# Der Einfluss des Neurokinin-1 Rezeptor Antagonismus auf die cholinerge Transmission in definierten Gehirngebieten und dessen Rolle bei Lern- und Gedächtnisprozessen in *Rattus Norvegicus*

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## Zusammenfassung

Substanz P (SP) ist ein Neuropeptid der Tachykinin-Familie, das an Neurokinin (NK) Rezeptoren bindet. Es hat die höchste Affinität zum NK-1 Rezeptor, bindet jedoch auch an den NK-2 und NK-3 Rezeptor. Für SP wurden promnestische, anxiolytische und verstärkende Effekte in *Rattus Norvegicus* nachgewiesen. In der vorliegenden Arbeit wurde die Rolle des NK-1 Rezeptors bei Lern- und Gedächtnisprozessen untersucht. Der selektive nicht-peptiderge NK-1 Rezeptor Antagonist SR140333 wurde in Konzentrationen von 1, 3 und 9mg/kg intraperitoneal (i.p.) verabreicht und mittels *in vivo* Mikrodialyse in anästhesierten Tieren dessen Einfluss auf die extrazelluläre Acetylcholin (ACh) –Konzentration in Zielstrukturen der cholinergen Neurone des basalen Vorderhirns, dem Frontalcortex, der Amygdala und dem Hippocampus gemessen. Diese Untersuchung ergab im Hippocampus einen dosisabhängigen Anstieg in der ACh-Konzentration, die 60 Minuten nach der Behandlung wieder auf das Aufgangsniveau abfiel. Die Verhaltensrelevanz dieses Befundes wurde in verschiedenen Paradigmen getestet. Eine Injektion von SR140333 nach dem Lerndurchgang hatte promnestische Effekte auf die Habituation an das Offenfeld und die Erinnerungsleistung im passiven Vermeidungslernen. Im Rahmen dieses Promotionsvorhabens wurde ein Paradigma zur Untersuchung des episodischen Gedächtnis bei Ratten entwickelt. In Kontrollstudien zu diesem Paradigma wurden durch eine einmalige Injektion von physiologischer Kochsalzlösung Defizite im episodischen Gedächtnis bei Ratten beobachtet, die durch eine Stressreaktion auf die Injektionsprozedur selbst erklärt werden können. Eine einmalige i.p.-Verabreichung von 1mg/kg SR140333 vor dem Lerndurchgang kompensierte die beobachteten stressinduzierten Defizite und führte zu einer vollständigen Wiederherstellung des episodischen Gedächtnis bei Ratten. Diese Ergebnisse deuten auf promnestische Effekte durch NK-1 Rezeptor Antagonismus hin. Die Substanzklasse der NK-1 Rezeptor Antagonisten könnte damit von therapeutischem Nutzen bei der Behandlung von dementiellen Erkrankungen wie Morbus Alzheimer sein, zu deren frühesten Symptomen z.B. Defizite des episodischen Gedächtnis gehören.

## Summary

Substance P, a neuropeptide of the tachykinin-family, preferentially binds to the neurokinin (NK) –1 receptor, but also has some affinity for the NK-2 and NK-3 receptors. It was shown, that SP exerts promnestic, anxiolytic and reinforcing effects in rats. In the studies presented here, the involvement of the NK-1 receptor in learning and memory processes have been investigated. Therefore the selektive non-peptidegic NK-1 receptor antagonist SR140333 was utilized and intraperitoneally (i.p.) applied in doses of 1, 3 and 9mg/kg. First the impact of a single i.p.-injection of SR140333 on extracellular acetylcholine (ACh)- levels in three major projection areas of the cholinergic basal forebrain, namely the frontal cortex, amygdala and hippocampus, was measured by means of *in vivo* microdialysis in anaesthetized rats and subsequent HPLC analysis. Immediately after injection of SR140333 a dose-dependent increase of ACh-levels was observed, which returned to baseline after 60 minutes. But SR140333 did not effect ACh-levels in the frotal cortex and amygdala. The behavioral effects of systemic injections of NK-1 receptor antagonist SR140333 were investigated in three different tasks. Post-trial injection of SR140333 had minor facilatory effects on the open-field habituation and the inhibitory avoidance learning. Based on an object exploration paradigm to investigate episodic-*like* memory in mice a new task for rats was adapted in the present doctoral thesis. It was shown that a single pre-trial injection of saline has detrimental effects on episodic-*like* memory in rats, which might have been due to stress-induced effects by means of the injections procedure itself. A single pre-trial administration of 1mg/kg SR140333 compensated the stress-induced deficits and reinstated episodic-*like* memory. In conclusion, the results of the present thesis demonstrated promnestic effects by NK-1 receptor antagonism. Thus, NK-1 receptor antagonism might have beneficial effects on the cognitive defictis observed in early stages of patients suffering from Alzheimer's disease, which should be elucidated by further investigations.

Zusammenfassender Überblick über die wissenschaftlichen Arbeiten der kumulativen Dissertation

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# 1 Einleitung

Der Schwerpunkt dieses Promotionsvorhabens liegt auf der Untersuchung der Rolle des Neurokinin (NK) -1 Rezeptors bei Lern- und Gedächtnisprozessen. Zur Untersuchung dieser Fragestellung wurde der spezifische und selektive NK-1 Rezeptor Antagonist SR140333 verwendet und 1) die *in vivo* Mikrodialyse als neurochemische Methode zur Messung der extrazellulären Acetylcholin-Konzentrationen in definierten Hirngebieten herangezogen, 2) aus der Literatur bekannte Paradigmen zur Messung der Lern- und Gedächtnisleistung und Verstärkungsprozessen eingesetzt und 3) ein neues Paradigma zur Untersuchung des episodischen Gedächtnisses in Labortieren konzeptuell eruiert und empirisch validiert.

### *Grundlagen des Lernens und der Gedächtnisbildung*

Auf den folgenden Seiten werden zunächst zwei Lernformen, das assoziative und das nicht-assoziative Lernen kurz besprochen. Anschließend werden verschiedene Gedächtnisformen beschrieben und die Probleme beim Nachweis des episodischen Gedächtnisses bei Säugetieren dargestellt.

#### *Assoziatives und nicht-assoziatives Lernen*

Das Lernen und die Gedächtnisbildung sind Prozesse, die bei Säugetieren das gesamte Leben überdauern. Im tierexperimentellen Bereich können zwei Formen des Lernens unterschieden werden: das assoziative oder relationale und das nicht-assoziative Lernen. Der Unterschied zwischen diesen beiden Lernformen liegt primär in der Komplexität des dargebotenen Reizes und der darauf folgenden Reaktion. Beim nicht-assoziativen Lernen wird ein Reiz einer Modalität, also z.B. ein Ton, ein visuelles Signal oder ein Schmerzreiz, dargeboten, auf den eine Reaktion erfolgt. Zum nicht-assoziativen Lernen gehören die Habituation und die Sensitivierung von Reaktionen. Die Habituation wurde z.B. an der Meerschnecke Aplysia untersucht, die auf bestimmte dargebotene Reize mit dem Rückzug von Kiemen und Siphon reagiert. Eine Reizung des Siphons löst bei der Meerschnecke einen Rückzugreflex von Siphon und Kiemen aus. Bei Repetition der Reizung in definierten Abständen, kann

eine Abschwächung des Rückzugreflexes beobachtet werden. Diese Abschwächung der Reaktion wird als Habituation bezeichnet (Bailey & Chen, 1989).

Auch die zweite Form des nicht-assoziativen Lernens, die Sensitivierung, kann an Aplysia untersucht werden, indem z.B. ein schmerzhafter Reiz, wie ein Elektroschock, am Schwanz der Schnecke verabreicht wird. Die Schnecke reagiert darauf mit dem reflexhaften Rückzug des Siphons. Bei wiederholter Reizung wird der Reflex verstärkt. Der Prozess der Verstärkung einer Reaktion auf einen spezifischen Reiz wird als Sensitivierung bezeichnet (Kandel & Schwartz, 1982). Die Erinnerung an diese Reizung kann mehrere Wochen anhalten.

Die Formen des assoziativen oder relationalen Lernens sind komplexer und meist beschränkt auf Wirbeltiere. Assoziatives Lernen beschreibt alle Formen des Lernens, die über eine einfache Reiz-Reflex-Kopplung hinausgehen. Dazu gehören die klassische und operante Konditionierung, das Habituationslernen und auch das räumliche Lernen, um nur einige Beispiele zu nennen. Bei der operanten Konditionierung z.B. wird ein zufällig auftretendes Verhalten durch die kontingente Darbietung eines Belohungsreizes (appetetiver Reiz) verstärkt oder durch eine Bestrafung (aversiver Reiz) abgeschwächt. Skinner hat z.B. Tauben darauf konditioniert auf eine Scheibe zu picken, um aus einem Spender Futter zu erhalten (Skinner, 1947). Bei der operanten Konditionierung ist meist eine Phase der Verhaltensformung, ein so genanntes „shaping“ notwendig. Im Falle der Tauben von Skinner, wurde das Tier sukzessive an die Pickvorrichtung herangeführt, indem z.B. bereits für die Annäherung an den Spender eine Futterbelohnung dargeboten wurde. Das Ziel dieses „shapings“ war, das Tier zum Picken auf eine Scheibe zu veranlassen. Sobald das Tier dieses Verhalten zeigte, erfolgte die kontingente Belohnung für das Picken auf die Scheibe. Bei der operanten Konditionierung können die Reize zur Verstärkung oder Abschwächung eines Verhaltens jeweils entweder dargeboten oder entzogen werden, so dass der Experimentator durch geschickten Einsatz von bestimmten Reizen, das Verhalten des Versuchstieres modulieren kann.

Eine der einfachsten Formen des assoziativen Lernens ist das Habituationslernen. Diese Form muss jedoch von der Habituation auf einen einfachen Reiz, wie bei Aplysia, unterschieden werden. Es wird angenommen, dass die Tiere bei der Habituation an eine neue Umgebung, z.B. ein Offenfeld, während der ersten Exposition die Umgebungsstimuli innerhalb und außerhalb des

Offenfeldes enkodieren und eine Repräsentation dieser Umgebung entwickeln. Bei der zweiten Exposition erinnert das Tier diese Umgebung als bekannt und reagiert mit einer Reduktion der Explorationsaktivität. Weil es sich bei diesem Paradigma um eine sehr einfache Form des assoziativen Lernens handelt und es lediglich auf dem nativen Explorationsverhalten von Ratten basiert, wurde es in einer Studie dieses Promotionsvorhabens eingesetzt.

Ein weiteres Paradigma, dass in diesem Promotionsvorhaben verwendet wurde, ist das passive Vermeidungslernen. Es stellt ebenfalls eine Form des assoziativen Lernen dar und enthält eine aversive, emotionale Komponente. Beim Paradigma der passiven Vermeidung wird das Tier in der Lernphase einer aversiven Situation ausgesetzt. Dies kann bei Ratten ein erhobenes Podest (Step-down Anordnung) oder eine helle, offene Umgebung (Step-through Anordnung) sein (Bures et al., 1983). Das Tier hat jedoch die Möglichkeit dieser Situation zu entgehen und sich in eine vermeintlich sichere Umgebung zu begeben, d.h. es kann vom Podest herabsteigen oder eine dunkle Umgebung aufsuchen. Das Aufsuchen dieser bevorzugten Umgebung wird mit einem Fußschock gepaart, d.h. die Äußerung der natürlichen Verhaltenstendenz des Tieres wird bestraft. Die Lernleistung des Tieres wird an der Fähigkeit zur Unterdrückung des Aufsuchens der vermeintlich sicheren Umgebung gemessen, also an der Fähigkeit zur Vermeidung der mit dem Fußschock assoziierten Situation. Das Tier befindet sich also in einer Konfliktsituation, einem Aversions-Aversions-Konflikt, in dem das beobachtbare Verhalten das Ergebnis der relativen Aversivität der beiden Situationen ist. In den meisten Fällen lernen die Tiere die Vermeidungsreaktion in nur einem einzigen Lerndurchgang.

Beim räumlichen Lernen ist das Erlernen von (komplexen) Reizkonstellationen notwendig. Ein Paradigma, dass in der tierexperimentellen Forschung zur Untersuchung des räumlichen Gedächtnis verwendet wird, ist das Wasserlabyrith von Morris (Morris, 1984). Hier wird eine Ratte in ein Wasserbecken gesetzt, in dem sich an einer bestimmten Position eine Plattform befindet, auf die sich das Tier retten kann. Diese Plattform ist jedoch für das Tier nicht sichtbar, da sie sich wenige Zentimeter unter der Wasseroberfläche befindet und das Wasser trüb ist. Nach wenigen Durchgängen erlernen die Tiere die Position der Plattform und schwimmen gezielt hin, selbst wenn sie an unterschiedlichen Positionen des Wasserbeckens eingesetzt werden. Es wird angenommen, dass das Tier eine Repräsentation des Raumes entwickelt, in dem sich das Wasserlabyrinth befindet, und die Position der

Plattform in Relation zu diesen räumlichen Hinweisreizen setzt, um so die Plattform zu finden. Dies sind nur einige wenige Beispiele für tierexperimentelle Lernmodelle, die einen Eindruck von der Vielseitigkeit des Lernens vermitteln sollen.

*Das implizite Gedächtnis*

Gelernte Inhalte können dem Individuum entweder für wenige Minuten, Stunden oder auch lebenslang zur Verfügung stehen. Das Gedächtnis lässt sich bei Säugetieren in seiner zeitlichen Ausdehnung in Kurz- und Langzeitgedächtnis unterscheiden. Da in diesem Promotionsvorhaben ausschließlich tierexperimentelle Studien zu Formen des Langzeitgedächtnis (LZG) durchgeführt wurden, wird auf das Kurzzeitgedächtnis nicht näher eingegangen. Das LZG kann speziesübergreifend in drei Gedächtnisformen unterschieden werden, die co-existieren, konkurrieren und interagieren: das emotionale, das prozedurale und das explizite/deklarative Gedächtnis (Eichenbaum, 2002). Das prozedurale und das emotionale Gedächtnis werden dem so genannten impliziten Gedächtnis zugeordnet, das sich vom expliziten Gedächtnis darin unterscheidet, dass der Zugriff auf implizite Gedächtnisinhalte unbewusst abläuft, während die Verarbeitung und der Abruf expliziter Inhalte bewusst geschieht. In Dissoziationsstudien wird versucht, diese drei Gedächtnisformen zu differenzieren, deren Interaktionen zu untersuchen und die relevanten Gehirnstrukturen zu identifizieren. Bisher wird angenommen, dass die Amygdala für das emotionale Gedächtnis eine wichtige Rolle spielt, die als Schlüsselstruktur für die Wahrnehmung und die Expression von Emotionen betrachtet wird (LeDoux & Phelps, 2000).

Das prozedurale Gedächtnis umfasst z.B. das Erlernen von Verhaltenssequenzen, die in einer bestimmten zeitlichen Abfolge stehen müssen, motorische Fähig- und Fertigkeiten und auch Gewohnheiten. Das Striatum und das Cerebellum stellen Schlüsselstrukturen für diese Gedächtnisform dar. Beispiele für prozedurale Gedächtnisinhalte sind die Fähigkeit zum Gehen/Laufen oder Fahrrad fahren. Dies sind Fähigkeiten, die dem impliziten Gedächtnis zugeordnet werden, weil diese Gedächtnisinhalte nicht bewusst verarbeitet und abgerufen werden. Ein tierexperimentelles Paradigma zur Testung des prozeduralen Gedächtnis ist z.B. das T-Labyrinth. Hier wird eine Ratte oder Maus in dem langen Arm eines Labyrinths ausgesetzt, das die Form eines T hat. In einem der beiden anderen Arme befindet sich eine Futterbelohnung, und das Tier soll die Position des Futters erlernen. Es gibt

prinzipiell zwei Strategien, die das Tier bei der Futtersuche anwenden kann: eine räumliche (allozentrische) und eine prozedurale (egozentirsche) Strategie. Bei der räumlichen Strategie erlernt das Tier die Futterposition aufgrund von räumlichen Reizen aus der Umgebung, ähnlich dem Lernen im Wasserlabyrinth. Bei der prozeduralen Strategie, lernt das Tier, dass es an der Gabelung z.B. immer nach rechts gehen muss, um an das Futter zu gelangen. Für diese Strategie sind lediglich interne Stimuli, propriozeptive Reize, notwendig. Allerdings ist die prozedurale Strategie sehr unflexibel anwendbar und kann nur dann erfolgreich genutzt werden, wenn sich das Labyrinth und das Futter an fixen Positionen befinden. Dieses Beispiel verdeutlicht auch, dass eine bestimmte Aufgabe über unterschiedliche Lernstrategien und unter Beteiligung distinkter Gedächtnissysteme gelöst werden kann, denn für die Anwendung der räumlichen Strategie wird u.a. eine wichtige Rolle des Hippocampus diskutiert.

*Das explizite Gedächtnis*

Im Gegensatz zu impliziten Gedächtnisinhalten werden die Inhalte des deklarativen/expliziten Gedächtnis bewusst verarbeitet und abgerufen. Das explizite Gedächtnis umfasst das Wissen um Fakten und Ereignisse. Für das explizite Gedächtnis scheinen u.a. die parahippocampale Region (PH) und der Hippocampus eine wichtige Rolle zu spielen. Die PH wird als Konvergenzstation für Informationen aus den sensorischen Assoziationscortices und motorischen Cortices betrachtet. Die PH umfasst den ento-, peri- und postrhinalen (bei Primaten parahippocampal genannt) Cortex, die bereits verarbeitete Informationen aus den Assoziationscortices erhalten, weiter verarbeiten und mittelfristig zur Lösung bestimmter Aufgaben speichern. Der perirhinale Cortex z.B. scheint eine wichtige Rolle bei der Wiedererkennung von Objekten zu spielen (Winters & Bussey, 2005), während der postrhinale Cortex möglicherweise bei der Enkodierung der egozentrischen Position von Objekten beteiligt ist (Gaffan et al., 2004). Zwischen der PH und dem Hippocampus bestehen reziproke Verbindungen, v.a. durch den Tractus perforans, ausgehend vom entorhinalen und perirhinalen Cortex (Amaral & Witter, 1989). Dies legt die Vermutung nahe, dass in der PH Informationen aufbereitet werden und diese dann unter Beteiligung des Hippocampus weiter verarbeitet und möglicherweise langfristig gespeichert werden. D.h. der Hippocampus spielt nicht nur eine wichtige Rolle für das räumliche Gedächtnis und die Bildung der von O'Keefe

und Nadel postulierten kognitiven Landkarte (O'Keefe & Nadel, 1979), sondern auch für deklarative / explizite Gedächtnisinhalte (Manns & Eichenbaum, 2006).

Das explizite/deklarative Gedächtnis wird unterteilt in ein semantisches und ein episodisches Gedächtnis. Während das semantische Gedächtnis Wissen und Fakten umfasst, stellt das episodische Gedächtnis einen Speicher von autobiografischen Ereignissen und Erlebnissen dar. In tierexperimentellen Studien konnte nachgewiesen werden, dass auch Säugetiere Fakten erlernen und Wissen erwerben können. Z.B. im Falle des oben beschriebenen Wasserlabyrinths erlernen Ratten und Mäuse in mehreren Lerndurchgängen sukzessive die Position der Plattform – sie erlernen eine räumliche Strategie.

Die Bildung eines episodischen Gedächtnis jedoch bedarf, einer kognitivistischen Definition nach, eines autonoetischen Bewusstseins, eines Selbst und eines Empfindes für die subjektive Zeit (Tulving, 2002), um diese mentale Zeitreise beim Abruf episodischer Gedächtnisinhalte mit all seinen sensorischen und emotionalen Komponenten wieder zu erleben und als das eigene vergangene Erleben erkennen zu können. Untersuchungen zum episodischen Gedächtnis erfolgen beim Menschen durch Befragung des Probanden zu einer bestimmten Episode seines Lebens. Um objektivierbare Antworten zu erhalten, handelt es sich in der Regel um eine Testsituation, in der Items gelernt werden sollen. Die semantischen Inhalte werden abgefragt, indem der Proband die gelernten Items wieder geben soll. Die episodische Komponente zielt auf das Erinnern an die Testsituation in all seinen Fassetten und das Erlernen der Items selbst ab. Diese Methodik zur Differenzierung des episodischen und semantischen Gedächtnis ist jedoch meist nicht adäquat, da z.B. auch amnestische Patienten, bei denen Defizite im episodischen Gedächtnis beobachtet werden, auch Schwierigkeiten haben können neue semantische Inhalte zu lernen. D.h. diese Patienten erinnern sich oft auch nicht mehr an die Items einer gelernten Wortliste. In diesem Fall ist auch durch die Methode der Befragung keine eindeutige Differenzierung von semantischem und episodischem Gedächtnis möglich.

Das Mittel der verbalen Kommunikation zur Untersuchung des episodischen Gedächtnis kann naturgemäß zur Untersuchung von (Labor)Tieren nicht angewendet werden, da sie nicht zu einer Episode ihres Lebens befragt werden können. Daher berufen sich Wissenschaftler der tierexperimentellen Forschung auf Kernelemente der Definition Tulving’s, die die Komponenten des Bewusstseins und des Selbst

zunächst nicht berücksichtigt und das episodische Gedächtnis als Gedächtnis für singuläre Ereignisse (was) in einem zeitlichen (wann) und räumlichen (wo) Kontext beschreibt (Clayton & Dickinson, 1998). Diese Definition ist weitaus pragmatischer und auf die tierexperimentelle Grundlagenforschung anwendbar. Da es sich bei dieser Definition um eine reduzierte Form handelt, wird die Gedächtnisform als „episodic-*like* memory“ bezeichnet. In dieser Arbeit wird der Begriff episodisches Gedächtnis beibehalten und jeweils die Untersuchungsspezies mit benannt.

Der Nachweis des episodischen Gedächtnis außerhalb der Spezies Mensch erwies sich bisher im Wesentlichen aus zwei Gründen als schwierig: 1) Die zeitliche Komponente. In einigen Versuchen ist es gelungen zu zeigen, dass Säugetiere nicht nur die Gegenwart wahrnehmen, sondern auch eine Erinnerung an die Vergangenheit und die Fähigkeit zur Planung für die unmittelbare Zukunft besitzen (Zentall, 2005; Clayton et al., 2003). Dennoch erwies es sich als schwierig diese zeitliche Komponente (wann) in einer Aufgabe mit der räumlichen (wo) und der inhaltlichen (was) Komponente zu integrieren. 2) Die Differenzierung von semantischen und episodischen Inhalten, war aufgrund des Versuchsdesigns schwer zu gewährleisten. In den meisten bisherigen Versuchen wurden intensive Trainingsphasen integriert. Z.B. sollten Vögel (Buschhäher) lernen, dass eine Form der Nahrung nach Ablauf einer bestimmten Zeit verdirbt, während eine andere frisch bleibt (Clayton & Dickinson, 1998). Für eine andere Versuchsreihe war es u.a. notwendig, dass Ratten die Futterbelohnung aus einem kleinen Gefäß ausgraben (Ergorul & Eichenbaum, 2004). Dieses Verhalten mussten die Ratten zunächst erlernen, bevor die eigentliche Testung zum episodischen Gedächtnis begann, in der die Tiere die gelernten Verhaltensweisen anwenden sollten. Durch die Einführung von intensiven Trainingsphasen hat das Tier jedoch die Möglichkeit Regeln / Strategien zu lernen, ähnlich dem Wasserlabyrinth oder dem „shaping“ bei der operanten Konditionierung. Daher kann in diesen Paradigmen nicht mehr die Verwendung von Faktenwissen („semantischen“ Gedächtnisinhalten) oder prozeduralen Reaktionsstrategien, zur Lösung der Aufgabe ausgeschlossen werden. Das Problem der prozeduralen Reaktionsstrategie kann an folgendem Beispiel verdeutlicht werden: In dem oben erwähnten T-Labyrinth z.B. beruht die erste Aktion des Tieres auf einem spontanen Wendeverhalten in Richtung des einen oder anderen Armes. Wird dieses Prozedere wiederholt und eine Futterbelohnung in einem der beiden Arme konstant dargeboten, so entwickelt das Tier zunächst eine

allozentrische räumliche Repräsentation der Umgebung und dem Wissen um die Position der Belohung. Dies stellt ein Faktenwissen dar. Wird die Lernphase verlängert, so wird ein Verhaltenschema derart induziert, dass das Tier z.B. immer nach rechts geht, um die Belohnung zu erhalten. In diesem Fall handelt es sich sogar um ein prozedurales Lernen, dass u.a. über das Striatum vermittelt wird (Packard & McGaugh, 1996). D.h. je nach Dauer der Lernphase kann das Tier unterschiedliche Strategien erwerben um dieselbe Aufgabe zu lösen. Um das episodische Gedächtnis von Säugetieren zu untersuchen, sollten Tests herangezogen werden, die auf eine Trainings- oder „Shaping"-Phase verzichten können, was sich jedoch bisher als schwierig erwies. Dennoch arbeiten die Vertreter der tierexperimentellen Forschung mit Nachdruck an einem adäquaten Modell zur Untersuchung des episodischen Gedächtnis bei Labortieren (eine Übersicht geben Dere et al., 2006). Im Rahmen dieses Promotionsvorhabens wurde daher ein Paradigma entwickelt, das zum ersten Mal diese Gedächtnisleistung bei Ratten testen kann, und somit als neues Werkzeug zur Untersuchung des episodischen Gedächtnisses bei Ratten dienen soll.

Besonders explizite Gedächtnisinhalte unterliegen altersbedingten Veränderungen. Zu den bekanntesten Veränderungen gehört der Abbau von geistigen Fähigkeiten bei dementiellen Erkrankungen, wie Morbus Alzheimer. Zu den Symptomen der Alzheimer Demenz gehören unter anderem die Dysphasie und Agnosie sowie Defizite im episodischen Gedächtnis, die sich in einer räumlichen und zeitlichen Desorientierung äußern. Als Ursachen der Alzheimer Demenz werden genetische Ursachen, die u.a. Mutationen an den Genen, die das β-Amyloid Precurser Protein (APP) oder das Pesenilin kodieren, postuliert. Eine der einflussreichsten Theorien zur Genese von Morbus Alzheimer ist die Amyloid-Kaskaden-Theorie (Hardy & Allsop, 1991). Sie postuliert Abnormalitäten bei der Produktion und dem Abbau des APP, die dann wiederum zu Amyloid-Ablagerungen führen. Die Folge sind intraneuronale Veränderungen, die dann zur Neurodegeneration führen (Kosik & Coleman, 1992). Die Amyloid-Ablagerungen scheinen meist im Temporallappen zu beginnen (Hardy & Higgins, 1992), welcher wiederum, wie oben beschrieben, im Zusammenhang mit dem expliziten Gedächtnis diskutiert wird. Ein Grund für das besondere Interesse am vermeintlich rein-menschlichen episodischen Gedächtnis rührt unter anderem von der Beobachtung her, dass neurodegenerative Erkrankungen wie Morbus Alzheimer als eines der frühesten Symptome Defizite des episodischen Gedächtnis auslösen (Small et al.,

2003). Neben den oben beschriebenen zellulären Veränderungen, die als mögliche Ursache dementieller Erkrankungen diskutiert werden, können aber auch neurochemische Veränderungen beobachtet werden. Eine derzeit weitestgehend anerkannte Theorie zur Entstehung einiger Symptome bei Morbus Alzheimer Patienten ist die „cholinerge Hypothese“, die die Degeneration cholinerger Neurone des basalen Vorderhirns als Ursache der kognitiven Beeinträchtigungen postuliert (Araujo et al., 2005; Bartus, 2000; Robbins et al., 1997). Im Folgenden wird die Rolle des cholinergen basalen Vorderhirns bei Prozessen wie Aufmerksamkeit, Lernen und Gedächtnis kurz dargestellt.

### *Das cholinerge basale Vorderhirn*

Das cholinerge basale Vorderhirn (basal forebrain, BF) der Ratte besteht aus Substrukturen, die sich aufgrund ihrer Afferenzen und Efferenzen in die Regionen Ch1 bis Ch6 einteilen lassen (Mesulam et al., 1983) und sich funktional unterscheiden.

Im Zusammenhang mit den hier vorgestellten Arbeiten sind die Regionen Ch1, das mediale septum (MS) und die Ch4, die unter anderem den Nucleus basalis magnocellularis (NBM), der als Analogstruktur zum Nucleus basalis Meynart bei Primaten verstanden wird (Lehmann et al., 1980), und die Substantia innominata (SI) umfasst, besonders hervor zu heben. Die cholinergen Neurone der Ch1 projizieren zum Hippocampus, während die Neurone der Ch4 Region in einer topographischen Organisation alle Schichten des Neocortex innervieren und auch in die Amygdala projizieren.

Die Ch1 Neurone projizieren in den Hippocampus, der v.a. bei räumlichen Aufgaben eine wichtige Rolle spielt. Seit der Entdeckung der so genannten Platzzellen gilt der Hippocampus als Repräsentationsgebiet der „kognitiven Landkarte“ innerhalb des Säugetiergehirns (O'Keefe & Nadel, 1979), und bildet so die Grundlage für die Navigation im Raum, die Assoziation von Ereignissen jeder Art (internale und externe Stimuli) mit einer bestimmten Umgebung. So spielt der Hippocampus z.B. eine wichtige Rolle bei Lernaufgaben wie dem Wasserlabyrinth von Morris.

Darüber hinaus deuten Untersuchungen an Amnesie-Patienten, die eine Läsion im medialen Temporallappen aufweisen, deren berühmtester Vertreter H.M. darstellt, auf eine Schlüsselfunktion dieser Region, im Speziellen des Hippocampus,

für das autobiographische oder episodische Gedächtnis hin. H.M., z.B., wies nach bilateraler Resektion des medialen Temporallappens, inklusive Hippocampus und Amygdala, unter anderem eine schwere anterograde Amnesie auf. Folgestudien haben dann ergeben, dass der Hippocampus bei der Integration der räumlich-zeitlichen Komponenten eines Ereignisses eine Schlüsselrolle hat, und dass Läsionen des Hippocampus somit zu Defiziten episodischer Gedächtnisinhalte führen (Aggleton & Brown, 1999). Dem Hippocampus wird auch bei Primaten und Ratten eine wichtige Rolle bei der Integration räumlicher, zeitlicher und inhaltlicher Komponenten eines Ereignisses zugesprochen (Manns & Eichenbaum, 2006). Es ist bekannt, dass die Modulation der cholinergen Transmission im Hippocampus Lernen und Gedächtnisprozesse bei Ratten (Sarter & Parikh, 2005c) und auch das episodische Gedächtnis beim Menschen beeinflusst (Hasselmo et al., 1996).

Die Projektionen der Ch4 in den Cortex modulieren Aufmerksamkeitsressourcen zur Optimierung des Signal-Rausch-Verhältnisses um ein Signal vor dem Hintergrund von irrelevanten Information zu detektieren (Sarter et al., 2005a). Cholinerge Projektionen in den Neocortex spielen damit eine wichtige Rolle bei allen Aufgaben, die Aufmerksamkeit verlangen, und eine Störung der cholinergen Transmission ist mit defizitärer Informationsverarbeitung verbunden (Sarter et al., 2005b; Sarter & Bruno, 1999).

