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Die Rolle von Neurokinin Rezeptoren bei der Modulation von Lern- und Gedächtnisprozessen und deren Einfluss auf das cholinerge System im basalen Vorderhirn

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III Zusammenfassung

Es finden sich vermehrt Hinweise, dass Neurokinine (NK) eine bedeutende Rolle bei der Modulation von Verhalten und der damit verbundenen neurochemischen Transmission einnehmen. Eine Beteiligung der Neurokinine an emotionalen Verhaltensweisen wie auch Lern- und Gedächtnisprozessen ist hierbei von besonderem Interesse. Bisher konnten im Gehirn drei Neurokinin-Rezeptorsubtypen identifiziert werden: NK_1-, NK_2- und NK_3-Rezeptoren, wobei sich zunächst die Erforschung der Funktion von Neurokininen und deren Rezeptoren auf den NK_1-Rezeptor fokussierte. Allerdings ist bisher wenig bekannt, über welche spezifischen NK-Rezeptorsubtypen und neurochemischen Mechanismen die Vermittlung emotionaler wie auch Lern- und Gedächtnisprozessen mediiert wird. Es besteht Evidenz, dass das cholinerge System des basalen Vorderhirns Lern- und Gedächtnisleistungen beeinflusst. Sowohl NK-Rezeptor Antagonisten als auch NK-Rezeptor Agonisten nehmen möglicherweise Einfluss auf kognitive Prozesse, wobei sich dabei ein Zusammenhang mit dem cholinergen System vermuten ließe. In den letzten Jahren wurden NK-Rezeptor Antagonisten entwickelt, die einen potentiellen Einsatz bei der Behandlung von Angststörungen, Depression und Schizophrenie verheißen.

In diesem Zusammenhang sollte diese Arbeit, die Rolle von NK_2- und NK_3-Rezeptoren bei Lern- und Gedächtnisprozessen, emotionalen Verhalten und den Einfluss dieser Rezeptoren auf die Aktivität des cholinergen Systems im basalen Vorderhirn bei Wistar Ratten klären. Dabei wurde an adulten Wistar Ratten untersucht, inwieweit eine intra-septale Injektion des NK_2-Rezeptor Antagonist Bz-Ala-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH_2 die Gedächtnisleistung bei einem Objektexplorations-Paradigma, welches räumliches und temporales Objektgedächtnis evaluiert, beeinflusst. Mit Hilfe der in vivo Mikrodialyse in anästhesierten Tieren wurde der Effekt dieses NK_2-Rezeptor Antagonisten auf die extrazelluläre

Acetylcholin (ACh) Konzentration in Frontalcortex, Amygdala und Hippocampus getestet. In einer weiteren Studie wurde die in vivo Mikrodialyse unter Anästhesie genutzt, um den Einfluss einer Applikation des NK_2-Rezeptor Agonisten NKA und NK_3-Rezeptor Agonisten NKB ins mediale Septum auf die cholinerge Neurotransmission in Frontalcortex, Amygdala und Hippocampus zu bestimmen. Basierend auf den Ergebnissen wurde im Anschluss analysiert, wie sich eine Vorbehandlung des NK_2-Rezeptor Antagonisten SR48968 auf die cholinerge Neurotransmission nach NK_2- bzw. NK_3-Rezeptoraktivierung auswirkt. Weiterhin sollte ermittelt werden, ob der NK_3-Rezeptor Agonist Senktide emotionale Verhaltensweisen im „Open-Field" Test und „Forced Swimming Test" alternder Wistar Ratten verändert. Außerdem war es Ziel, den Einfluss von Senktide auf die Gedächtnisleistung im „Episodic-like Memory" Paradigma bei alten Tieren zu erheben. Mittels in vivo Mikrodialyse sollte bei anästhesierten alternden Ratten untersucht werden, wie eine Injektion des NK_3-Rezeptor Agonisten die Konzentration von ACh in Frontalcortex, Amygdala und Hippocampus beeinflusst. In der vorliegenden Dissertation wurde ebenfalls untersucht, welchen Einfluss Senktide auf Objektwiedererkennung, räumliche und temporale Objektgedächtnisleistung nach Blockade des cholinergen Systems anhand des muscarinergen ACh-Rezeptor Antagonisten Scopolamin ausübt.

Die Ergebnisse ergaben, dass eine intra-septale Injektion von Bz-Ala-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH_2 ein Defizit beim Objektexplorations-Paradigma kompensierte, indem eine Komponente: das temporale Objektgedächtnis, wiederhergestellt werden konnte. Zudem konnte beobachtet werden, dass der NK_2-Rezeptor Antagonist die ACh Freisetzung in Frontalcortex und Hippocampus vermindert. Die intra-septale Applikation von NKA erhöhte die ACh Neurotransmission in Amygdala und Hippocampus, wohingegen die NKB Behandlung lediglich zu einem Anstieg der ACh Level im Hippocampus führte. Die Vorbehandlung mit dem NK_2-Rezeptor Antagonisten SR48968 resultierte in einer Verminderung der durch NKA bzw. NKB-induzierten ACh Freisetzung. Desweiteren

zeigte sich, dass die NK_3-Rezeptor Aktivierung durch Senktide ängstliches Verhalten im „Open-Field“ Test und depressionsähnliche Verhaltensweisen im „Forced Swimming Test“ bei alten Tieren reduziert. Der NK_3-Rezeptor Agonist war zwar nicht in der Lage eine altersabhängige Beeinträchtigung im „Episodic-like Memory“ Paradigma auszugleichen, gleichwohl konnte eine Komponente episodischer Gedächtnisleistung, nämlich räumliches Objektgedächtnis, herbeiführt werden. Darüber hinaus zeigte sich bei den alternden Ratten eine dosisabhängige Erhöhung der ACh Freisetzung in Frontalcortex, Amygdala oder Hippocampus nach Behandlung mit dem NK_3-Rezeptor Agonisten Senktide. Eine Behandlung von adulten Ratten mit dem ACh-Rezeptor Antagonisten Scopolamin erwies sich als effizient, um ein Defizit bei Objektwiedererkennung, räumlichen und temporalen Objektgedächtnis auszulösen. Die beeinträchtigte Gedächtnisleistung bei diesen Objektexplorations-Paradigmen konnte durch die NK_3-Rezeptor Aktivierung von Senktide dosisabhängig kompensiert werden.

Die vorliegenden Ergebnisse deuten darauf hin, dass NK_2-Rezeptoren im medialen Septum sowohl bei der Vermittlung von Lern- und Gedächtnisprozessen als auch der Aktivierung des cholinergen Systems im basalen Vorderhirn involviert sind. Ein Zusammenhang von Verhaltenseffekten und cholinerger Aktivität auf Basis der NK_3-Rezeptor Aktivierung kann ebenso postuliert werden. Dabei scheinen NK_3-Rezeptoren eine wichtige Rolle in Verbindung mit emotionsrelevanten Verhaltensweisen wie auch Lern- und Gedächtnisfunktionen einzunehmen.

IV Summary

There is considerable evidence for a prominent role of neurokinins (NK) in behavior and neurochemical neurotransmission. The work presented here focuses on their involvement in emotional behaviors as well as in learning and memory processes. Three distinct neurokinin receptors have been identified in the brain, namely the NK_1, NK_2- and NK_3-receptors. In the past, most studies dealing with neurokinin functions focused mainly on the NK_1-receptors. However, it is still unclear as to which NK receptors and neurotransmitter systems mediate emotionality and learning and memory processes. Accumulating evidence suggest that the cholinergic system of the basal forebrain is an important substrate for mnemonic processing. Both NK-receptor agonists and NK-receptor antagonists possibly influence cognitive function in association to the activity of the cholinergic system. NK-receptor antagonists have become available only recently and seem to have therapeutic potential for the treatment of anxiety, depression and schizophrenia.

The aim of this project was to ascertain the role of NK_2- and NK_3-receptors in processes governing learning and memory, emotional behavior and their impact on the activity of cholinergic system of the basal forebrain in Wistar rats. We investigated the effect of intra-septal injection of the NK_2-receptor antagonist Bz-Ala-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH_2 on object recognition memory using an object exploration paradigm combining memory for spatial location and temporal order. The in vivo microdialysis method in the anaesthetized preparation was used to determine the influence of NK_2-receptor antagonism on extracellular ACh levels in the frontal cortex, amygdala and hippocampus. In a further study we tested the effects of administrating an NK_2-receptor agonist (NKA) and NK_3-receptor agonist (NKB) into the medial septum on cholinergic neurotransmission in the frontal cortex, amygdala and hippocampus using in vivo microdialysis in anesthetized animals. Having found a facilitating effect, we examined whether intra-septal pre-treatment with the NK_2-

receptor antagonist SR48968 would affect the NKA- or NKB-induced cholinergic activity. Another study was conducted in aged Wistar rats to investigate the effects of Senktide, a highly potent NK_3-receptor agonist, on emotionality in an open field test and on depression-like behaviour in the forced swimming test. Furthermore, an episodic-like memory task was used to test the impact of senktide on learning and memory performance in the aged rat. By in vivo microdialysis, changes of ACh release in the frontal cortex, amygdala and hippocampus upon NK_3-receptor agonism was determined in aged animals. Furthermore, we investigated the influence of the NK_3-receptor agonist senktide on object, object-place as well as object recognition for temporal order after a scopolamine-induced blockade of muscarinergic ACh receptors.

The results show that administration of the NK_2-receptor antagonist Bz-Ala-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH_2 into the medial septum improved object recognition memory for temporal order. Intra-septal injection of this substance also decreased ACh neurotransmission in the frontal cortex and hippocampus. We found that injection into the medial septum of the NK_2-receptor agonist NKA increased ACh levels in the amygdala and hippocampus, whereas the NK_3-receptor agonist NKB enhanced ACh release only in the hippocampus. Moreover, pre-treatment with the NK_2-receptor antagonist SR48968 into the medial septum diminished the intra-septal NKA- or NKB-induced ACh neurotransmission in both the amygdala and hippocampus. In addition, the results demonstrated that NK_3-receptor activation by senktide produced dose-dependent anxiolytic and antidepressant-like effects in aged rats as assessed by the open field test and the forced swimming test, respectively. Senktide did not establish episodic-like memory in the aged animals, but reinstated components of episodic-like memory, namely object-place recognition. Treatment with senktide also increased ACh levels in the frontal cortex, amygdala and hippocampus, dose-dependently. Furthermore, the NK_3-receptor agonist senktide was found to alleviate memory deficits in object, object-place as well as object recognition

for temporal order induced by the muscarinergic ACh receptor antagonist scopolamine.

The results advance our understanding of selective behavioral and neurochemical functions of the NK receptors. They suggest that NK_2-receptors in the medial septum modulate learning and memory processes through an action on the cholinergic system of the basal forebrain. Given the profound effects of NK_3-receptor activation on behavior and cholinergic activity of the basal forebrain, suggests that also the NK_3-receptors mediate both, emotional as well as learning and memory processes, and that cholinergic mechanism underlie these effects.

V Abkürzungsverzeichnis

ACh	Acetylcholin
AChE	Acetylcholineesterase
Aβ	β-Amyloid
ChAT	Cholinacetyltransferase
ChO	Cholineoxidase
DA	Dopamin
EC	elektrochemischer Detektion
HK-1	Hemokinin 1
HPLC	hochauflösende Hochdruckflüssigkeits-Chromatographie
min	Minuten
NA	Noradrenalin
NK	Neurokinine
NKA	Neurokinin A
NKB	Neurokinin B
NK_1	Neurokinin 1
NK_2	Neurokinin 2
NK_3	Neurokinin 2
NPK	Neuropeptid K
NPγ	Neuropeptid γ
NBM	Nucleus basalis magnocellularis
5-HT	Serotonin
sog.	sogenannt
s.c.	subcutan
SP	Substanz P
u.a.	unter anderem

H_2O_2	Wasserstoffperoxid
ZNS	Zentralnervensystem

1 Einleitung

Eine Vielzahl von Studien zeigten bei neuropsychiatrischen und neurologischen Erkrankungen wie Depressionen, Schizophrenie, Demenzen, Multiple Sklerose und Epilepsie neben körperlichen Funktionsstörungen auch kognitive Beeinträchtigungen. Ebenso lässt mit zunehmendem Alter die Leistungsfähigkeit aufgrund körperlicher, sensorischer und kognitiver Defizite nach. Bezüglich kognitiver Veränderungen aufgrund neurologischer und neuropsychiatrischer Erkrankungen oder natürlicher Alterungsprozesse sind Beeinträchtigungen in diversen Bereichen wie Informationsverarbeitung, Reaktionsgeschwindigkeit, Aufmerksamkeit und Gedächtnis präsent. Der Focus der Forschung richtet sich dabei immer stärker auf strukturelle Veränderungen im Gehirn und eventuell darin begründet liegende Gedächtnisdefizite.

Ein großes Interesse besteht darin, verschiedene Möglichkeiten zur Modulation kognitiver Beeinträchtigungen zu evaluieren. Ein möglicher Ansatzpunkt bietet hierbei das cholinerge System im Gehirn. Bisherige Befunde weisen darauf hin, dass das cholinerge System Lern- und Gedächtnisleistungen beeinflusst (Gold, 2003a). In den letzten Jahren gelangte man aufgrund tierexperimenteller Studien zu der Annahme, dass Neuropeptide wie etwa die Neurokinine in der Lage sind, die Wirkung des Neurotransmitters Acetylcholin zu modulieren. Dabei nehmen Neurokinin-Rezeptoren möglicherweise Einfluss auf Lern- und Gedächtnisprozesse, wobei sich ein Zusammenhang mit dem cholinergen System vermuten ließe. Demnach stellen Neurokinine als Wirkstoffklasse bei Lern- und Gedächtnisbeeinträchtigungen ein interessantes und potentielles Einsatzgebiet dar.

Das übergeordnete Ziel dieser Dissertation ist die tierexperimentelle Untersuchung der Rolle von Neurokinin-Rezeptoren bei der Modulation von Lern- und Gedächtnisprozessen und deren Einfluss auf das cholinerge System im basalen Vorderhirn.

1.1. Neurokinine und Neurokinin-Rezeptoren

Neuropeptide mit der gemeinsamen Aminosäuresequenz Phe-X-Gly-Leu-Met-NH_2 (X steht für eine variable Aminosäure) am carboxyterminalen Ende werden als Tachykinine bezeichnet (Severini et al., 2002), die bei Säugetieren auch als Neurokinine (NK) benannt werden. Zur Familie der Neurokinine gehören Substanz P (SP), Neurokinin A (NKA), Neurokinin B (NKB), Neuropeptid K (NPK), Neuropeptid γ (NPγ) und Hemokinin 1 (HK-1). SP, NKA, NPK und NPγ werden vom selben Gen Preprotachykinin-A (Carter and Krause, 1990), NKB vom Preprotachykinin-B (Page et al., 2006) und HK-1 vom Preprotachykinin-C (Kurtz et al., 2002) Gen kodiert. NK agieren als Neurotransmitter oder Neuromodulatoren und binden an spezifische NK-Rezeptoren, derer bisher 3 Subtypen identifiziert wurden: NK_1, NK_2 und NK_3 (Maggi, 1995). Die endogenen Liganden für die NK_1-, NK_2- und NK_3-Rezeptoren sind SP, NKA bzw. NKB (Maggi, 2000;Mussap et al., 1993;Regoli et al., 1989). Es wurde nachgewiesen, dass die Neurokinine mit unterschiedlichen Affinitäten an den drei bekannten NK-Rezeptorsubtypen binden (Maggi, 1995;Regoli et al., 1994). Hierbei besitzt SP die höchste Affinität zum NK_1-Rezeptor, wohingegen NKA und NKB die höchste Affinität zum NK_2- bzw. NK_3-Rezeptor haben. Allerdings binden die NK an allen drei Rezeptorsubtypen (Maggi, 1995;Regoli et al., 1994). Die unterschiedlichen NK-Rezeptoren gehören zur Familie der G-Protein gekoppelten Rezeptoren und kommen sowohl in der Peripherie als auch im Zentralnervensystem (ZNS) in unterschiedlicher Dichte und Verteilung vor (Almeida et al., 2004). SP, NKA und NKB sind im Gehirn weit verteilt (Kanazawa et al., 1984;Ribeiro-da-Silva and Hökfelt, 2000), wobei SP am häufigsten vorkommt (Arai and Emson, 1986). Die NK_1-Rezeptoren werden in vielen Gehirnstrukturen wie u.a. in Basalganglien, Substantia nigra, Hypothalamus, Locus coeruleus, medialer Präfrontalcortex, Amygdala, Hippocampus, basales Vorderhirn (Maeno et al., 1993) exprimiert. NK_2-Rezeptoren hingegen kommen nur vorwiegend im Frontalcortex, Hippocampus, Thalamus und

medialen Septum vor (Hagan et al., 1993; Saffroy et al., 2003). NK_3-Rezeptoren sind in Arealen wie ventrales Pallidum, ventrales tegmentales Areal (VTA), medialer Präfrontalcortex, Amygdala, Nucleus accumbens, Substantia nigra und mediales Septum präsent (Ding et al., 1996;Shughrue et al., 1996).

