hin und legt nahe, dass Naloxon vermutlich keine klinische Wirksamkeit bei der Verhinderung von Cue-induzierten Rückfällen in Kokain- oder Morphinabhängigen aufweist.

3.1.3 Acamprosat + Naloxon (Kombination)

Auch die Kombination von Acamprosat und Naloxon zeigte, entgegen der beschriebenen synergistischen Wirkung in der Rückfallsprophylaxe bei Alkoholikern (Kiefer *et al.* 2003), keinen Effekt auf die Expression einer Kokain- oder Morphin-CPP (MS1). Somit scheint ein klinischer Einsatz dieser Substanzkombination bei Kokain- oder Morphinabhängigen aufgrund der vorliegenden Daten nicht gerechtfertigt.

3.1.4 MPEP

Bezüglich der Wirkung von MPEP auf die Entwicklung einer CPP stimmen die vorliegenden Ergebnisse (MS2) der Tests im drogenfreien Zustand („undrugged") mit denen aus der Literatur (Popik & Wrobel 2002; McGeehan & Olive 2003b) überein. In bisherigen Studien wurde für Mäuse eine Reduktion der Entwicklung sowohl einer Kokain- als auch einer Morphin-CPP beschrieben (beides ebenfalls im drogenfreien Zustand getestet). Die vorliegenden Ergebnisse aus dem Test unter dem Einfluss des jeweiligen Suchtmittels („drugged") stehen jedoch im Widerspruch zu den in der Literatur gezogenen Schlussfolgerungen, dass MPEP die Entwicklung einer Kokain- und Morphin-CPP blockiert. Es zeigte sich nämlich (in MS2) eine sehr starke CPP Expression, wenn unter dem Einfluss von MPEP und Kokain oder MPEP und Morphin getestet wurde, d.h. unter dem Einfluss der Konditionierungssubstanzen. Dies legt nahe, dass MPEP die Entwicklung der Suchtmittel-CPP nicht *per se* blockiert, sondern deren Expression state-dependent (siehe 1.3) macht. Folglich kann MPEP bei gleichzeitiger Gabe eine Kontext-konditionierte Suchtmittelbelohnung nicht reduzieren. Weiterhin ungeklärt bleibt die Frage, ob MPEP direkt an den Kontrollmechanismen für „State-dependent"-Lernen beteiligt ist, oder ob MPEP einfach nur den pharmakologischen Zustand („State") erzeugt, der die Voraussetzung für das „State-dependent"-Lernen bildet.

Bezüglich der Wirkung von MPEP auf die Expression einer Suchtmittel-CPP steht die gefundene Reduktion der Morphin-CPP Expression (MS2) in Einklang mit den Ergebnissen von Popik und Wrobel (2002), die bei hoher MPEP-Dosierung ebenfalls eine Reduktion beobachteten. Dagegen ist die Beobachtung, dass MPEP zwar die Expression einer Amphetamin-CPP (MS3), nicht jedoch die einer Kokain-CPP (MS2) blockieren kann, auf den ersten Blick überraschend. Aufgrund der ähnlichen Wirkungsweise beider Suchtmittel durch Blockade des DA-Reuptakes wäre zu vermuten, dass MPEP beide Verhaltensweisen in gleicher Weise beeinflusst. Allerdings konnten schon frühere Studien Unterschiede zwischen Amphetamin- und Kokain-induziertem Verhalten nachweisen. So wurde z.B. die Entwicklung einer Kokain-, nicht jedoch einer Amphetamin-CPP durch Läsion des prälimbischen Areals des medialen PFC (mPFC) blockiert (Tzschentke & Schmidt 1998b). Läsionen des infralimbischen mPFC zeigten ebenfalls keinen Effekt auf Amphetamin-CPP, blockierten jedoch die Entwicklung einer Morphin-CPP (Tzschentke & Schmidt 1999). Nimmt man nun an, dass nur glutamaterge Projektionen aus bestimmten Teilen des mPFC durch MPEP beeinflusst werden, lassen sich die gefundenen Unterschiede bzgl. Amphetamin, Kokain und Morphin erklären. Dies stellt jedoch nur eine mögliche Erklärung dar, insbesondere da beide Läsionsstudien nur die Beteiligung des mPFC an der Entwicklung, nicht aber an der Expression einer CPP untersuchten. Ferner zeigte der mPFC überhaupt keinen Einfluß auf die Entwicklung einer Amphetamin-CPP (Tzschentke & Schmidt 1999), so dass bei dem Effekt von MPEP auf die Amphetamin-CPP Expression vermutlich andere glutamaterge Afferenzen des Belohnungssytems eine Rolle spielen.

Die Beobachtung, dass MPEP keinen Einfluss auf die Expression einer MDMA-CPP hat (MS3), deutet ferner auf Unterschiede in der neuronalen Verarbeitung bei der Expression von Amphetamin- oder Morphin-konditioniertem Verhalten einerseits und Kokain- oder MDMA-konditioniertem Verhalten andererseits hin. Ein Vergleich der vorliegenden MDMA-Daten mit Ergebnissen aus der Literatur ist leider nicht möglich, da es sich hierbei offenbar um die erste Studie handelte, die den Effekt einer potentiellen Anti-Craving-Substanz auf die Expression einer MDMA-CPP untersuchte. Andererseits ergänzt sich die Tatsache, dass MPEP im gleichen Versuch keinen Effekt auf die Expression einer Futter-CPP ausübt, gut mit anderen Studien, welche zeigen konnten, dass MPEP keinen Effekt auf eine Futter-SA hat (Chiamulera *et al.* 2001; Paterson *et al.* 2003). Ein Einfluss von MPEP auf den Belohnungswert von Nahrungskonsum oder von Stimuli die mit der Nahrungsaufnahme assoziiert wurden, hätte ansonsten einer klinischen Anwendung von MPEP bei Suchtmittelabhängigen entgegengestanden. Ferner zeigte MPEP (in MS2) in Übereinstimmung mit bisherigen Veröffentlichungen (Popik & Wrobel 2002; McGeehan & Olive 2003b) bei keiner der getesteten Dosierungen eine CPP Entwicklung. Somit scheint ein Suchtpotential von MPEP unwahrscheinlich, was ebenfalls eine wichtige Voraussetzung für eine mögliche therapeutische Verwendung darstellt. Zusammenfassend lässt sich sagen, dass MPEP nützlich für die Rückfallprophylaxe bei Morphin- und Amphetaminabhängigkeit sein könnte, nicht jedoch bei MDMA- oder Kokainahängigkeit.

3.2 Rolle der Amygdala bei Kokain-CPP Expression

Die vorliegenden Ergebnisse (MS4) zeigen, dass bei der Expression einer Kokain-CPP nur die CeA, nicht jedoch die BLA, eine wichtige Rolle spielt. Dies stimmt mit anderen Ergebnissen überein, die zeigten dass Kokain-CPP Expression nicht durch eine BLA-Läsion beeinflusst wird (Fuchs *et al.* 2002). Die BLA scheint also beim Abrufen der konditionierten Belohnung keine Rolle zu spielen. Das kann dadurch erklärt werden, dass die BLA insbesondere während des Erlernens einer Suchtmittel-Kontext-Assoziation für die Evaluation eines belohnenden Stimulus oder für das Verknüpfen eines neutralen Stimulus mit einem Belohnungswert zuständig ist (zur Übersicht siehe Holland & Gallagher 1999; See 2002; Everitt *et al.* 2003; See *et al.* 2003). Dies zeigt sich darin, dass kein Erlernen einer Pavlovschen Konditionierung in BLA-lädierten Ratten gefunden wurde (Lindgren *et al.* 2003). Ferner wird die Extinktion einer Kokain-CPP durch eine BLA-Läsion blockiert (Fuchs *et al.* 2002), d.h. die jetzt neue Information (kein Suchtmittel mehr vorhanden) kann nicht mit dem Kontext assoziiert werden. Die Unabhängigkeit von der Kontrolle durch die BLA gilt jedoch nicht für die Expression von konditionierter Furcht, die durch Inaktivierung der BLA reduziert werden konnte (Helmstetter & Bellgowan 1994). Die CeA ist andererseits insbesondere wichtig bei der Kontrolle von Aufmerksamkeit und der Orientierung auf distale Stimuli wie z.B. den Umweltkontext (See 2002), was beim Abrufen (der Expression) einer Suchtmittel-Kontext-Assoziation entscheidend ist. Dies zeigt sich z.B. darin, dass das Abrufen einer konditionierten Assoziation nur durch CeA- nicht aber durch BLA-Läsion blockiert werden kann (Holland & Gallagher 2003). Allerdings ist aus einer weiteren Arbeit bekannt, dass an der Amphetamin-CPP Expression weder die BLA noch die CeA beteiligt ist, dafür jedoch die laterale Amygdala eine wichtige Rolle spielt (Hiroi & White 1991). Daher scheint sich die Beteiligung verschiedener Subareale der Amygdala bei verschiedenen Suchtmitteln zu unterscheiden.

Da kein Effekt einer temporären BLA-Inaktivierung gefunden wurde, scheinen Neurone aus der BLA (die hauptsächlich in die CeA projizieren) somit nicht an der Expression einer Kontext-konditionierten Kokain-Assoziation beteiligt zu sein. Es wurde jedoch gezeigt, dass DA-D1-Rezeptoren in der CeA eine modulatorische Rolle auf die Diskriminierung eines Kokain-Stimulus ausüben (Callahan *et al.* 1995). In Morphin-konditionierten Tieren konnte man ferner nachweisen, dass DA-Agonisten die Expression einer CPP hemmen (Zarrindast *et al.* 2003). Eine mögliche Erklärung wäre daher, dass die Präsentation eines Suchtmittel-assoziierten Kontextes DA in der CeA erhöht und somit die Kokain-CPP Expression reduziert. In Kokain-konditionierten Tieren konnte man tatsächlich zeigen, dass Kokain-assoziierte Stimuli die DA-Konzentration in der Amygdala erhöhen, allerdings wurde in dieser Arbeit nicht auf mögliche Unterschiede zwischen Subarealen der Amygdala eingegangen (Weiss *et al.* 2000). Eine solche Erhöhung der DA-Konzentration in der CeA könnte z.B. über das VTA vermittelt werden, welches dopaminerg in die CeA projiziert (Winnicka & WiSniewski 1999).

3.3 Rolle von Glutamat bei CPP Expression

3.3.1 Amphetamin

Eine Beteiligung von Glu bei der Expression einer Amphetamin-CPP konnte durch die Blockade von mGluR5 mittels MPEP (MS3) eindeutig nachgewiesen werden. Ergänzend dazu zeigten andere Arbeiten, dass auch ionotrope Glutamatrezeptoren an diesem Verhalten beteiligt sind. Der nicht-kompetitive NMDA-Rezeptor-Antagonist MK-801, der NMDA-Rezeptor-Antagonist (±)-CPP, der AMPA/Kainat-Rezeptor-Antagonist DNQX sowie der AMPA-Rezeptor selektive Antagonist GYKI 52466 (Layer *et al.* 1993; Bespalov 1996; Tzschentke & Schmidt 1997) blockierten ebenfalls die Amphetamin-CPP Expression. ACPC, ein partieller Agonist an der Glycin-Bindungsstelle des NMDA-Rezeptors, zeigte allerdings keinen Effekt auf die Expression einer Amphetamin-CPP (Papp *et al.* 2002). Zusammenfassend scheint Glu aber einen bedeutenden Einfluss auf die Expression einer Amphetamin-CPP zu haben, woran sowohl mGluR5, als auch ionotrope NMDA- und AMPA-Rezeptoren beteiligt sind.

3.3.2 Kokain

In der vorliegenden Arbeit (MS2) zeigte sich kein Einfluss von mGluR5 auf die Expression einer Kokain-CPP. Es ist jedoch bekannt, dass Glu generell eine Rolle bei der Expression einer Kokain-CPP spielt, da diese durch die NAALADase (einem Enzym zur Glu-Synthese)-Inhibitoren GPI 5693 und 2-PMPA blockiert werden kann (Slusher *et al.* 2001). Ferner reduziert der nicht-kompetitive NMDA-Rezeptor-Antagonist Memantin die Kokain-CPP Expression (Kotlinska & Biala 2000). Acamprosat, das über funktionelle NMDA-Rezeptor antagonistische Eigenschaften verfügt (al Qatari *et al.* 1998), hemmte ebenfalls die Kokain-CPP Expression in der vorliegenden Arbeit (MS1). Des Weiteren konnte die Kokain-CPP Expression durch den AMPA/Kainat-Rezeptor-Antagonist DNQX blockiert werden, woran insbesondere die Rezeptoren im Nac beteiligt sind (Cervo & Samanin 1995). In derselben Arbeit wurde hingegen gezeigt, dass der nicht-kompetitive NMDA-Rezeptor-Antagonist MK-801 keinen Effekt auf eine Kokain-CPP Expression hat. Ferner zeigten andere Arbeiten, dass sowohl ein Antagonist (L-701,324) als auch ein partieller Agonist (ACPC) der Glycin-Bindungsstelle des NMDA-Rezeptors keinen Effekt auf die Expression einer Kokain-CPP aufwiesen (Papp *et al.* 2002). Abschließend kann daher die Schlussfolgerung gezogen werden, dass Glu hauptsächlich über ionotrope Glu-Rezeptoren die Expression einer Kokain-CPP beeinflussen kann. Daran sind AMPA-/Kainat-Rezeptoren, vermutlich aber auch NMDA-Rezeptoren beteiligt. Für die Beteiligung von NMDA-Rezeptoren liegen jedoch zum Teil widersprüchliche Ergebnisse vor, so dass weitere Experimente für eine abschließende Beurteilung notwendig sind.

3.3.3 MDMA

Die Expression einer MDMA-CPP zeigte in der vorliegenden Arbeit (MS3) keine Beeinflussung durch eine mGluR5-Blockade mittels MPEP. In der Literatur wurden ebenfalls keine weiteren Arbeiten gefunden, welche die Wirkung einer (möglichen) Anti-Craving-Substanz auf die Expression einer MDMA-CPP untersuchten. Daher kann nicht abschließend geklärt werden, ob Glutamat bei der Expression einer MDMA-CPP überhaupt eine Rolle spielt und wenn dies so ist, über welche Rezeptoren dieser Effekt vermittelt wird.

3.3.4 Morphin

Auch die Morphin-CPP Expression, die durch den unspezifischen ionotropen Glu-Rezeptor-Antagonisten Kynurensäure blockiert werden kann (Bespalov *et al.* 1994), steht eindeutig unter dem Einfluss von Glu. Insbesondere durch die Modulation der ionotropen Glu-Transmission konnte eine Reduktion der Morphin-CPP Expression erreicht werden, wobei bisher die folgenden Substanzen als wirksam beschrieben worden sind: die nicht-kompetitiven NMDA-Rezeptor-Antagonisten Memantin (Popik & Danysz 1997; Popik *et al.* 2003) und MK-801 (Tzschentke & Schmidt 1997), der kompetitive NMDA-Rezeptor-Antagonist NPC 17742 (Popik & Kolasiewicz 1999), der AMPA/Kainat-Rezeptor-Antagonist DNQX bei Applikation in den Nac (Layer *et al.* 1993), sowie der AMPA-Rezeptor selektive Antagonist GYKI 52466 (Tzschentke & Schmidt 1997). Ferner wird die Morphin-CPP Expression, im Gegensatz zur Expression einer Amphetamin- oder Kokain-CPP, auch durch Modulation an der Glycin-Bindungsstelle des NMDA-Rezeptors mittels des Antagonisten L-701,324 (Kotlinska & Biala 1999) oder durch den partiellen Agonisten ACPC reduziert (Papp *et al.* 2002). Acamprosat zeigt, im Gegensatz zur Reduktion der Kokain-CPP Expression, keinen Effekt auf die Expression einer Morphin-CPP (MS1). Es muss daher in Frage gestellt werden, ob der Effekt von Acamprosat auf die Kokain-CPP Expression ausschließlich über seine funktionelle NMDA-Rezeptor antagonistische Wirkung erklärt werden kann. Ansonsten hätte auch die Morphin-CPP Expression, die eine starke Beeinflussung durch NMDA-Rezeptoren aufweist, durch Acamprosat reduziert sein müssen, was aber nicht beobachtet wurde (MS1). Eine genauere Untersuchung zeigt, dass NMDA-Rezeptoren im Nac und im VTA an der Morphin-CPP Expression beteiligt sind (Popik & Kolasiewicz 1999). Aus anderen Versuchen ist ferner bekannt, dass die NMDA-Rezeptor-induzierte Membrandepolarisation striataler Output-Neurone durch den selektiven mGluR5-Agonist CHPG potenziert wird, ein Effekt der durch MPEP blockiert werden kann (Pisani *et al.* 2001). Aufgrund der hohen mGluR5-Rezeptordichte im Nac (Spooren *et al.* 2003) kann vermutet werden, dass die Reduktion der Morphin-CPP Expression durch MPEP (MS2; Popik & Wrobel 2002) zumindest teilweise auf einer Blockade der durch mGluR5-potenzierten NMDA-Transmission beruht. Aus den bisherigen Erkenntnissen kann man zusammenfassen, dass Glu (ähnlich wie bei Amphetamin-CPP Expression) sowohl durch mGluR5, als auch über ionotrope NMDA- und AMPA-Rezeptoren die Expression einer Morphin-CPP beeinflusst.

3.3.5 Natürliche Belohnung

Konditionierte Futterbelohnung ist ein wichtiger Parameter, der zur Evaluation der Wirksamkeit einer möglichen Anti-Craving-Substanz verwendet werden kann. Sollte diese Testsubstanz eine negative Wirkung auf die belohnenden Eigenschaften von Futter zeigen, wäre sie damit für suchttherapeutische Zwecke unbrauchbar. Da Suchtmittelabhängige oftmals abgemagert und in einer schlechten körperlichen Gesamtverfassung sind, wäre die Gabe einer Substanz die zur Abnahme der Nahrungsaufnahme führen könnte fatal. In der vorliegenden Arbeit (MS3) ließ sich die Expression einer Futter-CPP allerdings nicht durch eine mGluR5-Blockade mittels MPEP beeinflussen. Andere Studien zeigen ferner, dass NMDA-Rezeptor-Blockade mittels Memantin keinen Einfluss auf die Futter-CPP Expression hat (Popik & Danysz 1997; Popik *et al.* 2003). Dies könnte bedeuten, dass Glu bei der Expression natürlicher Belohnung, im Gegensatz zu Suchtmittel-induzierter Belohnung, gar keine Rolle spielt. Allerdings sind noch weitere Studien, z.B. mit AMPA- oder Kainat-Rezeptor-Antagonisten notwendig, um eine abschließende Aussage darüber treffen zu können.

4 Literatur

Ahmed SH, Kenny PJ, Koob GF, Markou A (2002) Neurobiological evidence for hedonic allostasis associated with escalating cocaine use. *Nat.Neurosci.* **5,** 625-626.

al Qatari M, Bouchenafa O, Littleton J (1998) Mechanism of action of acamprosate. Part II. Ethanol dependence modifies effects of acamprosate on NMDA receptor binding in membranes from rat cerebral cortex. *Alcohol Clin.Exp.Res.* **22,** 810-814.

Amtage J, Schmidt WJ (2003) Context-dependent catalepsy intensification is due to classical conditioning and sensitization. *Behav.Pharmacol.* **14,** 563-567.

Bardo MT, Bevins RA (2000) Conditioned place preference: what does it add to our preclinical understanding of drug reward? *Psychopharmacology (Berl)* **153,** 31-43.

Bell K, Kalivas PW (1996) Context-specific cross-sensitization between systemic cocaine and intra- accumbens AMPA infusion in the rat. *Psychopharmacology (Berl)* **127,** 377-383.

Bespalov A, Dumpis M, Piotrovsky L, Zvartau E (1994) Excitatory amino acid receptor antagonist kynurenic acid attenuates rewarding potential of morphine. *Eur.J.Pharmacol.* **264,** 233-239.

Bespalov AY (1996) The expression of both amphetamine-conditioned place preference and pentylenetetrazol-conditioned place aversion is attenuated by the NMDA receptor antagonist (+/-)-CPP. *Drug Alcohol Depend.* **41,** 85-88.

Callahan PM, Bryan SK, Cunningham KA (1995) Discriminative stimulus effects of cocaine: Antagonism by dopamine D1 receptor blockade in the amygdala. *Pharmacol.Biochem.Behav.* **51,** 759-766.

Carlezon WA, Jr., Mendrek A, Wise RA (1995) MK-801 disrupts the expression but not the development of bromocriptine sensitization: a state-dependency interpretation. *Synapse* **20,** 1-9.

Carlezon WA, Jr., Boundy VA, Haile CN, Lane SB, Kalb RG, Neve RL, Nestler EJ (1997) Sensitization to morphine induced by viral-mediated gene transfer. *Science* **277,** 812-814.

Carlezon WA, Jr., Nestler EJ (2002) Elevated levels of GluR1 in the midbrain: a trigger for sensitization to drugs of abuse? *Trends Neurosci.* **25,** 610-615.

Carr DB, Sesack SR (2000) Projections from the rat prefrontal cortex to the ventral tegmental area: target specificity in the synaptic associations with mesoaccumbens and mesocortical neurons. *J.Neurosci.* **20,** 3864-3873.

Cervo L, Samanin R (1995) Effects of dopaminergic and glutamatergic receptor antagonists on the acquisition and expression of cocaine conditioning place preference. *Brain Res.* **673,** 242-250.

Chiamulera C, Epping-Jordan MP, Zocchi A, Marcon C, Cottiny C, Tacconi S, Corsi M, Orzi F, Conquet F (2001) Reinforcing and locomotor stimulant effects of cocaine are absent in mGluR5 null mutant mice. *Nat.Neurosci.* **4,** 873-874.

Childress AR, Mozley PD, McElgin W, Fitzgerald J, Reivich M, O'Brien CP (1999) Limbic activation during cue-induced cocaine craving. *Am.J.Psychiatry* **156,** 11-18.

Ciccocioppo R, Sanna PP, Weiss F (2001) Cocaine-predictive stimulus induces drug-seeking behavior and neural activation in limbic brain regions after multiple months of abstinence: reversal by D(1) antagonists. *Proc.Natl.Acad.Sci.U.S.A.* **98,** 1976-1981.

Cornish JL, Kalivas PW (2000) Glutamate transmission in the nucleus accumbens mediates relapse in cocaine addiction. *J.Neurosci.* **20,** RC89.

Di Ciano P, Cardinal RN, Cowell RA, Little SJ, Everitt BJ (2001) Differential involvement of NMDA, AMPA/kainate, and dopamine receptors in the nucleus accumbens core in the acquisition and performance of pavlovian approach behavior. *J.Neurosci.* **21,** 9471-9477.

Erb S, Shaham Y, Stewart J (1996) Stress reinstates cocaine-seeking behavior after prolonged extinction and a drug-free period. *Psychopharmacology (Berl)* **128,** 408-412.

Everitt BJ, Robbins TW (2000) Second-order schedules of drug reinforcement in rats and monkeys: measurement of reinforcing efficacy and drug-seeking behaviour. *Psychopharmacology (Berl)* **153,** 17-30.

Everitt BJ, Dickinson A, Robbins TW (2001) The neuropsychological basis of addictive behaviour. *Brain Res.Brain Res.Rev.* **36,** 129-138.

Everitt BJ, Cardinal RN, Parkinson JA, Robbins TW (2003) Appetitive behavior: impact of amygdala-dependent mechanisms of emotional learning. *Ann.N.Y.Acad.Scien.* **985,** 233-250.

Fuchs RA, Weber SM, Rice HJ, Neisewander JL (2002) Effects of excitotoxic lesions of the basolateral amygdala on cocaine- seeking behavior and cocaine conditioned place preference in rats. *Brain Res.* **929,** 15-25.

Ghasemzadeh MB, Nelson LC, Lu XY, Kalivas PW (1999) Neuroadaptations in ionotropic and metabotropic glutamate receptor mRNA produced by cocaine treatment. *J.Neurochem.* **72,** 157-165.

Grant S, London ED, Newlin DB, Villemagne VL, Liu X, Contoreggi C, Phillips RL, Kimes AS, Margolin A (1996) Activation of memory circuits during cue-elicited cocaine craving. *Proc.Natl.Acad.Sci.U.S.A.* **93,** 12040-12045.

Grimm JW, See RE (2000) Dissociation of primary and secondary reward-relevant limbic nuclei in an animal model of relapse. *Neuropsychopharmacology* **22,** 473-479.

Harris GC, Aston-Jones G (2003) Critical role for ventral tegmental glutamate in preference for a cocaine-conditioned environment. *Neuropsychopharmacology* **28,** 73-76.

Helmstetter FJ, Bellgowan PS (1994) Effects of muscimol applied to the basolateral amygdala on acquisition and expression of contextual fear conditioning in rats. *Behav.Neurosci.* **108**, 1005-1009.

Helmuth L (2001) Addiction. Beyond the pleasure principle. *Science* **294,** 983-984.

Heyman GM (2000) An economic approach to animal models of alcoholism. *Alc.Res.&Health* **24,** 132-139.

Hiroi N, White NM (1991) The lateral nucleus of the amygdala mediates expression of the amphetamine-produced conditioned place preference. *J.Neurosci.* **11,** 2107-2116.

Holland PC, Gallagher M (1999) Amygdala circuitry in attentional and representational processes. *Trends in Cognitive Sciences* **3,** 65-73.

Holland PC, Gallagher M (2003) Double dissociation of the effects of lesions of basolateral and central amygdala on conditioned stimulus-potentiated feeding and Pavlovian-instrumental transfer. *Eur.J.Neurosci.* **17**, 1680-1694.

Hotsenpiller G, Giorgetti M, Wolf ME (2001) Alterations in behaviour and glutamate transmission following presentation of stimuli previously associated with cocaine exposure. *Eur.J.Neurosci.* **14,** 1843-1855.

Hyman SE, Malenka RC (2001) Addiction and the brain: the neurobiology of compulsion and its persistence. *Nat.Rev.Neurosci.* **2,** 695-703.

Ito R, Dalley JW, Howes SR, Robbins TW, Everitt BJ (2000) Dissociation in conditioned dopamine release in the nucleus accumbens core and shell in response to cocaine cues and during cocaine-seeking behavior in rats. *J.Neurosci.* **20,** 7489-7495.

Jackson A, Koek W, Colpaert FC (1992) NMDA antagonists make learning and recall state-dependent. *Behav.Pharmacol.* **3,** 415-421.

Kalivas PW (2004) Glutamate systems in cocaine addiction. *Curr.Opin.Pharmacol.* **4,** 23-29.

Kiefer F, Jahn H, Tarnaske T, Helwig H, Briken P, Holzbach R, Kampf P, Stracke R, Baehr M, Naber D, Wiedemann K (2003) Comparing and combining naltrexone and acamprosate in relapse prevention of alcoholism: a double-blind, placebo-controlled study. *Arch Gen.Psychiatry* **60,** 92-99.

Klein A, Schmidt WJ (2003) Catalepsy intensifies context-dependently irrespective of whether it is induced by intermittent or chronic dopamine deficiency. *Behav.Pharmacol.* **14,** 49-53.

Koob GF, Le Moal M (1997) Drug abuse: hedonic homeostatic dysregulation. *Science* **278,** 52-58.

Koob GF, Nestler EJ (1997) The neurobiology of drug addiction. *J.Neuropsychiatry Clin.Neurosci.* **9,** 482-497.

Kotlinska J, Biala G (1999) Effects of the NMDA/glycine receptor antagonist, L-701,324, on morphine- and cocaine-induced place preference. *Pol.J.Pharmacol.* **51,** 323-330.

Kotlinska J, Biala G (2000) Memantine and ACPC affect conditioned place preference induced by cocaine in rats. *Pol.J.Pharmacol.* **52,** 179-185.

Kozell LB, Meshul CK (2001) The effects of acute or repeated cocaine administration on nerve terminal glutamate within the rat mesolimbic system. *Neuroscience* **106,** 15-25.

Kratzer U, Schmidt WJ (2003) Acamprosate does not induce a conditioned place preference and reveals no state-dependent effects in this paradigm. *Progr.Neuro-Psychopharmacol.& Biol.Psychiat.* **27,** 653-656.