Die Amygdala hat eine zentrale Bedeutung für emotionale Prozesse, da hier sensorische Informationen (z.B. Angst auslösende Stimuli) integriert und die motorischen und vegetativen Reaktionen auf diese Reize initiiert werden (LeDoux & Phelps, 2000; LeDoux & Muller, 1997). Die basaloamygdaloiden Projektionen der Ch4 modulieren Lern- und Gedächtnisprozesse in Konditionierungsaufgaben und konkurrieren scheinbar mit dem Hippocampus bei der Bewältigung dieser Aufgaben, wie sich in einer Reihe von Studien zur konditionierten Platzpräferenz zeigte (für eine Übersicht siehe Gold, 2003).

Die obigen Ausführungen deuten auf eine zentrale Rolle des cholinergen BF bei der Modulation von Aufmerksamkeitsressourcen und der Gedächtnisbildung hin. Die cholinergen Neurone des BF werden unter anderem von Substanz Pergen Terminalen innerviert, deren Ursprung größtenteils im Nucleus accumbens liegt (Napier et al., 1995; Zaborszky et al., 1991). Immunohistochemische und -cytochemische Analysen weisen darauf hin, dass sich funktionale NK-1 Rezeptoren auf cholinergen Neuronen des BF befinden (Chen et al., 2001; Gerfen, 1991). Die

Verabreichung des Substanz P (SP) analogen Stoffes, SP Methyl-Esther, erleichtert die cholinerge Transmission im Hippocampus in vitro (Kouznetsova & Nistri, 2000) und die Verabreichung von SP in den NBM erhöht die ACh-Konzentration im Frontalcortex der anästhesierten Ratte (De Souza Silva et al., 2000). Diese Befunde deuten auf einen modulatorischen Einfluss von Neurokinen auf die Aktivität cholinerger Neurone des BF bzw. in Zielstrukturen des BF hin. Neurokinine wie SP könnten auf diesem Wege auch Lern- und Gedächtnisprozesse beeinflussen. Im Folgenden wird eine Übersicht zu den bisherigen Befunden der Verhaltenseffekte von SP und der Inaktivierung des NK-1 Rezeptors gegeben.

### *Effekte von Substanz P und der Inaktivierung des Neurokinin-1 Rezeptors*

Substanz P (SP) ist ein Neuropeptid bestehend aus 11 Aminosäuren und gehört zur Klasse der Tachykinine. Im Zentralnervensystem (ZNS) von Säugetieren werden sie als Neurokinine (NK) bezeichnet. Bisher wurden sechs verschiedene NK nachgewiesen, die sich aufgrund ihrer gemeinsamen Aminosäuresequenz am C-Terminal klassieren lassen. SP ist das älteste bekannte (von Euler & Gaddum, 1931) NK und bindet an spezifische NK-Rezeptoren (NK-R), derer bisher drei verschiedene Typen identifiziert wurden, die im ZNS in unterschiedlicher Dichte und Verteilung vorkommen. SP weist die höchste Affinität zum NK-1 Rezeptor (NK-1-R) auf, bindet jedoch auch an den NK-2 und NK-3-R.

Die Verteilung der SP Terminale und die Präsenz von NK-1-Rezeptoren in Strukturen wie Amygdala, Cortex, Hippocampus und dem BF (Maeno et al., 1993) deuten auf den modulatorischen Einfluss von SP bei affektiven Prozessen, Gedächtnisbildung und Angstmechanismen hin (für eine Übersicht siehe Hasenöhrl et al., 2000). Diese Prozesse werden möglicherweise über eine Interaktion mit dem NK-1-R beeinflusst werden.

In einer Reihe von Studien wurde gezeigt, dass SP bei zentraler Applikation in den NBM eine anxiolytische Wirkung bei Ratten hat (Echeverry et al., 2001; Hasenöhrl et al., 1998), die durch die Verabreichung des NK-1-R Antagonisten Win 51,708 abgeschwächt werden konnte (Nikolaus et al., 1999). Es wurden aber auch anxiogene Effekte bei zentraler und systemischer Applikation von SP beobachtet (Gavioli et al., 1999; Aguiar & Brandão, 1996; Teixeira et al., 1996). Diese Diskrepanz kann auf unterschiedlichen Wirkprofilen der SP-Metaboliten beruhen. Während bei Primanten die systemische Applikation des N-terminalen Fragments

anxiolytische Effekte aufwies (Barros et al., 2002), konnte bei Nagetieren nach Injektion in den NBM jedoch sowohl durch das N- als auch das C-terminale Fragment eine anxiolytische Wirkung erzielt werden (Nikolaus et al., 2000). Verabreichungen in das dorsale periaquäductale Grau (PAG) haben sich jedoch als anxiogen erwiesen (de Araújo et al., 1999; de Araújo et al., 1998). Trotz der uneindeutigen Befundlage bei Studien mit NK Agonisten, weisen Versuche zum NK-1-R Antagonismus durch NK-1-R Blockade (McLean, 2005; Rupniak et al., 2003; Loiseau et al., 2003; Lieb et al., 2000; File, 2000; Vassout et al., 2000; Teixeira et al., 1996) oder durch Verwendung von knock-out Mäusen (Rupniak et al., 2000) auf einen anxiolytischen Effekt hin.

Auch bei der Genese der Depression scheint SP eine Rolle zu spielen, die jedoch nicht eindeutig geklärt ist, da Untersuchungen an depressiven Patienten uneinheitliche Befunde erzielt haben (McLean, 2005; eine Übersicht geben z.B. Herpfer & Lieb, 2005). Während in einigen Studien eine erhöhte SP-Konzentration in der Cerebrospinalflüssigkeit (CSF) depressiver Patienten nachgewiesen wurde (Bondy et al., 2003; Rimon et al., 1984), konnten in anderen Untersuchungen keine Veränderung nachgewiesen werden (Deuschle et al., 2005; Berrettini et al., 1985). Dennoch lieferten einige präklinische Studien solide antidepressive Effekte durch Inaktivierung des NK-1-R, entweder durch genetische knock-outs oder durch Verabreichung von NK-1-R Antagonisten (Rupniak et al., 2001; Rupniak & Kramer, 1999; Kramer et al., 1998). In klinischen Studien hat sich zunächst der NK-1-R Antagonist Aprepitant (auch bekannt als MK-869) bewährt (Kramer, 2000) und die Applikation eines weiteren NK-1-R Antagonisten L-759274 für eine Dauer von sechs Wochen führte ebenfalls zu einer signifikanten Verbesserung der Depressions-Symptomatik (Kramer et al., 2004). Der Wirkmechanismus der NK-1-R Inaktivierung zur Behandlung von depressiven Erkrankungen ist jedoch bislang nicht geklärt. Da die Behandlung mit klassischen Antidepressiva keinen Einfluss auf die SP Konzentration im Serum zu haben scheint und die SP-Konzentration in der CSF eher noch erhöht (Deuschle et al., 2005), wird ein modulatorischer Effekt über serotonerge (Haddjeri & Blier, 2001; Sergeyev et al., 1999) oder noradrenerge (Hahn & Bannon, 1998) Mechanismen zur Schwächung der Symptomatik bei affektiven Erkrankungen nicht ausgeschlossen. Neuere Untersuchungen deuten ebenfalls auf eine Beteiligung des NK-2-R bei der Ätiologie affektiver Erkrankungen und eine therapeutische

Implikation von NK-2-R Antagonisten hin (Herpfer & Lieb, 2005; Steinberg et al., 2001).

Seit der Entwicklung von selektiven und spezifischen nicht-peptidergen NK-R Antagonisten richtet sich der Focus der präklinischen und klinischen Studien auf die Untersuchung der Rolle derartiger Antagonisten bei affektiven Störungen. Es ist jedoch auch bekannt, dass die systemische oder zentrale Applikation von SP in den NBM das Lernen bei Ratten verbessert (Huston & Hasenöhrl, 1995; Hasenöhrl et al., 1994; Pelleymounter et al., 1988; Schlesinger et al., 1986; Schlesinger et al., 1983a; Schlesinger et al., 1983b). Bisherige Untersuchungen beschränkten sich neben der Verabreichung des Gesamtmoleküls auf die systemische oder zentrale Applikation seiner Metaboliten, d.h. dem C-terminalen oder N-terminalen Fragment. Während das C-terminale Fragment vor allem eine Rolle bei den verstärkenden Effekten von SP zu spielen scheint, wird das N-terminale Fragment für die Modulation der Lern- und Gedächtnisprozessen durch SP verantwortlich gemacht (Hasenöhrl et al., 2000). So wurde gezeigt, dass SP (1-7) passives Vermeidungslernen nach systemischer (Hasenohrl et al., 1990) oder zentraler Injektion in den NBM verbessert (Gerhardt et al., 1992). Welche Rezeptoren aber an der Vermittlung der promnestischen Effekte von SP und seinem Animofragment beteiligt sind, ist nicht bekannt. Daher wurde im Rahmen dieser Dissertation durch die Anwendung eines selektiven und spezifischen NK-1-R Antagonisten die Rolle des NK-1-R bei Lern- und Gedächtnisprozessen sowohl auf der Verhaltensebene als auch neurochemisch untersucht.

### *Fragestellungen*

In diesem Promotionsvorhaben wurde zunächst untersucht, ob die systemische Verabreichung des selektiven und spezifischen NK-1-R Antagonisten SR140333 einen Einfluss auf die extrazelluläre ACh-Konzentration in drei Zielstrukturen des BF, dem Frontalcortex, der Amygdala und dem Hippocampus, hat. Denn für das cholinerge BF wird eine zentrale Rolle bei Aufmerksamkeits-, Lern- und Gedächtnisprozessen postuliert und es gibt Hinweise auf eine Interaktion von Neurokinen und cholinergen Neuronen des BF.

Weiterhin wurde untersucht, ob sich die neurochemischen Befunde, die im Rahmen dieses Promotionsvorhabens beobachtet wurden, auch auf der Verhaltensebene manifestieren. Dazu wurden drei Lern- und Gedächtnistest herangezogen: 1) Die Habituation an eine neue Umgebung. Denn für diese Aufgabe

spielt unter anderem der Hippocampus eine Rolle. Es wurde gezeigt, dass die hippocampale cholinerge Aktivität während des Habituationslernens erhöht war und dass diese Erhöhung mit der Gedächtnisleistung korrelierte (Thiel et al., 1998). 2) Das passive Vermeidungslernen. Untersuchungen der neurochemischen Prozesse, die zur Lösung dieser Aufgabe notwendig sind, deuten auf eine sequenzielle Beteiligung von Hippocampus, Amygdala, enthorhinalem und parietalen Cortex bei der Bildung und dem Abruf der Lerninhalte hin (Izquierdo et al., 1997). 3) Ein Paradigma zur Untersuchung des episodischen Gedächtnis, dass im Rahmen dieses Promotionsvorhabens entwickelt wurde.

# 2 Überblick über die Methoden

Die detaillierte Beschreibung der verwendeten Methoden kann den einzelnen Originalarbeiten entnommen werden. Daher soll hier nur eine kurze Übersicht dieser Methoden gegeben werden.

***Neurochemie***

Zur Bestimmung der extrazellulären ACh-Konzentration wurde die Methode der *in vivo* Mikrodialyse in Kombination mit der Hochleistungs-Flüssigkeits-Chromatographie (high-perfromance liquid chromatography, HPLC) verwendet. Detaillierte Beschreibungen der Mikrodialyse-Prozedur finden sich bei Boix et al. (1994), während die HPLC-Analytik im Wesentlichen auf Damsma et al. (1987) zurück geht. In der *in vivo* Mikrodialyse wurden zunächst drei Proben á 20 Minuten erhoben, um die Ausgangslage des ACh-Spiegels zu bestimmen. Die unterschiedlichen Dosierungen, 0 (Vehikellösung, 0,01% Tween 80), 1, 3 oder 9mg/kg, des NK-1-R Antagonisten SR140333 wurden über eine fixierte Kanüle ins Peritoneum appliziert (i.p.) und die Veränderung der ACh-Konzentration ipsilateral im Frontalcortex, der Amygdala und dem Hippocampus bei anästhesierten Ratten über einen Zeitraum von einer Stunde gemessen. Anschließend wurde das Tier letal anästhesiert und zwecks Validierung der Kanülenplatzierung perfundiert. Die ausgewählten Hirnstrukturen stellen die drei Hauptprojektionsgebiete des

cholinergen BF dar. Es wurden anästhesierte Tiere gewählt, da die ACh-Konzentrationen v.a. im Frontalcortex mit dem Aktivitätsniveau schwanken.

***Behaviorale Tests***

Zur Untersuchung der Lern- und Gedächtnisleistung wurde zunächst ein Paradigma zum passiven Vermeidungslernen gewählt. Es wurde eine Apparatur verwendet, die aus einem hellen und einem dunklen Abteil bestand, die durch eine Guillotinentür voneinander getrennt waren (Step-through Anordnung) (Bures et al., 1983). Im Lerndurchgang wurde die Ratte in das helle Abteil gesetzt und die Zeit bis zum Übertritt in das dunkle Abteil gemessen. Die Guillotinentür wurde dann hinter dem Tier verschlossen und ein Fußschock verabreicht. Anschließend wurde das Tier aus der Apparatur genommen und erhielt je nach Gruppenzugehörigkeit eine i.p.-Injektion der Vehikellösung (0,01% Tween 80), oder der Testsubstanz SR140333 in einer Dosis von 1, 3 oder 9mg/kg. Da der Einfluss des NK-1-R Antagonisten SR140333 auf das Langzeitgedächtnis untersucht werden sollte, wurden Testdurchgänge 24 Stunden und 14 bzw. 15 Tage nach dem Lerndurchgang durchgeführt. Die Operationalisierung der Gedächtnisleistung erfolgte durch die Differenz der Übertrittslatenzen in das dunkle Abteil zwischen Lern- und Testdurchgang. Dieser Test basiert auf dem nativen Vermeidungsverhalten von Ratten vor hellen, offenen Räumen.

In einem weiteren Test wurde der Einfluss von SR140333 auf die Habituation an ein Offenfeld untersucht. Dieser Test bestand aus einer Lern- und einer Testphase, die identisch abliefen und 24 Stunden auseinander lagen. Das Tier wurde in das Offenfeld gesetzt und konnte für einen Zeitraum von 10 Minuten frei explorieren. Im Anschluss an die Lernphase erhielt die Ratte je nach Gruppenzugehörigkeit eine einmalige i.p.-Injektion (Vehikellösung, 1, 3, oder 9mg/kg SR140333). Das Verhalten des Tieres wurde in der Lern- und Testphase aufgezeichnet und post-hoc ausgewertet. Die Habituationsleistung wurde anhand der Differenz zwischen Test- und Lernphase in den Parametern Anzahl des Aufrichtverhaltens und zurückgelegte Strecke ermessen. Diese Aufgabe bedarf weder appetetiver noch aversiver Reize.

Die Messung der episodischen Gedächtnisleistung wurde durch die Kontaktzeiten mit Objekten in einem Paradigma der Objektexploration operationalisiert. Die Aufgabe bestand aus zwei Lern- und einem Testdurchgang, die

je 5 Minuten lang waren und je 50 Minuten auseinander lagen. In den beiden Lerndurchgängen wurden jeweils vier gleiche Objekte an bestimmten Positionen des Offenfeldes dargeboten. Die Objekte unterschieden sich jedoch zwischen den beiden Lerndurchgängen. Im Testdurchgang wurden je zwei Duplikate der in den beiden Lerndurchgängen dargebotenen Objektes erneut präsentiert. Je eines der Objekte befand sich auf einer bereits bekannten Position und das andere war räumlich verschoben. Die Behandlung der Ratten erfolgte 30 Minuten vor dem ersten Lerndurchgang, falls eine Behandlung vorgesehen war. Je nach Experiment waren unterschiedliche Behandlungen vorgesehen: In den Validierungsexperimenten gab es Versuchgruppen ohne Behandlung und solche, die entweder physiologische Kochsalzlösung oder 15mg/kg des partiellen N-Methyl-D-Aspartat-Rezeptor Agonisten D-Cycloserin (DCS) erhielten; in der Studie zur Rolle des NK-1-R beim episodischen Gedächtnis wurden Versuchsgruppen ohne Behandlung oder mit einer Injektion der Vehikellösung (0,01 % Tween 80), 1, 3 oder 9mg/kg SR140333 eingeführt. Die Aufgabe zur Untersuchung des episodischen Gedächtnis wurde im Rahmen des Promotionsvorhabens entwickelt und erfolgreich angewendet. Es wird angenommen, dass zur Lösung dieser Aufgabe die Integration verschiedener Dimensionen der Objektinformationen –*was* wurde *wann* und *wo* enkodiert- notwendig ist. Es wurden Objekte verwendet, die keine ethologische Relevanz für Ratten besaßen. Es sind zur Lösung ebenfalls keine appetetiven oder aversiven Reize notwendig, sondern lediglich das native Explorationsverhalten der Ratten. Dieses Paradigma bietet die Möglichkeit Mechanismen des episodischen Gedächtnisses am Tiermodell zu untersuchen.

Zusätzlich zu den Lern- und Gedächtnistests wurde zur Untersuchung eines eventuell vorliegenden Suchtpotentials auch eine Studie zu den verstärkenden Eigenschaften des NK-1-R Antagonisten SR140333 durchgeführt. Zu diesem Zweck wurde das „Corral Maze“ (Hasenohrl et al., 1989) verwendet. Bei diesem Paradigma handelt es sich um ein homogenes, rundes Offenfeld, das in Konditionierungsquadranten aufteilbar ist. Es wird angenommen, dass durch die Homogenität *a priori* vorliegende Präferenzen ausgeschlossen werden können, so dass eine Balancierung der Konditionierungsloci - wie dies in anderen Paradigmen wie der „two-compartment box“ der Fall ist - nicht mehr notwendig ist. Diese Aufgabe bestand aus fünf Durchgängen, einem Durchgang pro Tag. Im ersten Durchgang wurden Ausgangswerte ermittelt und die Tiere konnten sich frei in der Apparatur

bewegen. In den Durchgängen 2-4, den Konditionierungsdurchgängen, wurden die Trennwände eingesetzt. Das Tier erhielt je nach Gruppenzugehörigkeit 30 Minuten vor dem jeweiligen Durchgang eine i.p.-Injektion der Vehikellösung (0,01% Tween 80), 1, 3 oder 9mg/kg SR140333. Das Tier wurde an den Tagen 2-4 in den gleichen Quadranten gesetzt und konnte diesen auch nicht verlassen. Am fünften Tag, dem Testdurchgang, hatten die Tiere wieder freien Zugang zum gesamten Offenfeld. Die Verstärkung durch SR140333 wurde durch die Veränderung der Aufenthaltszeit im Konditionierungsquadranten zwischen dem ersten und dem letzten Tag operationalisiert.

# 3 Zusammenfassung und Diskussion der Ergebnisse

Auf den folgenden Seiten werden die Ergebnisse der vorgestellten Experimente kurz zusammen gefasst, in den aktuellen wissenschaftlichen Kontext eingeordnet und ein Ausblick gegeben.

### *Zusammenfassende Diskussion der Ergebnisse*

Die neurochemischen Effekte einer i.p. Applikation des NK-1-R Antagonisten SR140333 wurden im Rahmen einer *in vivo* Mikrodialyse Studie am anästhesierten Tier eruiert. In dieser Studie wurde die extrazelluläre ACh-Konzentration im Frontalcortex, der Amygdala und dem Hippocampus simultan untersucht. Im Frontalcortex und der Amygdala wurden keine Veränderungen gemessen. Im Hippocampus jedoch ergab die Analyse der ACh-Konzentration einen dosisabhängigen Anstieg, der innerhalb von 60 Minuten nach Applikation wieder das Ausgangsniveau erreicht hatte (1).

Substanzen, die den ACh-Spiegel gerade auch im Hippocampus erhöhen, sind potentiell als Nootropica interessant. Daher wurde eine Reihe von Gedächtnistests mit systemischer Applikation von SR140333 durchgeführt. SR140333 erwies sich dabei als schwach promnestisch im Habituationslernen. Beim passiven Vermeidungslernen konnte bei der Testung nach 14 Tagen eine bessere Gedächtnisleistung im Vergleich zur Kontrollgruppe nachgewiesen werden. Mit dem

Paradigma der konditionierten Platzpräferenz konnte für SR140333 keine verstärkende Wirkung gezeigt werden (1).

Im Rahmen dieses Promotionsvorhabens wurde ein Objektexplorationsparadigma zur Untersuchung des episodischen Gedächtnis entwickelt, das eine Variante des Paradigmas zur Messung des episodischen Gedächtnis bei Mäusen (Dere et al., 2005) darstellt. Mit diesem Objektexplorationsparadigma konnte zum ersten Mal hinreichend das episodische Gedächtnis bei Ratten nachgewiesen werden (2). In Validierungsexperimenten zu diesem Test konnten nach der Injektion von physiologischer Kochsalzlösung, die als übliche Kontrolllösung in pharmakologischen Studien gilt, replizierbare Defizite des episodischen Gedächtnis bei Ratten beobachtet werden (2). Diese Defizite konnten jedoch teilweise durch eine einmalige systemische Applikation von D-Cylcoserin (DCS), einem partiellen N-Methyl-D-Aspartat (NMDA) - Rezeptor Agonisten für die Glycin-Bindungsstelle, aufgehoben werden (2).

Anschließend wurde der Einfluss von SR140333 auf das episodische Gedächtnis bei Ratten getestet. SR140333 wurde einmalig vor dem ersten Lerndurchgang systemisch appliziert. Als Kontrollgruppen dienten eine Gruppe ohne Behandlung, als Positivkontrolle für den Versuchsaufbau, und eine Gruppe, die mit dem Lösungsmittel der Wirksubstanz, 0,01%ige Tween 80-Lösung, behandelt wurde. Wie bereits in Vorstudien beobachtet wurde (2), zeigten die unbehandelten Tiere eine normale Performanz in der Aufgabe zum episodischen Gedächtnis, während die Tiere, die eine Kontrollinjektion erhielten, erneut Defizite aufwiesen (3). Die Verabreichung des NK-1-R Antagonisten SR140333 in einer geringen Dosierung (1mg/kg SR140333, i.p.) kompensierte das Defizit jedoch vollständig (3), und war damit auch DCS in dieser Aufgabe überlegen.

Bei den Tieren, die eine höhere Dosierung des Antagonisten erhielten, 3mg/ kg und 9mg/kg SR140333, sind in der Studie zum episodischen Gedächtnis dosisabhängig Nebenwirkungen aufgetreten. Diese Tiere zeigten Stereotypien, eine bogenförmige Überstreckung des Körpers und ein stark vermindertes Explorationsverhalten, weshalb diese beiden Gruppen von der weiteren statistischen Analyse ausgeschlossen wurden. Eine mögliche Erklärung für diese Nebenwirkungen sind abdominale Krämpfe. In einigen Studien, die den Einfluss von NK-1-R Antagonisten auf die Darmaktivität untersuchten, wurde gezeigt, dass einige NK-1-R Antagonisten partiell agonistische Effekte aufweisen, w.z.B.

Dickdarmkontraktionen (Bailey & Jordan, 1984) und eine erhöhte Aktivität des Ileums (Featherstone et al., 1986; Hawcock et al., 1982). Die in der Studie zum episodischen Gedächtnis aufgetretenen Nebenwirkungen konnten jedoch in den vorangegangenen Verhaltensstudien mit SR140333 nicht beobachtet werden. In Folgestudien sollten diese Nebenwirkungen jedoch genauer untersucht werden.

Die hier dargestellten Ergebnisse zu den neurochemischen und behavioralen Effekten des NK-1-R Antagonisten SR140333 erscheinen zunächst überraschend und im Widerspruch zu den bisherigen Befunden, die mit einem endogenen Agonisten des NK-1-R, SP, erzielt wurden. Denn SP scheint promnestische, anxiolytische, verstärkende und neurogenerative Eigenschaften zu haben. Diese Effekte sind jedoch einerseits abhängig von Applikationsort und Dosis und andererseits auch spezifisch für die aktiven Metaboliten von SP (eine Übersicht geben Hasenöhrl et al., 2000). SP unterliegt nach seiner Ausschüttung in den synaptischen Spalt einer schnellen Metabolisierung durch Endopeptidasen in ein N- und ein C-terminales Fragment, deren Aminosäuresequenzlänge variabel ist (Couture & Regoli, 1981). Wie eingangs bereits erwähnt scheint das C-terminale Fragment die verstärkenden Effekte von SP zu vermitteln, während das N-terminale Fragment für die Modulation der Lern- und Gedächtnisprozesse durch SP verantwortlich gemacht wird (Hasenöhrl et al., 2000). Der scheinbare Widerspruch, dass sowohl ein Agonist, SP bzw. das Aminofragment von SP, und ein selektiver NK-1-R Antagonist, SR140333, über den gleichen Rezeptor promnestische Effekte vermitteln, kann durch folgende Befunde erklärt werden: Für das Aminofragment wurde eine spezifische Bindungsstelle, der so genannte SP-N Rezeptor, nachgewiesen (Igwe et al., 1990; Hanley et al., 1980) über den die Verhaltenseffekte des N-terminalen Fragments vermittelt werden. Es wurde gezeigt, dass das Aminofragment die Nocizeption durch SP in Mäusen antagonisiert (Kreeger & Larson, 1996; Yukhananov & Larson, 1994). D.h. möglicherweise beruhen die promnestischen Effekte des Aminofragments von SP auf intrazellulären Prozessen, ausgelöst durch die SP-N Bindungsstelle, die die Effekte des Gesamtmoleküls zu antagonisieren scheinen. Durch einen solchen Mechanismus würden die beobachteten Effekte von SP und dem N-terminalen Fragment von SP nicht mit den in dieser Dissertation vorgestellten promnestischen Effekten nach Verabreichung eines spezifischen und selektiven NK-1-R Antagonisten SR140333 in Widerspruch stehen.

In den drei hier behandelten Gedächtnistests, der passiven Vermeidung, der Habituation an eine neue Umgebung und dem episodischen Gedächtnis, wurden unterschiedlich starke promnestische Effekte nach i.p.-Applikation des NK-1-R Antagonisten SR140333 beobachtet. Abgesehen von den unterschiedlichen Anforderungen der verwendeten Tests, erfolgte auch die Substanzverabreichung zu unterschiedlichen Zeitpunkten. Bei den Aufgaben zur Habituation an eine neue Umgebung und beim passiven Vermeidungslernen wurde der NK-1-R Antagonist nach dem jeweiligen Lerndurchgang verabreicht. Auf diese Weise können die durch SR140333 verursachten Effekte weitestgehend auf den Prozess der Konsolidierung der gelernten Inhalte beschränkt werden. Bei einer Applikation vor dem Lerndurchgang, wie bei der Aufgabe zum episodischen Gedächtnis, ist keine klare Eingrenzung möglich, d.h. SR140333 kann die Akquisition, die Konsolidierung oder sogar den Abruf beeinflusst haben. Ausgehend von den neurochemischen Effekten von SR140333 erscheint zumindest ein Einfluss auf den Abruf als unwahrscheinlich, da der ACh-Spiegel nach ca. 60 Minuten wieder das Ausgangsniveau erreicht hat (1) und der Abruf der Informationen aus den beiden Lerndurchgängen erst 140 Minuten nach Substanzgabe im Testdurchgang erforderlich war (3). Der NK-1-R Antagonist SR140333 scheint sowohl die Akquisition als auch die Konsolidierung von Lerninhalten zu beeinflussen. Die relative Bedeutung des NK-1-R Antagonismus für die unterschiedlichen Phasen der Gedächtnisbildung, kann jedoch aufgrund der hier vorgestellten Ergebnisse nicht beantwortet werden. Zur Klärung dieser Frage sollte die Substanzverabreichung in einem konstanten Paradigma, z.B. dem passiven Vermeidungslernen, zu unterschiedlichen Zeitpunkten erfolgen.

In beiden Studien zum episodischen Gedächtnis bei Ratten deuten die Ergebnisse der Gruppen, die mit einer Kontrolllösung behandelt wurden, darauf hin, dass Stressoren, wie die Injektionsprozedur selbst, die erfolgreiche Lösung dieser Aufgabe beeinträchtigen (2 und 3). Diese Tiere differenzierten in der Testphase nicht zwischen den wahrgenommen Objekten, sondern wiesen eine Gleichverteilung der Explorationszeiten über alle vier Objekte auf. Daher wird angenommen, dass die Verabreichung von SR140333 vor dem Lerndurchgang die Stressreaktion, verursacht durch die Injektionsprozedur, kompensiert haben könnte. Hinweise auf einen modulatorischen Einfluss von Neurokininen und dem NK-1-R auf Stressreaktionen stützen sich auf eine ganze Reihe von Befunden. NK-1-R Antagonisten können unter anderem die Aktivität der Hypthalamus-Hypophysen-Nebennierenrinden-(nach dem

englischen hypothalamus-pituitary-adrenal, HPA) Achse direkt modulieren. Es wurden funktionale NK-1-R im Nebennierenmark nachgewiesen, das SPerge Afferenzen erhält (Hinson & Kapas, 1996; Hinson et al., 1994). SP hat exzitatorische Effekte auf die Nebennierenrinde und erleichtert die Sekretion von Glukokortikoiden, w.z.B. Kortikosteron, und wird als Reaktion auf wahrgenommene Stressoren, wie Fußschock, ausgeschüttet (Vaupel et al., 1988). Bei gesunden Probanden konnten nach intravenöser Verabreichung von SP Schlafstörungen, ein verschlechterter Gemütszustand und ein Anstieg der SP-Konzentration im Serum beobachtet werden (Lieb et al., 2002). Ausgehend von diesen Beobachtungen wird hypothetisiert, dass NK-1-R Antagonisten die Stress-Antwort auf Stressoren abschwächt (für eine Übersicht siehe Rupniak, 2005; Steinberg et al., 2002; Bester et al., 2001; Altier & Stewart, 1999). Folglich könnte die Verabreichung von SR140333 in einer geringen Dosierung die Stressreaktion, verursacht durch die Injektionsprozedur, abgeschwächt oder kompensiert haben und so zur Wiederherstellung des episodischen Gedächtnis bei Ratten beigetragen haben.