Neurokinine scheinen neben kognitiven (Hasenöhrl et al., 2000;Huston and Hasenöhrl, 1995;Siuciak et al., 2007) und emotionalen Prozessen (Chahl, 2006;Ebner and Singewald, 2006;Hasenöhrl et al., 2000;Louis et al., 2008) auch bei einer Vielzahl physiologischer Funktionen während der Immunantwort, Stress, Schmerzübertragung sowie Entzündungsprozessen (Almeida et al., 2004;Culman and Unger, 1995;Nussdorfer and Malendowicz, 1998;Severini et al., 2002) involviert zu sein. In der Vergangenheit wurde ein potentielles Anwendungsgebiet von NK_1-Rezeptor Antagonisten bei der Behandlung von Angst und Depressionen diskutiert (Czeh et al., 2006;Herpfer and Lieb, 2005;Kramer et al., 1998;Lieb et al., 2002). Weiterhin konnte gezeigt werden, dass sowohl NK_2-Rezeptor- (Dableh et al., 2005;Steinberg et al., 2001) als auch NK_3-Rezeptor Antagonisten (Salome et al., 2006) antidepressive Eigenschaften aufweisen und NK_3-Rezeptor Antagonisten effektiv in der Behandlung von Schizophrenie zu sein scheinen (Meltzer et al., 2004).

In der Literatur finden sich Studien, die eine Interaktion der Neurokinine mit verschiedenen Neurotransmittersystemen beobachteten. Neben Dopamin (DA) (Galarraga et al., 1999;Kombian et al., 2003;Marco et al., 1998), Serotonin (5-HT) (Guiard et al., 2006;Stoessl et al., 1990) und Noradrenalin (NA) (Gobbi and Blier, 2005;Jung et al., 1996) ließ sich der Einfluss der Neurokinine und deren Rezeptoren auch auf das cholinerge System nachweisen. Im folgenden Abschnitt wird der Zusammenhang von Neurokininen und dem Neurotransmitter Acetylcholin (ACh) näher erläutert.

1.2. Acetylcholin und Neurokinine

Das cholinerge System des basalen Vorderhirns scheint bei einer Reihe von Prozessen wie Aufmerksamkeit (Sarter and Bruno, 1997), Angst (Degroot and Treit, 2004) sowie Lern- und Gedächtnisprozessen (Blokland, 1995;Chang and Gold, 2004;Gold, 2003b;Sarter and Bruno, 1997) involviert zu sein. Das basale Vorderhirn besteht aus dem diagonalen Band von Broca, laterales und mediales Septum, Substantia innominata und Nucleus basalis magnocellularis und ist Hauptursprungsgebiet der primären cholinergen Projektionen zum Frontalcortex, Amygdala und Hippocampus (Mesulam et al., 1983). Das mediale Septum unterhält cholinerge Projektionen zum Hippocampus (Mesulam et al., 1983) und der Nucleus basalis magnocellularis (NBM) besitzt einige Projektionen zum Bulbus olfactorius und Amygdala, wobei die Hauptprojektionen jene zum Neocortex darstellen. Selektive cholinerge Läsionen in Subregionen des basalen Vorderhirns weisen auf eine unterschiedliche Beteiligung an kognitiven Prozessen hin. So zeigte sich, dass die septo-hippocampale Projektion bei Arbeitsgedächtnisprozessen, die NBM-Neocortex Projektion bei Aufmerksamkeitsprozessen und die Projektion vom NBM zur Amygdala beim Abruf affektbedingter Konditionierung eine Rolle spielt (Everitt and Robbins, 1997). Cholinerge Läsionen in Strukturen des basalen Vorderhirns führten zu einer Verringerung der ACh Neurotransmission und wurden von verschiedenen Verhaltensbeeinträchtigungen begleitet (Bartolini et al., 1996;Casamenti et al., 1988).

Verschiedene Studien zeigten altersbedingte morphologische und physiologische Veränderungen u.a. innerhalb des cholinergen Systems im Gehirn (Araujo et al., 1990;Mckinney and Jacksonville, 2005;Sarter and Bruno, 2004). Dahingehend sind während Alterungsprozesse die cholinerge Aktivität vermindert (Araujo et al., 1990;Baxter et al., 1999;Freo et al., 2005;Rossner, 1997), sowie eine entsprechende Abnahme der Aktivität des Enzyms Cholinacetyltransferase (ChAT) im Cortex sowohl beim Menschen (Feuerstein and Seeger, 1997;Schliebs and Arendt,

2011;Takei et al., 1989) wie auch bei Labortieren (Birthelmer et al., 2003;Fischer et al., 1987;1989;Herzog et al., 2003) nachweisbar. Weiterhin ist ausreichend bekannt, dass mit fortschreitenden Alter eine Reduktion der Anzahl, Hypertrophie und Hypotrophie cholinerger Neurone (Cooper and Sofroniew, 1996;De Lacalle et al., 1991;Finch, 1993;Schliebs and Arendt, 2011;Stroessner-Johnson et al., 1992) sowie eine Veränderung der Dichte und Affinität cholinerger Rezeptoren (Smith et al., 1995;Tribollet et al., 2004) stattfindet.

Einige Studien legen die Vermutung eines Einfluss von NK-Rezeptoren auf das cholinerge System im basalen Vorderhirn nahe. Untermauert wird dies durch den Befund, dass die Injektion des endogenen NK_1-Rezeptor Liganden SP ins NBM zu einem Anstieg der ACh Level im Frontalcortex führte (De Souza Silva et al., 2000). Außerdem zeigte sich eine Co-Lokalisation von NK_1-, NK_2- und NK_3-Rezeptoren und dem cholinergen Marker ChAT im medialen Septum bzw. diagonalen Band von Broca (Beaujouan et al., 1986;Mantyh et al., 1984;Saffroy et al., 2003;Shughrue et al., 1996;Steinberg et al., 1998). Ergänzend dazu wurden auf cholinergen Neuronen im medialen Septum sowohl NK_1- als auch NK_3-Rezeptoren identifiziert (Chen et al., 2001a;2001b;Gerfen, 1991), wobei 80 % cholinerger Neurone mit dem NK_1-Rezeptor assoziiert sind (Chen et al., 2001b;Gerfen, 1991) und 66 % der cholinergen Neurone NK_3-Rezeptoren exprimieren (Chen et al., 2001a). Das mediale Septum scheint eine wichtige Rolle bei der Interaktion von Neurokininen und dem cholinergen Systems des basalen Vorderhirns einzunehmen. Es ist die Hirnregion mit der höchsten Dichte an NK_2-Rezeptoren (Saffroy et al., 2001;2003). Daneben sind ebenfalls NK_1- (Maeno et al., 1993) und NK_3-Rezeptoren (Shughrue et al., 1996) im medialen Septum lokalisiert. Es gibt zudem Evidenz, dass die Aktivität cholinerger Neurone des septo-hippocampalen Systems mit Hilfe von NK_1-, NK_2- und NK_3-Rezeptor Agonisten stimuliert und dies wiederum anhand jeweiliger selektiver Antagonisten blockiert werden kann (Morozova et al., 2008). Weiterhin wird den NK_1-, NK_2- und NK_3-Rezeptoren eine Rolle innerhalb des septo-hippocampalen cholinergen Neurotransmission beigemessen. Hierbei zeigte sich in einigen Mikrodialyse Studien

ein Anstieg der ACh Freisetzung im Hippocampus nach intra-septaler Injektion von NK_1-, NK_2- und NK_3-Rezeptor Agonisten (Marco et al., 1998;Steinberg et al., 1998). Intra-septale Injektion des NK_2-Rezeptor Agonisten NKA steigerte die ACh Neurotransmission im Hippocampus und dieser Effekt konnte durch den NK_2-Rezeptor Antagonisten SR48968 inhibiert werden (Steinberg et al., 1998). Zusätzlich fanden Desvignes und Kollegen (2003), dass es durch taktile Stimulation oder Injektion des Corticotrophin-Releasing-Faktors (CRF) zu einer Erhöhung der ACh Level im Hippocampus kommt, und dies durch einen NK_2-Rezeptor Antagonisten blockiert werden konnte. Darüber hinaus berichteten verschiedene Autoren einen hippocampalen Anstieg der ACh Freisetzung nach intra-septaler Applikation des NK_3-Rezeptor Agonisten Senktide (Marco et al., 1998;Steinberg et al., 1998). Zusätzlich wurde auch eine Erhöhung ACh Level im Striatum nach Aktivierung der NK_3-Rezeptoren beschrieben (Steinberg et al., 1995).

Überdies gibt es verschiedene Hinweise, dass NK-Rezeptoren Einfluss auf die Abnahme cholinerger Aktivität während Alterungsprozesse nehmen. So verhinderte die Injektion des NK_3-Rezeptor Liganden NKB ins NBM den Verlust cholinerger Neurone (Wenk et al., 1997). Daneben zeigte sich, dass mit Hilfe von SP (Yankner et al., 1990) als auch NKB (Wenk et al., 1997;Yankner et al., 1990) die neurotoxische Wirkung von β-Amyloid (Aβ) Proteinen, die als neurotoxische Ablagerungen im Gehirn von Alzheimer Patienten gelten (Moreno et al., 2007), aufgehoben wurde.

1.3. Lernen und Gedächtnis, Neurokinine

Studien zu den neurobiologischen Grundlagen von Lernen und Gedächtnis haben ergeben, dass Substanzen, die die Aktivität des cholinergen Systems im basalen Vorderhirn erhöhen, ein promnestisches Wirkungsprofil aufweisen (Bruno et al., 1999;Gold, 2003a;Masuoka and Kamei, 2009;Puma et al., 1999). Indes resultierte eine Verminderung cholinerger Aktivität des basalen Vorderhirns in einer Vielzahl von Lern- und Gedächtnisdefiziten (Chang and Gold, 2004;Christensen et al., 1992;Everitt and Robbins, 1997;Fibiger et al., 1991;Walsh et al., 1996). Eine Zerstörung cholinerger Neurone zog Beeinträchtigungen bei Objektwiedererkennung (Vnek et al., 1996), räumlichen Lernen (Leanza et al., 1995) und räumliches Arbeitsgedächtnis (Shen et al., 1996) nach sich. Hierbei scheint es, dass das septo-hippocampale cholinerge System eine wichtige Rolle bei Lern- und Gedächtnisprozessen einnimmt (Hasselmo, 1999;2006;Kesner, 1988). Die Assoziation von cholinerger Aktivität und Lern- und Gedächtnismechanismen wird durch Studien untermauert, die zum einen nach einer Reduktion der ACh Neurotransmission im Hippocampus Defizite bei räumlichen Lernaufgaben („Water Maze“, „T-Maze“, „Radial Arm Maze“) und Vermeidungslernen fanden (Chang and Gold, 2004;Lehmann et al., 2000), zum anderen einen Zusammenhang zwischen dem Anstieg der ACh Level im Hippocampus und der Performance räumlicher Gedächtnisparadigmen postulierten (Fadda et al., 2000;Stancampiano et al., 1999). Dennoch besteht in der Literatur über die Rolle des septo-hippocampalen Systems bei Lern- und Gedächtnisprozessen eine Kontroverse. Zum einen beschreiben verschiedene Autoren das Vorhandensein von Lern- und Gedächtnisdefiziten aufgrund von Läsionen im medialen Septum (Chang and Gold, 2004;Janis et al., 1998;Lamprea et al., 2000;Leanza et al., 1995;1996;Walsh et al., 1996), andererseits gibt es Hinweise auf ein Fehlen von Lern- und Gedächtnisbeeinträchtigungen (Baxter et al., 1996;Bizon et al., 2003;Chappell et al., 1998;Perry et al., 2001).

Ebenso scheint ein Zusammenhang zwischen cholinerger Degeneration und kognitiver Beeinträchtigung zu bestehen, dabei zeigte sich eine starke Korrelation von cholinerger Aktivität und dem Ausmaß kognitiver Defizite (Bartus, 2000;Fischer et al., 1989;Gage et al., 1988;Hellweg et al., 1990;Martinez-Serrano et al., 1995). Mit zunehmendem Alter kommt es beim Menschen zu einer Abnahme der kognitiven Leistungen (Erickson and Barnes, 2003), was von morphologischen und biochemischen Veränderungen begleitet wird. Im Tiermodell wurden diverse altersabhängige Beeinträchtigungen in verschiedenen Lern- und Gedächtnisparadigmen wie „Water Maze" (Gage et al., 1984;Rapp et al., 1987;Schulz et al., 2002;Topic et al., 2005;van der Staay and de Jonge, 1993), „Inhibitory Avoidance" (Riccio and Schulenburg, 1969;Egger and Livesey, 1972;Miettinen et al., 1993;Schulz et al., 2002), klassische Furchtkonditionierung (Powell et al., 1991) und Objektwiedererkennung (de Lima et al., 2005;Liu et al., 2004;Pitsikas et al., 2005) beobachtet. Studien an alten Ratten ergaben, dass eine Degeneration cholinerger Neurone im basalen Vorderhirn von einer Lern- und Gedächtnisverminderung begleitet wird (Fischer et al., 1989;Hellweg et al., 1990;Martinez-Serrano et al., 1995). Ein ähnlicher Zusammenhang findet sich bei Alzheimer Patienten (Patel and Tariot, 1991;VanDenBerg et al., 2000), wo eine Degeneration cholinerger Neurone im Nucleus Basalis Meynert, vergleichbar mit dem NBM bei Ratten, mit den kognitiven Defiziten der Alzheimer Erkrankung in Verbindung gebracht wird (Bartus, 2000;Mesulam, 2004). Es ist hinreichend bekannt, dass sich bereits zu Beginn der Alzheimer Erkrankung eine progressive Verschlechterung episodischen Gedächtnisses zeigt (Lindeboom and Weinstein, 2004;Small et al., 2003). Bisher wurden noch keine Studien durchgeführt, die im Tiermodell Defizite im episodischen Gedächtnis bei alternden Tieren nachweisen konnten. Das „Episodic-like Memory" Paradigma, mit dem episodische Gedächtnisleistung bei Labortieren validiert werden kann, wurde erst kürzlich entwickelt (Dere and De Souza Silva, 2010;Kart-Teke et al., 2006).

Die Identifikation von NK-Rezeptoren u.a. im Frontalcortex, Amygdala und Hippocampus, Hirnareale (Ding et al., 1996;Maeno et al., 1993;Saffroy et al., 2003), die

mit Lernen und Gedächtnis assoziiert werden, und der Einfluss von NK_1-, NK_2- und NK_3-Rezeptor Agonisten auf die Aktivität des cholinergen Systems (Marco et al., 1998;Morozova et al., 2008;Steinberg et al., 1998), lassen eine Rolle der Neurokinine bei der Modulation von Lern- und Gedächtnisprozessen vermuten. In früheren Studien wurde bereits den NK-Rezeptoren ein Einfluss auf Lernen und Gedächtnis zugeschrieben. Zunächst zeigte sich, dass NK_1-Rezeptoren bei Lern- und Gedächtnisprozessen von Ratten (Hasenöhrl et al., 2000;Kart et al., 2004;Kart-Teke et al., 2007;Stäubli and Huston, 1980) und Mäusen (Morcuende et al., 2003) eine Rolle spielen. Die Injektion von SP, dem NK_1-Rezeptor Agonisten, hatte einen gedächtnisfördernden Effekt bei verschiedenen Lernaufgaben wie „Passive Avoidance" (Stäubli and Huston, 1980), „Inhibitory Avoidance" (Hasenöhrl et al., 1990a) und „Water maze" (Hasenöhrl et al., 1990b) zur Folge. Knockout des NK_1-Rezeptors führte zu einer besseren Performance im „Water maze" (Morcuende et al., 2003). Desweiteren begünstigte eine systemische Behandlung von Ratten mit einem NK_1-Rezeptor Antagonisten die Gedächtnisleistung beim „Inhibitory Avoidance" (Kart et al., 2004) und „Episodic-like Memory" (Kart-Teke et al., 2007). Befunde für eine Rolle von NK_2- und NK_3-Rezeptoren bei Lern- und Gedächtnisprozessen gibt es ebenso (Kameyama et al., 1998;Siuciak et al., 2007;Ukai et al., 1996;Zlomuzica et al., 2008). So wurde beschrieben, dass ein bestehendes Defizit in einer „Spontaneous Alternation" Aufgabe sowohl durch den NK_2-Rezeptor Agonisten NKA als auch den NK_3-Rezeptor Agonisten Senktide aufgehoben wurde (Kameyama et al., 1998;Ukai et al., 1998). Der NK_3-Rezeptor Agonist NKB war in der Lage, eine beeinträchtigte Referenzgedächtnisleisung im „Radial Maze" zu verhindern (Wenk et al., 1997). Zudem hatte die Injektion von Senktide bei Mäusen einen promnestischen Effekt in einer „Episodic-like memory" Aufgabe (Zlomuzica et al., 2008). Im Gegensatz dazu wurde nach NK_3-Rezeptor Knockout ein Lern- und Gedächtnisdefizit beobachtet (Siuciak et al., 2007).

2 Überblick über die Methoden

In diesem Abschnitt werden die Methoden und Paradigmen kurz erläutert, die in der vorliegenden Arbeit verwendet wurden. Die detaillierte Beschreibung kann dem Methodenteil der jeweiligen Publikationen entnommen werden.

