Die Rolle metabotroper Glutamatrezeptoren der Gruppe I beim Lernen und Gedächtnis der Ratte

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Andreas Gravius
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Dekan:	Prof. Dr. F. Schöffl
1. Berichterstatter:	Prof. Dr. W. Danysz
2. Berichterstatter:	Prof. Dr. W. J. Schmidt

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1. Einleitung

1.1. Das glutamaterge System

Glutamat spielt eine vorherrschende Rolle als Neurotransmitter im zentralen Nervensystem. Die neuronale glutamaterge Signalübertragung erfolgt über verschiedene Typen von Rezeptoren, ionotrope wie AMPA (α-Amino-3-hydroxy-5-methyl-4-isoxazol-4-propionat), Kainat und NMDA (N-Methyl-D-Aspartat) Rezeptoren, sowie metabotrope G-Protein gekoppelte Glutamatrezeptoren. Letztere werden anhand pharmakologischer Mechanismen und Homologien ihrer Aminosäuresequenzen in drei Untergruppen eingeteilt: Gruppe I umfasst die Subtypen 1 und 5 (mGluR1/5), die an die Hydrolyse von Phosphoinositol gekoppelt sind. Gruppe II beinhaltet die Rezeptorsubtypen 2 und 3 (mGluR2/3), wobei Gruppe III die Subtypen 4, 6, 7 und 8 (mGluR4/6/7/8) einschließt. Die Rezeptoren der Gruppen II und III wiederum sind negativ an eine Adenylat-Cyclase gekoppelt (Conn and Pin 1997).

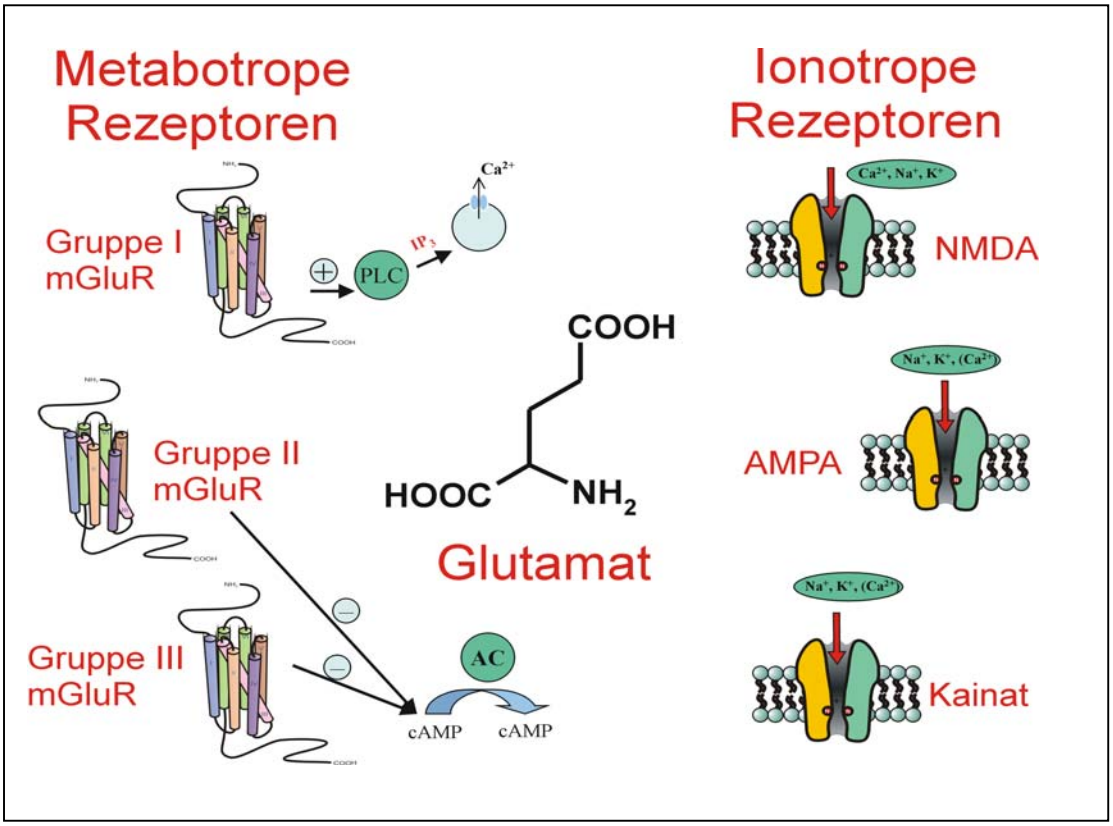

Abb. 1: Ionotrope und metabotrope Glutamatrezeptoren. MGlu Rezeptoren der Gruppe I sind gekoppelt an die Hydrolyse von Inositoltriphosphat; mGluR der Gruppe II und III sind negativ an eine Adenylatcyclase gekoppelt. Die ionotropen NMDA, AMPA und Kainatrezeptoren weisen eine Permeabilität für Ca^{2+}, K^+ und Na^+ auf. Modifiziert nach Danysz & Parsons.

1.2. mGluR Gruppe I in Lernen und Gedächtnis

Erste Hinweise auf eine Beteiligung metabotroper Glutamatrezeptoren an Lernprozessen ergab sich aus der Beobachtung, dass durch deren pharmakologische Hemmung die Langzeitpotenzierung (LTP) beeinträchtigt wurde (Bashir et al. 1993). LTP wird als physiologische Grundlage vieler Lern- und Gedächtnisprozesse betrachtet. Weitere Studien untersuchten daraufhin den Zusammenhang zwischen LTP und Lernen unter besonderer Berücksichtigung von mGlu Rezeptoren. So wurde bei mGluR1 Knock-out (KO)-Mäusen ein signifikantes Lerndefizit sowie eine Beeinträchtigung der LTP im Hippokampus festgestellt (Aiba et al. 1994). In ähnlicher Weise führte die pharmakologische Inhibition von mGlu5 Rezeptoren zu einer Verminderung der LTP sowie des räumlichen Lernens in der Ratte (Balschun & Wetzel 2002). In jüngster Zeit, gerade durch die Entwicklung spezifischer Antagonisten mit hoher Selektivität für die beiden Subtypen mGluR1 und mGluR5, konnten weitere Erkenntnisse bezüglich mGlu Rezeptoren der Gruppe I bei Lernen gewonnen werden. So wurde z.B. durch Administration eines selektiven mGluR5 Antagonisten vor der Konditionierung eine Verminderung der Furcht-potenzierten Schreckreaktion beobachtet (Schulz et al. 2001).

1.3. Verteilung im Gehirn

Entscheidend für die funktionelle Bedeutung von mGluR1 und mGluR5 ist deren Lokalisation in verschiedenen Hirnstrukturen. MGluR1 und mGluR5 finden sich in fast allen Bereichen des Gehirns, wobei hohe Expressionsmuster im Thalamus, Septum, im olfaktorischen System, sowie im Hippokampus, einer für viele Lernformen essentielle Struktur, zu finden sind. In letzterem variiert die Verteilung von mGluR1 jedoch je nach Substruktur. Während deren höchste Dichte im CA3 und Hilum gemessen wurde, fehlen sie fast vollständig in CA1;

mGluR5 hingegen sind in hoher Dichte in den hippokampalen Strukturen zu finden (Spooren et al. 2003). Ebenfalls unterschiedliche Expressionsmuster weisen die Nuklei der Amygdala auf, wobei die Zahl der mGlu5 Rezeptoren hier überwiegt (Spooren et al. 2003). Die Amygdala ist vor allem an der Akquisition und Expression von Furcht beteiligt (Phillips & LeDoux 1992).

1.4. Verwendete Lernmodelle

Die Furchtkonditionierung ist ein in der tierexperimentellen Verhaltenspharmakologie häufig angewandtes Versuchsmodell. Mit genau definierten Stimuli und innerhalb kurzer Trainingszeit wird ein Furchtgedächtnis geformt, welches über einen Zeitraum von mehreren Wochen abgerufen werden kann. In einer klassischen Versuchsprozedur dient meist ein aversiver elektrischer Reiz als unkonditionierter Stimulus (US), der mit einem neutralen Reiz, dem späteren konditionierten Stimulus („conditioned stimulus“, CS), durch gemeinsame Präsentation verknüpft wird. Das Versuchstier assoziiert beide Reize miteinander und speichert diese Erfahrung während der Konsolidierungsphase als Gedächtnisspur ab. Das Wiederabrufen dieses Gedächtnisinhaltes durch spezielle Reize („cues“) löst wiederum eine spezifische, je nach Modell unterschiedliche, Verhaltensantwort des Tieres aus. Die Quantifikation dieser Verhaltensreaktion während des Testes dient schließlich als Maß für die Intensität des zuvor geformten Gedächtnisses.

1.4.1. Passive Avoidance

Das Verhaltensmodell „Passive Avoidance“ (PA) beruht auf instrumentellem Lernen. Im Prinzip wird das natürlich angeborene Verhalten der Ratten ausgenutzt, von einer hellen in eine dunkle Umgebung zu flüchten. Die Versuchsapparatur besteht aus einer Startbox und zwei mit dieser durch Öffnungen verbundenen Kammern, von denen eine ausgeleuchtet wird und die

andere dunkel verbleibt. Wechselt das Tier während der Konditionierung in den dunklen Bereich der Versuchsapparatur, werden direkt danach milde elektrische Reize über ein Metallgitter am Boden verabreicht. Das Versuchstier assoziiert nun diesen dunklen, aber nicht den hellen Bereich mit einer negativen Erfahrung. Während des Tests, der meist 24 Stunden nach der Konditionierung stattfindet, wird das Tier in die Startbox platziert und die Latenzzeit, welche das Tier zum Überwechseln in den dunklen Bereich benötigt, dient als Maß für die Intensität des Furchtgedächtnisses.

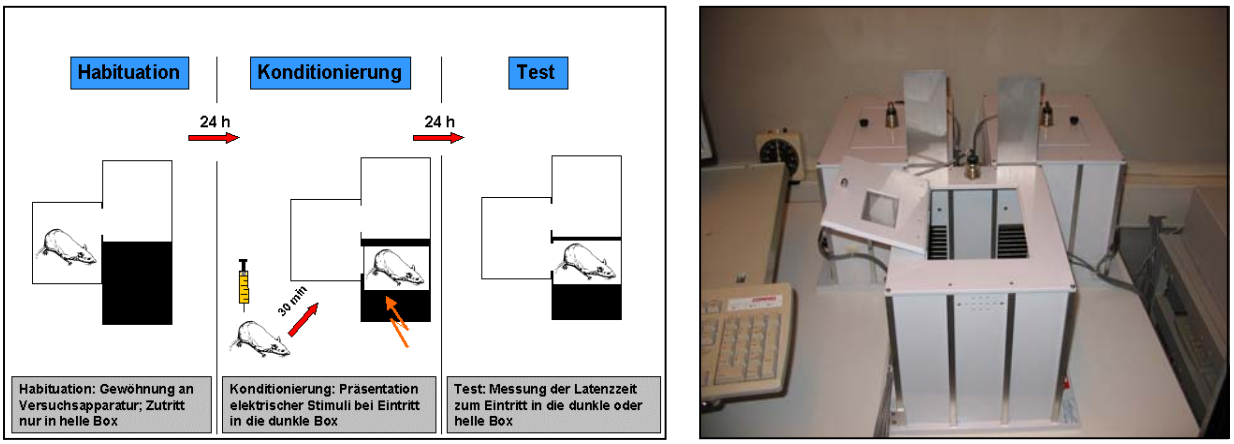

Abb. 2 Versuchsschema (links) und Versuchsapparatur von Passive Avoidance. 24 h nach der Gewöhnung an die Versuchsapparatur werden die Tiere zur Konditionierung in die Startbox platziert. Beim Eintritt in die dunkle Box werden elektrische Stimuli verabreicht. Zum Test 24 h danach werden die Versuchstiere erneut in die Startbox gesetzt und die Zeit zum Eintritt in die helle oder dunkle Box wird gemessen. Die Latenzzeit zum Eintritt in de dunkle Box dient als Maß für die Stärke des Furchtgedächtnisses.

1.4.2. Furcht-potenzierte Schreckreaktion (FPS)

In diesem experimentellen Versuchsansatz werden die Versuchstiere in einer klassischen Prozedur konditioniert. Hierbei wird ein aversiver Stimulus, ein elektrischer Reiz, mit einem neutralen Lichtreiz verknüpft, der nach wenigen Konditionierungseinheiten zum nunmehr konditionierten Stimulus wird. Nach der Konditionierung löst der Lichtreiz eine Furchtreaktion im Tier aus, die als Maß für die Stärke des Furchtgedächtnisses dient und quantifiziert werden

kann. In diesem Fall handelt es sich um eine Erhöhung der Schreckreaktion, welche in Gegenwart des Lichtreizes stärker ist als in dessen Abwesenheit.

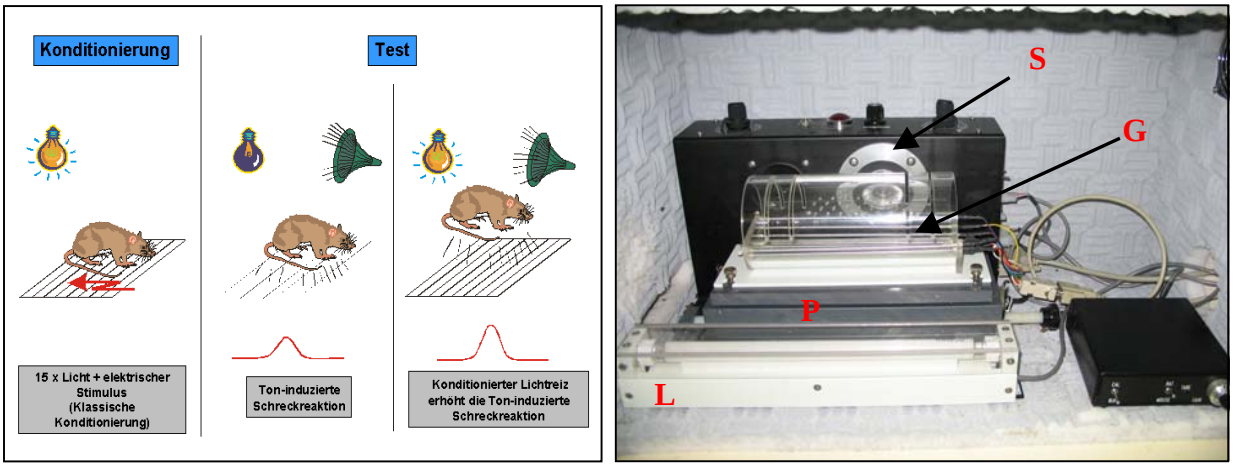

Abb. 3 Versuchsschema (links) sowie verwendete Gerätschaften zur Messung der FPS (rechts). Während der Konditionierung wird ein Lichtreiz (L) mit einem elektrischen Stimulus verknüpft, der über ein Schockgitter (G) übertragen wird. Zum Test der FPS werden über einen Lautsprecher akustische Schreckreize verabreicht, von denen die Hälfte zusammen mit dem Lichtreiz (konditionierter Stimulus), die andere Hälfte im Dunkeln gegeben wird. Die Schreckreaktionen werden über eine spezielle Plattform (P) als Spannungsänderungen durch einen Computer quantifiziert.

1.4.3. Kontext-abhängige und auditive Furchtkonditionierung

Im Falle der kontextabhängigen Furchtkonditionierung (KFK) wird das Versuchstier während des Trainings in eine spezielle Kammer platziert, die einen Metallgitterboden enthält. Nach wenigen Minuten, nachdem das Versuchstier den Kontext erfasst hat, wird ein elektrischer Reiz verabreicht, und das Tier assoziiert den zuvor neutralen Kontext nun mit einem schmerzhaften Reiz. Wird das Versuchstier erneut in die Trainingskammer gesetzt, löst diese eine sogenannte Verhaltensstarre („Freezing“) aus, eine spezifische Furchtreaktion. Die Dauer dieser Starre während des Testes dient hierbei als direktes Maß für die Stärke des Furchtgedächtnisses.

Bei der auditiven Furchtkonditionierung (AFK) wird ein neutraler Ton mit einem elektrischen Stimulus verknüpft. Nach einigen Trainingseinheiten wird der Ton zum konditionierten Stimulus. Während des Testes wird das Versuchstier in eine vom Trainingskontext verschiedene Versuchskammer platziert, in welchem dem Tier mehrmals der konditionierte

Ton präsentiert wird. Die Dauer der dadurch ausgelösten Verhaltensstarre während der Tonpräsentationen dient als Maß für die Intensität des Furchtgedächtnisses.

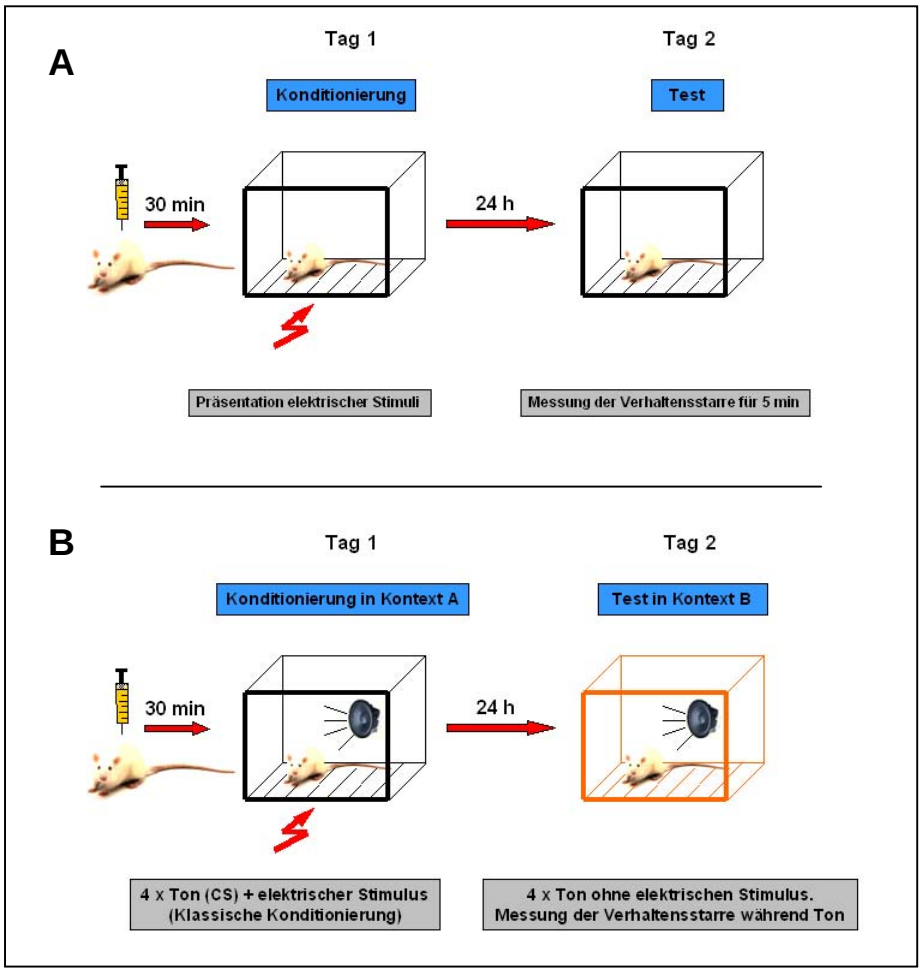

Abb. 4 Schemata der kontext-abhängigen (A) sowie auditiven Furchtkonditionierung (B). Hier jeweils dargestellt die Methodologie zur Untersuchung der Wirkungen von Substanzen auf die Akquisition. **A:** Nach einer Substanzeinwirkzeit von 30 min werden die Tiere in die Trainingskammer platziert, in der 3 elektrische Stimuli verabreicht werden. 24 h danach, zum Test der konditionierten Furcht, werden die Tiere erneut in die gleiche Kammer gesetzt und die Gesamtzeit der gezeigten Verhaltensstarre dient als Maß für die Ausprägung des Furchtgedächtnisses. **B:** Nach einer Einwirkzeit von 30 min werden die Tiere in die Trainingskammer (Kontext A) platziert, in der 4 elektrische Stimuli mit einem neutralen Ton präsentiert werden. 24 h danach, zum Test der konditionierten Furcht, werden die Tiere in eine andere Kammer (Kontext B) gesetzt und die Gesamtzeit der gezeigten Verhaltensstarre während der Tonpräsentationen dient als Maß für die Ausprägung des Furchtgedächtnisses.

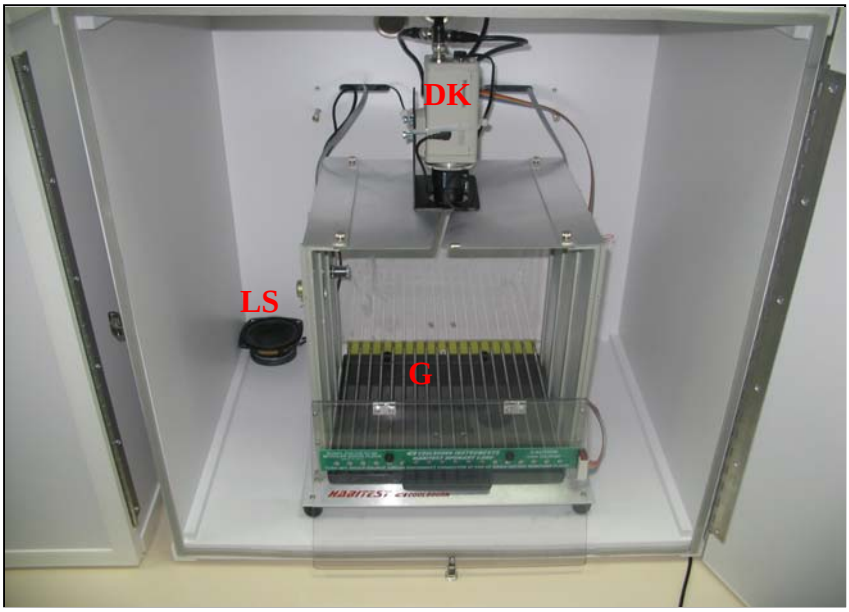

Abb. 5 Trainingskammer zur Messung kontext- und auditiver Furchtreaktionen. Die Kammer steht in einer schallabschwächenden Box. Die elektrischen Reize werden über ein Gitter (G) am Boden übertragen, ein Lautsprecher (LS) überträgt den auditiven Stimulus. Die Bewegungen der Versuchstiere werden durch eine Digitalkamera (DK) auf einen Computer übertragen und mit Hilfe spezieller Software quantifiziert.