Eine weitere Erklärung für die in dieser Dissertation vorgestellten Befunde, ist eine zentrale Wirkung des NK-1-R Antagonisten. NK-1-R wurden auf inhibitorischen Interneuronen im Hippocampus nachgewiesen (Sloviter et al., 2001), welcher von SPergen Terminalen unter anderem über eine septo-hippocampale Projektion direkt innerviert wird (Peterson & Shurlow, 1992). Die Erhöhung der Transmission inhibitorischer Interneurone auf Pyramidalzellen durch SP wird durch SR140333 geblockt (Ogier & Raggenbass, 2003). Die intra-septale Injektion des GABA-A Agonisten Muscimol löste Defizite in einer Aufgabe zur passiven Vermeidung aus, die durch intra-hippocampale Verabreichung von Acetylcholin-Esterase-Hemmer Physostigmin abgeschwächt wurden (Degroot & Parent, 2001). Da SR140333 nach systemischer Verabreichung den extrazellulären ACh-Spiegel im Hippocampus erhöhte (1), wird hypothetisiert, dass der NK-1-R Antagonist SR140333 promnestische Effekte durch Hemmung der Transmission inhibitorischer Interneurone und der daraus resultierenden Erhöhung der cholinergen Transmission vermittelt. Es ist bekannt, dass die Modulation cholinerger Transmission im Hippocampus Lernen und Gedächtnis in einer Reihe von Verhaltenstest beeinflusst (Sarter & Parikh, 2005), unter anderem das episodische Gedächtnis (Hasselmo et al., 1996). Folglich könnte die Kompensation der Defizite im episodischen Gedächtnis bei Ratten (3) durch eine Erhöhung der extrazellulären ACh-

Konzentration im Hippocampus nach Verabreichung des NK-1-R Antagonisten (1) erklärt werden. Da es sich bei den im Rahmen dieses Promotionsvorhabens durchgeführten Studien, um Versuche mit systemischer Applikation handelt, ist keine klare Eingrenzung der beteiligten Gehirnstrukturen möglich. Zur Erklärung der hier beschriebenen Befunde sind darüber hinaus jedoch auch andere Mechanismen als die Modulation der cholinergen Transmission möglich, w.z.B. ein Einfluss auf andere Neurotransmitter-Systeme, wie das glutamaterge oder serotonerge System, und bedürfen weiterer Untersuchungen.

Zusammenfassend lässt sich festhalten, dass die systemische Verabreichung von NK-1-R Antagonist SR140333 promnestische Effekte in drei unabhängigen Tests vermittelte, und dass diese Effekte möglicherweise durch die beobachtete Erhöhung im extrazellulären hippocampalen ACh-Spiegel erklärt werden können. Aus der Studie zum episodischen Gedächtnis bei Ratten ging hervor, dass die stress-induzierten Defizite, die in der Kontrollgruppe beobachtet wurden, durch eine einmalige Injektion von SR140333 vollständig kompensiert wurden. Diese Effekte können einerseits durch eine Modulation der extrazellulären ACh-Konzentration im Hippocampus oder aber durch einen direkten Einfluss auf die HPA-Achse erklärt werden. Außerdem konnten für SR140333 keine verstärkenden Effekte nachgewiesen werden. Die hier vorgestellten Effekte auf die Gedächtnisleistung von Ratten deuten auf solide Gedächtnis verbessernde Effekte durch den NK-1-R Antagonismus hin, und liefern erste Hinweise für einen potenziellen therapeutischen Nutzen der Substanzklasse der NK-1-R Antagonisten bei der Behandlung dementieller Erkrankungen wie der Alzheimer Demenz.

### *Ausblick*

In der letzten Dekade ist der Anteil an Studien, die die Rolle der Neuromodulatoren, wie SP, Neuokinin A und B, Cholezystokinin, Somatostatin oder Enkephalin, die mit klassischen Neurotransmittern, wie ACh, Serotonin, Dopamin oder GABA, ko-lokalisiert sind, stark gestiegen. Ein Grund wird sicherlich in den verbesserten technischen Nachweismöglichkeiten dieser Peptide, aber auch in der Erkenntnis, dass diese Neuromodulatoren eine eigenständige Rolle bei vielen Verhaltenseffekten spielen, liegen. Die Ergebnisse, die im Rahmen dieses Promotionsvorhabens gesammelt wurden, deuten auf promnestische Effekte durch NK-1-R Antagonismus hin. Die zugrunde liegenden Mechanismen sind jedoch keineswegs geklärt und

sollten in weiteren Studien untersucht werden. Durch die Prozedur der gezielten lokalen Applikation von NK-1-R Agonisten und Antagonisten in definierte Gehirngebiete, wie das mediale Septum, den Hippocampus oder den NBM, können diese Mechanismen sowohl neurochemisch als auch auf der Verhaltensebene eruiert werden.

Die Annahme, dass die Verabreichung von SR140333 aber auch Stressreaktionen kompensiert haben könnte, die zu Defiziten bei der Gedächtnisbildung führen können, lenkt die Erforschung des NK-1-R Antagonismus in eine Richtung, die auch für die Untersuchung von stress-bedingten Erkrankungen und auch affektiven Störungen von Bedeutung sein kann. In diesem Bereich gibt es bereits wie oben beschrieben eine Reihe von Untersuchungen, die auf einen modulatorischen Effekt von NK-1-R Antagonisten bei stress-bedingten Erkrankungen hindeuten (eine Übersicht gibt Rupniak, 2005).

Aus den Ergebnissen dieser Dissertation lassen sich Implikationen in eine weitere Richtung ableiten: Die Untersuchung des episodischen Gedächtnis bei Säugetieren. Das besondere Interesse an dieser Gedächtnisform resultiert zum einen aus der vermeintlichen Sonderstellung des Menschen für den Besitz eines derartigen Gedächtnis aber auch aus der Beobachtung, dass Defizite des episodischen Gedächtnis zu den frühen Symptomen bei dementiellen Erkrankungen, wie der Alzheimer Demenz, gehören. Im Jahre 2000 wurde die Anzahl der Personen mit dementiellen Erkrankungen (inkl. Alzheimer) in der Gruppe der 30-99 jährigen auf über 4,6 Millionen Erkrankte (12,3 je 1 000 Einwohner) europaweit geschätzt (Amt für amtliche Veröffentlichungen der Europäischen Gemeinschaften, 2002). Vor diesem Hintergrund besteht auch ein sozioökonomisches Interesse darin, die Mechanismen, die zur Entstehung von Demenzen führen, zu untersuchen und pharmakologische Behandlungsmöglichkeiten zu entwickeln. Mit Hilfe des im Rahmen dieses Promotionsvorhabens weiter entwickelten Objektexplorationsparadigmas könnten nun auch die Mechanismen des episodischen Gedächtnis in Labortieren direkt untersucht werden.

Bei diesem Promotionsvorhaben wurden einige Fragen zur Rolle des NK-1-R Antagonismus bei Lern- und Gedächtnisprozessen beantwortet, aber auch eine Reihe von neuen Fragen aufgeworfen und Implikationen für verschiedene Forschungsrichtungen dargestellt, die in Folgestudien weiter untersucht werden sollten.

# 4 Referenzliste


1. Aggleton,J.P. & Brown,M.W. (1999). Episodic memory, amnesia, and the hippocampal-anterior thalamic axis. The *Behavioral and Brain Sciences 22*, 425-444.

2. Aguiar,M.S. & Brandão,M.L. (1996). Effects of microinjections of the neuropeptide substance P in the dorsal periaquaductal gray on the behaviour of rats in the plus-maze test. *Physiology & Behavior 60*, 1183-1186.

3. Altier,N. & Stewart,J. (1999). The tachykinin NK-1 receptor antagonist, RP-67580, infused into the ventral tegmental area prevents stress-induced analgesia in the formalin test. *Physiology & Behavior 66*, 717-721.

4. Amaral,D.G. & Witter,M.P. (1989). The three-dimensional organization of the hippocampal formation: a review of anatomical data. *Neuroscience 31*, 571-591.

5. Amt für amtliche Veröffentlichungen der Europäischen Gemeinschaften. (2002). Statistiken zur Gesundheit. *Panorama der Europäischen Union*.

6. Araujo,J.A., Studzinski,C.M. & Milgram,N.W. (2005). Further evidence for the cholinergic hypothesis of aging and dementia from the canine model of aging. *Progress in Neuro-Psychopharmacolology and Biological Psychiatry 29*, 411-422.

7. Bailey,C.H. & Chen,M. (1989). Structural plasticity at identified synapses during long-term memory in Aplysia. *Journal of Neurobiology 20*, 356-372.

8. Bailey,S.J. & Jordan,C.C. (1984). A study of [D-Pro2, D-Phe7, D-Trp9]-substance P and [D-Trp7,9]-substance P as tachykinin partial agonists in the rat colon. *British Journal of Pharmacology 82*, 441-451.

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

10. Bartus,R.T. (2000). On neurodegenerative diseases, models, and treatment strategies: lessons learned and lessons forgotten a generation following the cholinergic hypothesis. *Experimental Neurology 163*, 495-529.

11. Berrettini,W.H., Rubinow,D.R., Nurnberger,J.I., Jr., Simmons-Alling,S., Post,R.M. & Gershon,E.S. (1985). CSF substance P immunoreactivity in affective disorders. *Biological Psychiatry 20*, 965-970.

12. Bester,H., De,F. & Hunt,S.P. (2001). The NK1 receptor is essential for the full expression of noxious inhibitory controls in the mouse. *The Journal of Neuroscience 21*, 1039-1046.

13. Boix,F., Pfister,M., Huston,J.P. & Schwarting,R.K. (1994). Substance P decreases extracellular concentrations of acetylcholine in neostriatum and nucleus accumbens in vivo: possible relevance for the central processing of reward and aversion. *Behavioral Brain Research 63*, 213-219.

14. Bondy,B., Baghai,T.C., Minov,C., Schule,C., Schwarz,M.J., Zwanzger,P., Rupprecht,R. & Moller,H.J. (2003). Substance P serum levels are increased in major depression: preliminary results. *Biological Psychiatry 53*, 538-542.

15. Bures,J., Buresova,O. & Huston,J.P. (1983). Techniques and basic experiments for the study of brain and behavior. (Amsterdam: Elsevier).

16. Chen,L.W., Wei,L.C., Liu,H.L., Qiu,Y. & Chan,Y.S. (2001). Cholinergic neurons expressing substance P receptor (NK(1)) in the basal forebrain of the rat: a double immunocytochemical study. *Brain Research 904*, 161-166.

17. Clayton,N.S., Bussey,T.J. & Dickinson,A. (2003). Can animals recall the past and plan for the future? *Nature Reviews Neuroscience 4*, 685-691.

18. Clayton,N.S. & Dickinson,A. (1998). Episodic-like memory during cache recovery by scrub jays. *Nature 395*, 272-274.

19. Couture,R. & Regoli,D. (1981). Inactivation of substance P and its C-terminal fragments in rat plasma and its inhibition by Captopril. *Canadian Journal of Physiology and Pharmacology 59*, 621-625.

20. Damsma,G., van Bueren,D.L., Westerink,B.H.C. & Horn,A.S. (1987). Determination of acetylcholine and choline in the femtomole range by means of HPLC, a post-column enzyme reactor, and electrochemical detection. *Chromatographia 24*, 827-831.

21. de Araújo,J.E., Huston,J.P. & Brandão,M.L. (1998). Aversive effects of the C-fragment of Substance P in the dorsal periaqueductal gray matter. *Experimental Brain Research 123*, 84-89.

22. de Araújo,J.E., Silva,R.C., Huston,J.P. & Brandão,M.L. (1999). Anxiogenic effects of substance P and its 7-11 C terminal, but not the 1-7 N terminal, injected into the dorsal periaqueductal gray. *Peptides 20*, 1437-1443.

23. De Souza Silva,M.A., Hasenöhrl,R.U., Tomaz,C., Schwarting,R.K.W. & Huston,J.P. (2000). Differential modulation of frontal cortex acetylcholine by injection of substance P into the nucleus basalis magnocellularis region in the freely-moving vs. the anesthetized preparation. *Synapse 38*, 243-253.

24. Degroot,A. & Parent,M.B. (2001). Infusions of physostigmine into the hippocampus or the entorhinal cortex attenuate avoidance retention deficits produced by intra-septal infusions of the GABA agonist muscimol. *Brain Research 920*, 10-18.

25. Dere,E., Huston,J.P. & De Souza Silva,M.A. (2005). Integrated memory for objects, places and temporal order: evidence for episodic-like memory in mice. *Neurobiology of Learning and Memory 84*, 214-221.

26. Dere,E., Kart-Teke,E., Huston,J.P. & De Souza Silva,M.A. (2006). The case for episodic memory in animals. *Neuroscience and Biobehavioral Reviews, 30 (6),* 1206-1224.

27. Deuschle,M., Sander,P., Herpfer,I., Fiebich,B.L., Heuser,I. & Lieb,K. (2005). Substance P in serum and cerebrospinal fluid of depressed patients: no effect of antidepressant treatment. *Psychiatry Research 136*, 1-6.

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

29. Eichenbaum,H. (2002). The Cognitive Neuroscience of Memory- an introduction. New York: Oxford University Press, Inc.

30. Ergorul,C. & Eichenbaum,H. (2004). The hippocampus and memory for "what," "where," and "when". *Learning & Memory 11*, 397-405.

31. Featherstone,R.L., Fosbraey,P. & Morton,I.K. (1986). A comparison of the effects of three substance P antagonists on tachykinin-stimulated [3H]-acetylcholine release in the guinea-pig ileum. *British Journal of Pharmacology 87*, 73-77.

32. File,S.E. (2000). NKP608, an NK1 receptor antagonist, has an anxiolytic action in the social interaction test in rats. *Psychopharmacology (Berl.) 152*, 105-109.

33. Gaffan,E.A., Healey,A.N. & Eacott,M.J. (2004). Objects and positions in visual scenes: effects of perirhinal and postrhinal cortex lesions in the rat. *Behavioral Neuroscience 118*, 992-1010.

34. Gavioli,E.C., Canteras,N.S. & De-Lima,T.C. (1999). Anxiogenic-like effect induced by substance P injected into the lateral septal nucleus. *Neuroreport 10*, 3399-3403.

35. Gerfen,C.R. (1991). Substance P (neurokinin-1) receptor mRNA is selectively expressed in cholinergic neurons in the striatum and basal forebrain. *Brain Research 556*, 165-170.

36. Gerhardt,P., Hasenöhrl,R.U. & Huston,J.P. (1992). Enhanced learning produced by injection of neurokinin substance P into the region of the nucleus basalis magnocellularis: mediation by the N-terminal sequence. *Experimental Neurology 118*, 302-308.

37. Gold,P.E. (2003). Acetylcholine modulation of neural systems involved in learning and memory. *Neurobiology of Learning and Memory 80*, 194-210.

38. Haddjeri,N. & Blier,P. (2001). Sustained blockade of neurokinin-1 receptors enhances serotonin neurotransmission. *Biological Psychiatry 50*, 191-199.

39. Hahn,M.K. & Bannon,M.J. (1998). Tachykinin NK1 receptor antagonists enhance stress-induced c-fos in rat locus coeruleus. *European Journal of Pharmacology 348*, 155-160.

40. Hanley,M.R., Sandberg,B.E., Lee,C.M., Iversen,L.L., Brundish,D.E. & Wade,R. (1980). Specific binding of 3H-substance P to rat brain membranes. *Nature 286*, 810-812.

41. Hardy,J. & Allsop,D. (1991). Amyloid deposition as the central event in the aetiology of Alzheimer's disease. *Trends in Pharmacological Sciences 12*, 383-388.

42. Hardy,J.A. & Higgins,G.A. (1992). Alzheimer's disease: the amyloid cascade hypothesis. *Science 256*, 184-185.

43. Hasenöhrl,R.U., De Souza Silva,M.A., Nika,E., Tomaz,C., Brandão,M.L., Schwarting,R.K.W. & Huston,J.P. (2000). Substance P and its role in neural mechanisms governing learning, anxiety and functional recovery. *Neuropeptides 34*, 272-280.

44. Hasenöhrl,R.U., Frisch,C., Nikolaus,S. & Huston,J.P. (1994). Chronic administration of neurokinin SP improves maze performance in aged Rattus norvegicus. *Behavioral & Neural Biology 62*, 110-120.

45. Hasenohrl,R.U., Gerhardt,P. & Huston,J.P. (1990). Substance P enhancement of inhibitory avoidance learning: mediation by the N-terminal sequence. *Peptides 11*, 163-167.

46. Hasenöhrl,R.U., Jentjens,O., De Souza Silva,M.A., Tomaz,C. & Huston,J.P. (1998). Anxiolytic-like action of neurokinin substance P administered systematically or into the nucleus basalis magnocellularis region. *European Journal of Pharmacology 354*, 123-133.

47. Hasenohrl,R.U., Oitzl,M.S. & Huston,J.P. (1989). Conditioned place preference in the corral: a procedure for measuring reinforcing properties of drugs. *Journal of Neuroscience Methods 30*, 141-146.

48. Hasselmo,M.E., Wyble,B.P. & Wallenstein,G.V. (1996). Encoding and retrieval of episodic memories: role of cholinergic and GABAergic modulation in the hippocampus. *Hippocampus 6*, 693-708.

49. Hawcock,A.B., Hayes,A.G. & Tyers,M.B. (1982). Agonist effects of [D-Pro2,D-Phe7,D-Trp9]substance P - evidence for different receptors. *European Journal of Pharmacology 80*, 135-138.

50. Herpfer,I. & Lieb,K. (2005). Substance P receptor antagonists in psychiatry: rationale for development and therapeutic potential. *CNS Drugs 19*, 275-293.

51. Hinson,J.P. & Kapas,S. (1996). Effect of splanchnic nerve section and compensatory adrenal hypertrophy on rat adrenal neuropeptide content. *Regulatory Peptides 61*, 105-109.

52. Hinson,J.P., Purbrick,A., Cameron,L.A. & Kapas,S. (1994). The role of neuropeptides in the regulation of adrenal zona fasciculata/reticularis function. Effects of vasoactive intestinal polypeptide, substance P, neuropeptide Y, Met- and Leu-enkephalin and neurotensin on corticosterone secretion in the intact perfused rat adrenal gland in situ. *Neuropeptides 26*, 391-397.

53. Huston,J.P. & Hasenöhrl,R.U. (1995). The role of neuropeptides in learning: focus on the neurokinin substance P. *Behavioral Brain Research 66*, 117-127.

54. Igwe,O.J., Kim,D.C., Seybold,V.S. & Larson,A.A. (1990). Specific binding of substance P aminoterminal heptapeptide [SP(1-7)] to mouse brain and spinal cord membranes. *The Journal of Neuroscience 10*, 3653-3663.

55. Izquierdo,I., Quillfeldt,J.A., Zanatta,M.S., Quevedo,J., Schaeffer,E., Schmitz,P.K. & Medina,J.H. (1997). Sequential role of hippocampus and amygdala, entorhinal cortex and parietal cortex in formation and retrieval of memory for inhibitory avoidance in rats. *European Journal of Neuroscience 9*, 786-793.

56. Kandel,E.R. & Schwartz,J.H. (1982). Molecular biology of learning: modulation of transmitter release. *Science 218*, 433-443.

57. Kosik,K.S. & Coleman,P.D. (1992). Is β-amyloid neurotoxic? *Neurobiology of Aging 13*, 535-630.

58. Kouznetsova,M. & Nistri,A. (2000). Facilitation of cholinergic transmission by substance P methyl ester in the mouse hippocampal slice preparation. *European Journal of Neuroscience 12*, 585-594.

59. Kramer,M.S. (2000). Update on Substance P (NK-1 receptor) antagonists in clinical trials for depression. *Neuropeptides 34*, 255.

60. Kramer,M.S., Cutler,N., Feighner,J., Shrivastava,R., Carman,J., Sramek,J.J., Reines,S.A., Liu,G., Snavely,D., Wyatt-Knowles,E., Hale,J.J., Mills,S.G., MacCoss,M., Swain,C.J., Harrison,T., Hill,R.G., Hefti,F., Scolnick,E.M., Cascieri,M.A., Chicchi,G.G., Sadowski,S., Williams,A.R., Hewson,L., Smith,D., Rupniak,N.M. & et al. (1998). Distinct mechanism for antidepressant activity by blockade of central substance P receptors. *Science 281*, 1640-1645.

61. Kramer,M.S., Winokur,A., Kelsey,J., Preskorn,S.H., Rothschild,A.J., Snavely,D., Ghosh,K., Ball,W.A., Reines,S.A., Munjack,D., Apter,J.T., Cunningham,L., Kling,M., Bari,M., Getson,A. & Lee,Y. (2004). Demonstration of the efficacy and safety of a novel substance P (NK1) receptor antagonist in major depression. *Neuropsychopharmacology 29*, 385-392.

62. Kreeger,J.S. & Larson,A.A. (1996). The substance P amino-terminal metabolite substance P(1-7), administered peripherally, prevents the development of acute morphine tolerance and attenuates the expression of withdrawal in mice. *The Journal of Pharmacology and Experimental Therapeutics 279*, 662-667.

63. LeDoux,J.E. & Muller,J. (1997). Emotional memory and psychopathology. *Philosophical Transactions of the Royal Society of London Series B - Biological Sciences 352*, 1719-1726.

64. LeDoux,J.E. & Phelps,E.A. (2000). Emotional networks in the brain. In Handbook of Emotions, M. Lewis and J. M. Haviland-Jones, eds. New York: Guilford Press, pp. 157-172.

65. Lehmann,J., Nagy,J.I., Atmadia,S. & Fibiger,H.C. (1980). The nucleus basalis magnocellularis: the origin of a cholinergic projection to the neocortex of the rat. *Neuroscience 5*, 1161-1174.

66. Lieb,K., Ahlvers,K., Dancker,K., Strohbusch,S., Reincke,M., Feige,B., Berger,M., Riemann,D. & Voderholzer,U. (2002). Effects of the neuropeptide substance P on sleep, mood, and neuroendocrine measures in healthy young men. *Neuropsychopharmacology 27*, 1041-1049.

67. Lieb,K., Fiebich,B.L. & Berger,M. (2000). Substanz P-Rezeptor-Antagonisten als neues antidepressives und anxiolytisches Wirkprinzip? [Substance P receptor antagonists - a new antidepressive and anxiolytic mechanism?]. *Nervenarzt 71*, 758-761.

68. Loiseau,F., Le Bihan,C., Hamon,M. & Thiebot,M.H. (2003). Distinct effects of diazepam and NK1 receptor antagonists in two conflict procedures in rats. *Behavioural Pharmacology 14*, 447-455.

69. Maeno,H., Kiyama,H. & Tohyama,M. (1993). Distribution of the substance P receptor (NK-1 receptor) in the central nervous system. *Molecular Brain Research 18*, 43-58.

70. Manns,J.R. & Eichenbaum,H. (2006). Evolution of declarative memory. *Hippocampus 16*, 795-808.

71. McLean,S. (2005). Do substance P & the NK1 receptor have a role in depression and anxiety? *Current Pharmaceutical Design 11*, 1529-1547.

72. Mesulam,M.M., Mufson,E.J., Wainer,B.H. & Levey,A.I. (1983). Central cholinergic pathways in the rat: an overview based on an alternative nomenclature (Ch1-Ch6). *Neuroscience 10*, 1185-1201.

73. Morris,R. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods 11*, 47-60.

74. Napier,T.C., Mitrovic,I., Churchill,L., Klitenick,M.A., Lu,X.-Y. & Kalivas,P.W. (1995). Substance P in the ventral pallidum: Projection from the ventral striatum, and electrophysiological and behavioral consequences of pallidal substance P. *Neuroscience 69*, 59-70.

75. Nikolaus,S., Huston,J.P. & Hasenöhrl,R.U. (1999). The neurokinin-1 receptor antagonist WIN51,708 attenuates the anxiolytic-like effects of ventralpallidal substance P injection. *Neuroreport 10*, 2293-2296.

76. Nikolaus,S., Huston,J.P. & Hasenohrl,R.U. (2000). Anxiolytic-like effects in rats produced by ventral pallidal injection of both N- and C-terminal fragments of substance P. *Neuroscience Letters 283*, 37-40.

77. O'Keefe,J. & Nadel,L. (1979). The hippocampus as a cognitive map. Oxford, UK: Clarendon Press.

78. Ogier,R. & Raggenbass,M. (2003). Action of tachykinins in the rat hippocampus: modulation of inhibitory synaptic transmission. *European Journal of Neuroscience 17*, 2639-2647.

79. Packard,M.G. & McGaugh,J.L. (1996). Inactivation of hippocampus or caudate nucleus with lidocaine differentially affects expression of place and response learning. *Neurobiology of Learning and Memory 65*, 65-72.

80. Pelleymounter,M.A., Schlesinger,K., Wehner,J., Hall,M.E. & Stewart,J.M. (1988). Nigral 5-HT and substance P-induced enhancement of passive avoidance retention. *Behavioral Brain Research 29*, 159-172.

81. Peterson,G.M. & Shurlow,C.L. (1992). Morphological evidence for a substance P projection from medial septum to hippocampus. *Peptides 13*, 509-517.

82. Rimon,R., Le Greves,P., Nyberg,F., Heikkila,L., Salmela,L. & Terenius,L. (1984). Elevation of substance P-like peptides in the CSF of psychiatric patients. *Biological Psychiatry 19*, 509-516.

83. Robbins,T.W., McAlonan,G., Muir,J.L. & Everitt,B.J. (1997). Cognitive enhancers in theory and practice: studies of the cholinergic hypothesis of cognitive deficits in Alzheimer's disease. *Behavioral Brain Research 83*, 15-23.

84. Rupniak,N.M., Carlson,E.C., Harrison,T., Oates,B., Seward,E., Owen,S., De-Felipe,C., Hunt,S. & Wheeldon,A. (2000). Pharmacological blockade or genetic deletion of substance P (NK(1)) receptors attenuates neonatal vocalisation in guinea-pigs and mice. *Neuropharmacology 39*, 1413-1421.

85. Rupniak,N.M., Carlson,E.J., Webb,J.K., Harrison,T., Porsolt,R.D., Roux,S., De Felipe,C., Hunt,S.P., Oates,B. & Wheeldon,A. (2001). Comparison of the phenotype of NK1R - / - mice with pharmacological blockade of the substance P ($NK_1$) receptor in assays for antidepressant and anxiolytic drugs. *Behavioural Pharmacology 12*, 497-508.

86. Rupniak,N.M. & Kramer,M.S. (1999). Discovery of the antidepressant and anti-emetic efficacy of substance P receptor (NK1) antagonists. *Trends in Pharmacological Science 20*, 485-490.

87. Rupniak,N.M., Webb,J.K., Fisher,A., Smith,D. & Boyce,S. (2003). The substance P (NK1) receptor antagonist L-760735 inhibits fear conditioning in gerbils. *Neuropharmacology 44*, 516-523.

88. Rupniak,N.M.J. (2005). Substance P ($NK_1$ receptor) antagonists. In Handbook of stress and the brain. Part 2: Stress: Integrative and Clinical Aspects, T. Steckler, N. H. Kalin, and J. M. H. M. Reul, eds. Amsterdam: Elsevier, pp. 423-435.

89. Sarter,M. & Bruno,J.P. (1999). Abnormal regulation of corticopetal cholinergic neurons and impaired information processing in neuropsychiatric disorders. *Trends in Neuroscience 22*, 67-74.

90. Sarter,M., Hasselmo,M.E., Bruno,J.P. & Givens,B. (2005a). Unraveling the attentional functions of cortical cholinergic inputs: interactions between signal-

driven and cognitive modulation of signal detection. *Brain Research Reviews 48*, 98-111.

91. Sarter,M., Nelson,C.L. & Bruno,J.P. (2005b). Cortical cholinergic transmission and cortical information processing in schizophrenia. *Schizophrenia Bulletin 31*, 117-138.

92. Sarter,M. & Parikh,V. (2005c). Choline transporters, cholinergic transmission and cognition. *Nature Reviews Neuroscience 6*, 48-56.

93. Schlesinger,K., Lipsitz,D.U., Peck,P.L., Pelleymounter,M.A., Stewart,J.M. & Chase,T.N. (1983a). Substance P enhancement of passive and active avoidance conditioning in mice. *Pharmacology, Biochemistry and Behavior 19*, 655-661.

94. Schlesinger,K., Lipsitz,D.U., Peck,P.L., Pelleymounter,M.A., Stewart,J.M. & Chase,T.N. (1983b). Substance P reversal of electroconvulsive shock and cycloheximide-induced retrograde amnesia. *Behavioral and Neural Biology 39*, 30-39.

95. Schlesinger,K., Pelleymounter,M.A., van de,K.J., Bader,D.L., Stewart,J.M. & Chase,T.N. (1986). Substance P facilitation of memory: effects in an appetitively motivated learning task. *Behavioral and Neural Biology 45*, 230-239.

96. Sergeyev,V., Hokfelt,T. & Hurd,Y. (1999). Serotonin and substance P co-exist in dorsal raphe neurons of the human brain. *Neuroreport 10*, 3967-3970.

97. Skinner,B.F. (1947). "Superstition" in the pigeon. *Journal of Experimental Psychology 38*, 168-172.

98. Sloviter,R.S., Ali-Akbarian,L., Horvath,K.D. & Menkens,K.A. (2001). Substance P receptor expression by inhibitory interneurons of the rat hippocampus: enhanced detection using improved immunocytochemical methods for the preservation and colocalization of GABA and other neuronal markers. *The Journal of Comparative Neurology 430*, 283-305.

99. Small,B.J., Mobly,J.L., Laukka,E.J., Jones,S. & Backman,L. (2003). Cognitive deficits in preclinical Alzheimer's disease. *ACTA Neurologica Scandinavica. Supplementum 179*, 29-33.

100. Steinberg,R., Alonso,R., Griebel,G., Bert,L., Jung,M., Oury-Donat,F., Poncelet,M., Gueudet,C., Desvignes,C., Le Fur,G. & Soubrie,P. (2001). Selective blockade of neurokinin-2 receptors produces antidepressant-like effects associated with reduced corticotropin-releasing factor function. *The Journal of Pharmacology and Experimental Therapeutics 299*, 449-458.

101. Steinberg,R., Alonso,R., Rouquier,L., Desvignes,C., Michaud,J.C., Cudennec,A., Jung,M., Simiand,J., Griebel,G., Emonds-Alt,X., Le Fur,G. & Soubrie,P. (2002). SSR240600 [(R)-2-(1-[2-[4-[2-[3,5-bis(trifluoromethyl)phenyl]acetyl]-2-(3,4-dichloro phenyl)-2-morpholinyl]ethyl]-4-piperidinyl)-2-methylpropanamide], a centrally active nonpeptide antagonist of the tachykinin neurokinin 1 receptor: II. Neurochemical and behavioral

characterization. *The Journal of Pharmacology and Experimental Therapeutics 303*, 1180-1188.

102. Teixeira,R.M., Santos,A.R.S., Ribeiro,S.J., Calixto,J.B., Rae,G.A. & de Lima,T.C.M. (1996). Effects of central administration of tachykinin receptor agonists & antagonists on plus-maze bahavior in mice. *European Journal of Pharmacology 311*, 7-14.

103. Thiel,C.M., Huston,J.P. & Schwarting,R.K.W. (1998). Hippocampal acetylcholine and habituation learning. *Neuroscience 85*, 1253-1262.

104. Tulving,E. (2002). Episodic memory: from mind to brain. *Annual Review of Psychology 53*, 1-25.

105. Vassout,A., Veenstra,S., Hauser,K., Ofner,S., Brugger,F., Schilling,W. & Gentsch,C. (2000). NKP608: a selective NK-1 receptor antagonist with anxiolytic-like effects in the social interaction and social exploration test in rats. *Regulatory Peptides 96*, 7-16.

106. Vaupel,R., Jarry,H., Schlomer,H.T. & Wuttke,W. (1988). Differential response of substance P-containing subtypes of adrenomedullary cells to different stressors. *Endocrinology 123*, 2140-2145.

107. von Euler,U.S. & Gaddum,J.H. (1931). An unidentified depressor substance in certain tissue extracts. *Journal of Physiology 72*, 74-87.

108. Winters,B.D. & Bussey,T.J. (2005). Glutamate receptors in perirhinal cortex mediate encoding, retrieval, and consolidation of object recognition memory. *The Journal of Neuroscience 25*, 4243-4251.