2.1. Verhaltensparadigmen

2.1.1. „Open-field" Test

Der „Open-field" Test basiert auf spontanen Verhaltensweisen und ist ein Paradigma bei dem Explorationsverhalten, lokomotorische Aktivität, emotionales Verhalten wie auch Habituationslernen von Versuchstieren untersucht werden kann. Die Apparatur, das sog. Offenfeld, ist eine nach oben hin offene Arena, die in Form (quadratisch, rund), Größe und Material variieren kann. Wird ein Versuchstier ins Offenfeld gesetzt (typischerweise ins Zentrum der Arena), so hat es die Möglichkeit dieses für eine festgelegte Zeit (5-20 min) frei zu explorieren. Die Exploration einer neuen Umgebung hat einen adaptiven Wert für den Organismus, indem es die Möglichkeit bietet neue Nahrungsressourcen, Fortpflanzungsmöglichkeiten, Schlupflöcher sowie potentielle Gefahrenquellen ausfindig zu machen. Sowohl der Aufenthalt in verschiedenen Bereichen des Offenfelds als auch die lokomotorische Aktivität dienen als ein Maß für Ängstlichkeit. Die Neuartigkeit unbekannter Umgebungen induziert einen Konflikt zwischen Annäherung und Vermeidung (Lister, 1990;Montgomery, 1955), d.h. einerseits den Drang aufgrund der Neuheit die Umgebung zu explorieren und anderseits eine potentiell gefährliche Situation zu meiden. Wird das Verhalten des Tieres im Sinne von Emotionalität analysiert, so wird angenommen, dass die offenen Bereiche im Zentrum der Arena aus Angst vermieden werden, sich das Tier länger in der Peripherie aufhält und eine geringere Aufenthaltszeit im Zentrum zeigt (Prut and

Belzung, 2003). Somit vereinigt der „Open-field“ Test eine natürliche unkonditionierte Aversion in Bezug auf offene, erleuchtete und ungeschützte Bereiche (File, 1985;Griebel et al., 1993;Treit et al., 1993a).

2.1.2. Objektexplorations-Paradigma

Das Objektexplorations-Paradigma basiert auf spontanen Explorationsverhalten von Tieren, dass durch neuartige Reize (unbekannte Objekte) induziert wird. Nagetiere zeigen für gewöhnlich eine angeborene Präferenz für neuartige gegenüber familiären Reizen, die sog. „Novelty Preference“ (Ballaz, 2009;Dellu et al., 1996). Der Vorteil gegenüber anderen Gedächtnisaufgaben besteht darin, dass die Tiere nicht erst durch Futter- oder Wasserdeprivation bzw. durch die Applikation von positiven oder negativen Verstärkern (Futtergabe bzw. elektrischer Schock) motiviert werden. Objektexploration sollte in einer bereits vertrauten Umgebung gemessen werden, um zu verhindern, dass ein Großteil der Exploration durch die Neuheit der Umgebung absorbiert wird. Dem kann durch eine vorherige, meist an drei aufeinander folgenden Tagen für jeweils 10 Minuten stattfindende, Habituation in der zu testenden Umgebung/Arena vorgebeugt werden. Bei der objektbezogenen Exploration nähern sich Ratten neuen Objekten und haben physischen Kontakt mit diesen, so dass man aus der Häufigkeit und Dauer der Kontakte auf den Neuheitswert der Objekte schließen kann. Als Objekte dienen Gegenstände, die sich in Form, Oberflächenstruktur, Größe und Farbe unterscheiden und für die Tiere keine ethologische Relevanz besitzen. Das Paradigma der Objektexploration kann je nach Länge des Retentionsintervalls sowohl zur Messung von Kurzzeitgedächtnis- als auch Langzeitgedächtnismechanismen eingesetzt werden und reagiert sensitiv auf Interventionen, die geeignet sind die Encodierung, Konsolidierung und Abruf von Gedächtnisinhalten zu modulieren.

Es existieren unterschiedliche Varianten des Objektexplorations-Paradigma (Abb. 1): Objektwiedererkennung („object recognition"), räumliches Objektgedächtnis („object-place recognition") und temporales Objektgedächtnis („object recognition for temporal order"). Objektwiedererkennung erhebt die Fähigkeit der Tiere zwischen bekannten und neuen Objekten zu unterscheiden (Ennaceur and Delacour, 1988). In einer bereits bekannten Umgebung wird ein bekanntes Objekt weniger exploriert als ein unbekanntes Objekt - dies ist ein Indiz dafür, dass die Tiere sowohl verschiedenartige Objekte diskriminieren können als auch ein Gedächtnisinhalt vorliegt, der die physikalischen Charakteristika der Objekte abbildet und bei erneuter Darbietung zu einem Abruf bzw. Wiedererkennen führt und somit erneute intensive Exploration inhibiert. Das räumliche Objektgedächtnis definiert die Fähigkeit der Tiere zwischen einem räumlich verschobenen und räumlich familiären Objekt zu unterscheiden (Ennaceur et al., 1997). Dabei explorieren die Tiere jenes Objekt vermehrt, welches gegenüber der vorher dargebotenen Position räumlich verschoben ist. Der Neuheitswert der Objekte spiegelt sich in der Veränderung der objektbezogenen räumlichen Präsentation wieder. Die zeitliche Abfolge der präsentierten Objekte spielt beim temporalen Objektgedächtnis eine entscheidende Rolle. Hierbei werden jene Objekte stärker exploriert, deren vormalige Darbietung länger zurück liegt, d.h. das ältere Objekt wird häufiger exploriert als das neuere Objekt (Mitchell and Laiacona, 1998).

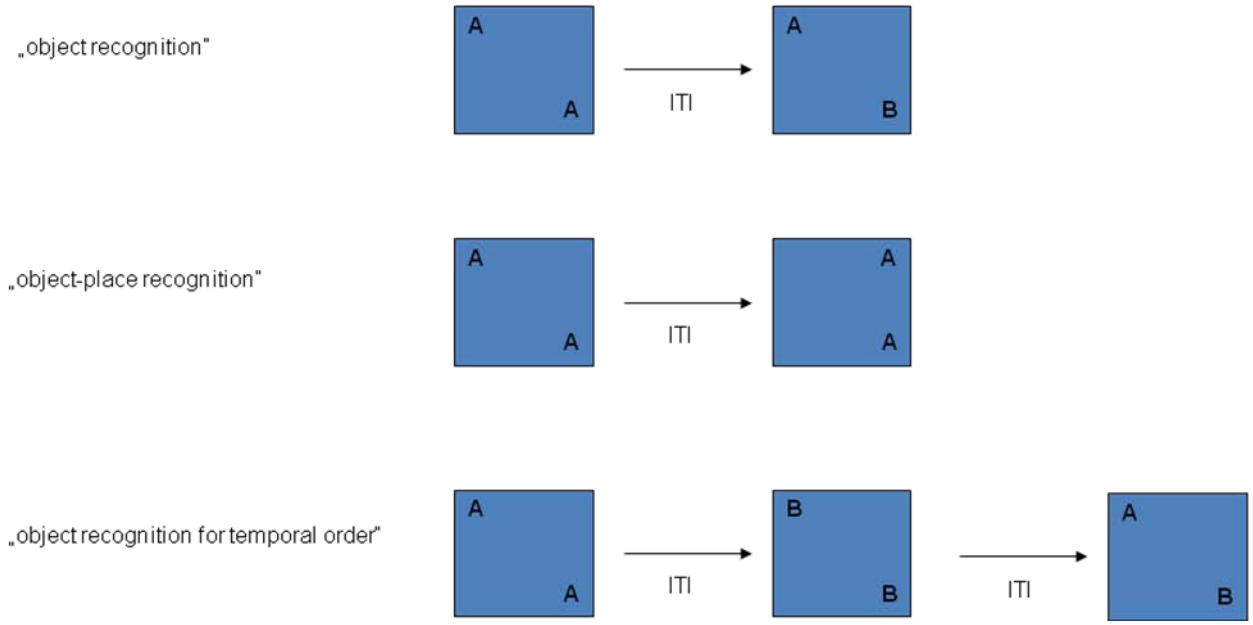

Abb. 1: Schematische Darstellung der Objektexplorations-Paradigmen Objektwiedererkennung („object recognition"), räumliches Objektgedächtnis („object-place recognition") und temporales Objektgedächtnis („object recognition for temporal order"). A = Objekt A, B = Objekt B, ITI = Intertrial-Intervall

2.1.3. „Episodic-like Memory" Paradigma

In der Vergangenheit konnte gezeigt werden, dass Tiere offenbar über ein episodisches Gedächtnis verfügen, d.h. sie sind in der Lage einen integrierten Gedächtnisinhalt für ein persönliches Erlebnis zu bilden der "Was", "Wann", und "Wo" Informationen einschließt (Clayton and Dickinson, 1998;Clayton et al., 2001;Eacott and Norman, 2004). In der Vergangenheit wurde ein „Episodic-like Memory" Paradigma (Abb. 2) für Nagetiere entwickelt, dass als Modell für human-analoge episodische Gedächtnisleistung dient (Dere et al., 2005;Kart-Teke et al., 2006). Dieses Paradigma basiert auf „Novelty Preference" (Ballaz, 2009;Dellu et al., 1996) und vereint verschiedene Objektexplorations-Paradigmen (Dere et al., 2007;Ennaceur and Delacour, 1988;Ennaceur et al., 1997;Mitchell and Laiacona, 1998).

Dabei besteht die Möglichkeit gleichzeitig objektspezifische, räumliche und zeitliche Informationen abzurufen (Dere et al., 2005). In diesem Zusammenhang wird angenommen, dass dabei eine Integration verschiedener Dimensionen während der Objektexploration stattfindet und die Tiere in der Lage sind zu erinnern, welches Objekt ("Was") sie an welcher Position ("Wo") und zu welcher Zeit ("Wann") vorgefunden haben (Dere et al., 2005;Dere and De Souza Silva, 2010;Kart-Teke et al., 2006).

Die Tiere durchlaufen 3 Durchgänge, von jeweils 5 Minuten Dauer. Zwei unterschiedliche Objekte aus verschiedenem Material in jeweils vierfacher Ausführung, die sich in ihrer Form, Oberflächentextur und Farbe unterscheiden, werden verwendet. Im 1. Durchgang werden 4 identische Objekte (Objekt A) in 8 möglichen Positionen des Offenfeldes platziert. Nach dem Inter-Trial-Intervall wird im 2. Durchgang ein anderes Objekt (Objekt B) in vierfacher Ausführung präsentiert, wobei 2 Objekte auf bisher noch nicht besetzten Positionen platziert sind. Während des Test-Durchgangs werden nun jeweils 2 identische Kopien von Objekt A und Objekt B im Offenfeld positioniert, wobei jeweils eines dieser Objekte an der Position platziert ist, an dem sich das jeweilige Objekt während des 1. bzw. 2. Durchgangs (Objekt A1 und Objekt B1) befand, und die anderen Objekte werden verglichen mit den vorherigen Durchgängen in die Position des jeweils anderen Objekts verschoben (Objekt A2 und Objekt B2). Bei funktionierender episodischer Gedächtnisleistung wird im Test-Durchgang Objekt A1 länger exploriert als Objekt B2, Objekt B2 wird länger exploriert als Objekt B1, und Objekt A1 wird länger exploriert als Objekt A2.

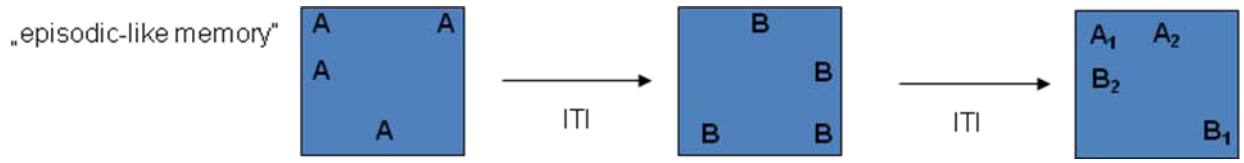

Abb. 2: Schematische Darstellung des „Episodic-like memory“ Paradigma. A = Objekt A, B = Objekt B, ITI = Intertrial-Intervall

2.1.4. „Forced Swimming Test“

Der „Forced Swimming Test“ ist ein Paradigma welches der Erfassung aber auch Induktion depressions-ähnlichen Verhaltens im Tiermodell dient (Porsolt et al., 1977;Castagne et al., 2011). Er wird meist zum Screening pharmakologischer Substanzen verwendet, um deren Wirksamkeit im Hinblick auf antidepressive Eigenschaften zu validieren. Das Prinzip des „Forced Swimming Test“ beruht darauf, dass Versuchstiere einer für sie nicht vermeidbare „aversive“ Situation ausgesetzt werden, indem sie in einem mit Wasser gefüllten Zylinder gesetzt werden. Anfänglich reagieren Tiere auf diese aversive Situation mit aktiven Fluchtverhalten wie Schwimmen und Klettern. Da es aber für die Tiere keine Fluchtmöglichkeit aus dem Zylinder gibt, ist das Tier zunehmend inaktiv und immobil. Die Tiere bewegen sich nur noch, um den Kopf über Wasser und die Balance zu halten. Wenn das Tier 24 Stunden nach der ersten Exposition erneut in den Zylinder mit Wasser gesetzt wird, fängt es früher an, passives Verhalten in Form von Immobilität zu zeigen. Dieses immobile Verhalten deutet auf eine gelernte Hilflosigkeit (Seligman and Beagley, 1975) und sog. „Behavioral Despair“ (Porsolt et al., 1977;Castagne et al., 2011) hin. Potentiell antidepressive Eigenschaften pharmakologischer Substanzen zeigen sich in einer Verminderung der Immobilität oder späteren Beginn der Immobilität und einer entsprechenden Zunahme aktiven Fluchtverhalten wie Schwimmen und Klettern.

2.2. In vivo Mikrodialyse

Die Methode der in vivo Mikrodialyse ermöglicht die Bestimmung der extrazellulären Konzentration von Acetylcholine in definierten Hirngebieten und findet am frei beweglichen oder, wie in den hier vorgestellten Experimenten, am anästhesierten Tier seine Anwendung. Das Funktionsprinzip beruht auf der Diffusion gelöster Moleküle durch eine semipermeable Membran entlang eines Konzentrationsgradienten. Zu diesem Zweck wird eine Mikrodialysesonde ins Gehirn implantiert. Die Mikrodialysesonde wird mit einer isotonen Perfusionsflüssigkeit durchspült, welche in ihrer Zusammensetzung der des extrazellulären Raumes ähnelt. Die gelösten Moleküle diffundieren kontinuierlich in die Sonde und werden im Perfusat aus der Sonde gepumpt. Die Proben (Dialysat) werden gesammelt und anschließend die ACh Konzentration ermittelt. Es wird angenommen, dass die Bestandteile des Dialysates ein zeitliches und räumliches Abbild der Neurotransmission im extrazellulären Raum liefert, und demzufolge die Konzentration der einzelnen Bestandteile die Zusammensetzung im extrazellulären Raum wiederspiegelt.

Die in vivo Mikrodialyse Prozedur der nachfolgend dargestellten Studien werden am anästhesierten Tier (Urethan-Anästhesie) durchgeführt. Dazu werden Mikrodialysesonden in die zuvor durch einen operativen Eingriff implantierten Führungskanülen im Frontalcortex, Amygdala und Hippocampus eingesetzt. Nach einer 2 stündigen Stabilisierungsphase werden die Basallevel der ACh Neurotransmission in 10 minütigen Probenintervallen für 1 Stunde ermittelt. Danach erhalten die Tiere die jeweilige pharmakologische Behandlung. Im Anschluss wird die Probenentnahme in 10 minütigen Abständen für weitere 90 Minuten fortgesetzt.

Zur Analyse der Dialysate wird die hochauflösende Hochdruckflüssigkeits-Chromatographie gekoppelt mit elektrochemischer Detektion (HPLC-EC) verwendet. Mit Hilfe einer mobilen Phase wird unter Druck das Dialysat durch eine Trennsäule gepumpt, die mit der stationären Phase (Füllmaterial) gefüllt ist. Dabei

treten die im Dialysat enthaltenen Bestandteile mit der stationären Phase in unterschiedliche Wechselwirkungen, die einzelnen Stoffe werden aufgetrennt und treten verzögert aus der Säule aus. Die Verweildauer der einzelnen Bestandteile in der Trennsäule ist methodenspezifisch abhängig von Faktoren, wie z.B. Größe der Moleküle oder elektrische Ladung. Der Trennsäule ist ein Enzymreaktor nachgeschaltet, der mit den Enzymen Acetylcholineesterase (AChE) und Cholineoxidase (ChO) beladen ist. Durch AChE wird das als erstes eluierte ACh zu Cholin und Acetat hydrolysiert und Cholin durch ChO zu Wasserstoffperoxid (H_2O_2) und Betain oxidiert. Die so getrennten Bestandteile werden durch die mobile Phase zum Detektor und der darin enthaltenen Arbeitselektrode, dem eine elektrische Spannung anliegt, transportiert. Dadurch wird H_2O_2 oxidiert, der dabei entstehende Elektronenfluss wird gegen eine Referenzelektrode gemessen, als elektrisches Signal an einen PC weitergeleitet und graphisch in Form von Peaks dargestellt. Je größer die Menge der oxidierten Substanz und der daraus resultierende Elektronenfluss ist, desto größer ist der dazugehörige Peak. Die quantitative Konzentration von ACh und Cholin im Dialysat wird mit Hilfe von Standardlösungen, die eine definierte Konzentration der einzelnen Substanzen enthalten, bestimmt.