Kruzich PJ, See RE (2001) Differential contributions of the basolateral and central amygdala in the acquisition and expression of conditioned relapse to cocaine-seeking behavior. *J.Neurosci.* **21,** RC155.

Lanis A, Schmidt WJ (2001) NMDA receptor antagonists do not block the development of sensitization of catalepsy, but make its expression state-dependent. *Behav.Pharmacol.* **12,** 143-149.

Layer RT, Uretsky NJ, Wallace LJ (1993) Effects of the AMPA/kainate receptor antagonist DNQX in the nucleus accumbens on drug-induced conditioned place preference. *Brain Res.* **617,** 267-273.

Leshner AI (1997) Addiction is a brain disease, and it matters. *Science* **278,** 45-47.

Lindgren JL, Gallagher M, Holland PC (2003) Lesions of basolateral amygdala impair extinction of CS motivational value, but not of explicit conditioned responses, in Pavlovian appetitive second-order conditioning. *Eur.J.Neurosci.* **17**, 160-166.

McFarland K, Ettenberg A (1997) Reinstatement of drug-seeking behavior produced by heroin-predictive environmental stimuli. *Psychopharmacology (Berl)* **131,** 86-92.

McFarland K, Kalivas PW (2001) The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. *J.Neurosci.* **21,** 8655-8663.

McFarland K, Lapish CC, Kalivas PW (2003) Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. *J.Neurosci.* **23,** 3531-3537.

McGeehan AJ, Olive MF (2003a) The anti-relapse compound acamprosate inhibits the development of a conditioned place preference to ethanol and cocaine but not morphine. *Brit.J.Pharmacol.* **138,** 9-12.

McGeehan AJ, Olive MF (2003b) The mGluR5 antagonist MPEP reduces the conditioned rewarding effects of cocaine but not other drugs of abuse. *Synapse* **47,** 240-242.

Morgan MJ (2000) Ecstasy (MDMA): a review of its possible persistent psychological effects. *Psychopharmacology (Berl)* **152,** 230-248.

Mueller D, Stewart J (2000) Cocaine-induced conditioned place preference: reinstatement by priming injections of cocaine after extinction. *Behav.Brain Res.* **115,** 39-47.

Neisewander JL, Pierce RC, Bardo MT (1990) Naloxone enhances the expression of morphine-induced conditioned place preference. *Psychopharmacology (Berl)* **100,** 201-205.

Noble F, Fournie-Zaluski MC, Roques BP (1993) Unlike morphine the endogenous enkephalins protected by RB101 are unable to establish a conditioned place preference in mice. *Eur.J.Pharmacol.* **230,** 139-149.

Olds J, Milner P (1954) Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *J.Comp.Physiol.Psychol.* **47,** 419-427.

Papp M, Gruca P, Willner P (2002) Selective blockade of drug-induced place preference conditioning by ACPC, a functional NDMA-receptor antagonist. *Neuropsychopharmacology* **27,** 727-743.

Parkinson JA, Robbins TW, Everitt BJ (2000) Dissociable roles of the central and basolateral amygdala in appetitive emotional learning. *Eur.J.Neurosci.* **12,** 405-413.

Paterson NE, Semenova S, Gasparini F, Markou A (2003) The mGluR5 antagonist MPEP decreased nicotine self-administration in rats and mice. *Psychopharmacology (Berl)* **167,** 257-264.

Phillips PE, Stuber GD, Heien ML, Wightman RM, Carelli RM (2003) Subsecond dopamine release promotes cocaine seeking. *Nature* **422,** 614-618.

Pierce RC, Bell K, Duffy P, Kalivas PW (1996) Repeated cocaine augments excitatory amino acid transmission in the nucleus accumbens only in rats having developed behavioral sensitization. *J.Neurosci.* **16,** 1550-1560.

Pisani A, Gubellini P, Bonsi P, Conquet F, Picconi B, Centonze D, Bernardi G, Calabresi P (2001) Metabotropic glutamate receptor 5 mediates the potentiation of N-methyl-D-aspartate responses in medium spiny striatal neurons. *Neuroscience* **106,** 579-587.

Popik P, Danysz W (1997) Inhibition of reinforcing effects of morphine and motivational aspects of naloxone-precipitated opioid withdrawal by N-methyl-D-aspartate receptor antagonist, memantine. *J.Pharmacol.Exp.Ther.* **280,** 854-865.

Popik P, Kolasiewicz W (1999) Mesolimbic NMDA receptors are implicated in the expression of conditioned morphine reward. *N.-S.Arch.Pharmacol.* **359,** 288-294.

Popik P, Wrobel M (2002) Morphine conditioned reward is inhibited by MPEP, the mGluR5 antagonist. *Neuropharmacology* **43,** 1210-1217.

Popik P, Wrobel M, Rygula R, Bisaga A, Bespalov AY (2003) Effects of memantine, an NMDA receptor antagonist, on place preference conditioned with drug and nondrug reinforcers in mice. *Behav.Pharmacol.* **14,** 237-244.

Reid MS, Berger SP (1996) Evidence for sensitization of cocaine-induced nucleus accumbens glutamate release. *Neuroreport* **7,** 1325-1329.

Robbins TW, Everitt BJ (1999) Drug addiction: bad habits add up. *Nature* **398,** 567-570.

Robins LN, Davis DH, Goodwin DW (1974) Drug use by U.S. Army enlisted men in Vietnam: a follow-up on their return home. *Am.J.Epidemiol.* **99,** 235-249.

Robinson TE, Berridge KC (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res.Brain Res.Rev.* **18,** 247-291.

Sanchez CJ, Sorg BA (2001) Conditioned fear stimuli reinstate cocaine-induced conditioned place preference. *Brain Res.* **908,** 86-92.

Schmidt WJ (1997) Biologische Grundlagen der Sucht. *B.I.F.Futura* **12,** 172-178.

See RE (2002) Neural substrates of conditioned-cued relapse to drug-seeking behavior. *Pharmacol.Biochem.Behav.* **71,** 517-529.

See RE, Fuchs RA, Ledford CC, McLaughlin J (2003) Drug Addiction, Relapse, and the Amygdala. *Ann.N.Y.Acad.Sci* **985,** 294-307.

Shaham Y, Erb S, Stewart J (2000) Stress-induced relapse to heroin and cocaine seeking in rats: a review. *Brain Res.Brain Res.Rev.* **33,** 13-33.

Shippenberg TS, Rea W, Slusher BS (2000) Modulation of behavioral sensitization to cocaine by NAALADase inhibition. *Synapse* **38,** 161-166.

Slusher BS, Thomas A, Paul M, Schad CA, Ashby CR, Jr. (2001) Expression and acquisition of the conditioned place preference response to cocaine in rats is blocked by selective inhibitors of the enzyme N- acetylated-alpha-linked-acidic dipeptidase (NAALADASE). *Synapse* **41,** 22-28.

Spanagel R, Zieglgänsberger W (1997) Anti-craving compounds for ethanol: new pharmacological tools to study addictive processes. *Trends Pharmacol.Sci.* **18,** 54-59.

Spanagel R, Sillaber I, Zieglgänsberger W, Corrigall WA, Stewart J, Shaham Y (1998) Acamprosate suppresses the expression of morphine-induced sensitization in rats but does not affect heroin self-administration or relapse induced by heroin or stress. *Psychopharmacology (Berl)* **139,** 391-401.

Spooren W, Ballard T, Gasparini F, Amalric M, Mutel V, Schreiber R (2003) Insight into the function of Group I and Group II metabotropic glutamate (mGlu) receptors: behavioural characterization and implications for the treatment of CNS disorders. *Behav.Pharmacol.* **14,** 257-277.

Strakowski SM, Sax KW, Setters MJ, Keck PE Jr. (1996) Enhanced response to repeated d-amphetamine challenge: evidence for behavioral sensitization in humans. *Biol.Psychiatry* **40,** 872-880.

Tiffany ST, Carter BL (1998) Is craving the source of compulsive drug use? *J Psychopharmacol.* **12,** 23-30.

Tiffany ST, Conklin CA (2000) A cognitive processing model of alcohol craving and compulsive alcohol use. *Addiction* **95,** S145-S153.

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

Tzschentke TM, Schmidt WJ (1997) Interactions of MK-801 and GYKI 52466 with morphine and amphetamine in place preference conditioning and behavioural sensitization. *Behav.Brain Res.* **84,** 99-107.

Tzschentke TM, Schmidt WJ (1998a) Does the noncompetitive NMDA receptor antagonist dizocilpine (MK801) really block behavioural sensitization associated with repeated drug administration? *Trends Pharmacol.Sci.* **19,** 447-451.

Tzschentke TM, Schmidt WJ (1998b) Discrete quinolinic acid lesions of the rat prelimbic medial prefrontal cortex affect cocaine- and MK-801-, but not morphine- and amphetamine- induced reward and psychomotor activation as measured with the place preference conditioning paradigm. *Behav.Brain Res.* **97,** 115-127.

Tzschentke TM, Schmidt WJ (1999) Functional heterogeneity of the rat medial prefrontal cortex: effects of discrete subarea-specific lesions on drug-induced conditioned place preference and behavioural sensitization. *Eur.J.Neurosci.* **11,** 4099-4109.

Tzschentke TM, Schmidt WJ (2003) Glutamatergic mechanisms in addiction. *Mol.Psychiatry* **8,** 373-382.

Ungless MA, Whistler JL, Malenka RC, Bonci A (2001) Single cocaine exposure in vivo induces long-term potentiation in dopamine neurons. *Nature* **411,** 583-587.

Vorel SR, Liu X, Hayes RJ, Spector JA, Gardner EL (2001) Relapse to cocaine-seeking after hippocampal theta burst stimulation. *Science* **292,** 1175-1178.

Weiss F, Maldonado-Vlaar CS, Parsons LH, Kerr TM, Smith DL, Ben Shahar O (2000) Control of cocaine-seeking behavior by drug-associated stimuli in rats: effects on recovery of extinguished operant-responding and extracellular dopamine levels in amygdala and nucleus accumbens. *Proc.Natl.Acad.Sci.U.S.A.* **97,** 4321-4326.

Winnicka MM, WiSniewski K (1999) Dopaminergic projection to the central amygdala mediates the facilitatory effect of CCK-8US and caerulein on memory in rats. *Pharmacol.Res.* **39,** 445-450.

Wise RA, Kelsey JE (1994) Behavioral Models Of Addiction. In “Principles of Addiction Medicine”, pp.1-11, Ed.: Miller NS, Chery Chase, Maryland, American Society of Addiction Medicine Inc.

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

Wolffgramm J, Heyne A (1995) From controlled drug intake to loss of control: the irreversible development of drug addiction in the rat. *Behav.Brain Res.* **70,** 77-94.

Zarrindast MR, Rezayof A, Sahraei H, Haeri-Rohani A, Rassouli Y (2003) Involvement of dopamine D1 receptors of the central amygdala on the acquisition and expression of morphine-induced place preference in rat. *Brain Res.* **965,** 212-221.

5 Abkürzungen und Fachbegriffe

AMPA	Alpha-Amino-3-Hydroxy-5-Methyl-4-Isoxazol-Propionsäure
BLA	basolaterale Amygdala
CeA	zentrale Amygdala
CPA	konditionierte Platzaversion („conditioned place aversion“)
CPP	konditionierte Platzpräferenz („conditioned place preference“)
Craving	(unwiderstehliches) Verlangen nach einem Suchtmittel
Cues	Umweltreize oder diskrete Stimuli, welche mit einem Suchtmittel assoziiert wurden
DA	Dopamin
GABA	Gammaaminobuttersäure („gamma amino butyric acid”)
Glu	Glutamat
ICSS	Intrakranielle Selbststimulation
MDMA	3,4-Methylendioxymethylamphetamin
mGluR5	metabotrope Glu-Rezeptoren des Subtyps 5
MPEP	2-Methyl-6-(Phenylethynyl)-Pyridin
mPFC	medialer präfrontaler Kortex
MS	Manuskript
Nac	Nucleus accumbens
NMDA	N-Methyl-D-Aspartat
Reinstatement	erneute Auslösung eines zuvor extingierten Verhaltens durch ein Suchtmittel, durch Cues oder durch Stress
SA	Selbstadministration („self-administration“; z.B. eines Suchtmittels)
VTA	Ventrales tegmentales Areal

6 Erklärung zum Eigenanteil an den einzelnen Manuskripten

Manuskript 1 Komplette konzeptionelle und inhaltliche Planung, komplette Versuchsvorbereitung, -durchführung und –auswertung sowie Erstellung des Manuskriptes.

Manuskript 2 Komplette konzeptionelle und inhaltliche Planung, komplette Versuchsvorbereitung, -durchführung und –auswertung sowie Erstellung des Manuskriptes.

Manuskript 3 Komplette konzeptionelle und inhaltliche Planung sowie komplette Versuchsauswertung incl. Erstellung des Manuskriptes. Ca. 40 % Beteiligung an der Versuchsvorbereitung und –durchführung.
Ca. 60% der Versuchsvorbereitung und -durchführung erfolgten unter meiner Anleitung durch Frau Eleonora M. I. Capuani (Studentin der Graduate School for Neuroscience, Tübingen) im Rahmen einer von mir betreuten Lab-Rotation.

Manuskript 4 Komplette konzeptionelle und inhaltliche Planung, komplette Versuchsvorbereitung, -durchführung und –auswertung sowie Erstellung des Manuskriptes.

Bei keinem der aufgeführten Manuskripte ging der Anteil von Herrn Prof. Dr. Werner J. Schmidt über das im Rahmen eines Betreuungsverhältnisses übliche Maß hinaus.

7 Lebenslauf

Name	Volker Herzig
Geburtsdatum	03.05.1974

Schulausbildung

1981 – 1985	Grundschule, Leonberg-Höfingen
1985 – 1991	Johannes-Kepler-Gymnasium, Leonberg; Abschluss: Mittlere Reife
1991 – 1994	Naturwissenschaftlich-Technisches Gymnasium, Kerschensteinerschule, Stuttgart-Feuerbach; Abschluss: Allgemeine Hochschulreife und staatlich geprüfter, chemisch-technischer Assistent (CTA)

Berufliche Tätigkeiten

1994-1996	Zivildienst
1996	Landeskriminalamt Baden–Württemberg, Referat Chemie: Anstellung als CTA für allgemein toxikologische Untersuchungen, insbesondere der Analyse von Rauschgift

Studium/ wissenschaftliche Ausbildung

1996	Grundstudium der Physik (Diplom), Eberhard-Karls-Universität Tübingen
1997-1999	Grundstudium der Biologie (Diplom), Eberhard-Karls-Universität Tübingen
1999	Vordiplom in Biologie
1999	Integriertes Auslandsstudium im Rahmen eines DAAD-Stipendiums an der Universität São Paulo (USP), Campus Ribeirão Preto (Brasilien)
2000-2001	Hauptstudium der Biologie (Diplom), Eberhard-Karls-Universität Tübingen (Hauptfach: Tierphysiologie, Nebenfächer: Zoologie und Pharmakologie).
2001	Diplomarbeit „Effects of state of nutrition, growth and sex on the quantity and composition of *Phoneutria nigriventer* (Keyserling, 1891) spider venom", angefertigt unter Anleitung von Prof. Dr. Wagner Ferreira dos Santos (Laboratorio de Neurobiologia e Peçonhas, Dept. de Biologia, FFCLRP-USP, Ribeirão Preto, Brasilien)
2001– 2004	Doktorarbeit „Die Rolle von Glutamat bei der Expression von konditioniertem Belohnungsverhalten unter Berücksichtigung des Einflusses der Amygdala", angefertigt unter Anleitung von Prof. Dr. Werner J. Schmidt (Abt. Neuropharmakologie, Zoologisches Institut, Fakultät für Biologie, Eberhard-Karls-Universität Tübingen, BRD)

Im Selbstverlag herausgegeben von:
Volker Herzig
Narzissenstr. 26
72108 Rottenburg

8 Anhang: einzelne Manuskripte

Testing the effectiveness of anti-craving drugs acamprosate and naloxone on expression of morphine or cocaine conditioned place preference by applying a repeated-testing schedule in rats

Volker Herzig and Werner J. Schmidt

Zoological Institute, Neuropharmacology, University of Tübingen, Germany

Abstract

Experiment 1:
It is well known that the social context powerfully influences behaviour. Here we examine whether it also influences drug-seeking. Further, acamprosate and naloxone have been discussed to affect different types of craving: acamprosate was reported to affect withdrawal-craving, while naloxone affects reward-craving. Conditioned drug-seeking was modelled using the conditioned place preference (CPP) paradigm. Socially isolated and group-housed rats were conditioned with morphine and their CPP expression was examined after treatment with acamprosate or naloxone. As a result, no difference in morphine-CPP expression was observed between differentially housed rats. Furthermore, CPP expression was not altered in both groups after treatment with acamprosate or naloxone. In conclusion, two weeks of isolation are not sufficient to alter the rewarding properties of morphine or the effects of treatment with acamprosate and naloxone in rats.

Experiment 2:
Clinical studies in weaned alcoholics revealed a superior effect of a joint administration of both acamprosate and naloxone in prevention of relapse. Furthermore, an effect of acamprosate on cocaine-conditioned behaviours has recently been reported. Emanating from the results of Exp. 1, only group-housed rats were either conditioned to cocaine or morphine. Thereafter, both groups were tested repeatedly for expression of CPP after acamprosate, naloxone or their joint administration. Additionally, the usefulness and suitability of repeated testing was elaborated. As a result, only acamprosate treatment inhibited expression of cocaine-CPP. Methodologically, repeated testing produced reasonable results and may therefore be considered a useful method to study the effects of anti-craving drugs on CPP expression, thereby reducing the number of experimental animals.

Keywords: conditioned place preference (CPP) expression, morphine, cocaine, naloxone, acamprosate, isolation.

Introduction

Acamprosate (calcium acetylhomotaurinate) has been shown to be effective against alcohol craving. In rodents, acamprosate suppressed the alcohol deprivation effect and reduced alcohol intake as well as some signs of physical alcohol withdrawal [30, for review see 31]. In weaned alcoholic patients, acamprosate was effective in reducing the probability of relapses. Animal experiments have shown that acamprosate may also counteract several aspects of morphine craving: acamprosate reduced the intensity of physical morphine-dependence in mice [28] and inhibited the motivational component of morphine withdrawal [12]. Furthermore, acamprosate suppressed expression [32] but not development [14] of morphine-

sensitization. This indicates that expression and development of morphine-sensitization are two different processes. A potential effect of acamprosate on development of cocaine-conditioned behaviours was recently reported [15]. As yet, acamprosate has not been tested on expression of cocaine- or morphine-conditioned behaviours.

Another drug with possible anti-craving properties is naloxone, a μ-opiate receptor antagonist that reduced alcohol-induced behaviours in animal experiments. Furthermore, the very similar μ-opiate receptor antagonist naltrexone was reported to reduce alcohol intake and to facilitate abstinence in patients [for review see 31]. Other animal experiments report that naloxone infusion into the ventral tegmental area or the periaqueductal gray blocked development of morphine place preference [23]. However, morphine-CPP expression was increased in both mice and rats after naloxone treatment [21, 22]. Additionally, cocaine-induced reinstatement of cocaine-seeking was not affected by naltrexone pretreatment [5]. The effectiveness of naloxone on expression of cocaine-conditioned behaviours has not yet been examined.

The joint administration of acamprosate and naloxone even produced a superior effect than both drugs given separately, resulting in a lower probability for relapse in alcoholic patients [9]. However, neither animal nor human studies examined a potential additive or synergistic effect of acamprosate plus naloxone in reduction of opiate- or cocaine-induced behaviors. Furthermore, acamprosate and naloxone are discussed to affect different types of craving: acamprosate was reported to affect withdrawal- or relief-craving craving, while naloxone affects reward-craving [3, 31]. Thus, both drugs may differ in their effectiveness within subgroups. Isolation that is also known as a factor that can induce alterations in the response to different drugs [16, 27, 36, 37] may therefore constitute a model to induce different types of craving.

In the present study a CPP paradigm was used as an animal model of context (cue)-induced drug-seeking. Experiment 1 addresses the question whether social isolation can affect morphine-seeking and whether different responses to treatment with acamprosate or naloxone can be observed compared to group-housed rats. Therefore, isolated and group-housed rats were conditioned to morphine and tested repeatedly for morphine-CPP expression after treatment with acamprosate or naloxone. Experiment 2 was designed to examine the effects of treatment with acamprosate, naloxone or their joint administration on expression of both cocaine- and morphine-CPP. For this purpose, rats were repeatedly tested for CPP expression, thereby elaborating the usefulness and suitability of repeated testing.

Material and methods

Animals

For experiment 1, 23 male Sprague-Dawley rats (animal breeding facility of the university of Tübingen, Germany; F1 generation of Charles River rats, Sulzfeld, Germany), weighing about 240-290 g at the experiment-start were used. For experiment 2, 24 male Sprague-Dawley rats (Charles River, Sulzfeld, Germany), weighing about 230-270 g were used. All rats were fed with 12 g of standard rat chow per rat and day, received water *ad libitum* and were kept under a 12 hour light-dark cycle (light-onset 8 a.m.) and the experiments were carried out during the light-phase. For experiment 1, a group of rats (isolated; n = 12) was individually housed (isolated two weeks prior to experiment), while the other group (group-housed; n = 11) and all rats for experiment 2 (morphine- and cocaine-group; both n = 12) were group-housed in groups of 5-6 rats.

CPP-apparatus

The experimental setup consisted of six CPP boxes (TSE Systems, Germany), each with three different coloured and textured chambers. One of the end chambers (both about 31 cm x 25 cm) had grey walls and a rough-textured floor, while the other had striped black and white walls and a smooth floor. The smaller, middle chamber (11 cm x 25 cm) had white walls and also a smooth floor. All chambers were equipped with photo sensors to detect the location of the rat and to measure the locomotion. Three CPP-boxes were placed with the grey-coloured chambers facing the room and the other three boxes with the grey-coloured chambers facing the wall. The walls separating the chambers (during conditioning), could be replaced by walls with open doors (during pretest and tests), to allow the rats to pass into the other chambers. During all tests, the middle chamber was always illuminated to reduce the time spent inside. According to the number of light-beam breaks, the locomotion and the times spent in each chamber were simultaneously determined. Thus, our CPP setup allows the analysis of treatment-effects on both CPP and locomotion (the latter might be important to recognize potential side-effects of the given treatment).

Drugs

All drugs were dissolved in physiological saline (0.9 % NaCl, Fresenius Kabi GmbH, Bad Homburg, Germany) and injected intraperitoneally (i.p.) in a volume of 1 ml/kg. Both morphine (morphine-sulphate, lot 20737, Th. Geyer, Renningen, Germany) and cocaine (cocaine-hydrochloride, lot L447362 931, Merck, Germany) were used doses of 10 mg/kg and applied 10 min prior to the start of the experiment. Acamprosate (Merck, Germany) was used at a dose of 200 mg/kg (injected 30 min before start) that proved most effective in preventing the motivational aspect of naloxone-induced morphine-withdrawal [12]. A dose of 2 mg/kg naloxone (naloxone hydrochloride, lot 16H1461, Sigma, Germany) was used (injected 10 min prior to the test), since it showed effectiveness in blocking morphine-CPP development [4, 18].

<u>Experiment 1: social isolation vs. group-housing</u>

Two-groups of rats underwent the same experimental treatment to examine the effect of different housing-conditions and to reveal potential differences in the effectiveness of acamprosate- or naloxone-treatment on expression of morphine-CPP.

Therefore, one group (n = 12) was individually housed (isolated 2 weeks prior to experiment), while the others were group-housed (n = 11). During pretest (day 1), all rats were placed for 20 min into the middle chamber without prior injection and with free access to all chambers. Thereafter, the later morphine-associated chamber was assigned to each rat in a counterbalanced manner. From days 2-9, eight days of conditioning sessions followed with closed doors for 30 min. During conditioning, all rats received 10 mg/kg of morphine and were then confined to one end-chamber on even days or to the other end-chamber on odd days after saline injection. The following tests were all carried out with open doors for 20 min. Expression of CPP was determined in the undrugged-state (i.e. after saline injection) on day 10. To account for the possible influence of extinction on the treatment outcome, half of the rats of each group received 200 mg/kg acamprosate before the test on day 11 and 2 mg/kg naloxone before the test on day 13, while the other rats of each group received both drugs in the opposite order. In between these tests, a challenge injection of morphine (10 mg/kg) was given on day 12 to maintain high CPP expression.

<u>Experiment 2: separate vs. joint treatment with acamprosate and naloxone</u>

The second experiment was designed to evaluate the effectiveness of acamprosate, naloxone or their joint administration on expression of cocaine- or morphine-CPP. Thereby, our newly

designed method for repeated testing the effect of different anti-craving drugs on CPP expression was established.

After three days of pretest, one group of rats (n = 12) was conditioned to morphine and the other group (n = 12) was conditioned to cocaine, both with five conditioning-pairings for 30 min. The other procedures were the same as in experiment 1. After conditioning, one day (day 14) without testing (rats remained in their home-cage without treatment) followed to overcome the regular schedule of drug and saline application that was used during conditioning. Thereafter, repeated tests were performed as shown in table 1.

***Table 1:* Repeated-testing-schedule**
The pretest-phase consisted of three pretests, followed by a conditioning-phase of ten days when cocaine or morphine (10 mg/kg i.p. of both) were applied alternately with saline to the respective groups. After one day (day 14) without testing (rats remained untreated in their home-cage), a repeated-testing schedule followed with four in-between drug- (= drug$_{1-4}$; i.e. treatment with 5 mg/kg i.p. of the respective conditioning drug) and saline- (= saline$_{1-4}$) tests and four tests with the potential anti-craving drug (= $\mathbf{X_{1-4}}$, i.e. saline (as internal control), acamprosate, naloxone or a combination of acamprosate plus naloxone; applied according to a Latin Square design, for details see materials and methods section).

Day	**1**	**2**	**3**	**4-13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**21**	**22**	**23**
tests	pretest1	pretest2	pretest3	conditioning	no test	test1	test2	test3	test4	test5	test6	test7	test8	test9	test10	test11	test12
treatment	-	-	-	drug or saline	-	drug$_1$	saline$_1$	$\mathbf{X_1}$	drug$_2$	saline$_2$	$\mathbf{X_2}$	drug$_3$	saline$_3$	$\mathbf{X_3}$	drug$_4$	saline$_4$	$\mathbf{X_4}$

pretest-phase (days 1–3) | conditioning-phase (days 4-13) | testing-phase (days 15–23)

The testing-schedule started with a test (day 15) in the drugged-state (i.e. after administration of the conditioning-drug), then a test (day 16) in the undrugged-state was performed and a test (day 17) after administration of one of four testing-drugs (i.e. saline, acamprosate, naloxone and combination of acamprosate plus naloxone) followed on the subsequent day. Thereafter, this testing scheme was repeated for three times that all rats received four tests in each the drugged- and the undrugged-state and four tests with the testing-drugs. The testing-drugs were treated according to a Latin square design in a way that one fourth of the rats of each group started the tests with one of the four testing-drugs. The Latin square design was applied in order to account for possible effects caused by CPP-extinction.

Data analysis
Statistical analysis was performed by using the program GB-Stat 7.0 (Dynamic Microsystems Inc.). In all statistical tests $p < 0.05$ was set as significant and $p < 0.01$ as high significant. Locomotion was calculated according to the number of light-beam breaks in all three chambers during the tests. The CPP values were calculated by subtracting the time spent in the saline-associated chamber from the time spent in the drug-associated chamber for each rat. For analysis of differences between the isolated and group-housed rats in experiment 1, a two-way ANOVA was used. Thereafter, locomotion and CPP data were analysed by repeated measures ANOVA followed by multiple post-hoc test (Dunn's Bonferroni correction) with pretest-CPP and saline-locomotion as references. The same statistical method was used for analysis of locomotion and CPP data of the cocaine- and morphine-conditioned rats in experiment 2. The average CPP of the three pretests in each group served as an internal control for within-group comparisons with the test-CPP (i.e. saline, acamprosate, naloxone and combination of acamprosate plus naloxone) and with the average CPP during the saline- and the cocaine- or morphine-sessions. For analysis of locomotion data (exp. 2), the average

saline-locomotion served as internal control. Rats that did not show stable CPP-expression during the saline-tests were excluded from statistical analysis. The criterion for stable CPP expression was reached if the average saline-CPP minus the average pretest-CPP was more than 100 s. Consequently, three rats from the isolated-group and four rats from the group-housed rats in experiment 1 were excluded from analysis, while four rats in the cocaine- and two rats in the morphine-group in experiment 2 were excluded.