109. Yukhananov,R.Y. & Larson,A.A. (1994). An N-terminal fragment of substance P, substance P(1-7), down-regulates neurokinin-1 binding in the mouse spinal cord. *Neuroscience Letters 178*, 163-166.

110. Zaborszky,L., Cullinan,W.E. & Braun,A. (1991). Afferents to basal forebrain cholinergic projection neurons: an update. In The basal forebrain: Anatomy to function, T. C. Napier, P. W. Kalivas, and I. Hanin, eds. New York: Plenum Press, pp. 43-100.

111. Zentall,T.R. (2005). Animals may not be stuck in time. *Learning and Motivation 36*, 208-225.

# 5 Anhang

Eigene Publikationen

(1) Kart,E., Jocham,G., Muller,C.P., Schlomer,C., Brandao,M.L., Huston,J.P. & Souza Silva,M.A. (2004). Neurokinin-1 receptor antagonism by SR140333: enhanced in vivo ACh in the hippocampus and promnestic post-trial effects. *Peptides 25*, 1959-1969

(2) Kart-Teke,E., Souza Silva,M.A., Huston,J.P. & Dere,E. (2006). Wistar rats show episodic-like memory for unique experiences. *Neurobiology of Learning and Memory 85*, 173-182.

(3) Kart-Teke,E., Dere,E., Brandao,M.L., Huston,J.P. & De Souza Silva,M.A. (2007). Reinstatement of episodic-like memory in rats by neurokinin-1 receptor antagonism. *Neurobiology of Learning and Memory*, *87 (3)*, 324-331.

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# Neurokinin-1 receptor antagonism by SR140333: enhanced in vivo ACh in the hippocampus and promnestic post-trial effects

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**Abstract**

Substance P (SP) has memory-promoting, reinforcing and anxiolytic-like effects when applied systemically or centrally. Such effects may be mediated by the neurokinin-1 (NK-1) receptor, since SP preferentially binds to this receptor. We measured the effects of a selective non-peptide NK-1 receptor antagonist, SR140333 (1, 3 and 9 mg/kg i.p.) on ACh levels in frontal cortex, amygdala and hippocampus by microdialysis and HPLC. Levels of ACh in the hippocampus increased dose-dependently immediately after treatment. The same doses of SR140333 given post-trial had minor facilitative effects on inhibitory avoidance learning and open-field habituation, but did not have reinforcing effects in a conditioned place preference (CPP) task. The selective action of NK-1 receptor antagonism on hippocampal ACh may be related to its positive influence on learning.


*Keywords:* NK-1 receptor antagonist; Acetylcholine; Amygdala; Frontal cortex; Hippocampus; In vivo microdialysis; Open-field; Habituation; Inhibitory avoidance; Memory; Conditioned place preference; Reinforcement; Rats

## 1. Introduction

Substance P (SP), an endogenous neuropeptide of the tachykinin family, is widely distributed throughout the mammalian brain. It binds to all three neurokinin (NK) receptor subtypes, NK-1, NK-2 and NK-3, with preference for the NK-1 receptor [28] which is distributed throughout the brain [49], and modulates neuronal activity in a variety of structures, brainstem nuclei [19], striatum [64], cortex [17], hippocampal formation [62], amygdala [61] and the basal forebrain [26,30,42].

SP has been implicated in the modulation of behavioral processes such as reinforcement, learning, memory, anxiety and fear, when applied centrally or systemically [26]. Systemic administration of SP in rodents facilitates inhibitory and active avoidance learning [55], discrimination learning [57], and reverses retrograde amnesia induced by electroconvulsive shock or cycloheximide [56]. Post-trial injections of SP had memory promoting effects in passive avoidance tasks after systemic [26,69] or central injection into either the nucleus basalis magnocellularis (NBM) or medial septal region [63]. It was also found to induce conditioned place preference (CPP), and to have anxiolytic-like effects in rats, with the nucleus basalis magnocellularis as one of the main sites for the behaviorally relevant action of SP [26,30].

Depletion of basal forebrain ACh plays a crucial role in age-related cognitive deficits [41]. Accordingly enhancement of cholinergic activity correlates with improved attention, learning and memory [51]. Modulation of cholinergic activity in cortical structures or the hippocampus has an impact on learning and memory processes [23,27]. Inhibitory avoidance learning is disrupted by post-trial i.p. injections of M1 receptor antagonists [52], and cholinergic activity at muscarinic

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receptors in the hippocampus facilitates glutamate-mediated LTP [34].

Due to its anatomical architecture and projection fields the basal forebrain is a key structure in attention, learning and memory processes (for a review see [24]), and appears to be a main site for the behavioral actions of SP, especially the subregion of the NBM [26,30]. The cholinergic neurons of the basal forebrain constitute the major source of cholinergic inputs to telencephalic regions through two main projections: (a) a basalocortical one, which projects from the NBM/substantia innominata to neocortex and amygdala, and (b) a septohippocampal projection from the medial septal area to the hippocampus [8].

The cholinergic cells of the NBM and medial septal region are innervated by SP-containing terminals that originate mostly from the nucleus accumbens [42,70]. They contact cholinergic neurons [4], which express mRNA encoding for SP (NK-1) receptors [10,20]. Immunohistochemical and immunocytochemical studies have also shown that neurokinin receptors are localised on cholinergic neurons of the basal forebrain [47].

Neurokinins have an excitatory action on cholinergic neurons: in the striatum neurokinins increase the activity of cholinergic interneurons via postsynaptic NK-1, NK-2 and NK-3 receptors [1]. Furthermore SP methylester, a selective NK-1 agonist, facilitates ACh release from mouse hippocampal slices in vitro [36]. Injections of SP into the NBM increased ACh levels in the frontal cortex of anaesthetised rats [13].

It follows that the behavioral effects of SP may be mediated by NK-1 receptors localized in cholinergic neurons of the NBM area. Using the non-peptide NK-1 receptor antagonist SR140333, which is a selective high affinity NK-1 receptor antagonist in different species, including rats [16], we here investigated the effects of NK-1 receptor blockade on extracellular ACh levels in the terminal fields of the cholinergic cells of the basal forebrain, namely frontal cortex, amygdala and hippocampus. We chose to study the anaesthetized rather than the freely moving preparation, since under the latter condition, motor activity can mask and confound the effects of pharmacological manipulation of cholinergic activity [13,22]. Based on the results of the neurochemical experiment, namely an increase in hippocampal ACh, we hypothesized that the NK-1 blocker could have facilitating effects on learning in hippocampus-dependent tasks [2,32,33,68]. Therefore, we assessed the effects of SR140333 on inhibitory avoidance and open-field habituation, and, since SP effects on memory are closely related to reinforcement [26,30] we also tested for conditioned place preference.

## 2. Materials and methods

### 2.1. Subjects

Thirty-one adult male Wistar rats weighing 250–300 g, obtained from TVA (Tierversuchsanlage, University of Düsseldorf), were used for the microdialysis experiment. Ninety-five male adult Wistar rats were used for behavioral testing. Animals were kept in a temperature controlled room (20–22 °C) under reversed 12/12 h day–night cycle, with lights off at 7:00 a.m. Animals submitted to microdialysis were housed in acrylic glass cages in groups of three to four until surgery; thereafter they were caged individually. Animals used for behavioral tests were kept in groups of two to four per cage. All animals had free access to food and water and were maintained according to the German Law of Animal Protection of 1998.

### 2.2. Drugs

SR140333 ((*S*)1-(2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl)piperidin-3-yl]ethyl)-4-phenyl-1-azoniabicyclo[2.2.2]octane chloride) was a gift from Sanofi Synthelabo, Chilly-Mazarin, France. It was diluted in vehicle (0.01% Tween 80 (Sigma–Aldrich, USA) in distilled water), which served as control. SR140333 was applied i.p. in doses of 1, 3 and 9 mg/kg (injection volume: 1 ml/kg) in all experiments.

### 2.3. Neurochemical measurement

#### 2.3.1. Surgery

Animals submitted to microdialysis experiments were anaesthetised with a mixture of Xylazinhydrochloride (8 mg/kg, Rompun, Bayer, Leverkusen, Germany) and Ketaminhydrochloride (90 mg/kg, Ketavet, Pharmacia & Upjohnm GmbH, Erlangen, Germany). With the aid of a stereotaxic apparatus (David Kopf Instruments, California, USA), three guide-cannulae (15 mm; 22 G, stainless steel) were placed according to a stereotaxic atlas [45] above the following areas: frontal cortex (A: +3.7 mm/L: ±3.0 mm/D: −1.3 mm), amygdala (A: −2.5 mm/L: ±5.7 mm/D: −7.8 mm, with an angle of 10° towards the midline) and hippocampus (A: −6.0 mm/L: ±4.8 mm/D: −3.2 mm), coordinates taken from bregma. All cannulae were placed unilaterally, counterbalanced into either the right or the left hemisphere. They were fixed with the aid of one screw and dental cement. After surgery the animals were allowed to recover for 3–5 days before they were subjected to the microdialysis procedure.

#### 2.3.2. Microdialysis procedure

Experiments were conducted between 9:00 a.m. and 6:00 p.m. Thirty-one animals were randomly assigned to one of the following groups: vehicle, 1, 3 or 9 mg/kg SR140333. They were anaesthetized with 1.25 g/kg urethane (Sigma–Aldrich, USA) i.p. and placed on a heating pad in an acrylic box (45 cm × 25 cm × 22 cm). Body temperature was maintained during the experiment between 36.5 and 37.5 °C by a temperature controller (CMA/150, Sweden). A catheter was inserted into the intraperitoneal cavity to allow injection of solutions without touching the animal. Dialysis probes

were prepared according to the procedure described previously [3]. They had an active membrane length of 4.0 mm for the hippocampus and 2.0 mm for frontal cortex and amygdala. Probes were connected via polyethylene tubes to microdialysis syringes attached to a microdialysis pump and perfused at a flow rate of 2 μl/min with Ringer's solution ($Na^+$ 146, $K^+$ 4, $Ca^{2+}$ 2.3 and $Cl^-$ 156 mmol/l, Braun, Melsungen, Germany) containing neostigmine (10 μmol) by a micropump (CMA/100, Sweden).

After a stabilisation period of 2 h, four baseline samples á 20 min were collected in Eppendorf cups containing 10 μl of ethylhomocholine (EHCh) solution, used as internal standard [48]. After each sampling interval the animal received an injection of 0.2 ml Ringer's solution through the intraperitoneally inserted catheter, and depth of anaesthesia was adjusted when necessary with urethane. At the end of the fourth sample the animals received an i.p. injection of vehicle or SR140333 (1, 3 or 9 mg/kg). Three more samples were collected and immediately analysed with HPLC-ECD-method for ACh concentrations.

#### 2.3.3. HPLC analysis

HPLC-ECD analysis was performed according to the procedure described by Damsma et al. [11], except for the internal standard, EHCh [48]. Briefly, ACh was analysed by a sequential process of chromatographic separation, enzymatic conversion of ACh to hydrogen peroxide and its electrochemical detection. Separation was achieved using an analytical column (75 mm length, 2 mm inner diameter) filled with ChromSpher 5C18 (Merck, Germany) and loaded with sodium dodecyl sulfate (Sigma–Aldrich, USA). An enzyme reactor filled with LiChrosorb $NH_2$ (Merck, Germany), activated by glutardialdehyde (Merck, Germany) and loaded with 80 units acetylcholinesterase (Sigma–Aldrich, USA) and 40 units choline oxidase (Sigma–Aldrich, USA), which were covalently bound to the stationary phase was attached to the end of the analytical column for enzymatic cleavage of the compounds. On passage through the enzyme reactor ACh is converted to hydrogen peroxide, which is electrochemically detected at a platinum electrode set at a potential of 0.5 mV versus an Ag/AgCl reference electrode. The mobile phase was composed of 1mM tetramethylammoniumchloride (Merck, Germany) and 0.18 M $K_2HPO_4$ (Merck, Germany), adjusted to pH 8.0 with $KH_2PO_4$ (Merck, Germany), and was delivered by a HPLC pump (L-7110, Merck, Germany) at 0.3 ml/min.

#### 2.3.4. Histology

After the experiment the animals were administered an overdose of pentobarbital (Nembutal, Sanofi Ceva, Libourne, France), perfused with physiological saline and 10% formalin through the left ventricle. The brains were removed and stored in formalin (10%) until sliced. Staining was done with cresyl violet (Sigma–Aldrich, USA), and cannula placement was verified with the help of a atlas [45].

### 2.4. Step-through inhibitory avoidance

The inhibitory avoidance apparatus was divided into two compartments, one illuminated (21 cm × 23 cm × 23 cm, 700 lx) and one dark (20 cm × 23 cm × 23 cm, 0.3 lx) compartment, separated by a guillotine door. The floor consisted of electrifiable stainless steel bars. A working air conditioning provided background masking noise.

Experiments were conducted between 9:00 a.m. and 4:00 p.m. Animals were randomly assigned to one of the following group: immediate post-trial injection of vehicle ($n$ = 10), 1 mg/kg ($n$ = 11), 3 mg/kg ($n$ = 11) or 9 mg/kg of SR140333 ($n$ = 11). On the experimental day, the animals were gently placed into the illuminated compartment of the inhibitory avoidance apparatus, facing the wall opposite to the dark compartment entry. Immediately after the animal stepped into the dark compartment, the door was closed, and a shock (0.2 mA) was applied for 1 s. The latency to step into the dark compartment was measured. After application of the shock the animal was gently taken out of the apparatus and given an injection of either vehicle or SR140333 according to its group assignment. The animals were tested for retention of the inhibitory avoidance task 24 h and 15 days after training. In the test trials the animal was placed into the illuminated compartment and the latency to step through to the dark compartment was measured. The cut-off value to step through was set at 300 s. To avoid olfactory cues the apparatus was cleaned with a 0.01% acetic acid solution and dried after each trial.

Based on the results of the first inhibitory avoidance experiment in which the level of learning was too high to allow expression of a facilitative treatment effect, a second such experiment was conducted. For this study we wished to have an overall lower level of learning in order to disclose possible promnestic effects of the NK-1 antagonist. An appropriate shock intensity was found to be 0.3 mA for 1 s. (This shock level is higher than the 0.2 mA/s used in the first experiment, yet led to a lower level of learning. It was conducted six months after the first experiment, under different atmospheric (humidity) conditions, which could account for this change in effectiveness.) The apparatus and experimental procedure were the same as in the first inhibitory avoidance experiment. Forty-six animals were randomly assigned to one of the following groups: post-trial injection of vehicle ($n$ = 10), 1 mg/kg ($n$ = 12), 3 mg/kg ($n$ = 12) or 9 mg/kg SR140333 ($n$ = 12). The procedure was the same as in the first experiment.

### 2.5. Open-field habituation learning

When an animal is re-exposed to a particular environment it will likely engage in less exploratory locomotor activity than upon initial exposure. The magnitude of such change in behavior has been used to gauge memory for the first exposure. This test of recognition memory can be used for post-trial administration of drugs, similar to the inhibitory avoidance task [21,54]. After a washout period of 2 months, 43

animals from the first inhibitory avoidance experiment were subjected to the open-field habituation task. The purpose of this experiment was to determine whether post-trial administration of the NK-1 receptor antagonist SR140333 would influence habituation to the open field. The open-field apparatus was a grey acryl box of 60 cm × 60 cm × 38 cm size with an open roof. Two meters above the center of the field a camera was mounted for the purpose of automated video-tracking. The experiment was conducted under white light, with light bulbs directed against the wall providing an illumination of 3–5 lx in the entire box.

The animals were randomly assigned to one of four groups: injection of vehicle ($n$ = 10), 1 mg/kg ($n$ = 11), 3 mg/kg ($n$ = 11), or 9 mg/kg SR140333 ($n$ = 11). On the first experimental day, the baseline trial, the animals were gently placed into the center of the open-field for 10 min of free exploration. Immediately thereafter, they received an i.p. injection according to their group assignment. In the test trial 24 h later the animals were again placed for 10 min into the open-field. Their behavior during baseline (day 1) and test trials (day 2) in the open field was recorded by an automated video-computer-system (EthoVision Tracking Software, Noldus, Netherlands) for subsequent analysis of the rearing frequency (number of times the animal raised up on its hind limbs, lifting the forelimbs up or against the wall) and locomotion (distance (cm) moved in the arena). Locomotion was analysed automatically by the software, and frequency of rearing was analysed by a trained experimenter unaware of the group assignment from the video-taped image.

### 2.6. Conditioned place preference

After a wash-out period of 2 months, 43 animals from the second inhibitory avoidance experiment plus nine naïve animals were used to test for possible reinforcing effects of the NK-1 receptor antagonist utilizing a conditioned place preference paradigm, the corral maze [25]. After treatment the animal is confined to one of the quadrants of a circular open field. A preference for this quadrant during testing with the whole field available is indicative of reinforcing properties of the treatment.

The place preference corral consisted of a circular black arena (83.5 cm diameter × 44 cm), which could be divided into four equal sized quadrants by placement of Plexiglas walls. The maze was positioned in a room provided with extra-maze cues for spatial orientation. White light bulbs directed against the wall supplied a dim illumination inside the corral maze varying from 1.9 lx (periphery) to 3.1 lx (center).

The experiments were conducted from 9:00 a.m. to 3:00 p.m. The animals were randomly assigned to one of the following groups: vehicle ($n$ = 13), 1 mg/kg ($n$ = 13), 3 mg/kg ($n$ = 13), 9 mg/kg SR140333 ($n$ = 13). On the first experimental day (baseline trial) the animal was gently placed into the center of the maze and allowed to explore it for 15 min. On the second to fourth experimental days, three conditioning trials were administered, during which the animal received an i.p. injection according to its group assignment. Five min later it was placed for 20 min into one of the two quadrants where it had spent neither the most nor the least time in the baseline trial. On the fifth day, the test trial, they again had free access to the entire maze for 15 min. The time spent in each quadrant was analysed automatically with the aid of the EthoVision tracking software (Noldus, Netherlands).

### 2.7. Statistical analysis

Data from the microdialysis study were analysed separately for each brain structure. ACh concentrations were converted into percentage of baseline (mean of the three baseline samples collected before the treatment was taken as 100%) and subjected to two-way repeated measures analysis of variance (ANOVA) based on the general linear model. Another two-way repeated measures ANOVA was calculated for the last three baseline measurements to check for baseline differences between the groups. Data from the CPP were also analysed by two-way repeated measures ANOVA, with time spent in the treatment quadrant on baseline trial and test trial and treatment as factors. Degrees of freedom were corrected according to the Huynh-Feldt procedure for repeated measures in univariate statistics, if the Mauchly-test of sphericity was significant. Post-hoc Tukey-HSD for between-group differences and paired $t$-tests for within-group differences were applied when appropriate. Data from the training trial of the inhibitory avoidance experiments and the first day in the open-field habituation task were analysed by Kruskal-Wallis tests for between-group differences. Data obtained from the inhibitory avoidance task and open-field habituation task were analysed by Mann–Whitney $U$-test for between-group and Wilcoxon-test for within-group differences. The software SPSS11.0 was used for all calculations. Unless specified otherwise, all $P$-values given are two-tailed and were considered significant if $\alpha < 0.05$.

## 3. Results

### 3.1. Neurochemistry

#### 3.1.1. Histology

After histological screening no animal had to be totally excluded from data analysis. However, leakage of probes during the dialysis procedure (three in the amygdala and one in hippocampus) and wrong cannula placement (two in hippocampus) led to the following sample sizes: frontal cortex: vehicle, $n$ = 9; SR140333 1 mg/kg, $n$ = 8; 3 mg/kg, $n$ = 9; 9 mg/kg, $n$ = 5; amygdala: vehicle, $n$ = 8; SR140333 1 mg/kg, $n$ = 7; 3 mg/kg, $n$ = 8; 9 mg/kg, $n$ = 5; hippocampus: vehicle, $n$ = 9; SR140333 1 mg/kg, $n$ = 6; 3 mg/kg, $n$ = 8; 9 mg/kg, $n$ = 5.

#### 3.1.2. Acetylcholine measurements

The mean ± S.E.M. basal concentrations of ACh in dialysates were: frontal cortex: 0.404 ± 0.033 pmol/40 μl;

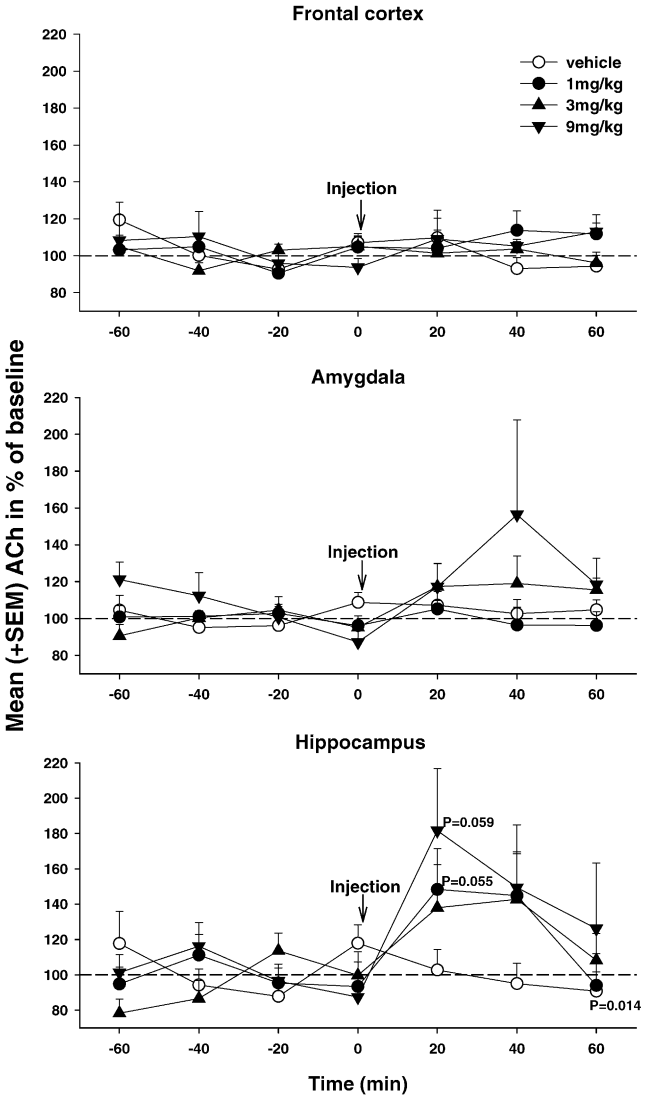


Fig. 1. Extracellular ACh level (20 min samples; mean + S.E.M.) in frontal cortex (upper panel), amygdala (middle panel) and hippocampus (lower panel) of anaesthetized animals. The values are expressed in percent of baseline (mean of the last three baseline samples (min −40 to 0) taken as 100%) collected before the i.p. injection (indicated by arrow) of vehicle (○), 1 mg/kg (●), 3 mg/kg (▲) or 9 mg/kg (▼) SR140333. *P*-values from paired *t*-test against the last baseline sample.

amygdala: 2.108 ± 0.429 pmol/40 μl; hippocampus: 1.245 ± 0.092 pmol/40 μl. Analysis of variance with Huynh-Feldt correction for degrees of freedom (d.f.) was conducted separately for each brain structure; thus, the results are presented separately for frontal cortex, amygdala and hippocampus. Levels of ACh (% of baseline, mean + S.E.M.) for the control group (vehicle) or experimental groups (1, 3 and 9 mg/kg SR140333) for the frontal cortex, amygdala and hippocampus are depicted in Fig. 1.

In the *frontal cortex* (Fig. 1), analysis of variance yielded neither a main effect of group ($F(3, 27) = 1.111$, $P > 0.05$) nor, after correction for d.f., a main effect of time ($F(4, 112) = 0.908$, $P > 0.05$) nor an interaction ($F(12, 112) = 0.969$, $P > 0.05$). The mean level of each group was stable, ranging between 92 and 114% across the entire experiment.

In the *amygdala* (Fig. 1) there was a weak tendency towards a main effect for time after correction for d.f. ($F(2, 61) = 2.406$, $P < 0.1$), but neither an interaction effect ($F(7, 61) = 1.186$, $P > 0.05$) nor a main effect of group ($F(3, 24) = 1.602$, $P > 0.05$) was found. The ACh levels ranged between 87 and 156% over the entire measurement period.

In the *hippocampus* (Fig. 1) there was neither a group ($F(3,25) = 1.838$, $P > 0.05$) nor an interaction effect ($F(15,120) = 1.290$, $P > 0.05$), but a significant main effect of time ($F(5,120) = 5.450$, $P < 0.001$), which reflected the dose-dependent increase of ACh extracellular levels immediately after injection of SR140333, with a return to control levels 1 h later. Two-way repeated measures ANOVA for the last three baseline samples yielded no significant difference (group-effect: $F(3, 24) = 0.639$, $P > 0.05$; sample-effect: $F(2, 48) = 0.079$, $P > 0.05$; group × sample interaction: $F(6, 48) = 1.457$, $P > 0.05$); thus, paired *t*-tests for within-group comparisons were conducted. In the vehicle group the ACh level declined to 91 ± 11% ($T(8) = 3.125$, $P = 0.014$) in the third sample after treatment (60 min). In the 1 mg/kg group ACh increased to 148 ± 23% ($T(5) = -2.492$, $P = 0.055$) and 144 ± 23% in the first and second samples after treatment (at 20 and 40 min post-injection), respectively. In the 3 mg/kg to 138 ± 24% and 143 ± 26% in the first and second samples after treatment, respectively and in the 9 mg/kg group it increased to 181 ± 35% ($T(4) = -2.618$, $P = 0.059$) in the first sample after treatment, while in this group a decline in relation to the first sample was observed in the second sample after treatment (149 ± 35%).

### 3.2. Inhibitory avoidance

Data from the inhibitory avoidance experiments are presented as median ($Q_{0.5}$), lower ($Q_{0.25}$) and upper ($Q_{0.75}$) quartile. For the first experiment (Fig. 2), where the foot-shock level resulted in high level of avoidance learning, the median training levels of all four groups were similar, ranging between $Q_{0.5} = 17$ s and $Q_{0.5} = 31$ s (Kruskal-Wallis, $\chi^2(3) = 2.827$, $P = 0.419$). Mann–Whitney *U*-test for comparison between the groups indicated no difference from the vehicle treated group for all three doses in the 24 h test (comparison against vehicle: 1 mg/kg: $P = 0.114$; 3 mg/kg: $P = 0.809$; 9 mg/kg: $P = 0.512$) and in the 15 days test (comparison against vehicle: 1 mg/kg: $P = 0.114$; 3 mg/kg: $P = 0.282$; 9 mg/kg: $P = 0.387$).

In order to test for the possibility that SR140333 could positively influence memory, in the second inhibitory avoidance experiment (Fig. 3) the intensity of shock was set in order to obtain an overall low level of avoidance learning. Across groups the training step-through median ranged from $Q_{0.5} = 13$ s to $Q_{0.5} = 20$ s (Kruskal-Wallis, $\chi^2(3) = 2.684$, $P = 0.443$). One-tailed Mann–Whitney *U*-test for between group

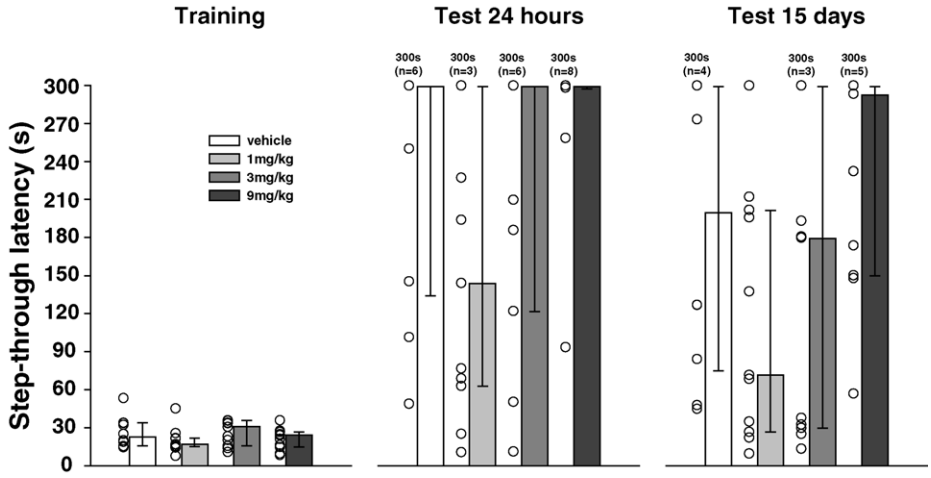


Fig. 2. Inhibitory avoidance performance of animals treated with post-trial vehicle, 1, 3 or 9 mg/kg SR140333 during training, test 24 h and test 15 days. Data are presented as median ($Q_{0.5}$), lower ($Q_{0.25}$) and upper ($Q_{0.75}$) quartile of the step-through latencies in the training trial, test 24 h and 15 days. During the training trail, the animals received a foot-shock (0.2 mA, 1 s) contingent on the step-through response ($n$ = 10–11).

comparisons indicated the following differences: in the 24 h test, none of the treatment groups differed from vehicle (one-tailed Mann–Whitney *U*-test against vehicle: 1 mg/kg: $P$ = 0.228; 3 mg/kg: $P$ = 0.337; 9 mg/kg: $P$ = 0.436). In the 14 days test, the 3 mg/kg treated group was different from vehicle (one-tailed Mann–Whitney *U*-test against vehicle: 1 mg/kg: $P$ = 0.270; 3 mg/kg: $P$ = 0.04; 9 mg/kg: $P$ = 0.386). Based on this trend for a group difference, within-group comparisons were performed for test 14 days against the training session. It revealed that only the animals treated with 3 mg/kg SR140333 had significantly higher latencies to step into the dark compartment (one-tailed Wilcoxon, test 14 days versus training, 3 mg/kg: $P$ = 0.012).