3 Durchgeführte Studien

Die vollständigen veröffentlichten Studien und das zur Veröffentlichung eingereichte Manuskript sind im Anhang dieser Arbeit zu finden.

3.1. Der Effekt einer intra-septalen Applikation eines NK2-Rezeptor Antagonisten in einem Objektexplorations-Paradigma und der acetylcholinergen Neurotransmission in Frontalcortex, Amygdala und Hippocampus

NK_2-Rezeptoren kommen vorwiegend im Frontalcortex, Hippocampus, Thalamus und medialen Septum vor (Hagan et al., 1993;Saffroy et al., 2001). In einer früheren Studie wurde gezeigt, dass eine Applikation des endogenen NK_2-Rezeptor-Liganden NKA ins mediale Septum zu einer Erhöhung der extrazellulären ACh-Level im Hippocampus führt und dies durch Applikation eines NK_2-Rezeptor Antagonisten blockiert werden konnte (Steinberg et al., 1998). Dies deutet darauf hin, dass NK_2-Rezeptoren die Aktivität des cholinergen Systems beeinflussen, und lässt eine mögliche Rolle der NK_2-Rezeptoren bei Lern- und Gedächtnisprozessen über eine Modulation des cholinergen Systems im basalen Vorderhirn vermuten.

Im vorliegenden Experiment untersuchten wir bei männlichen Wistar Ratten den Effekt einer Applikation des peptidergen NK_2-Rezeptor Antagonisten Bz-Ala-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH_2 ins mediale Septum auf Objektgedächtnis mit Hilfe eines Paradigmas, welches räumliches Objektgedächtnis und temporales Objektgedächtnis in einer Aufgabe miteinander kombiniert. Für die lokale Applikation ins mediale Septum wurde den Tieren in einer stereotaktischen Operation eine Führungskanüle ins Gehirn implantiert. Nach einer 5 tägigen

Erholung von der Operation werden die Tiere zunächst an drei aufeinander folgenden Tagen für jeweils 10 Minuten in das Offenfeld gesetzt und dürfen es frei explorieren (Habituation). Am Experimentaltag wird dem Tier 30 Minuten vor der Objektgedächtnisaufgabe mittels Injektionsnadel je nach Gruppenzugehhörigkeit Vehikel (phosphatgepufferte Kochsalzlösung), 1, 10 oder 100 pmol des peptidergen NK_2-Rezeptor Antagonisten im Volumen von 0.5 µl über einen Zeitraum von 30 Sekunden ins mediale Septum injiziert. Die Tiere durchlaufen 3 Durchgänge, von jeweils 5 Minuten Dauer und einem Inter-Trial-Intervall von 50 Minuten. Zwei unterschiedliche Objekte aus Plastik in jeweils vierfacher Ausführung, die sich in ihrer Form, Oberflächentextur und Farbe unterscheiden, wurden verwendet. Im 1. Durchgang wurden 4 identische Objekte in 8 möglichen Positionen des Offenfeldes (randomisiert) platziert. Das Tier wurde ins Zentrum des Offenfeldes gesetzt und konnte die Objekte für 5 Minuten explorieren. Nach einem 50 minütigen Intertrial-Intervall im Heimkäfig wurde im 2. Durchgang ein anderes Objekt in vierfacher Ausführung präsentiert. Wobei zwei Positionen denen aus dem ersten Durchgang entsprachen und zwei bisher noch nicht besetzte Positionen der insgesamt acht möglichen, gewählt wurden. Das Tier konnte erneut für fünf Minuten das Offenfeld explorieren. Im Test-Durchgang wurden die Objekte so platziert, dass zwei Objekte jeweils aus dem 1. und dem 2. Durchgang dargeboten wurden. Eines der jeweiligen zwei bekannten Objekte aus den dazugehörigen Durchgängen verblieb an der bereits bekannten Position, das zweite Objekt wurde verglichen mit den vorherigen Durchgängen in dessen Position im Offenfeld verschoben. Das Tier konnte diese erneut für 5 Minuten explorieren. Hierbei zeigte sich, dass Vehikel-behandelte Tiere nicht in der Lage waren sowohl zwischen räumlich verschobenen und räumlich familiären Objekten (räumliches Objektgedächtnis) als auch zwischen länger zurückliegenden und kürzlich dargebotenen Objekten (temporales Objektgedächtnis) zu differenzieren. Applikation des peptidergen NK_2-Rezeptor Antagonisten Bz-Ala-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH_2 in der Dosierung 1 pmol resultierte in einem intakten temporales Objektgedächtnis, was sich in einer vermehrten Exploration der

beiden Objekte darstellte, deren vormalige Darbietung länger zurücklag. Allerdings wiesen diese Tiere ein Defizit im räumlichen Objektgedächtnis auf. Die Tiere, die eine intra-septale Injektion von 10 und 100 pmol des NK_2-Rezeptor Antagonisten erhielten, zeigten keine unterschiedlichen Explorationszeiten der Objekte, d.h. sie waren im räumlichen sowie temporalen Objektgedächtnis beeinträchtigt. Diese Ergebnisse deuten darauf hin, dass eine Injektion von Vehikel ins mediale Septum zu einem Defizit sowohl beim räumlichen als auch beim temporales Objektgedächtnis führt. Der Effekt des NK_2-Rezeptor Antagonisten im vorliegenden Objektgedächtnis-Paradigma kann im Sinne einer protektiven Wirkung interpretiert werden, so dass der NK_2-Rezeptor Antagonist beispielsweise einer stress- und/oder auch mechanisch-induzierten Beeinträchtigung entgegenwirkte. Die Annahme der Beeinflussung eines stressinduzierten Defizits scheint insoweit naheliegend, da frühere Befunde mit Applikation eines NK_2-Rezeptor Antagonisten auf eine Reduktion ängstlichen Verhaltens, welches mit Stress assoziiert wird, hinweisen (Borelli et al., 2010;Griebel et al., 2001;Louis et al., 2008).

Weiterhin untersuchten wir mittels in vivo Mikrodialyse den Effekt der Applikation des peptidergen NK_2-Rezeptor Antagonisten Bz-Ala-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH_2 ins mediale Septum auf extrazelluläre ACh Neurotransmission in Frontalcortex, Amygdala und Hippocampus, Strukturen die in Lern- und Gedächtnisprozesse stark involviert sind. Nach einer 5 tägigen Erholung von der Implantation der Führungskanülen wurde am Experimentaltag die in vivo Mikrodialyse durchgeführt. Die Tiere erhielten ins mediale Septum je nach Gruppenzugehörigkeit eine Sham Injektion (keine Flüssigkeit, nur Einführen der Injektionsnadel), Vehikel, 1, 10 oder 100 pmol des NK_2-Rezeptor Antagonisten. Im Vergleich der Applikation von Vehikel und NK_2-Rezeptor Antagonisten ins mediale Septum wurde eine Abnahme der ACh Neurotransmission bei den mit 10 pmol NK_2-Rezeptor Antagonist behandelten Tieren deutlich. Hierbei zeigte sich, dass die ACh Level im Hippocampus und Frontalcortex 30 Minuten und im Frontalcortex auch zusätzlich 80 Minuten nach Applikation vermindert war. In der Amygdala gab es

keinen signifikanten Unterschied in der cholinergen Neurotransmission zwischen den einzelnen Gruppen. Basierend auf Befunden die zeigten, dass NK_2-Rezeptor Agonismus im medialen Septum zu einem Anstieg von ACh Level im Hippocampus führt (Steinberg et al., 1998) entsprachen die vorliegenden Ergebnisse den Erwartungen, dass der NK_2-Rezeptor Antagonist ACh Level vermindert. Der NK_2-Rezeptor Antagonist scheint einen Anstieg von ACh Level durch Vehikel Injektion entgegenzuwirken. Dies steht im Einklang mit in der Literatur beschriebenen Blockade einer erhöhten ACh Neurotransmission mit Hilfe systemischen und intra-septalen NK_2-Rezeptor Antagonismus (Desvignes et al., 2003;Steinberg et al., 1998). Die vorliegenden Ergebnisse verdeutlichen, dass das cholinerge System des basalen Vorderhirns sensitiv gegenüber lokaler Applikation von Vehikel reagiert. Der Effekt des NK_2-Rezeptor Antagonisten kann als Blockade des durch die Vehikel Injektion verursachten Anstiegs der ACh Neurotransmission verstanden werden. Dies kann durch die Tatsache gestützt werden, dass im Gegensatz zu Vehikel die Sham Injektion keinen Anstieg der ACh Neurotransmission zur Folge hat und kein Unterschied zwischen Sham Injektion und Applikation des NK_2-Rezeptor Antagonisten besteht.

3.2. Der Effekt einer intra-septalen Applikation eines NK2-Rezeptor Antagonisten auf die Aktivierung der acetylcholinergen Neurotransmission in Frontalcortex, Amygdala und Hippocampus durch NK2-Rezeptor oder NK3-Rezeptor Agonisten

Das mediale Septum ist einer der Hirnstrukturen mit der höchsten Dichte an NK_2-Rezeptoren (Saffroy et al., 2003) und zugleich Hauptursprungsgebiet der cholinergen Neurone des basalen Vorderhirns mit Projektionen zum Frontalcortex, Amygdala und Hippocampus (Mesulam et al., 1983). Zusätzlich konnten im medialen Septum auch NK_1- (Maeno et al., 1993) und NK_3-Rezeptoren (Shughrue et al., 1996) nachgewiesen werden. Eine Rolle aller 3 NK-Rezeptoren bei der Aktivierung von cholinergen Neuronen des septo-hippocampalen Systems wurde bisher deutlich (Morozova et al., 2008).

Mittels in vivo Mikrodialyse untersuchten wir den Effekt einer intra-septalen Applikation des NK_2-Rezeptor Agonisten NKA und NK_3-Rezeptor Agonisten NKB auf ACh Neurotransmission in Frontalcortex, Amygdala und Hippocampus. Es wurde Vehikel, der NK_2-Rezeptor Agonist NKA (0.1, 1 oder 10 μM) oder der NK_3-Rezeptor Agonist NKB (0.1, 1 oder 10 μM) ins mediale Septum appliziert. Applikation des NK_2-Rezeptor Agonisten NKA ins mediale Septum erhöhte in der Dosierung 1 μM innerhalb der ersten 10 Minuten und 10 μM stufenweise bis zum Ende der Probennahme die ACh Neurotransmission im Hippocampus. In der Amygdala zeigte sich ebenfalls eine graduelle Zunahme der ACh Neurotransmission in der höchsten Dosierung (10 μM) von NKA, wohingegen im Frontalcortex keine Veränderung der ACh Level zu beobachten war. Die vorliegenden Ergebnisse stehen im Einklang mit früheren Befunden von Steinberg und Kollegen (1998), wo bereits ein Anstieg der ACh Level im Hippocampus nach Applikation von NKA ins mediale Septum beschrieben wurde. Ein Effekt des NK_2-Rezeptor Agonisten auf die ACh Neurotransmission in der Amygdala wurde zuvor noch nicht beobachtet. Die Frage

die sich hierbei stellt, sind direkte oder indirekte Projektionen vom medialen Septum zur Amygdala für die Veränderung der ACh Level verantwortlich. Die aktuellen Ergebnisse stützen die Hypothese, dass cholinerge Neurotransmission im Hippocampus funktionell die Amygdala beeinflusst (Calandreau et al., 2006) und eine solche Interaktion über eine Aktivierung der NK_2-Rezeptoren im medialen Septum reguliert werden könnte. Die intra-septale Applikation des NK_3-Rezeptor Agonisten NKB resultierte in erhöhter ACh Neurotransmission im Hippocampus, wobei sich der stärkste Effekt bei den mit 1 μM NKB behandelten Tieren zeigte. Die Applikation von NKB hatte keinen signifikanten Effekt auf die ACh Neurotransmission in Frontalcortex und Amygdala. Diese Ergebnisse sind konform mit zuvor publizierten, die einen Anstieg der ACh Level im Hippocampus nach intra-septaler Injektion des NK_3-Rezeptor Agonisten Senktide fanden (Marco et al., 1998). Da 66 % der cholinergen Neurone im medialen Septum NK_3-Rezeptoren exprimieren (Chen et al., 2001a), kann ein enger Zusammenhang zwischen NKB und cholinergen Neuronen vermutet werden. Desweiteren gehen Morozova und Kollegen (2008) beim NK_3-Rezeptor Agonismus von einer Aktivierung cholinerger Neurone innerhalb des septo-hippocampalen Systems aus. Zusätzlich wurde bereits auch eine Aktivierung cholinerger Neurone im Striatum durch Applikation des NK_3-Rezeptor Agonisten Senktide berichtet (Steinberg et al., 1995).

Ausgehend von den oben beschrieben Ergebnissen untersuchten wir den Effekt einer intra-septalen Vorbehandlung mit dem nicht-peptidergen NK_2-Rezeptor Antagonisten SR48968 auf die erhöhte ACh Neurotransmission in Amygdala und Hippocampus. Während der in vivo Mikrodialyse wurde zunächst je nach Gruppenzugehörigkeit Vehikel oder SR48968 in den Dosierungen 1, 10 oder 100 pmol appliziert und im Anschluss der NK_2-Rezeptor Agonist NKA (1 μM) oder der NK_3-Rezeptor Agonist NKB (10 μM) ins mediale Septum injiziert. Die Vorbehandlung mit dem NK_2-Rezeptor Antagonisten SR48968 reduzierte dosisabhängig die durch 1 μM NKA induzierte ACh Neurotransmission in Amygdala und Hippocampus, wobei die Dosen von 1 pmol und 100 pmol SR48968

am effektivsten waren. Basierend auf den Befunden von Steinberg und Kollegen (1998), die eine Blockade cholinerger Aktivität nach Injektion des NK_2-Rezeptor Agonist NKA ins mediale Septum aufgrund systemischer Applikation des NK_2-Rezeptor Antagonisten SR48968 beobachteten, stellte sich die Frage von welcher Hirnstruktur aus die NKA-induzierte ACh Neurotransmission im Hippocampus mediiert wird. Die aktuellen Daten bestätigten die Hypothese, dass eine intra-septale Applikation von SR48968 in der Lage ist, eine durch NKA-induzierte cholinerge Aktivität im Hippocampus zu inhibieren. Dies liegt nahe, wenn man die Anwesenheit der NK_2-Rezeptoren im medialen Septum und Hippocampus (Saffroy et al., 2001;2003) berücksichtigt, die ihrerseits vermutlich den Effekt systemischer wie auch lokaler Applikation des NK_2-Rezeptor Antagonisten modulieren. Allerdings scheint der beschriebene Effekt eher durch die NK_2-Rezeptoren aus dem medialen Septum, statt über im Hippocampus vorkommende NK_2-Rezeptoren, vermittelt zu werden. Bislang wurde mehrfach ein inhibitorischer Effekt von NK_2-Rezeptor Antagonisten auf cholinerge Neurotransmission beschriebenen (Desvignes et al., 2003;Steinberg et al., 1995;1998). Beispielsweise konnte mit Hilfe des NK_2-Rezeptor Antagonisten SR48968 ein durch taktile Stimulation (Steinberg et al., 1998) oder Verabreichung des Corticotrophin-Releasing-Faktors (CRF) induzierter Anstieg der ACh Neurotransmission im Hippocampus (Steinberg et al., 1998) blockiert bzw. reduziert werden. Zudem wurde in einer elektrophysiologischen Studie eine Inhibition einer durch NK_2-Rezeptor Agonismus stimulierter cholinerger Aktivität mit Hilfe eines NK_2-Rezeptor Antagonisten nachgewiesen (Morozova et al., 2008).

Eine Vorbehandlung mit dem NK_2-Rezeptor Antagonisten SR48968 verringerte dosisabhängig die durch 10 μM NKB induzierte ACh Neurotransmission in Amygdala und Hippocampus. Dabei zeigten sich die Dosierungen von 10 pmol und 100 pmol SR48968 am effektivsten. Diese Befunde sind in der Hinsicht erstaunlich, da in der Vergangenheit gefunden wurde, dass zum einen eine Injektion des NK_3-Rezeptor Agonisten Senktide ins mediale Septum die Freisetzung von ACh im Hippocampus stimuliert und die systemische Verabreichung des NK_2-Rezeptor

Antagonisten SR144190 dies nicht blockierte (Steinberg et al., 1998) und zum anderen der NK_2-Rezeptor Antagonist SR48968 die durch Senktide induzierte ACh Neurotransmission im Striatum nicht verminderte (Steinberg et al., 1995). Die konträren Ergebnisse im Rahmen der hier durchgeführten Studie lassen vermuten, dass intra-septale NK_2-Rezeptoren auch die NKB-induzierte ACh Freisetzung vom medialen Septum aus modulieren. Hierdurch besteht die Möglichkeit einer Aktivierung cholinerger Neurone des septo-hippocampalen Systems anhand von NKB über die Vermittlung sowohl des NK_2- als auch NK_3-Rezeptors. In der Literatur finden sich auch bereits Hinweise auf eine Interaktion des NK_2-Rezeptor Antagonist SR48968 mit NK_3-Rezeptoren (Petitet et al., 1993;Suman-Chauhan et al., 1994) genauso wie eine bestehende Affinität von NK_3-Rezeptor Agonisten zum NK_2-Rezeptor bereits beschrieben wurde (Almeida et al., 2004;Regoli et al., 1989). Zudem bestärken die vorliegenden Ergebnisse die Annahme einer Kreuzreaktivität zwischen den endogenen NK-Agonisten und deren Rezeptoren (Almeida et al., 2004;Liu et al., 1994). Abschließend sollte festgehalten werden, dass der NK_2-Rezeptor Antagonist SR48968 durch seine Wechselwirkung sowohl mit dem NK_2- als auch NK_3-Rezeptor keinen differenzierbaren Effekt auf zuvor induzierte ACh Freisetzung innerhalb des septo-hippocampalen cholinergen Systems ausübt.