Results

Experiment 1:

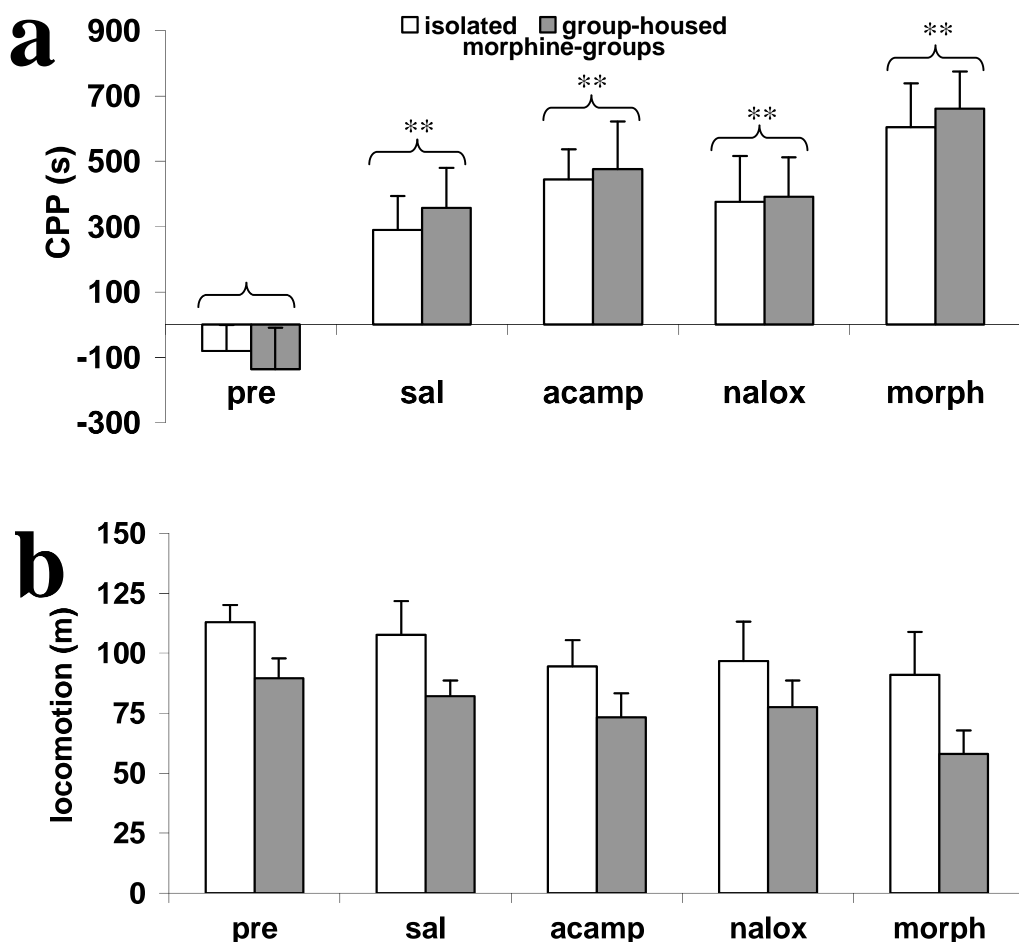

Figure 1: **Influence of housing conditions on morphine-CPP expression**
(**a**) Morphine-CPP expression (= time spent in morphine- minus time spent in saline-associated chamber) and (**b**) locomotion (in all three chambers) for isolated (n = 9) and group-housed (n = 7) rats during experiment 1. After pretest (pre) and conditioning (not indicated), rats were treated with saline (sal), acamprosate (acamp), naloxone (nalox) and morphine (morph). For detailed testing-order see materials and methods section. Data of isolated and group-housed rats have been merged for statistical analysis. ** $p < 0.01$ according to repeated measures ANOVA followed by Dunn's Bonferroni post-hoc comparison with pretest-CPP as reference; $^{++}$ $p < 0.01$ compared to saline-CPP. No significant difference in locomotion was observed after all treatments as compared to the saline-locomotion

The two-way ANOVA comparison of the CPP-expression during pretest and all tests between the group-housed and the isolated rats (fig. 1a) revealed a high significant effect of treatment ($F_{4,32} = 27.292$; $p < 0.0001$). However, no significant difference between both groups ($F_{1,8} = 0.035$; $p = 0.8566$) was observed and also no significant test vs. group interaction occurred

($F_{4,32}$ = 0.215; p = 0.9282). Because CPP responding to treatment did not differ between both groups, all rats were merged into one group for further statistical analysis. Thus, repeated measures ANOVA followed by multiple post-hoc tests (Dunn's Bonferroni correction) showed that a high significant morphine-CPP (p < 0.01) was expressed during all tests (i.e. saline, acamprosate, naloxone and morphine) as compared to the pretest CPP. As compared to the saline-CPP, high significant increase in CPP expression was observed during the morphine-test (p < 0.01).

In parallel to CPP results, a high significant effect of the tests (= different treatments) on locomotion (fig. 1b) was observed for both groups ($F_{4,32}$ = 6.439; p = 0.0006). Furthermore, a significant difference in locomotion between both groups occurred ($F_{1,8}$ = 7.159; p = 0.0281), but no test vs. group interaction was found ($F_{4,32}$ = 0.292; p = 0.8808). Since locomotion responding after different treatments did not differ between both groups, further statistical analysis was done with the merged group. Repeated measures ANOVA and post-hoc tests revealed no significant alteration in locomotion during all treatments (all p > 0.05) as compared to the saline locomotion.

Experiment 2:

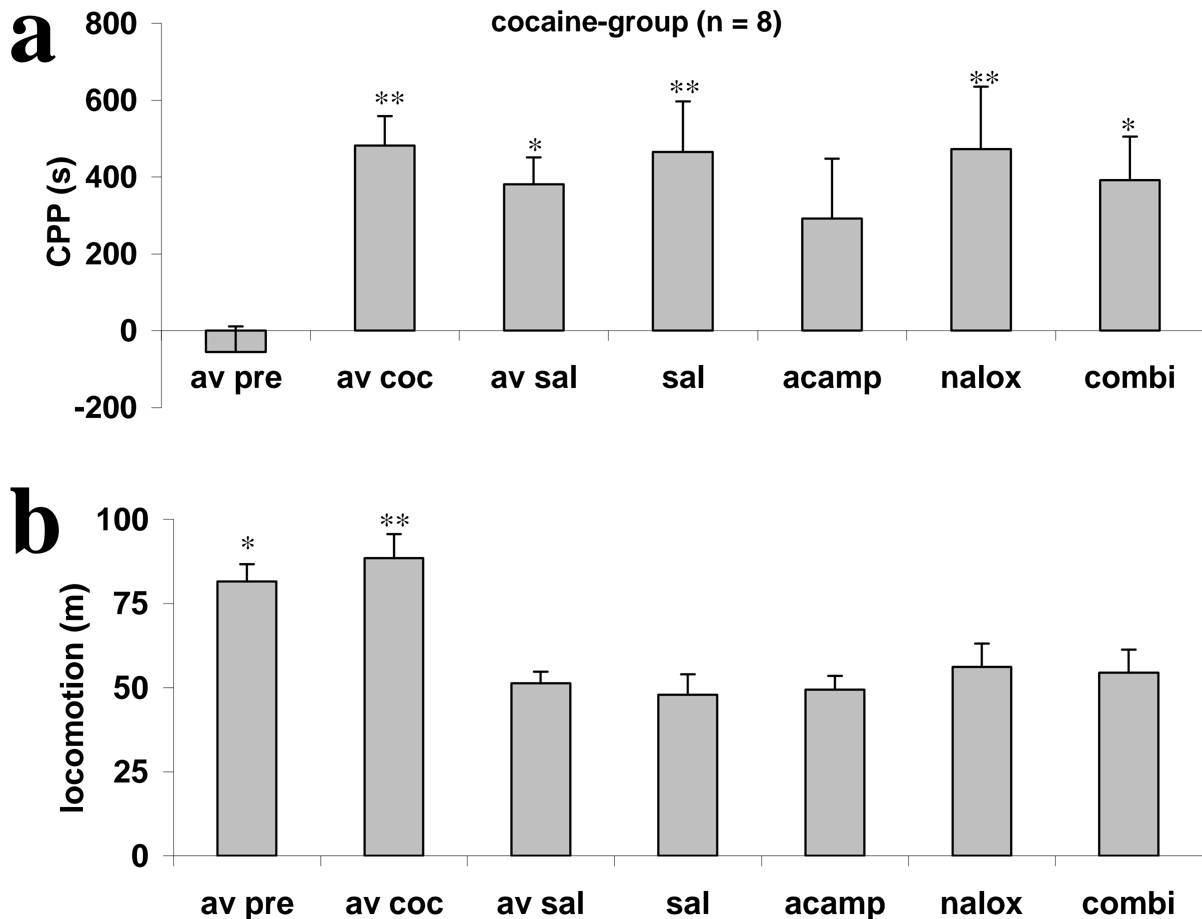

Figure 2: **Influence of treatment on cocaine-CPP expression**

(**a**) Cocaine-CPP expression (= time spent in cocaine- minus time spent in saline-associated chamber) and (**b**) locomotion (in all three chambers) in the cocaine-group (n = 8) during experiment 2. After three pretests (the average is indicated as av pre) and conditioning (not indicated), several repeated tests followed (for detailed testing-order see tab. 1 and materials and methods section). The average values of the in-between saline- (av sal) and cocaine-tests (av coc) are shown. As potential anti-craving drugs, acamprosate (acamp), naloxone (nalox), their joint administration (combi) and saline (sal, as internal control) have been tested. * p < 0.05 or ** p < 0.01 according to repeated measures ANOVA followed by Dunn's Bonferroni post-hoc comparison with (**a**) average pretest-CPP or (**b**) average saline-locomotion as reference.

In the cocaine-group (fig. 2a), repeated measures ANOVA comparing CPP expression of average pretest with CPP during the substance tests and during the average (of four) saline- and cocaine-tests, showed a high significant effect ($F_{6,42} = 4.888$; $p = 0.0007$). Multiple post-hoc tests (Dunn's Bonferroni correction) revealed significant CPP expression during the average saline- and the combination (acamprosate plus naloxone)-tests ($p < 0.05$) and high significant CPP expression ($p < 0.01$) during the average cocaine-, the saline- and the naloxone-tests. For the acamprosate-test, no significant difference compared to the average pretest CPP was found ($p > 0.05$). In the morphine-group (fig. 3a), a high significant effect of repeated testing was observed ($F_{6,54} = 9.486$; $p < 0.0001$). Multiple post-hoc tests (CPP during average pretest as reference) showed high significant CPP expression ($p < 0.01$) during all tests. A separate analysis (repeated measures ANOVA) of the in-between drug- and saline-tests revealed no time-dependent decrease in CPP-expression (= extinction) by repeated testing in both the cocaine- (cocaine-tests: $F_{3,21} = 0.166$; $p = 0.9181$; saline-tests: $F_{3,21} = 0.167$; $p = 0.9174$) and the morphine-group (morphine-tests: $F_{3,27} = 0.792$; $p = 0.5092$; saline-tests: $F_{3,27} = 1.891$; $p = 0.1549$).

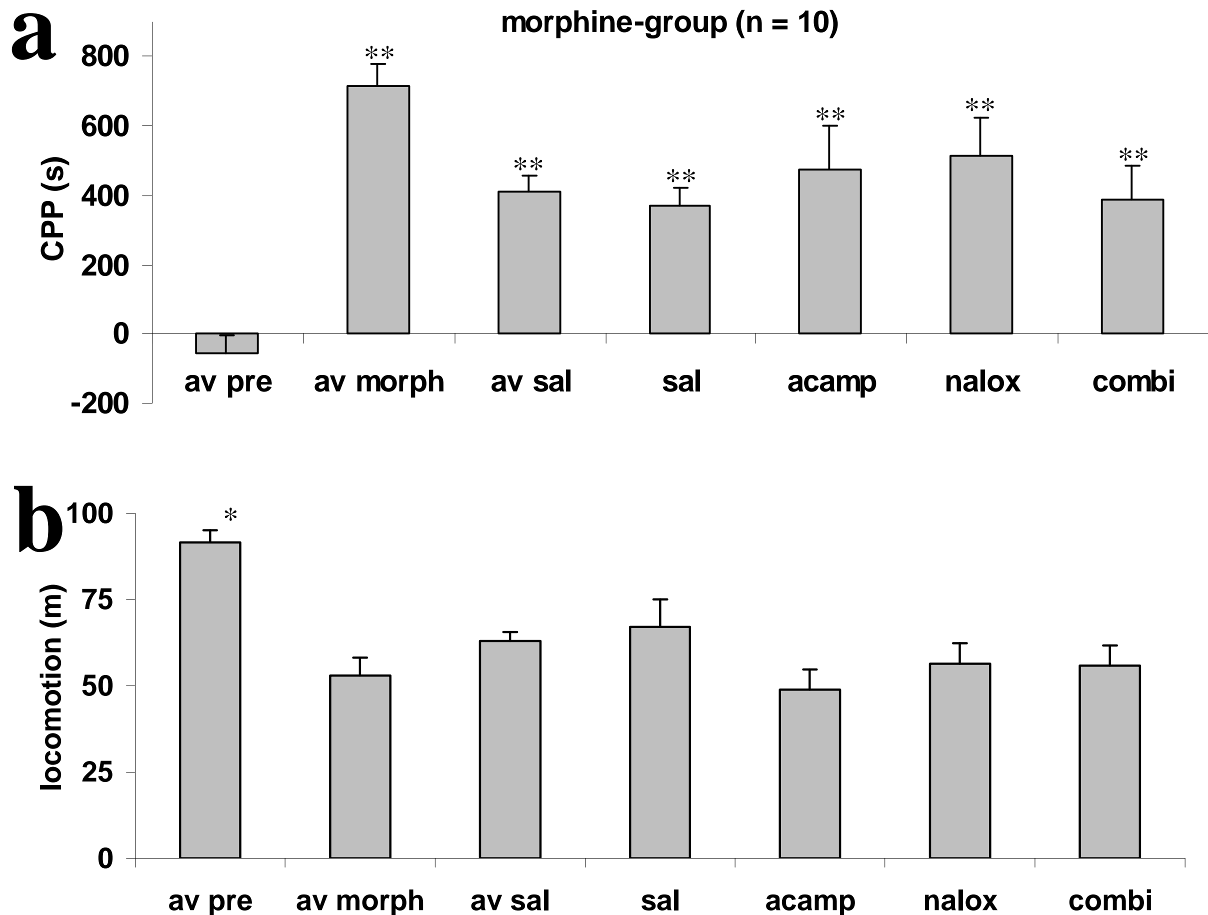

Figure 3: **Influence of treatment on morphine-CPP expression**

(**a**) Morphine-CPP expression (= time spent in morphine- minus time spent in saline-associated chamber) and (**b**) locomotion (in all three chambers) in the cocaine-group (n = 10) during experiment 2. After three pretests (the average is indicated as av pre) and conditioning (not indicated), several repeated tests followed (for detailed testing-order see tab. 1 and materials and methods section). The average values of the in-between saline- (av sal) and morphine-tests (av morph) are shown. As potential anti-craving drugs, acamprosate (acamp), naloxone (nalox), their joint administration (combi) and saline (sal, as internal control) have been tested. * $p < 0.05$ or ** $p < 0.01$ according to repeated measures ANOVA followed by Dunn's Bonferroni post-hoc comparison with (**a**) average pretest-CPP or (**b**) average saline-locomotion as reference.

The parallel analysis of the locomotion data in the cocaine-group (fig.2b) showed a high significant effect ($F_{6,42} = 6.161$; $p = 0.0001$) of the repeated tests and multiple post-hoc tests (Dunn's Bonferroni correction) revealed significantly increased locomotion (as compared to average saline locomotion) during the average pretest ($p < 0.05$) and high significant increased locomotion during the average cocaine-test ($p < 0.01$). In the morphine-group (fig. 3b), also a high significant effect of repeated testing was found ($F_{6,54} = 6.492$; $p < 0.0001$). Post-hoc tests revealed a high significant increase in locomotion only during the average pretest ($p < 0.05$).

Discussion

Usefulness of repeated-testing of CPP expression

In both experiments, the rats were repeatedly tested in the CPP paradigm for CPP-expression: Four repeated tests have been performed in experiment 1 and twelve repeated tests (see tab.1) were carried out in experiment 2. An advantage of repeated testing is that different anti-craving drugs can be compared for their effectiveness to reduce drug-CPP expression within the same rats. Additionally, different doses of one anti-craving drug may also be compared within the same rats, enabling a more favourable statistical comparison (within-group comparison instead of between group comparison). Importantly, repeated testing can reduce the overall number of required experimental animals. This argument becomes more and more important considering the increasingly difficult ethical justification of animal experiments.

One important argument against repeated testing of the same rats in a CPP paradigm is that drug-CPP expression declines continuously due to extinction processes. For experiment 1, four subsequent CPP tests have been carried out, that are not expected to produce large extinction. For extinction of cocaine-CPP expression, Mueller and Stewart [19] showed that significant extinction-induced effects were only observed after eight subsequent tests under saline. For experiment 2, twelve subsequent tests have been carried out. However, no significant extinction was observed (as controlled during the in-between drug- and saline-tests) in both groups. The observed stability of CPP expression might be explained by the rather high conditioning-doses of cocaine and morphine (both 10 mg/ kg) and the rather long conditioning-phase (5 pairings) that produced a strong conditioning. Furthermore, the lack of extinction could have been caused by the application of intermediate drug-tests that maintained CPP-expression on a high level [19,20]. However, one could argue that by repeatedly testing CPP expression in the drugged-state (when rats have access to all chambers), the entire CPP-box becomes conditioned to the drug. Yet, this should result in a decrease of CPP-expression after repeated tests that was not observed. In experiment 2, no significant extinction of CPP expression was observed during the undrugged- and the drugged-tests in both the cocaine- and the morphine-group, what reflects the stability of CPP expression. Thus, we conclude that intermediate tests in the drugged-state can maintain strong CPP expression (for a certain period of time, in our study for at least 12 testing days) enabling to conduct several repeated tests. Since always one test in the undrugged-state separated the tests in the drugged-state from the anti-craving drug tests, possible effects induced by acute drug-withdrawal are minimized. Furthermore, the undrugged-tests together with the drugged-tests provide control-values for a stable CPP-expression.

Another argument against repeated testing of different anti-craving drugs (in the present study: acamprosate, naloxone and the combination of acamprosate and naloxone) is that the drug tested first might influence (e.g. due to induction of tolerance or sensitization) the effect for the drug tested afterwards. Such effects cannot be completely excluded by the present

testing-schedule (tab. 1), but the applied Latin square design (with internal saline-control tests) minimizes the influence that such effects exert on the behavioural outcome (i.e. on expression of CPP). Since there were always a drugged and an undrugged test between two anti-craving drug tests, only anti-craving drugs with very long half-lifes can still be present in a behaviourally relevant amount to directly affect the outcome of the subsequent substance test. However, in that case the CPP-expression during the in-between drug- and saline-tests (that function as controls), should also be affected. In summary, we conclude that the presented repeated-testing schedule may be useful for repeatedly testing different anti-craving drugs (or different doses of these drugs) on their effect on CPP-expression. Regarding the observed effects after anti-craving drug treatment (see the following discussion for the effects of acamprosate or naloxone), reasonable results as compared to the literature were obtained. Thus, the application of repeated testing may be helpful in reducing the overall number of experimental animals used for future CPP studies.

Influence of the housing-conditions on CPP expression

The main result of experiment 1 is that the housing conditions do not influence development or expression of morphine-CPP. Furthermore, neither acamprosate nor naloxone significantly altered expression of morphine-CPP. The significant effect of housing-conditions on locomotion reflects that group-housed rats exhibit a more pronounced decrease in locomotion due to habituation (caused by repeated testing) than isolated-rats. This result is consistent with previous findings that reported a slower habituation in isolated rats [17], indicating that the novelty of a testing environment stimulates isolated rats for a longer time. Regarding the effects of different housing conditions on morphine-reward, in contrast to our findings, a decrease of morphine’s reinforcing properties by social isolation was reported [37]. A similar decrease in the reinforcing properties was reported for amphetamine [36] and heroin [27]. However, these studies [27, 36, 37] examined (male Long Evans or Lister hooded) rats that have been isolated from weaning and doses of 1 and 5 mg/kg (s.c.) morphine have been used. Thus, the observed lack of effect of social isolation on development of CPP in the present study could be due to the rather short isolation-period that was applied (two weeks), or due to the higher dose of morphine, or because another strain of rats has been used. However, by using a two week isolation period, another study from our laboratory already showed differences in development of MDMA- (“ecstasy”) CPP [16]. In that study, a CPP for MDMA was only found in isolated rats, but not in the group-housed. Yet, for mice conditioned to morphine, only 30 but not 15 days of isolation were effective in reducing CPP development [6]. Thus, we conclude that the two week isolation period in the present study was not sufficient to alter the rewarding properties of morphine, at least in male Sprague-Dawley rats. Acamprosate or naloxone did also not show any effect on morphine-CPP expression in both the isolated and group-housed rats. Thus, further experiments using a longer isolation period are suggested to clarify possible differences due to housing conditions in the effectiveness of these substances to affect morphine-CPP expression.

Influence of acamprosate on CPP expression

In the present studies, acamprosate reduced cocaine- but not morphine-CPP expression. The effects of acamprosate on cocaine-CPP expression cannot be explained by an altered locomotion due to acamprosate. Neither acamprosate, nor naloxone or combination treatment significantly altered locomotion in the cocaine- or the morphine-conditioned rats (as compared to the locomotion during the average saline-tests). The only significant effects on locomotion that have been observed in experiment 2 are the increased locomotion during the average pretest in both groups and the increased locomotion during the average cocaine-test in the cocaine-group. An increased locomotion during pretest (compared to saline) can be explained by the initial novelty of the CPP boxes, resulting in a high exploration behaviour

that vanishes due to habituation after repeated testing in the same environment. The increased locomotion during the cocaine-tests on the other hand is caused by the acute psychostimulant properties of cocaine. Thus, we conclude that the observed effects regarding the locomotion do not correlate with the observed reduction of cocaine-CPP expression by acamprosate. Furthermore, the reduction of cocaine-CPP expression by acamprosate seems rather weak, since CPP expression was not significantly different from the average saline CPP expression ($p > 0.05$). Nevertheless, acamprosate-treatment inhibited cocaine-CPP expression (i.e. not significantly different from average pretest CPP), therefore we assume a weak effect of acamprosate on cocaine-CPP expression.

Acamprosate has been tested as a potential anti-craving drug in cocaine- and morphine-conditioned rats, because it proved to be effective in clinical trials with alcoholic patients as well as in several animal experiments on alcohol-induced behaviours [for review see 31]. Acamprosate by itself neither produces CPP, nor conditioned place aversion in both mice [15] and rats [13], indicating a low abuse potential and underlining the clinical safety of acamprosate. The observed inhibition of cocaine-CPP expression is consistent with results from mice showing that acamprosate can inhibit development of CPP to cocaine and ethanol, but not to morphine [15]. Consequently, acamprosate inhibits both development and expression of cocaine-CPP, while both aspects of morphine-CPP are not affected [see 15 and the present data]. Additionally, acamprosate showed no effect on heroin self-administration, heroin- and stress-induced relapse and development of morphine-sensitization [14, 32] and the discriminative stimulus properties of morphine were also not affected by acamprosate [24]. However, acamprosate suppressed expression of morphine-sensitization [32] and inhibited the motivational component of morphine withdrawal [12]. Furthermore, the intensity of physical morphine-dependence was also reduced by acamprosate in mice [28]. Thus, acamprosate only alters specific aspects of opiate-induced behaviours, while leaving others unaffected. However, our findings that acamprosate does not affect expression of morphine-CPP fit to the conclusion drawn by Spanagel et al. [32] that acamprosate may not be effective in treatment of cue-induced relapse behavior in opiate addicts.

A possible mechanism of acamprosate that accounts for the inhibition of cocaine-CPP could be its functional NMDA-receptor antagonistic profile, as it has been suggested for ethanol-dependent rats [1]. In line with this hypothesis, the non-competitive NMDA-receptor antagonists MK-801 and memantine prevented expression of psychostimulant-CPP (amphetamine-CPP in [35]; cocaine-CPP in [11]). Furthermore, acamprosate, memantine and MK-801 showed an almost identical up-regulation pattern of NMDA-receptor subunits [26]. The weak antagonism of acamprosate on NMDA-receptors [26] may fit to the weak effect observed on cocaine-CPP expression in the present study. However, MK-801 and memantine also blocked morphine-CPP expression [25, 35]. Hence, the hypothesis that the observed effects of acamprosate on cocaine-CPP expression are only due to its NMDA-receptor antagonistic properties is challenged. Another possible way for acamprosate to affect cocaine-CPP could be via the discussed interaction with voltage-gated calcium channels [31]. However, the calcium channel antagonist isradipine did not affect expression of cocaine-CPP [7]. Yet, in combination with naltrexone, that by itself was not effective, isradipine blocked cocaine-CPP expression [7]. Thus, calcium channel blockade alone does not seem to be sufficient to affect cocaine-CPP expression. In summary, based on the present study and the cited literature, it is difficult to draw a clear conclusion concerning the possible mechanism of acamprosate and neither its NMDA-receptor antagonistic properties nor the calcium channel blockade alone can completely explain why cocaine- but not morphine-CPP expression is inhibited by acamprosate. However, distinct neural mechanisms for secondary cocaine- and morphine-reward are implicated.

Influence of naloxone on CPP expression

In some studies, naloxone itself did not alter place preference [4] while it induced conditioned place aversion in other studies [8, 18]. Cocaine-induced reinstatement of cocaine-seeking was not affected by naltrexone pretreatment [5], while morphine- [33] and heroin-induced [29] relapse to heroin self-administration was reduced by naltrexone. On the other hand, heroin-seeking was not affected by naloxone pre-treatment [2]. Furthermore, an increase of morphine-CPP expression in mice and rats after naloxone treatment has been demonstrated [21, 22]. Therefore, the present findings that naloxone-treatment did not reduce CPP expression to both cocaine and morphine and the slightly (but not significantly) increased morphine-CPP expression after naloxone treatment nicely fit to the quoted literature. A possible explanation why naloxone, if showing any effect at all, increases morphine-CPP expression is given by Noble et al. [22]: they stated that this illustrates the negative motivational properties of morphine withdrawal or the establishment of psychic drug dependence. We agree that naloxone-induced withdrawal is likely to increase craving for the drug, which is reflected by increased morphine-CPP expression.

Influence of acamprosate-naloxone-combination on CPP expression

Concerning the acamprosate-naloxone combination treatment, a recent study demonstrated that the combination had a superior effect in relapse prevention of alcoholism than both drugs given separately [9]. On the other hand, preclinical studies using rats did not find any additive or synergistic effects of the combined treatment in reducing alcohol consumption [34]. However, a recent study found that in mice, only a high dose of naltrexone alone was effective in reducing alcohol consumption, but by combining it with acamprosate, also the lower naltrexone dose reduced alcohol consumption [10]. Thus, a possible facilitating effect of acamprosate on the naltrexone effect may be assumed. The results of the present study, however, suggest that the combined treatment may not show a superior effect in relapse prevention of cocaine- and morphine-addiction.

In summary, two weeks of isolation were not sufficient to alter the rewarding properties of morphine in rats. Acamprosate reduced cocaine- but not morphine-CPP expression without altering locomotion. Naloxone and the joint administration of acamprosate and naloxone did not significantly affect expression of both cocaine- and morphine-CPP. Methodologically, repeated-testing produced reasonable results and may help in reducing the number of experimental animals.

Acknowledgements

This study was funded by the federal ministry for education and research 01EB0110-Baden-Württemberg consortium for addiction research.