### 3.3. Open-field habituation learning

The frequency of rearing and locomotion were considered for the statistical analysis. No differences across the groups were observed on the baseline trial for any of the referred parameters (Kruskal-Wallis, rearing frequency: $\chi^2(3) = 1.048$, $P$ = 0.790 and locomotion: $\chi^2(3) = 0.188$, $P$ = 0.974). Therefore, the scores obtained in the test are expressed as the mean (+S.E.M.) percentage of baseline behavior and represented in Fig. 4. Within-group comparisons indicated that the 3 mg/kg and 9 mg/kg treated groups showed habituation on the test trial, that is, a decrease in number of rearings (Wilcoxon, day 1 versus day 2, rearing frequency: 3 mg/kg: $P$ = 0.013

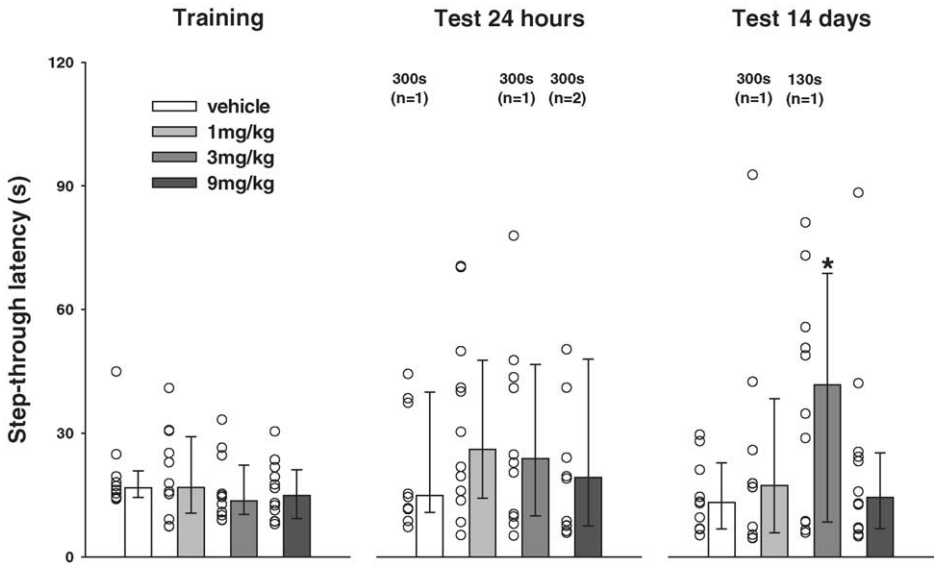


Fig. 3. Inhibitory avoidance performance of animals treated with post-trial injection of vehicle, 1, 3 or 9 mg/kg SR140333. Data are presented as median ($Q_{0.5}$), lower ($Q_{0.25}$) and upper ($Q_{0.75}$) quartile of the step-through latencies during training, test 24 h and test 14 days. The animals received a foot-shock (0.3 mA, 1 s) contingent on the step-through response during the training trail ($n$ = 10–12). Difference from training trial is indicated by $^*$($P$ < 0.05) according to one-tailed Wilcoxon test.

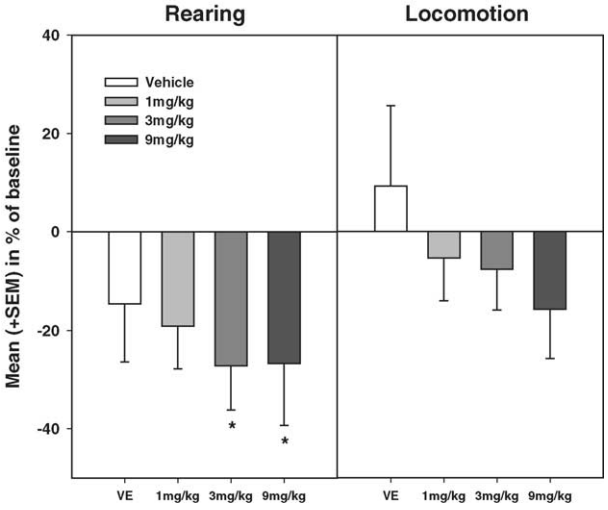


Fig. 4. Mean (+S.E.M.) percentage of baseline (day 1) rearing and locomotion in the open-field during the test trial on day 2. Animals received a post-trial injection of vehicle, 1, 3 or 9 mg/kg SR140333 immediately after the training trial and were tested 24 h later ($n$ = 10–11). Differences from baseline are indicated by $^{*}(P < 0.05)$ according to Wilcoxon test.

and 9 mg/kg: $P = 0.041$). A tendency for habituation was observed for the groups that received either vehicle or 1 mg/kg of SR140333 (Wilcoxon, day 1 versus day 2, rearing frequency: vehicle: $P = 0.066$, 1 mg/kg: $P = 0.091$). No differences were observed in locomotion (Wilcoxon, day 1 versus day 2, $P > 0.05$ for all the comparisons). Mann-Whitney $U$-test for comparison between the groups indicated no difference from the vehicle treated group for all three doses on day 2 in any of the parameters considered for analysis ($P > 0.05$ for all the comparisons).

### 3.4. Conditioned place preference

The time spent in the conditioning quadrant during baseline and test trials was compared by repeated measures ANOVA (Fig. 5). Neither a significant main group ($F(3,48) = 1.103$, $P > 0.05$) nor trial-effect ($F(1,48) = 0.818$, $P > 0.05$), and no interaction of trial × group ($F(3,48) = 0.998$, $P > 0.05$) were observed. Therefore, these results provide no evidence for place preference or place aversion induced by SR140333.

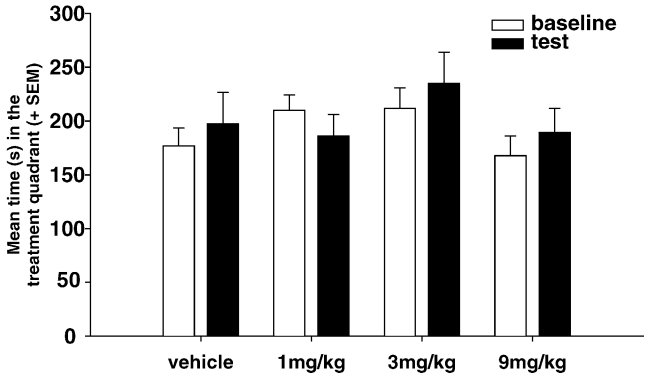


Fig. 5. Mean (+S.E.M.) of time spent in the treatment quadrant of the corral maze during baseline (white bars) and test trial (black bars). During the three consecutive conditioning trials the animals were treated with i.p. injection of vehicle, 1, 3 or 9 mg/kg SR1403335 min before being confined into one of the quadrants of the coral maze. In the baseline and test trial animals were permitted access the entire arena ($n$ = 13 per group).

## 4. Discussion

The intent of this study was to investigate the action of systemic application of the NK-1 receptor antagonist SR140333 on the levels of ACh in three projection areas of the cholinergic cells of the basal forebrain, namely the frontal cortex, amygdala and hippocampus, and to assess its possible influence on inhibitory avoidance learning and on open-field habituation learning, and its potential reinforcing properties in a conditioned place preference task.

Systemic injection of SR140333 increased ACh levels in the hippocampus in a dose-dependent manner, but not in the frontal cortex nor in the amygdala. The first inhibitory avoidance experiment in which a high shock-level led to optimal avoidance learning showed no evidence for amnestic effects of the NK-1 antagonist. In the second inhibitory avoidance study the shock level was adjusted to lead to minimal avoidance learning. The results indicated no effects in the test 24 h after training but in the test 14 days after training only the 3 mg/kg group exhibited avoidance learning. These results can be interpreted in terms of a promnestic effect of the NK-1 antagonist and provide evidence for behavioral activity of this compound, namely an improvement of inhibitory avoidance learning when injected post-trial during the presumed memory consolidation period.

In the open-field habituation learning paradigm an improvement in between-trial behavioral habituation was observed in rearing behavior for the 3 and 9 mg/kg groups. These results corroborate the results obtained in the inhibitory avoidance learning paradigm, providing evidence that NK-1 antagonism can have promnestic effects. There was no evidence for reinforcing or aversive effects of SR140333 in the conditioned place preference paradigm.

Since the drug was delivered systemically, it is possible that the effects observed were due to an action of the compound in the periphery. Recently a comparative study of a variety of NK-1 receptor antagonists pointed towards high selectivity of SR140333 towards the NK-1 receptor in vitro but low brain penetration of this compound, when applied systemically to gerbils [53]. However, other studies have indicated a central action of SR140333 when applied peripherally. For example, systemic injection of this compound blocked thalamic activity to a noxious stimulus applied to the hind paw of rats [16]. Furthermore, i.p. injected SR140333 inhibited turning behavior induced by intrastriatal apomorphine injection and scratching induced by i.c.v. injection of SP and septide [35]. Based on these studies, which indicate brain penetration of systemically injected SR140333, the results of the present study can be interpreted in terms of direct

central effects, although peripheral influences cannot be ruled out.

Although the underlying central mechanisms are unknown, one can hypothesize the following:

(1) It is possible that the observed results were induced by blockade of NK-1 receptors in the NBM or medial septum. The cholinergic neurons of the NBM receive SP-ergic input mainly from the ventral striatum [40,42], while the input to lateral and medial septum of the basal forebrain arises from intrinsic neurons, and a variety of hypothalamic and tegmental nuclei [65]. Injections of SP into the vicinity of the NBM, or medial septal nucleus, have been shown to promote learning as well as to be reinforcing in place preference tasks (for a review see [26]). Injection of SP into the NBM also increased ACh levels in the frontal cortex, either immediately after injection or with 2 h delay, depending on the dose injected [13]. The neural mechanisms underlying these effects are still poorly understood. It is known that SP is the preferred endogenous ligand of the NK−1 receptor [28]. SP-containing terminals synapse cholinergic neurons of the basal forebrain, which express mRNA encoding for NK−1 receptors [4,20]. Neurons expressing NK−1 and NK−3 like-immunoreactivity are also choline acetyltransferase (ChAT) positive in this area [9,10]. Given the role of the cholinergic cells in memory processes, the memory-promoting effect of SP is hypothesized to be mediated by the activation of cholinergic cells, primarily through NK−1 receptors. Blockade of NK-1 receptors would be expected to abolish such SP-mediated effects, since SP binds preferentially to the NK-1 receptor. However, instead of the expected decrease in ACh levels, we found no effect of the selective non-peptide NK-1 receptor antagonist SR140333 within 1 h of dialysis in the frontal cortex and amygdala, but an increase of ACh levels in the hippocampus immediately after the injection, returning to baseline levels within 1 h.
Injection of the N-terminal fragments of SP into the NBM region, but not C-terminal fragments, had memory-promoting effects. The inverse structure-activity relationship was observed for the reinforcing effects: the C-terminal, but not the N-terminal sequence of SP was shown to be reinforcing [26,30]. The present results do not contradict predictions based on these results, since it is likely that the memory-promoting effects of SP are due to its N-terminal fragment action on the SPN binding site [31]. SP1−7 increased the expression of NMDA receptor subunits NR1, NR2A and NR2B [71]. The overexpression of the NR2B subunit in the forebrain of transgenic mice improves memory [66]. It is also likely that the reinforcing effects of SP are not mediated only by the NK−1 receptor. The C-terminal fragment SP5−11 (DiMe-C7) which binds to both NK−1 and NK−3 receptors, with higher affinity to NK−3 than to NK−1 receptors [50], induces reinforcement and increases DA levels in the NAc when injected into the NBM region; the reinforcing effects of DiMe-C7 could not be completely antagonized by the NK−1 receptor antagonists WIN51,708 [43]. Therefore, it is possible that both NK-1 and NK-3 receptors are involved in the mediation of the reinforcing effects of SP.

(2) Peripherally administered NK-1 receptor antagonists may act directly on NK-1 receptors present on inhibitory interneurons in the hippocampus [60]. The hippocampus is richly innervated by SP terminals [5,58] and there is evidence for a direct septo-hippocampal SP-ergic projection [46]. SP enhances inhibitory interneuron transmission on pyramidal cells of the hippocampus and this effect is blocked by application of SR140333 [44]. Thus, the NK-1 receptor modulates GABAergic transmission in the hippocampus and may be implicated in learning and memory processes. It has been suggested that high levels of septal GABAergic activity might impair memory for an inhibitory avoidance task by down-regulating ACh levels in the hippocampal region, and that such memory impairments can be ameliorated by increasing ACh levels in the hippocampus [15]. Since the same doses of SR140333 that induced promnestic effects in the present study also increased the extracellular level of ACh in the hippocampus, it is tempting to link the memory-promoting effect of the NK-1 antagonist to its effectiveness in increasing levels of ACh in the hippocampus. Therefore, it is possible that promnestic effects of the NK-1 receptor antagonists may be due to an inhibition of GABAergic and consequently increased cholinergic activity in the hippocampus. Nicotininc cholinergic activity in this brain area has been implicated in the retrieval of long-term avoidance learning [38,39].

(3) It is also possible that the increase in ACh levels observed was due to an action by SR140333 on other neurotransmitter systems interacting with cholinergic cells of the basal forebrain.

Besides its memory promoting and positive reinforcing actions [26,30], SP has been reported to have both anxiolytic [26] and anxiogenic [12,67] effects, depending on the dose and site of injection. These effects of SP can be differentiated in those mediated by its N- and C-terminal fragments, i.e. anxiety, learning- and memory-, and reinforcement-related properties, respectively (for a review see [26]). Recently NK-1 receptor antagonists have been suggested to have antidepressant and anxiolytic properties [6,18,29,37]. Chronic treatment with antidepressants led to a decrease in SP levels in certain brain structures [59] and repeated electroconvulsive shocks increased NK-1 receptor densities in cortical structures, while leaving mRNA levels for this receptor unchanged [7]. Furthermore NK-1 receptor knock-out mice were less aggressive than wild-type mice [14]. These studies show that NK-1 blockade by itself is involved in a variety of behavioral effects and might have therapeutical efficiency in the treatment of mood and anxiety disorders. The rising

interest in modulation of central peptidergic systems by selective agonists or antagonists is encouraged by lesser side effects compared to classical agents. As for the clinical application of anxiolytics, it would be advantageous to develop effective compounds without amnestic side effects, perhaps on the basis of NK-1 antagonism.

In conclusion, the results of this study provide evidence for an effect of systemic NK-1 antagonist injection on central ACh levels and a tendency towards promnestic effects. To our knowledge, this is the first demonstration of changes of ACh levels in the rat brain mediated by a systemic injection of a NK-1 antagonist and its relation to memory performance. These data indicate possible neuro-behavioral effects of NK-1 antagonists other than the antidepressant [37] and anxiolytic [6,18] actions described so far. The brain-site dependent actions of SR140333 may also have relevance for understanding neurokinin-cholinergic interactions in terms of neuronal and behavioral functions of these transmitter systems.

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## References

[1] Bell MI, Richardson PJ, Lee K. Characterization of the mechanism of action of tachykinins in rat striatal cholinergic interneurons. Neuroscience 1998;87:649–58.
[2] Bevilaqua LR, Kerr DS, Medina JH, Izquierdo I, Cammarota M. Inhibition of hippocampal Jun N-terminal kinase enhances short-term memory but blocks long-term memory formation and retrieval of an inhibitory avoidance task. Eur J Neurosci 2003;17:897–902.
[3] Boix F, Pfister M, Huston JP, Schwarting RK. Substance P decreases extracellular concentrations of acetylcholine in neostriatum and nucleus accumbens in vivo: possible relevance for the central processing of reward and aversion. Behav Brain Res 1994;63:213–9.
[4] Bolam JP, Ingham CA, Izzo PN, Levey AI, Rye DBM, Smith AD, et al. Substance P-containing terminals in synaptic contact with cholinergic neuron in the neostriatum and basal forebrain: a double immunocytochemical study in the rat. Brain Res 1986;397:279–89.
[5] Borhegyi Z, Leranth C. Substance P innervation of the rat hippocampal formation. J Comp Neurol 1997;384:41–58.
[6] Boyce S, Smith D, Carlson E, Hewson L, Rigby M, O'Donnell R, et al. Intra-amygdala injection of the substance P [NK(1) receptor] antagonist L-760735 inhibits neonatal vocalisations in guinea-pigs. Neuropharmacology 2001;41:130–7.
[7] Burnet PW, Miller R, Lewis LJ, Pei Q, Sharp T, Harrison PJ. Electroconvulsive shock increases tachykinin NK(1) receptors, but not the encoding mRNA, in rat cortex. Eur J Pharmacol 2001;413:213–9.
[8] Butcher LL. Cholinergic neurons networks. In: Paxinos G, editor. The rat nervous system. San Diego: Academic Press; 1995. p. 1003–37.
[9] Chen LW, Wei LC, Liu HL, Ding YQ, Zhang H, Rao ZR, et al. Cholinergic neurons expressing neuromedin K receptor (NK3) in the basal forebrain of the rat: a double immunofluorescence study. Neuroscience 2001;103:413–22.
[10] Chen LW, Wei LC, Liu HL, Qiu Y, Chan YS. Cholinergic neurons expressing substance P receptor (NK(1)) in the basal forebrain of the rat: a double immunocytochemical study. Brain Res 2001;904:161–6.
[11] Damsma G, van Bueren DL, Westerink BHC, Horn AS. Determination of acetylcholine and choline in the femtomole range by means of HPLC, a post-column enzyme reactor, and electrochemical detection. Chromatographia 1987;24:827–31.
[12] De Araújo JE, Silva RC, Huston JP, Brandão ML. Anxiogenic effects of substance P and its 7–11 C terminal, but not the 1–7 N terminal, injected into the dorsal periaqueductal gray. Peptides 1999;20:1437–43.
[13] De Souza Silva MA, Hasenöhrl RU, Tomaz C, Schwarting RKW, Huston JP. Differential modulation of frontal cortex acetylcholine by injection of substance P into the nucleus basalis magnocellularis region in the freely-moving vs. the anesthetized preparation. Synapse 2000;38:243–53.
[14] De-Felipe C, Herrero JF, O'Brien JA, Palmer JA, Doyle CA, Smith AJ, et al. Altered nociception, analgesia and aggression in mice lacking the receptor for substance P. Nature 1998;392:394–7.
[15] Degroot A, Parent MB. Infusions of physostigmine into the hippocampus or the entorhinal cortex attenuate avoidance retention deficits produced by intra-septal infusions of the GABA agonist muscimol. Brain Res 2001;920:10–8.
[16] Emonds-Alt X, Doutremepuich JD, Heaulme M, Neliat G, Santucci V, Steinberg R, et al. In vitro and in vivo biological activities of SR140333, a novel potent non-peptide tachykinin NK1 receptor antagonist. Eur J Pharmacol 1993;250:403–13.
[17] Feuerstein TJ, Gleichauf O, Landwehrmeyer GB. Modulation of cortical acetylcholine release by serotonin: the role of substance P interneurons. Naunyn Schmiedebergs Arch Pharmacol 1996;354:618–26.
[18] File SE. NKP608 an NK1 receptor antagonist, has an anxiolytic action in the social interaction test in rats. Psychopharmacology (Berl) 2000;152:105–9.
[19] Froger N, Gardier AM, Moratalla R, Alberti I, Lena I, Boni C, et al. 5-Hydroxytryptamine (5-HT)1A autoreceptor adaptive changes in substance P (neurokinin 1) receptor knock-out mice mimic antidepressant-induced desensitization. J Neurosci 2001;21:8188–97.
[20] Gerfen CR. Substance P (neurokinin-1) receptor mRNA is selectively expressed in cholinergic neurons in the striatum and basal forebrain. Brain Res 1991;556:165–70.
[21] Gerhardt P, Voits M, Fink H, Huston JP. Evidence for mnemotropic action of cholecystokinin fragments Boc-CCK-4 and CCK-8S. Peptides 1994;15:689–97.
[22] Giovannini MG, Bartolini L, Kopf SR, Pepeu G. Acetylcholine release from the frontal cortex during exploratory activity. Brain Res 1998;784:218–27.
[23] Giovannini MG, Rakovska A, Benton RS, Pazzagli M, Bianchi L, Pepeu G. Effects of novelty and habituation on acetylcholine, GABA, and glutamate release from the frontal cortex and hippocampus of freely moving rats. Neuroscience 2001;106:43–53.
[24] Givens BS, Sarter M. Modulation of cognitive processes by transsynaptic activation of the basal forebrain. Behav Brain Res 1997;84:1–22.
[25] Hasenohrl RU, Oitzl MS, Huston JP. Conditioned place preference in the corral: a procedure for measuring reinforcing properties of drugs. J Neurosci Methods 1989;30:141–6.
[26] Hasenohrl RU, Souza-Silva MA, Nikolaus S, Tomaz C, Brandao ML, Schwarting RK, et al. Substance P and its role in neural mechanisms governing learning, anxiety and functional recovery. Neuropeptides 2000;34:272–80.
[27] Hironaka N, Tanaka K, Izaki Y, Hori K, Nomura M. Memory-related acetylcholine efflux from rat prefrontal cortex and hippocampus: a microdialysis study. Brain Res 2001;901:143–50.
[28] Hokfelt T, Pernow B, Wahren J. Substance P: a pioneer amongst neuropeptides. J Intern Med 2001;249:27–40.

[29] Holmes A, Heilig M, Rupniak NM, Steckler T, Griebel G. Neuropeptide systems as novel therapeutic targets for depression and anxiety disorders. Trends Pharmacol Sci 2003;24:580–8.
[30] Huston JP, Hasenöhrl RU. The role of neuropeptides in learning: focus on the neurokinin substance P. Behav Brain Res 1995;66:117–27.
[31] Igwe OJ, Kim DC, Seybold VS, Larson AA. Specific binding of substance P aminoterminal heptapeptide [SP(1-7)] to mouse brain and spinal cord membranes. J Neurosci 1990;10:3653–63.
[32] Izquierdo I, Medina JH. Memory formation: the sequence of biochemical events in the hippocampus and its connection to activity in other brain structures. Neurobiol Learn Mem 1997;68:285–316.
[33] Izquierdo LA, Barros DM, Ardenghi PG, Pereira P, Rodrigues C, Choi H, et al. Different hippocampal molecular requirements for short- and long-term retrieval of one-trial avoidance learning. Behav Brain Res 2000;111:93–8.
[34] Jerusalinsky D, Kornisiuk E, Izquierdo I. Cholinergic neurotransmission and synaptic plasticity concerning memory processing. Neurochem Res 1997;22:507–15.
[35] Jung M, Calassi R, Maruani J, Barnouin MC, Souilhac J, Poncelet M, et al. Neuropharmacological characterization of SR 140333, a non peptide antagonist of NK1 receptors 1994;33:167–79.
[36] Kouznetsova M, Nistri A. Facilitation of cholinergic transmission by substance P methyl ester in the mouse hippocampal slice preparation. Eur J Neurosci 2000;12:585–94.
[37] Kramer MS, Cutler N, Feighner J, Shrivastava R, Carman J, Sramek JJ, et al. Distinct mechanism for antidepressant activity by blockade of central substance P receptors. Science 1998;281:1640–5.
[38] Luft T, Pereira GS, Cammarota M, Izquierdo I. Different time course for the memory facilitating effect of bicuculline in hippocampus, entorhinal cortex, and posterior parietal cortex of rats. Neurobiol Learn Mem 2004;82:52–6.
[39] Marti BD, Ramirez MR, Dos Reis EA, Izquierdo I. Participation of hippocampal nicotinic receptors in acquisition, consolidation and retrieval of memory for one trial inhibitory avoidance in rats. Neuroscience 2004;126:651–6.
[40] Mitrovic I, Napier TC. Substance P attenuates and DAMGO potentiates amygdala glutamatergic neurotransmission within the ventral pallidum. Brain Res 1998;792:193–206.
[41] Muir JL. Acetylcholine, aging, and Alzheimer's disease. Pharmacol Biochem Behav 1997;56:687–96.
[42] Napier TC, Mitrovic I, Churchill L, Klitenick MA, Lu X-Y, Kalivas PW. Substance P in the ventral pallidum: projection from the ventral striatum, and electrophysiological and behavioral consequences of pallidal substance P. Neuroscience 1995;69:59–70.
[43] Nikolaus S, Huston JP, Hasenohrl RU. Reinforcing effects of neurokinin substance P in the ventral pallidum: mediation by the tachykinin NK1 receptor. Eur J Pharmacol 1999;370:93–9.
[44] Ogier R, Raggenbass M. Action of tachykinins in the rat hippocampus: modulation of inhibitory synaptic transmission. Eur J Neurosci 2003;17:2639–47.
[45] Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 2nd ed. Sydney: Academic Press; 1986.
[46] Peterson GM, Shurlow CL. Morphological evidence for a substance P projection from medial septum to hippocampus. Peptides 1992;13:509–17.
[47] Pickel VM, Douglas J, Chan J, Gamp PD, Bunnett NW. Neurokinin 1 receptor distribution in cholinergic neurons and targets of substance P terminals in the rat nucleus accumbens. J Comp Neurol 2000;423:500–11.
[48] Potter PE, Meek JL, Neff NH. Acetylcholine and choline in neuronal tissue measured by HPLC with electrochemical detection. J Neurochem 1983;41:188–94.
[49] Quartara L, Maggi CA. The tachykinin $NK_1$ receptor. Part II: Distribution and pathophysiology. Neuropeptides 1998;32:1–49.
[50] Regoli D, Boudon A, Fauchere JL. Receptors and antagonists for substance P and related peptides. Pharmacol Rev 1994;46:551–99.
[51] Robbins TW, McAlonan G, Muir JL, Everitt BJ. Cognitive enhancers in theory and practice: studies of the cholinergic hypothesis of cognitive deficits in Alzheimer's disease. Behav Brain Res 1997;83:15–23.
[52] Roldan G, Bolanos-Badillo E, Gonzalez-Sanchez H, Quirarte GL, Prado-Alcala RA. Selective M1 muscarinic receptor antagonists disrupt memory consolidation of inhibitory avoidance in rats. Neurosci Lett 1997;230:93–6.
[53] Rupniak NM, Carlson EJ, Shepheard S, Bentley G, Williams AR, Hill A, et al. Comparison of the functional blockade of rat substance P (NK1) receptors by GR205171, RP67580, SR140333 and NKP-608. Neuropharmacology 2003;45:231–41.
[54] Schildein S, Huston JP, Schwarting RK. Open field habituation learning is improved by nicotine and attenuated by mecamylamine administered posttrial into the nucleus accumbens. Neurobiol Learn Mem 2002;77:277–90.
[55] Schlesinger K, Lipsitz DU, Peck PL, Pelleymounter MA, Stewart JM, Chase TN. Substance P enhancement of passive and active avoidance conditioning in mice. Pharmacol Biochem Behav 1983;19:655–61.
[56] Schlesinger K, Lipsitz DU, Peck PL, Pelleymounter MA, Stewart JM, Chase TN. Substance P reversal of electroconvulsive shock and cycloheximide-induced retrograde amnesia. Behav Neural Biol 1983;39:30–9.
[57] Schlesinger K, Pelleymounter MA, van de KJ, Bader DL, Stewart JM, Chase TN. Substance P facilitation of memory: effects in an appetitively motivated learning task. Behav Neural Biol 1986;45:230–9.
[58] Senut MC, Menetrey D, Lamour Y. Cholinergic and peptidergic projections from the medial septum and the nucleus of the diagonal band of Broca to dorsal hippocampus, cingulate cortex and olfactory bulb: a combined wheatgerm agglutinin-apohorseradish peroxidase-gold immunohistochemical study. Neuroscience 1989;30:385–403.
[59] Shirayama Y, Mitsushio H, Takashima M, Ichikawa H, Takahashi K. Reduction of substance P after chronic antidressants treatment in the striatum, substantia nigra and amygdala of the rat. Brain Res 1996;739:70–8.
[60] Sloviter RS, Ali-Akbarian L, Horvath KD, Menkens KA. Substance P receptor expression by inhibitory interneurons of the rat hippocampus: enhanced detection using improved immunocytochemical methods for the preservation and colocalization of GABA and other neuronal markers. J Comp Neurol 2001;430:283–305.
[61] Smith DW, Hewson L, Fuller P, Williams AR, Wheeldon A, Rupniak NM. The substance P antagonist L-760,735 inhibits stress-induced NK(1) receptor internalisation in the basolateral amygdala. Brain Res 1999;848:90–5.
[62] Stacey AE, Woodhall GL, Jones R-SG. Activation of neurokinin-1 receptors promotes GABA release at synapses in the rat entorhinal cortex. Neuroscience 2002;115:575–86.
[63] Stäubli U, Huston JP. Facilitation of learning by post-trial injection of substance P into the medial septal nucleus. Behav Brain Res 1980;1:245–55.
[64] Steinberg R, Rodier D, Souilhac J, Bougault I, Emonds-Alt X, Soubrié P, et al. Pharmacological characterization of tachykinin receptors controlling acetylcholine release from rat striatum: an in vivo microdialysis study. J Neurochem 1995;65:2543–8.
[65] Szeidemann Z, Jakab RL, Shanabrough M, Leranth C. Extrinsic and intrinsic substance P innervation of the rat lateral septal area calbindin cells. Neuroscience 1995;69:1205–21.
[66] Tang YP, Shimizu E, Dube GR, Rampon C, Kerchner GA, Zhuo M, et al. Genetic enhancement of learning and memory in mice. Nature 1999;401:63–9.
[67] Teixeira RM, Santos ARS, Ribeiro SJ, Calixto JB, Rae GA, de Lima TCM. Effects of central administration of tachykinin receptor agonists and antagonists on plus-maze bahavior in mice. Eur J Pharmacol 1996;311:7–14.

[68] Thiel CM, Huston JP, Schwarting RKW. Hippocampal acetylcholine and habituation learning. Neuroscience 1998;85:1253–62.
[69] Tomaz C, Huston JP. Facilitation of conditioned inhibitory avoidance by post-trial peripheral injection of substance P. Pharmacol Biochem Behav 1986;25:469–72.
[70] Zaborsky L, Cullinan WE, Braun A. Afferents to basal forebrain cholinergic projection neurons: an update. In: Napier TC, Kalivas PW, Hanin I, editors. The basal forebrain: anatomy to function. New York: Plenum Press; 1991. p. 43–100.
[71] Zhou Q, Le Greves P, Ragnar F, Nyberg F. Intracerebroventricular injection of the N-terminal substance P fragment SP(1–7) regulates the expression of the N-methyl-D-aspartate receptor NR1, NR2A and NR2B subunit mRNAs in the rat brain. Neurosci Lett 2000;291:109–12.

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# Wistar rats show episodic-like memory for unique experiences

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**Abstract**

Human episodic memory refers to the recollection of an unique past experience in terms of its details, its locale, and temporal occurrence. Episodic memory, even in principle, has been difficult to demonstrate in non-verbal mammals. Previously, we provided evidence that mice are able to form an integrated memory for "what," "where," and "when" aspects of single experiences by combining different versions of the novelty-preference paradigm, i.e., object recognition memory, the memory for locations in which objects were explored, and the temporal order memory for objects presented at distinct time points. In the present series of experiments we evaluated whether this paradigm, with minor modifications, also works with rats. We found that rats spent more time exploring an "old familiar" object relative to a "recent familiar" object, suggesting that they recognized objects previously explored during separate trials and remembered their order of presentation. Concurrently, the rats responded differentially to spatial object displacement dependent on whether an "old familiar" or "recent familiar" object was shifted to a location, where it was not encountered previously. These results provide strong evidence that the rats established an integrated memory for "what," "where," and "when." We also found that acute stress impaired the animal's performance in the episodic-like memory task, which, however, could be partially reversed by the *N*-Methyl-D-aspartate-receptors agonist D-cycloserine.