1.5. Zielsetzung der Arbeit

Die vorliegende Arbeit hatte zum Ziel, die Rolle metabotroper Glutamatrezeptoren der Gruppe I in Lern- und Gedächtnisvorgängen bei der Ratte näher zu bestimmen. Dazu dienten vier verschiedene Verhaltensmodelle, um den Einfluss von mGluR1 und mGluR5 auf die verschiedenen Stadien zu untersuchen. Alle verwendeten Modelle basieren auf aversiver Konditionierung. Der Vorteil dabei liegt in der sehr kurzen Trainingszeit und in der starken Ausprägung der Furchtgedächtnisse. Weiterhin werden hier wenige, klar definierte Stimuli benutzt, wodurch eine hohe Reproduzierbarkeit der Ergebnisse gegeben ist. Von grundlegender Bedeutung für die Studie war die Verwendung spezifischer Rezeptorantagonisten, um mögliche Unterschiede in der Funktion von mGluR1 und mGluR5 feststellen zu können. (*3-ethyl-2-methyl-quinolin-6-yl)-(4-methoxy-cyclohexyl)-methanone methanesulfonate*

(EMQMCM, JNJ16567083) diente als Antagonist für mGluR1 und *[(2- methyl-1,3-thiazol-4-yl)ethynyl]pyridine* (MTEP) für mGluR5.

In Manuskript 1 wurde der Einfluss von mGluR1 und mGluR5 auf die Akquisition, Konsolidierung sowie Expression in PA untersucht. Des weiteren sollte geklärt werden, ob eine Aktivierung mGluR1 und mGluR5 zur Akquisition nötig ist. In weiteren Experimenten wurde eine Beteiligung besagter Rezeptortypen bei der Akquisition in FPS untersucht, einem Modell klassischer Konditionierung.

Die Versuche in Manuskript 2 folgten der Fragestellung, ob eine mögliche Interaktion zwischen metabotropen Rezeptoren der Gruppe I und NMDA Rezeptoren bei der Akquisition in PA und FPS besteht. Dazu dienten die Ergebnisse aus Manuskript 1 als Grundlage zur Wahl geeigneter Dosierungen. Zur pharmakologischen Blockade der NMDA Rezeptoren fand der spezifische Antagonist (+)-MK-801 Verwendung. In weiteren Versuchen wurde untersucht, ob durch die gemeinsame Applikation der Rezeptorantagonisten mögliche analgetische Wirkung auftreten, welche die Interpretation der Ergebnisse der Akquisition beeinflussen könnten.

In Manuskript 1 und 2, wurde beobachtet, dass mGluR1 und mGluR5 unterschiedlich an der Akquisition und Expression beteiligt zu sein scheinen. Dies sollte nun in einer näheren Betrachtung in Manuskript 3 weiter untersucht werden. Dazu wurde die Beteiligung von mGlu Rezeptoren der Gruppe I bei der Akquisition sowie der Expression in KFK im Vergleich zu AFK getestet. Dazu wurden auch hier EMQMCM und MTEP als pharmakologische Mittel zur Hemmung von mGluR1 beziehungsweise mGluR5 verwendet.

2. Zusammenfassung der Ergebnisse der einzelnen Manuskripte

2.1. Manuskript 1

A. Gravius, M. Pietraszek, D. Schäfer, W.J. Schmidt, W. Danysz: "Effects of mGluR1 and mGluR5 antagonists on negatively reinforced learning."

Veröffentlicht in *Behavioural Pharmacology*

Der Prozess der Gedächtnisbildung lässt sich vereinfacht in drei aufeinanderfolgende Abschnitte unterteilen: während der Akquisition, des eigentlichen Lernens, werden bestimmte sensorische Informationen verknüpft. Im speziellen Falle der Furchtkonditionierung umfasst die Akquisition eine Assoziation zwischen einem bestimmten räumlichen Kontext oder einem spezifischen Stimulus und einem elektrischen Fußschock. Während der anschließenden Konsolidierung wird das Erlernte im Langzeitgedächtnis gespeichert. Das Wiederabrufen („Retrieval") der zuvor gespeicherten Gedächtnisspuren durch spezifische Stimuli umfasst den letzten Abschnitt der Gedächtnisbildung.

Während der spezifische mGluR5 Antagonist MTEP die Akquisition in PA und FPS verminderte, beeinträchtigte EMQMCM (mGluR1 Antagonist) nur die Akquisition in PA. Interessanterweise bewirkte die gleichzeitige Verabreichung beider Antagonisten in jeweils nicht wirksamer Dosierung eine signifikante Verschlechterung der Akquisition in PA. Weiterhin wurden beide Antagonisten auf ihre Beeinflussung hinsichtlich der Konsolidierung untersucht, jedoch bewirkten die Sunstanzen keine Verhaltensänderung im Vergleich zur Kontrollgruppe während des Testes. Nach Gabe von EMQMCM vor dem Test verschlechterte sich die Expression in PA, während MTEP keinen Einfluss auf die Ausführung des erlernten Verhaltens hatte.

Außerdem wurde in weiteren Experimenten untersucht, ob die unter Substanzeinfluss erlernte Furcht einer „State-dependency“ unterliegt, d.h., die Tiere befinden sich während der Konditionierung in einem anderen pharmakologischen Zustand als während des Testes, und es besteht die Möglichkeit, das die Verhaltensweisen unter Substanzeinfluss zwar erlernt, aber ohne diesen Einfluss nicht abgerufen werden können. Dieses wurde ist zum Beispiel für NMDA Rezeptorantagonisten berichtet (Jackson et al. 1992). In Manuskript 1 konnte jedoch beobachtet werden, dass die während des Testes festgestellte Lernbeeinträchtigung unabhängig vom pharmakologischen Zustand der Tiere erfolgte. Gleichsam zeigte eine frühere Arbeit, dass die Furchtkonditionierung unter Einfluss des mGluR5 Antagonisten MPEP (*2-methyl-6-(phenylethynyl)-pyridine*) keiner „State-dependency“ unterlag (Schulz et al. 2001).

Zusammenfassend zeigt sich, dass mGluR1 sowie mGluR5 an der Akquisition von Hippokampus-abhängigen PA Verhaltens beteiligt sind. Zudem scheint eine gemeinsame Aktivierung der Rezeptoren zum kontext-spezifischen Lernen nötig zu sein. Hingegen konnte kein Einfluss beider Rezeptortypen auf die Konsolidierung der erlernten Gedächtnisinhalte nachgewiesen werden. Im Falle des Wiederabrufens scheint nur der Subtyp mGluR1 beteiligt zu sein. An der Akquisition von FPS, welche unabhängig vom Hippokampus abläuft, scheint jedoch nur mGluR5 beteiligt zu sein.

2.2. Manuskript 2

A. Gravius, M. Pietraszek, W.J. Schmidt, W. Danysz: “Functional interaction of NMDA and group I metabotropic glutamate receptors in negatively reinforced learning in rats”
Veröffentlicht in *Psychopharmacology*

Es ist mittlerweile weitestgehend akzeptiert, dass NMDA Rezeptoren eine Schlüsselrolle bei der Induktion der LTP und beim Lernen zukommt. Neuere Daten belegen ebenfalls eine

Funktion von mGluRs der Gruppe I in diesen Prozessen, und aus neuesten Studien geht hervor, dass eine gemeinsame Aktivierung von NMDA und mGlu Rezeptoren der Gruppe I zur Induktion der LTP sowie Lernen notwendig ist.

In der vorliegenden Studie wurde die Interaktion von NMDA und mGlu Rezeptoren der Gruppe I bei der Akquisition in zwei Tiermodellen, PA und FPS, untersucht. Dazu wurden zum einen der mGluR5-spezifische Antagonist MTEP, zum anderen der mGluR1 Antagonist EMQMCM gemeinsam mit dem NMDA Rezeptor Antagonisten (+)-MK-801 in jeweils unwirksamen Einzeldosierungen systemisch verabreicht. Im Falle von PA zeigte sich eine eindeutige Verschlechterung der Akquisition nach gemeinsamer Verabreichung von EMQMCM mit (+)-MK-801 sowie MTEP mit (+)-MK-801. Im Falle von FPS konnte keine solche Interaktion nach Koadministration der jeweiligen Antagonisten beobachtet werden. In weiteren Experimenten zeigte sich, dass die gemeinsame Verabreichung von EMQMCM mit (+)-MK-801 und MTEP mit (+)-MK-801 keinen Einfluss auf die Empfindlichkeit gegenüber den eingesetzten elektrischen Stimuli nahm.

Abschließend kann man festhalten, dass eine Interaktion zwischen mGluR1 und NMDA Rezeptoren sowie zwischen mGluR5 und NMDA Rezeptoren nur bei kontext-abhängigen Lernen von PA festzustellen war, wobei die Akquisition der FPS unbeeinflusst blieb.

2.3. Manuskript 3

A. Gravius, C. Barberi, D. Schäfer, W.J. Schmidt, W. Danysz: “The role of group I metabotropic glutamate receptors in acquisition and expression of contextual and auditory fear conditioning in rats – a comparison”

Veröffentlicht in *Neuropharmacology*

Viele neuere Studien belegen, dass mGluR1 und mGluR5 an verschiedenen Lern- und Gedächtnisprozessen beteiligt sind. Unklar ist jedoch, ob beide Rezeptortypen generell zum Lernen notwendig sind, oder ob sie nur in bestimmten Modellen eine Funktion ausüben. In der vorliegenden Studie sollte daher untersucht werden, welchen Einfluss metabotrope Glutamatrezeptoren der Gruppe I auf das Lernen sowie den Widerabruf von Gedächtnisspuren im direkten Vergleich zwischen kontext-abhängiger und auditiver Furchtkonditionierung haben. Dabei dient in beiden Fällen die Furcht-konditionierte Verhaltensstarre als Maß der Intensität des Furchtgedächtnisses während des Testes. Die Effekte beider Rezeptortypen wurden mithilfe systemisch applizierter selektiver Antagonisten getestet; EMQMCM für mGluR1 und MTEP für mGluR5. Deren Einflüsse auf die Akquisition wurden mit dem NMDA Rezeptorantagonisten (+)-MK-801 und Skopolamin, einem nicht selektiven muskarinischen Rezeptorantagonisten verglichen. Die anxiolytisch wirksamen Substanzen Diazepam und Citalopram, ein selektiver Serotonin-Wiederaufnahmehemmer, dienten als Referenzsubstanzen zum Test der Expression des Furchtgedächtnisses.

EMQMCM beeinträchtigte die Akquisition kontextabhängiger Furchtkonditionierung, hatte aber keinen Einfluss auf die Akquisition auditiver Furchtkonditionierung. In gleicher Weise war die Gabe von MTEP, als auch (+)-MK-801 vor der Akquisition auditiver Furchtkonditionierung ohne Wirkung, wobei die kontextabhängige Konditionierung signifikant gehemmt wurde. Im Gegensatz zu diesen Befunden hemmte Skopolamin die Akquisition auditiver Furchtkonditionierung. Erfolgte die Applikation direkt vor dem Test, wurde die Expression kontextabhängiger Furcht von EMQMCM, MTEP und Citalopram, aber nicht von Diazepam gehemmt. Im Unterschied dazu verminderte nur MTEP, aber nicht alle anderen getesteten Substanzen die Expression konditionierter auditiver Furcht.

Interessanterweise zeigte sich, dass mGluR1 und mGluR5 an der Akquisition der Hippokampus-abhängigen Kontext-Konditionierung, aber nicht an der Hippokampus-unabhängigen auditiven Konditionierung beteiligt sind. Während mGluR1 und mGluR5 die

Expression kontext-konditionierter Furcht hemmen, scheint nur mGluR5 an der Expression auditiv-konditionierter Furcht beteiligt zu sein.

3. Diskussion

3.1. Einfluss von mGluR1 und mGluR5 auf instrumentelles Lernen in PA

In Manuskript 1 konnte gezeigt werden, dass eine pharmakologische Blockade durch den mGluR1-Antagonisten EMQMCM und den mGluR5-Antagonisten MTEP dosisabhängig zu einer Verschlechterung der Akquisition von PA führte. Dies deutet darauf hin, dass eine Aktivierung beider Rezeptorsubtypen zum kontext-abhängigem instrumentellen Lernen notwendig ist. Die Ergebnisse mit mGluR5 stehen im Einklang mit vorangegangenen Studien. Zum Beispiel führte die systemische Gabe von MPEP, einem mGluR5-Antagonisten, vor der Furchtkonditionierung in PA zu einer Verminderung des Lernens (Schachtman et al. 2004). In einer weiteren Arbeit wurde gezeigt, dass AIDA (*1-aminoindan-1,5-dicarboxylic acid*), ein mGlu Rezeptorantagonist der Gruppe I mit höherer Selektivität für mGluR1 ebenfalls die Akquisition von PA hemmte (Nadlewska et al. 2003). In Bezug auf mGluR1 gibt es jedoch keine vergleichbaren Studien mit mGluR1-spezifischen Antagonisten.

Interessanterweise führte die gemeinsame Applikation unwirksamer Einzeldosierungen von MTEP und EMQMCM vor der Konditionierung zu einer starken Verschlechterung der Akquisition. Das Ausmaß dieser Inhibition könnte auf eine synergistische Wirkung beider Rezeptortypen hindeuten, jedoch konnte dies aufgrund der nicht-parametrischen Verteilung der Daten statistisch nicht belegt werden. Ähnliche Ergebnisse diesbezüglich sind nicht bekannt, jedoch ergeben sich Hinweise für eine synergistische Wirkung beider Rezeptoren aus der Literatur. Es wurde zum Beispiel nach gemeinsamer Gabe von MPEP und LY367385 (*(S)-(+)-*

alpha-amino-4-carboxy-2-methylbenzeneacetic acid), einem mGluR1 Antagonisten, entdeckt, dass die LTP in kortiko-striatalen Bereichen blockiert ist und eine Reduktion der LTP wurde ebenfalls in mGluR1 und mGluR5 KO Mäusen beobachtet (Gubellini et al. 2003).

Es ist bekannt, dass der Hippokampus beim kontext-spezifischen Lernen beteiligt ist. So führte eine funktionelle Inaktivierung des Hippokampus zu einer Blockade der Akquisition von PA (Ambrogi Lorenzini et al. 1997). Weiterhin konnte gezeigt werden, dass MPEP die LTP im Hippokampus sowie räumliches Lernen verschlechterte (Balschun & Wetzel 2002).
Zudem zeiget sich eine Verminderung kontext-abhängigen Lernens sowie eine Reduktion der LTP im Hippokampus von mGluR1 KO Mäusen (Aiba et al. 1994). Auch die Amygdala ist an den Lernprozessen von PA beteiligt. So führten zum Beispiel Infusionen von Tetrodotoxin in die Amygdala nach der Konditionierung zu einer Verschlechterung des PA Verhaltens während des Testes (Bucherelli et al. 1992). Diese Befunde deuten darauf hin, dass eine Aktivierung von mGluR1 sowie mGluR5 der Amygdala und des Hippokampus zum Erlernen von PA notwendig ist.

Gleichfalls wurde in Manuskript 1 untersucht, ob metabotrope Rezeptoren der Gruppe I an der Konsolidierung von PA beteiligt sind. Dazu wurden systemische Applikationen von MTEP, bzw. EMQMCM direkt nach der Konditionierung durchgeführt. Jedoch hatte keine der Behandlungen einen Einfluss auf das Wiederabrufen des Furchtgedächtnisses während des Tests im Vergleich zur Kontrollgruppe. Dazu finden sich unterschiedliche Ergebnisse in der Literatur. Es zeigte sich, dass MPEP die Konsolidierung von PA verstärkte (Nadlewska et al. 2002). Eine Verbesserung der Konsolidierung fand sich ebenfalls nach Gabe von AIDA (Nadlewska et al. 2003).

In weiteren Experimenten wurde der Einfluss von mGluR1 und mGluR5 auf den Wiederabruf des PA Gedächtnisses getestet. Dazu wurden EMQMCM oder MTEP jeweils direkt vor dem Test verabreicht. Interessanterweise führte die Gabe des mGluR1-Antagonisten EMQMCM zu einer Verschlechterung des Wiederabrufs, wohingegen MTEP keinen Effekt

diesbezüglich zeigte. Vorangegangene Studien zeigten gegensätzliche Ergebnisse, so hatte zum Beispiel die Gabe von AIDA vor dem Test keine Auswirkung auf den Wiederabruf des Gedächtnis (Nadlewska et al. 2003). Wohingegen MPEP den Wiederabruf verbesserte (Nadlewska et al. 2002). Zusammenfassend kann man anmerken, dass metabotrope Glutamatrezeptoren der Gruppe I am Prozess des Wiederabrufs von Gedächtnisspuren involviert sind, jedoch konnte dies in unserer Studie nur für mGluR1 gezeigt werden.

Bezüglich Konsolidierung und Wiederabruf muss angemerkt werden, dass eine potentielle Verbesserung durch die getesteten Substanzen nicht nachgewiesen werden kann, da nach unserem PA-Versuchsprotokoll die meisten Kontrolltiere die dunkle Box während des Testes nicht betreten.

3.2. Einfluss von mGluR1 und mGluR5 auf die klassische Furchtkonditionierung im speziellen Fall der Furcht-potenzierten Schreckreaktion

Aus früheren Studien ergaben sich Hinweise, dass mGlu Rezeptoren der Gruppe I an der Akquisition der FPS beteiligt sind. Die Applikation von MPEP vor der Konditionierung führte zum Beispiel zu einer Verschlechterung der Akquisition (Schulz et al. 2001). Dies entspricht unseren Ergebnissen mit MTEP aus Manuskript 1, wonach ebenfalls die Akquisition vermindert wurde. Im Gegensatz dazu hatte die Gabe von EMQMCM in unserer Arbeit keine Auswirkung auf die Akquisition der FPS, jedoch existieren nach unserem Wissen keine vergleichbaren Studien, welche die Wirkung von mGluR1-Antagonisten bei der FPS untersuchten .

Im Gegensatz zu PA ist die FPS unabhängig vom Konditionierungskontext. Demzufolge unterscheiden sich beide Modelle auch durch die beteiligten Hirnregionen. Studien deuten darauf hin, dass kontext-abhängige Furchtkonditionierung sowohl den Hippokampus als auch die Amygdala einschließt (Kim & Fanselow 1992), wohingegen

klassische Konditionierung unabhängig vom Hippokampus gesteuert wird (Phillips & LeDoux 1992).

Die Aktivierung von mGlu5 Rezeptoren innerhalb der Amygdala scheint für die Akquisition klassischer Konditionierung notwendig zu sein. So führten Infusionen von MPEP in die Amygdala zu einer Inhibition der Akquisition von auditiver Furchtkonditionierung (Rodrigues et al. 2002). Vergleichbare Studien sind für mGluR1-Antagonisten nicht bekannt. Jedoch zeigte eine Arbeit, dass AIDA kontext-abhängige, aber nicht auditive Furchtkonditionierung hemmte (Nielsen et al. 1997). Weiterhin war kontext-abhängige, aber nicht auditive Furchtkonditionierung in mGluR1 Knock-out-Mäusen beeinträchtigt (Aiba et al. 1994). Zusammen mit unserem Ergebnis bezüglich mGluR1 und FPS deuten diese Befunde darauf, dass eine Aktivierung von mGlu1 Rezeptoren, im Gegensatz zu mGluR5, zum Erlernen klassisch-konditionierter Furcht nicht notwendig zu sein scheint.

3.3. Vergleich des Zusammenspiels von mGlu Rezeptoren der Gruppe I und NMDA Rezeptoren beim Erlernen von PA und FPS

In Manuskript 2 konnte gezeigt werden, dass eine gemeinsame Aktivierung von mGlu der Gruppe I und NMDA Rezeptoren zur Akquisition von PA, aber nicht der FPS, notwendig ist. Aufgrund der nichtparamterischen Daten konnte jedoch nicht berechnet werden, ob die Interaktion der jeweiligen Rezeptoren beim Erlernen von PA additiver oder synergistischer Natur ist. Aus früheren *in vitro* Studien ergaben sich erste Hinweise für eine Interaktion besagter Rezeptortypen. Interessanterweise wurde gezeigt, dass eine kombinierte Aktivierung von mGlu sowie von NMDA Rezeptoren im Hippokampus zur Induktion der LTP erforderlich ist (Musgrave et al. 1993; Fuji et al. 2004). Zudem war in mGluR5-KO Mäusen die Stärke der LTP signifikant reduziert in NMDA Rezeptor-abhängigen Signalwegen wie CA1 und Gyrus

dentatus, nicht aber in CA3, ein von NMDA Rezeptoren unabhängiger Signalweg (Lu et al. 1997).

Diese funktionale Interaktion konnte in neueren Studien auch *in vivo* belegt werden. Zum Beispiel führten Kombinationen von NMDA Rezeptor-Antagonisten (Phencyclidin oder (+)-MK-801) mit dem mGluR5-Antagonisten MPEP zu einer Verschlechterung räumlichen und instrumentellen Lernens sowie zu einer Verminderung des Arbeitsgedächtnis (Campbell et al. 2004; Homayoun et al. 2004). Diese Erkenntnisse werden durch die Ergebnisse der PA-Versuche aus Manuskript 2 erweitert, so dass zum ersten Mal auch ein Zusammenspiel zwischen mGluR1 oder mGluR5 mit NMDA Rezeptoren in aversivem, instrumentellen Lernen beobachtet wurde.

Überraschend allerdings waren die Ergebnisse für die FPS, da hier weder unwirksame Einzeldosierungen von MTEP zusammen mit (+)-MK-801, noch EMQMCM mit (+)-MK-801 einen Effekt auf die Akquisition zeigten. Es ist bekannt, dass NMDA Rezeptoren an der Akquisition der FPS beteiligt sind. So verminderte die Applikation des NMDA Rezeptorantagonisten AP5 (*amino-5-phosphonopentanoic acid*) direkt in die Amygdala die FPS (Miserendino et al. 1990). Ein ähnliches Ergebnis wurde in unserem Falle durch systemische Applikation von (+)-MK-801 erzielt. Gleichfalls an der Akquisition der FPS beteiligt sind mGluR5. Zum Beispiel wurde die FPS durch systemische Gabe von MPEP gehemmt (Schulz et al. 2001), als auch nach direkter Applikation in die Amygdala (Fendt and Schmid, 2002). Daher ist es erstaunlich, dass zum einen die gemeinsame Applikation von MTEP und (+)-MK-801 in unwirksamen Einzeldosierungen keinen Einfluss auf die Akquisition der FPS nahm, und zum anderen, da sich eine überlappende Verteilung der betreffenden Rezeptortypen in der Amygdala findet (Spooren et al. 2003). Im Gegensatz zu PA sind bei FPS unterschiedliche Hirnregionen beteiligt, was diesen Unterschied bei der Akquisition erklären könnte. Weiterhin ist nicht bekannt, ob eine Wechselwirkung zwischen NMDA und mGluR der Gruppe I generell in allen Hirnstrukturen stattfindet.

Weniger erstaunlich hingegen sind die Ergebnisse der Ko-Applikation von EMQMCM mit (+)-MK-801, da erstere Substanz zuvor in allen getesteten Dosierungen keine Wirkung auf die Akquisition der FPS hatte (Gravius et al. 2005). Zudem deuten einige Arbeiten darauf hin, dass mGlu1 Rezeptoren an kontext-abhängiger, aber nicht an der Akquisition klassischer Konditionierung beteiligt zu sein scheinen (Aiba et al. 1994; Nielsen et al. 1997).