References

[1] al Qatari M, Bouchenafa O, and Littleton J. Mechanism of action of acamprosate. Part II. Ethanol dependence modifies effects of acamprosate on NMDA receptor binding in membranes from rat cerebral cortex. Alcohol Clin Exp Res 1998;22: 810-814.

[2] Alderson HL, Robbins TW, and Everitt BJ. Heroin self-administration under a second-order schedule of reinforcement: acquisition and maintenance of heroin-seeking behaviour in rats. Psychopharmacol 2000;153: 120-133.

[3] Anton RF, Kranzler HR, and Meyer RE. Neurobehavioral aspects of the pharmacotherapy of alcohol dependence. Clin Neurosci 1995;3: 145-154.

[4] Bardo MT, and Neisewander JL. Single-trial conditioned place preference using intravenous morphine. Pharmacol Biochem Behav 1986;25: 1101-1105.

[5] Comer SD, Lac ST, Curtis LK, and Carroll ME. Effects of buprenorphine and naltrexone on reinstatement of cocaine- reinforced responding in rats. J Pharmacol Exp Ther 1993;267: 1470-1477.

[6] Coudereau J-P, Debray M, Monier C, Bourre J-M, and Frances H. Isolation impairs place preference conditioning to morphine but not aversive learning in mice. Psychopharmacol 1997;130:117-123.

[7] Cramer CM, Hubbell CL, and Reid LD. A combination of isradipine and naltrexone blocks cocaine's enhancement of a cocaine place preference. Pharmacol Biochem Behav 1998;60: 847-853.

[8] Iwamoto ET. Place-conditioning properties of mu, kappa, and sigma opioid agonists. Alcohol Drug Res 1985;6: 327-339.

[9] Kiefer F, Jahn H, Tarnaske T, Helwig H, Briken P, Holzbach R, Kampf P, Stracke R, Baehr M, Naber D, and Wiedemann K. Comparing and combining naltrexone and acamprosate in relapse prevention of alcoholism: a double-blind, placebo-controlled study. Arch Gen Psychiat 2003;60: 92-99.

[10] Kim SG, Han BD, Park JM, Kim MJ, and Stromberg MF. Effect of the combination of naltrexone and acamprosate on alcohol intake in mice. Psychiat Clin Neurosci 2004;58: 30-36.

[11] Kotlinska J, and Biala G. Memantine and ACPC affect conditioned place preference induced by cocaine in rats. Pol J Pharmacol 2000;52: 179-185.

[12] Kratzer U, and Schmidt WJ. The anti-craving drug acamprosate inhibits the conditioned place aversion induced by naloxone-precipitated morphine withdrawal in rats. Neurosci Lett 1998;252: 53-56.

[13] Kratzer U, and Schmidt WJ. Acamprosate does not induce a conditioned place preference and reveals no state-dependent effects in this paradigm. Progr Neuro-Psychopharmacol & Biol Psychiat 2003;27: 653-656.

[14] Kratzer U, Spanagel R, and Schmidt WJ. The effect of acamprosate on the development of morphine-induced behavioral sensitization in rats. Behav Pharmacol 2003;14: 351-356.

[15] McGeehan AJ, and Olive MF. The anti-relapse compound acamprosate inhibits the development of a conditioned place preference to ethanol and cocaine but not morphine. Br J Pharmacol 2003;138: 9-12.

[16] Meyer A, Mayerhofer A, Kovar KA, and Schmidt WJ. Rewarding effects of the optical isomers of 3,4-methylenedioxy- methylamphetamine ('Ecstasy') and 3,4-methylenedioxy-ethylamphetamine ('Eve') measured by conditioned place preference in rats. Neurosci Lett 2002;330: 280-284.

[17] Morgan MJ, Einon DF, and Nicholas DJ. The effects of isolation rearing on behavioural inhibition in the rat. Q J Exp Psychol 1975;27: 615-634.

[18] Mucha RF, van der KD, O'Shaughnessy M, and Bucenieks P. Drug reinforcement studied by the use of place conditioning in rat. Brain Res 1982;243: 91-105.

[19] Mueller D, and Stewart J. Cocaine-induced conditioned place preference: reinstatement by priming injections of cocaine after extinction. Behav Brain Res 2000;115: 39-47.

[20] Mueller D, Perdikaris D, and Stewart J. Persistence and drug-induced reinstatement of a morphine-induced conditioned place preference. Behav Brain Res 2002;136: 389-397.

[21] Neisewander JL, Pierce RC, and Bardo MT. Naloxone enhances the expression of morphine-induced conditioned place preference. Psychopharmacol 1990;100: 201-205.

[22] Noble F, Fournie-Zaluski MC, and Roques BP. Unlike morphine the endogenous enkephalins protected by RB101 are unable to establish a conditioned place preference in mice. Eur J Pharmacol 1993;230: 139-149.

[23] Olmstead MC, and Franklin KB. The development of a conditioned place preference to morphine: effects of microinjections into various CNS sites. Behav Neurosci 1997;111: 1324-1334.

[24] Pascucci T, Cioli I, Pisetzky F, Dupre S, Spirito A, and Nencini P. Acamprosate does not antagonise the discriminative stimulus properties of amphetamine and morphine in rats. Pharmacol Res 1999;40: 333-338.

[25] Popik P, Wrobel M, Rygula R, Bisaga A, and Bespalov AY. Effects of memantine, an NMDA receptor antagonist, on place preference conditioned with drug and nondrug reinforcers in mice. Behav Pharmacol 2003;14: 237-244.

[26] Rammes G, Mahal B, Putzke J, Parsons C, Spielmanns P, Pestel E, Spanagel R, Zieglgansberger W and Schadrack J. The anti-craving compound acamprosate acts as a weak NMDA-receptor antagonist, but modulates NMDA-receptor subunit expression similar to memantine and MK-801. Neuropharmacology 2001;40: 749-760.

[27] Schenk S, Hunt T, Colle L, and Amit Z. Isolation versus grouped housing in rats: differential effects of low doses of heroin in the place preference paradigm. Life Sci 1983;32: 1129-1134.

[28] Sepulveda J, Ortega A, Zapata G, and Contreras E. Acamprosate decreases the induction of tolerance and physical dependence in morphine-treated mice. Eur J Pharmacol 2002;445: 87-91.

[29] Shaham Y, and Stewart J. Effects of opioid and dopamine receptor antagonists on relapse induced by stress and re-exposure to heroin in rats. Psychopharmacol 1996;125: 385-391.

[30] Spanagel R, Holter SM, Allingham K, Landgraf R, and Zieglgansberger W. Acamprosate and alcohol: I. Effects on alcohol intake following alcohol deprivation in the rat. Eur J Pharmacol 1996;305: 39-44.

[31] Spanagel R, and Zieglgansberger W. Anti-craving compounds for ethanol: new pharmacological tools to study addictive processes. Trends Pharmacol Sci 1997;18: 54-59.

[32] Spanagel R, Sillaber I, Zieglgansberger W, Corrigall WA, Stewart J, and Shaham Y. Acamprosate suppresses the expression of morphine-induced sensitization in rats but does not affect heroin self-administration or relapse induced by heroin or stress. Psychopharmacol 1998;139: 391-401.

[33] Stewart J. Conditioned and unconditioned drug effects in relapse to opiate and stimulant drug self-adminstration. Prog Neuropsychopharmacol Biol Psychiat 1983;7: 591-597.

[34] Stromberg MF, Mackler SA, Volpicelli JR, and O'Brien CP. Effect of acamprosate and naltrexone, alone or in combination, on ethanol consumption. Alcohol 2001;23: 109-116.

[35] Tzschentke TM, and Schmidt WJ. Interactions of MK-801 and GYKI 52466 with morphine and amphetamine in place preference conditioning and behavioural sensitization. Behav Brain Res 1997;84: 99-107.

[36] Wongwitdecha N, and Marsden CA. Isolation rearing prevents the reinforcing properties of amphetamine in a conditioned place preference paradigm. Eur J Pharmacol 1995;279: 99-103.

[37] Wongwitdecha N, and Marsden CA. Effect of social isolation on the reinforcing properties of morphine in the conditioned place preference test. Pharmacol Biochem Behav 1996;53: 531-534.

Effects of MPEP on locomotion, sensitization and conditioned reward induced by cocaine or morphine

Volker Herzig and Werner J. Schmidt

Zoological Institute, Neuropharmacology, University of Tübingen, Germany

Abstract

Exposure to environmental cues is considered a major cause of relapse in detoxified addicts. Recent findings showed an involvement of glutamate in cue-induced relapse and suggest that subtype 5 of metabotropic glutamate receptors (mGluR5) is involved in conditioned drug-reward. The present study applied the conditioned place preference (CPP) paradigm to examine the involvement of mGluR5 in cocaine- and morphine-induced behaviours. Results of previous mice-studies were extended into rats by using the selective mGluR5 antagonist 2-methyl-6-(phenylethynyl)pyridine (MPEP). As a result, the evaluated behavioural parameters were dose-relatedly affected by MPEP. Low-dosed MPEP (10 mg/kg, i.p.) did not affect spontaneous locomotion, reduced cocaine-induced hyperlocomotion and produced sensitized locomotion, while showing no effect on sensitized locomotion induced by repeated cocaine or morphine. Low-dosed MPEP did not genuinely block development of cocaine- and morphine-CPP, but rendered CPP expression state-dependent. The medium MPEP-dose (30 mg/kg) was most effective in reducing spontaneous locomotion. The high MPEP-dose (50 mg/kg) was most effective in reducing both body-weight and morphine-CPP expression. Cocaine-CPP expression was not affected by any MPEP-dose. In conclusion, mGluR5 are involved in modulation of spontaneous and cocaine-induced locomotion, in state-dependent learning and in expression of morphine-CPP. Thus, MPEP may be beneficial for relapse prevention in morphine-addicts.

Keywords: MPEP, metabotropic glutamate receptors subtype 5 (mGluR5), cocaine, morphine, locomotion, sensitization, conditioned place preference (CPP), secondary reward, state-dependency, body-weight.

Introduction

For a long time in addiction research, dopamine was considered as the most important neurotransmitter involved. Recent research, however, demonstrates that glutamate plays an equally important role in processes underlying addiction (for reviews see Tzschentke and Schmidt, 2003; Kalivas, 2004). Additionally, glutamate plays a role in sensitization, i.e. the intensification of behaviour upon repeated treatment (Pierce et al., 1996; Wolf, 1998; Shippenberg et al., 2000; Vanderschuren and Kalivas, 2000). Sensitization has been linked to addiction by the hypothesis that repeated drug administration increases the incentive salience of the administered drug and of the context, finally leading to loss of control of drug taking (for reviews see Wolf, 1998; Robinson and Berridge, 1993; Tzschentke and Schmidt, 2003). However, despite many studies showing sensitization in animal experiments, only few studies provide evidence for sensitization in humans (see for example Strakowski et al., 1996).

An involvement of glutamate in secondary cocaine-reward was reported (Slusher et al., 2001) and a direct effect of cocaine-associated stimuli on the glutamate release in the Nucleus accumbens (Nac) was demonstrated by Hotsenpiller et al. (2001). This is important, since

presentation of environmental stimuli associated with a drug can induce relapse (Childress et al., 1999; Ito et al., 2000), which is a major problem for detoxified drug addicts trying to stay clean. Repeated cocaine administration followed by extinction induced changes in the expression of mRNA for ionotropic glutamate receptors of the AMPA- and NMDA- subtype in reward related brain regions such as Nac core and shell, striatum, ventral tegmental area and prefrontal cortex (Ghasemzadeh et al., 1999). The expression of drug-conditioned behaviours depends on AMPA/Kainate receptors in the Nac (Cornish and Kalivas, 2000; Di Ciano et al., 2001; Hotsenpiller et al., 2001). NMDA receptors on the other hand are involved in the acquisition of conditioned behaviours (Tzschentke and Schmidt, 1997; Di Ciano et al., 2001). Recently metabotropic glutamate receptors (mGluR) came into focus of addiction research, when Chiamulera et al. (2001) demonstrated on mGluR subtype 5 (mGluR5) knockout (k.o.) mice that mGluR5 are essential for both cocaine-induced hyperactivity and cocaine's primary rewarding properties.

The involvement of mGluR5 in secondary rewarding effects of cocaine and morphine is less clear. A recent study demonstrated that 2-methyl-6-(phenylethynyl)pyridine (MPEP), a selective antagonist for mGluR5, blocked acquisition and expression of conditioned place preference (CPP) for morphine in mice (Popik and Wrobel, 2002). McGeehan and Olive (2003) on the other hand reported no effect of MPEP on development of morphine-CPP in mice, while development of cocaine-CPP was inhibited. In order to provide more experimental data about the involvement of mGluR5 in development and expression of secondary drug reward and to extent previous findings from mice into rats, the present study used MPEP in the place preference paradigm to selectively block mGluR5 transmission in rats. MPEP has so far been reported to selectively block mGluR5 while neither affecting other types of glutamatergic receptors nor several other types of neurotransmitter receptors (see Spooren et al., 2000). Systemic MPEP rapidly penetrates the blood-brain-barrier and shows full mGluR5 blockade in rats 5-60 min after application and keeps its selectivity for mGluR5 up to doses of 50 mg/kg (Anderson et al., 2002). The present study used MPEP to determine the involvement of mGluR5 in spontaneous and drug-induced locomotion and in development of cocaine- and morphine-induced sensitization of locomotion. Furthermore, the involvement of mGluR5 in development and expression of cocaine- or morphine-CPP was studied.

Material and methods

Animals

All animal experiments were conducted in accordance with the principles of animal care and the national laws on animal experiments. All efforts were made to minimise animal suffering and to reduce the number of animals used. For this study 64 male Sprague-Dawley rats (Charles River, Sulzfeld, Germany), weighing about 220-270 g at the beginning of the experiment, were used. Rats were fed with 12 g of standard rat chow per rat and day and received water ad libitum. All rats were naïve and housed in groups of 5-7 animals. They were kept under a 12 hour light-dark cycle with lights on at 7 a.m. and the experiments were carried out during the light-phase. All rats were allowed to habituate to the colony room for nearly two weeks, and they were habituated to handling three times prior to the experiments.

Drugs

All drugs were dissolved in physiological saline (0.9 % NaCl, Fresenius Kabi GmbH, Bad Homburg, Germany) and injected i.p. in a volume of 1 ml/kg. For determination of the appropriate amount of drug that had to be injected, the weight of each rat was measured every day before the experiment-start. The selective mGluR5 antagonist MPEP was supplied by

Novartis Pharma AG (Basel, Switzerland) and doses of 10, 30 and 50 mg/kg were used. MPEP was always injected 30 min prior to the start of the CPP-experiment. Both morphine (morphine-sulphate, lot 20737, Th. Geyer, Renningen, Germany) and cocaine (cocaine-hydrochloride, lot L447362 931, Merck, Germany) were used at a dose of 10 mg/kg and applied 10 min prior to the start of the experiment. Generally each rat received two daily injections and in case no drug was applied (e.g. on saline conditioning-days), two saline-injections were applied.

CPP-apparatus

The experimental CPP setup consisted of six boxes (TSE Systems, Germany), each with three different coloured and textured chambers. One of the end chambers (both about 31 cm x 25 cm) had grey walls and a rough-textured floor, while the other had striped black and white walls and a smooth floor. The smaller, middle chamber (11 cm x 25 cm) had white walls and a smooth floor. The walls separating the chambers (during conditioning), could be replaced by walls with open doors (during tests), to allow the rats to pass into the other chambers. The size of the doors was about 11.5 cm in height and 10 cm in width. Three CPP-boxes were placed with the grey-coloured chambers facing the room and the other three boxes with the grey-coloured chambers facing the wall. All chambers were equipped with photo sensors (four in the middle chamber and 11 in each of the end chambers) to detect the location of the rat to analyse the time spent in each chamber. Locomotion for each rat was calculated according to the number of light-beam breaks.

Experiment 1: CPP development

The rats were randomly assigned to six equal-sized groups (n = 8). For all groups the experiment consisted of three phases: pretest (days 1-3), conditioning (days 4-13) and two subsequent tests (starting on day 15). One day without testing (day 14, rats remained untreated in their home-cage) was performed to overcome the regular drug-saline application order that was present during conditioning. Every day each rat was placed into the same CPP-box and after the end of each run the CPP-boxes were cleaned with water and wiped dry. Each pretest lasted for 20 min and rats were placed into the middle chamber without prior injection and with free access to all chambers to reveal potential unconditioned place preferences. During ten days of conditioning, each rat was confined for 30 min to one end chamber after injection of the conditioning drug on odd conditioning days and to the other end chamber after saline injection on even conditioning days. The assignment of the drug-associated chamber was counterbalanced within each group respective to differentially oriented and coloured chambers. Conditioning drugs were applied as followed: Saline-group: saline; MPEP10-group: 10 mg/kg MPEP; MPEP30-group: 30 mg/kg MPEP; MPEP50-group: 50 mg/kg MPEP; MPEP-cocaine-group: 10 mg/kg MPEP plus 10 mg/kg cocaine; MPEP-morphine-group: 10 mg/kg MPEP plus 10 mg/kg morphine. The two tests that followed after conditioning lasted for 20 min with free access to all chambers and for the start of the tests the rats were placed into the middle chamber. Before the first test, all groups received a saline injection (= undrugged-test), whereas the conditioning drug was applied before the second test (= drugged-test) to reveal possible state-dependent effects of MPEP on CPP expression.

Experiment 2: CPP expression

Two groups of rats (each n = 8) underwent pretests and conditioning as in experiment 1 by using either 10 mg/kg cocaine (cocaine-group) or 10 mg/kg morphine (morphine-group) as conditioning drugs. Starting from day 15, several repeated tests (only one test per day) for CPP expression followed. First an undrugged test (after saline injection) was carried out, followed by a test after injection of 10 mg/kg MPEP. The other MPEP-doses (30 and 50 mg/kg) were tested subsequently, always preceded by a saline-test and a test in the drugged-

state. Additionally, one saline-test was carried out (as a control) after a test in the drugged-state (termed as “saline-after-drug”). For ease of comprehensibility, the three tests in the drugged-state were averaged (termed as “average drug” or “av drug”) and also the saline-test on day 15 and the saline-after-drug test were averaged (termed as “average saline” or “av sal”) for analysis and data presentation. The three drugged-tests that were intended to maintain high CPP expression (see Mueller and Stewart, 2000) were analysed for a time-dependent decrease of CPP expression to exclude possible effects caused by extinction.

Data analysis
Statistical analysis was performed by using the program GB-Stat 7.0 (Dynamic Microsystems Inc.). Significance-levels were set as * $p < 0.05$ (significant) and ** $p < 0.01$ (high significant). To analyse the effects (i.e. acute effects, sensitization) of conditioning on locomotion, a two-way repeated measures ANOVA was used within each group to compare saline-treated vs. conditioning-drug-treated conditioning days. The first saline-treated conditioning day thereby served as reference for post-hoc comparison according to Dunnett’s procedure (two-tailed). Locomotion during all tests was calculated as the sum of the locomotion in all three chambers and statistically analysed in the same way as the CPP data for the respective tests (as explained below). CPP was calculated for each rat by subtracting the time spent in the saline-associated chamber from the time spent in the drug-associated chamber. For experiment 1, a two-way ANOVA (randomized blocks) was used to compare the CPP data of the six groups. A post-hoc comparison according to Dunn’s Bonferroni correction enabled within-group comparison (av pre of the respective group as reference) and between group comparison (CPP of the saline-group on the respective test-day as reference). For experiment 2, only rats that showed stable CPP expression were used for statistical analysis. For each rat, the criterion for stable CPP expression was reached if the average CPP (of the three drugged-tests and the two-saline-tests) minus the CPP during the average pretests was ≥ 100 s. Consequently, 2 rats in the cocaine- and 1 rat in the morphine-group were excluded from analysis since testing the effects of MPEP on CPP-expression makes no sense in rats that do not really express CPP. The CPP values during the repeated tests in experiment 2 were analysed by applying a repeated-measures ANOVA, followed by multiple post-hoc Fisher’s LSD comparison. Average pretest of the respective group was used as reference to detect significant CPP expression whereas the average saline CPP was used as reference to detect possible effects of MPEP on CPP expression.

Results

Locomotion during the conditioning-phase
The locomotion data obtained during conditioning in experiment 1 and 2 were analysed for acute effects of treatment on locomotion and for possible development of sensitized locomotion after repeated treatment (fig. 1). In the saline-group (fig. 1a), no significant difference in locomotion was found between odd and even saline-conditioned days ($F_{1,14} = 0.146$; $p = 0.7082$). In all three MPEP-groups (fig. 1a, b), also no significant difference in locomotion was observed between saline- and MPEP-treated conditioning days (MPEP10-group: $F_{1,14} = 1.878$; $p = 0.1922$ / MPEP30-group: $F_{1,14} = 0.711$; $p = 0.4134$ / MPEP50-group: $F_{1,14} = 0.029$; $p = 0.8683$). However, post-hoc analysis revealed high, significantly increased locomotion ($p < 0.01$) on the last three MPEP-treated conditioning days (MPEP10-group), a high, significantly decreased locomotion ($p < 0.01$) on the first and second MPEP-treated conditioning days (MPEP30-group) and a significantly decreased locomotion ($p < 0.05$) on the fourth MPEP-treated day (MPEP30-group). In the cocaine-group (fig. 1c), locomotion on cocaine-conditioning days was high and significantly differed from saline-

conditioned days (F1,10 = 18.28; p = 0.0016). Post-hoc comparisons showed significantly increased locomotion during all cocaine-treated days (first and second cocaine-treated days: p < 0.05; third to fifth cocaine-treated days: p < 0.01). In the MPEP-cocaine-group (fig. 1c), locomotion on the drug-conditioning days significantly differed from saline-days (F1,14 = 22.21; p = 0.0003), and post-hoc test revealed significantly increased (p < 0.01) locomotion during the last three drug-treated days. In the morphine-group (fig. 1d), no significant difference was observed between morphine- and saline-days (F1,12 = 0.590; p = 0.4574), but the post-hoc test revealed significantly increased (p < 0.05) locomotion during the third morphine-treated day. In the MPEP-morphine-group (fig. 1d), also no significant difference was observed between drug- and saline-days (F1,14 = 2.938; p = 0.1086), but the post-hoc test revealed significantly increased locomotion on the last drug-treated day (p < 0.01).

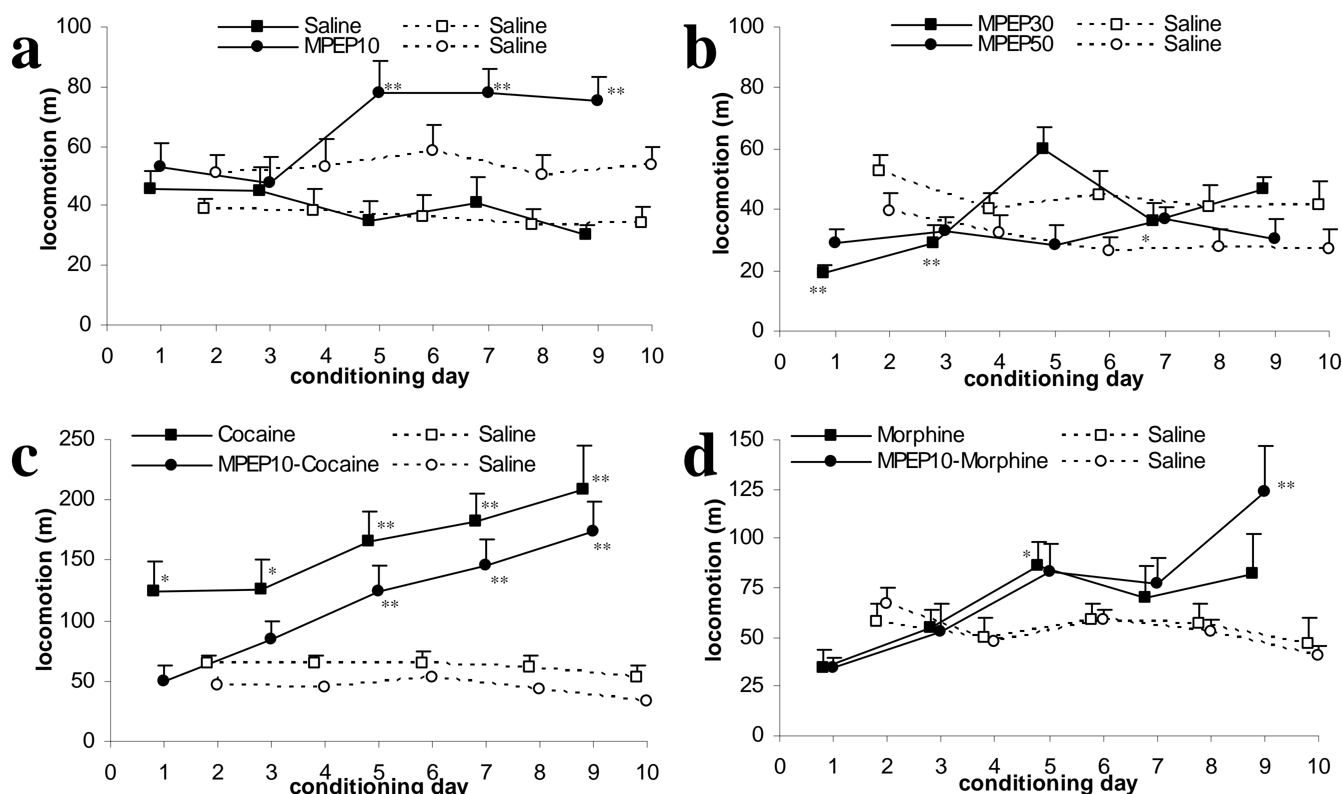

***Figure 1*: Spontaneous and sensitized locomotion during conditioning**
The average locomotion (and SEM) during the drug- and saline-treated conditioning days is presented for all eight groups of experiments 1 and 2. On drug-treated days, the groups received **a:** saline or 10 mg/kg MPEP; **b:** 30 or 50 mg/kg MPEP; **c**: 10 mg/kg cocaine or a combination of 10 mg/kg MPEP plus 10 mg/kg cocaine; **d**: 10 mg/kg morphine or a combination of 10 mg/kg MPEP plus 10 mg/kg morphine. On saline-treated days, all groups received only saline-treatment. *****(p < 0.05) and ****** (p < 0.01) indicate significant or high significant difference from the first saline-treated day (= conditioning day 2) of the respective group according to repeated measures ANOVA followed by Dunnett's post-hoc comparison.

<u>Effect of the MPEP-dose on body-weight</u>

Two-way ANOVA (randomized blocks) comparison of the body-weight (fig. 2) of experimental day 3 (one day before the first MPEP treatment) with experimental day 17 (first test after the last MPEP treatment) revealed no significant effect of group (i.e. saline- vs. three MPEP-groups; F3,21 = 2.553; p = 0.0829) and also no significant effect of day (i.e. day 3 vs. day 17; F1,7 = 2.643; p = 0.148), but a significant group-day interaction (F3,21 = 4.319; p = 0.0161). Only in the MPEP50-group, a significant decrease (p < 0.05) in body-weight on experimental day 17 was found (by Dunnett's two-tailed post-hoc comparison with body-weight of the saline-group on experimental day 3 as reference).