*Keywords:* *N*-Methyl-D-aspartate-receptors; D-Cycloserine; Animal models of episodic memory; Neuroplasticity; Stress; Mental time travel

## 1. Introduction

Episodic memory refers to the conscious recollection of personal experiences. Humans not only remember "what" happened to them, but also "where" and "when" they experienced it. It has been proposed that this type of memory requires autonoetic awareness and a sense of subjective time (Tulving, 2002). In humans, conscious recollection is generally tested by asking the subjects whether they *remember* studying an item, or if they simply *know* whether the particular item was part of the learning material presented, because it appears somewhat familiar to them (Yonelinas, 2002). This procedure obviously cannot be applied to non-verbal animals. Furthermore, it has been suggested that animals, being devoid of a sense of subjective time, might only remember the facts of a past event (equivalent to semantic memory), while being unaware that they remember facts about a personal experience (Roberts, 2002). However, factoring out the issue of conscious recollection, non-verbal animals may bear an implicit form of episodic memory, i.e., the ability to integrate and remember the "what," "where," and "when" elements of a personal experience, which can be inferred from behavior. Such an integrated memory has been shown in food-caching scrub jays (Clayton & Dickinson, 1998). Unequivocal demonstrations of episodic-like memory in non-human mammals were hampered by the fact, that it proved to be difficult to operationalize the "when" component in addition to the "what" and "where" components of an episodic memory in a single task (Hampton & Schwartz, 2004), without using extensive training procedures, which might induce rule learning, and, consequently semantic memory recall during the test trials. In this regard, Eacott and Norman (2004) proposed that the temporal component of an episodic memory is

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negligible, even in humans, and should be replaced by an "occasion-specifying context" similar to the discriminative stimuli provided by distinct background scenes enabling non-human primates to perform food rewarded object discrimination tasks (Gaffan, 1994). However, Ergorul and Eichenbaum (2004) acknowledged the importance of the "when" aspect of an episodic memory, and showed that rats extensively trained to remember a series of odors presented in different places, use a combination of spatial "where" and olfactory "what" cues to distinguish "when" events occurred. We recently designed an three-trial object exploration task for mice, which does not require food or water deprivation, the application of reinforcers or extensive rule learning. In this task different versions of the novelty-preference paradigm were combined, that are presumed to measure object recognition memory (Ennaceur & Delacour, 1988), the memory for locations in which objects were explored (Ennaceur, Neave, & Aggleton, 1997), and the temporal order memory for object presentations (Mitchell & Laiacona, 1998). Our results suggest that mice are able to form an integrated memory for "what," "where," and "when" after unique experiences. In the present series of experiments we evaluated whether this paradigm (Dere, Huston, & De Souza Silva, 2005a; Dere, Huston, & De Souza Silva, 2005b), with minor modifications, can also be applied to rats and whether the *N*-Methyl-D-aspartate-receptors (NMDA-R) agonist D-cycloserine (DCS) has promestic effects on episodic-like memory.

## 2. Experiment 1: Episodic-like memory in Wistar rats

### 2.1. Introduction

The object exploration task consists of a sample trial, during which rats or mice explore two equal objects, followed by a delayed test trial, in which a novel object is presented together with one familiar object already presented during the sample trial. One generally finds that rats and mice spend more time exploring the novel object, indicating that the familiar object was recognized (Ennaceur & Delacour, 1988). A modification of the paradigm allows to measure the memory for locations, where objects were initially explored, by presenting two equal and familiar objects during the test trial, with one of the objects shifted to a novel location. Here, animals spend more time exploring the object encountered in the novel location (Ennaceur et al., 1997). Another variant of the novelty-preference paradigm measures the memory for the temporal order in which two different objects were presented in the past. Unlike the object recognition and object-place memory versions, this task is a three-trial procedure, composed of two sample trials, with an inter-trial interval of about 1, during which two copies of a novel object are presented. During the test trial one "old familiar" object known from sample trial one, and another "recent familiar" object from sample trial two are presented together. Here, one finds that the animals spend more time exploring the "old familiar" object relative to the "recent familiar" object, indicating that the previously explored objects are recognized and discriminated in terms of their relative recency (Mitchell & Laiacona, 1998).

We combined these different procedures into a single spontaneous exploration task consisting of two sample trials and one test trial. This task has the advantage that it does not require food or water deprivation, the application of reinforcers or extensive rule learning. Episodic memory, in its narrowest sense, might be defined as the ability to remember the "what," "where," and "when" of a past experience. Previously, we provided the first evidence that mice are able to form an integrated memory for "what," "where," and "when" aspects of single experiences by combining different versions of the novelty-preference paradigm, i.e., object recognition memory, the memory for locations in which objects were explored, and the temporal order memory for objects presented at distinct time points (Dere et al., 2005a, 2005b). In the present experiment we evaluated whether this paradigm, with minor modifications, also works with rats. In terms of the neurobiology of learning and memory, rats are the best studied mammalian species. Thus, a valid rat model of episodic-like memory would be especially valuable.

### 2.2. Subjects

Subjects were 20 naïve male Wistar rats, weighting 270–360 g. Rats were group housed with $n = 5$ per cage. They were maintained in a temperature and humidity controlled room (20–22 °C) on a reversed 12 h light–dark cycle with lights off at 07:00 a.m., and had free access to rodent chow and tap water. Testing occurred during the dark phase. Animals were habituated to the housing conditions for two weeks. Before testing, the animals were handled for 5 days to reduce possible stress from testing procedure. All experiments were performed according to the guidelines of the German Animal Protection Law and were approved by the North Rhine Westphalia State Authority.

### 2.3. Apparatus and object stimuli

Behavior was assessed in a sound-attenuated experimental room with a noise-generator providing masking noise. The open-field ($60 \times 60 \times 30$ cm) used was made of gray polyvinyl chloride and illuminated by four 75 W bulbs providing a light density of approximately 17 lx at the center of the field. Its floor was virtually divided into 9 quadrants of equal size. A video camera, connected to a video recorder, was mounted 160 cm above the field to store sample and test trials on video tapes for off-line analysis. The open-field had an open roof, so that the rats could perceive external distal cues. After each trial, the apparatus was thoroughly cleaned with water containing 0.1% acetic acid.

Three different objects (in quadruplicate) made of glass, which differed in terms of height (22–25 cm), base diameter (8–10 cm) and color (red, green, white), shape

(octagon, circular, rectangle) and surface texture (plain, grooved) were used. Since the objects were made of the same material, they could not be distinguished by olfactory cues during the test trial. The objects had sufficient weight to ensure that the rats could not displace them. After each trial, the objects were thoroughly cleaned with an 0.1% acetic acid solution to remove odor cues. The objects had no known ethological significance for the rats and had never been paired with a reinforcer. Pilot studies ensured that rats could discriminate the three objects, and there was no per se preference for one of these objects. It is difficult to specify the sensory modality, tactile vs. visual, primarily used by the rats to explore the objects. Since the objects differ in terms of form, dimension, and surface texture, it is likely that the rat's use a combination of both sensory input's to form a mental representation of the objects. In this regard it was shown that neurons in the central trigeminal nuclei, which receive input from the large facial whiskers, tuned to the direction of whisker deflection (Jones, Lee, Trageser, Simons, & Keller, 2004) and that rat's use their whiskers to discriminate objects (Polley, Rickert, & Frostig, 2005). Rats which attempted to climb onto the objects were excluded from data analysis.

### 2.4. Habituation to the open-field

Rats were familiarized with the test apparatus for 3 consecutive days. The rats were placed into the central part of the open-field and allowed to explore for 10 min.

### 2.5. Simultaneous assessment of memory for "what," "where," and "when" by combining different versions of the novelty-preference paradigm

One day after the last habitation session, the "what," "where," and "when" task was performed. Each rat received two sample trials and a test trial. After placement of the rat into the open-field, the experimenter left the room to avoid interactions with the animal during testing. On the first sample trial the rats were placed into the center of the open-field containing 4 copies of a novel object randomly placed in four out of eight possible locations, and allowed to explore them for 5 min. After a delay of 50 min, the rats received a second sample trial identical to the first, except that four novel objects were present. Two of these objects were placed randomly in two out of four possible locations, which already contained objects during sample trial one, while the remaining two objects were randomly placed in two out of four locations, which were not occupied by objects during the first sample trial. The particular objects presented on sample trials 1 and 2 were randomly determined for each rat. After an additional delay of 50 min, the rats received a test trial, lasting 5 min, identical to the sample trials, except that two copies of the object from sample trial 1 ("*old familiar*" objects) and two copies of the object known from sample trial 2 ("*recent familiar*" objects) were present. One object of each type was presented in a randomly chosen location, in which it was already encountered during the respective sample trial, while the other object of the same type was presented in a randomly chosen location, where it was not encountered during the sample trial. Thus, one "old familiar" object was kept in place ("*old familiar*" *stationary* object $= A_1$), while the other was displaced to a novel location ("*old familiar*" *displaced* object $= A_2$). In contrast to the study performed with mice (Dere et al., 2005a, 2005b) the same was done for the "recent familiar" objects termed "*recent familiar*" *stationary* object $= B_1$ and "*recent familiar*" *displaced* object $= B_2$ to know whether the exploration pattern exhibited by the rats would indicate an interaction between the factors recency and spatial displacement. All four objects were presented at familiar locations, that is at locations which already contained objects during sample trial one and/or two. Fig. 1 gives an example for sample and test trials of the "what," "where," and "when" task.

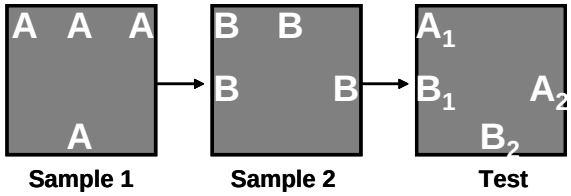


Fig. 1. Experimental design. This schematic drawing shows one example of a possible object arrangement for the "what," "where," and "when" task. The rats received three 5 min trials with a 50 min inter-trial interval. During the test trial, two "old familiar" and two "recent familiar" objects known from the sample trials were presented at familiar and novel locations relative to the respective sample trials. $A_1$, "old familiar-stationary"; $A_2$, "old familiar-displaced"; $B_1$, "recent familiar-stationary"; and $B_2$, "recent familiar-displaced."

### 2.6. Dependent variables and data acquisition

For each rat the time spent exploring the objects during sample and test trials was scored off-line from video tapes. Exploration of an object was assumed when the rat approached an object and had physical contact with it, either with its, snout and/or forepaws.

### 2.7. Predictions

Based on our (Dere et al., 2005a, 2005b) and others previous research (Ennaceur & Delacour, 1988; Ennaceur et al., 1997; Mitchell & Laiacona, 1998), we hypothesized that on the test trial the rats would spend more time exploring the "old familiar" stationary object initially explored during sample trial 1 relative to the "recent familiar" stationary object first presented during sample trial 2, reflecting memory for "what" and "when." Furthermore, we hypothesized that the rats would spend more time exploring the displaced relative to the stationary object of the same type, reflecting memory for "what" and "where."

### 2.8. Statistics

Within-subject differences in the time spent exploring the "old familiar" and "recent familiar" stationary objects and in the time spent exploring the displaced and stationary objects for each type of object were analyzed by means of sign tests. All $p$ values given represent measures of effect. For the sake of simplicity all data will be presented as mean + SEM, although the appropriate form of data presentation for non-parametric analyses would be median and interquartile ranges.

### 2.9. Results

As hypothesized, during the test trial, the rats spent more time exploring the stationary "old familiar" object relative to the stationary "recent familiar" object ($p = .038$, one-tailed sign test; Fig. 2). To know whether this effect was due to differences in the amount of learning, we also compared the time spent exploring the objects on sample trial 1 with sample trial 2. The rats showed similar exploration times on both sample trials ($p > .05$; data not shown). Thus, differences in the amount of learning cannot account for the above finding.

We further predicted that the rats, for each object type separately, would spent more time exploring displaced object relative to the stationary object. The rats indeed spent more time exploring the displaced "recent familiar" compared to the stationary "recent familiar" object ($p = .038$; Fig. 2). However, contrary to the prediction, the displaced "old familiar" object was explored for a shorter period relative to the stationary "old familiar" object ($p = .038$, Fig. 2). To know whether this exploration pattern ($A_1 > A_2$, $B_1 < B_2$) was due to chance we performed a binominal test. The data were compared to a binomial distribution, with four possible cases ($C_1$: $A_1 < A_2$, $B_1 < B_2$; $C_2$: $A_1 < A_2$, $B_1 > B_2$; $C_3$: $A_1 > A_2$, $B_1 > B_2$; $C_4$: $A_1 > A_2$, $B_1 < B_2$), which is a rather conservative test in terms of getting a statistical significant result, since more cases could be defined. The binominal test revealed that the exploration pattern found ($C_4$: $A_1 > A_2$, $B_1 < B_2$) was not due to chance [$B(16, 0.25)$: $p = .028$].

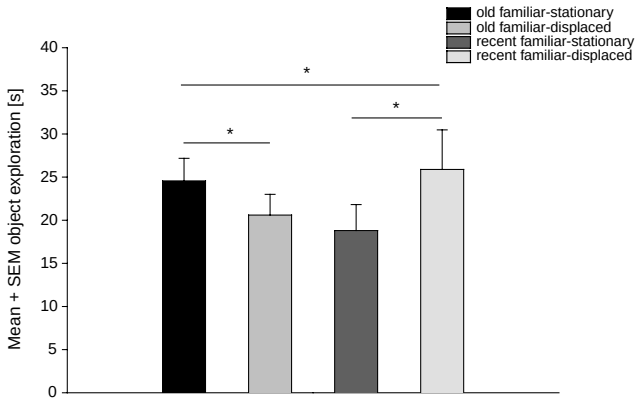


Fig. 2. Episodic-like object memory in rats; experiment 1: bars represent mean (+SEM) object exploration [s] of indicated objects. *$p < .05$.

### 2.10. Discussion

In line with previous work from other groups (Hannesson, Howland, & Phillips, 2004; Hotte, Naudon, & Jay, 2005) the rats spent more time exploring a stationary "old familiar" object relative to a stationary "recent familiar" object, suggesting that they both recognized previously explored objects and remembered their order of appearance. But, more importantly, our results suggest that rats are also able to simultaneously discriminate whether the objects were spatially displaced or stationary compared to their first appearance, reflecting knowledge of "what" and "where" in addition to "what" and "when." We predicted that the rats, for each object type separately, would spend more time exploring displaced object relative to the stationary object. However, while the rats indeed preferred the displaced "recent familiar" compared to the stationary "recent familiar" object, they, in contrast, preferred the stationary "old familiar" object relative to the displaced "old familiar" object, suggesting an interaction between the factors recency and spatial displacement. This interaction, although not explicitly predicted, actually provides strong evidence that the rats indeed established an integrated memory for "what," "where," and "when," since it excludes the possibility that the temporal- and spatial object information were encoded, stored, and retrieved independently from each other.

## 3. Experiment 2: Replication of the findings of experiment 1 and the effects of pre-sample trial 1 saline injection on episodic-like memory

### 3.1. Introduction

In this second experiment we investigated whether the exploration pattern observed in experiment 1, especially in terms of the interaction between the factors recency and spatial displacement, is replicable using a new batch of naïve rats. In pilot work with mice we noticed that the stress associated with a single i.p. saline injection prior to sample trial 1 had a detrimental effect on the test trial performance of the mice (unpublished observations). Furthermore, it has been reported that a simple saline injection procedure (Nagel & Huston, 1988) and other stressful manipulations can impair memory (Baker & Kim, 2002). Since we would like to investigate the effects of pharmacological treatments on this episodic memory model, it would be important to know the effects of the injection procedure alone on the rat's performance in this task. Therefore, our second aim was to evaluate whether a single saline injection prior to sample trial 1 would, per se, have an effect on the rat's test trial performance.

### 3.2. Subjects

Subjects were 24 naïve male Wistar rats, which were maintained and handled as described under experiment 1.

### 3.3. Drugs and application procedure

The animals were randomly assigned to one of two groups, either an untreated group ($n = 12$) or a saline-treated group ($n = 12$). Physiological saline was injected in a volume of 1 ml/kg 30 min prior to the first sample trial.

### 3.4. Predictions

Based on the results of experiment 1, we hypothesized that on the test trial the untreated rats would spend more time exploring the "old familiar" stationary object relative to the "recent familiar" stationary object. Furthermore, we hypothesized that the untreated rats would spend more time exploring the displaced "recent familiar" object relative to the stationary "recent familiar" object. According to the findings from experiment 1 we hypothesized, that the untreated rats would spent less time exploring the displaced "old familiar" object relative to the stationary "old familiar" object. For the saline-treated group no exact hypotheses were formulated, since in contrast to mice, as mentioned above, rats might be less susceptible to stress associated with an i.p. needle injection.

### 3.5. Statistics

Sample trial data were analysed by means of Wilcoxon tests. For the test trial, data within-subject differences in the time spent exploring the "old familiar" and "recent familiar" stationary objects and in the time spent exploring the displaced and stationary objects for each type of object were analyzed by means of Wilcoxon tests. The pattern of object exploration during the test trial was analysed by means of a $B(n, 0.25)$ binomial test with four possible cases ($C_1$: $A_1 < A_2$, $B_1 < B_2$; $C_2$: $A_1 < A_2$, $B_1 > B_2$; $C_3$: $A_1 > A_2$, $B_1 > B_2$; $C_4$: $A_1 > A_2$, $B_1 < B_2$), which is a rather conservative condition in terms of statistical significance since more cases are possible. Four rats, which attempted to climb onto the objects had to be excluded from data analysis, yielding an sample size of $n = 10$ rats per group.

### 3.6. Results

The untreated rats showed similar exploration times on both sample trials ($p > .05$; Wilcoxon test, data not shown) and spent more time exploring the stationary "old familiar" object relative to the stationary "recent familiar" object ($p = .032$, one-tailed Wilcoxon test; Fig. 3). The untreated rats also spent more time exploring the displaced "recent familiar" compared to the stationary "recent familiar" object ($p = .014$; Fig. 3). As predicted from experiment 1, the untreated rats explored the displaced "old familiar" object for a shorter period compared to the stationary "old familiar" object ($p = .003$, Fig. 3). According to a binominal distribution [$B(10, 0.25)$], the probability of finding the above exploration pattern ($C_4$) by chance would be $p = .003$. Thus, the results of experiment 1 were replicated in an independent experiment using experimentally naïve rats.

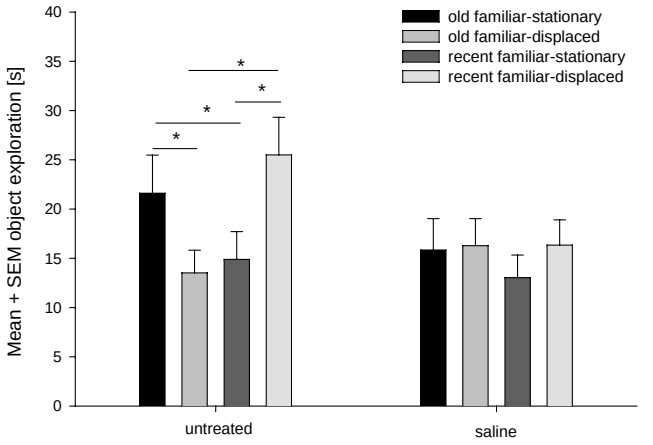


Fig. 3. Experiment 2: effects of a pre-sample trial 1 saline injection on episodic-like memory in rats. Bars represent mean (+SEM) object exploration [s] of indicated objects. $^*p < .05$.

In contrast to the untreated group, the rats which received an i.p. saline injection failed to discriminate both the recency and spatial displacement of objects (all $p$'s $> .05$; Fig. 3). The probability that the exploration pattern of the saline-injected rats was due to chance was calculated as $p = .47$.

### 3.7. Discussion

In this second experiment the results of experiment 1 were replicated, demonstrating that the exploration pattern observed in experiment 1 is a robust finding. Our results suggest that rats, similar to mice (Dere et al., 2005a), are able to establish an integrated memory for "what," "where," and "when." Compared to the study performed with mice (Dere et al., 2005a), the design of the episodic-like memory task in the present series of experiments with rats was slightly modified. In the mouse study only one of the "old familiar" objects was spatially displaced during the test trial, while the two "recent familiar" objects were kept in place. Consequently, the mice spent more time exploring the displaced "old familiar" object relative to the stationary "old familiar" object, while there was no difference regarding the two "recent familiar" objects. In the present experiments also one of the "recent familiar" objects was displaced in order to know whether it makes a difference if an "old" or a "recent" object is spatially displaced. An interaction between temporal- and spatial object information, as presently found in two independent experiments, would suggest that temporal and spatial characteristics of an object are integrated into a single memory. In contrast, if we had instead found that the displaced objects regardless of their order of presentation were explored for a longer or shorter time compared to the respective stationary objects of the same type (e.g., $A_2 > A_1$ and $B_2 > B_1$ or $A_2 < A_1$ and $B_2 < B_1$), this would have left the possibility that the temporal- and spatial object information given during the sample trials were encoded, stored, and retrieved independently from each other. The exploration pattern found here, i.e.,

$A_2 < A_1$ and $B_2 > B_1$, argues against such an interpretation. The hippocampus is critical for object-place associations (Gilbert & Kesner, 2004; Mumby, Gaskin, Glenn, Schramek, & Lehmann, 2002) but possibly not for object recognition memory, which rather depends on the perirhinal cortex (Winters & Bussey, 2005). Temporal order memory for objects is mediated by the medial prefrontal cortex (Chiba, Kesner, & Reynolds, 1994; Hannesson et al., 2004). An integrated object memory for "what," "where," and "when" as found here raises the possibility that these three structures are part of a distributed neuronal network which might participate in episodic-like memory encoding, consolidation, and retrieval.

To utilize our episodic-like memory task for pharmacological research, e.g., the screening for promnestic drugs, which might be used to treat neuropsychiatric diseases such as Alzheimer's disease, affecting episodic memory, we investigated whether the stress caused by an i.p. saline injection would per se have an effect on episodic-like memory performance. Unfortunately, an i.p. saline injection disturbed the performance of the rats. The rats which received an injection failed to discriminate both the recency and spatial displacement of objects. Thus, it must be assumed that performance in our task is highly sensitive to stressful stimuli, such as a needle puncture. It is known that a simple saline injection procedure (Nagel & Huston, 1988) and other stressful manipulations, such as restraint, impair memory performance (Baker & Kim, 2002). In the next experiment we asked whether the memory impairing effect of an injection could be ameliorated or even reversed by a promnestic dose of the cognitive enhancer DCS. Furthermore, it is also conceivable that DCS improves task performance above the level of untreated rats.

## 4. Experiment 3: Effects of a pre-sample trial 1 DCS injection on episodic-like memory

### 4.1. Introduction

NMDA-R have been implicated in certain types of long-term synaptic plasticity and memory consolidation (Martin & Morris, 2002). Pre- or post-sample trial systemic application of the NMDA-R antagonist MK-801 impairs object recognition memory (De Lima, Laranja, Bromberg, Roesler, & Schroder, 2005). Daily systemic administration of the NMDA-R antagonists phencyclidine and MK-801 for 5 days impaired object-place associations (Mandillo, Rinaldi, Oliverio, & Mele, 2003). Furthermore, the temporal order memory for spatial locations is impaired after systemic phencyclidine application (Long & Kesner, 1995). Thus, blockade of NMDA-R impairs different types of memory (object recognition, object-place associations, and temporal order memory), which are all required for solving our object exploration task. DCS is a partial agonist at the glycine binding site on the NMDA-R and facilitates NMDA-R mediated responses by increasing the mean channel opening frequency (Johnson & Ascher, 1987). DCS has promnestic effects in normal rats and mice in diverse learning tasks (Hughes, 2004; Land & Riccio, 1999; Lelong, Dauphin, & Boulouard, 2001; Matsuoka & Aigner, 1996; Pussinen & Sirvio, 1999; Quartermain, Mower, Rafferty, Herting, & Lanthorn, 1994), reverses memory deficits induced by anticholinergic treatment (Ohno & Watanabe, 1996; Pitkanen, Sirvio, MacDonald, Ekonsalo, & Riekkinen, 1995; Zajaczkowski & Danysz, 1997), alleviates memory deficits after septal (Riekkinen, Ikonen, & Riekkinen, 1998) and hippocampal lesions (Schuster & Schmidt, 1992), and following traumatic brain injury (Temple & Hamm, 1996), counteracts aging-induced learning impairments (Baxter et al., 1994) and seems to be effective in the treatment of cognitive deficits due to Alzheimer's disease (Schwartz, Hashtroudi, Herting, Schwartz, & Deutsch, 1996; Tsai, Falk, Gunther, & Coyle, 1999). Finally, it has been shown that the behavioral effects of DCS depend on the anxiety level of rats, having task-dependent behavioral consequences (Ho et al., 2005). Given that NMDA-R are involved in object recognition-, object-place, and temporal order memory, and, given that DCS enhances cognition in normal rats, it might ameliorate or reverse the memory impairing effects of injection-induced stress, as we found in experiment 2, or even promote episodic-like memory performance.

### 4.2. Subjects

Subjects were 30 naïve male Wistar rats, which were maintained and handled as described under experiment 1.

### 4.3. Drugs and application procedure

Animals received either an i.p. injection of physiological saline or 15 mg/kg DCS (Sigma, Steinheim, Germany), a partial *N*-methyl-D-aspartate NMDA-R agonist at the glycine site, in an injection volume of 1 ml/kg. DCS was diluted in physiological saline. All injections were made 30 min prior to the first sample trial. Nine rats had to be excluded from data analysis, since they attempted to climb onto the objects, yielding an sample sizes of $n = 9$ rats in the saline group and $n = 12$ rats in the DCS group.

### 4.4. Predictions

Based on the results of experiment 2, we hypothesized that on the test trial the saline-treated rats would fail to show episodic-like memory as described above. The rats treated with DCS should instead spend more time exploring the "old familiar" stationary object relative to the "recent familiar" stationary object. Furthermore, we hypothesized that the DCS group should spend more time exploring the displaced "recent familiar" object relative to the stationary "recent familiar" object, while the opposite should be the case for the displaced "old familiar" object relative to the stationary "old familiar" object.

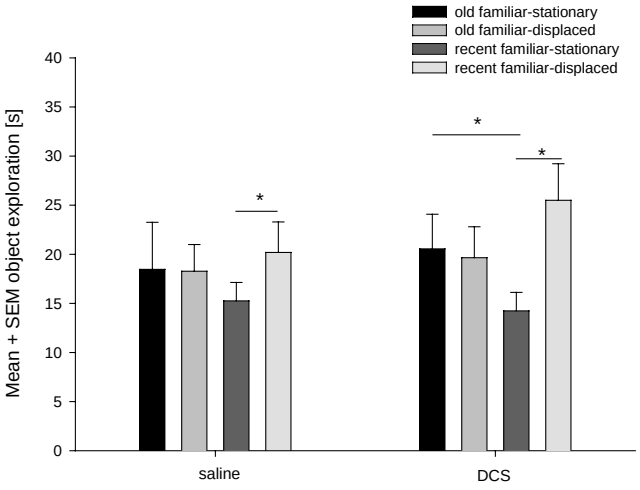


Fig. 4. Experiment 2: effects of a pre-sample trial 1 saline and DCS injection on episodic-like memory in rats. Bars represent mean (+SEM) object exploration [s] of indicated objects. $^{*}p < .05$.

### 4.5. Results

Both groups showed similar exploration times on both sample trials ($p$'s > .05; Wilcoxon test, data not shown). The saline-treated rats showed similar exploration of the "old familiar" and "recent familiar" stationary objects ($p > .05$; one-tailed Wilcoxon test; Fig. 4). Also, no statistical difference between the displaced "old familiar and stationary "old familiar" object ($p > .05$; Fig. 4) was found for this group. However, contrary to the saline group in experiment 2, the present saline group spent more time exploring the displaced "recent familiar" compared to the stationary "recent familiar" object ($p = .01$; Fig. 4). A binominal test revealed that the probability of yielding this exploration pattern by chance would be equivalent to a $p$ value of .166 [$B(9, 0.25)$].

The DCS group spent more time exploring the stationary "old familiar" object relative to the stationary "recent familiar" object ($p = .031$, one-tailed Wilcoxon test; Fig. 4). The DCS-treated rats also spent more time exploring the displaced "recent familiar" compared to the stationary "recent familiar" object ($p = .005$; Fig. 4). However, the exploration times of the displaced "old familiar"- and stationary "old familiar" object were similar in the DCS group ($p = .259$, Fig. 4). Nevertheless, according to a binominal distribution [$B(12, 0.25)$], the probability of finding the above exploration pattern ($C_4$) by chance would be $p = .002$. Thus, as predicted, the DCS administration indeed ameliorated the detrimental effects of injection stress on episodic-like memory performance in rats.

### 4.6. Discussion

Similar to the results of experiment 2, the rats receiving a saline injection failed to exhibit episodic-like memory in terms of recency judgements, i.e., failed to discriminate between the stationary "old familiar" and "recent familiar" objects. Furthermore, the spatial displacement of one "old familiar" object relative to a stationary "old familiar" object was likewise not detected by the saline groups in both experiments 2 and 3. Whereas the statistical analysis of saline-treated group in experiment 2 suggested similar exploration times of the displaced "recent familiar" compared to the stationary "recent familiar" object (although a trend towards a difference was nevertheless evident), the saline group in experiment 3 spent more time exploring the displaced "recent familiar" compared to the stationary "recent familiar" object. The latter finding suggests that the information given on sample trial 2 was somewhat better remembered by the saline-treated rats compared to the information given on sample trial 1. Since the rats received a saline injection prior to sample trial 1 it is possible that the injection stress more strongly affected the encoding and/or consolidation of the information given on sample trial 1 compared to sample trial 2. It is reasonable to assume that the stress response, e.g., the activation of the hypothalamus–pituitary–adrenal (HPA)-axis inducing the release of stress hormones (Roozendaal, 2002), progressively declined until the second sample trial, thus, having a lower impact on sample trial 2. Since the saline-injected rats showed a similar amount of exploration during sample trials 1 and 2, the impaired retrieval of sample 1 information was not due to different amounts of learning, but might be instead explained by impaired encoding and/or consolidation of the sample trial 1 information. Consequently, DCS, a NMDA-R agonist, which has been shown to have promnestic action, should ameliorate or even reverse the memory impairing effects of injection-induced stress. As predicted, DCS was able to ameliorate the detrimental effects of injection stress on the encoding and/or consolidation of sample trial 1 information as evidenced by an intact recency discrimination. However, the DCS group still failed to discriminate between the displaced "old familiar"- and the stationary "old familiar" object, suggesting that the spatial information given on sample trial one was not memorized. Thus, DCS at a dose of 15 mg/kg was not fully effective in reversing the detrimental effects of injection stress on memory formation. In this regard, it is possible that higher doses of DCS would be more effective.