Um auszuschließen, dass die beobachteten Effekte nicht aus einer möglichen analgetischen Wirkung der betreffenden Substanzen resultieren, wurde eine Schock-Titration durchgeführt. Dazu wurden diejenigen Dosierungen von MTEP, EMQMCM sowie (+)-MK-801 gewählt, die eine Verschlechterung der Akquisition in PA zeigten. Jedoch war kein Unterschied in der Empfindlichkeit gegenüber den applizierten elektrischen Stimuli zwischen den einzelnen Gruppen festzustellen. Eine frühere Studie zeigte gleichfalls keinen Einfluss von MTEP und auch EMQMCM auf akuten Schmerz (Sevostianova & Danysz 2006). Dies lässt vermuten, dass die beobachteten Effekte in PA nach gemeinsamer Applikation von (+)-MK-801 mit MTEP oder (+)-MK-801 mit EMQMCM nur durch Hemmung der Akquisition, nicht aber durch analgetische Wirkung auftraten.

3.4. Vergleich der Rolle von mGlu Rezeptoren der Gruppe I bei der Akquisition und Expression in kontext-abhängiger und auditiver Furcht

Einige Ergebnisse in den ersten beiden Manuskripten deuten darauf hin, dass mGluR1 und mGluR5 in unterschiedlicher Weise an Lernen und an der Gedächtnisbildung beteiligt zu sein scheinen. Aus diesem Grund wurde in einer weiteren Versuchsreihe die Beteiligung der beiden Rezeptorsubtypen an der Akquisition sowie Expression von kontext-abhängigen im Vergleich zu auditiven Furchtgedächtnissen untersucht. Dazu dienten die spezifischen Antagonisten EMQMCM für mGluR1 und MTEP für mGluR5. Da NMDA und muskarinische cholinerge Rezeptoren an vielen Lernvorgängen beteiligt sind, wurden die Effekte von EMQMCM und

MTEP auf die Akquisition mit dem NMDA Rezeptorantagonisten (+)-MK-801 sowie dem unspezifischen muskarinischen Antagonisten Skopolamin verglichen. Da in beiden verwendeten Verhaltensmodellen konditionierte Furcht als Maß des Gedächtnisses dient, fanden im Falle der Expression der selektive Serotonin-Wiederaufnahmehemmer Citalopram und Diazepam Verwendung. Während Citalopram die Expression in KFK hemmte, wurde die Expression in AFC nicht beeinflusst. Interessanterweise verbesserte Citalopram die Akquisition in KFK, hatte aber keinen Effekt auf diese in AFK. Frühere Arbeiten liefern diesbezüglich kontroverse Ergebnisse, wobei ein ausschlaggebender Punkt für die Wirkung ist, ob Citalopram chronisch oder akut appliziert wurde. In einer Arbeit wurde beobachtet, dass Citalopram nach akuter Behandlung die Akquisition verbesserte, nach chronischer Applikation jedoch verschlechterte (Burghardt et al. 2004). Im Bezug auf die Expression zeigten verschiedene Studien, dass Citalopram nach akuter Gabe das Ausmaß der Verhaltensstarre reduzierte (Hashimoto et al. 1996; Inoue et al. 1996; Izumi et al. 2006), was mit unseren Beobachtungen übereinstimmt. Zur Überraschung zeigte Diazepam in beiden Modellen keinen anxiolytischen Effekt, in KFK wurde das Ausmaß der Verhaltensstarre sogar gesteigert. Letzteres rührt höchstwahrscheinlich von sedativen Effekten von Diazepam her, was eine mögliche anxiolytische Wirkung verschleiert haben könnte. Im Gegensatz dazu zeigte sich in früheren Studien, dass Diazepam in ähnlichem Dosisbereich in verschiedenen Verhaltensmodellen anxiolytisch wirksam war (Brodkin et al. 2002; Tizzano et al. 2002). Im speziellen Falle von KFK zeigte Midazolam, ebenfalls eine Substanz aus der Gruppe der Benzodiazepine, eine Verminderung der Ausprägung der Verhaltensstarre (Sienkiewicz-Jarosz et al. 2003; Pietraszek et al. 2005), wobei hier jedoch Ratten des Wistar-Stammes benutzt wurden und in unserem Fall Sprague-Dawley-Ratten Verwendung fanden.

Sowohl EMQMCM als auch MTEP beeinträchtigten die Akquisition in KFK, aber nicht in AFK. Interessanterweise führte die Applikation von (+)-MK-801 zum gleichen Ergebnis, lediglich Skopolamin reduzierte die Akquisition in AFK in dosisabhängiger Weise. Die Gabe

von Diazepam führte zu einer Verminderung der Akquisition in KFK, aber nicht von AFK. Im Gegensatz dazu steigerte Citalopram sogar die Akquisition in KFK, hatte aber keinen Einfluss auf die Akquisition in AFK. Während MTEP die Expression in beiden Verhaltensmodellen hemmte, reduzierte EMQMCM nur die Expression in KFK. Überraschenderweise wurde die Expression in AFK durch Diazepam nicht beeinflusst, in KFK wurde das Ausmaß der Verhaltensstarre sogar noch erhöht. Citalopram hingegen reduzierte die Expression konditionierter Furcht in KFK, aber nicht AFK.

Ausgehend von der weitestgehend akzeptierten Rolle von NMDA Rezeptoren bei verschiedenen Lernvorgängen und weiter ausgehend davon, dass eine Interaktion zwischen mGluR1 und mGluR5 mit NMDA Rezeptoren bei einigen Formen des Lernens stattfindet (Campbell et al. 2004; Gravius et al. 2006), ist es nicht überraschend, dass sowohl (+)-MK-801, EMQMCM als auch MTEP die Akquisition in KFK hemmten. Weiterhin belegen diese Ergebnisse frühere Arbeiten, die zeigten, dass sowohl mGluR1 als auch mGluR5 an aversivem, kontext-abhängigen Lernen beteiligt sind (Aiba et al. 1994; Lu et al. 1997; Schachtman et al. 2003). Im Gegensatz dazu stehen die Ergebnisse der Akquisition in AFK. Hierbei zeigte keiner der oben genannten Antagonisten einen Effekt. Skopolamin hingegen hemmte die Akquisition in AFK, ein Ergebnis, dass frühere Arbeiten bestätigten (Rudy 1996; Anagnostoras et al. 1999). Vorige Studien mit NMDA Rezeptorantagonisten in Lernmodellen mit einem spezifischen Stimulus liefern kontroverse Ergebnisse. So wurde die Akquisition von FPS durch NMDA Rezeptorantagonisten blockiert (Miserendino et al. 1990; Gravius et al. 2006). In anderen Studien beeinträchtigten Antagonisten wie in unserem Falle die Akquisition von KFK, aber nicht AFK (Fanselow et al. 1994; Gould et al. 2002). Dies lässt darauf schließen, dass NMDA Rezeptoren nicht generell an der Akquisition klassisch konditionierter Furcht beteiligt sind. So erscheint die Akquisition von FPS, aber nicht AFK, von NMDAR abhängig zu sein. Betrachtet man die Interaktion zwischen den Rezeptortypen, so scheint es nicht verwunderlich, dass EMQMCM und MTEP die Akquisition in KFK, aber nicht AFK hemmten. Frühere Arbeiten

unterstützen diese Befunde. So wurde in mGluR1 als auch mGluR5 KO-Mäusen eine Beeinträchtigung von KFK, aber nicht AFK beobachtet (Aiba et al. 1994; Lu et al. 1997). Jedoch sind mGluR5 an der Akquisition von FPS beteiligt (Schulz et al. 2001; Fendt & Schmid 2002; Gravius et al. 2005).

Zusammenfassend betrachtet bleibt unklar, warum in den Experimenten die Akquisition von AFK nicht beeinflusst wurde. Das lässt vermuten, dass hierbei unterschiedliche neuronale Signalwege als beispielsweise bei FPS beteiligt sind. Zudem unterscheiden sich die Modelle in ihren verwendeten konditionierten Stimuli, sowie in der Art der konditionierten Verhaltensantwort (Schreckreaktion – Verhaltensstarre). Dies könnte ebenfalls zu den unterschiedlichen Befunden beitragen.

Ein anderes Bild ergab bei Untersuchung der Effekte von EMQMCM und MTEP auf die Expression der konditionierten Furcht. Während EMQMCM nur die Expression in KFK hemmte, verminderte die Applikation von MTEP die konditionierte Furcht sowohl in KFK als auch AFK. In beiden Modellen wird die konditionierte Furcht der Tiere als Starre angezeigt, eine Verhaltensantwort, deren Ausprägung durch anxiolytisch wirksame Substanzen vermindert werden kann. Diesbezüglich zeigten frühere Arbeiten, dass sich MTEP sowie MPEP in verschiedenen Verhaltensmodellen als anxiolytisch wirksam erwiesen haben (Klodzinska et al. 2004; Steckler et al. 2005b; Pietraszek et al. 2005). Von besonderem Interesse der Daten aus Manuskript 3 ist die unterschiedliche Wirkung von MTEP auf die Akquisition und Expression, wobei erstere nicht beeinflusst wurde. Dies deutet auf unterschiedlich beteiligte Hirnbereiche bei Akquisition und Expression von AFK hin. Im Gegensatz dazu wurde sowohl die Akquisition als auch die Expression der FPS, einem anderen Lernmodell nach klassischer Konditionierung, durch MPEP und MTEP gehemmt (Schulz et al. 2001; Pietraszek et al. 2005). Weniger ist über die Rolle von mGluR1 in diesem Hinblick bekannt. Jedoch finden sich in der Literatur deutliche Hinweise für anxiolytische Wirkungen

von mGluR1-Antagonisten, wobei dies nicht in allen Tests gezeigt werden konnte (Steckler et al. 2005b; Pietraszek et al. 2005).

Im Hinblick auf die Expression sollten nicht nur anxiolytische Effekte in Betracht gezogen werden, sondern auch mögliche Wirkungen auf den Wiederabruf der Gedächtnisspuren. Aus einigen Arbeiten ist bekannt, dass mGluRs hierbei beteiligt zu sein scheinen. Zum Beispiel wurde der Wiederabruf von PA-Gedächtnis durch den selektiven Gruppe I mGluR-Antagonisten DHPG dosisabhängig verschlechtert oder verbessert (Car et al. 2000), und MCPG hemmte den Wiederabruf von PA nach Applikation in den Hippokampus (Izquierdo et al. 2000). Im gleichen Lernmodell verschlechterte EMQMCM deutlich den Wiederabruf nach Applikation vor dem Test (Gravius et al. 2005). Im Falle der Furchtkonditionierung lassen sich anxiolytische Wirkungen von Effekten auf den Wiederabruf nicht klar voneinander trennen, so dass weitere Untersuchungen nötig sind, um genauere Aussagen über die Wirkungsweise von mGluR der Gruppe I an der Expression machen zu können.

3.5. Zusammenfassende Diskussion

Die Rolle von mGluRs der Gruppe I in der Ratte wurde in verschiedenen Lern- und Gedächtnismodellen mit Hilfe spezifischer Rezeptorantagonisten getestet. Die verwendeten Modelle bieten den Vorteil, dass nach nur wenigen Trainingseinheiten sowie kurzer Trainingszeit stabile Furchtgedächtnisse geformt werden, und zudem können die verschiedenen Stadien wie Akquisition, Konsolidierung und Expression getrennt untersucht werden. Allen Modellen liegt aversives Lernen zu Grunde, jedoch variieren sie in der unterschiedlichen Beteiligung verschiedener Hirnbereiche sowie in der Expression der Verhaltensantwort.

EMQMCM, MTEP sowie (+)-MK-801, verminderten die Akquisition in den beiden kontext-abhängigen Modellen PA und KFK, jedoch hatte keine dieser genannten Substanzen

einen Einfluss auf die Akquisition in AFK. Diese wurde lediglich durch Skopolamin vermindert. Jedoch konnte gezeigt werden, dass sowohl MTEP als auch (+)-MK-801 die Akquisition in FPS hemmten, ein wie AFK klassisches Konditionierungsmodell. Dies deutet darauf hin, dass das glutamaterge System bei der Akquisition in AFK möglicherweise nicht involviert ist. Interessanterweise konnte für mGluR1 keine Beteiligung an der Akquisition in FPS nachgewiesen werden, was für eine unterschiedliche Rezeptorbeteiligung beim Erlernen von FPS spricht.

In der vorliegenden Arbeit konnte zum ersten Mal gezeigt werden, dass zum einen eine gemeinsame Aktivierung von mGluR1 und mGluR5, sowie NMDAR und mGluR1 und NMDAR und mGluR5, zur Akquisition von Hippokampus-abhängigem PA nötig ist. Dies konnte für die Akquisition in Hippokampus-unabhängigem FPS nicht beobachtet werden.

	Kontext-abhängige Konditionierung					**Klassische Konditionierung**		
	Passive Avoidance			**KFK**		**AFK**		**FPS**
Substanz	**Akquisition**	**Konsolidierung**	**Expression**	**Akquisition**	**Expression**	**Akquisition**	**Expression**	**Akquisition**
EMQMCM	↓	k. E	↓	↓	↓	k. E.	k. E.	k. E.
MTEP	↓	k. E	k. E.	↓	↓	k. E	↓	↓
(+)-MK-801	↓	-	-	↓	-	k. E	-	↓
EMQMCM + MTEP	↓	-	-	-	-	-	-	-
EMQMCM + (+)-MK-801	↓	-	-	-	-	-	-	k. E
MTEP + (+)-MK-801	↓	-	-	-	-	-	-	k. E
Diazepam	-	-	k. E	↓	↑	k. E	k. E	-
Citalopram	-	-	-	↑	↓	k. E	k. E	-
Skopolamin	-	-	-	-	-	↓	-	-

Tab. 1: Zusammenfassung der Ergebnisse aus allen Manuskripten (↓: Verminderung; ↑: Verbesserung; k.E.: kein Effekt; - nicht getestet)

Ein weiterer Unterschied zwischen mGluR1 und mGluR5 war bei der Expression von PA zu beobachten, wobei der Gedächtniswiederabruf nur durch EMQMCM, aber nicht durch MTEP gestört wurde. Im Gegensatz dazu wurde die Expression in AFK durch MTEP gehemmt, wobei EMQMCM keinen Effekt zeigte. Im Unterschied dazu konnte eine Beteiligung von sowohl mGluR1 als auch mGluR5 bei der Expression in KFK gezeigt werden.

Zusammenfassend kann man festhalten, dass das glutamaterge System nicht generell an allen Lernprozessen in verschiedenen Verhaltensmodellen der Ratte beteiligt zu sein scheint. Im Hinblick auf mGlu Rezeptoren der Gruppe I zeigte sich eine unterschiedliche Beteiligung an der Akquisition von FPS sowie Expression von PA und AFK. Hingegen scheint die Akquisition in kontext-abhängigen Modellen wie PA und KFK von einer Aktivierung von NMDA als auch von mGlu Rezeptoren der Gruppe I abhängig zu sein.

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5. Abkürzungen

AFK	auditive Furchtkonditionierung
AIDA	*1-aminoindan-1,5-dicarboxylic acid*
AMPA	*α-amino-3-hydroxy-5-methyl-4-isoxazol-4-propionic acid*
AP5	*amino-5-phosphonopentanoic acid*
CS	*conditioned stimulus,* konditionierter Stimulus
EMQMCM	*3-ethyl-2-methyl-quinolin-6-yl)-(4-methoxy-cyclohexyl)-methanone methanesulfonate*
FPS	Furcht-potenzierte Schreckreaktion
KFK	kontext-abhängige Furchtkonditionierung
KO	*Knock-out*
LTP	Langzeitpotenzierung
MGluR	metabotroper Glutamatrezeptor
(+)-MK-801	Dizocilpin
MPEP	*2-methyl-6-(phenylethynyl)-pyridine*
MTEP	*[(2- methyl-1,3-thiazol-4-yl)ethynyl]pyridine*
NMDA	N-Methyl-D-Aspartat
PA	*Passive Avoidance*
US	*unconditioned stimulus,* unkonditionierter Stimulus

6. Erklärung zum Eigenanteil an den Manuskripten

Manuskript 1: Etablierung der Methoden, vollständige konzeptionelle Planung und Anfertigung des Manuskriptes, 80 % der Versuchsvorbereitung und Durchführung sowie komplette Versuchsauswertung. Die Methode FPS wurde gemeinsam mit M. Pietraszek etabliert. Etwa 20 % der Versuchsvorbereitung und- Versuchsdurchführung erfolgten unter meiner Anleitung durch D. Schäfer.

Manuskript 2: Vollständige konzeptionelle Planung und Anfertigung des Manuskriptes, 80 % der Versuchsvorbereitung und Durchführung sowie komplette Versuchsauswertung. Etwa 20 % der Versuchsvorbereitung und- Versuchsdurchführung wurden von M. Pietraszek ausgeführt.

Manuskript 3: Etablierung der Methoden, vollständige konzeptionelle Planung und Anfertigung des Manuskriptes, 70 % der Versuchsvorbereitung sowie Durchführung und komplette Versuchsauswertung. Etwa 30 % der Versuchsvorbereitung und- Versuchsdurchführung erfolgten unter meiner Anleitung durch C. Barberi und D. Schäfer.

Bei keinem der angeführten Manuskripte ging der Anteil von Prof. Dr. W. J. Schmidt sowie Prof. Dr. W. Danysz über das im Rahmen eines Betreuungsverhältnisses übliche Maß hinaus.

7. Anhang: Manuskripte

Effects of mGlu1 and mGlu5 receptor antagonists on negatively reinforced learning

A. Gravius[a], M. Pietraszek[a,b], D. Schäfer[a], W. J. Schmidt[c] and W. Danysz[a]

Effects on aversive learning of the novel highly selective mGlu5 receptor antagonist [(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP) and mGlu1 receptor antagonist (3-ethyl-2-methyl-quinolin-6-yl)-(4-methoxy-cyclohexyl)-methanone methanesulfonate (EMQMCM) were tested, after systemic administration, in the passive avoidance (PA) and fear potentiated startle (FPS) paradigms. Both MTEP at 10 mg/kg and EMQMCM at 5 and 10 mg/kg, given 30 min before training, impaired acquisition of the passive avoidance response (PAR). Co-administration of MTEP and EMQMCM at doses ineffective when administered alone, produced anterograde amnesia when given 30 min before the acquisition phase. Neither EMQMCM (5 mg/kg) nor MTEP (10 mg/kg) impaired retention of the PAR after direct post-training injections. EMQMCM (5 mg/kg), but not MTEP (10 mg/kg) blocked the PAR when given 30 min before testing. Pre-training administration of MTEP at doses of 2.5 and 5 mg/kg inhibited fear conditioning in the FPS when tested 24 h later. In contrast, EMQMCM was ineffective. Our findings suggest diverse involvement of mGlu1 and mGlu5 receptors in negatively reinforced learning.
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Keywords: mGlu1 receptor antagonist, mGlu5 receptor antagonist, learning, fear potentiated startle, passive avoidance, rat

[a]Preclinical R & D, Merz Pharmaceuticals, Frankfurt am Main, Germany, [b]Institute of Pharmacology, Polish Academy of Sciences, Cracow, Poland and [c]University of Tübingen, Department of Neuropharmacology, Zoological Institute, Tübingen, Germany.

Correspondence and requests for reprints to Professor Wojciech Danysz, Merz Pharmaceuticals GmbH, Eckenheimer Landstrasse 100, 60318 Frankfurt am Main, Germany.
E-mail: wojciech.danysz@merz.de

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The results described here have been partially presented at a Society for Neuroscience meeting (Gravius *et al.*, 2004).

Introduction

Glutamate plays a very significant role in neurotransmission in the central nervous system. Fast transmission is mediated by ionotropic glutamate receptors such as DL-α-amino-3-hydroxy-5-methylisoxazolepropionic acid (AMPA), kainate and *N*-methyl-D-aspartate (NMDA) receptors, while the transmission of the G-protein-coupled metabotropic glutamate (mGlu) receptors is more modulatory in nature (Parsons *et al.*, 1998; Pin and Acher, 2002). Until now, eight subtypes of mGlu receptors have been identified, and divided in three groups based on pharmacological and transmission mechanisms, as well as sequence homologies. Group I mGlu receptors (mGlu1 and mGlu5) are coupled to phosphoinositol hydrolysis. Group II (mGlu2/3) and group III (mGlu4/6/7/8) are negatively linked to adenylate cyclase (Conn and Pin, 1997).

Many studies have addressed the involvement of mGlu group I in cognitive function (reviewed by Spooren *et al.*, 2003). Recent studies confirmed the involvement of group I mGlu receptors in long-term potentiation (LTP) (Bashir *et al.*, 1993) and learning and memory processes, e.g. in spatial learning in rats (Balschun and Wetzel, 2002; Petersen *et al.*, 2002).

Most studies of aversive learning have focused especially on the role of mGlu5 receptors. For example, 2-methyl-6-(phenylethynyl)-pyridine (MPEP), a mGlu5 receptor noncompetitive antagonist, was shown to block fear conditioning (Schulz *et al.*, 2001; Rodrigues *et al.*, 2002) and attenuated conditioned taste aversion (Schachtman *et al.*, 2003).

In contrast, little is known about mGlu1 in this respect (Maciejak *et al.*, 2003). However, mice lacking the mGlu1 receptor show deficits in LTP and learning (Aiba *et al.*, 1994). This is not surprising given positive coupling between mGlu1 and NMDA receptors, and the accepted role of the latter type in synaptic plasticity (Collingridge, 1987; Danysz *et al.*, 1995). Until recently, studies using systemically active mGlu1 antagonists have been very limited, due to a lack of antagonists with satisfactory penetration to the CNS. In the present study, a systemically active mGlu1 antagonist EMQMCM [(3-ethyl-2-methyl-quinolin-6-yl)-(4-methoxy-cyclohexyl)-methanone methanesulfonate; Lesage *et al.*, 2002] was compared with the recently described mGlu5 receptor antagonist MTEP [(2-methyl-1,3-thiazol-4-yl)ethynyl]-pyridine (Busse *et al.*, 2004).

Thus, the present study was designed to assess the role of mGlu1 and mGlu5 receptors in learning in two negatively reinforced tests, fear potentiated startle (FPS) and passive avoidance (PA), using systemically active, selective antagonists of these receptors, i.e. EMQMCM and

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MTEP. In the FPS test, a form of Pavlovian conditioning, usually a footshock (US) is paired with light (CS), which later potentiates the startle response to an acoustic stimulus (Walker and Davis, 2002).

In PA, subjects learn to avoid a dark compartment after receiving a footshock, while in FPS enhancement of startle response is assessed upon presentation of a conditioned cue (light) previously paired with a footshock. In both paradigms, stable fear memories are formed after short training, which provides the advantage of investigating acquisition, consolidation and retrieval. During the consolidation phase, the learned experience is transferred to stable memory traces. Retrieval is the reactivation process of the stored traces by reminders such as context or cue (light), which were present during acquisition. To study the role of mGlu receptors on this phase, the substances were given before retrieval. In this case, possible anxiolytic effects cannot be excluded. Therefore, as a control, diazepam was used at a dose showing anxiolytic effects in several models for anxiety (Davis, 1979; Pietraszek *et al.*, 2004).