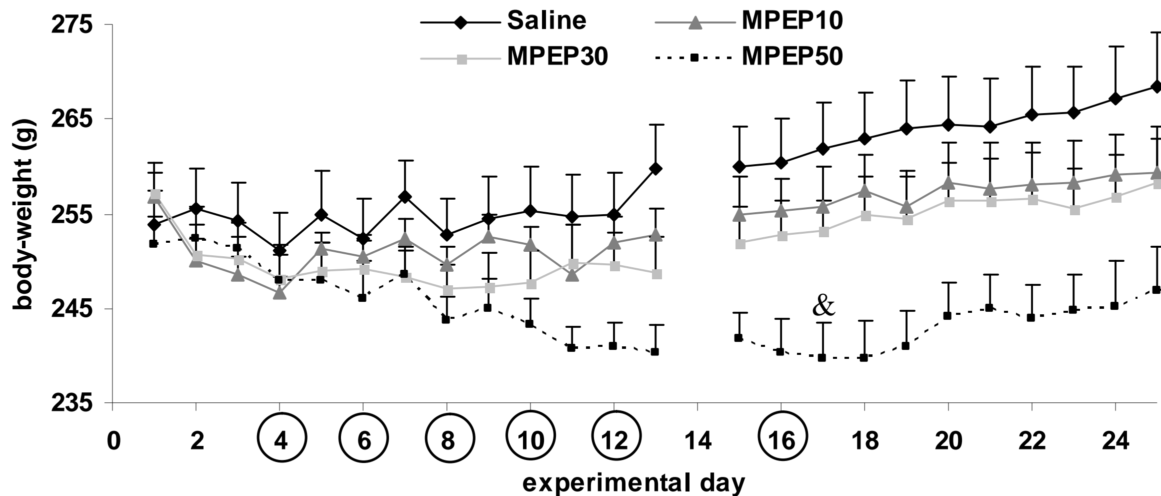

Figure 2**:** **Effects of MPEP on the body-weight**
Average body-weight (and SEM) of rats in the saline-group and the three groups receiving different doses of MPEP in experiment 1 during the three pretests (days 1-3), the conditioning-phase (days 4-13) and the testing-phase (days 15-16), followed by a post-experimental (days 17-25) body-weight control. On the encircled days MPEP was administered to all MPEP-groups. [&] ($p < 0.05$) indicates significant difference from the body-weight of the saline-group on experimental day 3 (= first day before MPEP-administration) according to a two-way ANOVA followed by Dunnett's post-hoc comparison.

<u>Experiment 1: CPP development</u>
Analysis of the CPP data from the six groups (fig. 3a) showed a significant effect of group ($F_{5,35} = 4.310$; $p = 0.0037$) and also a significant treatment- (i.e. average pretest and two tests) effect ($F_{2,14} = 23.76$; $p < 0.0001$). Multiple post-hoc comparison (Dunn's Bonferroni correction) did not show any significant differences (using saline-group CPP of the respective day or the average pretest of the respective group as reference) for all groups during average pretest or during the undrugged-test. However, CPP in the MPEP-cocaine- and in the MPEP-morphine-group was significantly increased ($p < 0.01$) on the drug-test (with respect to both the saline-group CPP on the respective day or to the average pretest of the respective group).

Similar analysis for the locomotion of the six groups (fig. 3b) showed a significant effect of group ($F_{5,35} = 9.635$; $p < 0.0001$) and also a significant treatment-effect ($F_{2,14} = 8.068$, $p = 0.0047$). In the MPEP10-group locomotion was significantly increased ($p < 0.01$) during the undrugged- and the drugged-test (according to a multiple post-hoc comparison with the saline-group locomotion of the respective day). In the MPEP50-group locomotion was significantly decreased ($p < 0.01$) during the undrugged- and the drugged-test (as compared to the average pretest of this group) while in the MPEP-cocaine-group locomotion was found to be significantly increased ($p < 0.01$) during the drugged-state (as compared to both the saline-group locomotion of the respective day and the average pretest locomotion of this group). In the MPEP-morphine-group locomotion in the undrugged-state was significantly increased ($p < 0.01$) (as compared to the saline-group locomotion of the respective day).

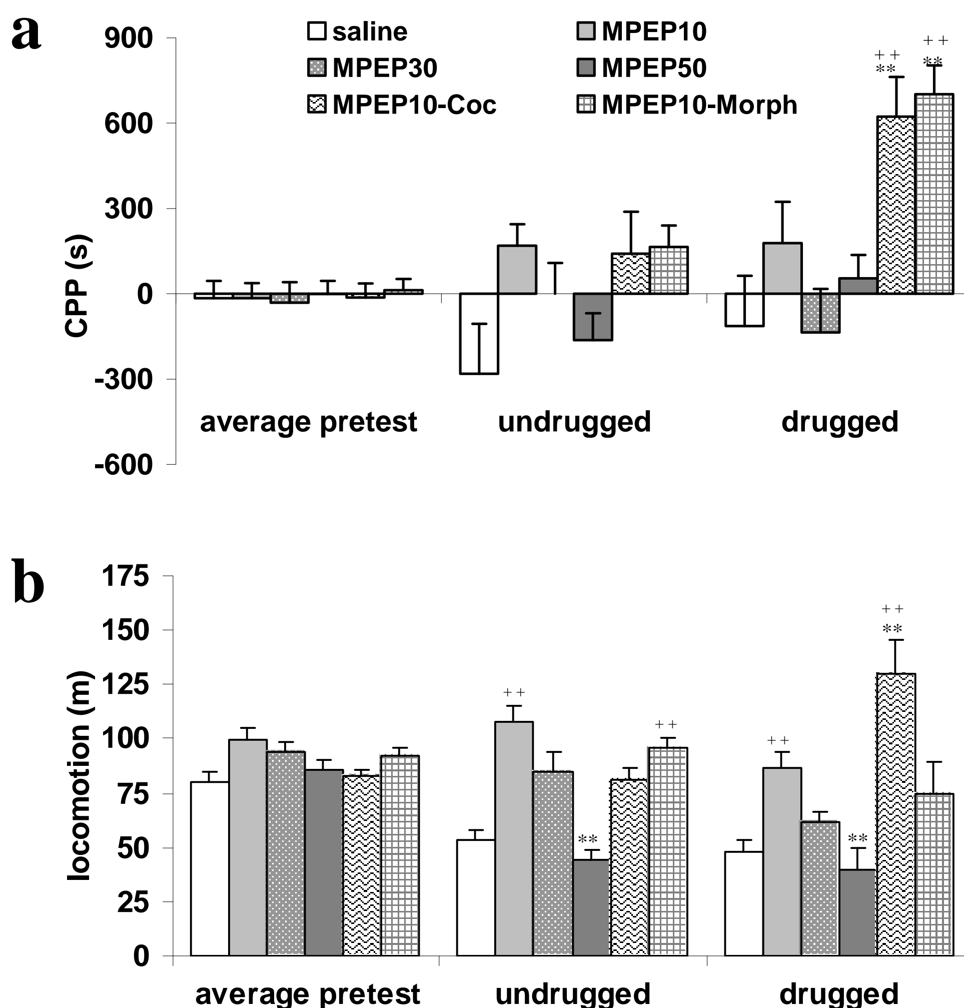

Figure 3: **Experiment 1: CPP development**
Results of experiment 1 for development of CPP and respective locomotion data are shown for the six groups that were treated with different conditioning drugs (i.e. the saline-group, three groups receiving different doses (10, 30 or 50 mg/kg) of MPEP, the cocaine-MPEP-group and the morphine-MPEP-group). **a:** Average CPP-values (calculated as difference of time spent in the drug- and the saline-associated chamber) and SEM during the average pretest and the tests in the undrugged- and the drugged-state. ** ($p < 0.01$) indicates high significant difference from the average pretest CPP of the respective group while $^{++}$ ($p < 0.01$) indicates high significant difference from the saline-group CPP of the respective day according to a two-way ANOVA followed by Dunn's Bonferroni post-hoc comparison. **b:** Respective average locomotion (as measured in all three chambers) and SEM for experiment 1. ** ($p < 0.01$) indicates high significant difference from the average pretest locomotion of the respective group while $^{++}$ ($p < 0.01$) indicates high significant difference from the saline-group locomotion of the respective day according to a two-way ANOVA followed by Dunn's Bonferroni post-hoc comparison.

Experiment 2: CPP expression

Analysis of the CPP data of experiment 2 (fig. 4a) showed a significant effect of repeated treatment (i.e. average pretest, average drug, average saline, 10, 30 and 50 mg/kg MPEP) on CPP expression in both the cocaine- ($F_{5,25} = 5.131$; $p = 0.0023$) and the morphine- ($F_{5,30} = 8.495$; $p < 0.0001$) group. In the cocaine-group, Fisher's post-hoc test (using the average pretest CPP as reference) revealed significant CPP expression ($p < 0.05$) during the average saline- and the MPEP50-tests and also significant CPP expression ($p < 0.01$) during the average cocaine- and the MPEP10- and MPEP30-tests. Additionally, the average cocaine-test was significantly increased ($p < 0.05$) as compared to the average saline-test in the cocaine-group. In the morphine-group, the post-hoc test showed significant CPP expression ($p < 0.01$) during the average saline-, the average morphine- and the MPEP10-tests and no significant CPP expression ($p > 0.05$) during the MPEP30- and the MPEP50-tests. Additionally, the

MPEP50-test in the morphine-group was significantly decreased ($p < 0.05$) and the average morphine-test was significantly increased ($p < 0.05$) as compared to the average saline-test in the morphine-group.

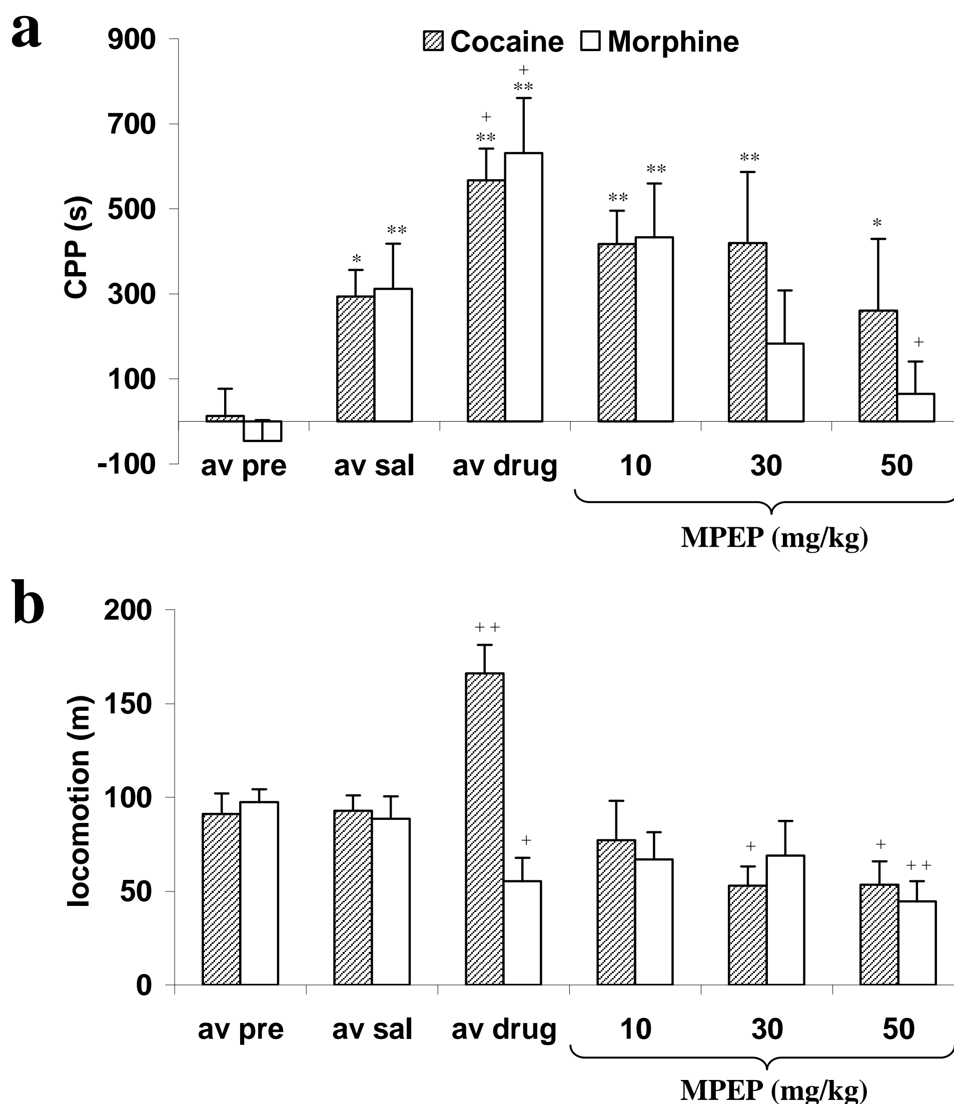

***Figure 4*: Experiment 2: CPP expression**
Results of experiment 2 for expression of CPP and respective locomotion data for the cocaine- and morphine-group during several repeated tests. For details of the repeated testing schedule see materials and methods section. The average of three pretests ("av pre"), the average of two saline-tests ("av sal"), the average of three drug-reinstatements ("av drug", see also tab. 1) and the tests with three different doses of MPEP (MPEP10, MPEP30 and MPEP50) are indicated. **a:** Average CPP-values (calculated as difference of time spent in the drug- and the saline-associated chamber) and SEM during all tests. * ($p < 0.05$) and ** ($p < 0.01$) indicate significant or high significant difference from the average pretest CPP while $^{+}$ ($p < 0.05$) indicates significant difference from the average saline-CPP of the respective group according to a two-way ANOVA followed by Fisher's LSD post-hoc comparison. **b:** Respective average locomotion (as measured in all three chambers) and SEM for experiment 2. $^{+}$ ($p < 0.05$) and $^{++}$ ($p < 0.01$) indicate significant or high significant difference from the average saline locomotion of the respective group according to a two-way ANOVA followed by Fisher's LSD post-hoc comparison.

***Table 1*: Drug-reinstatement sessions**
Average CPP expression and locomotion (each with respective SEM) is shown during the tests in the drugged-state that were carried out to maintain high CPP expression. Repeated-measures ANOVA did not yield significant extinction-like decline in CPP expression during the drugged-tests in both the cocaine- and the morphine-group.

group	measurement	drug-reinstatement		
		1	2	3
cocaine	**CPP (s)**	**587.6**	**585.6**	**527.6**
	SEM	67.2	95.7	120.6
	locomotion (m)	**161.5**	**187.0**	**149.7**
	SEM	18.2	20.2	28.1
morphine	**CPP (s)**	**692.6**	**505.1**	**697.9**
	SEM	147.2	302.2	163.7
	locomotion (m)	**61.5**	**50.5**	**53.9**
	SEM	11.1	13.9	13.0

To account for extinction, the three drugged-tests within the cocaine- and morphine-group were separately analysed for a possible extinction-like decrease in CPP expression after repeated testing. However, no significant decrease in CPP expression was observed in the cocaine- ($F_{2,10} = 0.207$; $p = 0.8167$) and in the morphine-group ($F_{2,12} = 0.267$; $p = 0.7697$) according to the respective repeated-measures ANOVA. Fisher's post hoc comparison did not reveal any significant difference in CPP expression between any of the three drugged-tests within both groups either.

Analysis of the locomotion data of experiment 2 (fig. 4b) revealed a significant effect of the repeated treatment on CPP expression in the cocaine-group ($F_{5,25} = 9.946$; $p < 0.0001$) and a significant effect in the morphine-group ($F_{5,30} = 3.169$; $p = 0.0205$). In the cocaine-group locomotion was significantly increased ($p < 0.01$) during the average cocaine-test and significantly decreased ($p < 0.05$) during the MPEP30- and the MPEP50-tests (comparisons according to Fisher's post-hoc test using the average saline-locomotion as reference). In the morphine-group, significantly decreased locomotion was found during the average morphine-test ($p < 0.05$) and during the MPEP50-test ($p < 0.01$).

Discussion

Spontaneous locomotion

The locomotion data during conditioning in the saline-group (fig. 1a) indicate that our CPP setup does not per se produce different locomotion on different conditioning days. Furthermore, our results show that systemically administered MPEP at a dose of 30 mg/kg reduced spontaneous locomotion (fig. 1b). This acute inhibition of spontaneous locomotion by MPEP is in accordance with the results of another study (Spooren et al., 2000). A role of the group I mGluR subtype1 in regulation of motor activity in the ventral tegmental area and Nac has already been demonstrated by Swanson and Kalivas (2000), therefore the observed effectiveness of mGluR5- (that also belong to group I mGluR) blockade in reduction of locomotion extents these previous findings. The effective dose of 30 mg/kg MPEP was also recommended by Anderson et al. (2002) for maximum blockade of mGluR5 in rats. The lack of effect of the low and the high MPEP doses on locomotion may indicate that a specific degree of mGluR5 blockade is required to alter locomotion. However, 50 mg/kg MPEP was at least to some extent effective in affecting spontaneous locomotion, since it reduced locomotion in the cocaine- and morphine-group (fig. 4b). mGluR5 are found in particular high abundance in Nac and striatum (Testa et al.,1994; Romano et al., 1995; Spooren et al.,

2000), thus a possible mechanism of MPEP to alter locomotion could be a blockade of mGluR5, that are postsynaptically localized on spiny I neurons in the striatum (Tallaksen-Greene et al., 1998). Group I mGluR were reported to specifically interact with D1-like (but not D2-like) dopamine receptors to control locomotion (David and Abraini, 2001). A localization of mGluR5 on striatal neurons of the direct basal ganglia pathway, bearing D1-receptors (Schmidt, 1998), could therefore provide an explanation for the acute inhibitory effects of MPEP on locomotion.

Cocaine-induced hyperlocomotion
The observed blockade of cocaine-induced hyperlocomotion (fig. 1c) by MPEP co-treatment on the initial conditioning day is a new finding in rats and consistent with findings of Chiamulera et al. (2001) and McGeehan et al. (2004) that were obtained mice. As selectively the cocaine-induced hyperlocomotion, but not the sensitization of locomotion after repeated cocaine (see next paragraph) was blocked by MPEP, one could assume that MPEP only blocks hyperlocomotion up to a certain magnitude. If the cocaine-induced hyperlocomotion is increased by sensitization processes, MPEP no longer possesses the ability to fully reduce the locomotion to a saline-level. However, another way to explain these findings is to assume that distinct mechanisms are involved in cocaine-induced hyperlocomotion and cocaine-induced sensitization of locomotion and that mGluR5 are only involved in cocaine-induced hyperlocomotion. An interesting observation is that even 10 mg/kg MPEP were effective in blocking the cocaine-induced hyperlocomotion, while the same dose did not show any effect on spontaneous locomotion. This implies different sensitivities of both mechanisms to mGluR5-blockade.

Development of sensitized locomotion
The present data on sensitization should be treated with caution, since the drug was applied every second day and not on a daily scheme, as usually done in studies dealing with sensitization. Furthermore, the photo sensors in our CPP-boxes only detect locomotion parallel to the length of the box, while the usually applied activity boxes detect locomotion parallel and perpendicular to the length of the box. Nevertheless, we found development of sensitized locomotion, at least in the cocaine-group (fig. 1c), a finding that is usually observed after repeated administration of cocaine (Vanderschuren and Kalivas, 2000). Therefore, we conclude that our CPP-setup is at least sensitive for detecting the strong sensitization observed after repeated cocaine.

In contrast to its effect on cocaine-induced hyperlocomotion, MPEP did not affect development of cocaine-induced sensitized locomotion which is reflected in the increased locomotion on the last three conditioning days in the MPEP-cocaine-group. On the one hand this result is surprising, since Chiamulera et al. (2001) reported that mGluR5 k.o. mice showed no sensitized locomotion after repeated cocaine. On the other hand the induction of sensitized locomotion by repeated conditioning with this low dose of MPEP (see discussion below) may account for the lack of effect of this MPEP dose on drug-induced sensitization. Therefore, we propose further experiments for a clarification of the effect of higher doses of MPEP on drug-induced sensitization. Regarding the results of the morphine-group, sensitization was not finally proved as locomotion was only increased during the third morphine-conditioning, but not during the fourth or fifth. However, due to the significantly increased locomotion on the last conditioning day in the MPEP-morphine-group (fig. 1d), it can be concluded that MPEP does not reduce development of morphine-induced sensitized locomotion. In summary, low MPEP doses do not affect development of cocaine- or morphine-induced sensitization of locomotion.

Another interesting and new finding is the sensitization after repeated treatment with 10 mg/kg MPEP. Despite the "ceiling effect" on the last three MPEP-treatments (fig. 1a) that is unusual for sensitization, the data of experiment 1 (fig. 3b) indicate that the MPEP10-group exhibits a permanent increase in locomotion after conditioning already finished. Additionally, a more detailed analysis of the locomotion in each chamber (data not shown) in the MPEP10-group during the undrugged test revealed that this increased locomotion was context-dependent, because mainly locomotion in the MPEP-associated chamber was increased. As context-dependency also plays an important role in sensitization (Amtage and Schmidt, 2003), we suggest that the increased locomotion in the MPEP10-group can be considered as sensitization. Additionally, the decrease in locomotion induced by the medium MPEP dose (fig. 1b) was only an initial effect that was overcome (probably by sensitization or tolerance) after repeated conditioning. Importantly, the observed induction of sensitized locomotion after repeated treatment with low MPEP-doses is in accordance with a proposed therapeutic benefit of mGluR5 antagonists in Parkinson's disease (Ossowska et al., 2001; Breysse et al., 2002). In conclusion, conditioning with a low MPEP dose does not prevent development of drug-induced sensitization of locomotion but can itself produce sensitized locomotion.

Effect on body-weight

We observed that high MPEP-dose significantly reduced the body-weight of rats after 6 applications of MPEP (fig. 2). With the end of MPEP-administration, these rats again gained weight in parallel to the saline-group. According to our knowledge, this is a new observation and no effect of MPEP or other drugs acting on mGluR5 on the body-weight has been reported so far. Nevertheless, this effect is interesting, because MPEP did not affect food self-administration (Chiamulera et al., 2001). Thus, the effect of MPEP on the body-weight is unlikely caused by an alteration of the positive rewarding properties of food. As the present study was not designed to analyse the involvement of mGluR5 in control of the body-weight and the effectiveness of MPEP was only detected as a "side-action", we suggest that a thorough investigation aiming at this effect may be valuable, since drugs that potentially reduce body-weight without producing strong side-effects may for example be useful in the therapy of obesity.

CPP development and state-dependency

As one outcome of experiment 1, no significant CPP developed in the saline-group and in the groups conditioned with three different doses of MPEP (fig. 3a). These findings in rats are consistent with the reported lack of CPP-development in mice after conditioning with three lower doses (1, 5 and 20 mg/kg) of MPEP (McGeehan and Olive, 2003) or with 30 mg/kg MPEP (Popik and Wrobel, 2002). For MPEP, this further implicates a low abuse-liability if used as medication.

Another result of experiment 1 is that no CPP development was observed in the undrugged-test of the MPEP-cocaine- and MPEP-morphine-groups. This observation fits to the recent report of McGeehan and Olive (2003) showing that MPEP dose-dependently (up to 20 mg/kg) reduced the development of cocaine-CPP in mice as measured in the undrugged-state. It is also in accordance with a study demonstrating that 30 mg/kg MPEP inhibited development of morphine-CPP in mice (Popik and Wrobel, 2002). However, the very strong CPP expression (fig. 3a) observed in the drugged-state (i.e. after treatment with the MPEP-drug-combination) in both the MPEP-cocaine- and the MPEP-morphine-group is an unprecedented finding (respective to the effects of MPEP) and it challenges the explanation given by these authors. Such strong CPP, observed only in the drugged-state, does not fit to the suggested blockade of CPP development by MPEP, but rather points to a state-dependent effect. According to the state dependency hypotheses, a behaviour that has been acquired in a distinct state (e.g. under

a specific drug or drug-combination), can only be expressed if the animal is in the same state. The neuronal basis of state-dependency is still unclear, but an involvement of NMDA glutamate receptors has been demonstrated. NMDA receptor antagonists can make learning and recall (Jackson et al., 1992) as well as the expression of behavioural sensitization state-dependent (Carlezon et al., 1995; Wise et al., 1996; Tzschentke and Schmidt, 1998; Lanis and Schmidt, 2001). Thus, we suggest a new interpretation of the present and previous studies whereas the lack of CPP expression in the undrugged-state of both combination-groups is caused by state-dependent mechanisms, i.e. the absence of treatment with the respective MPEP-drug-combination (i.e. MPEP plus cocaine or morphine) that is required for expression of CPP. This further implies that MPEP actually does not affect the rewarding effects of the drug, what would have been expected if it had really blocked CPP development. Therefore it will probably not be effective when applied together with one of these abused drugs. In conclusion, MPEP co-administration does not genuinely block development of CPP, but provides an internal-state to which CPP development is conditioned to. For expression of this behaviour, the same internal-state has to be present. Future research has to elucidate if mGluR5 are directly involved in control of state-dependent learning or if they are only indirectly involved by providing an internal-state for state-dependent learning.

Expression of CPP

As the main outcome of experiment 2, only morphine- but not cocaine-CPP expression can be reduced by MPEP (fig. 4a). The high MPEP-dose was most effective in this respect, but also the middle dose showed some effect. As we already discussed, these doses also reduced spontaneous locomotion. However, this cannot account for the reduction of morphine-CPP by MPEP, because locomotion in the cocaine-group was also significantly reduced without affecting cocaine-CPP expression. Additionally, in case the effects on locomotion would have contributed to the effect on CPP expression, extremely increased standard errors should have been found. Such effects are usually observed if a drug for example produces sedative effects and the rat (after being placed into the middle chamber) randomly moves to one of the end chambers or even stays in the middle chamber, where it rests the whole time. However, the standard errors in the morphine-group were comparatively small after the MPEP-treatments what excludes sedative effects as potential explanation for the effects of MPEP on morphine-CPP expression. Additionally, reduction of body-weight by MPEP can also not account for the acute effect on morphine-CPP expression effect, since it was only observed after repeated application of high MPEP-doses. Another possible explanation is that the reduction in morphine-CPP expression was caused by extinction that progressively decreased responding after repeated tests. However, as the cocaine-group was tested according to the same schedule, their CPP expression should have also declined. Furthermore, in order to prevent extinction, both the MPEP30- and MPEP50-tests were preceded by tests in the drugged state that are known to reinstate previously extinguished CPP responding (Mueller and Stewart, 2000). The high CPP expression and the complete absence of extinction during the three drugged-tests (tab. 1) suggest that repeated tests in the drugged-state indeed maintained CPP expression in both the cocaine- and the morphine-group on a high level and prevented extinction. This is especially interesting, since the drugged-tests were carried out with open doors and access to all chambers, thereby conditioning the effects of the drug to the whole CPP box instead of the prior conditioning to a defined end chamber. Therefore, such a "new-conditioning" should have also decreased CPP expression after repeated drug-sessions. However, this was probably not observed because only three drugged-tests were used and these tests lasted only for 20 min while the initial five conditionings lasted for 30 min each. Nevertheless, this effect may restrict the number of drug-reinstatements that can be used to prevent extinction. Additionally, the strength of conditioning (rather high doses of cocaine and morphine and five pairings have been used) may also contribute to the observed stability

of CPP expression. Based on the above made considerations, we suggest that repeatedly testing CPP-expression, always interrupted by drugged-tests, may be useful to save rats in future CPP experiments.

The ineffectiveness of MPEP in preventing expression of cocaine-CPP (fig. 4a) is a new finding and it is surprising, since McGeehan and Olive (2003) showed that MPEP reduces development of cocaine-CPP in mice. However, as discussed above, their results may be due to state-dependent effects induced by MPEP. Thus, in contrast to the effectiveness of MPEP in primary cocaine reward as tested in the self-administration paradigm (Chiamulara et al., 2001) secondary cocaine-reward seems not to be affected by MPEP. On the other hand, the observed reduction of morphine-CPP expression in rats perfectly fits to the results of Popik and Wrobel (2002), showing that the higher dose (30 mg/kg) but not the lower dose (10 mg/kg) of MPEP reduced CPP-expression in mice. In the present study, 50 mg/kg MPEP even produced a stronger reduction in morphine-CPP expression in the rats than the 30 mg/kg dose. Regarding the selectivity of MPEP, Anderson et al. (2002) suggested that by systemic application into rats, even the high dose of 50 mg/kg MPEP lacks unspecific effects on other receptors than mGluR5. However, recent studies also reported unspecific effects of MPEP that acts as a positive allosteric modulator for human mGluR4 (Mathiesen et al., 2003) and as an inhibitor of the human norepinephrine transporter (Heidbreder et al., 2003). Furthermore, noncompetetive NMDA-receptor antagonistic properties have also been reported for MPEP (O'Leary et al., 2000). Thus, based on the present data we cannot finally decide whether the reduction of morphine-CPP expression by MPEP (especially the effect observed with the high MPEP dose) can be solely attributed to the blockade of mGluR5. Unfortunately, no studies are published that examined the effects of mGluR4 or elevated norepinephrine on expression of cocaine- or morphine-CPP. According to other studies examining the effects of noncompetetive NMDA-receptor-antagonists on CPP expression, it has been reported that memantine reduces expression of cocaine-CPP (Kotlinska and Biala, 2000) while expression of morphine-CPP is reduced by memantine (Popik et al., 2003) or MK-801 (Tzschentke and Schmidt, 1997). Thus, in case NMDA-receptor antagonistic effects would have contributed to the reduction of CPP expression by MPEP, both cocaine- and morphine-CPP expression should have been affected. As this was not the case, the effectiveness of MPEP on morphine-CPP expression is more likely due to mGluR5-blockade. In conclusion, medium and high doses of MPEP reduce expression of morphine- but not cocaine-CPP.