3.3. Der Effekt einer systemischen Applikation des NK_3-Rezeptor Agonisten Senktide auf emotionales Verhalten, Lernen und Gedächtnis sowie acetylcholinerge Neurotransmission in Frontalcortex, Amygdala und Hippocampus bei alten Tieren

NK_3-Rezeptoren konnten in Hirnarealen identifiziert werden, die sowohl an emotionalen und an Lern- und Gedächtnisprozessen beteiligt sind: wie Frontalcortex, Amygdala, Hypothalamus und Hippocampus (Ding et al., 1996;Duarte et al., 2006;Shughrue et al., 1996;Mileusnic et al., 1999). Senktide besitzt eine hohe Affinität zum NK_3-Rezeptor verschiedener Spezies wie Ratten, Mäuse, Mongolische Wüstenrennmäuse (Gerbils) und Meerschweinchen (Massi et al., 2000) und agiert als potenter Agonist am NK_3-Rezeptor. In wenigen Studien konnte ein Einfluss des NK_3-Rezeptor Agonist Senktide auf emotionales Verhalten (Ribeiro and De Lima, 2002) sowie Lern- und Gedächtnisprozesse (Kameyama et al., 1998;Ukai et al., 1996;Zlomuzica et al., 2008) belegt werden. So berichteten zum einen Ribeiro und De Lima (2002) eine durch Senktide-induzierte anxiolytische Wirkung, zum anderen wurden promnestische Eigenschaften von Senktide bei einer „Spontaneous Alternation“ (Kameyama et al., 1998;Ukai et al., 1998) und „Episodic-like Memory“ (Zlomuzica et al., 2008) Aufgabe bei Mäusen beobachtet. Außerdem weisen frühere Resultate darauf hin, dass Senktide Einfluss auf altersbedingte Defizite nehmen könnte (Wenk et al., 1997;Yankner et al., 1990).

Der „Open-field“ und „Forced Swimming” Test wurde verwendet, um den Effekt von systemischer Applikation des NK_3-Rezeptor Agonisten Senktide auf emotionales Verhalten in alten Wistar Ratten zu evaluieren. Den Tieren wurde 30 Minuten vor der Verhaltenstestung subcutan (s.c.) Vehikel, 0.2 mg/kg oder 0.4 mg/kg Senktide appliziert. Im „Open-field“ Test hatten die Tiere die Möglichkeit das Offenfeld für 15 Minuten frei zu explorieren. Als Maß für Ängstlichkeit wurde hierbei die Aufenthaltszeit im Zentrum (Bereich 30x30 cm) des Offenfelds erhoben. Um

potentiell antidepressive Eigenschaften von Senktide zu testen, wurde der „Forced Swimming" Test durchgeführt. Hierzu wurden bei einer ersten Exposition die Tiere 15 Minuten der „Forced Swimming" Apparatur ausgesetzt, ohne dass die Tiere eine pharmakologische Behandlung erhielten. Am nachfolgenden Testtag wurde den Tieren 30 Minuten vor der erneuten Verhaltenstestung s.c. Vehikel, 0.2 mg/kg oder 0.4 mg/kg Senktide appliziert und für 5 Minuten in den wassergefüllten Zylinder eingesetzt. Die Dauer von Immobilität und aktiven Fluchtverhalten (Schwimmen, Klettern) wurde in beiden Versuchsdurchgängen erhoben. Im „Open-field" Test zeigte sich ein anxiolytischer Effekt durch Senktide in den Dosierungen 0.2 mg/kg und 0.4 mg/kg im Vergleich zu den Vehikel-behandelten Tieren. So erhöhte Senktide die Aufenthaltszeit im Zentrum des Offenfelds, was auf verminderte Ängstlichkeit der Tiere hindeutet. Im „Forced Swimming" Test bewirkte die 0.2 mg/kg Senktide Dosierung eine Verminderung der Dauer von Immobilität im Vergleich zu den Vehikel-behandelten Tieren. Demzufolge konnten antidepressive Eigenschaften von Senktide nachgewiesen werden. Bezüglich aktiven Fluchtverhaltens, also der Dauer von Schwimmen und Klettern, hatte die Behandlung mit Senktide keinen signifikanten Einfluss. Die Ergebnisse aus dem „Open-field" Test stehen im Einklang mit einer früheren Studie an Mäusen bei der Senktide im „Elevated Plus Maze" Paradigma einen anxiolytischen Effekt hatte und dieser durch NK_3-Rezeptor Antagonismus aufgehoben werden konnte (Ribeiro et al., 1999). Indessen beschrieben Salome und Kollegen (2006) anxiolytische Eigenschaften nach Blockade des NK_3-Rezeptors in Gerbils. Die dosisabhängigen antidepressiven Eigenschaften von Senktide im „Forced Swimming" Test bekräftigen die Vermutung, dass eine Aktivierung der NK_3-Rezeptoren diese Eigenschaften modulieren oder beteiligen. Eine Vermittlung der antidepressiven Eigenschaften ist über eine Interaktion der NK_3-Rezeptoren mit verschiedenen Neurotransmittersystemen u.a. 5-HT, NA oder DA möglich. Diese Neurotransmitter wurden bereits mit veränderten neurophysiologischen Mechanismen und Dysbalance bei Depression in Verbindung gebracht (Moret and Briley, 2011;D'Aquila et al., 2000). Intra-cerebrale Applikation

von Senktide löste vergleichbare Verhaltensweisen in Mäusen (Stoessl et al., 1987) und Ratten (Stoessl et al., 1990) aus, die bekanntermaßen nach Stimulation des serotonergen Systems beobachtet werden, und wiederum durch 5-HT Rezeptor Antagonisten inhibiert wurden (Stoessl et al., 1987;Stoessl et al., 1990). Zudem gibt es Evidenz eines Zusammenhangs zwischen NK_3-Rezeptor Aktivierung und dem noradrenergen System (Bert et al., 2002;Jung et al., 1996). Lokale Injektion des NK_3-Rezeptor Agonisten Senktide erhöhte die NA Neurotransmission im Präfrontalcortex von Meerschweinchen, wobei dieser Effekt mit Hilfe eines NK_3-Rezeptor Antagonisten blockiert wurde (Jung et al., 1996). Daneben erhöhte Senktide die Feuerrate noradrenerger Neurone (Jung et al., 1996). Außerdem wurde in einigen Studien beobachtet, dass die Aktivierung von NK_3-Rezeptoren zum einen die Ausschüttung von Dopamin im Nucleus accumbens und Putamen (Marco et al., 1998), und weiterhin die Feuerrate dopaminerger Neurone verstärkt (Nalivaiko et al., 1997).

Weiterhin untersuchten wir den Einfluss Senktide Applikation (s.c.) auf die Gedächtnisleistung im „Episodic-like Memory“ Paradigma alter Wistar Ratten. Vor Beginn der eigentlichen Verhaltenstestung wurden die alten Tiere an drei aufeinander folgenden Tagen für jeweils 10 Minuten an das Offenfeld habituiert. Am Experimentaltag wurde dem Tier 30 Minuten vor Beginn der „Episodic-like Memory“ Aufgabe je nach Gruppenzugehörigkeit s.c. Vehikel, 0.2 mg/kg oder 0.4 mg/kg Senktide appliziert. Die Tiere durchliefen 3 Durchgänge, von jeweils 5 Minuten Dauer und einem Intertrial-Intervall von 25 Minuten. Hierbei ergab sich, dass sowohl Vehikel- als auch Senktide-behandelte Tiere eine Beeinträchtigung des episodischen Gedächtnisses aufwiesen. Intakte episodische Gedächtnisleistung beinhaltet die Einzelkomponenten räumliches und temporales Objektgedächtnis sowie der Interaktion räumlicher und temporaler Objektinformationen zu einem spezifischen Muster. Betrachtet man einzelne Komponenten des „Episodic-like Memory“ Paradigma, also räumliches oder temporales Objektgedächtnis, führte die systemische Applikation von 0.2 mg/kg Senktide bei den alten Tieren zur Darbietung

intakten räumlichen Objektgedächtnisses. Die vorliegenden Ergebnisse beschreiben erstmals ein Defizit im „Episodic-like Memory“ Paradigma bei alten Tieren. In der Vergangenheit wurde bereits mit Hilfe des „Episodic-like Memory“ Paradigma episodische Gedächtnisleistung bei adulten Ratten nachgewiesen (Kart-Teke et al., 2006). Die Behandlung der hier im „Episodic-like Memory“ Paradigma beeinträchtigten alten Tiere mit Senktide führte zu keiner episodischen Gedächtnisleistung in dieser Aufgabe, hingegen konnte durch 0.2 mg/kg Senktide eine gedächtnisfördernde Performance bei der räumlichen Objektgedächtniskomponente, d.h. „was“ und „wo“ erreicht werden. In einer Studie mit adulten Mäusen, die ein Defizit im „Episodic-like Memory“ Paradigma aufwiesen, war ebenfalls 0.2 mg/kg Senktide die Dosierung bei der die Tiere in der Lage waren sich an einzelne Komponenten der „Episodic-like Memory“ Aufgabe zu erinnern (Zlomuzica et al., 2008).

Mittels der in vivo Mikrodialyse untersuchten wir zudem den Effekt einer s.c. Applikation von Senktide auf die ACh Neurotransmission in Frontalcortex, Amygdala und Hippocampus. Die pharmakologische Behandlung durch s.c Applikation von Vehikel, 0.2 mg/kg oder 0.4 mg/kg Senktide erfolgte während in vivo Mikrodialyse. Die in vivo Mikrodialyse Daten ergaben einen dosisabhängigen graduellen Anstieg der ACh Neurotransmission in Frontalcortex, Amygdala und Hippocampus nach Senktide Applikation. Dabei zeigte sich, dass die Dosierung 0.2 mg/kg Senktide in Frontalcortex wie Amygdala und im Hippocampus 0.4 mg/kg Senktide am stärksten die ACh Level im Vergleich zu den Vehikel Kontrolltieren erhöhte. Aufgrund neuroanatomischer (Mesulam et al., 1983) und immunhistochemischer (Chen et al., 2001a) Befunde wird angenommen, dass der Effekt von Senktide auf cholinerge Aktivität in Frontalcortex, Amygdala und Hippocampus über eine direkte Modulation cholinerger Neurone mediiert wird. Bestätigung dieser Annahme findet sich in der Literatur, wo zuvor ein Anstieg der ACh Neurotransmission im Hippocampus infolge einer Injektion von NK_3-Rezeptor

Agonisten ins mediale Septum beschrieben wurde (Marco et al., 1998;Steinberg et al., 1998).

3.4. Der Effekt einer systemischen Applikation des NK_3-Rezeptor Agonisten Senktide auf ein Scopolamin-induziertes Defizit bei verschiedenen Objektgedächtnis-Paradigmen

Basierend auf den oben beschriebenen Resultaten, bei denen der NK_3-Rezeptor Agonist Senktide promnestische Eigenschaften aufwies und zudem zu einem Anstieg der ACh Freisetzung in Frontalcortex, Amygdala sowie Hippocampus führte, kann NK_3-Rezeptoren eine mögliche Rolle bei der Modulation von Lern- und Gedächtnisprozessen durch Aktivierung des cholinergen Systems zugeschrieben werden. Diese Annahme wird zunächst gestützt durch den immunhistochemischen Nachweis von NK_3-Rezeptoren, die von cholinergen Neuronen exprimiert wurden (Chen et al., 2001a) und desweiteren durch Aktivierung cholinerger Neurone innerhalb des septo-hippocampalen Systems aufgrund NK_3-Rezeptor Agonismus (Morozova et al., 2008). Weiterhin ist hinreichend bekannt, dass einerseits eine Verminderung der cholinergen Aktivität im basalen Vorderhirn in Lern- und Gedächtnisdefiziten resultiert (Chang and Gold, 2004;LeBlanc et al., 1999;Lehmann et al., 2000;Walsh et al., 1996) und andererseits cholinerge Agonisten in einer Vielzahl von Lern- und Gedächtnisparadigmen promnestisch wirken (Gold, 2003a;Masuoka and Kamei, 2009;Puma et al., 1999). Durch Blockade des cholinergen Systems mit Hilfe des muscarinergen ACh-Rezeptor Antagonisten Scopolamin können kognitive Defizite im Tiermodell induziert werden (Klinkenberg and Blokland, 2010). In verschiedenen Lern- und Gedächtnisparadigmen, wie „Inhibitory Avoidance“ (Botton et al., 2010), „Radial Maze“ (Ennaceur and Meliani, 1992), „Water Maze“ (Hodges, Jr. et al., 2009) und Objektgedächtnisaufgaben (Dodart et al., 1997;Ennaceur

and Meliani, 1992;Sambeth et al., 2007), wurden durch Scopolamin vormals Beeinträchtigungen herbeigeführt.

Wir untersuchten den Einfluss einer s.c. Applikation des NK_3-Rezeptor Agonisten Senktide auf ein durch Scopolamin-induziertes Defizit bei Objektwiedererkennung, räumliches sowie temporales Objektgedächtnis adulter Wistar Ratten. Die Tiere wurden vor Beginn der eigentlichen Verhaltenstestung für 10 Minuten an das Offenfeld habituiert. Am jeweiligen Experimentaltag wurde dem Tier je nach Gruppenzugehörigkeit sowohl 1 Stunde vor Beginn der Aufgabe Vehikel oder 0.75 mg/kg Scopolamin (s.c.) als auch 30 Minuten vorher Vehikel, 0.2 mg/kg oder 0.4 mg/kg Senktide (s.c.) appliziert. Alle Tiere durchliefen mit einem 7 tägigen Abstand voneinander die einzelnen Objektexplorations-Paradigmen. Das Objektwiedererkennungs- und räumliches Objektgedächtnis-Paradigma beinhaltete jeweils 2 Durchgänge, das temporale Objektgedächtnis hingegen 3 Durchgänge von je 5 Minuten Dauer und einem Intertrial-Intervall von 25 Minuten. Zwei unterschiedliche Objekte, die sich in ihrer Höhe, Form, Oberflächentextur und Farbe unterscheiden, wurden verwendet. Dabei zeigte sich, dass 0.75 mg/kg Scopolamin Objektwiedererkennung beeinträchtigt, indem diese Tiere nicht zwischen neuen und bekannten Objekten unterscheiden konnten. Die Behandlung mit 0.2 mg/kg Senktide verhinderte das durch Scopolamin induzierte Defizit bei der Objektwiedererkennung, so dass das neue Objekt signifikant länger als das bekannte Objekt exploriert wurde. Weiterhin behinderte Scopolamin die Performance im räumlichen Objektgedächtnis durch ein Defizit bei der Unterscheidung zwischen räumlich verschobenen und räumlich familiären Objekten, während 0.2 mg/kg wie auch 0.4 mg/kg Senktide diese Beeinträchtigung inhibierte. Beim temporalen Objektgedächtnis induzierte Scopolamin ebenso ein Defizit, wodurch die Tiere nicht fähig waren zwischen Objekten, deren vormalige Darbietung länger zurück lag oder neueren Objekten zu diskriminieren. Die Applikation von 0.4 mg/kg Senktide blockierte die Beeinträchtigung des temporalen Objektgedächtnisses, so dass diese Tiere das länger zurückliegende Objekt vermehrt explorierten als das neuere Objekt.