## General discussion

Although, it has been shown that rodents are able to remember "what" and "when" (Hannesson et al., 2004) or "what" and "where" information (Dix & Aggleton, 1999) given on unique trials, it proved to be difficult to demonstrate memory for "what," "where," and "when" simultaneously in rodents and primates without using extensive training procedures, which might induce semantic rather than episodic memory recall (Bird, Roberts, Abroms, Kit, & Crupi, 2003; Gaffan, 1994; Hampton & Schwartz, 2004 for review). Previously, we showed that mice are able to remember the exact location and order of presentation of two different objects reflecting memory for "what,"

"where," and "when" using a novel object exploration task (Dere et al., 2005a, 2005b). Here, we demonstrate "what," "where," and "when" memory in a second species. In experiments 1 and 2 we showed that rats spent more time exploring an "old familiar" object relative to a "recent familiar" object, suggesting that they both recognized previously explored objects and remembered their order of appearance. Furthermore, the rats preferred a displaced "recent familiar" object compared to a stationary "recent familiar" object, while the opposite was found for the displaced and stationary "old familiar" object. This interaction provides strong evidence that the rats indeed established an integrated memory for "what," "where," and "when," since it excludes the possibility that the temporal- and spatial object information were encoded, stored, and retrieved independently from each other. Thus, our results suggest that rats are able to form an integrated memory for objects, places, and temporal order. In experiments 2 and 3 we showed that an i.p. saline injection prior to sample trial 1 impaired episodic-like memory, which, however, could be partially reversed by the NMDA-R agonist DCS. DCS is a partial agonist at the glycine binding site on the NMDA-R, facilitates NMDA-R mediated responses (Johnson & Ascher, 1987), has promnestic effects in normal rats and mice in diverse learning tasks (Hughes, 2004; Land & Riccio, 1999; Lelong et al., 2001; Matsuoka & Aigner, 1996; Pussinen & Sirvio, 1999; Quartermain et al., 1994) and seems to be effective in the treatment of cognitive deficits due to Alzheimer's disease (Schwartz et al., 1996; Tsai et al., 1999). In experiment 3 we showed that the NMDA-R agonist DCS was able to ameliorate the memory impairing effects of a single pre-sample trial 1 injection with saline. Since it is unlikely that physiological saline itself impairs episodic-like memory, we assume that the acute stress associated with the injection procedure had a detrimental effect on either encoding or consolidation of the information given on sample trial one. Our results suggest that DCS counteracted stress-induced deficits of an injection on either encoding or consolidation of the information given on sample trial 1.

Tulving (2002) proposed that human's ability to mentally travel back in time and consciously remember and re-experience personal experiences, thought to require autonoetic awareness, a self concept and a sense of subjective time, is a human-specific ability, which has no analogue in animal kingdom. Currently, this assumption is questioned. It has been shown that food-caching scrub jays not only remembered what kind of food they had cached and where they placed it, but also how long ago they did this (Clayton & Dickinson, 1998). Furthermore, it has been demonstrated that the "what," "where," and "when" memory of these birds meets the criteria of being integrated and flexible (De Kort, Dickinson, & Clayton, 2005). A food-caching radial maze task similar to the one in the scrub jay experiments showed that rats remember where and when a favored reward was previously presented (Babb & Crystal, 2005). Ergorul and Eichenbaum (2004) showed that when rats are presented with a series of odors in different places, and thereafter were tested for memory of the order in which these odors were encountered, they use a combination of spatial "where" and olfactory "what" cues to distinguish "when" events occurred. While these studies suggest that birds and rodents may bear an implicit form of episodic memory, that is, the ability to integrate and remember the "what," "where," and "when" elements of a personal experience, these tasks do not measure the memory for unique experiences, since they require extensive training and thus rule learning. In this regard, our spontaneous object memory task has the advantage to assess the "what," "where," and "when" memory for single experiences, which does not require the application of reinforcers or extensive rule learning.

The 50 min inter-trial interval was chosen on the basis of experiments assessing temporal order memory for objects using rats and our previous episodic-like memory study using mice (Dere et al., 2005a, 2005b). Mitchell and Laiacona (1998) showed that rats can discriminate between an old familiar object presented 130 min ago and a recent familiar object presented 65 min ago. Similarly, Hannesson et al. (2004) showed significant recency discrimination for an old familiar object, which was presented 113 min ago, and a recent familiar object presented 49 min ago. Although longer inter-trial intervals might be also possible, it has been shown that rats can recognize objects only for retention intervals up to 3 h (King et al., 2004).

One objection against animal models of human episodic memory is the possibility that the animal's performance in delayed memory tasks might be guided by relative familiarity judgements rather than explicit or conscious recollection of a personal experience (Yonelinas, 2002). This view implies that memory traces decay passively over time unless the memory is reactivated and thus strengthened. An animal might therefore judge the order of two objects presented one after the other by comparing their memory trace strengths. In this regard, Fortin, Agster, and Eichenbaum (2002) showed that normal rats do not use the relative strengths of memories to identify the order of a series of odors. While it is still possible that a delay of only 50 min led to substantial differences in memory trace strength that would account for the discrimination of "when" an object was presented by our control rats, the concomitant discrimination of "where" an particular object ("what") was previously encountered argues against this interpretation. Because the spatial information was gathered on a single occasion, relative strengths of memory traces could not be used to decide whether an object was spatially displaced or not. Moreover, we found an interaction between "what," "where" and "when" information, excluding the possibility that the temporal- and spatial object information were encoded, stored, and retrieved independently from each other and suggesting that the "what," "where" and "when" memory was integrated. While further research is required to resolve the issue of conscious recollection, our present results at least demonstrate that rats bear an implicit form of episodic memory, that is the ability to integrate and

subsequently remember the "what," "where," and "when" information of a past experience.

Our episodic-like memory task meets several criteria proposed to be essential for an animal model of episodic memory (Clayton, Bussey, & Dickinson, 2003; Eichenbaum, Fortin, Ergorul, Wright, & Agster, 2005; Hampton & Schwartz, 2004; Zentall, 2005; Zentall, Clement, Bhatt, & Allen, 2001). In our task, the rats have to remember a specific episode rather than applying an invariant rule acquired over multiple trials. Furthermore, the task requires binding or integration of "what," "where," and "when" information. The inter-trial interval (50 min) excludes the possibility that the rat's performance during the test trial relies on short-term memory. The test trial constitutes a novel situation, which cannot be anticipated by the rats, thus requiring retrospective memory retrieval. Thus, our task is a good approximation to the behavioral criteria proposed to be essential for an animal model of episodic-like memory. However, other more strict criteria for episodic memory in animals were proposed, such as the demonstration of conscious recollection (Tulving, 2002), prospective memory (using episodic memories to organize behavior to satisfy future needs), or a flexible expression of episodic memories (De Kort et al., 2005). It remains to be determined, whether it is possible to design a task which satisfies all of these criteria.

In humans, deficits in episodic memory are found after damage to the medial temporal lobe, the hippocampus, the frontal cortex, and the mammilary bodies in the diencephalon (Aggleton & Brown, 1999). In terms of object memory, animal studies indicate that the hippocampus is critical for object-place associations (Gilbert & Kesner, 2004; Mumby et al., 2002), but possibly not for object recognition memory, which rather depends on the perirhinal cortex (Winters & Bussey, 2005). Temporal order memory for objects is mediated by the medial prefrontal cortex (Chiba et al., 1994; Hannesson et al., 2004). NMDA-R have been implicated in object recognition memory (De Lima et al., 2005), object-place associations (Mandillo et al., 2003), and the temporal order memory for spatial locations (Long & Kesner, 1995). In the present study, we showed that enhancement of NMDA-R function by DCS can ameliorate acute stress-induced impairments in episodic-like memory. Therefore, it is possible that episodic memory in humans as well as episodic-like memory in rodents relies on NMDA-R-induced neuroplasticity in the perirhinal cortex, hippocampus, and medial prefrontal cortex.

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## References

Aggleton, J. P., & Brown, M. W. (1999). Episodic memory, amnesia, and the hippocampal-anterior thalamic axis. *Behavioral Brain Sciences, 22*, 425–444.

Babb, S. J., & Crystal, J. D. (2005). Discrimination of what, when, and where: Implications for episodic-like memory in rats. *Learning and Motivation, 36*, 177–189.

Baker, K. B., & Kim, J. J. (2002). Effects of stress and hippocampal NMDA receptor antagonism on recognition memory in rats. *Learning and Memory, 9*, 58–65.

Baxter, M. G., Lanthorn, T. H., Frick, K. M., Golski, S., Wan, R. Q., & Olton, D. S. (1994). D-Cycloserin, a novel cognitive enhancer, improves spatial memory in aged rats. *Neurobiology of Aging, 15*, 207–213.

Bird, L. R., Roberts, W. A., Abroms, B., Kit, K. A., & Crupi, C. (2003). Spatial memory for food hidden by rats (*Rattus norvegicus*) on the radial maze: Studies of memory for where, what, and when. *Journal of Comparative Psychology, 117*, 176–187.

Chiba, A. A., Kesner, R. P., & Reynolds, A. M. (1994). Memory for spatial location as a function of temporal lag in rats: Role of hippocampus and medial prefrontal cortex. *Behavioral and Neural Biology, 61*, 123–131.

Clayton, N. S., Bussey, T. J., & Dickinson, A. (2003). Can animals recall the past and plan for the future? *Nature Reviews Neuroscience, 4*, 685–691.

Clayton, N. S., & Dickinson, A. (1998). Episodic-like memory during cache recovery by scrub jays. *Nature, 395*, 272–274.

De Kort, S. R., Dickinson, A., & Clayton, N. S. (2005). Retrospective cognition by food-caching western scrub-jays. *Learning and Motivation, 36*, 159–176.

De Lima, M. N., Laranja, D. C., Bromberg, E., Roesler, R., & Schroder, N. (2005). Pre- or post-training administration of the NMDA receptor blocker MK-801 impairs object recognition memory in rats. *Behavioural Brain Research, 156*, 139–143.

Dere, E., Huston, J. P., & De Souza Silva, M. A. (2005a). Integrated memory for objects, places and temporal order: Evidence for episodic-like memory in mice. *Neurobiology of Learning and Memory, 84*, 214–221.

Dere, E., Huston, J. P., & DeSouzaSilva, M. A. (2005b). Episodic-like memory in mice: Simultaneous assessment of object, place and temporal order memory. *Brain Research Protocols,* in press, doi:10.1016/j.brainresprot.2005.08.001.

Dix, S. L., & Aggleton, J. P. (1999). Extending the spontaneous preference test of recognition: Evidence of object-location and object-context recognition. *Behavioural Brain Research, 99*, 191–200.

Eacott, M. J., & Norman, G. (2004). Integrated memory for object, place, and context in rats: A possible model of episodic-like memory? *Journal of Neuroscience, 24*, 1948–1953.

Eichenbaum, H., Fortin, N. J., Ergorul, C., Wright, S. P., & Agster, K. L. (2005). Episodic recollection in animals: „ If t walks like a duck and quacks like a duck…". *Learning and Motivation, 36*, 190–207.

Ennaceur, A., & Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behavioural Brain Research, 31*, 47–59.

Ennaceur, A., Neave, N., & Aggleton, J. P. (1997). Spontaneous object recognition and object location memory in rats: The effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. *Experimental Brain Research, 113*, 509–519.

Ergorul, C., & Eichenbaum, H. (2004). The hippocampus and memory for "what," "where," and "when". *Learning and Memory, 11*, 397–405.

Fortin, N. J., Agster, K. L., & Eichenbaum, H. B. (2002). Critical role of the hippocampus in memory for sequences of events. *Nature Neuroscience, 5*, 458–462.

Gaffan, D. (1994). Dissociated effects of perirhinal cortex ablation, fornix transection and amygdalectomy: Evidence for multiple memory systems in the primate temporal lobe. *Experimental Brain Research, 99*, 411–422.

Gilbert, P. E., & Kesner, R. P. (2004). Memory for objects and their locations: The role of the hippocampus in retention of object-place associations. *Neurobiology of Learning and Memory, 81*, 39–45.

Hampton, R. R., & Schwartz, B. L. (2004). Episodic memory in nonhumans: What, and where, is when? *Current Opinion in Neurobiology, 14*, 192–197.

Hannesson, D. K., Howland, J. G., & Phillips, A. G. (2004). Interaction between perirhinal and medial prefrontal cortex is required for tempo-

ral order but not recognition memory for objects in rats. *Journal of Neuroscience, 24*, 4596–4604.

Ho, Y. J., Hsu, L. S., Wang, C. F., Hsu, W. Y., Lai, T. J., Hsu, C. C., et al. (2005). Behavioral effects of D-cycloserine in rats: The role of anxiety level. *Brain Research, 1043*, 179–185.

Hotte, M., Naudon, L., & Jay, T. M. (2005). Modulation of recognition and temporal order memory retrieval by dopamine D(1) receptor in rats. *Neurobiology of Learning and Memory, 84*, 85–92.

Hughes, R. N. (2004). Responsiveness to brightness change in male and female rats following treatment with the partial agonist of the *N*-methyl-D-aspartate receptor, D-cycloserine. *Behavioural Brain Research, 152*, 199–207.

Johnson, J. W., & Ascher, P. (1987). Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature, 325*, 529–531.

Jones, L. M., Lee, S., Trageser, J. C., Simons, D. J., & Keller, A. (2004). Precise temporal responses in whisker trigeminal neurons. *Journal of Neurophysiology, 92*, 665–668.

King, M. V., Sleight, A. J., Woolley, M. L., Topham, I. A., Marsden, C. A., & Fone, K. C. (2004). 5-HT6 receptor antagonists reverse delay-dependent deficits in novel object discrimination by enhancing consolidation—an effect sensitive to NMDA receptor antagonism. *Neuropharmacology, 47*, 195–204.

Land, C., & Riccio, D. C. (1999). D-Cycloserine: Effects on long-term retention of a conditioned response and on memory for contextual attributes. *Neurobiology of Learning and Memory, 72*, 158–168.

Lelong, V., Dauphin, F., & Boulouard, M. (2001). RS 67333 and D-cycloserine accelerate learning acquisition in the rat. *Neuropharmacology, 410*, 517–522.

Long, J. M., & Kesner, R. P. (1995). Phencyclidine impairs temporal order memory for spatial locations in rats. *Pharmacology, Biochemistry and Behavior, 52*, 645–648.

Mandillo, S., Rinaldi, A., Oliverio, A., & Mele, A. (2003). Repeated administration of phencyclidine, amphetamine and MK-801 selectively impairs spatial learning in mice: A possible model of psychotomimetic drug-induced cognitive deficits. *Behavioural Pharmacology, 14*, 533–544.

Martin, S. J., & Morris, R. G. (2002). New life in an old idea: The synaptic plasticity and memory hypothesis revisited. *Hippocampus, 12*, 609–636.

Matsuoka, N., & Aigner, T. G. (1996). D-Cycloserin, a partial agonist at the glycine site coupled to *N*-methyl-D-aspartate receptors, improves visual recognition memory in rhesus monkeys. *Journal of Pharmacology and Experimental Therapeutics, 278*, 891–897.

Mitchell, J. B., & Laiacona, J. (1998). The medial frontal cortex and temporal memory: Tests using spontaneous exploratory behaviour in the rat. *Behavioural Brain Research, 97*, 107–113.

Mumby, D. G., Gaskin, S., Glenn, M. J., Schramek, T. E., & Lehmann, H. (2002). Hippocampal damage and exploratory preferences in rats: Memory for objects, places, and contexts. *Learning and Memory, 9*, 49–57.

Nagel, J. A., & Huston, J. P. (1988). Enhanced inhibitory avoidance learning produced by post-trial injections of substance P into the basal forebrain. *Behavioural and Neural Biology, 49*, 374–385.

Ohno, M., & Watanabe, S. (1996). D-Cycloserine, a glycine site agonist, reverses working memory failure by hippocampal muscarinic receptor blockade in rats. *European Journal of Pharmacology, 318*, 267–271.

Pitkanen, M., Sirvio, J., MacDonald, E., Ekonsalo, T., & Riekkinen, P. (1995). The effects of D-cycloserine, a partial agonist at the glycine binding site, on spatial learning and working memory in scopolamine-treated rats. *Journal of Neural Transmission. Parkinson Disease and Dementia Section, 9*, 133–144.

Polley, D. B., Rickert, J. L., & Frostig, R. D. (2005). Whisker-based discrimination of object orientation determined with a rapid training paradigm. *Neurobiolory of Learning and Memory, 83*, 134–142.

Pussinen, R., & Sirvio, J. (1999). Effects of D-cycloserine, a positive modulator of *N*-methyl-D-aspartate receptors, and ST 587, a putative alpha-1 adrenergic agonist, individually and in combination, on the non-delayed and delayed foraging behaviour of rats assessed in the radial arm maze. *Journal of Psychopharmacology, 13*, 171–179.

Quartermain, D., Mower, J., Rafferty, M. F., Herting, R. L., & Lanthorn, T. H. (1994). Acute but not chronic activation of the NMDA-coupled glycine receptor with D-cycloserine facilitates learning and retention. *European Journal of Pharmacology, 257*, 7–12.

Riekkinen, P., Ikonen, S., & Riekkinen, M. (1998). D-cycloserine, a partial NMDA receptor-associated glycine-B site agonist, enhances reversal learning, but a cholinesterase inhibitor and nicotine has no effect. *Neuroreport, 9*, 3647–3651.

Roberts, W. A. (2002). Are animals stuck in time? *Psychological Bulletin, 128*, 473–489.

Roozendaal, B. (2002). Stress and memory: Opposing effects of glucocorticoids on memory consolidation and memory retrieval. *Neurobiology of Learning and Memory, 78*, 578–595.

Schuster, G. M., & Schmidt, W. J. (1992). D-Cycloserine reverses the working memory impairment of hippocampal-lesioned rats in a spatial learning task. *European Journal of Pharmacology, 224*, 97–98.

Schwartz, B. L., Hashtroudi, S., Herting, R. L., Schwartz, P., & Deutsch, S. I. (1996). D-Cycloserine enhances implicit memory in Alzheimer patients. *Neurology, 46*, 420–424.

Temple, M. D., & Hamm, R. J. (1996). Chronic, post-injury administration of D-cycloserine, an NMDA partial agonist, enhances cognitive performance following experimental brain injury. *Brain Research, 741*, 246–251.

Tsai, G. E., Falk, W. E., Gunther, J., & Coyle, J. T. (1999). Improved cognition in Alzheimer's disease with short-term D-cycloserine treatment. *American Journal of Psychology, 156*, 467–469.

Tulving, E. (2002). Episodic memory: From mind to brain. *Annual Reviews in Psychology, 53*, 1–25.

Winters, B. D., & Bussey, T. J. (2005). Transient inactivation of perirhinal cortex disrupts encoding, retrieval, and consolidation of object recognition memory. *Journal of Neuroscience, 25*, 52–61.

Yonelinas, A. P. (2002). The nature of recollection and familiarity: A review of 30 years of reseach. *Journal of Memory and Language, 46*, 441–517.

Zajaczkowski, W., & Danysz, W. (1997). Effects of D-cycloserine and aniracetam on spatial learning in rats with entorhinal cortex lesions. *Pharmacology, Biochemistry and Behavior, 56*, 21–29.

Zentall, T. R. (2005). Animals may not be stuck in time. *Learning and Motivation, 36*, 208–225.

Zentall, T. R., Clement, T. S., Bhatt, R. S., & Allen, J. (2001). Episodic-like memory in pigeons. *Psychonomic Bulletin Reviews, 8*, 685–690.

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# Reinstatement of episodic-like memory in rats by neurokinin-1 receptor antagonism

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**Abstract**

We previously showed that a systemic administration of the selective non-peptide neurokinin-1-receptor (NK-1-R) antagonist SR140333 increases hippocampal acetylcholine levels and facilitates long term memory. In the present study, we investigated whether systemic SR140333 has beneficial effects on episodic-like memory for unique experiences. Rats received either no injection, a vehicle injection or SR140333 at doses of 1, 3 and 9 mg/kg (i.p.) prior to the acquisition of an object memory for *what, where* and *when*. In line with previous results, untreated rats showed episodic-like memory, while vehicle-injected rats were impaired. A low dose of 1 mg/kg SR140333 reinstated episodic-like memory. This result might be related to the effects of SR140333 on hippocampal cholinergic transmission and/or on the stress-response elicited by the injection procedure. Higher doses of SR140333 (3 and 9 mg/kg) induced psychomotor effects, including stereotypic behaviors and arched posture. Since NK-1-R antagonists have anxiolytic and promestic properties and induce hippocampal acetylcholine release at lower doses, they might be effective in the alleviation of the cognitive deficits and increased anxiety seen in early stages of Alzheimer's disease.


*Keywords:* SR140333; Acetylcholine; Hippocampus; Object memory; Stress; Cognitive enhancer; Alzheimer's disease

## 1. Introduction

The neuropeptide substance P is a centrally active neurotransmitter or neuromodulator, which, *inter alia*, has been implicated in nociception (Harrison & Geppetti, 2001), synaptic plasticity (Langosch et al., 2005), learning and memory (Hasenöhrl et al., 2000) brain reward and reinforcement (Nikolaus, Huston, & Hasenohrl, 1999), emotionality (Hasenöhrl, Jentjens, De Souza Silva, Tomaz, & Huston, 1998) and affective disorders (Herpfer & Lieb, 2003).

Substance P stimulates neurokinin-receptors, with the highest affinity to the neurokinin-1-receptor (NK-1-R) (Hokfelt, Pernow, & Wahren, 2001; Quartara & Maggi, 1998). In rats, high densities of the NK-1-R have been found in the neocortex, hippocampal formation, basal forebrain, amygdala and brainstem (Mantyh, Gates, Mantyh, & Maggio, 1989). Antinociceptive, antidepressant as well as anxiolytic effects have been reported after application of NK-1 receptor antagonists to rats (McLean, 2005; Pitcher & Henry, 2004). In line with this evidence, genetic inactivation of the NK-1-R gene in mice reduced the susceptibility to stress in a variety of tasks, similar to mice treated with antidepressants (Rupniak et al., 2001; Santarelli et al., 2001). In terms of learning and memory, NK-1-R knockout mice showed normal trace fear conditioning and a slight improvement in the hidden-platform version of the water maze task. Furthermore, these mice showed increased neurogenesis and brain-derived neurotrophic factor levels in the hippocampus (Morcuende et al., 2003). Similar changes have been reported after chronic treatment with

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antidepressants. NK-1-R knockout mice also showed reduced pain sensitivity in the hot plate-test (Mansikka, Shiotani, Winchurch, & Raja, 1999).

SR140333 is a potent and selective non-peptidergic antagonist at the NK-1-R in various species including rats and humans (Emonds-Alt et al., 1993). In vitro, SR140333 blocks substance P-induced endothelium-dependent relaxation of rabbit pulmonary artery and contraction of guinea-pig ileum (Emonds-Alt et al., 1993). In rats, intra-cerebroventricular administration of SR140333 reduces infarct volume after focal cerebral ischemia (Yu, Cheng, Huang, Li, & Cao, 1997). Furthermore, systemic administration of SR140333 blocks the processing of pain stimuli in the thalamus (Emonds-Alt et al., 1993) and inhibits scratching behavior elicited by i.c.v. application of substance P (Jung et al., 1994).

Previously, we showed that systemic post-trial SR140333 injections in rats had minor facilitative effects on inhibitory avoidance learning and behavioral habituation to a novel environment. Systemic application of SR140333 also dose-dependently increased extracellular acetylcholine levels in the hippocampus as measured by in vivo microdialysis in anesthetized rats (Kart et al., 2004). It is well established that modulation of hippocampal cholinergic neurotransmission influences synaptic plasticity (Ovsepian, Anwyl, & Rowan, 2004) and learning performance in rats (Sarter & Parikh, 2005). Therefore, the promnestic effects of SR140333 might be related to increased cholinergic neurotransmission in the hippocampus.

Recently, we developed an episodic-like memory task for rats, in which different versions of the novelty-preference paradigm have been combined in order to simultaneously assess object, place and temporal order memory (Kart-Teke, De Souza Silva, Huston, & Dere, 2006). This task is highly sensitive to the memory impairing effects of stress, e.g. a single i.p. saline injection impaired the acquisition of a memory for *what, where and when* in rats. Given that NK1-R antagonism has moderate promnestic effects, counteracts the behavioral effects of various stressful stimuli and increases hippocampal acetylcholine release, we hypothesized that systemic injections of SR140333 would also reinstate episodic-like memory in mildly stressed rats.

## 2. Materials and methods

### 2.1. Animals

Seventy-five male Wistar rats weighting 270–320 g were used, and were group housed with $n = 5$ per cage. They were maintained in a temperature and humidity controlled room (20–22 °C) and kept on a reversed 12 h light–dark cycle with lights off at 07:00 a.m. Animals had free access to rodent chow and tap water. Testing occurred during the dark phase. Before testing the animals were handled for 5 days. All experiments were performed according to the guidelines of the German Animal Protection Law and were approved by the North Rhine Westphalia State Authority.

### 2.2. Apparatus

Episodic-like memory was tested in an open-field. The open-field was a rectangular acryl box (60 × 60 × 39 cm), with an open roof, so that the rats could perceive distal visual cues. Four 75 W bulbs provided an illumination intensity of 11 lx in the corners and 17 lx in the center of the open-field. A video camera was placed 1.6 m above the center of the open-field to record sample and test trials. Video recordings were used for off-line data collection. The open-field was placed in a sound attenuating chamber. Masking noise was delivered by a white noise-generator.

### 2.3. Object stimuli

Four exact copies of three distinct translucent glass objects were used, which had no ethological significance to the rats. The objects varied in shape (octagon, rectangular and square), texture (plain or grooved), color and height (22–25 cm). The objects had sufficient weight to ensure that the rats could not displace them. Previous work ensured that Wistar rats could discriminate the two objects, and there was no per se preference for one of these objects (see Kart-Teke et al., 2006). For each animal two of these three objects were randomly chosen, and the order of presentation during the sample trials was randomized.

### 2.4. Experimental procedure

Each animal was subjected to three habituation trials (each lasting 10 min) to the open-field for three consecutive days. On the fourth day, animals received two sample trials followed by the test trial. The rats were always placed in the central part of the open-field. Each trial lasted 5 min. The inter-trial interval was 50 min. After each trial, the objects and the open-field were thoroughly cleaned with 0.1% acetic acid solution in order to remove odor cues. The open-field was virtually divided into nine squares by 2 × 2 parallel lines. The central square was not used for object placement. For each animal, four out of eight squares were randomly chosen to position the four copies of the "old familiar" object in the first sample trial (Fig. 1). The second sample trial was identical to the first except that four copies of another object ("recent familiar") were present. Two copies of the "recent familiar" object were randomly placed onto positions that had been occupied in the first sample trial and two copies were positioned in new positions, that were randomly chosen from the remaining four peripheral positions. In the test trial (trial 3), two copies of both objects were present in either stationary or displaced positions, i.e. one of the copies of each object was presented in a position encountered in the respective sample trial, i.e. sample trial 1 ("old familiar-stationary" object, A1) or sample trial 2 ("recent familiar-stationary" object, B1). The remaining objects were presented in new positions ("old familiar-displaced", A2 and "recent familiar-displaced", B2). All four objects were placed onto positions previously encountered in the sample trials (Kart-Teke et al., 2006).

### 2.5. Drugs and application procedure

SR140333 ((*S*)1-{3-(3,4-dichloro-phenyl)-3-[2(4-phenyl-1-aza-bicyclo[2.2.2]oct-1-yl]-ethyl]-piperidin-1-yl}-2-(3-isopropoxy-phenyl)-ethanone benzenesulfonate) was a gift from Sanofi-Aventis, Chilly-Mazarin,

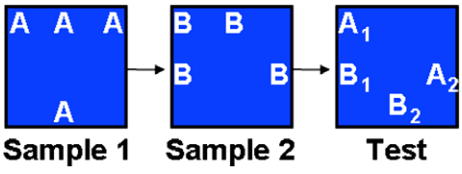


Fig. 1. Episodic-like object memory in rats. Experimental design. The schematic drawing shows an example of a possible object arrangement for the what, where, and when task. The rats received three 5 min trials with a 50 min inter-trial interval. During the test trial two "old familiar" and two "recent familiar" objects known from the sample trials were presented at familiar and novel locations relative to the respective sample trials. A1: "old familiar-stationary"; A2: "old familiar-displaced"; B1: "recent familiar-stationary" and B2: "recent familiar-displaced".

France. SR140333 was diluted in distilled water containing 0.01% Tween 80 (Sigma–Aldrich, USA). Doses of 0, 1, 3 and 9 mg/kg i.p. (injection volume: 1 ml/kg) were applied 30 min prior to the first sample trial. After the injection the animal was returned to its home cage. As a positive control for episodic-like memory in Wistar rats one group did not receive an injection prior to testing.

### 2.6. Dependent variables and data acquisition

The following behaviors were scored during the sample trials and the test trial: (1) Rearing: the total duration (s) an animal stood upon its hind legs with forelegs in the air or against the wall. (2) Locomotion: the total distance (cm) moved. (3) Velocity: the mean running speed (cm/s). (4) Grooming: the total duration (s) of fur cleaning. (5) Psychomotor effects: the frequency and duration of stereotype movement and arched posture. (6) Total time spent (s) exploring the objects. Exploration of an object was assumed when the rat had physical contact with an object, with its snout and/or forepaws. Sitting next to the object or rearing against the object while exploring the wall of the open-field were not considered as object exploration. The behavioral parameters were scored by an experienced observer, who was blind with respect to group assignment. Behaviors were scored semi-automatically using the EthoVision tracking system (Noldus, The Netherlands) run under the 'manually record behaviors' option.

### 2.7. Predictions

Based on our previous studies on episodic-like memory in rodents (Dere, Huston, & De Souza Silva, 2005a, 2005b; Kart-Teke et al., 2006), we hypothesized that untreated rats (no injection) would spend more time exploring the "old familiar-stationary" object relative to the "recent familiar-stationary" object. They should also spend more time exploring the "recent familiar-displaced" object relative to the "recent familiar-stationary" object and should spend less time exploring the "old familiar-displaced" object relative to the "old familiar-stationary" object. Such an exploration pattern suggests that the animals have established an integrated object memory for *what, where and when* information acquired on unique sample trials (see Kart-Teke et al., 2006 for discussion). In our previous work (Kart-Teke et al., 2006) we found that rats injected with vehicle failed to discriminate both the temporal order and displacement of objects. Therefore, we predicted that episodic-like memory should be impaired in rats injected with vehicle prior to sample trial 1. Finally, we hypothesized that the treatment with SR140333 would dose-dependently reinstate episodic-like memory.

### 2.8. Statistics

Rats, which attempted to climb onto the objects were excluded from data analysis, yielding the following sample sizes: untreated: $n = 15$; vehicle: $n = 14$; 1 mg/kg SR140333: $n = 14$; 3 mg/kg SR140333: $n = 11$; 9 mg/kg SR140333: $n = 4$. During the experiment we noticed severe psychomotor effects in animals treated with 3 and 9 mg/kg, but not with 1 mg/kg of SR140333. Since the psychomotor effects were especially severe in the group treated with 9 mg/kg SR140333, this treatment was terminated after four subjects. The a posteriori observed drug-induced psychomotor effects and their impact on object exploration, distance moved, running velocity, rearing and grooming duration was analyzed by means of Jonckheere-Terpstra statistics. Since the 3 and 9 mg/kg SR140333 groups showed dose-dependent impairments in the above behaviors, especially in object exploration, they were excluded from the statistical analysis of the episodic-like memory test.

The parameters, time spent exploring objects, distance moved, running velocity, rearing and grooming duration, were analyzed by means of Kruskal–Wallis tests for independent groups. The Mann–Whitney *U*-test was used for pair-wise group comparisons. Within-group comparisons were performed with the Wilcoxon-test. Binominal tests were used in order to assess whether the group's performance during the test trial was above chance level. The test trial performance of each group was analyzed by means of a B($n$, 0.25) binomial test with four possible cases (C1: A1 < A2, B1 < B2; C2: A1 < A2, B1 > B2; C3: A1 > A2, B1 > B2; C4: A1 > A2, B1 < B2), which is a rather conservative condition in terms of statistical significance since more cases are possible. All $p$-values given represent measures of effect. The data are presented as median with inter-quartile ranges as it is the appropriate form of data presentation for non-parametric analyses.