Methods

Subjects

Experimentally naive adult male Sprague–Dawley rats (240–280 g; Janvier, France) were housed in groups of four per cage. Colony room temperature and humidity were maintained respectively at $20 \pm 1^{\circ}C$ and $60 \pm 3\%$. Food and water were freely available and the animals were kept under an alternating 12 h/12 h day–night cycle (lights on at 07.00 hours) for at least 6 days before the experiments started. All experiments were conducted during the light period of the day–night cycle. Each animal was used only once. The study was approved by the Ethical Committee, Regierungspraesidium Darmstadt, Hessen and performed in accordance with the recommendations and policies of the US National Institutes of Health Guidelines for the Use of Animals.

Passive avoidance

Apparatus

The two-choice dark avoidance apparatus consisted of three compartments of identical size ($32 \times 25.5 \times 25.5\,cm^3$; width $\times$ depth $\times$ height) connected so that vertically sliding doors ($6 \times 9\,cm^2$; height $\times$ width) controlled access from the start compartment to both choice compartments. Each compartment was equipped with a source of light and a grid floor connected to a scrambling shock generator (ENV 414, Med Associates, St. Albans, Vermont, USA). The detection system consisted of six pairs of photobeams, located 3.5 cm above the floor and 4 cm from the doors. The system was remotely controlled through an interface (Med Associates) connected to an IBM-PC operating Med Associates software version IV.

Procedure

The experiments lasted 4 days. On the first day each animal received 5 min of handling. On the second day rats were handled again for 5 min and placed into the start box compartment for 5 min. During the habituation period only the lighted compartment was available, on the right or the left (balanced between the rats in each group). For training on day 3, rats were placed into the start box with free access to both choice compartments. The time of entering the light and/or dark compartment was recorded. When the rat entered the dark compartment the door was closed and foot electroshocks (1 mA, 1 s) were applied 3 times at 2 s intervals. Five seconds later, the rat was returned to the home cage. Responses to shock (vocalization, jumping) were observed to confirm that the shock had been administered. If the rat failed to enter the dark compartment within the first 180 s, it was excluded from the experiment. On the fourth retention day the rat was placed into the start box with free access to both choice compartments, and the time of entering the light and/or dark compartment was recorded. During retention no shocks were delivered. A cut-off time of 300 s was used.

Fear potentiated startle

Apparatus

For training, subjects were placed in acrylic animal holders [19 cm long, 7.6 cm internal diameter (i.d.)] with a grid floor consisting of nine stainless-steel bars (3 mm i.d.). Holders were fixed onto a startle platform (Med Associates, Model PHM-250B). The grid floor was connected to a scrambling shock generator, through which a 0.6 mA footshock could be delivered. The complete equipment was placed into a sound-attenuating chamber. Startle-eliciting noise bursts lasting 50 ms were generated by a noise generator (Med Associates, Model PHM-255A). The speaker was placed 7 cm from the animal holder in the back of the chamber. A fan attached on the side wall of the chamber produced a background noise of 62 dB, and a noise generator produced additional noise, so that the overall background noise was 64 dB. A 3.7 s visual cue was produced by an 8 W fluorescent stimulator (Med Associates, Model PHM-258L) consisting of an 8 W bulb, placed in front of the chamber. The output of the accelerometer was connected through an interface to an IBM-PC running Startle Reflex software (Med Associates, version 5.1).

Procedure

Pre-test. On the first day, in order to obtain groups with similar startle responses, a pre-test was performed. The subjects were placed into the acrylic holders, and after a 5 min acclimation period, six initial startle stimuli (2 of 95 dB, 2 of 100 dB and 2 of 105 dB, 50 ms duration) were presented to induce a stable startle baseline. Then, each subject received 30 startle stimuli, 10 of 95 dB, 10 of 100 dB and 10 of 105 dB. (7–23 s inter-stimulus interval).

Conditioning. For training, 24 h later, rats were placed back into the animal holders. After a 5 min acclimation period, 15 pairings of light with a 0.6 mA footshock were presented. The unconditioned stimulus was presented during the last 500 ms of the 3.7 s of light, so that both stimuli terminated together. The mean inter-trial interval was 60 s, with a range of 30–90 s.

Test. Twenty-four hours after training, rats were again placed into the animal holders. After a 5 min acclimation period, animals received 6 initial noise bursts of 95, 100 and 105 dB to establish a stable startle baseline before recording. Then, 30 startle stimuli (50 ms, of 95, 100 and 105 dB) were presented, one half of each type 3200 ms after the onset of the light (light–noise trials), and one half in dark (noise-alone trials). Inter-trial interval was 15–45 s Differences of light–noise and noise-alone trials were calculated.

Drugs

EMQMCM [(3-ethyl-2-methyl-quinolin-6-yl)-(4-methoxy-cyclohexyl)-methanone methanesulfonate, synthesized by Merz, Frankfurt, Germany] and MTEP [(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine, synthesized by Merz Frankfurt, Germany were dissolved in 10% Tween 80/water. Diazepam (Ratiopharm, Ulm, Germany) was diluted from a stock solution (10 mg/2 ml) to the final concentration with 1% of Tween 80/water. All substances were administered 30 min before the test, unless stated otherwise. Control animals received respective vehicles and all compounds were injected into the peritoneum (i.p.) in a volume of 2 ml/kg.

Statistical analysis

In the case of the FPS test, the mean startle amplitude for each trial type presentation was calculated as the mean of peaks, measured as the maximum value recorded in 100 ms beginning with the onset of the startle stimulus. FPS was calculated as the difference of the mean startle amplitude in the light (light–noise trials) minus the mean startle amplitude in the dark (noise-alone trials). The differences were analyzed by one- or two-way ANOVA, followed, if significant, by Duncan's test.

PA data, i.e. latencies to leave the start box (time to enter the dark or lighted box, whichever occurred first), and latencies to enter the dark box, were analyzed by nonparametric ANOVA (Kruskal–Wallis test), followed, if significant, by Dunn's or Mann–Whitney test. The results are expressed as medians with interquartile ranges.

Results

Effects of MTEP and EMQMCM on acquisition in PA

The latencies to leave the start box during training under acute influence of the drugs can serve as a measurement of general behavioral inhibition. On the other hand, latencies to enter the dark box during the retention test are taken as a direct measurement of memory impairment. MTEP at doses of 2.5, 5 and 10 mg/kg and EMQMCM at doses of 2.5, 5 and 10 mg/kg, administered 30 min before training, did not change the latency to leave the start box during training (Fig. 1). MTEP at a dose of 10 mg/kg significantly impaired the PAR tested 24 h later (Fig. 1). EMQMCM at doses of 5 and 10 mg/kg (but not 2.5 mg/kg) also impaired the acquisition of PA (Fig. 2).

Next a combination of selected, inactive doses of both agents was tested. MTEP at 5 mg/kg and EMQMCM at 2.5 mg/kg were ineffective when administered alone, but the combination significantly impaired acquisition compared to controls, or to the MTEP- or EMQMCM-alone groups (Fig. 3).

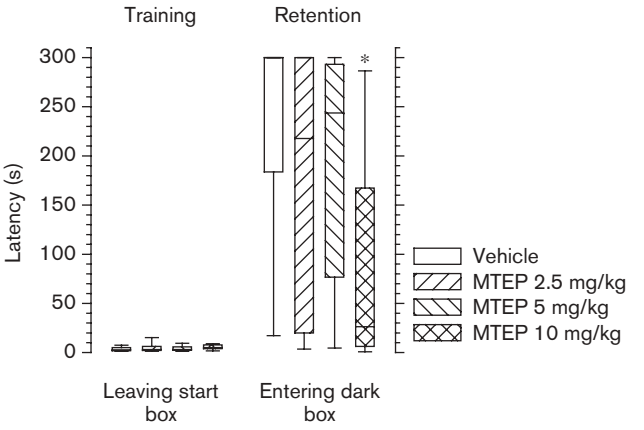
Effects of MTEP and EMQMCM on consolidation in PA

To evaluate the effect of MTEP and EMQMCM on memory consolidation, substances were administered directly after completion of the training trial (Fig. 4). Doses were selected that produced clear effects when given before the training, i.e. 10 and 5 mg/kg respectively. Retention tests took place 24 h later. Neither treatment changed latencies to enter the dark box on the retention day.

Effects of MTEP and EMQMCM on retrieval of PAR

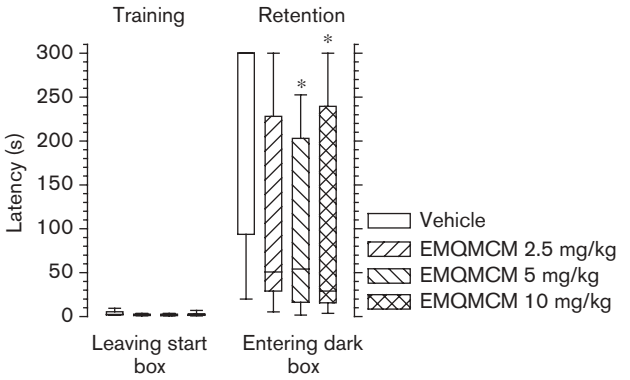
To compare the effect on retrieval of PA memory, MTEP and EMQMCM were given 30 min before the retention

Fig. 1



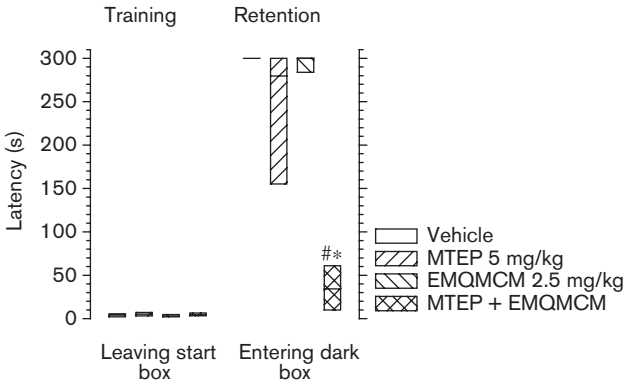
Effect of [(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP) on acquisition of two-choice passive avoidance. MTEP was given i.p. 30 min before training (acquisition). Retention of the passive avoidance response (PAR) was tested 24 h after training. The graphs show the latencies of leaving the start box and entering the dark box during training and test, respectively. Results are expressed as medians and interquartile ranges. *$P<0.05$ versus control, computed by ANOVA on ranks followed by Dunn's test ($n=12$ per group).

Fig. 2



Effect of (3-ethyl-2-methyl-quinolin-6-yl)-(4-methoxy-cyclohexyl)-methanone methanesulfonate (EMQMCM) on acquisition of two-choice passive avoidance. The substance was administered 30 min before training. EMQMCM at 5 and 10 mg/kg impaired retention of passive avoidance (for details see Fig. 1). *$P<0.05$ versus control computed by ANOVA on ranks followed by Dunn's test ($n=17$ per group).

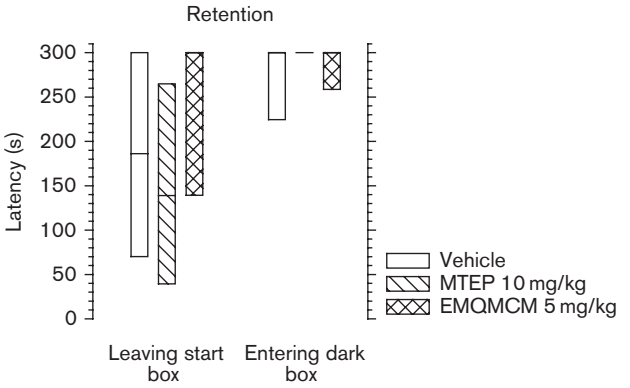
Fig. 3



Effect of [(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP) ($n=8$), (3-ethyl-2-methyl-quinolin-6-yl)-(4-methoxy-cyclohexyl)-methanone methanesulfonate (EMQMCM) ($n=8$), control ($n=8$) and combination ($n=7$) on acquisition of passive avoidance. Substances were administered 30 min before training (for details see Fig. 1). *$P<0.05$ versus control; #$P<0.05$ versus EMQMCM and MTEP computed by ANOVA on ranks followed by Dunn's test.

test, 24 h after training (Fig. 5). Latencies for leaving the start box are taken as a measure of general behavioral inhibition under the acute influence of the drugs during the test. MTEP at a dose of 10 mg/kg caused no significant delay in leaving the start box and entering the dark box. EMQMCM at 5 mg/kg did not influence latency of leaving the start box, but significantly reduced the latency of entering the dark box. Diazepam at 2 mg/kg influenced neither leaving the start box nor entering the dark box. Additionally, to evaluate possible state-dependent effects on learning, MTEP at 10 mg/kg was given

Fig. 4



Effect of [(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP) and (3-ethyl-2-methyl-quinolin-6-yl)-(4-methoxy-cyclohexyl)-methanone methanesulfonate (EMQMCM) ($n=8$ per group) on consolidation of passive avoidance. Substances were administered directly after training. Neither substance showed an effect on memory consolidation (for details see Fig. 1).

30 min before training, before test and before both training and test (Fig. 6). MTEP given before training or before both training and test significantly reduced latency to enter the dark box. MTEP administered before the test did not influence latency to enter the dark box. Similarly, EMQMCM at a dose of 5 mg/kg significantly reduced latency to enter the dark box when given before training, before test or before both training and test (Fig. 7).

Effects of MTEP and EMQMCM on acquisition of fear potentiation of startle

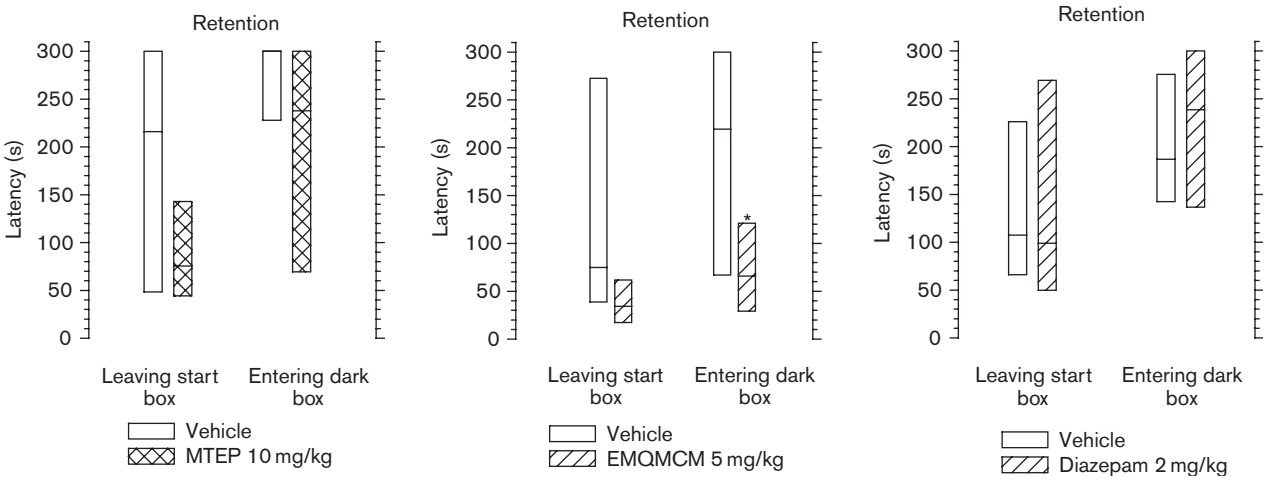
To evaluate the effect of MTEP and EMQMCM on acquisition of fear potentiation of acoustic startle, substances were administered 30 min before the start of conditioning. MTEP dose-dependently reduced startle amplitudes in light–noise trials, showing significant effects at doses of 2.5 and 5 mg/kg. No difference in mean startle amplitudes was observed in noise-alone trials (Fig. 8).

EMQMCM tended to attenuate the acquisition of FPS at doses 2.5 and 5 mg/kg, but this effect failed to reach statistical significance. No difference was found in startle amplitudes in noise-alone trials (Fig. 9).

Discussion

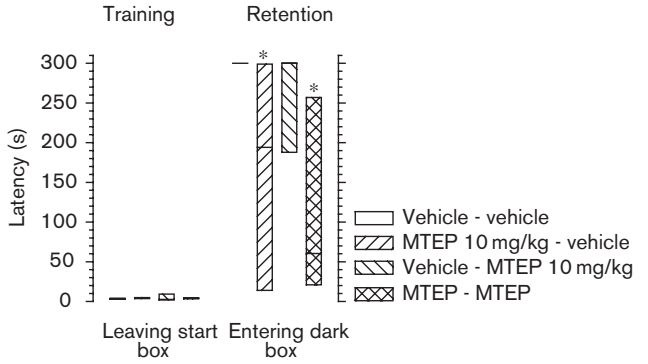
The present study assessed the involvement of mGlu receptor subtypes 1 and 5 in negatively reinforced learning, using PA and FPS. In the PA test, both the mGlu5 receptor antagonist MTEP and the mGlu1 antagonist EMQMCM produced dose-dependent amnesia when given before acquisition, but had no effect on

Fig. 5



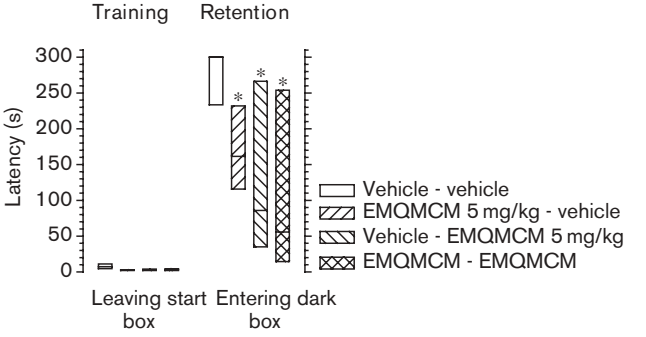
Effect of [(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP) ($n=8$), (3-ethyl-2-methyl-quinolin-6-yl)-(4-methoxy-cyclohexyl)-methanone methanesulfonate (EMQMCM) ($n=8$) and diazepam ($n=8$) on retrieval of passive avoidance. Substances were administered 30 min before the retention test. Diazepam and MTEP were ineffective, EMQMCM significantly reduced latency to enter the dark box (for details see Fig. 1). *$P<0.05$ versus control computed by ANOVA on ranks followed by Mann–Whitney test.

Fig. 6



Effect of [(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP) on passive avoidance. MTEP was administered before training, test and both training and test, to evaluate state-dependent memory impairment during retrieval. Both groups given MTEP before training and before both training and test showed a significantly reduced latency to enter the dark box. MTEP given before the test had no effect on latency to enter the dark box. Also, there was no difference between the groups given MTEP before training and before both training and test. (For details see Fig. 1.) *$P<0.05$ versus control, computed by ANOVA on ranks followed by Mann–Whitney test ($n=8$).

Fig. 7



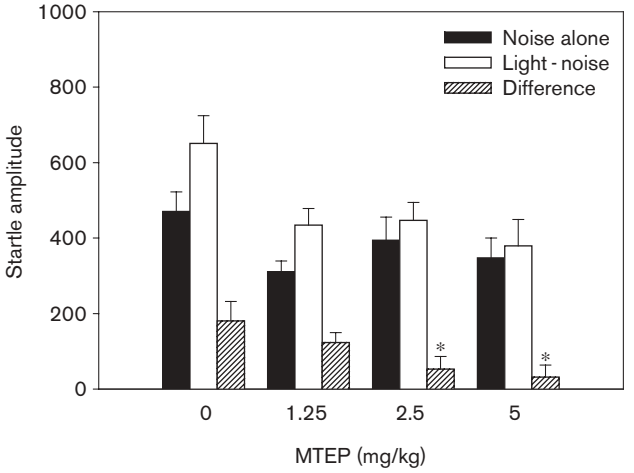
Effect of (3-ethyl-2-methyl-quinolin-6-yl)-(4-methoxy-cyclohexyl)-methanone methanesulfonate (EMQMCM) on passive avoidance. EMQMCM was administered before training, test or before both training and test, to evaluate state-dependent memory impairment. All EMQMCM groups showed a significantly reduced latency to enter the dark box. There was no difference between the three groups. (For details see Fig. 1.) *$P<0.05$ versus control, computed by ANOVA on ranks followed by Mann–Whitney tests ($n=8$ per group; for group EMQMCM before training $P=0.05$ versus control).

consolidation. EMQMCM, but not MTEP, impaired memory retrieval when given before the retention test in PA. In contrast, in FPS only the effect of MTEP reached statistical significance.

The choice of antagonists providing high selectivity and appropriate pharmacokinetics, such as CNS penetration and half-life, was of particular importance in this study. In this respect, MTEP was selected instead of MPEP (Novartis) as the latter substance was shown to inhibit the norepinephrine transporter and to interact with monoamine oxidase (MAO) (Heidbreder *et al.*, 2003). Further, for MTEP, the ED_{50} value for 50% of receptor occupancy 1 h after i.p. administration is 1.2 mg/kg, which is in the dose range used in the present work (Busse *et al.*, 2004).

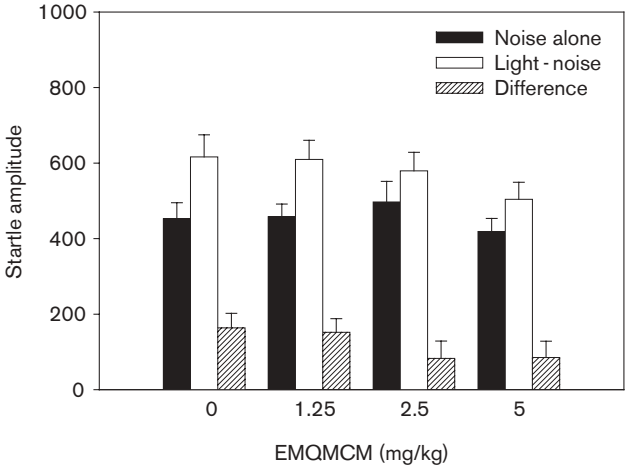
In contrast, available data on EMQMCM are very limited. Its IC_{50} value at mGlu1 is about 3.5 nmol/l, and related

Fig. 8



Effect of [(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP) on fear potentiation of startle. The diagram shows the mean startle amplitudes in the absence (black bars) and presence of the conditioned stimulus (white bars). Striped bars represent the differences between mean startle amplitudes in the light and in the dark. MTEP, given i.p. 30 min before the training, produced a dose-dependent reduction of startle amplitudes in the light ($F(3,37)=3.189$; $P<0.05$). No difference in startle amplitudes was found in noise-alone trials ($F(3,37)=1.88$; NS). *$P<0.05$ versus control, computed by one-way ANOVA followed by Duncan's test.