Vehikel- oder Senktide-behandelte Tiere ohne Vorbehandlung mit Scopolamin zeigten eine intakte Gedächtnisleistung bei den verwendeten Objektexplorations-Paradigmen. Diese Daten stehen im Einklang mit vorherigen Studien an Mäusen, bei denen eine intra-cerebrale Injektion von Senktide eine Scopolamin-induzierte Beeinträchtigung in der „Spatial Alternation" Aufgabe verhinderte (Kameyama et al., 1998;Ukai et al., 1996). Eine Beeinträchtigung bei der Performance der hier verwendeten Objektexplorations-Paradigmen nach Scopolamin Verabreichung bekräftigt bisherige Studien, bei denen ein Defizit in Objektwiedererkennung (Bartolini et al., 1996;Botton et al., 2010;Ennaceur and Meliani, 1992), räumlichen (Barker and Warburton, 2009;Pitsikas, 2007) sowie temporalen Objektgedächtnis (Barker and Warburton, 2011a) beobachteten. In der Literatur finden sich Hinweise auf eine unterschiedliche Beteiligung von Perirhinalen Cortex, medialer Präfrontalcortex und Hippocampus bei Objektexplorations-Paradigmen. Der Perirhinale Cortex scheint bei der Verarbeitung objektspezifischer Informationen (Aggleton et al., 1997;Barker and Warburton, 2011b), der mediale Präfrontalcortex bei der Ausbildung einer Assoziation zwischen objektspezifischen und räumlichen Informationen (Barker et al., 2007) und der Hippocampus bei der Verarbeitung räumlicher Informationen (Barker and Warburton, 2011b) involviert zu sein. Davon ausgehend liegt die Vermutung nahe, dass die promnestischen Eigenschaften des NK_3-Rezeptor Agonisten Senktide in den Paradigmen zur Objektwiedererkennung, räumliches sowie temporales Objektgedächtnis anhand NK_3-Rezeptor Aktivierung in den eben erwähnten Hirnregionen moduliert wurde. In der aktuellen Studie konnte durch 0.2 mg/kg Senktide das Objektwiedererkennungsdefizit möglicherweise über die Vermittlung vom medialen Präfrontalcortex und perirhinalen Cortex aufgehoben werden. Im Gegensatz dazu war die Dosierung 0.4 mg/kg effektiv in der Wiederherstellung des räumlichen wie temporalen Objektgedächtnisses nach Scopolamin-induzierter Beeinträchtigung, was über den Hippocampus mediiert wurden sein könnte.

4 Zusammenfassende Diskussion

Bisherige Forschungsergebnisse weisen Neurokininen eine beachtliche Rolle bei der Modulation von Verhalten zu. Allerdings ist bisher nicht ausreichend geklärt, über welche spezifischen NK-Rezeptorsubtypen und neurochemischen Mechanismen die Vermittlung von Lern- und Gedächtnisprozessen mediiert wird. Ein neurochemischer Erklärungsansatz für einen verhaltensrelevanten Effekt der Neurokinine besteht über eine Modulation des cholinergen Systems. Es besteht Anlass zur Vermutung eines Zusammenhangs zwischen dem cholinergen System im basalen Vorderhirn und Lern- und Gedächtnisleistungen (Gold, 2003a). Hierbei wurden u.a. Lern- und Gedächtnisbeeinträchtigungen nach verminderter hippocampaler ACh Neurotransmission deutlich (Chang and Gold, 2004;Lehmann et al., 2000). Cholinerge Neurone innerhalb des septo-hippocampalen Systems konnten mit Hilfe von NK-Rezeptor Agonisten stimuliert werden (Morozova et al., 2008). Die vorliegenden Studien sollten zu einem besseren Verständnis zur Rolle von NK_2- und NK_3-Rezeptoren bei Lern- und Gedächtnisprozessen und den Einfluss dieser Rezeptoren auf die Aktivität des cholinergen Systems im basalen Vorderhirn bei Wistar Ratten beitragen.

Aus der ersten Studie ging hervor, dass eine intra-septale Injektion von Vehikel zu Defiziten beim Objektexplorations-Paradigma führte und der NK_2-Rezeptor Antagonist Bz-Ala-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH_2 dies teilweise kompensierte, indem zumindest temporales Objektgedächtnis wiederhergestellt werden konnte. Zudem konnte gezeigt werden, dass der NK_2-Rezeptor Antagonist die Freisetzung von ACh im Frontalcortex und Hippocampus verminderte. Die Teilkompensation des Objektgedächtnisdefizites durch Applikation des NK_2-Rezeptor Antagonisten kann nicht ausschließlich als promnestischer Effekt angesehen werden, da vehikel-behandelte Tiere im Objektexplorations-Paradigma beeinträchtigt waren. Hierbei könnten ebenso die Modulation emotionaler Verhaltensweisen eine Rolle gespielt

haben. Eine mögliche Erklärung in diesem Zusammenhang könnte sein, dass die Injektionsprozedur selbst eine stressvolle Situation für die Tiere darstellt und das Defizit beim Objektgedächtnis-Paradigma daraus resultiert. Die Möglichkeit eines stress-induzierten Defizits wird durch frühere Befunde gestützt, bei denen ebenfalls Stress als mögliche Ursache für defizitäre Performance in Objektgedächtnis-Paradigmen sowohl nach systemischer (Kart-Teke et al., 2007;Zlomuzica et al., 2008) als auch nach intracerebraler Applikation (Winters and Bussey, 2005) genannt wurde. Beeinträchtigungen nach Vehikel Applikation wurden auch bereits für andere Lern- und Gedächtnisparadigmen wie das sog. „Delayed Non Matching To Sample" im Radial Arm Maze (Chrobak and Napier, 2002) oder „Inhibitory Avoidance" (Elrod and Buccafusco, 1988;Nagel and Huston, 1988) beschrieben. Die Teilkompensation des Defizites beim Objektgedächtnis-Paradigma mit Hilfe des NK_2-Rezeptor Antagonisten lässt vermuten, dass NK_2-Rezeptoren im Hinblick auf eine stress- induzierte Beeinträchtigung involviert sein könnten. Frühere Befunde bei denen bereits nach Applikation von NK_2-Rezeptor Antagonisten ängstliche oder stressrelevante Verhaltensweisen reduziert werden konnten (Borelli et al., 2010;Dableh et al., 2005;Griebel et al., 2001;Louis et al., 2008;Rogacki et al., 2011;Stratton et al., 1993;Teixeira et al., 1996;Walsh et al., 1995) unterstreichen solch eine Interpretation. Ebenso unterstützt diese Hypothese das Vorhandensein von NK_2-Rezeptoren im Cortex, Amygdala, Hippocampus, Thalamus und dorsale Raphekerne (Saffroy et al., 2001;2003), Hirnregionen die sowohl mit Lernen und Gedächtnis als auch emotionalen Prozessen assoziiert werden.

Es kann ebenfalls in Betracht gezogen werden, dass mechanische Stimulation, hervorgerufen durch die intra-septale Injektion von Vehikel, zu physiologischen Veränderungen im Gehirn führte und dies wiederum in einer Aktivierung oder Inhibition neuronaler Aktivität resultierte. Die sog. „Spreading Depression" (Leao, 1986) könnte ein Erklärungsansatz hierzu sein. Bei diesem Phänomen kommt es zu einer eigenständig fortschreitenden Welle elektrophysiologsicher Hyperaktivität durch neuronale Depolarisation, die von einer Welle der Inhibition gefolgt ist.

Beeinträchtigungen des räumlichen und temporalen Objektgedächtnisses könnten somit auch eine Folge mechanisch-induzierter Depolarisation sein. Frühere Studien beschrieben bereits einen Zusammenhang zwischen einem Verhaltenseffekt und dem Auftreten von „Spreading Depression" (Bures and Lansky, 2004;Oitzl and Huston, 1984;Siegfried et al., 1975). Ebenso ist es denkbar, dass das cholinerge System sensitiv gegenüber lokaler Vehikel-Applikation reagiert, wie es bereits durch verschieden Autoren postuliert wurde, nachdem Vehikel Injektionen zu einer erhöhten ACh Freisetzung führte (Boix et al., 1994;De Souza Silva et al., 2000). Die Vehikel Injektion scheint dabei ursächlich für erhöhte neuronale Aktivität und einem damit einhergehenden Anstieg cholinerger Neurotransmission verantwortlich zu sein. Der NK_2-Rezeptor Antagonist inhibiert folglich die physiologischen Auswirkungen der postulierten mechanischen Stimulation des septo-hippocampalen cholinergen Systems.

Auch wenn in der Literatur Hinweise bestehen, dass ein Anstieg der ACh Freisetzung durch sensorische Stimulation oder Injektion des Corticotrophin-Releasing-Faktors (CRF), ein Hormon was eine primäre Rolle bei Stressreaktionen einnimmt, eine stress-induzierte Aktivierung des septo-hippocampalen cholinergen Systems zugrundeliegt (Desvignes et al., 2003;Steinberg et al., 1998), scheint es anhand der vorliegenden neurochemischen Ergebnissen wahrscheinlicher, dass der Effekt des NK_2-Rezeptor Antagonisten eher mechanisch- als stress-induziert begründet liegt. Die Messung der ACh Freisetzung mit Hilfe der in vivo Mikrodialyse wurde im anästhesierten Tier durchgeführt und Stress als Einflussfaktor sowie dessen Minimierung dabei, scheint dabei vielmehr eine untergeordnete Rolle zu spielen.

Die Ergebnisse der zweiten Studie stehen im Einklang mit Befunden aus der Literatur, bei denen ebenfalls nach NK_2-Rezeptor Aktivierung durch NKA im medialen Septum (Steinberg et al., 1998) ein Anstieg der ACh Freisetzung im Hippocampus beobachtet wurde. Ergänzend dazu konnte in der vorliegenden Studie gezeigt werden, dass die intra-septale Applikation von NKA auch in der Amygdala zu erhöhter ACh Freisetzung führte. Die Injektion des NK_2-Rezeptor Antagonisten

SR48968 ins mediale Septum und die damit einhergehende Abnahme der ACh Neurotransmission im Hippocampus und Amygdala bestätigte gleichermaßen die in früheren Studien erzielten Befunde bei denen durch Blockade des NK_2-Rezeptors die cholinerge Aktivität verringert werden konnte (Desvignes et al., 2003;Steinberg et al., 1995;1998). Der NK_2-Rezeptor Antagonist SR48968 scheint in der Lage, einer Stimulation des septo-hippocampalen cholinergen Systems entgegenzuwirken. Vermutlich interagiert die cholinerge Neurotransmission im Hippocampus funktionell mit die Amygdala (Calandreau et al., 2006) und dies scheint über eine NK_2-Rezeptoraktivierung im medialen Septum reguliert zu werden. Die vorliegenden Resultate deuten demnach darauf hin, dass NK_2-Rezeptoren im medialen Septum eine wichtige Rolle bei der Regulation der ACh Neurotransmission im Hippocampus aber auch Amygdala einnehmen. Dabei besteht die Möglichkeit, dass cholinerge Projektionen vom medialen Septum ausgehend durch NK_2-Rezeptoren moduliert werden und die Annahme einer direkten Involvierung der NK_2-Rezeptoren bei Lern- und Gedächtnisprozessen über septo-hippocampale aber auch septo-amygdalare Projektionen bekräftigen.

Obwohl sich einerseits vermehrt angstkorrelierte Verhaltensweisen in verschiedenen Testparadigmen bei alten Ratten (Boguszewski and Zagrodzka, 2002;Darwish et al., 2001;Frussa-Filho et al., 1992) und Mäusen (Narita et al., 2006) zeigten, und es andererseits scheint, dass während des Alterns eine höhere Sensitivität gegenüber Stress besteht (Mabry et al., 1996), fokussierte sich die Forschung in Bezug auf eine Beteiligung von NK-Rezeptoren an emotionalem Verhalten im Vorfeld auf adulte Tiere. Die Ergebnisse der dritten Studie bestätigen Anhaltspunkte zu einer Beteiligung von NK_3-Rezeptoren an emotionalen Verhaltensweisen. So wurde in der Vergangenheit gefunden, dass eine NK_3-Rezeptor Aktivierung und damit einhergehende anxiolytische Wirkung mit Hilfe von NK_3-Rezeptor Antagonisten blockiert wurde, wodurch angenommen werden kann, dass NK_3-Rezeptoren Mechanismen der Angst modulieren (Ribeiro et al., 1999). Diese Hypothese erhält Unterstützung vom Nachweis der NK_3-Rezeptoren u.a. in der

Amygdala (Langlois et al., 2001;Mileusnic et al., 1999;Stoessl and Hill, 1990), einer Hirnregion die eine wichtige Rolle bei der Vermittlung von Angst spielt (Davis, 1992;Pesold and Treit, 1995;Treit et al., 1993b). Der Umstand, dass die NK_3-Rezeptor Aktivierung durch Senktide bei alten Ratten ängstliches Verhalten im „Open-Field" Test verminderte, steht im Einklang mit Befunden bei denen ebenfalls anxiolytische Eigenschaften von Senktide bei Mäusen (Ribeiro and De Lima, 2002;Ribeiro et al., 1999) berichtet wurden. Die vorliegenden Resultate liefern einen entscheidenden Hinweis für eine Beeinflussung von ängstlichem Verhalten durch NK_3-Rezeptoren in alten Tieren.

Gerade im Hinblick darauf, dass Depressionen zu den häufigsten psychischen Erkrankungen im Alter zählen, sind Studien bei alten Tieren von großem Interesse. Verschiedene Befunde deuten auf eine Beteiligung von Neurokininen bei der Pathophysiologie der Depression hin (Bondy et al., 2003;Shirayama et al., 1996;Sergeyev et al., 1999). Aufgrund der Identifikation von NK_3-Rezeptoren in Hirnarealen wie Frontalcortex, Amygdala und Hypothalamus (Ding et al., 1996;Duarte et al., 2006;Shughrue et al., 1996;Mileusnic et al., 1999), kann ihnen eine mögliche Rolle bei der Regulation der affektiven Störung Depression und bei Stress (Culman and Unger, 1995;Kramer et al., 1998;Smith et al., 1999) beigemessen werden. Außerdem interagieren NK_3-Rezeptoren mit dem serotonergen (Stoessl et al., 1987), dopaminergen (Marco et al., 1998) und noradrenergen System (Bert et al., 2002;Jung et al., 1996) im Gehirn, die klassischerweise an den neurobiologischen Mechanismen der Depression beteiligt sind (Kramer et al., 1998;Markou et al., 1998). Zusätzlich zur anxiolytischen Wirkung von Senktide bei alten Ratten, konnten ebenfalls antidepressive Eigenschaften des NK_3-Rezeptor Agonisten im „Forced Swimming Test" nachgewiesen werden. Eine Studie an Mäusen mit dem NK_3-Rezeptor Agonist Aminosenktide bestätigt die mit alten Ratten erzielten Befunde zu antidepressiv-assoziierten Verhalten nach NK_3-Rezeptor Aktivierung (Panocka et al., 2001). Jedoch bestehen auch konträre Resultate, bei denen ein antidepressiver Effekt nach NK_3-Rezeptor Blockade dokumentiert ist (Salome et al., 2006). Ein möglicher

Erklärungsansatz für diese gegenteiligen Ergebnisse bei NK_3-Rezeptor Aktivierung oder Blockade könnte in der Wahl der Tierspezies liegen. So können konträre Resultate auf bestehende Interspeziesunterschiede bei der Expression (Langlois et al., 2001;Mileusnic et al., 1999), beim pharmakologischen Bindungsprofil (Emonds-Alt et al., 1995;Maggi, 1995;Suman-Chauhan et al., 1994) sowie Struktur der NK_3-Rezeptoren (Maggi, 1995) zurückgeführt werden. Diese Unterschiede existieren zwischen 2 Speziesgruppen (Gruppe 1: Meerschweinchen, Gerbils und Primaten; Gruppe 2: Ratten und Mäuse), wobei innerhalb der Gruppen große Ähnlichkeiten bestehen. Die Verwendung von Gerbils als Versuchstiere bei der Studie zum antidepressiven Effekt nach Injektion eines NK_3-Rezeptor Antagonisten (Salome et al., 2006) unterstreicht die Vermutung bestehender Interspeziesunterschiede bei der Wirksamkeit antidepressiver Eigenschaften.