## 3. Results

### 3.1. Dose-dependent psychomotor effects of SR140333

In the course of the experiment, we noticed high levels of psychomotor effects in animals treated with 3 and 9 mg/kg, but not with 1 mg/kg of SR140333 (Fig. 2A and B). These effects were most prominent in the first sample trial 30 min after treatment, and, although reduced in magnitude, still apparent 140 min post-injection in the test trial (Jonckheere-Terpstra test: frequency: sample trial 1, $p < .01$; sample trial 2, $p < .01$; test trial, $p = .050$; total duration: $p < .01$; $p < .01$ and $p = .053$ respectively). Compared to the group treated with 1 mg/kg of SR140333, the 3 and 9 mg/kg groups showed reduced levels of object exploration throughout the sample trials (sample trial 1, $p < .01$; sample trial 2, $p < .01$), but not in the test trial ($p > .05$) performed 140 min after the injection (Fig. 2C). The 3 mg/kg group showed increases in the object exploration scores across the three trials, suggesting a slight recovery from psychomotor effects over time. In contrast, object exploration remained low throughout testing in the 9 mg/kg group. Furthermore, compared to the 1-mg/kg group, the 3 and 9 mg/kg groups also showed reduced locomotion (sample trial 1, $p = .01$; sample trial 2, $p < .001$; Fig. 2D) and running velocity (sample trial 1, $p = .01$; sample trial 2, $p < .001$; Fig. 2F) in the sample trials, but not in the test trial (both p's > .05). Again, the 9 mg/kg group showed the strongest impairment. An even stronger impairment of the 3 and 9 mg/kg groups relative to the 1 mg/kg group was found for rearing activity (sample trial 1, $p < .01$; sample trial 2, $p < .001$; test trial, $p < .01$; Fig. 2E) in the sample and test trials). There were no statistical differences in grooming behavior across groups and trials (all $p$'s > .05; data not shown). Because of the detrimental effects of the 3 and 9 mg/kg dose of SR140333 on object exploration in the sample trials and the test trial, these groups were excluded from the statistical analysis of the episodic-like memory test.

### 3.2. Sample trials

The performance of the untreated, vehicle injected, and 1 mg/kg SR140333 treated groups in the sample trials was highly similar in terms of the total time spent exploring the objects, the distance moved, the mean running velocity, the number of rearings and grooming behavior (Kruskal–Wallis test: all $p$'s > .05; data not shown). These results suggest that all groups showed comparable levels of explorative activity towards the sample objects and

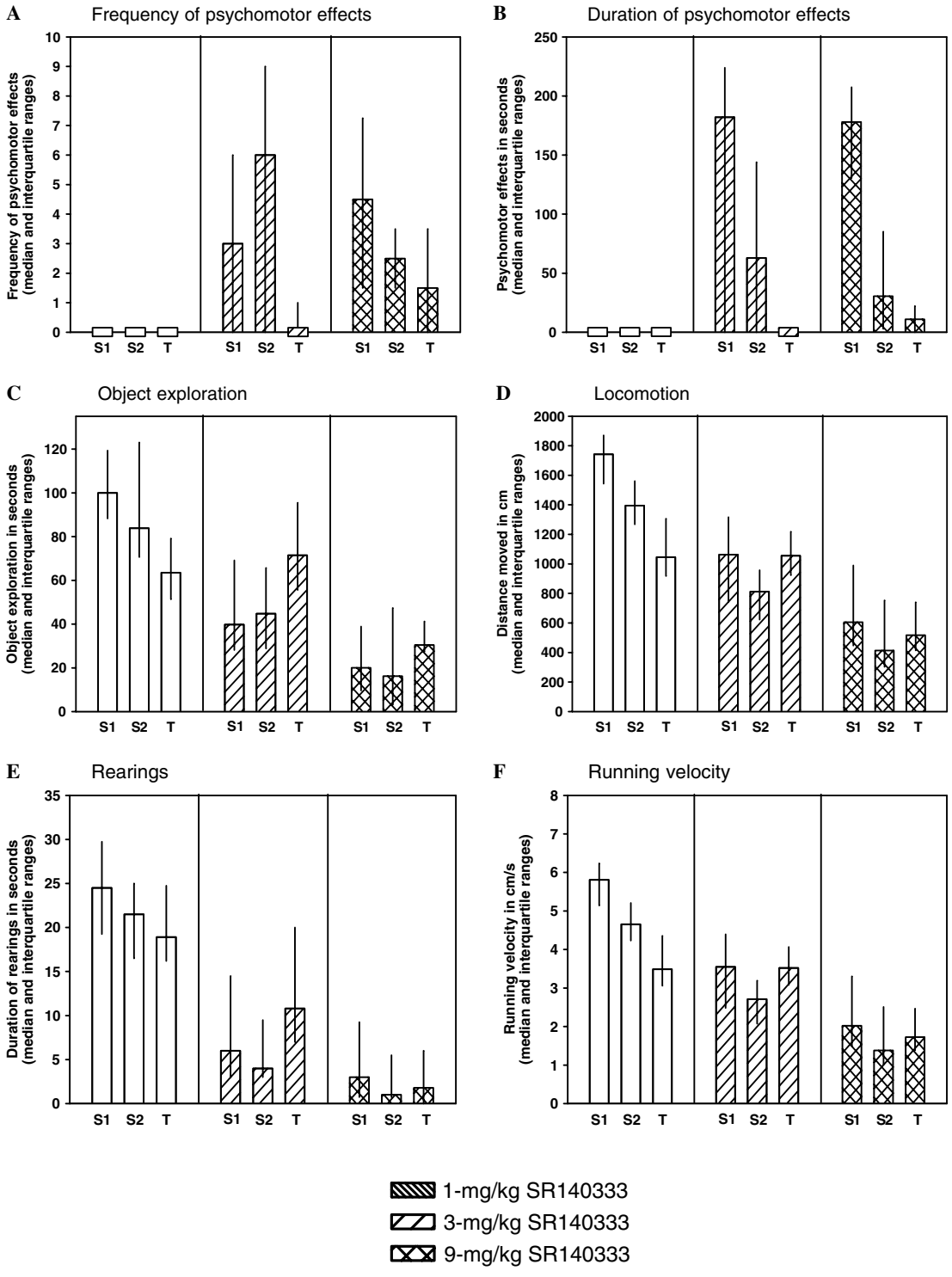


Fig. 2. Dose-dependent effects of SR140333 on different behaviors in the sample trials 1 (S1) and 2 (S2) and the test trial (T). Bars represent medians and inter-quartile ranges for the indicated groups. (A) Frequency of psychomotor effects. (B) Duration of psychomotor effects. (C) Effects of SR140333 on the total time spent exploring the objects. (D) Effects of SR140333 on Locomotion. (E) Effects of SR140333 on rearing behavior. (F) Effects of SR140333 on running velocity.

the open-field as well as motivation to explore the objects and the environment. Therefore, it appears unlikely that possible effects on episodic-like memory are due to different levels of familiarization with the sample objects or the spatial environment.

### 3.3. Episodic-like memory

As hypothesized, the untreated rats displayed episodic-like memory in terms of an integrated memory for *what, where and when* information acquired during unique

occasions (Kart-Teke et al., 2006). The untreated rats showed increased exploration of the "old familiar-stationary" object compared to the "recent familiar-stationary" object (one-tailed Wilcoxon test: $p = .032$; Fig. 4), reflecting a temporal order memory for *what and when* (Mitchell & Laiacona, 1998). Since the time spent in exploring the objects on sample trials 1 and 2 was highly similar (two-tailed Wilcoxon-test: $p > .05$), different levels of learning or familiarization with the objects cannot account for this effect (Fig. 3).

As predicted, the rats of the untreated group spent more time exploring the "recent familiar-displaced" object relative to the "recent familiar-stationary" object (one tailed Wilcoxon test: $p = .003$), reflecting a spatial memory for *what and where* (Ennaceur, Neave, & Aggleton, 1997). In contrast, the "old familiar-displaced" object was explored for a shorter time compared to the "old familiar-stationary" object ($p = .011$), suggesting that the *what, where and when* information was integrated (Kart-Teke et al., 2006). A binominal test confirmed that the number of subjects in the untreated group exhibiting this object exploration pattern (C4) was much higher compared to the number expected just by chance ($p < .001$).

As predicted, the stress induced by a vehicle injection impaired episodic-like memory in rats (Fig. 4). The vehicle-injected rats failed to detect both the temporal order in which objects have been presented and where they have been placed during the sample trials (one-tailed Wilcoxon test: all $p$'s $> .05$).

An injection of 1 mg/kg SR140333 30 min prior to the first sample trial blocked the stress-induced detrimental effect on episodic-like memory. Similar to untreated rats, the 1 mg/kg SR140333 group displayed temporal order memory as deduced from the comparison of the exploration times of the "old familiar-stationary" and "recent familiar-stationary" object (one-tailed Wilcoxon test: $p = .001$). This recency discrimination cannot be explained by a difference in the level of

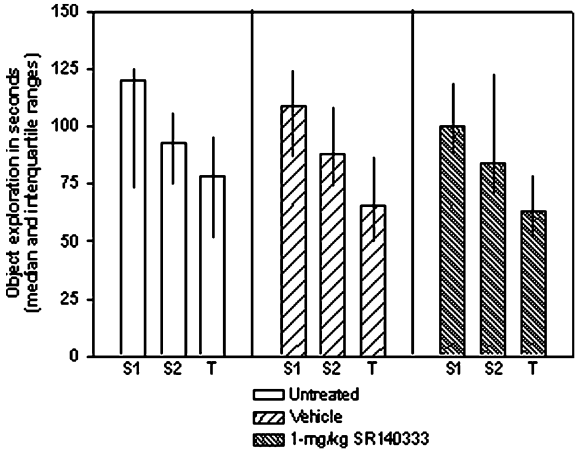


Fig. 3. The total time spend exploring the objects in the sample and test trials of the undrugged, vehicle-injected and 1 mg/kg SR140333 treated group. Bars represent medians and inter-quartile ranges for the indicated groups.

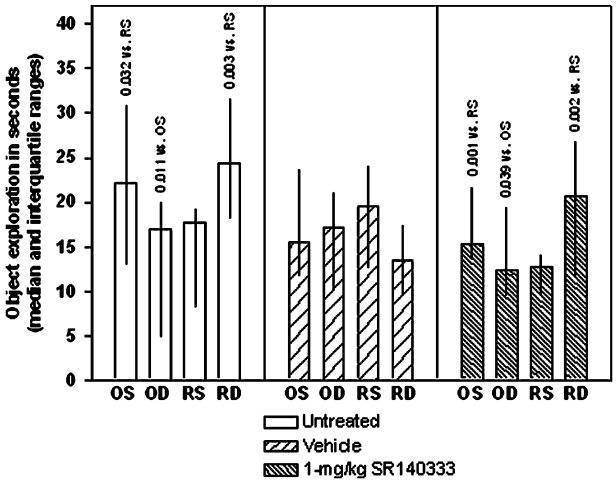


Fig. 4. Effects of SR140333 on episodic-like object memory in rats. Bars represent medians and inter-quartile ranges for the indicated groups. $p$-values refer to within-group differences in object exploration scores analyzed via the Wilcoxon test. *Abbreviations:* OS, old familiar stationary object; OD, old familiar displaced object; RS, recent familiar stationary object; RD, recent familiar displaced object.

learning, as exploration times in sample trials 1 and 2 were similar (two-tailed Wilcoxon-test: $p > .05$; Fig. 3). Spatial object memory, evidenced by the increased exploration of the "recent familiar-displaced" compared to the "recent familiar-stationary" object (one-tailed Wilcoxon test: $p = .002$) was also reinstated by SR140333. Finally, animals treated with 1 mg/kg of SR140333 showed an interaction of the factors *where and when* as suggested by the decreased exploration of the "old familiar-displaced" object compared to the "old familiar-stationary" object (one-tailed Wilcoxon test: $p = .039$). A binominal test confirmed that in the 1 mg/kg SR140333 group, the number of subjects exhibiting this object exploration pattern (C4) was much higher compared to the number of subjects expected just by chance ($p = .002$). Thus, these results suggest that the NK-1 receptor antagonist SR140333 given in a dose of 1 mg/kg reinstated episodic-like memory.

## 4. Discussion

We investigated whether SR140333 has beneficial effects on episodic-like memory for unique experiences. Rats received no injection, a vehicle injection or SR140333 at doses of 1, 3 and 9 mg/kg (i.p.) prior to the acquisition of an object memory for *what, where and when*. Untreated rats showed episodic-like memory, while vehicle-injected rats were impaired. A low dose of 1 mg/kg SR140333 reinstated episodic-like memory.

Higher doses of SR140333 (3 and 9 mg/kg) induced psychomotor effects, decreased locomotion and running velocity, as well as impairments in rearing behavior. The behavioral abnormalities of rats treated with 3 and 9 mg/kg doses of SR140333, which included reduced locomotion, running speed, rearings, and object exploration, might be due to abdominal seizures, especially during the first sample

trial. NK-1 receptor antagonists have been shown to affect gut motility. Some NK-1 receptor antagonists induce colon contraction (Bailey & Jordan, 1984) and increase the activity of the ileum (Featherstone, Fosbraey, & Morton, 1986).

It is known that a simple saline injection procedure (Nagel & Huston, 1988) and other stressful manipulations such as restraint or tail shock can impair memory by interfering with hippocampal synaptic plasticity (Baker & Kim, 2002). We previously showed that rats receiving a single saline injection prior to sample trial 1 failed to show episodic-like memory (Kart-Teke et al., 2006). The manipulations required to perform an i.p. vehicle injection include restraint and painful needle puncture. It is reasonable to assume that these manipulations trigger a stress response, e.g. the activation of the hypothalamus–pituitary–adrenal-axis inducing the release of stress hormones (Roozendaal, 2000), which, in turn, might impair subsequent learning. Animal studies suggest that stress affects memory performance in dependence on the reinforcement contingencies of the task. The learning of aversively motivated tasks is generally facilitated by prior stress, while the learning of appetitively motivated tasks, as well as tasks without explicit reinforcement of behavior, such as object recognition, is impaired by prior stress (Joels, Pu, Wiegert, Oitzl, & Krugers, 2006). In humans, a psychological stressor administered prior to the study phase of an episodic memory experiment enhanced the memory for emotional aspects of an event, but disrupted the memory for non-emotional aspects of the same event (Payne et al., 2006). Since our episodic-like memory task is a non-reinforced memory task, which presumably induces only little or no stress by itself, prior stress is likely to have detrimental effects on the performance in our task.

There is evidence that SR140333 blocks the central processing of nociceptive stimuli (Emonds-Alt et al., 1993). Furthermore, SR140333 has been shown to block the effects of restraint stress on ovarian steroid-dependent histamine release by substance P at the colonic level (Bradesi, Eutamene, Fioramonti, & Bueno, 2002) and antagonized stress-induced visceral hypersensitivity in female rats (Bradesi, Eutamene, Garcia-Villar, Fioramonti, & Bueno, 2003). Other NK-1-R antagonists have been reported to have antinociceptive, antidepressant and anxioytic effects (McLean, 2005; Pitcher & Henry, 2004) and NK-1-R knockouts are less susceptible to various stressors (Rupniak et al., 2001; Santarelli et al., 2001). Functional NK-1-R has been described in the adrenal gland, which receives substance P input via the splanchnic nerve (Hinson & Kapas, 1996; Hinson, Purbrick, Cameron, & Kapas, 1994). Substance P has excitatory effects on the adrenal cortex and facilitates glucocorticoid secretion, such as corticosterone, and is released in response to stressors, such as foot-shock (Vaupel, Jarry, Schlomer, & Wuttke, 1988). Thus, it is possible that the ability of a 1 mg/kg dose of SR140333 to reinstate episodic-like memory, which is normally impaired in vehicle treated rats, might be due to the attenuation of the stress response that is induced by the injection procedure.

Recently, the NK-1-R has also been implicated in hippocampal synaptic plasticity in guinea pigs (Langosch et al., 2005) and learning and memory in rats (Kart et al., 2004) and mice (Morcuende et al., 2003). NK-1-R knockout mice showed a slight improvement in the place version of the water maze task (Morcuende et al., 2003). In rats, systemic post-trial SR140333 injections had minor facilitative effects on inhibitory avoidance learning and behavioral habituation to a novel environment (Kart et al., 2004). Therefore, another possible explanation for the beneficial effect of SR140333 on episodic-like memory under stressful conditions might be that SR140333 facilitated the acquisition of information rather than diminishing the stress-response induced by the injection procedure.

Nevertheless, it should be noted that the evidence implicating the NK-1-R in learning and memory processes is not clear-cut. For example, systemically administered substance P, which preferentially stimulates the NK-1-R, has memory-promoting effects (reviewed in Hasenöhrl et al., 2000), similar to genetic inactivation (Morcuende et al., 2003) or pharmacological blockade of the NK-1-R (Kart et al., 2004). Moreover, substance P-induced potentiation of hippocampal LTP in guinea pigs is blocked by co-administration of the NK-1-R antagonist L-733060 (Langosch et al., 2005). Furthermore, substance P-induced potentiation of inhibitory neurotransmission in the hippocampus, which should rather impair learning and memory (Corcoran, Desmond, Frey, & Maren, 2005), is blocked by SR140333 (Ogier & Raggenbass, 2003). However, it should be noted that, in vivo, substance P is cleaved into biologically active N- and C-terminal hepta- and hexapeptide fragments. In terms of learning and memory and emotional behavior these fragments can have different effects (Huston & Hasenöhrl, 1995). The NK-1 receptor has two ligand binding sites, termed NK-1-major and NK-1-minor. The non-fragmented substance P molecule binds to the NK-1-major binding site and stimulates the adenylyl cyclase-cAMP pathway, while the C-terminal hexa- and heptapeptide fragments of substance P bind to the NK-1-minor site and stimulate the phospholipase C pathway (Alves et al., 2006). It is possible that NK-1-R antagonists either differ in their affinity regarding NK-1-major and NK-1-minor binding sites or that they stimulate both receptive sites, while the non-fragmented substance P molecule binds only to the NK-1-major site. Such differences might explain why stimulation of NK-1-R by substance P as well as blockade of the NK-1-R by NK-1-R antagonists can have similar behavioral effects, e.g. on learning and memory, while having opposing roles in hippocampal LTP.

It is known that modulation of hippocampal cholinergic neurotransmission affects synaptic plasticity (Ovsepian et al., 2004), learning and memory in rats (Sarter & Parikh, 2005) and episodic memory in humans (Hasselmo, Wyble, & Wallenstein, 1996). Progressive degeneration of cholinergic cells, especially those which innervate the frontal cortex and hippocampus, is a major symptom of Alzheimer's disease, possibly contributing to the early onset episodic

memory deficits in this disease (Riepe, 2005). We previously showed that an i.p. application of 1 mg/kg of SR140333 increased extracellular acetylcholine levels in the hippocampus to 148 ± 23% and 144 ± 23% of control levels after 20 and 40 min post-injection, respectively, while a saline injection reduced hippocampal acetylcholine levels only marginally by 91 ± 11% of control levels, measured 60 minutes post-injection (Kart et al., 2004). Thus, the 1 mg/kg i.p. dose of SR140333 has both promnestic effects on episodic-like memory and increases hippocampal acetylcholine efflux for at least 40 min post-injection. The hippocampus is thought to be a key structure for the encoding, consolidation and retrieval of episodic memories (Aggleton & Brown, 1999; Vargha-Khadem et al., 1997). NK-1 receptors have been detected in the hippocampus (Maeno, Kiyama, & Tohyama, 1993). It is therefore conceivable that the beneficial effect of SR140333 on stress-induced episodic-like memory deficits in rats relates to this enhancement of cholinergic neurotransmission in the hippocampus.

Since NK-1-R antagonists induce hippocampal neurogenesis (Rupniak, 2005), have anxiolytic and promestic properties, and induce hippocampal acetylcholine release at lower doses in anesthetized rats, they might be effective in the alleviation of the cognitive deficits and affective disorders present in early stages of Alzheimer's disease.

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## References

Aggleton, J. P., & Brown, M. W. (1999). Episodic memory, amnesia, and the hippocampal-anterior thalamic axis. *Behavioral Brain Science, 22*, 425–444.

Alves, I. D., Delaroche, D., Mouillac, B., Salamon, Z., Tollin, G., Hruby, V. J., et al. (2006). The two NK-1 binding sites correspond to distinct, independent, and non-interconvertible receptor conformational states as confirmed by plasmon-waveguide resonance spectroscopy. *Biochemistry, 45*, 5309–5318.

Bailey, S. J., & Jordan, C. C. (1984). A study of [D-Pro2, D-Phe7, D-Trp9]-substance P and [D-Trp7,9]-substance P as tachykinin partial agonists in the rat colon. *British Journal of Pharmacology, 82*, 441–451.

Baker, K. B., & Kim, J. J. (2002). Effects of stress and hippocampal NMDA receptor antagonism on recognition memory in rats. *Learning & Memory, 9*, 58–65.

Bradesi, S., Eutamene, H., Garcia-Villar, R., Fioramonti, J., & Bueno, L. (2003). Stress-induced visceral hypersensitivity in female rats is estrogen-dependent and involves tachykinin NK1 receptors. *Pain, 102*, 227–234.

Bradesi, S., Eutamene, H., Fioramonti, J., & Bueno, L. (2002). Acute restraint stress activates functional NK1 receptor in the colon of female rats: involvement of steroids. *Gut, 50*, 349–354.

Corcoran, K. A., Desmond, T. J., Frey, K. A., & Maren, S. (2005). Hippocampal inactivation disrupts the acquisition and contextual encoding of fear extinction. *The Journal of Neuroscience, 25*, 8978–8987.

Dere, E., Huston, J. P., & De Souza Silva, M. A. (2005a). Integrated Memory for objects, places and temporal order: evidence for episodic-like memory in mice. *Neurobiology of Learning and Memory, 84*, 214–221.

Dere, E., Huston, J. P., & De Souza Silva, M. A. (2005b). Protocol: episodic-like memory in mice: simultaneous assessment of object, place and temporal order memory. *Brain Research Protocols, 16*, 10–19.

Emonds-Alt, X., Doutremepuich, J. D., Heaulme, M., Neliat, G., Santucci, V., Steinberg, R., et al. (1993). In vitro and in vivo biological activities of SR140333, a novel potent non-peptide tachykinin NK1 receptor antagonist. *European Journal of Pharmacology, 250*, 403–413.

Ennaceur, A., Neave, N., & Aggleton, J. P. (1997). Spontaneous object recognition and object location memory in rats: the effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. *Experimental Brain Research, 113*, 509–519.

Featherstone, R. L., Fosbraey, P., & Morton, I. K. (1986). A comparison of the effects of three substance P antagonists on tachykinin-stimulated [3H]-acetylcholine release in the guinea-pig ileum. *British Journal of Pharmacology, 87*, 73–77.

Harrison, S., & Geppetti, P. (2001). Substance P. *International Journal of Biochemistry and Cell Biology, 33*, 555–576.

Hasenöhrl, R. U., De Souza Silva, M. A., Nikolaus, S., Tomaz, C., Brandao, M. L., Schwarting, R. K., et al. (2000). Substance P and its role in neural mechanisms governing learning, anxiety and functional recovery. *Neuropeptides, 34*, 272–280.

Hasenöhrl, R. U., Jentjens, O., De Souza Silva, M. A., Tomaz, C., & Huston, J. P. (1998). Anxiolytic-like action of neurokinin substance P administered systemically or into the nucleus basalis magnocellularis region. *European Journal of Pharmacology, 354*, 123–133.

Hasselmo, M. E., Wyble, B. P., & Wallenstein, G. V. (1996). Encoding and retrieval of episodic memories: role of cholinergic and GABAergic modulation in the hippocampus. *Hippocampus, 6*, 693–708.

Herpfer, I., & Lieb, K. (2003). Substance P and substance P receptor antagonists in the pathogenesis and treatment of affective disorders. *The World Journal of Biological Psychiatry: The Official Journal of the World Federation of Societies of Biological Psychiatry, 4*, 56–63.

Hinson, J. P., & Kapas, S. (1996). Effect of splanchnic nerve section and compensatory adrenal hypertrophy on rat adrenal neuropeptide content. *Regulatory Peptides, 61*, 105–109.

Hinson, J. P., Purbrick, A., Cameron, L. A., & Kapas, S. (1994). The role of neuropeptides in the regulation of adrenal zona fasciculata/reticularis function. Effects of vasoactive intestinal polypeptide, substance P, neuropeptide Y, Met- and Leuenkephalin and neurotensin on corticosterone secretion in the intact perfused rat adrenal gland in situ. *Neuropeptides, 26*, 391–397.

Hokfelt, T., Pernow, B., & Wahren, J. (2001). Substance P: a pioneer amongst neuropeptides. *Journal of Internal Medicine, 249*, 27–40.

Huston, J. P., & Hasenöhrl, R. U. (1995). The role of neuropeptides in learning: focus on the neurokinin substance P. *Behavioural Brain Research, 66*, 117–127.

Joels, M., Pu, Z., Wiegert, O., Oitzl, M. S., & Krugers, H. J. (2006). Learning under stress: how does it work? *Trends in Cognitive Sciences, 10*, 152–158.

Jung, M., Calassi, R., Maruani, J., Barnouin, M. C., Souilhac, J., Poncelet, M., et al. (1994). Neuropharmacological characterization of SR 140333, a non peptide antagonist of NK1 receptors. *Neuropharmacology, 33*, 167–179.

Kart, E., Jocham, G., Muller, C. P., Schlömer, C., Brandao, M. L., Huston, J. P., et al. (2004). Neurokinin-1 receptor antagonism by SR140333: enhanced in vivo ACh in the hippocampus and promnestic post-trial effects. *Peptides, 25*, 1959–1969.

Kart-Teke, E., De Souza Silva, M. A., Huston, J. P., & Dere, E. (2006). Wistar rats show episodic-like memory for unique experiences. *Neurobiology of Learning and Memory, 85*, 173–182.

Langosch, J. M., Kupferschmid, S., Heinen, M., Walden, J., Herpfer, I., Fiebich, B. L., et al. (2005). Effects of substance P and its antagonist L-733060 on long term potentiation in guinea pig hippocampal slices. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 29*, 315–319.

Maeno, H., Kiyama, H., & Tohyama, M. (1993). Distribution of the substance P receptor (NK-1 receptor) in the central nervous system. *Molecular Brain Research, 18*, 43–58.

Mansikka, H., Shiotani, M., Winchurch, R., & Raja, S. N. (1999). Neurokinin-1 receptors are involved in behavioral responses to high-intensity heat stimuli and capsaicin-induced hyperalgesia in mice. *Anesthesiology, 90*, 1643–1649.

Mantyh, P. W., Gates, T., Mantyh, C. R., & Maggio, J. E. (1989). Autoradiographic localization and characterization of tachykinin receptor binding sites in the rat brain and peripheral tissues. *The Journal of Neuroscience, 9*, 258–279.

McLean, S. (2005). Do substance P and the NK1 receptor have a role in depression and anxiety? *Current Pharmaceutical Design, 11*, 1529–1547.

Mitchell, J. B., & Laiacona, J. (1998). The medial frontal cortex and temporal memory: tests using spontaneous exploratory behaviour in the rat. *Behavioral Brain Research, 97*, 107–113.

Morcuende, S., Gadd, C. A., Peters, M., Moss, A., Harris, E. A., Sheasby, A., et al. (2003). Increased neurogenesis and brain-derived neurotrophic factor in neurokinin-1 receptor gene knockout mice. *European Journal of Neuroscience, 18*, 1828–1836.

Nagel, J. A., & Huston, J. P. (1988). Enhanced inhibitory avoidance learning produced by post-trial injections of substance P into the basal forebrain. *Behavioral and Neural Biology, 49*, 374–385.

Nikolaus, S., Huston, J. P., & Hasenohrl, R. U. (1999). Reinforcing effects of neurokinin substance P in the ventral pallidum: mediation by the tachykinin NK1 receptor. *European Journal of Pharmacology, 370*, 93–99.

Ogier, R., & Raggenbass, M. (2003). Action of tachykinins in the rat hippocampus: modulation of inhibitory synaptic transmission. *European Journal of Neuroscience, 17*, 2639–2647.

Ovsepian, S. V., Anwyl, R., & Rowan, M. J. (2004). Endogenous acetylcholine lowers the threshold for long-term potentiation induction in the CA1 area through muscarinic receptor activation: in vivo study. *European Journal of Neuroscience, 20*, 1267–1275.

Payne, J. D., Jackson, E. D., Ryan, L., Hoscheidt, S., Jacobs, J. W., & Nadel, L. (2006). The impact of stress on neutral and emotional aspects of episodic memory. *Memory, 14*, 1–16.

Pitcher, G. M., & Henry, J. L. (2004). Nociceptive response to innocuous mechanical stimulation is mediated via myelinated afferents and NK-1 receptor activation in a rat model of neuropathic pain. *Experimental Neurology, 186*, 173–197.

Quartara, L., & Maggi, C. A. (1998). The tachykinin NK1 receptor. Part II: distribution and pathophysiology. *Neuropeptides, 32*, 1–49.

Riepe, M. W. (2005). Cholinergic treatment: what are the early neuropathological targets? *European Journal of Neurology, 12*(Suppl 3), 3–9.

Roozendaal, B. (2000). Glucocorticoids and the regulation of memory consolidation. *Psychoneuroendocrinology, 25*, 213–238.

Rupniak, N. M. J. (2005). Substance P (NK1 receptor) antagonists. In T. Steckler, N. H. Kalin, & J. M. H. M. Reul (Eds.), *Handbook of Stress and the Brain. Part 2: Stress: Integrative and Clinical Aspects* (pp. 423–435). Amsterdam: Elsevier.

Rupniak, N. M. J., Carlson, E. J., Webb, J. K., Harrison, T., Porsolt, R. D., Roux, S., et al. (2001). Comparison of the phenotype of NK1R/ mice with pharmacological blockade of the substance P (NK1) receptor in assays for antidepressant and anxiolytic drugs. *Behavioural Pharmacology, 12*, 497–508.

Santarelli, L., Gobbi, G., Debs, P. C., Sibille, E. L., Blier, P., Hen, R., et al. (2001). Genetic and pharmacological disruption of neurokinin 1 receptor function decreases anxiety-related behaviors and increases serotonergic function. *Proceedings of the National Academy of Sciences of the United States of America, 98*, 1912–1917.

Sarter, M., & Parikh, V. (2005). Choline transporters, cholinergic transmission and cognition. *Nature Reviews Neuroscience, 6*, 48–56.

Vargha-Khadem, F., Gadian, D. G., Watkins, K. E., Connelly, A., Van Paesschen, W., & Mishkin, M. (1997). Differential effects of early hippocampal pathology on episodic and semantic memory. *Science, 277*, 376–380.

Vaupel, R., Jarry, H., Schlomer, H. T., & Wuttke, W. (1988). Differential response of substance P-containing subtypes of adrenomedullary cells to different stressors. *Endocrinology, 123*, 2140–2145.

Yu, Z., Cheng, G., Huang, X., Li, K., & Cao, X. (1997). Neurokinin-1 receptor antagonist SR140333: a novel type of drug to treat cerebral ischemia. *Neuroreport, 8*, 2117–2119.

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Düsseldorf, den 18.12.2006

(Emriye Teke)