Fig. 9



Effect of (3-ethyl-2-methyl-quinolin-6-yl)-(4-methoxy-cyclohexyl)-methanone methanesulfonate (EMQMCM) on fear potentiation of startle. The diagram shows the mean startle amplitudes in the absence (black bars) and presence of the conditioned stimulus (white bars). Striped bars represent the differences between mean startle amplitudes in the light and in the dark. No significant reduction of startle amplitudes was observed in the presence of the light ($F(3,67)=1.08$; NS) and no differences in startle amplitudes were found in noise-alone trials ($F(3,67)=0.56$; NS).

substances have been demonstrated to have a good CNS penetration (Lesage *et al.*, 2002; Steckler, 2003).

Group I mGlu receptors play a key role in LTP (Bashir *et al.*, 1993; Aiba *et al.*, 1994; Lu *et al.*, 1997), a type of synaptic plasticity underlying some forms of learning and memory. For example, MPEP impairs LTP and spatial learning in the radial maze (Naie and Manahan-Vaughan, 2004). Further, it was shown that MPEP and a mGlu1 antagonist LY367385 ((S)-(+)-alpha-amino-4-carboxy-2-methylbenzeneacetic acid), reduced LTP amplitude in corticostriatal regions, and co-administration of both drugs fully suppressed LTP (Gubellini *et al.*, 2003). In the same report, reduction of LTP amplitude was observed in mGlu1 and mGlu5 receptor knockout mice. The latter results would support our findings that co-administration of MTEP and EMQMCM blocked acquisition in PA at doses which were ineffective when administered alone.

Step-through PA is a widely used model to study memory processes, in which animals are punished by a mild footshock after entering a dark compartment. Memory can be tested later by change of latency to enter the dark box.

The hippocampus is well known to play a key role in learning and memory. It was found that i.c.v. injections of MPEP impaired hippocampal LTP *in vivo* and learning (Balschun and Wetzel, 2002; Naie and Manahan-Vaughan, 2004). Also, mGlu1 knockout mice showed reduced hippocampal LTP and impaired associative learning (Aiba *et al.*, 1994). The hippocampus has been demonstrated to be involved in processing of the PAR. For example, functional inactivation of the hippocampus impaired acquisition, consolidation and retrieval of PA (Ambrogi Lorenzini *et al.*, 1997). With respect to mGlu receptors, a study revealed that intra-hippocampal post-training infusions of the mGlu agonist ACPD (1S, 2R-aminocyclopentane dicarboxylate) enhanced memory in a step-down PA task, while infusion of MCPG (methylcarboxyphenylglycine), a mGlu antagonist, impaired the PAR (Bianchin *et al.*, 1994).

Another important brain structure involved in PA is the amygdala. Post-training infusions of lidocaine into the basolateral complex of the amygdala impaired retention performance (Parent and McGaugh, 1994). Also, complete amygdala block by post-acquisition infusions of tetrodotoxin impaired learning in this task (Bucherelli *et al.*, 1992). Further, pre-training infusions of MCPG into the basolateral amygdala impaired retention in a continuous multiple-trial inhibitory avoidance (Barros *et al.*, 2000).

Schachtman *et al.* (2004) found that systemic administration of MPEP dose-dependently (3 and 10 mg/kg, i.p.)

impaired acquisition in PA when given before training. In contrast, Nadlewska *et al.* (2002) showed that MPEP, at a dose of 1 mg/kg (i.v.) improved consolidation and retrieval in a step-through PA, i.e. given after training and before retrieval respectively; however, they did not test the effect of MPEP on acquisition. The same group (Nadlewska *et al.*, 2003) investigated effects of AIDA (1-aminoindan-1,5-dicarboxylic acid) (i.c.v.), a group I mGlu antagonist with higher selectivity at mGlu1 in the same test paradigm. This study demonstrated that AIDA impaired acquisition and facilitated consolidation. In the present study, both MTEP and EMQMCM, given before acquisition in the PA test, significantly impaired retention. Co-administration of MTEP and EMQMCM before training, using doses ineffective when administered alone, significantly impaired retention. The fact that the combination was significantly different to control, or to EMQMCM- and MTEP-treated groups, suggests synergistic or additive effects of the combination treatment. (However, this could not be assessed due to the nonparametric nature of the distribution).

In the present study, administration of both MTEP and EMQMCM directly after training had no effect on expression of the PAR. Taken together, these data suggest an involvement of mGlu1 and mGlu5 receptors in acquisition, but not in consolidation of PA at the doses tested.

Next we examined the role of mGlu1 and mGlu5 receptors on retrieval of the PAR, and evaluated whether memory impairment was due to state-dependent retrieval during the retention test. EMQMCM, but not MTEP, given before memory reactivation, significantly decreased latency to enter the dark box, suggesting mGlu1 but not mGlu5 receptors may be involved in memory retrieval process. To evaluate potential anxiolytic effects, diazepam, at 2 mg/kg, a dose showing anxiolytic effects in several anxiety models (Davis, 1979; Pietraszek *et al.*, 2004), failed to decrease latencies to enter dark box. These findings would support mnemonic rather than anxiolytic effects of EMQMCM in this experimental setup. A limited number of studies confirm involvement of mGlu receptors in retrieval of PAR. For example, intrahippocampal injection of MCPG blocked retrieval when tested both 3 h and 31 days after training (Izquierdo *et al.*, 2000). Further, DHPG [(S)-3,5-dihydroxyphenylglycine], a selective group I mGlu agonist, at doses of 0.01 and 1.0 nmol i.c.v, facilitated and, at the dose of 0.1 nmol, impaired retrieval of the PAR (Car *et al.*, 2000). In contrast, AIDA was shown to be ineffective on retention when administered before retrieval (Nadlewska *et al.*, 2003). Taken together, the data are controversial; however, the present data obtained with systemically active compounds support the involvement of mGlu1 in retrieval of aversive memory.

To evaluate possible state-dependent learning, MTEP was given before acquisition, before the retrieval test and before both training and retrieval. No difference was found between groups receiving MTEP before acquisition and before acquisition and test, indicating that the apparent amnesic effect of MTEP might not be due to state-dependent learning. Similarly, EMQMCM induced an impairment when administered either before training, before test or before both training and test, suggesting that the impairment was not due to state dependency.

It should be mentioned that our criterion was that most control animals should avoid the dark box during retention. Thus, possible improvements in consolidation and retrieval could not be evaluated.

The amygdala has been shown to play a crucial role in acquisition and expression of fear memories measured with FPS (Davis *et al.*, 1993) and fear-potentiated freezing (Fendt and Fanselow, 1999). A study revealed that acquisition, but not consolidation and expression, of conditioned fear was impaired by intra-amygdala injections of MPEP (Fendt and Schmid, 2002). Moreover, infusion of MPEP into the lateral amygdala impaired fear conditioning to contextual and auditory stimuli (freezing reaction) when administered before acquisition, but not before test (Rodrigues *et al.*, 2002). In our study, the subtype-specific mGlu5 receptor antagonist MTEP significantly impaired fear conditioning in the FPS test at doses of 2.5 and 5 mg/kg tested 24 h later. Thus, since the substance is given before training during the acquisition phase, this would indicate an impairment of the association of light (CS) with footshock (US). Our results confirm previous studies showing involvement of mGlu5 receptors in the acquisition of conditioned fear. In a previous experiment, it has been demonstrated that MPEP (p.o.) impaired the acquisition of FPS when administered before training (Schulz *et al.*, 2001). Also, the authors presented data showing that MPEP given before the test blocked expression of FPS; however, this effect could well be due to anxiolytic activity rather than impairment of retrieval (Spooren and Gasparini, 2004). Schachtman *et al.* (2003) found that MPEP (i.p.) impaired conditioned taste aversion.

Maciejak *et al.* (2003) found that neither a mGlu5 receptor antagonist (MPEP) nor a mGlu5 receptor agonist (RS)-2-chloro-5-hydroxyphenylglycine (CHPG) injected directly into hippocampus affected consolidation of contextual fear conditioning. In contrast, a group I antagonist (AIDA) improved, while a group I agonist (DHPG) decreased, consolidation of fear conditioning. Systemic administration of AIDA impaired contextual fear conditioning given before the training session (Riedel *et al.*, 2002). In a previous study, the same authors showed that AIDA blocked context-, but not

cue-dependent fear conditioning (Nielsen *et al.*, 1997). Similar results were obtained from mGlu1 knockout mice, which were impaired in context-dependent, but not cue-dependent fear conditioning. In the present study, EMQMCM failed to impair fear potentiation of startle. These findings would support the idea that mGlu1 receptors are involved in learning to an unspecific context, but not to discrete cues. In line with this, EMQMCM impaired acquisition in the context-dependent PA.

Possible analgesic effects of the substances administered could influence our results in the acquisition experiments in FPS and PA. However, most studies examining the involvement of mGlu5 receptors in acute pain did not reveal any effect. For example, MPEP after systemic administration was shown to have no effect on responses to noxious stimulation (Walker *et al.*, 2001; Kozela *et al.* 2003). Regarding mGlu1 receptors, due to the sparse availability of highly selective antagonists, little is known about acute pain. AIDA was shown to have an effect in the hot-plate test when injected i.c.v. in mice (Moroni *et al.*, 1997). However, in the Hargreaves test of plantar pain (acute pain), we did not observe antinociceptive effects of either EMQMCM or MTEP at the doses used in the present study (Sevostianova, personal communication). With respect to FPS, it was previously shown that MPEP did not influence sensitization of the startle response induced by footshocks (Schulz *et al.*, 2001). These data suggest that memory impairments in PA and fear potentiation of startle are not due to possible analgesic or sensitization effects of MTEP.

Also, apparent mnemonic effects could be obscured by possible general inhibition by acute drug treatment. However, we found no differences from controls in leaving the start box under influence of the respective substances. In the acquisition experiments, acute treatment before training had no effect on leaving the start box, suggesting no effect on general inhibition, at least at the level of sensitivity of the method. There was also no difference found between controls and treatment groups in leaving the start box under the influence of the drugs when given before the retention test in the retrieval experiments. Additionally, motor-impairing effect of both agents have been studied in a rota-rod test (data not shown). Experiments revealed that the minimal statistically effective MTEP dose was 20 mg/kg (*ca.* 25% decrease in latency to fall off the rod) and for EMQMCM 10 mg/kg (*ca.* 30% decrease in the latency to fall off the rod). This indicates that, at a dose effective in either learning task (10 mg/kg or lower for MTEP and 5 mg/kg for EMCMCM), it is unlikely that motor disturbances could interfere with performance.

In conclusion, the present data suggest that mGlu5 receptors seem to play a role in both context- and cue-dependent acquisition, while mGlu1 receptors are not involved in acquisition involving discrete cues. Moreover, in contrast to mGlu5 receptors, mGlu1 receptors may contribute to the retrieval of aversive memory, but this finding requires further elucidation.

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ORIGINAL INVESTIGATION

A. Gravius · M. Pietraszek · W. J. Schmidt · W. Danysz

Functional interaction of NMDA and group I metabotropic glutamate receptors in negatively reinforced learning in rats

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Abstract *Rationale:* The role of glutamatergic system in learning and memory has been extensively studied, and especially *N*-methyl-D-aspartate (NMDA) receptors have been implicated in different learning and memory processes. Less is known, however, about group I metabotropic glutamate (mGlu) receptors in this field. Recent studies indicated that the coactivation of both NMDA and group I mGlu receptors is required for the induction of long-term potentiation (LTP) and learning. *Objective:* The purpose of the study is to evaluate if there is a functional interaction between NMDA and group I mGlu receptors in two different models of aversive learning. *Methods:* Effects of NMDA, mGlu1, and mGlu5 receptor antagonists on acquisition were tested after systemic coadministration of selected ineffective doses in passive avoidance (PA) and fear-potentiated startle (FPS). *Results:* Interaction in aversive learning was investigated using selective antagonists: (3-ethyl-2-methyl-quinolin-6-yl)-(4-methoxy-cyclohexyl)-methanone methanesulfonate (EMQMCM) for mGlu1, [(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP) for mGlu5, and (+)-5-methyl-10,11-dihydro-5H-dibenzocycloheptene-5,10-imine maleate [(+)MK-801] for NMDA receptors. In PA, the coapplication of MTEP at a dose of 5 mg/kg and (+)MK-801 at a dose of 0.1 mg/kg 30 min before training impaired the acquisition tested 24 h later. Similarly, EMQMCM (2.5 mg/kg) plus (+)MK-801 (0.1 mg/kg), given during the acquisition phase, blocked the acquisition of the PA response. In contrast, neither the combination of MTEP (1.25 mg/kg) nor EMQMCM (5 mg/kg) plus (+)MK-801 (0.05 mg/kg) was effective on the acquisition assessed in the FPS paradigm. *Conclusion:* The findings suggest differences in the interaction of the NMDA and mGlu group I receptor types in aversive instrumental conditioning vs conditioning to a discrete light cue.

Keywords mGlu1 receptor antagonist · mGlu5 receptor antagonist · NMDA receptor antagonist · Acquisition · Fear-potentiated startle · Passive avoidance

A. Gravius · M. Pietraszek · W. Danysz (✉)
Preclinical R&D, Merz Pharmaceuticals,
Eckenheimer Landstrasse 100,
60318 Frankfurt am Main, Germany
e-mail: wojciech.danysz@merz.de
Tel.: +49-69-1503564
Fax: +49-69-5962150

M. Pietraszek
Institute of Pharmacology,
Polish Academy of Sciences,
Smetna 12,
31-343 Cracow, Poland

W. J. Schmidt
Department of Neuropharmacology,
Zoological Institute,
University of Tübingen,
Tübingen, Germany

Introduction

Glutamate plays a major role as a neurotransmitter in the central nervous system. While rapid transmission is mediated via ionotropic glutamate receptors such as alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate, and *N*-methyl-D-aspartate (NMDA) receptors, transmission of the G-protein-coupled metabotropic glutamate (mGlu) receptors is more modulatory in nature (Parsons et al. 1998; Pin and Acher 2002). Eight subtypes of mGlu receptors have been identified and divided in three groups on the basis of pharmacological and signal transduction mechanisms and homologies in amino acids sequences. While group I mGlu receptors (mGlu1 and mGlu5) are linked to phosphoinositol hydrolysis, group II (mglu2/3) and group III (mglu4/6/7/8) are negatively coupled to adenylate cyclase (Conn and Pin 1997).

Interestingly, the close interaction between NMDA and group I mGlu receptors has been demonstrated. For example, the activation of mGlu1 potentiated NMDA receptor currents in *Xenopus oocytes* involving an increase in intracellular calcium and activation of protein kinase C (Lan et al. 2001). Furthermore, in mice cortical slices, (*S*)-3,5-dihydroxyphenylglycine, a selective group I mGlu agonist, enhanced NMDA-induced depolarization, while

the selective mGlu5 antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) reduced this enhancement (Attucci et al. 2001). Interestingly, it has been found that a coactivation of hippocampal mGlu and NMDA receptors is required for the induction of long-term potentiation (LTP) (Musgrave et al. 1993; Fujii et al. 2004). In mice lacking mGlu5, LTP was significantly reduced in NMDA receptor-dependent pathways like the CA1 region and dentate gyrus of the hippocampus; however, LTP was not altered in the CA3 region, an NMDA receptor-independent pathway (Lu et al. 1997).

In contrast to in vitro studies, in vivo data examining coupling of NMDA and group I mGlu receptors in learning are very limited. Recently, it has been reported that coapplication of inactive doses of MPEP and (+)-5-methyl-10,11-dihydro-5H-dibenzocyclohepten-5,10-imine maleate [(+)MK-801] impaired working memory and instrumental learning (Homayoun et al. 2004). In addition, combination treatment using inactive doses of phencyclidine (PCP) and MPEP impaired spatial learning in a radial maze task (Campbell et al. 2004). However, so far, no data are available on aversive learning, investigating coupling between group I mGlu and NMDA receptors.

Thus, the present study was designed to investigate the interaction between group I mGlu receptors and NMDA receptors on the acquisition of aversive learning in passive avoidance (PA) and fear-potentiated startle (FPS) paradigms using selective antagonists. 3-Ethyl-2-methyl-quinolin-6-yl)-(4-methoxy-cyclohexyl)-methanone methanesulfonate (EMQMCM, JNJ16567083), [(2-methyl-1, 3-thiazol-4-yl)ethynyl]pyridine (MTEP), and (+)MK-801 were used to block mGlu1, mGlu5, and NMDA receptors, respectively. The doses for EMQMCM and MTEP were selected from our previous study (Gravius et al. 2005a).

In PA, subjects learn to connect a preferred dark environment with an aversive experience (electric foot shock). Thus, the latency to enter the dark chamber can be taken as a direct measurement of memory. FPS is a model of classical conditioning, in which a light cue as a conditioned stimulus (CS) is connected with an unconditioned stimulus (electric foot shock). By using startle-eliciting noise bursts in the presence and absence of the CS, memory can be directly assessed by the difference of the startle behavior with and without the CS. While PA learning highly depends on contextual information, FPS does not. Thus, both paradigms differ in their neuronal substrates. Studies revealed the involvement of both the hippocampus and amygdala in contextual conditioning (Kim and Fanselow 1992), while learning to a discrete cue appears to be hippocampus independent (Phillips and LeDoux 1992).

The working hypothesis of the present study was that blockade of either mGlu receptor together with the NMDA receptor should result in enhanced learning impairment given the role of each receptor subtype in neuronal plasticity in the brain. It was however not clear whether this can be generalized to diverse learning paradigms (aversive vs appetitive) and forms of learning (Pavlovian vs instrumental). Results of this study have been presented previously in the abstract form (Gravius et al. 2005b).

Materials and methods

Subjects

Experimentally naive adult male Sprague–Dawley rats (240–280 g; Janvier, France) were housed in groups of four per cage. Colony room temperature and humidity were maintained, respectively, at 20±1°C and 60±3%. Food and water were available ad libitum, and the animals were kept under an alternating 12 h/12 h day–night cycle (lights on at 07.00) for at least 6 days before the experiments started. All experiments were conducted during the light period of the day–night cycle. Each animal was used only once. The study was approved by the Ethical Committee, Regierungspraesidium Darmstadt, Hessen and performed in accordance with the recommendations and policies of the US National Institutes of Health Guidelines for the Use of Animals.

Passive avoidance

Apparatus

The two-choice dark avoidance apparatus consisted of three identical size compartments (w×d×h 32×25.5×25.5 cm^3) that were connected, so that from the start compartment, vertically sliding doors (h×w 6×9 cm^2) controlled the connection to both choice compartments. Each compartment was equipped with a source of light and a grid floor connected to an electric shock generator and scrambler (ENV 414, Med Associates, St. Albans, VT, USA). The animal detection was controlled by six pairs of photobeams, located 3.5 cm above the floor and 4 cm from the doors. The system was remotely controlled through an interface (MED Associates) connected to an IBM-PC operating MED Associates software version IV.

Procedure

The experiments lasted 4 days. On the first day, each animal received 5 min of handling. On the second day, rats were handled again for 5 min and placed into the start box compartment for 5 min. During the habituation period, only the lighted compartment was available, the right or left one (balanced between the rats in each group). For training on day 3, rats were placed into the start box with free access to both choice compartments. The time of entering the light and/or dark compartment was recorded. When the rat entered the dark compartment, the door was closed, and electric foot shocks (1 mA, 1 s) were applied three times with 2-s intervals. Five seconds later, the rat was returned to the home cage. The reactions to the foot shocks

(vocalizations, jumping) were considered as confirmation of their efficiency. If the rat did not enter the dark compartment within the first 180 s, it was excluded from the experiment. On the fourth retention day, the rat was placed in the start box with free access to both choice compartments. The time of entering the light and/or dark compartment was recorded. During retention, no shocks were delivered. A cutoff time of 300 s was used.

Fear-potentiated startle

Apparatus

For training, the subjects were placed in acrylic animal holders [19-cm length, 7.6-cm internal diameter (ID) with a grid floor consisting nine stainless steel bars (3-mm ID)]. The holders were fixed onto a startle platform (Med Associates, Model PHM-250B). The grid floor was connected to an electric shock generator and scrambler, in which a 0.6 mA foot shock could be delivered. The complete equipment was placed in a sound-attenuating chamber. Startle-eliciting noise bursts (50 ms) were generated by a noise generator (Med Associates, Model PHM-255A). The speaker was placed 7 cm from the animal holder in the back of the chamber. A fan attached on the sidewall of the chamber produced a background noise of 62 dB, and a noise generator produced additional noise; hence, the overall background noise was 64 dB. A 3.7-s visual cue was produced by an 8-W fluorescent stimulator (Med Associates, Model PHM-258L), consisting of an 8-W bulb, placed in front of the chamber. The output of the accelerometer was connected through an interface to an IBM-PC running Startle Reflex software (Med Associates, version 5.1).

Procedure

Pretest On the first day, to obtain groups with similar startle responses, a pretest was performed. The subjects were placed in the acrylic holders, and after a 5-min acclimation period, six initial startle stimuli (white noise, 100 dB, 50 ms duration) were presented to induce a stable startle baseline. Then, each subject received 15 startle stimuli of 100 dB (7–23 s interstimulus interval).

Conditioning For training 24 h later, the rats were placed back in the animal holders containing the grid floors. After a 5-min acclimation period, 15 pairings of light with a 0.6-mA foot shock were presented. The unconditioned stimulus was presented during the last 500 ms of the 3.7 s of light, hence both stimuli terminated together. The mean intertrial interval was 2 min with a range of 90–150 s.

Test Twenty-four hours after training, the rats again were placed in the animal holders. After a 5-min acclimation period, the animals received six initial noise bursts of 100 dB to establish a stable startle baseline before recording. Then, 24 startle stimuli (50 ms, 100 dB) were presented, one half of each type 3,200 ms after the onset of light (light–noise trials), and one half in dark (noise-alone trials). Intertrial interval was 15–45 s. Differences of light–noise and noise-alone trials were calculated.

Shock titration

To measure the shock responsivity, the same apparatus as described for FPS was used. The subjects were placed in the animal holders containing the grid floors. After a 5-min acclimation period, a total of ten electric foot shocks (1-s duration) were presented, every two at 0, 0.1, 0.2, 0.5, and 1 mA current intensity. The interval of the shock administration per intensity was 10 s, the interval to the next higher intensity was 60 s. The reactions (jumping) to the electric foot shocks were measured for the duration of the shock administration (1 s). The output of the accelerometer was connected through an interface to an IBM-PC running Advanced Startle software (Med Associates, version 1.03). The mean of each two shock reactions per current intensity was taken for calculation.

Statistical analysis

PA data, i.e., latencies to leave the start box (time to enter dark or lighted box, whichever occurred first) and latencies to enter the dark box were analyzed by nonparametric analysis of variance (ANOVA) (Kruskal–Wallis test), followed, if significant, by Mann–Whitney tests. The results are expressed as medians with interquartile ranges. For the shock titration experiment, the response was measured as the maximum peak value during the duration of the shock presentation. The values were analyzed by two-way ANOVA. In case of FPS, the test mean startle amplitude for each trial type presentation was calculated as the mean of peaks measured as the maximum value recorded in 100 ms beginning with the onset of the startle stimulus. FPS was calculated as the difference of mean startle amplitude in light (light–noise trials) minus mean startle amplitude in dark (noise-alone trials). The differences were analyzed by *t* test or one-way ANOVA followed, if significant, by the Duncan's test.