Ergänzend zum Einfluss auf emotionales Verhalten, konnte ebenso eine Beteiligung von NK_3-Rezeptoren an Lern- und Gedächtnisprozessen nachgewiesen werden. Bekanntermaßen resultierte NK_3-Rezeptor Knockout in einem Gedächtnisdefizit bei „Passive Avoidance“ und einer räumlichen Lernaufgabe (Siuciak et al., 2007) und eine Behandlung mit dem NK_3-Rezeptor Agonist Senktide wirkt in verschiedenen Paradigmen gedächtnisfördernd (Kameyama et al., 1998;Ukai et al., 1996;Zlomuzica et al., 2008). Außerdem war der NK_3-Rezeptor Agonist NKB ebenso in der Lage einer Beeinträchtigung beim Referenzgedächtnis entgegenzuwirken (Wenk et al., 1997). In der vorliegenden dritten Studie wurde erstmals ein „Episodic-like Memory“ Gedächtnisdefizit bei alten Tieren dokumentiert. Dieser Befund steht in Übereinstimmung mit Humanstudien, bei denen Beeinträchtigungen episodischer Gedächtnisleistung im Alter beschrieben wurden (Lindeboom and Weinstein, 2004;Small et al., 2003;Souchay et al., 2007). Zudem scheint das „Episodic-like Memory“ Paradigma eine anspruchsvolle Aufgabe mit umfangreicher Gedächtnisleistung darzustellen, bei der gleichzeitig objektspezifische, räumliche und zeitliche Informationen abgerufen werden müssen (Dere et al., 2005). Versuchstiere, die beim „Episodic-like Memory“ Paradigma unbeeinträchtigt sind,

integrieren vermeintlich verschiedene Informationskomponenten während der Objektexploration, so dass sie in der Lage sind zu erinnern, welches Objekt sie an welcher Position und zu welcher Zeit vorgefunden haben (Dere et al., 2005;Dere and De Souza Silva, 2010;Kart-Teke et al., 2006). Da jedoch altersabhängige Defizite bereits bei verschiedenen Objektgedächtnisaufgaben beschrieben wurden (de Lima et al., 2005;Robitsek et al., 2008), entsprach das vorliegende Defizit in der episodischen Gedächtnisleistung der alten Ratten den Erwartungen. Offenbar ist die Integration objektspezifischer, räumlicher und zeitlicher Informationen essentiell zur Generierung episodischen Gedächtnisleistung, so dass eine Beeinträchtigung bereits einer dieser Komponenten Defizite im „Episodic-like Memory" Paradigma verursachen könnte. Es ist hinreichend bekannt, dass altersbedingte Gedächtnisdefizite stark mit morphologischen und physiologischen Veränderungen im Gehirn korrelieren (Erickson and Barnes, 2003;Sarter and Bruno, 2004). Dabei kann beispielsweise das Defizit der alten Tiere auf altersspezifische neurobiologische Veränderungen im Hippocampus zurückgeführt werden (Lister and Barnes, 2009). Diese Annahme findet Unterstützung von einer Studie, in der nach hippocampaler Läsion Tiere in der „Episodic-like Memory" Performance defizitär waren (Li and Chao, 2008). Mit Hilfe des NK_3-Rezeptor Agonisten Senktide war es nicht möglich die Beeinträchtigung episodischer Gedächtnisleistung aufzuheben. Allerdings konnte eine Komponente episodischer Gedächtnisleistung, das räumliche Objektgedächtnis, herbeigeführt werden. Ausgehend von der Integrität des Hippocampus für räumliches Gedächtnis (Eichenbaum et al., 1999;Martin and Clark, 2007) und dem gedächtnisfördernden Effekt von 0.2 mg/kg Senktide bei der räumlichen Objektgedächtniskomponente, besteht die Möglichkeit einer Aktivierung der NK_3-Rezeptoren im Hippocampus und eine darüber vermittelte Wirkungsweise. Es kann zudem nicht ausgeschlossen werden, dass der gedächtnisfördernde Effekt von Senktide bei den alten Tieren auch in dessen oben beschriebenen anxiolytischen Eigenschaften begründet liegt. Diese Vermutung wird von einem in der Literatur bereits beschriebenen Zusammenhang von Gedächtnisleistung in Abhängigkeit von Emotionalität und Angst getragen

(Kalueff, 2007). Darüber hinaus wurde von einigen Autoren eine Wechselbeziehung zwischen Lern- und Gedächtnisdefiziten, wie sie während Alterungsprozessen beschrieben werden, und emotionalen Verhaltensweisen diskutiert (Kucuk et al., 2008;Rowe et al., 1998;Schulz et al., 2007). Da Angst und Stress eng miteinander verknüpft (Blazer et al., 1987) sind und Beeinträchtigungen von Lern- und Gedächtnisprozessen infolge von Stress bereits häufiger beobachtet wurden (Chrobak and Napier, 2002;Kart-Teke et al., 2007;Mabry et al., 1996;Stillman et al., 1998), könnte der Effekt von Senktide im „Episodic-like Memory" Paradigma auch im Sinne eines stressminimierenden Faktors interpretiert werden. In der Vergangenheit wurde bereits angenommen, dass episodische Gedächtnisleistung im verwendeten Paradigma anfällig gegenüber Stressoren zu sein scheint (Kart-Teke et al., 2007;Zlomuzica et al., 2008). Bezugnehmend auf die Resultate, dass Senktide in den Dosierungen 0.2 mg/kg und 0.4 mg/kg anxiolytische Eigenschaften besitzt damit einhergehend Stress reduziert würde, wäre zu erwarten gewesen, dass neben 0.2 mg/kg Senktide ebenso die höhere Dosierung, also 0.4 mg/kg Senktide, die Gedächtnisleistung im „Episodic-like Memory" Paradigma beeinflusst.

Einen entscheidenden Hinweis, ob der NK_3-Rezeptor Agonist Senktide Lern- und Gedächtnisprozesse fördert oder vielmehr ein stress-bezogenes Erklärungsmodell bei der Beurteilung des Effekts von Senktide auf Gedächtnisleistungen in Frage kommt, lieferte die vierte Studie. Hierbei zeigte sich bei adulten Ratten, dass Senktide ein mit Hilfe des ACh-Rezeptor Antagonisten Scopolamin induziertes Defizit bei Objektwiedererkennung, räumlichen und temporalen Objektgedächtnis kompensierte. Interessanterweise entfaltet Senktide bei Defizitmodellen eine promnestische Wirkung. Dementsprechend sind die vorliegenden Ergebnisse, dass Senktide zum einen eine altersabhängige und anderseits eine pharmakologisch-induzierte (Scopolamin) Gedächtnisbeeinträchtigung bei Ratten verhinderte, konform. Gerade diese Befunde sprechen für eine Förderung der Gedächtnisleistungen nach NK_3-Rezeptor Aktivierung durch Senktide. Weiter zurückliegende Befunde zeichneten ein ähnliches

Muster, indem die gedächtnisfördernden Eigenschaften des NK_3-Rezeptor Agonisten unter suboptimalen Bedingungen in einer „Episodic-like Memory" Aufgabe (Zlomuzica et al., 2008) oder bei der Arbeitsgedächtnisbeeinträchtigung infolge einer Scopolamin-Behandlung (Kameyama et al., 1998;Ukai et al., 1996) bei Mäusen zum Tragen kamen.

Verschiedene Autoren verdeutlichen einen Einfluss des cholinergen Systems bei der Modulation der NK_3-Rezeptor mediierten Gedächtnisleistung. Sowohl die Co-Lokalisation von cholinergen Neuronen und NK_3-Rezeptoren (Chen et al., 2001a), als auch ein Anstieg der ACh Freisetzung in verschiedenen Hirnregionen nach NK_3-Rezeptor Aktivierung (Marco et al., 1998;Steinberg et al., 1995) bekräftigten zunehmend diese Hypothese. Zudem dokumentierten einige Autoren nach Stimulation cholinerger Aktivität durch Nicotin gesteigerte Lern-und Gedächtnisleistungen (Puma et al., 1999;Uzüm et al., 2004). Die vorliegenden Studien ergaben, dass sowohl der NK_3-Rezeptor Agonist NKB als auch Senktide die ACh Freisetzung in Hirnarealen erhöhten, die mit Lern- und Gedächtnisprozessen assoziiert werden. Der dosisabhängige Effekt von Senktide auf die ACh Freisetzung in Frontalcortex und Hippocampus sowie die dosisabhängigen gedächtnisfördernden Eigenschaften auf Scopolamin-induzierte Gedächtnisdefizite stehen im Einklang. Die niedrigere Dosierung von Senktide (0.2 mg/kg) erhöhte ACh Level im Frontalcortex und wirkte einer Beeinträchtigung der Objektwiedererkennung entgegen. Die höhere Dosierung von Senktide (0.4 mg/kg) hingegen hatte einen Anstieg der ACh Neurotransmission im Hippocampus und eine Blockade von Scopolamin-induzierten räumlichen wie temporalen Objektgedächtnisdefizit zur Folge. Diese Befunde deuten auf eine unterschiedliche Beteiligung bestimmter Hirnareale des cholinergen Systems bei NK_3-Rezeptor vermittelten Verhaltensweisen hin. So scheint Objektwiedererkennung über eine cholinerge Aktivierung des Frontalcortexes, räumliches und temporales Objektgedächtnis über den Hippocampus moduliert zu werden. Eine Wechselwirkung von NK_3-Rezeptor Aktivierung und ACh Freisetzung scheint

demnach naheliegend. Basierend auf dieser Annahme kann die cholinerge Aktivität in Frontalcortex und Hippocampus als Grundlage der promnestischen Wirkung von Senktide angesehen werden.

Die Ergebnisse der vorliegenden Studien erweitern das Verständnis um die Beteiligung der NK_2- und NK_3-Rezeptoren an der Modulation von Verhalten und cholinerger Neurotransmission innerhalb des basalen Vorderhirns. Zudem stützen die vorliegenden Befunde Hinweise auf potentielle Anwendungsgebiete von NK_2 -Rezeptor Antagonisten und NK_3-Rezeptor Agonisten bei kognitiven und emotionalen Störungen.

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6 Veröffentlichungen

Nachfolgend sind die veröffentlichten Studien aufgeführt, auf denen diese Dissertation beruht.

1. S. Schäble, J. P. Huston, M. L. Brandao, E. Dere, and M. A. De Souza Silva. Neurokinin-2 receptor antagonism in medial septum influences temporal-order memory for objects and forebrain cholinergic activity. *Peptides* 31 (1):108-115, 2010.

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3. S. Schäble, B. Topic, T. Buddenberg, D. Petri, J. P. Huston, and M. A. De Souza Silva. Neurokinin3-R agonism in aged rats has anxiolytic-, antidepressant-, and promnestic-like effects and stimulates ACh release in frontal cortex, amygdala and hippocampus. *Eur. Neuropsychopharmacol.* 21 (6):484-494, 2011.

4. S. Schäble, J. P. Huston, M. Barros, C. Tomaz and M. A. De Souza Silva. The NK3 receptor agonist senktide ameliorates scopolamine-induced deficits in memory for object, place and temporal order. *Neurobiol. Learn. Mem.* 97 (2):235-240, 2012.

7 Anhang

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Neurokinin-2 receptor antagonism in medial septum influences temporal-order memory for objects and forebrain cholinergic activity

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ABSTRACT

In the mammalian brain the neurokinin NK_2 receptors are predominantly located in the hippocampus, thalamus, septum and frontal cortex. It has been shown that administration of the NK_2 receptor agonist, neurokinin A (NKA), into the medial septum of rats increases extracellular levels of acetylcholine (ACh) in the hippocampus and that NK_2 receptor antagonism blocks this increase. Therefore, given the prominent role of hippocampal ACh in information processing, we hypothesized that NK_2 receptor antagonism in the medial septum would negatively affect learning and memory via its influence on the cholinergic neurons of the basal forebrain. We investigated the action of local application of the peptidic NK_2 receptor antagonist, Bz-Ala-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH (1, 10 and 100 pmol), into the medial septum on object memory for temporal order and spatial location using an object novelty paradigm. By means of in vivo microdialysis and HPLC analyses, we also examined the influence of NK_2 receptor antagonism in the medial septum on ACh in major cholinergic projection areas of the basal forebrain, namely, hippocampus, frontal cortex and amygdala. *Results*: Injection of vehicle alone into the medial septum impaired memory for temporal order and spatial location of objects. Application of 1 pmol of the NK_2 receptor antagonist partially reversed this deficit by reinstating memory for temporal order. Injection of 10 pmol of the NK_2 receptor antagonist into the medial septum decreased levels of ACh in the hippocampus (at 30 min post-injection), and frontal cortex (at 30 and 80 min post-injection) in comparison to vehicle. However, this apparent decrease was the result of the blockade of a saline-induced increase in ACh levels.

1. Introduction

Three neurokinin (NK) receptors have been identified in the brain: the neurokinin-1 (NK_1), NK_2 and NK_3 receptors. While NK_1 and NK_3 receptors are widespread in the brain, the NK_2 receptors are found in restricted brain areas, i.e., in the frontal cortex, hippocampus, shell of the nucleus accumbens, septum and thalamus. Neurokinin A (NKA), neuropeptide K (NPK) and neuropeptide γ (NPγ) are the preferred endogenous ligands for NK_2 receptors, although substance P and neurokinin B also bind with lower affinity [39,40].

Relatively little is known about the functions of NK_2 receptors. Based on receptor localization, NK_2 receptors are suggested to be involved in drug sensitization, addiction and schizophrenia [39]. NK_2 receptor antagonists have also been proposed for the treatment of depression and some forms of anxiety disorders [19,20,43].

The septo-hippocampal cholinergic system has been implicated in a number of behavioral processes, including arousal, memory and emotion [17,45,47]. It has also been proposed to function as a gating system in the processing and integration of signals derived from several sensory inputs [31]. NK agonists exert an excitatory influence on the septo-hippocampal cholinergic system [28]. Post-trial injection of SP into the medial septum was found to facilitate passive avoidance learning [42]. Administration of the NK_2 receptor agonist, neurokinin A (NKA), into the medial septum of rats increased extracellular levels of acetylcholine (ACh) in the hippocampus and NK_2 receptor antagonism blocked this increase [44]. A highly selective and potent NK_2 receptor antagonist significantly blocked the NKA-induced release of ACh [21]. The enhancing effects of NKA were mediated by the activation of NK_2 receptors, since they were antagonized by SR48968. These results indicate that the NK_2 receptor may be critically involved in regulating hippocampal ACh release. This finding, and the prominent role attributed to forebrain ACh in learning and memory processes [3,18,30], is suggestive of a role for the NK_2 receptors in learning and memory processes via cholinergic

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modulation. We, therefore, hypothesized that NK_2 receptor antagonism in the medial septum would negatively affect learning and memory by influencing the activity of the cholinergic neurons of its main projection area, the hippocampus. The effects of intra-septal NK_2 receptor antagonism on learning and memory performance have so far not been investigated.

In the present study, we first assessed the effects of pre-trial administration of a peptidergic NK_2 receptor antagonist on object recognition memory in a novelty-preference paradigm, which combines memory for temporal order [32] and memory for spatial location [15]. Based on the suppressive effects of NK_2 receptor antagonism on hippocampal ACh release induced by medial septal NK_2 receptor agonism [44], we expected that NK_2 receptor antagonism in the medial septum would disrupt performance on the object recognition tasks. Furthermore, we tested whether intra-septal NK_2 receptor antagonism would modulate extracellular ACh levels under basal non-stimulated conditions, as assessed by vivo microdialysis and high performance liquid chromatography with electrochemical detection (HPLC–ECD). Again, based on the findings of Steinberg et al. above [44], we expected this treatment to decrease levels of ACh, especially in the hippocampus, the main cholinergic projection from the medial septal area. ACh levels were also measured concurrently in other basal forebrain cholinergic projection areas, namely the frontal cortex and amygdala.

2. Methods

2.1. Subjects

Adult male Wistar rats (260–300 g at the beginning of the experiments) from the breeding colony of the University of Düsseldorf were used for all experiments. Animals were housed in translucent plastic cages (60.0 cm × 20.0 cm × 38.0 cm; length × depth × height) under controlled laboratory conditions (temperature: 20 ± 2 °C) with free access to food and water under an artificial reversed 12:12 light–dark cycle (light off at 07:00 am). They were housed in groups of five animals per cage until the implantation of the guide-cannulae was carried out. The animals were allowed to adjust to the housing conditions for 2 weeks and were handled daily for 5 days preceding the experiments. Experiments were performed during the animals' active period between 8:00 am and 5:00 pm. All experiments were carried out according to the German Law of Animal Protection of 1998.

2.2. Drugs

The peptidic NK_2 receptor antagonist used, Bz-Ala-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH_2 (Bachem, USA), is extremely potent and selective [29]. It was diluted in phosphate-buffered saline (PBS) and administered in three doses: 1, 10, and 100 pmol. The vehicle, PBS, served as a control condition. For drug administration, an injection needle (17.1 mm long, 0.20 gauge) was inserted into the medial septum through a guide-cannula (16.3 mm long, 0.22 gauge) and the substance was applied in 0.5 μl volume with a flow rate of 1.0 μl/min, using a microinfusion pump (CMA/100, Sweden). After the end of the injection, the needle was left in place for another 30 s.

2.3. Object memory for temporal order and spatial location

We examined the effects of pre-trial administration of the NK_2 receptor antagonist, Bz-Ala-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH_2, into the medial septal area on object recognition memory in a novelty-preference paradigm, which combines memory for temporal order and memory for spatial location of objects.

2.3.1. Surgery

For local application of drugs, 5–7 days prior to the beginning of the behavioral testing, the animals were anesthetized with a mixture of ketaminhydrochloride (90.0 mg/kg; Pharmacia & Upjohn GmbH, Germany) and xylazinhydrochloride (8.0 mg/kg; Bayer, Germany). With the help of a stereotaxic apparatus (David Kopf Instruments, USA) and according to a stereotaxic atlas [36], a stainless steel guide-cannula for microinjection of the antagonist (16.3 mm long, 0.22 mm gauge) was placed above the medial septum (AP: +0.82 cm; ML: ±0.16 cm; DV: −0.60 cm, with 15° angle toward the midline); coordinates were taken from bregma. The guide-cannulae were placed unilaterally, counterbalanced into the right or left hemispheres and were fixed with two stainless steel screws and dental cement. After surgery the animals were allowed to recover for 1 week before being subjected to behavioral testing. They were housed individually for 2 days and afterwards, two animals per cage.

2.3.2. Apparatus

Memory for temporal order of presentation and spatial location of objects was tested in an open-field arena. The open-field used was a rectangular box (60 cm × 60 cm × 30 cm) with grey acrylic walls and open roof, located in a sound-attenuating room with masking noise. The arena was illuminated by four 40 W bulbs that provided a light density of approximately 13 lx at the center of the field. Its floor was divided into virtual nine quadrants of equal size, which were the possible positions of object placement, except for the central square, which was not used for object placement. A video camera, connected to a video recorder, was mounted 1.6 m above the arena to record the experiment on video tapes for off-line data acquisition.