Due to systemic application of MPEP, it remains speculative to name brain areas involved in the effect of MPEP on secondary morphine reward. As mGluR5 are highly abundant in Nac (Testa et al., 1994; Romano et al., 1995; Spooren et al., 2000), it is likely that the effects of MPEP are mediated within this important part of the brain reward system. Additionally, the prefrontal cortex, the hippocampus and the amygdala are also involved in drug addiction and constitute important sources of glutamatergic input to the Nac (Tzschentke and Schmidt, 2000; Vorel et al., 2001; See et al., 2003). The different effect of MPEP on expression of cocaine- and morphine-CPP might even be explained by the involvement of different sub-structures of these areas. The infralimbic medial prefrontal cortex is for example involved in morphine-CPP, while the prelimbic area mediates cocaine-CPP (Tzschentke and Schmidt, 1999).

In summary, our results suggest that mGluR5 are involved in modulation of spontaneous locomotion and cocaine-induced hyperlocomotion. A blockade of mGluR5 with a low dose (10 mg/kg) of MPEP is not sufficient to inhibit development of sensitization to cocaine or morphine, but can itself produce sensitized locomotion after repeated application. Furthermore, a low MPEP dose does not affect development of cocaine- and morphine-CPP,

but renders their expression state-dependent. Medium MPEP-doses (30 mg/kg) most effectively reduce spontaneous locomotion. Interestingly, repeated treatments with the high MPEP dose (50 mg/kg) reduced the body-weight of the rats. Importantly the high MPEP-dose most effectively reduced expression of morphine CPP, while expression of cocaine-CPP was not affected. This implicates mGluR5-blockade as a potential treatment for morphine addiction by reducing the rewarding effects of environmental stimuli, thereby preventing relapse.

Acknowledgements

We thank Drs. Rainer Kuhn and Fabrizio Gasparini (Novartis, Switzerland) for the kind supply of MPEP and Dr. Thomas Tzschentke for his comments on the manuscript. This study was funded by the federal ministry for education and research 01EB0110-Baden-Württemberg consortium for addiction research.

References

Amtage,J., Schmidt,W.J., 2003. Context-dependent catalepsy intensification is due to classical conditioning and sensitization. Behavioural Pharmacology 14, 563-567.

Anderson,J.J., Rao,S.P., Rowe,B., Giracello,D.R., Holtz,G., Chapman,D.F., Tehrani,L., Bradbury,M.J., Cosford,N.D., Varney,M.A., 2002. [3H]Methoxymethyl-3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine binding to metabotropic glutamate receptor subtype 5 in rodent brain: in vitro and in vivo characterization. Journal of Pharmacology and Experimental Therapeutics 303, 1044-1051.

Breysse,N., Baunez,C., Spooren,W., Gasparini,F., Amalric,M., 2002. Chronic but not acute treatment with a metabotropic glutamate 5 receptor antagonist reverses the akinetic deficits in a rat model of parkinsonism. Journal of Neuroscience 22, 5669-5678.

Carlezon,W.A., Jr., Mendrek,A., Wise,R.A., 1995. MK-801 disrupts the expression but not the development of bromocriptine sensitization: a state-dependency interpretation. Synapse 20, 1-9.

Chiamulera,C., Epping-Jordan,M.P., Zocchi,A., Marcon,C., Cottiny,C., Tacconi,S., Corsi,M., Orzi,F., Conquet,F., 2001. Reinforcing and locomotor stimulant effects of cocaine are absent in mGluR5 null mutant mice. Nature Neuroscience 4, 873-874.

Childress,A.R., Mozley,P.D., McElgin,W., Fitzgerald,J., Reivich,M., O'Brien,C.P., 1999. Limbic activation during cue-induced cocaine craving. American Journal of Psychiatry 156, 11-18.

Cornish,J.L., Kalivas,P.W., 2000. Glutamate transmission in the nucleus accumbens mediates relapse in cocaine addiction. Journal of Neuroscience 20, RC89.

David,H.N., Abraini,J.H., 2001. The group I metabotropic glutamate receptor antagonist S-4-CPG modulates the locomotor response produced by the activation of D1-like, but not D2-like, dopamine receptors in the rat nucleus accumbens. European Journal of Neuroscience 13, 2157-2164.

Di Ciano,P., Cardinal,R.N., Cowell,R.A., Little,S.J., Everitt,B.J., 2001. Differential involvement of NMDA, AMPA/kainate, and dopamine receptors in the nucleus accumbens core in the acquisition and performance of pavlovian approach behavior. Journal of Neuroscience 21, 9471-9477.

Ghasemzadeh,M.B., Nelson,L.C., Lu,X.Y., Kalivas,P.W., 1999. Neuroadaptations in ionotropic and metabotropic glutamate receptor mRNA produced by cocaine treatment. Journal of Neurochemistry 72, 157-165.

Heidbreder, C. A., Bianchi, M., Lacroix, L. P., Faedo, S., Perdona, E., Remelli, R., Cavanni, P., Crespi, F, 2003. Evidence that the metabotropic glutamate receptor 5 antagonist MPEP may act as an inhibitor of the norepinephrine transporter in vitro and in vivo. Synapse 50, 269-276.

Hotsenpiller,G., Giorgetti,M., Wolf,M.E., 2001. Alterations in behaviour and glutamate transmission following presentation of stimuli previously associated with cocaine exposure. European Journal of Neuroscience 14, 1843-1855.

Ito,R., Dalley,J.W., Howes,S.R., Robbins,T.W., Everitt,B.J., 2000. Dissociation in conditioned dopamine release in the nucleus accumbens core and shell in response to cocaine cues and during cocaine-seeking behavior in rats. Journal of Neuroscience 20, 7489-7495.

Jackson,A., Koek,W., Colpaert,F.C., 1992. NMDA antagonists make learning and recall state-dependent. Behavioural Pharmacology 3, 415-421.

Kalivas,P.W., 2004. Glutamate systems in cocaine addiction. Current Opinion in Pharmacology 4, 23-29.

Kotlinska, J., Biala, G, 2000. Memantine and ACPC affect conditioned place preference induced by cocaine in rats. Polish Journal of Pharmacology 52, 179-185.

Lanis,A., Schmidt,W.J., 2001. NMDA receptor antagonists do not block the development of sensitization of catalepsy, but make its expression state-dependent. Behavioural Pharmacology 12, 143-149.

Mathiesen, J. M., Svendsen, N., Brauner-Osborne, H., Thomsen, C., Ramirez, M. T., 2003. Positive allosteric modulation of the human metabotropic glutamate receptor 4 (hmGluR4) by SIB-1893 and MPEP. British Journal of Pharmacology 138, 1026-1030.

McGeehan,A.J., Olive,M.F., 2003. The mGluR5 antagonist MPEP reduces the conditioned rewarding effects of cocaine but not other drugs of abuse. Synapse 47, 240-242.

McGeehan,A.J., Janak,P.H., Olive,M.F., 2004. Effect of the mGluR5 antagonist 6-methyl-2-(phenylethynyl)pyridine (MPEP) on the acute locomotor stimulant properties of cocaine, d-amphetamine, and the dopamine reuptake inhibitor GBR12909 in mice. Psychopharmacology online first, DOI: 10.1007/s00213-003-1733-2.

Mueller,D., Stewart,J., 2000. Cocaine-induced conditioned place preference: reinstatement by priming injections of cocaine after extinction. Behavioural Brain Research 115, 39-47.

O'Leary, D. M., Movsesyan, V., Vicini, S., Faden, A. I., 2000. Selective mGluR5 antagonists MPEP and SIB-1893 decrease NMDA or glutamate-mediated neuronal toxicity through actions that reflect NMDA receptor antagonism. British Journal of Pharmacology 131, 1429-1437.

Ossowska,K., Konieczny,J., Wolfarth,S., Wieronska,J. , Pilc,A., 2001. Blockade of the metabotropic glutamate receptor subtype 5 (mGluR5) produces antiparkinsonian-like effects in rats. Neuropharmacology 41, 413-420.

Pierce,R.C., Bell,K., Duffy,P. , Kalivas,P.W., 1996. Repeated cocaine augments excitatory amino acid transmission in the nucleus accumbens only in rats having developed behavioral sensitization. Journal of Neuroscience 16, 1550-1560.

Popik,P., Wrobel,M., 2002. Morphine conditioned reward is inhibited by MPEP, the mGluR5 antagonist. Neuropharmacology 43, 1210-1217.

Popik, P., Wrobel, M., Rygula, R., Bisaga, A., Bespalov, A. Y., 2003. Effects of memantine, an NMDA receptor antagonist, on place preference conditioned with drug and nondrug reinforcers in mice. Behavioural Pharmacology 14, 237-244.

Robinson,T.E., Berridge,K.C., 1993. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Research Brain Research Reviews 18, 247-291.

Romano,C., Sesma,M.A., McDonald,C.T., O'Malley,K., van den Pol,A.N., Olney,J.W., 1995. Distribution of metabotropic glutamate receptor mGluR5 immunoreactivity in rat brain. Journal of Comparative Neurolology 355, 455-469.

Schmidt,W.J., 1998. Basal Ganglia Dopamine and Glutamate in Motor Activation and Plasticity. Neurology, Psychiatry and Brain Research 6, 155-160.

See,R.E., Fuchs,R.A., Ledford,C.C., McLaughlin,J., 2003. Drug Addiction, Relapse, and the Amygdala. Annals of the New York Academy of Sciences 985, 294-307.

Shippenberg,T.S., Rea,W., Slusher,B.S., 2000. Modulation of behavioral sensitization to cocaine by NAALADase inhibition. Synapse 38, 161-166.

Slusher,B.S., Thomas,A., Paul,M., Schad,C.A., Ashby,C.R., Jr., 2001. Expression and acquisition of the conditioned place preference response to cocaine in rats is blocked by selective inhibitors of the enzyme N-acetylated-alpha-linked-acidic dipeptidase (NAALADASE). Synapse 41, 22-28.

Spooren,W.P., Vassout,A., Neijt,H.C., Kuhn,R., Gasparini,F., Roux,S., Porsolt,R.D., Gentsch,C., 2000. Anxiolytic-like effects of the prototypical metabotropic glutamate receptor 5 antagonist 2-methyl-6-(phenylethynyl)pyridine in rodents. Journal of Pharmacology and Experimental Therapeutics 295, 1267-1275.

Strakowski, S. M., Sax, K. W., Setters, M. J., Keck, P. E., Jr., 1996. Enhanced response to repeated d-amphetamine challenge: evidence for behavioral sensitization in humans. Biological Psychiatry 40, 872-880.

Swanson,C.J., Kalivas,P.W., 2000. Regulation of locomotor activity by metabotropic glutamate receptors in the nucleus accumbens and ventral tegmental area. Journal of Pharmacology and Experimental Therapeutics 292, 406-414.

Tallaksen-Greene,S.J., Kaatz,K.W., Romano,C., Albin,R.L., 1998. Localization of mGluR1a-like immunoreactivity and mGluR5-like immunoreactivity in identified populations of striatal neurons. Brain Research 780, 210-217.

Testa,C.M., Standaert,D.G., Young,A.B., Penney,J.B., Jr., 1994. Metabotropic glutamate receptor mRNA expression in the basal ganglia of the rat. Journal of Neuroscience 14, 3005-3018.

Tzschentke,T.M., Schmidt,W.J., 1997. Interactions of MK-801 and GYKI 52466 with morphine and amphetamine in place preference conditioning and behavioural sensitization. Behavioural Brain Research 84, 99-107.

Tzschentke,T.M., Schmidt,W.J., 1998. Does the noncompetitive NMDA receptor antagonist dizocilpine (MK801) really block behavioural sensitization associated with repeated drug administration? Trends in Pharmacological Sciences 19, 447-451.

Tzschentke,T.M., Schmidt,W.J., 1999. Functional heterogeneity of the rat medial prefrontal cortex: effects of discrete subarea-specific lesions on drug-induced conditioned place preference and behavioural sensitization. European Journal of Neuroscience 11, 4099-4109.

Tzschentke,T.M., Schmidt,W.J., 2000. Functional relationship among medial prefrontal cortex, nucleus accumbens, and ventral tegmental area in locomotion and reward. Critical Reviews in Neurobiology 14, 131-142.

Tzschentke,T.M., Schmidt,W.J., 2003. Glutamatergic mechanisms in addiction. Molecular Psychiatry 8, 373-382.

Vanderschuren,L.J., Kalivas,P.W., 2000. Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. Psychopharmacology 151, 99-120.

Vorel,S.R., Liu,X., Hayes,R.J., Spector,J.A., Gardner,E.L., 2001. Relapse to cocaine-seeking after hippocampal theta burst stimulation. Science 292, 1175-1178.

Wise,R.A., Mendrek,A., Carlezon,W.A., Jr., 1996. MK-801 (dizocilpine): synergist and conditioned stimulus in bromocriptine-induced psychomotor sensitization. Synapse 22, 362-368.

Wolf,M.E., 1998. The role of excitatory amino acids in behavioral sensitization to psychomotor stimulants. Progress in Neurobiology 54, 679-720.

Effects of the mGluR5 antagonist MPEP on expression of conditioned place preference to natural (food) or drug (amphetamine, MDMA) reward in rats

Volker Herzig[1,*], Eleonora M. I. Capuani[2], Karl-Artur Kovar[3] and Werner J. Schmidt[1]

[1] Zoological Institute, Neuropharmacology, University of Tübingen, Germany
[2] International Max Planck Research School, Graduate School of Neural and Behavioural Sciences, University of Tübingen, Germany
[3] Pharmaceutical Institute, Department of Pharmaceutical Chemistry/Analysis, University of Tübingen, Germany

Abstract

Rationale: Recent studies revealed an involvement of metabotropic glutamate receptors of subtype 5 (mGluR5) in different models of drug addiction. mGluR5-blockade was reported to inhibit expression of context-conditioned morphine reward. *Objectives:* The present study examines if mGluR5 are also involved in expression of context-conditioned reward to other drugs of abuse (amphetamine or MDMA) or to context-conditioned natural (food) reward. *Methods:* Three groups of rats were conditioned to amphetamine, MDMA or food in the conditioned place preference (CPP) paradigm. After conditioning, expression of CPP was examined in the drug-free- or the rewarded-state, or after application of 50 mg/kg (i.p.) MPEP, a highly selective antagonist of mGluR5. *Results:* MPEP reduced locomotion in all groups. Furthermore, expression of amphetamine-CPP was reduced by MPEP, while expression of food- or MDMA-CPP was not affected. *Conclusions:* We suggest that mGluR5 are involved in modulation of spontaneous locomotion and in the expression of the context-conditioned rewarding effects of amphetamine. Furthermore, mGluR5 do not seem to be involved in context-conditioned natural or MDMA reward. In summary, mGluR5-blockade might be a useful treatment strategy to prevent context-induced amphetamine craving leaving natural reward unaffected.

Keywords: MPEP, metabotropic glutamate receptors of subtype 5 (mGluR5), amphetamine, MDMA (ecstasy), natural reward, locomotion, conditioned place preference (CPP) expression, context-conditioned reward.

Introduction

The last years of research revealed the importance of glutamate in processes underlying drug addiction (for review see Tzschentke and Schmidt 2003). Several recent studies suggest an involvement of metabotropic glutamate receptors of subtype 5 (mGluR5) in different models of drug addiction (Chiamulera et al. 2001; Popik and Wrobel 2002; McGeehan and Olive 2003). The selective mGluR5 antagonist 2-methyl-6-(phenylethynyl)pyridine (MPEP) was reported to block cocaine self-administration as well as development of cocaine conditioned place preference (CPP) in mice (Chiamulera et al. 2001; McGeehan and Olive 2003). The CPP paradigm, a model for context-induced drug-seeking, is especially relevant for addiction research, since contextual stimuli can induce craving that might finally lead to relapse (Childress et al. 1999). Additionally, substance dependence (= addiction) has been defined by the DSM IV (1994) as a maladaptive pattern of substance use that leads to several clinically significant impairments, e.g. that a great deal of time is spent in activities to obtain the

substance. Thus, the increase of time spent in the drug-associated chamber after conditioning with a drug of abuse as measured in the CPP paradigm is in line with the DSM IV (1994) definition of addiction.

It was shown that both development and expression of morphine-CPP was reduced by MPEP in mice (Popik and Wrobel 2002) and a previous study in our laboratory demonstrated that MPEP also inhibited expression of morphine-CPP in rats (Herzig and Schmidt 2003). Regarding other drugs of abuse, a reduction of ethanol-seeking behaviour and a reduction of the alcohol deprivation effect by MPEP has been reported (Marcon et al. 2003; Bäckström et al. 2004). Additionally, MPEP had no effect on development of CPP to amphetamine, nicotine and ethanol (McGeehan and Olive 2003). According to our opinion, the effects of MPEP on expression rather than on development of CPP are more suited to evaluate its usefulness as a potential anti-craving drug. As far as we know, no data exist about the effect of MPEP on expression of CPP to amphetamine or MDMA. Regarding MDMA, previous results of our laboratory indicate that isolated but not group-housed rats develop MDMA-CPP (Meyer et al. 2002). However, it remains to be determined if a subgroup within the group-housed rats also develops MDMA-CPP.

Additionally, the involvement of mGluR5 in natural reward is poorly understood. Chiamulera et al. (2001) reported that lever-pressing for food was not affected by MPEP. The effect of MPEP on context-conditioned food reward has not yet been examined. Nevertheless, an effect of MPEP on context-conditioned food reward would question its selectivity to drug-reward and its potential usefulness for relapse prevention in addiction therapy. In the present study a CPP paradigm was used in rats to examine the effects of MPEP on context-conditioned drug (amphetamine or MDMA) or natural (food) reward. A dose of 50 mg/kg MPEP was used to selectively block mGluR5-mediated transmission, because it proved most effective in inhibiting expression of morphine-CPP in our previous study (Herzig and Schmidt 2003), while still keeping its selectivity for mGluR5 receptors (Anderson et al. 2002).

Materials and methods

Animals

All animal experiments were conducted in accordance with the principles of animal care and the national laws on animal experiments. For this study 54 male Sprague-Dawley rats (F1 generation of Charles River rats, Sulzfeld, Germany) that were about seven weeks of age at the experiment start have been used. Rats were fed with 12 g of standard rat chow per rat and day and received water *ad libitum*. All rats were naïve and housed in groups of 6 animals. They were kept under a 12 hour light-dark cycle with lights on at 8 a.m. and the experiments were carried out during the light-phase. All rats were allowed to habituate to the colony room for three weeks, and they were habituated to handling three times prior to the experiments.

Drugs

For determination of the appropriate amount of drug that had to be injected, the weight of each rat was measured daily before the experiment-start. Amphetamine (D,L-amphetamine-sulphate, lot 23097, Th. Geyer, Renningen, Germany) was dissolved in physiological (0.9 %) saline (Fresenius Kabi GmbH, Bad Homburg, Germany) and injected i.p. 10 min prior to the start of the experiment at a dose of 4 mg/kg during conditioning or 2 mg/kg during the amphetamine-test. MDMA (R,S-3,4-Methylenedioxymethamphetamine, supplied by Pharmaceutical Institute, Department of Pharmaceutical Chemistry/Analysis, University of Tübingen, Germany) was dissolved in PBS-solution and injected s.c. 10 min prior to the

experiment at a dose of 5 mg/kg during conditioning or 2.5 mg/kg during the MDMA-test. The selective mGluR5 antagonist MPEP was supplied by Novartis Pharma AG (Basel, Switzerland) and a dose of 50 mg/kg, dissolved in physiological saline, was injected i.p. 30 min prior to the start of the MPEP-test. On conditioning or testing days when no drug was injected, only saline (i.p.) or PBS-solution (s.c) was administered as substitute to the respective groups. All doses were based on the salt form of each drug.

CPP-apparatus

The CPP apparatus consisted of six boxes (TSE Systems, Germany), each with three different coloured and textured chambers. One of the end chambers (both about 31 cm x 25 cm) had grey walls and a rough-textured plastic floor, while the other had striped black and white walls and a smooth plastic floor. The smaller, middle chamber (11 cm x 25 cm) had white walls and a smooth metal floor. All chambers were equipped with photo sensors to detect the location of the rat to analyse the time spent in each chamber. Locomotion was determined according to the number of light-beam breaks. Three CPP-boxes were placed with the grey-coloured chambers facing the room and the other three boxes with the grey-coloured chambers facing the wall. Each lid of each chamber contained a bulb for illumination and always the middle bulb was illuminated to reduce the time spent in the middle chamber. The walls separating the chambers (during conditioning) were replaced by walls with open doors (during the pretests and the tests) to allow the rats entering the other chambers.

CPP-experiment

An unbiased CPP procedure was used and the rats were randomly assigned to three groups: amphetamine- (n = 12), MDMA- (n = 24) and food-group (n = 18). For the food-group, more rats were used since food-conditioning might produce a weaker CPP than drug-conditioning. In the MDMA-group, even more rats were used to reveal potential individual differences in respect to the sensitivity to MDMA reward. For all groups, the experiment consisted of three phases: pretest (days 1-3), conditioning (days 4-13) and three tests (days 15-17). One day without testing (day 14, rats remained untreated in their home-cage) was performed after conditioning to overcome the regular reward – non-reward order. Every day each rat was placed into the same CPP-box and after the end of each run the CPP-boxes were cleaned with water and wiped dry. Within each group, the assignment of the rewarded chamber was counterbalanced respective to the differentially coloured and oriented chambers.

During three days of pretest each rat was placed for 20 min into the middle chamber without prior injection and with free access to all chambers. The average unconditioned place preference (UCPP) during three pretests served as a within-group control for the CPP expression during the tests. Then a conditioning session of ten days followed with closed doors for 30 min. On rewarded days the rats were always confined to the same end chamber and on non-rewarded days to the other. During conditioning-days, the amphetamine- and MDMA-groups received the respective drug on odd days and saline or PBS on even days before being confined to the either rewarded or non-rewarded chamber. The food-group was injected with saline on all conditioning days, but received a food reward inside the rewarded chamber only on odd conditioning-days. The food reward consisted of two (of the usual) food-pellets that had been macerated in water for 30 min. Thereafter (after the experiment at their normal feeding time on rewarded conditioning days), the food-group received the normal amount of food minus the amount of the food reward to maintain their normal daily food amount.

The following three tests were all carried out for 20 min and for the start the rats were placed into the middle chamber with free access to all chambers. Before each test, each rat always

received two injections, i.e. either 50 mg/ kg MPEP or saline 30 min prior to the test plus the drug or the respective substitutes (saline for the amphetamine- and food-groups, PBS for the MDMA-group) 10 min prior to the test. Half of the rats of each group received 50 mg/kg MPEP before the first test and the respective substitute before the second test, while the other half received the opposite the tests. The third test consisted of a challenge injection with the conditioning drug at the half dose (i.e. 2 mg/kg amphetamine, 2.5 mg/kg MDMA or one macerated food pellet placed inside the middle chamber). In the food-group, a fourth test under saline was carried out.

Data analysis

For all statistical analysis the program GB-Stat 7.0 (Dynamic Microsystems Inc.) was used and significance levels were set as $p < 0.05$ (significant) and $p < 0.01$ (high significant). CPP was calculated as difference of time spent in rewarded and non-rewarded chamber. Based on our previous experience with the CPP paradigm (data not presented), we observed that not all rats that were for example conditioned with amphetamine, cocaine, morphine or MDMA show CPP expression in the tests. This might be explained by the use of outbread Sprague Dawley rats that obviously show a large variation in their susceptibility to addictive drugs. Because rats that do not show CPP expression may falsify the outcome of the tests, each rat was analysed whether it really showed CPP expression or not. The criterion for CPP expression in the drug-conditioned groups was reached if the average CPP under the saline- and the drugged-test minus the UCPP during the average pretests was more than 100 s. In other words, each rat has to spend at least 100 s more time in the rewarded chamber after conditioning than before to meet the criterion. For the food-group, the average CPP under both saline-tests minus the UCPP during the average pretests has to be more than 100 s to reach criterion. Two saline-tests (instead of a saline- and a food-test) were used as reference in the food-group since the act of consuming the food may affect CPP expression and may therefore not be used as a reference. Rats that did not reach the criterion for CPP-expression were excluded from all statistical analysis. Consequently, 4 rats in the amphetamine-, 11 rats in the MDMA- and 8 rats in the food-group did not show stable CPP expression and were therefore not used for analysis. Locomotion was calculated according to the number of light-beam breaks during the pretests and tests. Locomotion and CPP data were analysed by repeated measures ANOVA followed by multiple post-hoc tests (Fisher's LSD) with average pretest-UCPP or saline-locomotion as respective references.

Results

CPP

For the food-group (n = 10), repeated measures ANOVA revealed a significant effect of the tests on CPP expression ($F = 3.27$; $p = 0.022$; fig. 1a). Within-group comparison (Fisher's LSD post-hoc test) with the average pretest-UCPP as reference showed significant CPP expression during both saline-tests ($p < 0.05$) and a high significant CPP expression during the MPEP-test ($p < 0.01$), but not during the food-challenge ($p > 0.05$). Thus, MPEP (50 mg/kg) did not block the expression of food-CPP.

Locomotion

Repeated measures ANOVA for the food-group revealed a high significant effect of the tests on locomotion ($F = 35.66$; $p < 0.0001$; fig. 1b). Fisher's LSD post-hoc test (saline-test locomotion as reference) showed high significant decreased locomotion during the MPEP-test ($p < 0.01$) and during the food-challenge ($p < 0.01$). Thus, MPEP (50 mg/kg) and food intake reduced the locomotion in the food-group

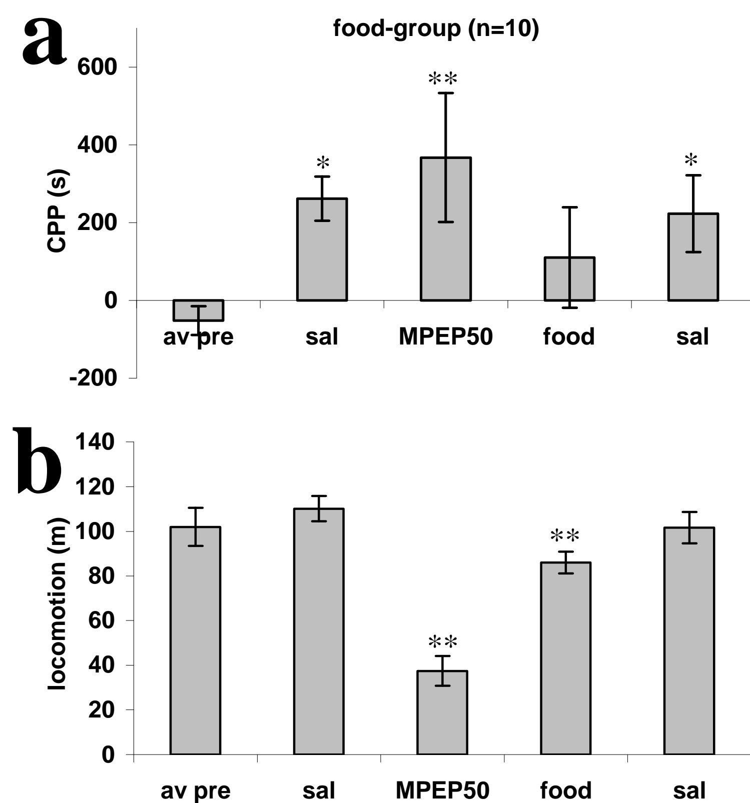

Figure 1: **Food-group**

The average **a:** conditioned place preference (CPP = time difference between rewarded and non-rewarded chamber) and **b:** locomotion (= locomotion in all three chambers) during the average of three pretests (av pre), during two tests after saline (sal), a test after 50 mg/kg MPEP (MPEP50) and the food challenge (food) consisting of a single macerated food pellet placed inside the middle chamber. Each test lasted for 20 min using three-chambered CPP-boxes. The SEM are indicated for each value. *($p < 0.05$) and ** ($p < 0.01$) indicate significant differences from the average pretest (for CPP data) or from the saline-test (for locomotion-data).