Drugs

EMQMCM (JNJ16567083) and MTEP, synthesized by Merz Pharmaceuticals (Frankfurt, Germany), were dissolved in 10% of Tween 80/water. (+)MK-801 (Tocris, Avonmouth, UK) was dissolved in physiological saline. All substances were administered 30 min before the training. Control animals received respective vehicles, and all compounds were injected intraperitonealy (i.p.) in a volume of 2 ml/kg.

Results

Passive avoidance test

The latencies to leave the start box during training are used as a measurement of general inhibition under short-term influence of the drugs. Latencies to enter the dark box during retention test are taken as direct measurement of memory impairment. (+)MK-801 at the dose of 0.2 mg/kg (but not 0.1 mg/kg) given 30 min before training significantly impaired the PA response tested 24 h later (Fig. 1). At either dose of 0.1 or 0.2 mg/kg, (+)MK-801 had no effect on time to leave the start box during training (Fig. 1).

To evaluate the effect of the combination of (+)MK-801 and EMQMCM on acquisition, selected, inactive doses of both substances were administered 30 min before the training trial. EMQMCM was given at 2.5 mg/kg, a dose shown in a recent study to be ineffective in this task when administered alone (Gravius et al. 2005a), while (+)MK-801 was given at 0.1 mg/kg (present study, Fig. 1). In the combinations experiment, as expected, neither (+)MK-801 nor EMQMCM impaired the PA response; however, the combination of both significantly reduced latency to enter the dark box tested 24 h after training (Fig. 2). Neither of the treatments changed the latencies to leave start box during the training trials (Fig. 2).

The same dose of (+)MK-801 like in the former experiment was used to examine the effect of coadministration with MTEP. A dose of 5 mg/kg of MTEP was selected based on our previous study (Gravius et al. 2005a). None of the treatments altered time to leave the start box during acquisition (Fig. 3). However, the coadministration of MTEP and (+)MK-801 during training significantly impaired the PA response tested 24 h later, while neither MTEP nor (+)MK-801 given alone was effective (Fig. 3).

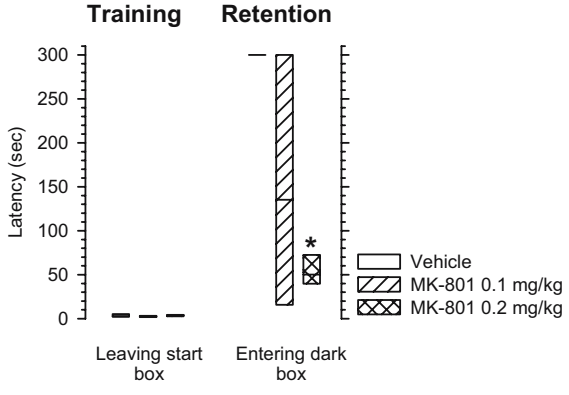

Fig. 1 Effect of (+)MK-801 on the acquisition of two-choice PA. (+)MK-801 was administered 30 min before training (acquisition). Retention of the PA response was tested 24 h after training. The graphs show the latencies of leaving the start box and entering the dark box during training and test, respectively. Results are expressed as medians and interquartile ranges. *p<0.05 vs control computed by ANOVA on ranks followed by Mann–Whitney tests (n=8 per group)

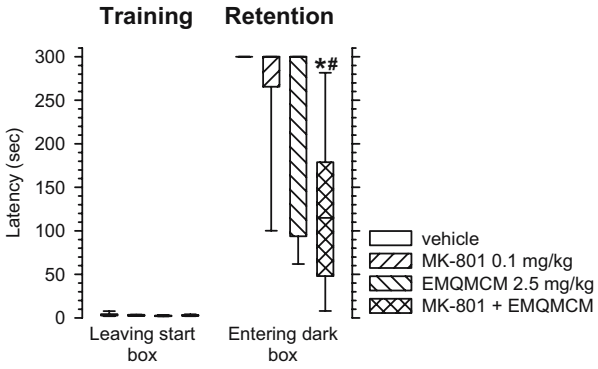

Fig. 2 Effect of EMQMCM, (+)MK-801, and combination of both on the acquisition of two-choice PA. Substances were administered 30 min before training. The combination of EMQMCM and (+)MK-801 significantly impaired retention of PA tested 24 h later (for details, see Fig. 1). *p<0.05 vs control computed by ANOVA on ranks followed by Mann–Whitney tests. $^{\#}p$<0.05 vs (+)MK-801 0.1 mg/kg (n=9 per group)

Shock titration

To test if MTEP, EMQMCM, or (+)MK-801 and the respective combinations change the responsivity of the animals to the foot shocks, we performed a shock titration. Therefore, the same doses inducing a retention impairment in PA were used. The combination of EMQMCM (2.5 mg/kg) and (+)MK-801 (0.1 mg/kg) had no effect on the responsivity to electric foot shocks (Fig. 4a). Likewise, coadministration of MTEP (5 mg/kg) and (+)MK-801 (0.1 mg/kg) also was ineffective on the shock responsivity of the animals (Fig. 4b).

Fear-potentiated startle

To evaluate if the light cue changes the startle response in untrained rats, one group of rats underwent the FPS procedure described above, however without receiving electric shocks. In this experiment, startle amplitudes were

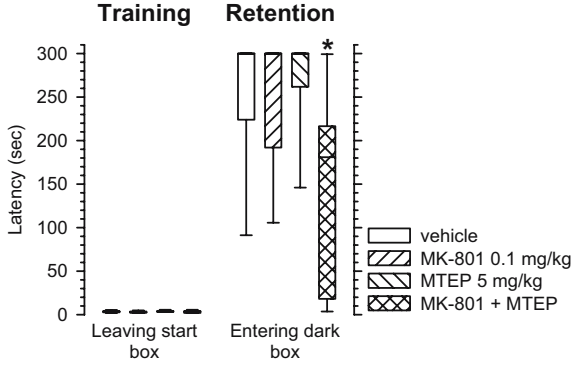

Fig. 3 Effect of MTEP, (+)MK-801, and combination on the acquisition of PA. Substances were administered 30 min before training. The combination of MTEP and (+)MK-801 significantly impaired retention of PA tested 24 h later (for details, see Fig. 1). *p<0.05 vs all other groups computed by ANOVA on ranks followed by Mann–Whitney tests (n=8–9)

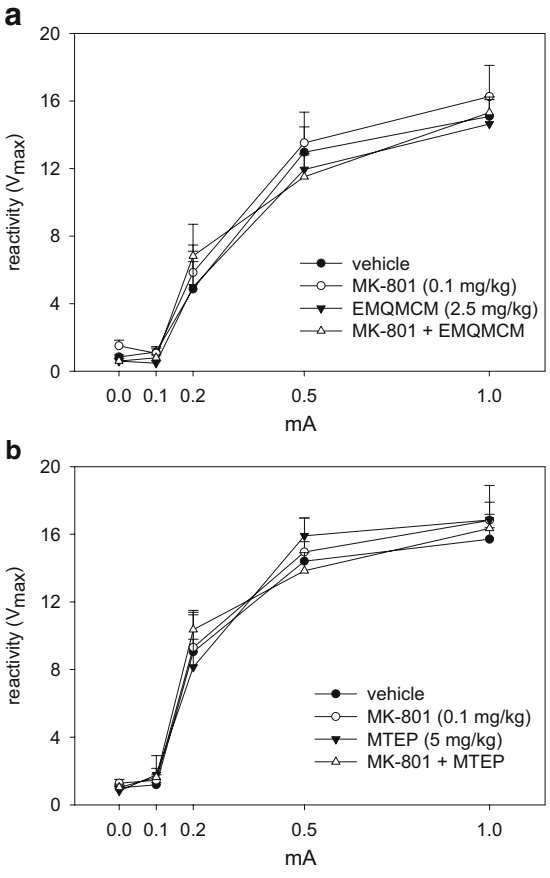

Fig. 4 a Effect of (+)MK-801, EMQMCM, and combination of both on responsivity to electric foot shocks. The graph shows the responsivity (jumping) to the different current intensities. Substances were administered 30 min before the test. Results are expressed as means ± SEM. Neither (+)MK-801, EMQMCM, nor the combination of both changed the responsivity of the subjects (n=8 per group, NS). **b** Effect of MTEP, (+)MK-801, and combination of both on responsivity to electric foot shocks. The graph shows the responsivity (jumping) to the different current intensities. Substances were administered 30 min before the test. Results are expressed as means ± SEM. Neither (+)MK-801, MTEP, nor the combination of both changed the responsivity of the subjects (n=8 per group, NS)

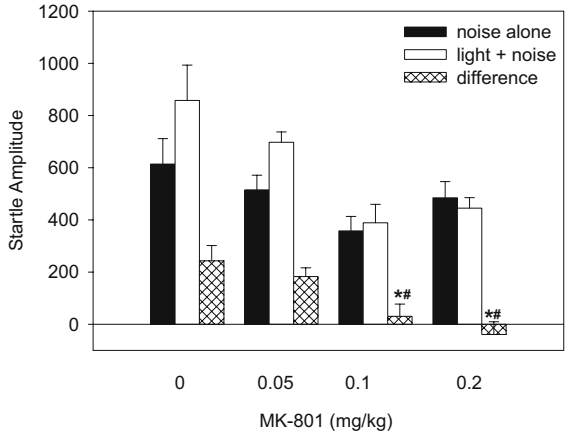

Fig. 5 Effect of (+)MK-801 on fear potentiation of startle. The diagram shows the mean startle amplitudes in the absence (*black bars*) and presence of the CS (*white bars*). *Striped bars* represent the differences between mean startle amplitudes in light and in dark. (+) MK-801, given intraperitoneally 30 min before the training, produced dose-dependent reduction of startle amplitudes in light ($F_{3,36}$=8.246, p<0.001). No difference of startle amplitudes was found in noise-alone trials ($F_{3,36}$=1.963, p=0.137). *p<0.05 vs control computed by one way ANOVA followed by Duncan's test

not changed in the presence of the light stimulus (data not shown).

The effect of (+)MK-801 given 30 min before conditioning was examined on the acquisition of fear potentiation of startle. (+)MK-801 produced a dose-dependent acquisition impairment, as indicated by reduction of startle amplitudes in light–noise trials compared with noise-alone trials during test. Both doses 0.1 and 0.2 mg/kg significantly impaired acquisition tested 24 h after conditioning. No difference was observed on mean startle amplitudes in noise-alone trials (Fig. 5).

In this experiment, selected, inactive doses of EMQMCM and (+)MK-801 were tested. EMQMCM was given at 5 mg/kg, a dose shown to be inactive in the previous study (Gravius et al. 2005a). (+)MK-801 was administered at 0.05 mg/kg based on dose response performed in the present study. Neither the single treatments nor the coadministration of both substances was effective given before acquisition (data not shown).

MTEP at dose 1.25 mg/kg, which was found to have no effect on the acquisition of fear potentiation of startle (Gravius et al. 2005a), was coadministered with (+)MK-801 at dose 0.05 mg/kg. This combined treatment did not impair fear conditioning when given 30 min before training during the acquisition phase (data not shown).

Discussion

The present experiments examined the interaction of NMDA receptors with mGlu1 and mGlu5 in the acquisition of aversive learning assessed in PA and FPS using selective antagonists. In our previous studies, we observed that MTEP impaired acquisition in both PA and FPS, while EMQMCM impaired learning only in PA (Gravius et al. 2005a). Thus, selected inactive doses of MTEP and EMQMCM were chosen from these experiments. In the present study, (+)MK-801 produced a dose-dependent acquisition impairment in both paradigms. In PA, both administration of MTEP and EMQMCM in combination with (+)MK-801 impaired retention when given before training, whereas each agent given alone was ineffective. Interestingly, in FPS, neither the combination of MTEP nor EMQMCM plus (+)MK-801 produced amnesia when administered before training.

Selection of appropriate antagonists for the respective receptor types providing satisfactory brain penetration as well as high selectivity is of basic importance for the interpretation of the present work. For MTEP, the ED_{50} value for 50% of receptor occupancy in vivo is 1.2 mg/kg 1 h after i.p. application (Busse et al. 2004). The dose of EMQMCM producing 50% receptor occupancy is not

known; however, the IC_{50} value for EMQMCM at the mGlu1 receptor is about 2–3 nM (Parsons, personal communication), and a derivative was shown to have good penetration to the brain (Steckler et al. 2005a). Furthermore, clear effects of both antagonists have been demonstrated in several animal models, e.g., anxiety and learning at the doses used in the present study (Pietraszek et al. 2005; Gravius et al. 2005a; Steckler et al. 2005b).

Involvement of NMDA receptors in learning was initially suggested on the basis of LTP experiments. LTP is a form of synaptic plasticity, widely accepted to be the physiological substrate for many forms of learning and memory (Aiba et al. 1994; Lu et al. 1997; Zajaczkowski et al. 1997; Balschun and Wetzel 2002). A number of publications confirmed that NMDA receptors play a prominent role in at least some forms of LTP and learning, as the blockade of NMDA receptor prevents the induction of hippocampal LTP and impairs learning (Morris et al. 1986; for review, see Danysz et al. 1995). In line with this, in the present study, the NMDA receptor antagonist (+) MK-801 impaired learning in both PA and FPS given 30 min before training. More recently, also mGlu receptors have been implicated in the induction of LTP (Bashir et al. 1993; Manahan-Vaughan 1997). Especially group I mGlu receptors seem to be involved, because after blockade of mGlu5 by MPEP, impairment of hippocampal LTP and spatial learning were observed (Balschun and Wetzel 2002; Naie and Manahan-Vaughan 2004). Likewise, mGlu1 mutant mice exhibited reduced LTP and impaired context-specific learning (Aiba et al. 1994). These data suggest that the combination of both group I mGlu receptor antagonists and NMDA antagonists should result in enhanced learning impairment, and such assumption is supported by in vitro studies.

Coactivation of hippocampal mGlu and NMDA receptors seems to be required for the induction of LTP (Musgrave et al. 1993; Fujii et al. 2004). Furthermore, in mice lacking mGlu5, LTP was significantly reduced in NMDA receptor-dependent pathways like the CA1 region and dentate gyrus of the hippocampus (Lu et al. 1997). In line with these findings, the present study revealed a clear interaction of mGlu5 and NMDA receptors in PA, a hippocampus-dependent learning task. These results extend previous in vivo findings showing that combination treatment using inactive doses of NMDA receptor antagonists [PCP or (+)MK-801] and mGlu5 receptor antagonist MPEP impaired spatial and instrumental learning and induced working memory deficit (Campbell et al. 2004; Homayoun et al. 2004). To the best of our knowledge, for the first time, the present work also revealed acquisition impairment in PA after combined treatment with mGlu1 and NMDA receptor antagonists.

Apart from the hippocampus, amygdala glutamate receptors also are involved in context-dependent learning. In PA, the mGlu antagonist methylcarboxyphenylglycine, injected into the amygdala before training, impaired the PA response (Barros et al. 2000). Furthermore, pretraining intra-amygdala injections of the NMDA receptor antagonists 3((+/−)-2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP), DL-2-amino-5-phosphonopentanoic acid (D,L-AP5), and D-AP5 blocked learning of the PA response when tested 48 h later (Kim and McGaugh 1992).

Amygdala glutamate receptors are also involved in cue-dependent aversive learning (Miserendino et al. 1990; Campeau et al. 1992; Fendt and Schmid 2002). However, in spite of the fact that NMDA and group I mGlu receptors are present in the amygdala and show overlapping distribution (for review, see Spooren et al. 2003), neither combined treatment of mGlu1 and NMDA nor mGlu5 and NMDA receptor antagonists produced acquisition impairment in FPS. Regarding NMDA and mGlu5 receptors, this is surprising, since direct injection of AP5 into the amygdala impaired acquisition of fear conditioning (Miserendino et al. 1990; Campeau et al. 1992). Likewise, MPEP was shown to impair the acquisition of fear potentiation of startle after intra-amygdala infusions (Fendt and Schmid 2002). Furthermore, it was shown previously that systemic administration of MTEP dose dependently impaired acquisition when given before training of FPS (Gravius et al. 2005a). In line with this, MPEP (p.o.) also impaired acquisition of FPS when administered before training (Schulz et al. 2001). Consistent with earlier findings, our present work also showed that the NMDA receptor antagonist (+)MK-801 produced a clear dose-dependent acquisition impairment in this task. Taken together, at least a reciprocal potentiation of acquisition impairment was expected after coadministration of selected ineffective doses of (+)MK-801 and MTEP. Instead, only a trend for an attenuation of acquisition was observed without reaching statistical significance. This finding might suggest that mGlu5 and NMDA receptors contribute to a different degree in PA and FPS learning, which is underlined by the different lowest active doses of (+)MK-801 and MTEP in both tasks. This could be explained by the different task requirements and thus by different involvement of the underlying neuronal substrates. While contextual learning depends both on the hippocampus and amygdala (Kim and Fanselow 1992), cue-dependent conditioning has been shown to be a hippocampus-independent process (Phillips and LeDoux 1992). With respect to the combination treatment, it is not known if there is a potential hypoadditive, additive, or synergistic effect of NMDA and mGlu5 receptors in learning, and whether this effect varies in different brain structures, which could explain the different results. Apparently, the range in which an effect of (+)MK-801 and MTEP can be seen is lower in FPS than in PA. Thus, in FPS, the use of doses twice as high as the inactive one (i.e., 0.1 mg/kg) results in nearly complete inhibition of startle potentiation, precluding demonstration of any enhancement. Therefore, a possible interaction at higher doses was not assessed. It is possible, that a combination of precisely selected doses based on extensive dose relationship study would allow showing enhancement of the impairing effect. However, using a simple combination of half of the minimal active doses, which usually is most likely to demonstrate enhancement of the combination effect, failed in the FPS test but was successful in PA test.

Unlike mGlu5, mGlu1 has not been implicated in the acquisition of FPS. In our previous study, EMQMCM failed to affect acquisition of FPS at the highest tested active dose of 5 mg/kg in other behavioral tests (Gravius et al. 2005a). Similarly, 1-aminoindan-1,5-dicarboxylic acid (AIDA), a group I mGlu antagonist with higher selectivity at mGlu1, blocked context but not cue-dependent acquisition of fear (Nielsen et al. 1997), and mGlu1 knockout mice were found to be impaired in context-dependent but not cue-dependent fear conditioning (Aiba et al. 1994). Also interesting in this context is the finding that induction of late-phase LTP was prevented after selective blockade of NMDA and mGlu5 receptors, but not after blockade of mGlu1 receptors (Lee et al. 2002). A latter study was performed at thalamic input synapses to the lateral amygdala, which are considered to be important for cue-dependent conditioning (Lee et al. 2002). However, in our study, (+)MK-801 clearly blocked fear conditioning, and the observation that EMQMCM failed to potentiate (+)MK-801-induced acquisition impairment may suggest a lack of interaction of both receptor types in cue-dependent conditioning.

To clarify if the observed retention impairments in PA did not result from the analgetic effect of the given substances, we performed a shock titration using the same doses for MTEP, EMQMCM, and (+)MK-801 which induced retention impairment in PA. Intensities of 0.1 and 0.2 mA were selected to detect drug effects at the responsivity threshold intensity. To test the responsivity at intermediate, 0.5 mA was selected, and 1 mA to evaluate the effect at the same intensity used in the PA experiment. Taken together, there was no difference in responsivity at all tested current intensities, suggesting that neither MTEP, EMQMCM, and (+)MK-801 alone nor the respective combinations suppress shock responsivity, at least at the tested doses. In line with these findings, the majority of studies examining involvement of mGlu5 on acute pain could not reveal any effect. For example, it was found that MPEP after systemic administration had no effect on responses to noxious stimulation (Walker et al. 2001; Kozela et al. 2003). In the case of mGlu1, because of scarce availability of selective antagonists, little data are available on acute pain. For example, AIDA was shown to have an analgetic effect in the hot plate test in mice after intracerebroventricular application (Moroni et al. 1997). However, we did not observe analgetic effects of EMQMCM in the Hargreaves test (acute pain), as well as MTEP at doses used in the present study after systemic application (Sevostianova and Danysz 2005). Taken together, the role of group I mGlu in acute pain is controversial; however, the majority of the studies did not reveal an involvement of mGlu1 and mGlu5 in the transmission of acute noxious stimuli (for recent review, see Lesage 2004). It was previously shown that MPEP did not influence sensitization of startle response induced by foot shocks (Schulz et al. 2001). Similar findings were observed after administration of NMDA receptor antagonists, showing that there is no change in reaction to foot shock (Danysz et al. 1988; DeNoble et al. 1990) and that they do not induce antinociceptive effects in acute pain tests (Nishiyama 2000; Gould et al. 2002). However, data on acute pain after coadministration of group I metabotropic and NMDA receptor antagonists have not been available. Therefore, in the present study, an additional test was performed to address this aspect and showed that the combination of mGlu and NMDA antagonists does not result into change in the reaction to foot shock as demonstrated using the shock titration paradigm. Apart from the above, it seems highly unlikely that there is a task-specific analgetic effect of the given substances, taken into consideration that only PA learning was impaired after the coapplication of the substances. Another possible interference with the PA performance could result from general activity inhibition by drug treatment which could obscure apparent mnemonic effects. However, short-term treatment before training did not influence the latencies of leaving the start box in PA, suggesting no effect on general inhibition, at least at the level of sensitivity of the method. The present results support recent evidence that activation of mGlu1 is not crucial for the acquisition of cue-dependent aversive conditioning. Regarding mGlu5, in spite of proved involvement in this field, a possible interaction with NMDA receptors could not be detected in cue-dependent learning; thus, this needs further investigation.

In conclusion, mGlu1 and mGlu5 receptors seem to work in concert with NMDA receptors in the acquisition of aversive instrumental learning. In the case of conditioning to a discrete light cue, no indication of such interaction was observed.

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The role of group I metabotropic glutamate receptors in acquisition and expression of contextual and auditory fear conditioning in rats – a comparison

A. Gravius [a], C. Barberi [a], D. Schäfer [a], W.J. Schmidt [b], W. Danysz [a,*]

[a] *Preclinical R & D, Merz Pharmaceuticals GmbH, Eckenheimer Landstrasse 100, 60318 Frankfurt am Main, Germany*
[b] *University of Tübingen, Department of Neuropharmacology, Zoological Institute, Tübingen, Germany*

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Abstract

Glutamatergic neurotransmission in the CNS plays a predominant role in learning and memory. While NMDA receptors have been extensively studied, less is known about the involvement of group I metabotropic glutamate receptors in this area. The purpose of the present study was to evaluate the contribution of mGluR1 and mGluR5 to both acquisition and expression of behaviours in contextual and auditory fear conditioning models. The effects of both receptor types were tested using selective antagonists: (3-ethyl-2-methyl-quinolin-6-yl)-(4-methoxy-cyclohexyl)-methanone methanesulfonate (EMQMCM) for mGluR1, and [(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP) for mGluR5. Their effects on acquisition were compared to those of the NMDA receptor antagonist (+)MK-801, and the unselective muscarinic antagonist scopolamine, while diazepam and citalopram served as reference compounds in the expression experiments. EMQMCM (1.25 to 5 mg/kg) impaired acquisition of contextual fear conditioning (CFC), but not auditory fear conditioning (AFC). Similarly, administration of MTEP during the acquisition phase impaired learning in CFC at doses of 2.5 to 10 mg/kg, but was ineffective in AFC. When given before the retention test, both EMQMCM (1 and 3 mg/kg) and MTEP (3 mg/kg) impaired expression of CFC. In contrast, MTEP (2.5 and 5 mg/kg) blocked the expression of AFC, while EMQMCM was ineffective. In conclusion, group I mGlu receptors are shown to be involved in the acquisition of hippocampus-dependent CFC, but not hippocampus-independent AFC. Unlike mGluR5, mGluR1 does not seem be involved in expression of AFC.

Keywords: mGluR1; mGluR5; NMDA receptor; Contextual fear conditioning; Auditory fear conditioning; Acquisition; Expression

1. Introduction

Glutamatergic neurotransmission in the brain is mediated via two types of receptors, ionotropic glutamate receptors and G-protein coupled metabotropic glutamate (mGlu) receptors. Ionotropic glutamate receptors are classified as *N*-methyl-D-aspartate (NMDA), kainate and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. Based on transmission profiles and sequence homologies, mGluRs form a family of eight subtypes which are divided into three different groups. Group I consists of mGluR1 and mGluR5, group II of mGluR2 and mGluR3, while mGluR4, mGluR6, mGluR7 and mGluR8 form group III (Conn and Pin, 1997).

Group I mGluRs have been shown to be involved in long-term potentiation (LTP) (Bashir et al., 1993) and various forms of learning and memory (Balschun and Wetzel, 2002; Petersen et al., 2002; Schulz et al., 2001; Steckler et al., 2005b). With regards to aversive learning, most studies have primarily focused on the role of mGluR5. For example, 2-methyl-6-(phenylethynyl)-pyridine (MPEP), a selective mGluR5 antagonist, was shown to inhibit fear potentiated startle (Schulz et al., 2001) and to attenuate conditioned taste aversion (Schachtman et al., 2003; for review see Simonyi et al., 2005). However, less is known about the role of mGluR1 in

* Corresponding author. Tel.: +49 69 150 3564; fax: +49 69 596 2150.
E-mail address: wojciech.danysz@merz.de (W. Danysz).

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negatively reinforced learning (Maciejak et al., 2003). Mice lacking mGluR1 show impairments in learning and LTP (Aiba et al., 1994). Furthermore, acquisition of passive avoidance (PA) is inhibited by (3-ethyl-2-methyl-quinolin-6-yl)-(4-methoxy-cyclohexyl)-methanone methanesulfonate (EMQ MCM, JNJ16567083), a selective antagonist of mGluR1 (Gravius et al., 2005). This effect might be well explained by coupling between mGluR1 and NMDA receptors, since the latter receptor subtype is widely accepted to play a role in synaptic plasticity (Collingridge, 1987; Danysz et al., 1995) However, the role of mGluR1 and mGluR5 receptors in context-induced vs. cue-induced learning has not yet been studied.

In contextual fear conditioning (CFC), the subjects learn to connect a neutral environment (the training chamber) with an aversive experience (an electric foot shock). Thus, during re-exposure to the training chamber, the animals express a freezing behaviour which is taken as a direct measure of fear. In auditory fear conditioning (AFC), which is a form of Pavlovian conditioning, a neutral tone (the conditioned stimulus, CS) is paired with an electric foot shock (the unconditioned stimulus, US). During the test, the rats are placed into a different context from that of training and are exposed to the CS alone. Freezing behaviour during CS presentation is taken as measure of fear. CFC and AFC differ in their neuronal substrates. Previous investigations revealed the involvement of both the amygdala and hippocampus in contextual conditioning (Kim and Fanselow, 1992), while cue-dependent learning was found to be independent of the hippocampus (Phillips and LeDoux, 1992).

The present study was therefore designed to examine the involvement of the group I receptor subtypes mGluR1 and mGluR5 in the acquisition and expression of both CFC and AFC, using the selective mGluR1 antagonist EMQMCM and the recently described selective mGluR5 antagonist [(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine) (MTEP) (Busse et al., 2004).

Given the accepted role of both NMDA receptors and the cholinergic system in most forms of learning, the effects of these mGluR antagonists upon acquisition were compared to those of (+)MK-801, an uncompetitive NMDA receptor antagonist, and scopolamine, a non-selective cholinergic antagonist. The effects on expression of CFC and AFC were compared to the selective serotonin reuptake inhibitor (SSRI) citalopram and the GABAergic agonist diazepam.

The present study was also designed to further evaluate and compare the effects of MTEP and EMQMCM upon the expression of conditioned fear with their effects on learning.

The results of this study have previously been partially presented in abstract form (Gravius et al., 2006b,c).

2. Materials and methods

2.1. Subjects

Experimentally naive adult male Sprague–Dawley rats (200–220 g at arrival; Janvier, Le Genest Saint Isle, France) were housed in groups of four per cage. Colony room temperature and humidity were maintained respectively at 20 ± 1 °C and 60 ± 3%. Food and water was available ad libitum and the animals were kept under an alternating 12 h/12 h day-night cycle (lights on at 07:00 h) for at least 6 days before the experiments started. All experiments were conducted during the light period of the day-night cycle. Each animal was used only once. The number of animals used per dose ranged from 7 to 16. The study was approved by the Ethical Committee, Regierungspraesidium Darmstadt, Hessen and performed in accordance with the recommendations and policies of the U.S. National Institutes of Health Guidelines for the Use of Animals.

2.2. Auditory fear conditioning

2.2.1. Apparatus

For training, subjects were placed in acrylic animal holders (19 cm long, 8.9 cm ID) with a grid floor consisting of 9 stainless steel bars (3 mm diameter). Holders were fixed onto a startle platform (Med Associates, St. Albans, VT, USA, Model PHM-250B). The grid floor was connected to an electric shock generator and scrambler, by which a 1 s electric foot shock of magnitude 1 mA could be delivered. The complete equipment was placed into a sound-attenuating chamber. A tone (80 dB SPL, 5 KHz, 30 s) was generated by a sound generator (Med Associates, Model PHM-255A). The speaker was placed 7 cm from the animal holder in the back of the chamber. A fan attached to the side wall of the chamber produced a background noise of 62 dB SPL and a noise generator produced additional noise, so that the overall background noise was 65 dB SPL. An 8 W fluorescent stimulator (Med Associates, Model PHM-258L) consisting of an 8 W bulb, placed in front of the chamber, provided light throughout the training session. The reactions to the electric foot shocks (jumping movements) were measured for the duration of the shock administration (1 s) and were taken as confirmation of their efficacy. The output of the accelerometer was connected through an interface to an IBM PC running Advanced Startle software (Med Associates, version 1.03).

Testing took place in a different context from that used for training. Here, the animals were placed in a test cage (Coulbourn Instruments, Allentown, PA, USA, Model H10-11R-TC, 30.5 × 25.4 × 30.5 cm^3, w × d × h) with a grey, plain plastic floor. The complete equipment was placed into a sound-attenuating isolation cubicle (Coulbourn Instruments, Model H10-24T, 63.5 × 50.8 × 66 cm^3, w × d × h). A 30 s tone (80 dB SPL, 5 KHz) was produced by a soundcard of an IBM PC, connected to a speaker (Coulbourn Instruments, Model H12-01R) which was attached on the sidewall of the test cage. Background noise of 63 dB SPL was produced by a fan (Coulbourn Instruments, Model ACT-130), which was fixed to the back wall of the isolation cubicle and a noise generator produced additional white noise; thus, the overall background noise was 65 dB SPL. A CCD camera (Panasonic, Secaucus, NJ, USA, Model WV-BP334) was mounted on top of the test chamber so that it could detect the movements of the subjects. The output signal of the camera was connected through an interface to an IBM PC running FreezeFrame software (version 1.62, Actimetrics, Wilmette, IL, USA).

2.2.2. Procedure

The experiment was performed over three consecutive days. For habituation, the subjects were placed for 10 min into the acrylic holders containing the grid floors. For conditioning, 24 h later, the rats were again placed into these same holders. After a 5 min acclimation period, 4 pairings of the tone (30 s, 80 dB SPL, 5 KHz) were presented with an 1 mA electric foot shock (US). The US was presented during the last second of the tone, so that both stimuli terminated together. The mean inter-trial interval was 120 s with a range of 90–150 s. 30 s after the last foot shock, the rat was placed back into the home cage. 24 h after training, the rats were placed into the test cages. After a 2 min acclimation period, 5 tones (30 s, 80 dB SPL, 5 KHz) were presented to the animals with 120 s intervals (90–150 s range). Freezing to the tone presentation was taken as a measure of the strength of the fear memory. Freezing was defined as the absence of all movements except those required for respiration.

2.3. Contextual fear conditioning

2.3.1. Apparatus

The same apparatus was used as for the testing of AFC. However, during both the training and the test, the plastic floor was replaced with a shock floor

consisting of 16 grids (Coulbourn Instruments, Model H10-11R-TC-SF). The grids were connected to an electric shock generator and scrambler, by which an 1 s electric foot shock of magnitude 0.45 mA could be delivered. Administration of the foot shocks and movement detection of the animals were provided by an IBM PC running FreezeFrame software (Actimetrics).

2.3.2. Procedure

The subjects were placed into the test cages and were allowed to acclimate to the apparatus for 2 min. For conditioning, 24 h later, rats were placed back into the test chambers and after 5 min acclimation period, 3 electric foot shocks (0.45 mA, 1 s) were delivered. The interval between the shocks was 60 s. Three min after the last shock, the animal was removed from the test. The animals behaviour was recorded throughout the training session. Testing took place 24 h after contextual conditioning. The subjects were placed back into the test cages and their freezing behaviour was recorded for a total of 5 min, starting from the time the animals were placed into the chambers.

2.4. Statistical analysis

For AFC, the mean percentage of time spent freezing during each of the 5 test tone presentations was calculated. In CFC, the percentage of time spent freezing during the 5 min test was measured. One way analysis of variance (ANOVA) followed by post-hoc Duncan's test was used for statistical analysis. The confidence limits at the 5% level were considered statistically significant. All data are shown as mean ± S.E.M

2.5. Drugs

EMQMCM (JNJ16567083) and MTEP were dissolved in a 10% solution of Tween 80 in water. (+)MK-801 (Tocris, Avonmouth, UK), citalopram (Lundbeck, Copenhagen, Denmark) and scopolamine (Tocris, Avonmouth, UK) were dissolved in physiological saline and diazepam (Ratiopharm, Ulm, Germany) was dissolved in a 1% solution of Tween in water. All substances were administered 30 min before the training or test, if not stated otherwise. EMQMCM, MTEP, (+)MK-801 and scopolamine were injected into the peritoneum (i.p.) in a volume of 2 ml/kg. Citalopram was injected subcutaneously (s.c.) in a volume of 1 ml/kg. Control animals received the respective vehicles.

3. Results

3.1. AFC

The percentage of time spent freezing during each tone presentation during the test was taken as a direct measure of the acquired fear. In the expression experiments, when animals are under the acute influence of the respective substances during testing, spontaneously occurring freezing behaviour before the first tone presentation was taken as a measure of non-specific effects. (+)MK-801, MTEP (1.25 to 5 mg/kg; $F_{3.36} = 0.374$, *NS*), EMQMCM (1.25 to 5 mg/kg; $F_{3.36} = 0.129$, *NS*), diazepam (0.5 to 2 mg/kg; $F_{3.36} = 0.613$, *NS*) and citalopram (1 to 10 mg/kg; $F_{3.36} = 2.826$, $p = 0.052$, *NS*), all failed to significantly affect acquisition of conditioned fear when given before conditioning (data not shown except for the example showing the effects of (+)MK-801; Fig. 1A). Scopolamine, however, significantly reduced freezing during the test when administered 30 min before training and at doses of 1 and 10 mg/kg (Fig. 1B).

When given 30 min before the test, EMQMCM (0.63 to 5 mg/kg) did not affect freezing behaviour to the tone CS ($F_{4,34} = 1.999$, *NS*), nor did it alter freezing before the first tone presentation ($F_{4,34} = 1.328$, *NS*) (data not shown). Likewise, administration of diazepam (0.5, 1 and 2 mg/kg) before expression failed to affect freezing behaviour 2 min before the first CS ($F_{3,43} = 0.482$, *NS*) although it showed a strong trend to increase freezing behaviour to the tone at the highest dose ($F_{3,43} = 2.74$, $p = 0.055$, *NS*) (data not shown). When administered before testing, citalopram (1, 3 and 10 mg/kg) did not affect freezing behaviour during the 2 min pre-tone phase ($F_{3,52} = 0.121$, *NS*) and failed to alter conditioned freezing compared to vehicle treated animals ($F_{3,52} = 1.329$, *NS*) (data not shown). However, when given 60 min before the retention test, MTEP significantly reduced freezing behaviour at the doses of 2.5 and 5 mg/kg (Fig. 2), while it did not affect freezing behaviour 2 min before the tone presentations ($F_{3,36} = 5.34$, *NS*).

3.2. CFC

The percentage of time spend freezing during the 5 min context re-exposure served as the measure of the strength of the fear memory. (+)MK-801 (at 0.1 and 0.2, but not at 0.05 mg/kg) significantly impaired learning when given during the acquisition phase (Fig. 3A) without affecting freezing

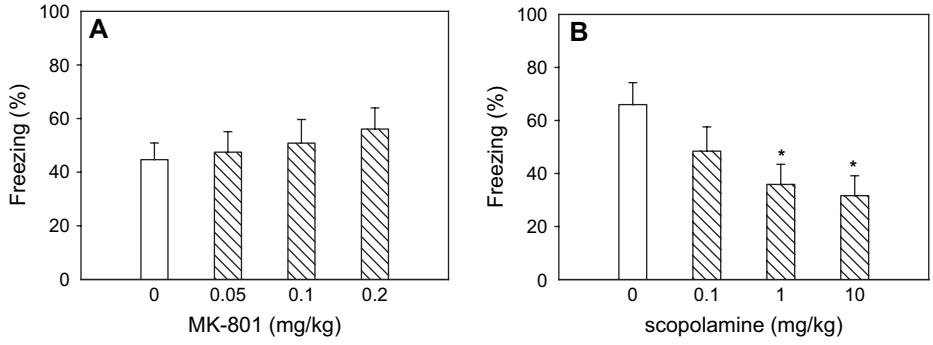

Fig. 1. (A) The effect of (+)MK-801 on acquisition of AFC. (+)MK-801 was administered 30 min before training (acquisition). Freezing behaviour to the tone CS was tested 24 h after training. The graphs show the mean percent of freezing during 5 subsequent presentations of the tone CS. Results are expressed as mean ± SEM ($n = 10$ per group). (B) The effect of scopolamine on acquisition of AFC. Scopolamine was administered 30 min before training (acquisition). Scopolamine, at 1 and 10 mg/kg, significantly reduced freezing to the tone CS when tested 24 h after conditioning ($F_{3,34} = 3.561$, $p = 0.024$). *$p < 0.05$ vs. group scopolamine 0 mg/kg computed by one-way ANOVA followed by Duncan's test. ($n = 9$–10 per group). For details see (A).

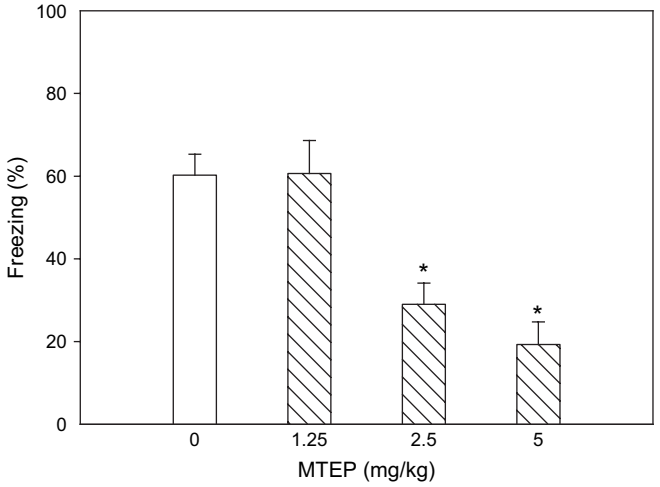

Fig. 2. The effect of MTEP on expression of AFC. MTEP was administered 60 min before the tone test (expression). MTEP, at 2.5 and 5 mg/kg, significantly reduced freezing to the tone CS when tested 24 h after conditioning ($F_{3,36} = 12.485$, $p < 0.001$). *$p < 0.05$ vs. groups MTEP 0 and 1.25 mg/kg computed by one-way ANOVA followed by Duncan's test. ($n = 10$ per group). For details see Fig. 1(A).

behaviour before the presentation of the first shock ($F_{3,27} = 2.038$, *NS*). MTEP dose-dependently reduced freezing behaviour, from 2.5 mg/kg to 10 mg/kg (Fig. 3B). However, at doses of 5 and 10 mg/kg, MTEP induced spontaneous freezing during the 5 min pre-shock phase ($F_{5,66} = 8.024$, $p = 0.004$). EMQMCM also impaired acquisition of contextual fear conditioning, at the doses of 1.25, 2.5 and 5 mg/kg (Fig. 3C) and displayed a trend to induce spontaneous freezing ($F_{4,53} = 14.466$, $p = 0.087$). In contrast, citalopram, given 30 min before conditioning, significantly increased the time spent freezing during the retention test 24 h later (Fig. 3D), but spared pre-shock freezing behaviour ($F_{3,36} = 1.993$, *NS*) Freezing behaviour during the retention test was significantly reduced after administration of diazepam at the dose of 2 mg/kg 30 min before contextual conditioning (Fig. 3E) while the same dose, diazepam also induced spontaneous freezing during the 5 min before the first shock ($F_{3,34} = 28.942$, $p < 0.001$).

When administered before the retention test, citalopram inhibited the expression of the freezing behaviour at the doses of 3 and 10 mg/kg (Fig. 4A). MTEP also reduced freezing at the highest tested dose, 3 mg/kg, when given before the context re-exposure (Fig. 4B). Similarly, EMQMCM decreased the expression of freezing, at doses of both 1 and 3 mg/kg (Fig. 4C). In contrast, diazepam (2 mg/kg) was shown to increase the time spend freezing when given 30 min before the retention test (Fig. 4D).

4. Discussion

Group I mGluRs are promising targets for the treatment of several neurological disorders, such as substance abuse, pain, depression and anxiety related disorders (Spooren et al., 2003) and some beneficial effects of mGluR1 and mGluR5 antagonists have previously been demonstrated. These receptor subtypes also play a role in some forms of learning and memory. Interestingly, MTEP and EMQMCM blocked acquisition of contextual conditioning in the present study, without affecting learning to the tone CS. While MTEP inhibited the expression of both AFC and CFC, EMQMCM only inhibited expression of the latter one.

The NMDA receptor antagonist (+)MK-801 failed to affect acquisition of fear conditioning to the auditory stimulus, while it clearly blocked context conditioning. In contrast, scopolamine impaired acquisition of AFC. Surprisingly, diazepam was ineffective in AFC when given before the retention test, yet in CFC, diazepam even significantly increased the freezing response when administered before retention test, albeit at the highest dose. When given during the acquisition phase in CFC, diazepam was found to impair learning, while again having no effect upon the acquisition of AFC. In contrast, administration of citalopram during the acquisition phase of CFC significantly increased fear responding when tested 24 h after conditioning. However, when administered before the retention test of CFC, citalopram inhibited the expression of fear. Citalopram did not significantly affect either acquisition nor expression of AFC.

The selection of suitable antagonists for the respective receptor subtypes is of basic importance for the present study. The compounds should combine good penetration to the CNS with high selectivity for the receptor subtype. For EMQMCM, the IC_{50} value at the mGlu1 receptor is about 2–3 nM (Parsons, personal communication) and a related substance has been shown to have good penetration to the brain (Steckler et al., 2005a). Therefore, at the doses used in the present investigation, EMQMCM would be expected to provide close to 100% receptor occupancy (Lavreysen et al., 2004). For MTEP, the ED_{50} value for 50% of receptor occupancy is 1.2 mg/kg 1 h after i.p. application (Busse et al., 2004). In addition, clear effects of both of these antagonists have been shown in several rodent models, such as those for learning and anxiety (Busse et al., 2004; Gravius et al., 2005, 2006a; Pietraszek et al., 2005; Steckler et al., 2005a,b).