CPP

For the amphetamine-group (n = 8), a significant effect of the tests on CPP expression was observed (F = 4.22; p = 0.0175; fig. 2a). As compared to the average pretest-UCPP (Fisher's LSD), a high significant CPP was expressed after saline ($p < 0.01$) and a significant CPP was also found after the amphetamine-challenge injection ($p < 0.05$). CPP expression during the MPEP-test was not significantly different from average pretest UCPP ($p > 0.05$), but significantly reduced ($p < 0.05$) as compared to the saline test. Thus, MPEP (50 mg/kg) blocked the expression of amphetamine-CPP.

Locomotion

In the amphetamine-group, also a high significant effect of the tests on locomotion was found (F = 36.61; $p < 0.0001$, fig. 2b). Compared to the saline locomotion, amphetamine produced a high significant increase ($p < 0.01$) while MPEP produced a high significant decrease ($p < 0.01$) of locomotion. Thus, amphetamine (2 mg/kg) increased and MPEP (50 mg/kg) reduced the locomotion in the amphetamine-group.

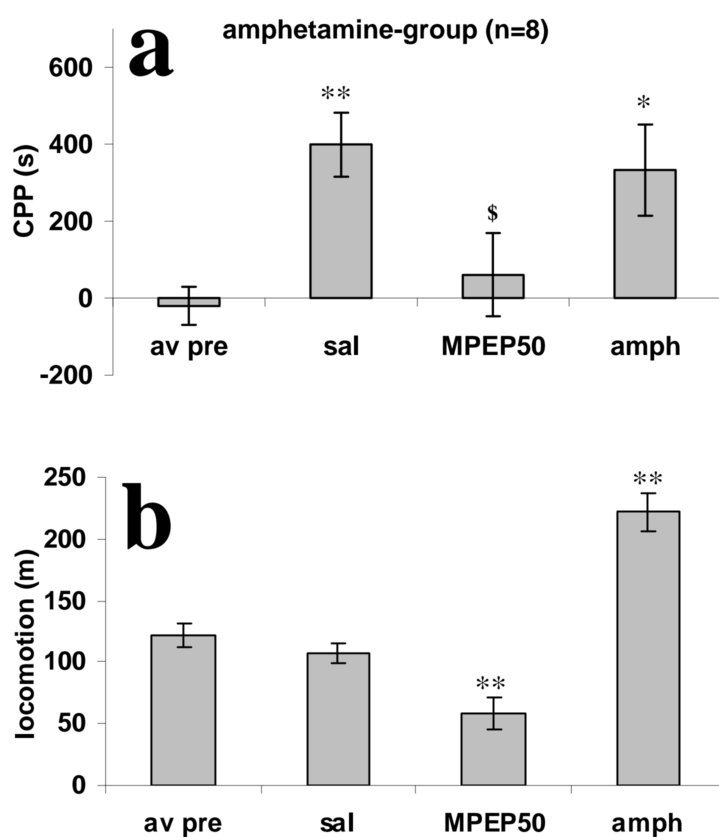

***Figure 2*: Amphetamine-group**

The average **a:** conditioned place preference (CPP = time difference between rewarded and non-rewarded chamber) and **b:** locomotion (= locomotion in all three chambers) during the average of three pretests (av pre) and during the tests after saline (sal), 50 mg/kg MPEP (MPEP50) and the challenge injection of 2 mg/kg amphetamine (amph). Each test lasted for 20 min using three-chambered CPP-boxes. The SEM are indicated for each value. *($p < 0.05$) and ** ($p < 0.01$) indicate significant differences from the average pretest (for CPP data) or from the saline-test (for locomotion-data). $ ($p < 0.05$) indicates significant reduction of CPP expression as compared to the saline-test.

<u>CPP</u>

For the MDMA-group (n = 13), a high significant effect of the tests on CPP expression was found (F = 8.07; p = 0.0003; fig. 3a). Post-hoc tests (Fisher's LSD) revealed high significant (p < 0.01) CPP expression after all tests (saline, MPEP50 and MDMA) as compared to the average pretest UCPP. Thus, MPEP (50 mg/kg) did not block the expression of MDMA-CPP.

<u>Locomotion</u>

In the MDMA-group, the tests showed a high significant effect on locomotion (F = 30.07; p < 0.0001; fig. 3b). Post-hoc comparison showed that locomotion in the saline-test was significantly lower than during the average pretests (p < 0.05). Furthermore, a high significant reduction of locomotion during the MPEP50 test (p < 0.01) as compared to the saline locomotion was observed. Thus, MPEP (50 mg/kg) reduced the locomotion in the MDMA-group.

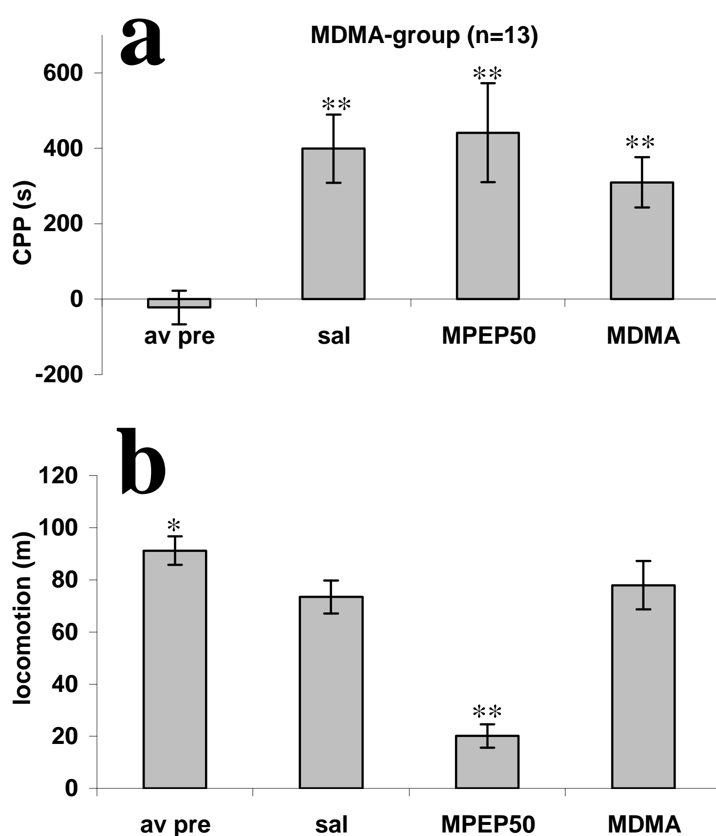

***Figure 3*: MDMA-group**

The average **a:** conditioned place preference (CPP = time difference between rewarded and non-rewarded chamber) and **b:** locomotion (= locomotion in all three chambers) during the average of three pretests (av pre) and during the tests after saline (sal), 50 mg/kg MPEP (MPEP50) and the challenge injection of 2.5 mg/kg MDMA (MDMA). Each test lasted for 20 min using three-chambered CPP-boxes. The SEM are indicated for each value. *(p < 0.05) and ** (p < 0.01) indicate significant differences from the average pretest of the respective subgroup (for CPP data) or from the respective saline-test (for locomotion-data).

Discussion

The main finding of the present study is the reduction of amphetamine-CPP expression by 50 mg/kg of the selective mGluR5 antagonist MPEP in rats, while food- and MDMA-CPP expression were not affected. Additionally, MPEP reduced the locomotion in all groups.

The observed reduction of locomotion by 50 mg/kg MPEP is in accordance with a previous report that showed an inhibition of spontaneous locomotion by high doses of MPEP (30 and 100 mg/kg, p.o.) in rats (Spooren et al. 2000). However, it contrasts a recent study that reported an increased locomotion by MPEP (5 and 20 mg/kg, i.p.) in mice (McGeehan et al. 2004). A possible explanation for these contradictory results may be the use of different rodent species or different doses of MPEP. Additionally, also procedural issues might play a role, since MPEP was always injected 30 min prior to measurement of locomotion in the present study, while it was applied immediately prior to the experiment in the McGeehan et al. (2004) study. Consequently, the contrasting results might be explained by a time-dependent change in the effect of MPEP, being first a positive modulator of locomotion that produces reduction of locomotion after a longer time. At least, it may be concluded that mGluR5 are involved in modulation of spontaneous locomotion.

Despite the CPP paradigm depends to a large extent on locomotor activity, it’s unlikely that the observed reduction of locomotion by MPEP is the cause for the reduction of amphetamine-CPP expression, especially since expression of MDMA- and food-CPP were not affected by MPEP despite a similar reduction of locomotion. Furthermore, locomotion was not completely abolished in all groups, thereby still allowing the expression of the preference behaviour. Additionally, the relatively low standard error for the CPP observed in the amphetamine-group during the MPEP-test does not fit to an effect that was only caused by altered locomotion. In that case the standard error would be expected to be very high, e.g. if a drug produces sedative effects and rats randomly move to one or the other chamber to sleep there. Thus, we conclude that the general effects of MPEP on locomotion cannot be a major reason for the selective effects on amphetamine-CPP expression.

By discussing the present results in respect to the published literature, it has to be kept in mind that only a subpopulation of “responders” was analysed. Furthermore, one might argue that by applying two subsequent tests (counterbalanced between saline- and MPEP- tests within each group), extinction of CPP expression could have influenced the selection of the responders. However, a closer look onto the selected responders showed that they were more or less equally distributed among both tests. Among the responders in the amphetamine-group (n = 8), 5 rats were first treated with MPEP and 3 rats received saline first. In the MDMA-group (n = 13), the relationship “MDMA-test : saline-test” was “6:7” and in the food-group (n = 10) it was “4:6”. Thus, it’s unlikely that the selection of the responders was based on extinction effects.

Regarding the lack of effect of MPEP on natural food reward, the present findings complement the previous results of other groups (Chiamulera et al. 2001; Paterson et al. 2003). MPEP did not affect food self-administration in these studies and we found no effect of MPEP on expression of context-conditioned food reward in the present study. The observed lack of food-CPP expression during the food-test may be explained by the impairment CPP expression due to food-consumption. Thus, the effects that contextual stimuli exert on behaviour seem to be masked by the act of consuming food. In summary, mGluR5 do not seem to be involved in the mechanisms regulating natural reward in rodents. However, other glutamate receptors are involved in natural reward since performance of

Pavlovian conditioned food approach was impaired by AMPA/kainate receptor antagonist LY293558 in the Nac core, while the NMDA receptor antagonist AP-5 impaired only the acquisition (Di Ciano et al. 2001). Additionally, the low affinity NMDA channel blocker memantine did not affect expression of food-CPP (Popik and Danysz 1997; Popik et al. 2003). A brain structure that might be especially important for natural reward is the ventral pallidum, since it is only involved in food-induced reinstatement and not in drug-induced reinstatement of cocaine-seeking (McFarland and Kalivas 2001). Conversely, the increased glutamate in the Nucleus accumbens initiates responding selectively for drug reinforcement and was not observed with food-induced responding (McFarland et al. 2003). Thus, a possible hypothesis could be that increased glutamate in the nucleus accumbens is prerequisite for MPEP to be effective.

The observed reduction of amphetamine-CPP expression by MPEP is in contrast to the finding that amphetamine-CPP development is not affected by MPEP in mice (McGeehan and Olive 2003). This may be explained by the lower doses (1-20 mg/kg) used by McGeehan and Olive (2003) or by a prominent role of dopaminergic transmission in amphetamine-CPP development (Spyraki et al. 1982; Hiroi and White 1991). However, results from our laboratory demonstrated that besides dopamine glutamate also plays a role for amphetamine-CPP development, since it was blocked by the glutamate release inhibitor riluzole (Tzschentke and Schmidt 1998). Nevertheless, we suggest that the glutamatergic mechanisms involved in development and expression of amphetamine-CPP are different, since MPEP reduced amphetamine-CPP expression but did not affect amphetamine-CPP development.

A search of the relevant literature showed that like MPEP, other drugs acting on glutamate receptors also reduced expression of amphetamine-CPP, e.g. the non-competitive NMDA-receptor antagonist MK-801, the NMDA-receptor antagonist (±)-3-(2-carboxy-piperazine-4-yl)-propyl-1-phosphonic acid ((±)-CPP), the AMPA/kainate-receptor antagonist DNQX or the AMPA-receptor selective antagonist GYKI 52466 (Layer et al. 1993; Bespalov 1996; Tzschentke and Schmidt 1997). This clearly demonstrates the importance of glutamatergic transmission for expression of context-conditioned amphetamine reward. Glutamate has also been shown to play a crucial role regarding expression of cocaine- and morphine-CPP. Especially NMDA receptors seem to be involved, since expression of cocaine-CPP is prevented by the non-competitive NMDA-receptor antagonist memantine (Kotlinska and Biala 2000) while expression of morphine-CPP is inhibited by memantine (Popik et al. 2003), by the non-competitive NMDA-receptor antagonist MK-801 (Tzschentke and Schmidt 1997), by the NMDA-receptor glycine-site antagonist L-701,324 (Kotlinska and Biala 1999) and by the competitive NMDA-receptor antagonist NPC 17742 (Popik and Kolasiewicz 1999). The latter study showed that NMDA-receptors in the nucleus accumbens and the ventral tegmental area are involved in morphine-CPP expression. Additionally, the NMDA-receptor-induced membrane depolarisation of striatal medium spiny neurons is potentiated by the selective mGluR5-agonist CHPG, an effect that was blocked by MPEP administration (Pisani et al. 2001). Repeated cocaine has been shown to increase mGluR5 mRNA levels in the dorsolateral striatum and the shell of the nucleus accumbens (Ghasemzadeh et al. 1999), both regions that already show a very high mGluR5 density in naïve individuals (for review see Spooren et al. 2003). Thus, we hypothesize that the effect of MPEP on expression of drug-CPP can be at least partially attributed to a blockade of the positive modulatory properties of mGluR5 on NMDA-receptor mediated transmission in the nucleus accumbens. This would also explain why MPEP showed no effect on expression of food-CPP that does not involve NMDA-receptors (Popik and Danysz 1997; Popik et al. 2003).

Examination of the literature published about MDMA did not reveal any study that tested the effect of a potential anti-craving drug on expression of MDMA-CPP. Therefore, no comparison of outcome of the present study with previous results can be drawn. Regarding development of MDMA-CPP, it has been suggested that the dopaminergic system is responsible for the rewarding properties of MDMA (Marona-Lewicka et al. 1996). The lack of effect of MPEP on expression of context-conditioned MDMA reward suggests that the mechanisms for conditioned reward differ between MDMA on one side and amphetamine (see present study) or morphine (Popik and Wrobel 2002) on the other side. An involvement of glutamate in expression of context-conditioned MDMA reward cannot be completely excluded, but at least mGluR5 do not seem to play a role.

The fact that only 13 of 24 rats in the MDMA-group developed MDMA-CPP may explain the difficulties of previous studies to detect MDMA-CPP by using a lower number (n = 10) of group-housed rats (Meyer et al. 2002). However, since MDMA-CPP was found in isolated rats in the latter study, isolation-stress seems to render the rats more sensitive to MDMA. An influence of isolation-stress on the serotonergic system (as the primary target of MDMA) has already been reported for isolated aggressive mice that exhibit lower serotonin content in comparison to group-housed mice (for review see Miczek et al. 2002). Additionally, reduction of serotonin metabolism and upregulation of cortical 5-HT2A receptors were reported from socially isolated mice (Rilke et al. 1998). We hypothesize that increased sensitivity to the rewarding effects of MDMA might be caused by a reduction of serotonergic transmission in stressed animals and the subsequent compensatory upregulation of the serotonin 5HT2 receptor density, since MDMA shows a strong affinity to 5HT2 receptors as reported by Battaglia et al. (1988). Regarding the present study, it might be assumed that social-stress in some of the group-housed rats altered their serotonergic system in a similar way as reported for isolation-stress. A behavioural differentiation due to social pressure within a group of rats has recently been described (Grasmuck and Desor 2002), and it might be speculated that the emergence of specialized roles within a group also involves alterations in neuronal transmission. In general, the influence of stress (either due to social-interaction or due to isolation) on the sensitivity for MDMA would correspond to results of Matuszewich et al. (2004), showing that chronic stress increases the pharmacological effects of methamphetamine. Another possible explanation why MDMA only produces CPP in stressed animals may be that (by some unknown mechanisms) MDMA alleviates the negative effects of stress, which is perceived as rewarding and hence induces CPP. This fits to human studies reporting that MDMA reduced the responsiveness to stress (Gerra et al. 2003). By use of a neurotoxic dosing regimen of MDMA in rats, it has also been shown that the stress-induced increase of serotonin in the hippocampus is prevented by MDMA (Matuszewich et al. 2002).

In summary, we suggest that mGluR5 are involved in modulation of spontaneous locomotion. Regarding the expression of context-conditioned food- or MDMA-reward, mGluR5 do not seem to play a significant role. However, mGluR5 are involved in expression of context-conditioned amphetamine reward. Thus, mGluR5-blockade is suggested as a promising treatment strategy to prevent context-induced amphetamine craving.

Acknowledgements

We thank Drs. Rainer Kuhn and Fabrizio Gasparini (Novartis, Switzerland) for the kind supply of MPEP. This study was funded by the federal ministry for education and research 01EB0110-Baden-Württemberg consortium for addiction research.

References

Anderson JJ, Rao SP, Rowe B, Giracello DR, Holtz G, Chapman DF, Tehrani L, Bradbury MJ, Cosford ND, Varney MA (2002) [3H]Methoxymethyl-3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine binding to metabotropic glutamate receptor subtype 5 in rodent brain: in vitro and in vivo characterization. J.Pharmacol.Exp.Ther. 303: 1044-1051

Bäckström P, Bachteler D, Koch S, Hyytiä P, Spanagel R (2004) mGluR5 antagonist MPEP reduces ethanol-seeking and relapse behaviour. Neuropsychopharmacol. 29: 921-928.

Battaglia G, Brooks BP, Kulsakdinun C, de Souza EB (1988) Pharmacologic profile of MDMA (3,4-methylenedioxymethamphetamine) at various brain recognition sites. Eur.J.Pharmacol. 149: 159-163

Bespalov AY (1996) The expression of both amphetamine-conditioned place preference and pentylenetetrazole-conditioned place aversion is attenuated by the NMDA receptor antagonist (±)-CPP. Drug Alc.Dep. 41: 85-88.

Chiamulera C, Epping-Jordan MP, Zocchi A, Marcon C, Cottiny C, Tacconi S, Corsi M, Orzi F, Conquet F (2001) Reinforcing and locomotor stimulant effects of cocaine are absent in mGluR5 null mutant mice. Nat.Neurosci. 4: 873-874

Childress AR, Mozley PD, McElgin W, Fitzgerald J, Reivich M, O'Brien CP (1999) Limbic activation during cue-induced cocaine craving. Am.J.Psychiat. 156: 11-18

Di Ciano P, Cardinal RN, Cowell RA, Little SJ, Everitt BJ (2001) Differential involvement of NMDA, AMPA/kainate, and dopamine receptors in the nucleus accumbens core in the acquisition and performance of pavlovian approach behavior. J.Neurosci. 21: 9471-9477

DSM-IV, Diagnostic and Statistical Manual of Mental Disorders (1994), ed. 4. Washington DC: American Psychiatric Association (AMA)

Gerra G, Bassignana S, Zaimovic A, Moi G, Bussandri M, Caccavari R, Brambilla F, Molina E (2003) Hypothalamic-pituitary-adrenal axis responses to stress in subjects with 3,4-methylenedioxy-methamphetamine ('ecstasy') use history: correlation with dopamine receptor sensitivity. Psychiatry Res. 120: 115-124

Ghasemzadeh MB, Nelson LC, Lu XY, Kalivas PW (1999) Neuroadaptations in ionotropic and metabotropic glutamate receptor mRNA produced by cocaine the tests. J.Neurochem. 72: 157-165

Grasmuck V, Desor D (2002) Behavioural differentiation of rats confronted to a complex diving-for-food situation. Behav.Processes 58: 67-77

Herzig V, Schmidt WJ (2003) Effects of the mGluR5 antagonist MPEP on locomotion, sensitization and secondary reward by cocaine and morphine. Behav.Pharmacol. 14: P83 (Abstract)

Hiroi N, White NM (1991) The amphetamine conditioned place preference: differential involvement of dopamine receptor subtypes and two dopaminergic terminal areas. Brain Res. 552: 141-152

Kotlinska J, Biala G (1999) Effects of the NMDA/glycine receptor antagonist, L-701,324, on morphine- and cocaine-induced place preference. Pol.J.Pharmacol. 51: 323-330

Kotlinska J, Biala G (2000) Memantine and ACPC affect conditioned place preference induced by cocaine in rats. Pol.J.Pharmacol. 52: 179-185

Layer RT, Uretsky NJ, Wallace LJ (1993) Effects of the AMPA/kainate receptor antagonist DNQX in the nucleus accumbens on drug-induced conditioned place preference. Brain Res. 617: 267-273

Marcon C, Andreoli M, Pilla M, Tessari M, Heidbreder CA (2003) A new model to assess drug and cue-induced relapse to ethanol self-administration in mice. Behav.Pharmacol. 14: S66

Marona-Lewicka D, Rhee GS, Sprague JE, Nichols DE (1996) Reinforcing effects of certain serotonin-releasing amphetamine derivatives. Pharmacol.Biochem.Behav. 53: 99-105

Matuszewich L, Filon ME, Finn DA, Yamamoto BK (2002) Altered forebrain neurotransmitter responses to immobilization stress following 3,4-methylenedioxymethamphetamine. Neuroscience 110: 41-48

Matuszewich L, Yamamoto BK (2004) Chronic stress augments the long-term and acute effects of methamphetamine. Neuroscience 124: 637-646

McFarland K, Kalivas PW (2001) The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. J.Neurosci. 21: 8655-8663

McFarland K, Lapish CC, Kalivas PW (2003) Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. J.Neurosci. 23: 3531-3537

McGeehan AJ, Olive MF (2003) The mGluR5 antagonist MPEP reduces the conditioned rewarding effects of cocaine but not other drugs of abuse. Synapse 47: 240-242

McGeehan AJ, Janak PH, Olive MF (2004) Effect of the mGluR5 antagonist 6-methyl-2-(phenylethynyl)pyridine (MPEP) on the acute locomotor stimulant properties of cocaine, d-amphetamine, and the dopamine reuptake inhibitor GBR12909 in mice. Psychopharmacology, online First, DOI: 10.1007/s00213-003-1733-2

Meyer A, Mayerhofer A, Kovar KA, Schmidt WJ (2002) Rewarding effects of the optical isomers of 3,4-methylenedioxy- methylamphetamine ('Ecstasy') and 3,4-methylenedioxy-ethylamphetamine ('Eve') measured by conditioned place preference in rats. Neurosci.Lett. 330: 280-284

Miczek KA, Fish EW, De Bold JF, De Almeida RM (2002) Social and neural determinants of aggressive behavior: pharmacotherapeutic targets at serotonin, dopamine and gamma- aminobutyric acid systems. Psychopharmacology 163: 434-458

Paterson NE, Semenova S, Gasparini F, Markou A (2003) The mGluR5 antagonist MPEP decreased nicotine self-administration in rats and mice. Psychopharmacology 167: 257-264

Pisani A, Gubellini P, Bonsi P, Conquet F, Picconi B, Centonze D, Bernardi G, Calabresi P (2001) Metabotropic glutamate receptor 5 mediates the potentiation of N-methyl-D-aspartate responses in medium spiny striatal neurons. Neuroscience 106: 579-587

Popik P, Danysz W (1997) Inhibition of Reinforcing Effects of Morphine and Motivational Aspects of Naloxone-Precipitated Opioid Withdrawal by N-Methyl-D-aspartate Receptor Antagonist, Memantine. J.Pharmacol.Exp.Ther. 280: 854-865

Popik P, Kolasiewicz W (1999) Mesolimbic NMDA receptors are implicated in the expression of conditioned morphine reward. N.-S.Arch.Pharmacol. 359: 288-294

Popik P, Wrobel M (2002) Morphine conditioned reward is inhibited by MPEP, the mGluR5 antagonist. Neuropharmacology 43: 1210-1217

Popik P, Wrobel M, Rygula R, Bisaga A, Bespalov AY (2003) Effects of memantine, an NMDA receptor antagonist, on place preference conditioned with drug and nondrug reinforcers in mice. Behav.Pharmacol. 14: 237-244

Rilke O, Freier D, Jahkel M, Oehler J (1998) Dynamic alterations of serotonergic metabolism and receptors during social isolation of low- and high-active mice. Pharmacol.Biochem.Behav. 59: 891-896

Spooren WP, Gasparini F, Bergmann R, Kuhn R (2000) Effects of the prototypical mGlu(5) receptor antagonist 2-methyl-6- (phenylethynyl)-pyridine on rotarod, locomotor activity and rotational responses in unilateral 6-OHDA-lesioned rats. Eur.J.Pharmacol. 406: 403-410

Spooren W, Ballard T, Gasparini F, Amalric M, Mutel V, Schreiber R (2003) Insight into the function of Group I and Group II metabotropic glutamate (mGlu) receptors: behavioural characterization and implications for the the tests of CNS disorders. Behav.Pharmacol. 14: 257-277

Spyraki C, Fibiger HC, Phillips AG (1982) Dopaminergic substrates of amphetamine-induced place preference conditioning. Brain Res. 253: 185-193

Tzschentke TM, Schmidt WJ (1997) Interactions of MK-801 and GYKI 52466 with morphine and amphetamine in place preference conditioning and behavioural sensitization. Behav.Brain Res. 84: 99-107

Tzschentke TM, Schmidt WJ (1998) Blockade of morphine- and amphetamine-induced conditioned place preference in the rat by riluzole. Neurosci.Lett. 242: 114-116

Tzschentke TM, Schmidt WJ (2003) Glutamatergic mechanisms in addiction. Mol.Psychiatry 8: 373-382

Reduction of cocaine conditioned place preference expression in rats by temporal inactivation of central but not basolateral amygdala

Volker Herzig and Werner J. Schmidt

Zoological Institute, Neuropharmacology, University of Tübingen, Germany

Abstract

Contextual stimuli are one important factor that can induce relapse in detoxified drug addicts and the amygdala is known to be important for stimulus-reinforcer associations. Closer examination revealed dissociable roles of the central and the basolateral amygdala in development of Pavlovian contextual conditioning to drugs of abuse. The role of both nuclei in expression of contextual conditioning, however, is not well understood. Therefore, we applied the conditioned place preference paradigm, a procedure based on Pavlovian conditioning, to elucidate the roles of these amygdalar nuclei in expression of contextual conditioning. Rats were implanted with microinfusion cannulas in either the central or basolateral amygdala and conditioned with cocaine. Rats that developed cocaine place preference were tested for expression after microinfusion of saline or a combination of baclofen and muscimol to induce temporal inactivation of the target nuclei. Our results show that temporal inactivation of the central but not the basolateral amygdala reduce expression of cocaine place preference while locomotion, as a measurement of general activity, was not affected. In summary, we suggest that central amygdala is involved in expression of contextual conditioning to cocaine.

Key words: temporal inactivation, microinfusion, contextual stimuli, secondary reward.

Introduction

Drug addiction has been characterized as a chronic relapsing disease (Leshner, 1997), because relapse to drug-taking is considered a major problem that opposes treatment of addiction. Drug associated stimuli, either discrete (syringe, crack-pipe, etc…) or predictive (environment, context), can induce craving that finally results in relapse (See, 2002). The amygdala, a structure implicated in emotional (both aversive and rewarding) behaviour (Holland and Gallagher, 1999), has been proposed as key regulator for discrete stimulus-reinforcer associations (See, 2002). Closer examination of amygdalar subregions using operant conditioning procedures (e.g. self-administration (SA)) demonstrated that basolateral amygdala (BLA) but not central amygdala (CeA) is important for development of reinforcing effects of drug-conditioned stimuli (Kruzich and See, 2001). However, both the BLA and the CeA seem to be important for expression of conditioned-stimulus induced relapse to drug-seeking (Kruzich and See, 2001). Though a large number of studies used operant conditioning to examine the roles of CeA and BLA, only few studies used Pavlovian conditioning models in this respect. Since operant conditioning models (e.g. SA-paradigm) and Pavlovian conditioning models (e.g. conditioned place preference (CPP)) are measuring fundamentally different processes (Bardo and Bevins, 2000) both models may help to clarify different aspects of drug-addiction.