The model of LTP is commonly believed to be the neural substrate for most kinds of learning and memory processes (Aiba et al., 1994; Lu et al., 1997; Zajaczkowski et al., 1997). Many investigations have confirmed the involvement of NMDA receptors in at least some forms of LTP and learning (Morris et al., 1986; for review see Danysz et al., 1995). More recently, mGluRs have also been implicated in the induction of LTP and the mechanism of learning (Bashir et al., 1993; Manahan-Vaughan, 1997) and group I mGluRs seem to be particularly involved. The mGluR5 antagonist MPEP impairs hippocampal LTP and spatial learning (Balschun and Wetzel, 2002; Naie and Manahan-Vaughan, 2004) and similar effects have been shown for mGluR1 antagonists. Also, mGluR1 KO mice show reduced LTP and impairments in contextual fear conditioning (Aiba et al., 1994). Given the accepted role of NMDA receptors in acquisition, and considering that both mGluR1 and mGluR5 seem to play in concert with NMDA receptors in some forms of learning and memory

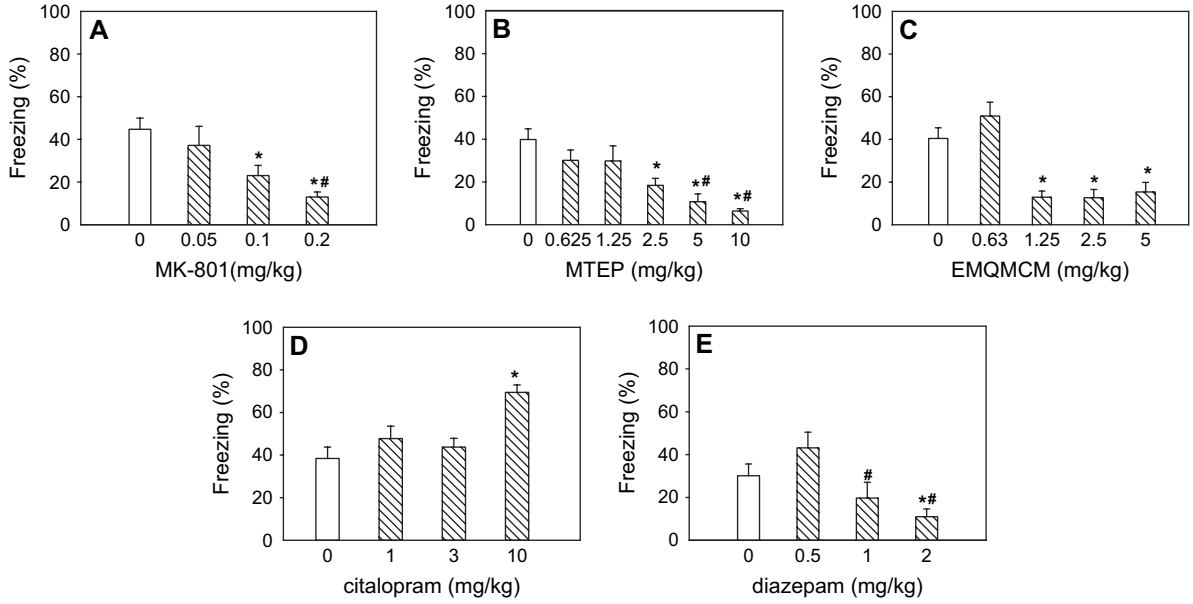

Fig. 3. (A) The effect of (+)MK-801 on acquisition of CFC. (+)MK-801 was administered 30 min before training (acquisition). (+)MK-801, at 0.1 and 0.2 mg/kg, significantly reduced the percentage of time spent freezing during context reexposure, tested 24 h after conditioning ($F_{3,27} = 5.976$, $p = 0.003$). *$p < 0.05$ vs. groups (+)MK-801 0 and 0.05 mg/kg. #$p < 0.05$ vs. (+)MK-801 0.05 mg/kg computed by one-way ANOVA followed by Duncan's test. ($n = 7$–8 per group). The graphs show the mean percent of time spent freezing during the 5 min retention test. Results are expressed as mean ± SEM. (B) The effect of MTEP on acquisition of CFC. MTEP was administered 30 min before training (acquisition). MTEP, at 2.5, 5 and 10 mg/kg, significantly reduced the percentage of time spent freezing during context reexposure, tested 24 h after conditioning ($F_{5,66} = 8.024$, $p < 0.001$). *$p < 0.05$ vs. group MTEP 0 mg/kg. #$p < 0.05$ vs. groups MTEP 0.63 and 1.25 mg/kg computed by one-way ANOVA followed by Duncan's test. ($n = 12$ per group). For details see Fig. 3(A). (C) The effect of EMQMCM on acquisition of CFC. EMQMCM was administered 30 min before training (acquisition). EMQMCM, at 1.25, 2.5 and 5 mg/kg, significantly reduced the percentage of time spent freezing during context reexposure, tested 24 h after conditioning ($F_{4,53} = 14.466$, $p < 0.001$). *$p < 0.05$ vs. group EMQMCM 0 and 0.63 mg/kg computed by one-way ANOVA followed by Duncan's test. ($n = 11$–12 per group). For details see Fig. 3(A). (D) The effect of citalopram on acquisition of CFC. Citalopram was administered 30 min before training (acquisition). Citalopram, at 10 mg/kg, significantly increased the percentage of time spent freezing during context reexposure, tested 24 h after conditioning ($F_{3,36} = 8.069$, $p < 0.001$). *$p < 0.05$ vs. all other groups computed by one-way ANOVA followed by Duncan's test. ($n = 10$ per group). For details see Fig. 3(A). (E) The effect of diazepam on acquisition of CFC. Diazepam was administered 30 min before training (acquisition). Diazepam, at 10 mg/kg, significantly increased the percentage of time spent freezing during context reexposure, tested 24 h after conditioning ($F_{3,34} = 5.334$, $p = 0.004$). *$p < 0.05$ vs. group diazepam 0 mg/kg, #$p < 0.05$ vs. diazepam 0.5 mg/kg computed by one-way ANOVA followed by Duncan's test. ($n = 9$–10 per group). For details see Fig. 3(A).

(Campbell et al., 2004; Gravius et al., 2006a) it is not surprising that both EMQMCM and MTEP impaired the acquisition of CFC in the present study. These results support and extend previous investigations demonstrating the involvement of both mGluR1 and mGluR5 in aversive context-specific learning (Aiba et al., 1994; Lu et al., 1997; Schachtman et al., 2003).

However, the results from experiments concerned with the acquisition of AFC are in contrast to those discussed above. In these experiments, neither (+)MK-801, EMQMCM nor MTEP had any significant effect when given before fear conditioning to the tone. For validation of the model, the non-specific cholinergic antagonist scopolamine was given before acquisition of AFC and was seen to significantly impair learning when tested 24 h later. This is in line with previous findings demonstrating the disruptive effects of scopolamine upon both contextual and auditory fear conditioning (Rudy, 1996; Anagnostaras et al., 1999).

With respect to learning to a discrete cue, previous studies using NMDA receptor antagonists are somewhat controversial. For example, acquisition of fear potentiated startle (FPS) was blocked by intra-amygdala infusions of NMDA receptor antagonists (Miserendino et al., 1990). Furthermore, FPS learning was inhibited by systemic administration of the NMDA receptor antagonist (+)MK-801 (Gravius et al., 2006a). In contrast, systemic application of (+)MK-801, at even higher doses than used in the present study, was shown to impair contextual conditioning, but spared acquisition of fear conditioning to a tone CS (Gould et al., 2002). Likewise, i.c.v. injections of the NMDAR antagonist DL-2-amino-5-phosphonovalerate (APV) blocked contextual fear conditioning without affecting AFC (Fanselow et al., 1994).

Our findings support these data and it appears that not all cue dependent fear conditioning paradigms can be generalized with regards to NMDA receptor involvement. While conditioning to a auditory stimulus appears to be NMDA receptor independent, FPS learning, in which light serves as the CS, seems to be NMDAR dependent. Considering the close interaction between NMDA receptors and group I mGluRs in learning, this might explain the finding that EMQMCM and MTEP did block only acquisition of CFC but not AFC. It has previously been shown that MPEP and MTEP impair FPS learning (Schulz et al., 2001; Fendt and Schmid, 2002; Gravius et al., 2005). Further, one study showed that pre-training intra-amygdala infusions of MPEP impaired both

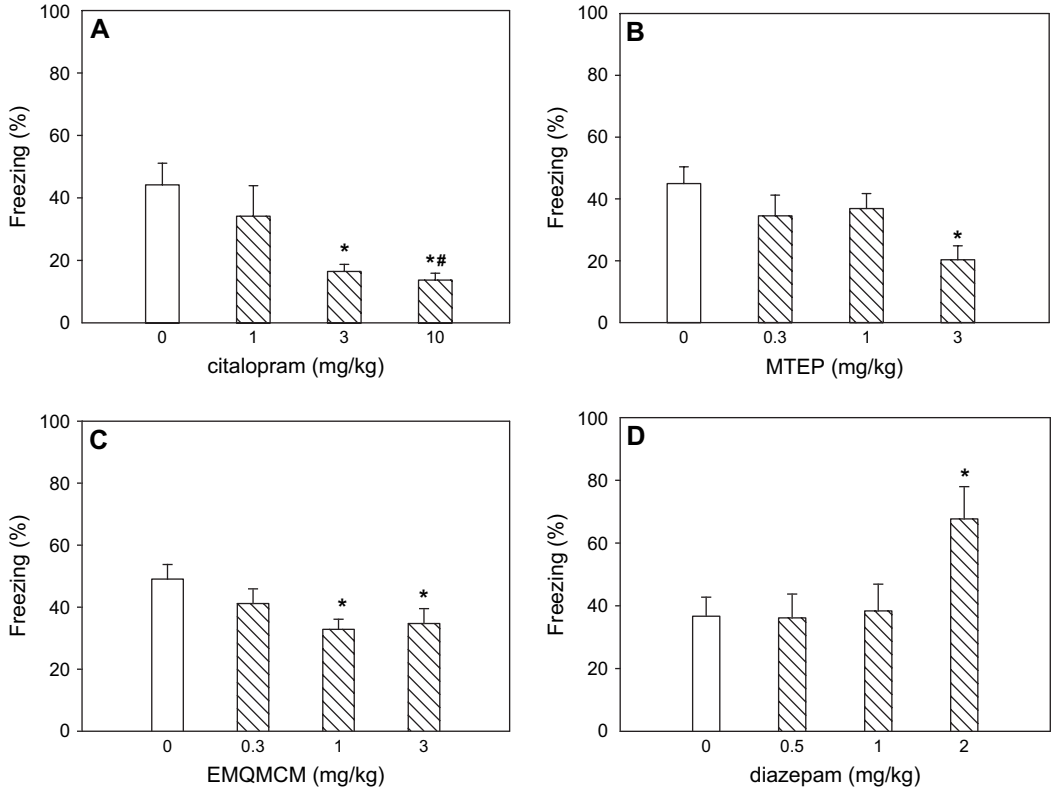

Fig. 4. (A) The effect of citalopram on expression of CFC. Citalopram was administered 30 min before context reexposure (expression). Citalopram dose-dependently decreased the percentage of time spent freezing, tested 24 h after conditioning ($F_{3,27} = 5.265$, $p = 0.005$). *$p < 0.05$ vs. group citalopram 0 mg/kg, #$p < 0.05$ vs. group citalopram 1 mg/kg computed by one-way ANOVA followed by Duncan's test. ($n = 7$–8 per group). For details see Fig. 3(A). (B) The effect of MTEP on expression of CFC. MTEP was administered 60 min before context reexposure (expression). MTEP, at 3 mg/kg, decreased the percentage of time spent freezing, tested 24 h after conditioning ($F_{3,52} = 3.776$, $p = 0.016$). *$p < 0.05$ vs. group MTEP 0 and 1 mg/kg computed by one-way ANOVA followed by Duncan's test. ($n = 14$ per group). For details see Fig. 3(A). (C) The effect of EMQMCM on expression of CFC. EMQMCM was administered 30 min before context reexposure (expression). EMQMCM dose-dependently decreased the percentage of time spent freezing, tested 24 h after conditioning ($F_{3,57} = 2.920$, $p = 0.042$). *$p < 0.05$ vs. group EMQMCM 0 mg/kg computed by one-way ANOVA followed by Duncan's test. ($n = 15$–16 per group). For details see Fig. 3(A). (D) The effect of diazepam on expression of CFC. Diazepam was administered 30 min before context reexposure (expression). Diazepam, at 2 mg/kg, increased the percentage of time spent freezing, tested 24 h after conditioning ($F_{3,28} = 3.448$, $p = 0.03$). *$p < 0.05$ vs. all other groups computed by one-way ANOVA followed by Duncan's test. ($n = 8$ per group). For details see Fig. 3(A).

acquisition in CFC and AFC (Rodrigues et al., 2002). However, mGluR5 KO mutant mice were found to be impaired in CFC, but not AFC as measured by time spent freezing (Lu et al., 1997), and the same finding has been observed in mGluR1 KO mice (Aiba et al., 1994). It should be noted though, that data examining the role of mGluR1 in cue dependent fear conditioning are scarce. In contrast to mGluR5, mGluR1 have not been implicated to play a role in FPS learning (Gravius et al., 2005). The differential disruptive effects of respective antagonists observed in both types of learning might be related to the different neural substrates underlying cued and contextual conditioning. Earlier reports indicated the involvement of both the amygdala and hippocampus in contextual conditioning (Kim and Fanselow, 1992), while cue-dependent fear conditioning appeared to be independent of the hippocampus (Phillips and LeDoux, 1992). Both NMDA and group I metabotropic glutamate receptors are distributed in high density in both the hippocampus and amygdala and show overlapping distribution (for review see Spooren et al., 2003).

Hippocampal NMDA receptors have been shown to play a role in CFC (Quinn et al., 2005). Pre-training injections of AP5 into the entorhinal cortex, a main input and output structure to the hippocampus, impair contextual but not auditory fear conditioning (Schenberg et al., 2005). Hippocampal group I mGluRs are also believed to be involved in the processing of PA learning, a hippocampus-dependent, context-specific learning task. It has been demonstrated that post-training intrahippocampal infusions of MPEP impair performance in the retention test (Coulibaly et al., 2005). Furthermore, reduced hippocampal LTP and impaired CFC was observed in mGluR1 KO mutant mice (Aiba et al., 1994). Glutamate receptors expressed in the amygdala have also been shown to play a role in CFC. For example, pre-training administration of the mGluR antagonist methylcarboxyphenylglycine (MCPG) via intra-amygdala injections impaired PA learning (Barros et al., 2000). Moreover, pre-training injections of the NMDA receptor antagonists CPP (3((±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid), D,L-AP5 (DL-2-amino-5-phosphonopentanoic acid) and D-AP5 into the amygdala blocked

acquisition of PA (Kim and McGaugh, 1992). NMDA receptors of the amygdala are also involved in cue-dependent aversive learning (Miserendino et al., 1990; Campeau et al., 1992). In accordance with this, administration of the mGluR5 antagonist MPEP via intra-amygdala infusions was shown to impair the acquisition of fear potentiation of startle (Fendt and Schmid, 2002).

In summary, it is not clear why, in the present experiments, only context and not conditioning to the tone was impaired by the respective antagonists. However, the data may suggest that the neural circuit involved is different from that implicated in processing the acquisition of, for example, FPS. It should also be noted that factors other than the CS vary in the different models. Different forms of expression of conditioned fear are measured, such as time spent freezing versus startle response, and this may contribute to the differential findings. One could argue that different electric stimuli were applied (3 times 0.45 mA in CFC versus 4 times 1 mA in AFC) which may have caused differences. However, different types of electric shockers were used in both models, and the parameters were established in such a way that the amount of time spent freezing was in a similar range in both models, with a slightly higher percentage in the AFC model. On the basis of this and under the presumption that the amount of time spent freezing directly reflects the strength of the fear memory (Blanchard and Blanchard, 1969), the drug effects should be comparable, with similar freezing responses.

In another set of experiments, both MTEP and EMQMCM were administered before the retention test in CFC and AFC. In both models, the conditioned fear memory is expressed as freezing, a behavioural response which can be reduced by compounds with potential anxiolytic effects. The effects of MTEP and EMQMCM were compared to those of the SSRI citalopram and the widely used benzodiazepine diazepam. While citalopram dose-dependently reduced freezing in CFC when given before the retention test, it was however ineffective on the expression of AFC. To further elucidate its possible effects on memory, citalopram was also administered before acquisition of both CFC and AFC. Interestingly, citalopram clearly enhanced acquisition of CFC and, in contrast, showed a trend to impair acquisition of auditory conditioning, although this effect failed to reach statistical significance. Previous studies investigating citalopram are controversial and a crucial factor seems to be whether its effects are tested after acute or chronic administration. For example, one study revealed that citalopram (10 mg/kg, i.p.) enhanced acquisition of AFC after acute treatment, but impaired acquisition after chronic administration (Burghardt et al., 2004). With respect to the expression of CFC, citalopram significantly reduced freezing when given systemically (acute) before the test (Hashimoto et al., 1996; Inoue et al., 1996b; Izumi et al., 2006), which is in line with the present findings. In contrast to our results which show facilitation of acquisition of CFC, citalopram has previously been shown to impair acquisition in a context-dependent conditioned fear stress paradigm (Inoue et al., 1996a). Taken together, the findings are confusing. However, acutely administered citalopram seems to be useful as a reference compound in models involving contextual conditioning. Surprisingly, diazepam failed to reduce the freezing response in both paradigms, and even increased the percentage of time spent freezing during the test at the highest dose of 2 mg/kg, an effect which reached statistical significance in CFC although not in AFC. This finding is most likely due to the sedative effect of diazepam, which might obscure its anxiolytic action. This result was unexpected, as diazepam in the selected dose-range has previously been seen to be active in several rodent models for anxiety (Brodkin et al., 2002; Tizzano et al., 2002). Furthermore, the related benzodiazepine midazolam was demonstrated to reduce fear conditioned freezing in CFC (Sienkiewicz-Jarosz et al., 2003; Pietraszek et al., 2005). However, Wistar rats were used in the latter studies, while rats of the Sprague–Dawley strain were used in the present study.

Antagonists of mGluR5 have previously been shown to produce anxiolytic-like effects in various rodent models assessing anxiety. For example, the anxiolytic action of MTEP was observed in the elevated plus maze (EPM) (Klodzinska et al., 2004a) and MPEP dose-dependently increased number of licks in the conditioned lick suppression test (Steckler et al., 2005b). In line with our findings, administration of MTEP before the expression of CFC has previously been shown to significantly reduce fear conditioned freezing (Pietraszek et al., 2005). A more recent study revealed, that intra-amygdala injections of MPEP showed anxiolytic-like effects in the EPM, Black and White box test and shock probe burying test (Pérez de la Mora et al., 2006). The present study extends these findings, as administration of MTEP before expression not only reduced conditioned freezing to the context, but also to the auditory stimulus in a dose-dependent manner. The latter finding is interesting because MTEP did not influence AFC when given before acquisition, which suggests a possible involvement of different neural substrates in the acquisition and the expression of AFC. In contrast, both the acquisition and expression of FPS, another cue-dependent task, have been demonstrated to be impaired by MPEP and MTEP (Schulz et al., 2001; Pietraszek et al., 2005). Interestingly, intra-amygdala infusions of MPEP impaired acquisition, but not expression of AFC (Rodrigues et al., 2002).

Fewer data, however, are available concerning the role of mGlu1 receptors in this field. AIDA, a selective group I mGluR antagonist with higher selectivity at the mGlu1 receptor, showed anxiolytic effects in both conflict drinking and the EPM test (Klodzinska et al., 2004b) and JNJ16259685, a selective antagonist of the mGlu1 receptor, exerted anxiolytic-like effects in the rat lick suppression test (Steckler et al., 2005b) A structurally related compound, EMQMCM, failed to show anxiolytic action in the Geller-Seifter test, but was effective in FPS and CFC (Pietraszek et al., 2005). This latter finding is in line with the present results showing the effect of EMQMCM on expression of CFC. However, EMQMCM failed to reduce fear conditioned freezing when given before the test of AFC in the present study. Taken together, antagonists of mGluR1 do not induce consistent anxiolytic-like effects in all models, and thus a generalized anxiolytic

potential can not be expected, in contrast to mGluR5. This may therefore suggest that mGluR1 and mGluR5 antagonists exert their anxiolytic-like effects possibly via different pathways.

The discussion concerning the effects of these compounds upon expression should be extended to include one further point. When the respective substances are administered before the test, the possibility remains that the observed effects on retention could be due to impairment of memory retrieval rather than an anxiolytic mechanism. A limited number studies have investigated the involvement of mGluR in retrieval process. MCPG blocked retrieval of the PA response when injected into the hippocampus (Izquierdo et al., 2000) and the selective group I mGluR agonist DHPG was shown to impair retrieval of PA at the dose of 0.1 nmol (i.c.v.), while it facilitated retrieval at doses 0.01 and 1 nmol (Car et al., 2000). Furthermore, in our previous work, we demonstrated that EMQMCM, given at a dose of 5 mg/kg before the retention test, impaired retrieval of PA (Gravius et al., 2005). However, the same dose of EMQMCM was ineffective on memory retrieval in a spatial reference memory task (unpublished data). In summary, there is some evidence for the involvement for group I mGluRs in the process of retrieval, while data suggest stronger involvement of the mGluR1 receptor subtype rather than mGluR5. Thus, we can not exclude the possibility that the observed impairments in the expression experiments could be due to retrieval deficits.

Any acute effects of administered substances on noxious sensitivity might influence the acquisition of the association of the painful stimulus with the context or tone. However, neither EMQMCM nor MTEP altered acute noxious responses in the Hargreaves test (Sevostianova and Danysz, in press) at the same dose range used in the present study. General behavioural inhibition under the acute influence of substances might obscure any effects upon acquisition or expression. In the experiments testing the substances on acquisition of CFC, neither EMQMCM, (+)MK-801 nor citalopram induced spontaneous freezing behaviour during the fist 5 min before the first shock presentation. In contrast, MTEP (at doses of 5 and 10 mg/kg) showed general inhibition, measured as immobility before the first shock. This finding is surprising, however, as we did not previously observe such effects at these doses. For example, MTEP impaired rota-rod performance at 20 mg/kg, but not at lower doses (data not shown), and did not affect responsivity to foot shocks (jumping reaction) at the dose of 5 mg/kg (Gravius et al., 2006a). However, administration of MTEP impaired acquisition at 2.5 mg/kg, whereas general inhibition was induced by a dose of 5 and 10 mg/kg, which would suggest that the effect of MTEP is indeed upon learning rather than simply being due to sedative effect. Diazepam also induced immobility during the first 5 min of the training at 2 mg/kg, and this dose also reduced freezing behaviour during the test 24 h later. This is in line with previous reports showing that diazepam, besides having anxiolytic properties, also exerts sedative and amnesic side effect (Helton et al., 1998). When given before the retention test of AFC, none of the agents tested induced spontaneous freezing behaviour 2 min before the first presentation of the tone, suggesting that expression was not influenced by possible general inhibition.

In conclusion, the present study revealed for the first time the differential effects of mGluR1 and mGluR5 antagonists in context vs. auditory fear conditioning. While EMQMCM only reduced conditioned freezing in CFC, MTEP also inhibited freezing to the tone when given before the retention test, which suggests a possible differential involvement of the receptor types in expression of conditioned fear. However, further work is needed to determine if the observed effects on expression are due to retrieval impairment or anxiolytic-like effects.

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Lebenslauf

Name	Andreas Gravius
Geburtsdatum	10.03.1975
Geburtsort	Kirn / Nahe
Familienstand	ledig
Wohnhaft in	Mainzer Straße 32, 65185 Wiesbaden

Schulausbildung

1981 - 1985	Grundschule, Meisenheim/Glan
1985 - 1994	Paul-Schneider-Gymnasium, Meisenheim/Glan

Berufliche Tätigkeiten

1994 - 1995	Zivildienst in der Altenpflege
1995 - 2003	Nebentätigkeit als Altenpflegehelfer (19,5 Wochenstunden)

Studium / wissenschaftliche Ausbildung

1996 - 2000	Grundstudium der Biologie, Johannes-Gutenberg-Universität Mainz
2000 - 2003	Hauptstudium der Biologie (Diplom), Johannes-Gutenberg-Universität Mainz (Botanik, Pharmakologie und Mikrobiologie)
2002	mündliche Diplomprüfungen
2002 - 2003	Diplomarbeit „Spc98 in *Nicotiana tabacum*: Isolation einer homologen Gensequenz und Messung der Expression während Zellteilung und Zellelongation“ im Fachbereich Botanik unter der Anleitung von Prof. Dr. W. Wernicke.
seit 2003	Doktorarbeit „Metabotrope Glutamatrezeptoren der Gruppe I beim Lernen und Gedächtnis der Ratte“ unter Anleitung von Prof. Dr. W. Danysz (Merz Pharmaceuticals GmbH, Abteilung In Vivo Pharmakologie) und Prof. W.J. Schmidt (Abteilung Neuropharmakologie, Zoologisches Institut, Fakultät für Biologie, Eberhard-Karls-Universität Tübingen).