Development of Pavlovian conditioned food-approach was impaired by CeA-, but not by BLA-lesions (Parkinson et al., 2000). To our knowledge, there is no study that examined the role of both amygdalar nuclei in expression of conditioned food-approach. Studies using the CPP paradigm showed that amygdalar lesions block development of cocaine CPP (Browns and Fibiger, 1993). Pre- and post-training infusions of the local anaesthetic bupivacaine into the amygdala blocked development, consolidation and expression of amphetamine CPP (Hsu et al., 2002). By lesions or inactivation of amygdalar sub-nuclei, it was shown that the BLA is not involved in expression of reinforcing effects of food- (Schroeder and Packard, 2000), amphetamine- (Hiroi and White, 1991) or cocaine- (Fuchs et al., 2002) associated contexts which is in contrast to results obtained from SA-studies. In order to investigate potentially different roles of both CeA and BLA in expression of contextual conditioning, we used a CPP paradigm in cocaine-conditioned rats that obtained temporal inactivation of either the CeA or the BLA. To account for specificity of the temporal inactivation to contextual reward, general activity as measured by locomotion was additionally observed during the tests.

Material and methods

Subjects

The subjects were 24 male Sprague-Dawley rats (F1 generation of Charles River rats, Sulzfeld, Germany, supplied from animal facility, University of Tübingen), divided into two equal-sized groups: the CeA-group and the BLA-group. Rats, weighing 220-320 g before surgery, were fed with 12 g standard rat chow (sniff Spezialdiäten GmbH, Soest, Germany) per rat and day and received water ad libitum. All rats were naïve and housed in groups of six animals in a temperature-controlled room (23°C). They were kept under a 12 hour light-dark cycle with lights on at 8 a.m. and experiments were carried out during light-phase. All rats were habituated to handling several times prior to experiments.

Surgery

Before surgery, rats were anesthetized with ketamine (75 mg/kg, intraperitoneally (i.p.), CuraMed Pharma GmbH, Karlsruhe, Germany) and xylazine (Rompun, 12 mg/kg, i.p., Bayer, Leverkusen, Germany). Guide cannulae were constructed from disposable, stainless steel needles (TSK Sterijekt, Geislingen, Germany, 0.8 mm diameter (= 21 g), cut to a length of 13 mm for CeA or 14 mm for BLA). A stereotaxic apparatus was used for bilateral implantation of guide cannulae into CeA (anterior-posterior -2.3; lateral ± 4.1; ventral -8.0) or BLA (anterior-posterior -2.6; lateral ± 4.8; ventral -8.6), all coordinates relative to the skull surface and bregma according to the atlas of Paxinos and Watson (1998). To enable group-housing by preventing the rats from drawing out each others stylets, a metal tube (12 x 5 mm inner diameter, 8 mm length) that surrounded the guide cannulae and jeweller screws that served as anchors were fixed to the skull with dental cement (Paladur, Heraeus Kulzer GmbH, Hanau, Germany). Stylets (disposable needles of 0.45 mm diameter (= 26 g), Neopoint, servoprax GmbH, Wesel, Germany; same length as the guide cannulae with the upper end bent) were inserted into guide cannulae to prevent obstruction by debris. After surgery, rats were housed individually for three days before group-housed again. All rats were allowed to recover from surgery for at least eight days before the place preference experiment started.

Apparatus

The CPP apparatus consisted of six boxes (TSE Systems, Germany), each with three different coloured and textured chambers. One of the end chambers had grey walls and a rough-textured plastic floor, while the other had striped black and white walls and a smooth plastic floor. The smaller, middle chamber had white walls and a smooth metal floor. The walls

separating the chambers (during conditioning) were replaced by walls with open doors (during pretests and testing) to allow the rats to pass into the other chambers. All chambers were equipped with photo sensors to detect the location of the rat to analyse the time spent in each chamber. Locomotion was determined according to the number of light-beam breaks. Three CPP-boxes were placed with the grey-coloured chambers facing the room and the other three boxes with the grey-coloured chambers facing the wall. Each lid of each chamber contained a bulb for illumination and always the bulb in the middle chamber was illuminated to reduce the time inside.

Place preference procedure

For both groups experiment consisted of three phases: pretest (days 1-3), conditioning (days 4-13), and three tests (days 15-17). During the pretests each rat was placed for 20 min into the middle chamber without prior injection and with free access to all chambers to reveal potential unconditioned place preferences. During the conditioning phase all rats received five pairings of 10 mg/kg cocaine (cocaine-hydrochloride, lot L447362 931, Merck, Germany, dissolved in saline) on odd and saline (Fresenius Kabi GmbH, Bad Homburg, Germany) on even days. On cocaine-treated days rats were always confined to the same chamber and on saline-treated days to the other chamber, respectively (with closed doors for 30 min). The assignment of the rewarded compartment was counterbalanced within each group according to different coloured and oriented (room or wall) chambers. Each rat was always placed into the same CPP-box and CPP-boxes were wetly cleaned and wiped dry after each run. All injections during the conditioning and the subsequent testing phase were administered i.p. and applied five minutes prior to placement of rats into CPP-boxes. On day 14, a day in the home-cage without testing was performed to overcome the regularity of drug- and saline-administration during conditioning. The three testing-days were carried out under the same conditions as during pretest (i.e. duration of 20 min with access to all chambers). During the first testing day both the CeA- and the BLA group received SHAM-treatment before determining CPP-expression that consisted of bilateral saline microinfusion and a subsequent i.p. saline injection. The microinfusion was applied through injection-cannulae (constructed from disposable needles, 0.45 mm diameter, Neopoint, servoprax GmbH, Wesel, Germany) inserted into the guide-cannulae and extended as far as the guide-cannulae into the target region. A volume of 0.5 µl saline/ side was infused bilaterally into the respective brain area over a period of 60 s via two 1 µl Hamilton syringes (SGE, Australia) that were connected to the injection cannulae by polyethylene tubing. Injection cannulae remained in place for additional 90 s after saline-microinfusion to allow time for diffusion before replacing them with stylets. Thereafter, a saline-injection was given to all rats and they were immediately placed into the CPP-boxes. The SHAM-treatment served as an internal control for the respective group to allow comparison with the microinfusion of GABA-agonists on the following day. On the second testing day, both groups received bilateral microinfusion of a combination of 1 nmol/µl of the GABAB-agonist baclofen (lot 14H0520, Sigma, Germany) + 0.1 nmol/µl of the GABAA-agonist muscimol (lot 71H4009, Sigma, Germany) dissolved in saline in a volume of 0.5 µl/ side according to the same microinfusion-procedure of the previous day. The combination of baclofen/muscimol was used to hyperpolarize the target nuclei and to produce a reversible inactivation (see McFarland et al., 2003). Before the last testing day, both groups received no microinjection but 10 mg/kg cocaine (i.p.) 5 min before the test.

Histology

After the end of the experiment, rats were sacrificed with CO_2 and their brains were removed and stored in 4% paraformaldehyde (Merck, Darmstadt, Germany) for at least seven days. After one day in 5% sucrose-solution the brains were kept for additional three days in a 30%

sucrose-solution before sectioning. Coronal cryo-sections of 40 µm were made by using a microtome (2800 Frigocut, Reichert-Jung, Cambridge Instruments GmbH, Nussloch, Germany). The slices were mounted on gelatinized slides and stained with Luxol Fast Blue and thionine. Thereafter, cannula placement was verified under a binocular microscope.

Data analysis
For statistical analysis the programs GB-Stat 7.0 (Dynamic Microsystems Inc.) and JMP 3.2.6 (SAS Institute Inc.) were used. In all statistical tests $p < 0.05$ was set as significant and $p < 0.01$ as high significant. Comparison of the time-difference spent in the drug-associated chamber during post- vs. pretest (as usually done in CPP-studies) was not sufficient to calculate the place preference, since it does not account for possible changes in the time spent in the middle compartment of our three-chambered CPP-boxes. Thus, CPP values were calculated as difference of the time spent in the cocaine- and the saline-associated chamber. A within subjects paradigm was used for evaluation of CPP expression and the average unconditioned place preference of the three pretests served as an internal reference (baseline) for the tests in the respective groups. Repeated measures ANOVA followed by Dunnett's post-hoc procedure was used (average pretest CPP vs. CPP of test-days) for analysis of CPP data. In both groups, rats that did not develop CPP (criteria: CPP after saline and CPP after cocaine < 100 s), rats that lost their microinfusion-assembly and rats with wrong cannula placement (i.e. one or both cannulae missed target) were excluded from analysis. In total, six rats in the CeA-group and four rats in the BLA-group were excluded from analysis. Statistical analysis of the locomotion data was done analogous to the CPP data analysis.

Results

Expression of cocaine-CPP
Analysis of the CPP data revealed significant effect of treatment in the CeA-group ($F = 5.11$; $p = 0.0123$). Multiple post-hoc comparisons for each testing-day (vs. average pretest) demonstrated that a cocaine-CPP has developed (fig. 1a), since high significant ($p < 0.01$) CPP was expressed during the first test (SHAM-treatment), and a significant ($p < 0.05$) CPP was expressed after cocaine-challenge on third test. During second test after baclofen/muscimol microinfusion, CeA-group lacked significant CPP expression ($p > 0.05$). A closer look onto the data for the individual rats revealed that CPP expression was reduced during baclofen-muscimol-infusion in each rat (as compared to the SHAM-test) within the CeA- but not the BLA-group. In comparison with the data of the SHAM-treatment, this reduction was due to a significant decrease in time spent in the cocaine-paired chamber ($F = 10.51$; $p = 0.0229$) and a significant increase in time spent in the saline-paired chamber ($F = 13.82$; $p = 0.0137$), while time spent in the middle chamber was not significantly affected ($F = 0.78$; $p = 0.4148$). For the BLA-group, a high significant effect of treatment was found ($F = 11.43$; $p < 0.0001$). Multiple post-hoc comparisons demonstrated a high significant ($p < 0.01$) cocaine-CPP expression (vs. average pretest) during all three treatment days (fig. 1a).

Locomotion
A high significant effect of treatment on locomotion was found in the CeA-group ($F = 54.14$; $p < 0.0001$). Multiple post-hoc comparisons (fig. 1b) revealed no significant change in locomotion during the first (after SHAM-treatment) and during the second test (after baclofen/muscimol-microinfusion) ($p > 0.05$), but a high significant increase in locomotion after cocaine-challenge during the third test ($p < 0.01$). In BLA-group, also a high significant effect of treatment on locomotion was found ($F = 14.93$; $p < 0.0001$). In agreement with results of the CeA-group, locomotion in BLA-group was not significantly altered during the

first and second test, but highly significant increased after cocaine-challenge during the third test ($p < 0.01$; fig. 1b).

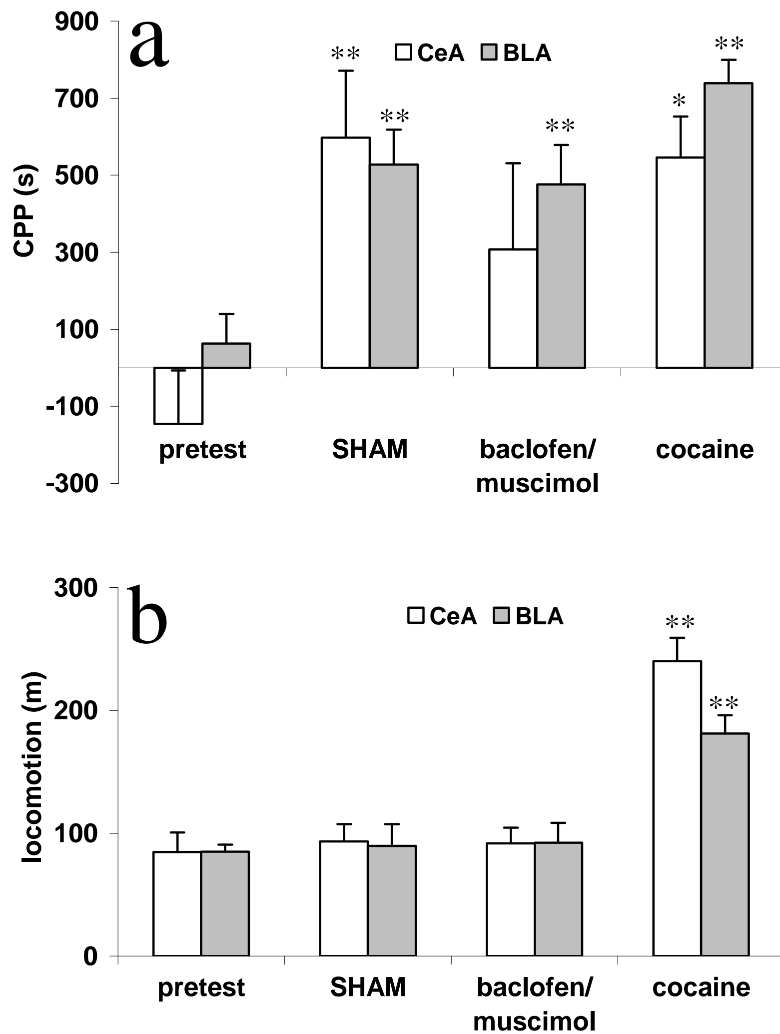

***Figure 1*:** **Place preference and locomotion**
Average place preference (CPP = difference of time spent in the cocaine- and the saline-associated chamber) (**a**) and locomotion (**b**) is presented for both the central amygdala- (CeA, n = 6) and the basolateral amygdala (BLA, n = 8) -group. CPP and locomotion of both groups were measured during pretest (no treatment, average of 3 pretests), after SHAM-treatment (i.e. saline microinfusion plus i.p. saline injection) or baclofen/muscimol microinfusion and after a challenge injection of cocaine. Statistical differences from the pretest-value of the respective group are indicated as * ($p < 0.05$) or ** ($p < 0.01$).

Histology
The schematic diagrams (according to the atlas of Paxinos and Watson, 1998) in figure 2 indicate the localization of the cannula tips for rats that were used for analysis. In both groups, three rats were excluded from analysis due to incorrect cannula placement.

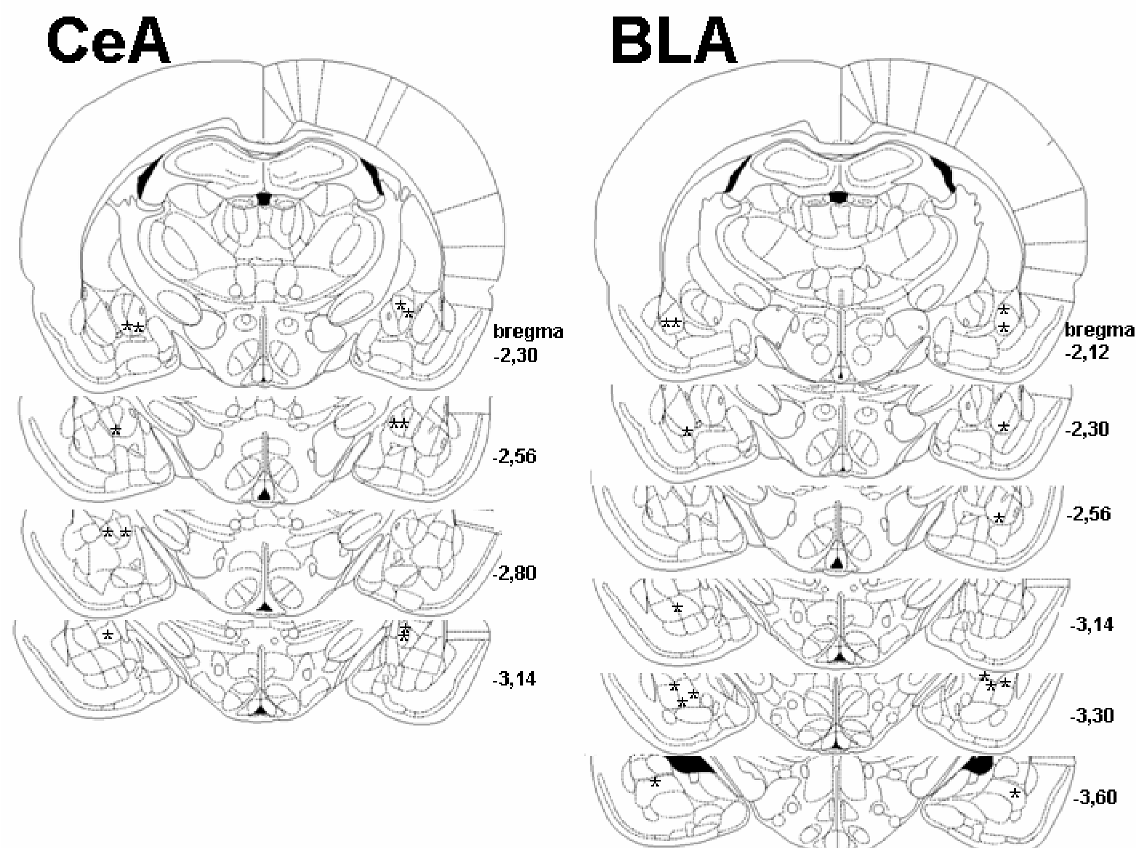

***Figure 2*:** **Microinfusion**
Localization of each microinfusion cannula tip in the central amygdala (CeA) or basolateral amygdala (BLA) is indicated by an asterisk. Schematic coronal brain sections were adapted from Paxinos and Watson (1998).

Discussion

Both the CeA- and the BLA-group exhibited a strong cocaine-CPP expression after SHAM-treatment (i.e. saline-microinfusion plus saline injection) on the first test day and after the cocaine-challenge injection on the third test day, which has been expected as a result of five conditioning-pairings with cocaine. Based on this two control tests a potential negative effect of the surgery or the microinfusion-procedure on development and expression of cocaine-CPP can be excluded. Consequently, the reduced CPP-expression that was observed during second test after baclofen/muscimol microinfusion in CeA-group was really caused by GABA-agonist-induced hyperpolarisation of the respective brain area. The finding that CPP expression in the BLA-group was not reduced by the GABA-agonist infusion furthermore excludes extinction as a possible cause for the reduction in the CeA-group otherwise CPP expression in both the CeA- and the BLA-group would have been reduced. Additionally, our previous experience and results from other groups (Mueller and Stewart, 2000) argue against an extinction-induced reduction in cocaine CPP-expression that is already significant on the second test-day, since cocaine-CPP expression is usually quite stable during the initial two testing days (if tested in the undrugged state). Since the cocaine-challenge injection on the

third day reinstated cocaine-CPP expression in the CeA-group, permanent impairment of the target area caused by GABA-agonists can also be ruled out. The observed increase in locomotion in both groups after cocaine-challenge further requires the integrity of neural circuits, at least those involved in cocaine-induced hyperlocomotion. Furthermore, locomotion in both groups after baclofen/muscimol microinfusion was not significantly different from average pretest locomotion or from locomotion observed after SHAM-treatment. Thus, we suggest that temporal CeA- but not BLA-inactivation can reduce reward of a cocaine-conditioned context and does not influence general activity.

A dissociable role of amygdalar sub-nuclei has been suggested, as only CeA-but not BLA-lesion impaired development of appetitive Pavlovian conditioning (Parkinson et al., 2000), unfortunately expression was not analysed in this study. Lesions or inactivation of the BLA did not reduce CPP expression to food (Schroeder and Packard, 2000), cocaine (Fuchs et al., 2002) or amphetamine (Hiroi and White, 1991). However, another study showed that expression of amphetamine-CPP can be blocked by a temporal inactivation of the amygdala (Hsu et al., 2002). This result becomes especially important, since they've infused the local anaesthetic drug (bupivacaine) into the BLA. Thus, amphetamine-CPP expression was blocked by BLA-inactivation in the latter study while no effect of BLA-inactivation on cocaine-CPP expression was found in the present study. However, Hsu et al. (2002) used an injection volume of 1µl per side (in contrast to 0.5 µl in the present study) and therefore stated that "it is unlikely that the infusions were limited to a specific amygdalar nucleus". Thus, we conclude that the CeA but not the BLA is involved in expression of psychostimulant CPP and that the findings of Hsu et al. (2002) can be explained by the co-inactivation of the CeA due to the large volume of bupivacaine injected into the BLA. However, there is another possible explanation for these different results. As Hiroi and White (1991) showed, only lesions of the lateral, but not BLA or CeA reduced expression of amphetamine-CPP. Therefore, different neural substrates involved in expression of amphetamine- vs. cocaine-CPP could also account for the differences between the quoted studies and the present results. Nevertheless, the involvement of the CeA in recall of associative memory is strengthened by the report of Holland and Gallagher (2003), showing a lack of Pavlovian-instrumental transfer that requires recall of conditioned associations in CeA-lesioned but not BLA-lesioned rats. The dissociative roles of the amygdalar sub-nuclei may be explained by the hypothesis that BLA is especially involved in reward evaluation/ devaluation and in attribution of neutral stimuli with incentive salience during conditioning (for reviews see Holland and Gallagher, 1999; See, 2002; Everitt et al., 2003; See et al., 2003), but not during recall of this association. On the other hand, only the CeA that is involved in control of attention and orientation to distal stimuli (See, 2002) seems to play a critical role in recall of cocaine-context-associations.

Another problem that becomes evident is the obvious difference between results from SA- and CPP-studies, showing that BLA is involved in cue-induced relapse to cocaine-seeking (Kruzich and See 2001) but not in expression of cocaine-CPP (Fuchs et al., 2002 and present results). According to our opinion, this may be due to different (but probably overlapping) neural circuits involved in instrumental vs. Pavlovian conditioning. This would explain both the concordances and discordances between CPP and SA as pointed out by Bardo and Bevins (2000) in their CPP-review article. We assume that the differences between CPP- and SA-studies are caused by the different proportion of instrumental vs. Pavlovian conditioning elements in both paradigms. During CPP conditioning environmental stimuli processed via Pavlovian mechanisms play the main role and if at all, only the act of moving from one chamber into the other can be considered as the instrumental part. On the other hand the instrumental lever-pressing plays the main role in SA-studies while environmental stimuli are only of secondary importance. Additionally, the different results regarding BLA-involvement

may be explained by the use of different cues, as See (2002) suggested that BLA is especially important for discrete cues (cue-light, tone, etc. that are used during SA-studies), while CeA plays a role for predictive cues (e.g. environmental cues that are present during CPP-studies). Another possible explanation for the differences between the present study and the study of Kruzich and See (2001) is that the latter study applied extinction-periods to analyse relapse while no extinction has been carried out in the present study.

The CeA is the major target of efferents from BLA, however, these projections do not play a role in expression of contextual drug reward, since inactivation of the BLA did not alter expression of cocaine-CPP. It was demonstrated, that dopamine D1-receptors in the CeA can exert a modulatory role upon discriminative-stimulus properties of cocaine (Callahan et al., 1995). On morphine-conditioned animals it was further demonstrated that dopamine-agonists in the CeA facilitate CPP-acquisition, but inhibit CPP-expression (Rezayof et al., 2002; Zarrindast et al., 2003). Thus, presentation of a drug-conditioned context may increase dopamine in the CeA that subsequently reduces expression of cocaine-CPP. This explanation is supported by a study showing that presentation of cocaine-discriminative stimuli increases dopamine-levels in the amygdala, however, differences in dopamine-increase between amygdalar sub-nuclei were not analysed (Weiss et al., 2000). The increase in dopamine in CeA might be caused by efferents from VTA, that project dopaminergically to CeA (Winnicka and WiSniewski, 1999). In summary, we conclude that a temporal lesion of CeA but not BLA reduces expression of contextual cocaine-reward without affecting general activity.

Acknowledgements

This study was funded by the federal ministry for education and research 01EB0110-Baden-Württemberg consortium for addiction research.

References

Bardo M.T. and Bevins R.A. (2000) Conditioned place preference: what does it add to our preclinical understanding of drug reward? *Psychopharmacology* 153, 31-43.

Browns E.E. and Fibiger H.C. (1993) Differential effects of excitotoxic lesions of the amygdala on cocaine-induced locomotion and conditioned place preference. *Psychopharmacology* 113, 123-130.

Callahan P.M., Bryan S.K., and Cunningham K.A. (1995) Discriminative stimulus effects of cocaine: Antagonism by dopamine D1 receptor blockade in the amygdala. *Pharmacol. Biochem. Behav.* 51, 759-766.

Everitt B.J., Cardinal R.N., Parkinson J.A., and Robbins T.W. (2003) Appetitive behavior: impact of amygdala-dependent mechanisms of emotional learning. *Ann. N. Y. Acad. Sci.* 985, 233-250.

Fuchs R.A., Weber S.M., Rice H.J., and Neisewander J.L. (2002) Effects of excitotoxic lesions of the basolateral amygdala on cocaine- seeking behavior and cocaine conditioned place preference in rats. *Brain Res.* 929, 15-25.

Hiroi N. and White N.M. (1991) The lateral nucleus of the amygdala mediates expression of the amphetamine-produced conditioned place preference. *J. Neurosci.* 11, 2107-2116.

Holland P.C. and Gallagher M. (1999) Amygdala circuitry in attentional and representational processes. *Trends Cogn. Sci.* 3, 65-73.

Holland P.C. and Gallagher M. (2003) Double dissociation of the effects of lesions of basolateral and central amygdala on conditioned stimulus-potentiated feeding and Pavlovian-instrumental transfer. *Eur. J. Neurosci.* 17, 1680-1694.

Hsu E.H., Schroeder J.P., and Packard M.G. (2002) The amygdala mediates memory consolidation for an amphetamine conditioned place preference. *Behav. Brain Res.* 129, 93-100.

Kruzich P.J. and See R.E. (2001) Differential contributions of the basolateral and central amygdala in the acquisition and expression of conditioned relapse to cocaine- seeking behavior. *J. Neurosci.* 21, RC155.

Leshner A.I. (1997) Addiction is a brain disease, and it matters. *Science* 278, 45-47.

McFarland K., Lapish C.C., and Kalivas P.W. (2003) Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. *J. Neurosci.* 23, 3531-3537.

Parkinson J.A., Robbins T.W., and Everitt B.J. (2000) Dissociable roles of the central and basolateral amygdala in appetitive emotional learning. *Eur. J. Neurosci.* 12, 405-413.

Paxinos G. and Watson C. (1998) The rat brain in Stereotaxic Coordinates. Academic Press, New York.

Rezayof A., Zarrindast M.R., Sahraei H., and Haeri-Rohani A.H. (2002) Involvement of dopamine D2 receptors of the central amygdala on the acquisition and expression of morphine-induced place preference in rat. *Pharmacol. Biochem. Behav.* 74, 187-197.

See R.E. (2002) Neural substrates of conditioned-cued relapse to drug-seeking behavior. *Pharmacol. Biochem. Behav.* 71, 517-529.

See R.E., Fuchs R.A., Ledford C.C., and McLaughlin J. (2003) Drug Addiction, Relapse, and the Amygdala. *Ann. N. Y. Acad. Sci* 985, 294-307.

Weiss F., Maldonado-Vlaar C.S., Parsons L.H., Kerr T.M., Smith D.L., and Ben Shahar O. (2000) Control of cocaine-seeking behavior by drug-associated stimuli in rats: effects on recovery of extinguished operant-responding and extracellular dopamine levels in amygdala and nucleus accumbens. *Proc. Natl. Acad. Sci. U. S. A.* 97, 4321-4326.

Winnicka M.M. and WiSniewski K. (1999) Dopaminergic projection to the central amygdala mediates the facilitatory effect of CCK-8US and caerulein on memory in rats. *Pharmacol. Res.* 39, 445-450.

Zarrindast M.R., Rezayof A., Sahraei H., Haeri-Rohani A., and Rassouli Y. (2003) Involvement of dopamine D1 receptors of the central amygdala on the acquisition and expression of morphine-induced place preference in rat. *Brain Res.* 965, 212-221.