2.3.3. Objects

Two different objects (in quadruplicate), made of plastic material, differing in color (violet, colorless), shape (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 emanating from the material with which the objects were made, during the test trial. The objects had a height of 28 cm and a sufficient weight (1500 g) to ensure that the rats could not displace them. Pilot studies ensured that rats could discriminate the two objects, and that there was no *a priori* preference for any of the objects.

2.3.4. Experimental procedure

This task combines different versions of the novelty-preference paradigm that are presumed to measure (a) object recognition memory [14], (b) memory for temporal order of presentation of objects [32] and (c) memory for locations in which objects were encountered [15].

Each animal was exposed to the open-field on 3 consecutive days (habituation). It was placed into the central part of the open-field and allowed to explore for 10 min. One day after the last habituation trial, the object memory test was administered. The animals received two sample trials, followed by the test trial (Fig. 1). The rats were always placed into the central part of the open-field, facing the same direction. Each trial lasted 5 min, with inter-trial intervals of 50 min. For each animal, four out of eight squares were randomly chosen to position the four copies of the "old" object in the first sample trial (see top of Fig. 1). During the second sample trial, four copies of a different object ("recent") were present. Two copies of the "recent" object were randomly placed onto positions that had been occupied in the first sample trial and two copies were placed in new positions, which were randomly chosen from the remaining six positions. In the test trial, two copies of both "old" (A1 and A2) and recent (B1 and B2) objects

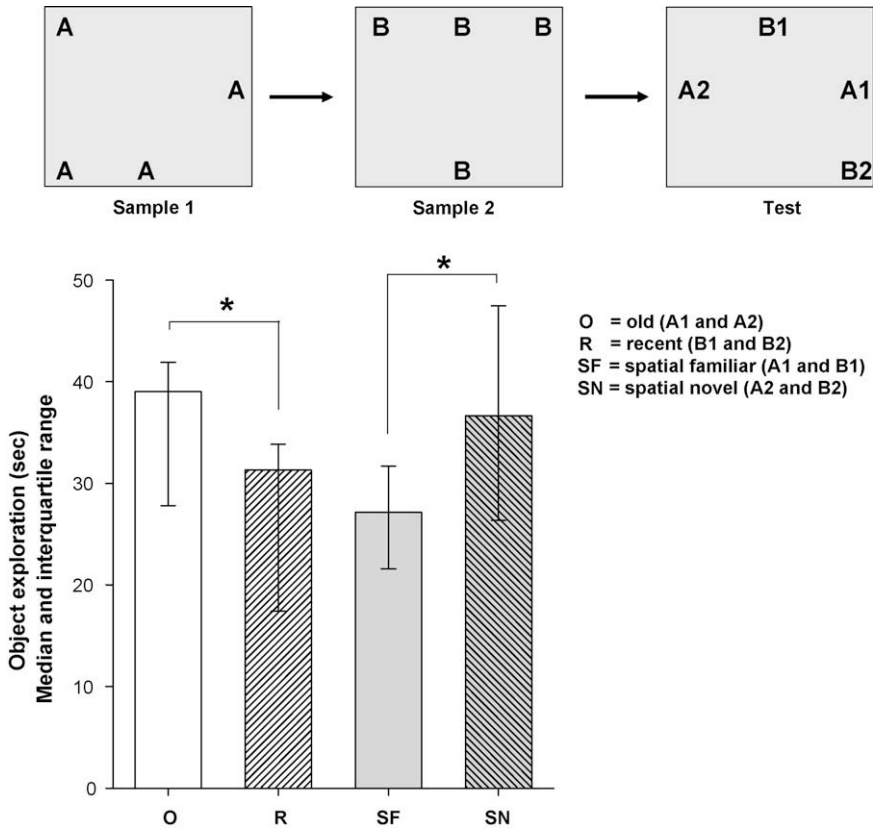

Fig. 1. (Top) Schematic representation of possible placement of objects in object memory task. The animals received three trials with 5 min duration and an inter-trial interval of 50 min. During the test trial, two "old" (A1 and A2) and two "recent" (B1 and B2) objects known from the sample trials were presented at "spatial familiar" (A1 and B1) and "spatial novel" (A2 and B2) locations relative to the sample trials. (Bottom) Object memory for temporal order and spatial location in untreated rats. The data are represented as median and interquartile range of exploration time (s) of "old" (O) and "recent" (R) as well as of "spatial familiar" (SF) and "spatial novel" objects. $^*p < 0.001$.

were present in either a spatial familiar (A1 and B1) or a spatial novel (A2 and B2) position. The time spent exploring the objects (in seconds) was scored by an experienced observer, who was blind to the experimental conditions. Object exploration was defined as a physical contact with an object either with the nose, vibrissae or forepaws. The experiments were carried out between 8:00 am and 4:00 pm. After each trial, the objects and the open-field were cleaned with a 0.1% acetic acid solution in order to remove odor cues. Animals that did not explore all four objects in each sample trial were excluded from the analyses.

2.3.5. Experiment 1: memory for temporal order of presentation and spatial location of objects in untreated rats

This experiment was carried out to determine whether the animals would exhibit discrimination of temporal order ("what" and "when") and spatial location ("what" and "where") of objects. Fifteen adult experimentally naïve male Wistar rats were used.

During the test trial (see top of Fig. 1), an "old" object known from sample trial 1 and another "recent" object from sample trial 2 were presented together. Here, we expected the animals to spend more time exploring the "old" object relative to the "recent" object, indicating that the previously explored objects were recognized and discriminated in terms of their relative recency, namely, temporal order [32]. Furthermore, the animals were tested for memory for spatial location during the same test trial by presenting copies of one "old" and of one "recent" object in a "novel" location which never contained objects. Here, we expected the animals to spend more time exploring the object encountered in the "spatial novel" location, indicating that they remember the positions in which the objects had been encountered during the corresponding sample trials [14,15].

2.3.6. Experiment 2: effect of pre-sample trial 1 injection of the NK_2 receptor antagonist into the medial septum on object memory for temporal order and spatial location

Forty-two adult, experimentally naïve male Wistar rats were randomly assigned to one of the following treatment groups: vehicle, 1, 10 or 100 pmol NK_2 receptor antagonist. The micro-injections of the peptidergic NK_2 receptor antagonist into the medial septum were made 30 min prior to the first sample trial, which initiated the object memory test described above.

2.3.7. Statistical analysis

For both experiments, repeated measures ANOVAs were used for within-group analysis of object exploration time during sample trials 1 and 2. In experiment 2, between-groups comparisons of exploration time during sample trials 1 and 2 were performed by one-way ANOVA, followed by Bonferroni post hoc tests, where indicated. The data obtained on the exploration time during the test trial in both experiments are expressed as medians (and interquartile range) (see Figs. 1 and 2). Within-group differences between the exploration times of the four objects during the test trial were calculated by the Wilcoxon test. For between-group comparison in experiment 2, exploration ratios were calculated: the temporal-order exploration ratio was calculated by dividing the time spent exploring the "old" object by the sum of the time spent exploring the "old" and the "recent" objects; the spatial location exploration ratio was calculated by dividing the time spent exploring the "spatial novel" object by the sum of the time spent exploring the "spatial novel" and the "spatial familiar" objects. The data were then analyzed by the Mann–Whitney *U*-test with pair-wise group comparisons against the vehicle group. The *p*-values given are

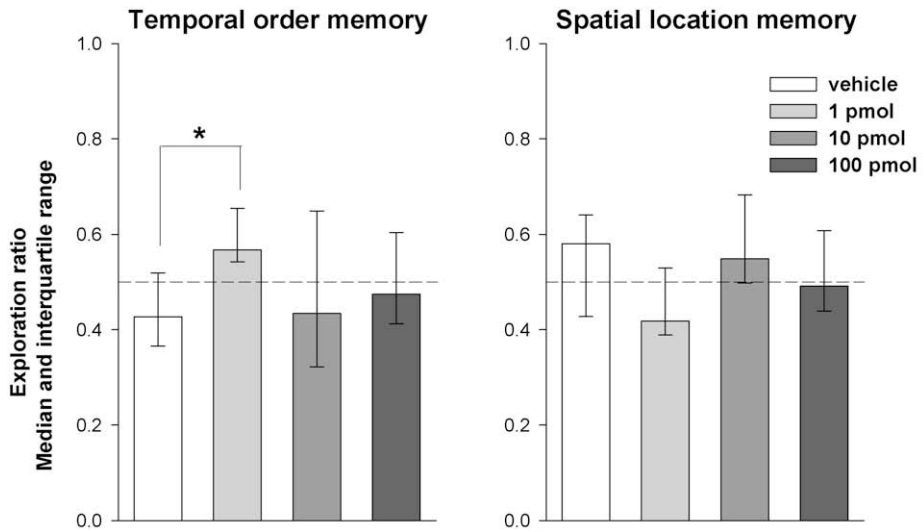

Fig. 2. Effect of vehicle or 1, 10 and 100 pmol NK_2 receptor antagonist injection into the medial septum on object exploration. (A) The ratio between the duration of exploration of the "old" and "recent" objects was calculated by dividing the time spent exploring the "old" object by the sum of the time spent exploring the "old" and "recent" objects. (B) The spatial location exploration ratio was calculated by dividing the time spent exploring the "spatial novel" object by the sum of the time spent exploring the "spatial novel" and the "spatial familiar" objects. The data are represented as median and interquartile range. $^*p < 0.05$.

two-tailed, and are considered to be significant when $p < .05$. The software SPSS 16.0 was used for the analysis.

2.4. Neurochemical effects

2.4.1. Experiment 3: medial septal NK_2 receptor antagonism and ACh in hippocampus, frontal cortex and amygdala

We examined the influence of injecting the NK_2 receptor antagonist, Bz-Ala-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH_2 into the medial septal area on extracellular ACh levels in the frontal cortex, amygdala and hippocampus under basal non-stimulated conditions.

2.4.2. Surgery

For local application of drugs and in vivo microdialysis, the surgery method used was identical to the one described above. With the help of a stereotaxic apparatus (David Kopf Instruments, USA) and according to a stereotaxic atlas [36] three stainless steel guide-cannulae for the microdialysis probes (15 mm long, 0.22 mm gauge) were implanted above the following brain areas: frontal cortex (AP: +3.7 mm, ML: ± 3.0 mm, DV: −1.3 mm), amygdala (AP: −2.5 mm, ML: ± 5.7 mm, DV: −7.8 mm, with 10° angle toward the midline) and hippocampus (AP: −6.0 mm, ML: ± 4.8 mm, DV: −3.2 mm); coordinates were taken from bregma. A guide-cannula for microinjection of the drug (16.3 mm long, 0.22 gauge) was placed above the medial septum (AP: +8.2 mm; ML: ± 1.6 mm; DV: −6.0 mm, with 15° angle toward the midline). All guide-cannulae were implanted unilaterally, alternately into the right or left hemisphere. The animals were allowed to recover from surgery for 1 week before being subjected to the microdialysis procedure and were housed individually.

2.4.3. Microdialysis procedure

The experiments were carried out between 8:00 am and 4:00 pm. Fifty-four adult male Wistar rats were randomly assigned to one of following groups: vehicle, sham, 1, 10 or 100 pmol NK_2 receptor antagonist injections into the medial septum. The microdialysis probes were prepared according to the procedure described elsewhere [2]. Five to seven days after guide-cannulae implantation, they were anesthetized with urethane (1.25 g/kg, Sigma–Aldrich, USA). Microdialysis probes were inserted through the guide-cannulae. They had an active membrane length of 4.0 mm for the hippocampus and 2.0 mm for frontal cortex and amygdala. A catheter was placed into the intraperitoneal (i.p.) cavity to allow fluid supply without physical contact with the animal. The animal was placed on a heating pad in an acrylic box (45 cm × 25 cm × 22 cm) and its body temperature was maintained at 37.5 ± 0.5 °C by a temperature controller (CMA/150, Sweden). The probes were perfused at a flow rate of 2 μl/min with ringer's solution (Braun, Germany) containing neostigmine (10 μmol), by a microinfusion pump (CMA/100, Sweden).

After a stabilization period of 2 h, six baseline samples, each corresponding to a time-window of 10 min, were collected in cups containing 10 μl of ethylhomocholine (EHCh) solution, used as the internal standard. Afterwards, either a sham injection (the injection needle was inserted into the medial septum through the guide-cannula and left in place for 1 min, but there was no fluid injection), vehicle or 1, 10 or 100 pmol NK_2 receptor antagonist was injected into the medial septum. Another 10 samples (10 min per sample) were collected in cups containing 10 μl EHCh. After each sampling the animal received an injection of 0.1 ml Ringer's solution through the inserted i.p. catheter and the depth of anesthesia was adjusted with urethane if necessary.

2.4.4. HPLC analysis

After collection, the samples were analyzed for the concentration of acetylcholine (ACh) by HPLC-EC according to standard method [7]. Ethylhomocholine (EHCh) was used as internal standard [37]. Briefly, ACh separation was achieved using an analytical column (75 mm length, 2 mm inner diameter) filled with ChromSpher 5C18 (Merck, Germany) and loaded with sodiumdodecylsulfate (Sigma–Aldrich, USA). The enzyme reactor was filled with LiChrosorb-NH_2 (Merck, Germany), activated by glutardialdehyde (Merck, Germany) and loaded with acetylcholine-esterase (Sigma–Aldrich, USA) and cholineoxidase (Sigma–Aldrich, USA), which are covalently bound to the stationary phase and attached to the end of the analytical column for enzymatic cleavage of ACh. On passage through the enzyme reactor, ACh is converted to hydrogenperoxide, which is electrochemically detected by a platinum electrode set at a potential of 0.350 mV versus an Ag/AgCl reference electrode (Antec Leyden, Netherlands). The mobile phase is composed of

1 mM tetramethylammoniumchloride (Merck, Germany) and 0.18 M K_2HPO_4 (Merck, Germany), adjusted to pH 8.0 with KH_2PO_4 (Merck, Germany), and is delivered by a HPLC pump (Merck, Germany) at a flow rate of 0.3 ml/min. The data were collected and processed and stored with the help of computer software (Chrom Perfect, Justice Laboratory Software).

2.4.5. Statistical analysis

Neurochemical data were analyzed for each brain area separately. ACh concentrations were converted into percentage of baseline (the mean of six baseline samples taken as 100%). ANOVAs with post hoc Tukey HSD test were calculated. The *p*-values given are two-tailed and were taken as measures of effect. The software SPSS 16.0 was used for the analyses.

2.5. Histology

After the microdialysis procedure or behavioral testing, the animals were deeply anesthetized with pentobarbital (Sanofi Ceva, France), and perfused with PBS and 10% formalin through the left heart ventricle. The brain was removed and stored in 10% formalin. After slicing the brain with a cryostat (Leica, Germany), slices were stained with cresylviolet (Sigma–Aldrich, USA). The accuracy of the injection site and placement of the microdialysis probes was verified, according to the atlas of Ref. [36]. Only those animals in which the placement of microdialysis and/or injection site probes were correct, were considered for statistical analysis.

3. Results

3.1. Experiment 1: memory for temporal order and spatial location of objects in untreated rats

Analysis of the data of sample trials 1 and 2 indicated no significant differences in duration of exploration of the objects (repeated measures ANOVA, $p > 0.05$). The duration of exploration of the object types "old", "recent", "spatial familiar" and "spatial novel" during the test trial are presented in the bottom of Fig. 1. During the test trial the untreated rats spent more time exploring the "old" object relative to the "recent" object ($p \leq 0.0001$, Wilcoxon test), indicating that these animals could distinguish the objects (sample 1) that were seen in time scale before the other ones (sample 2). This suggests that they had an intact memory for temporal order of object presentation. They also spent more time exploring the "spatial novel" object compared to the "spatial familiar" object ($p \leq 0.001$), indicating that they were able to distinguish between the objects, both from sample trials 1 and 2, that were presented in the same position from the ones presented in a position not seen before. This indicates that these animals had an intact spatial memory.

3.2. Experiment 2: effect of injecting the NK_2 receptor antagonist into the medial septum on memory for temporal order and spatial location

After the histological screening for injection site, 39 animals were considered suitable for statistical analysis. The sample sizes were as follows: vehicle, $n = 11$; 1 pmol, $n = 11$; 10 pmol, $n = 9$ and 100 pmol NK_2 receptor antagonist, $n = 8$.

Repeated measures ANOVA for between-group comparisons did not indicate any significant differences in duration of exploration of the objects during the sample trials 1 and 2 ($ps > 0.05$).

During the test trial, unlike the untreated animals tested above (Fig. 1), the group treated with vehicle was not able to distinguish the "old" from the "recent" objects and also not the "spatial familiar" from the "spatial novel" objects. This suggests that injection of the vehicle into the medial septum disrupted memory for temporal order of presentation and spatial location of the objects (Wilcoxon test, $ps > 0.05$). However, the group that received 1 pmol of the NK_2 receptor antagonist into the medial septum distinguished the "old" from the "recent" objects ($p = 0.007$), but not the "spatial familiar" from the "spatial novel" objects. This indicates that the NK_2 receptor antagonist re-established memory for temporal order, but not memory for spatial location. Neither the 10 nor 100 pmol treated group displayed significant differences in duration of exploration of the objects during the test trial ($ps > 0.05$). Comparisons between exploration ratios indicated that the exploration ratio for temporal order of the group treated with 1 pmol of the NK_2 receptor

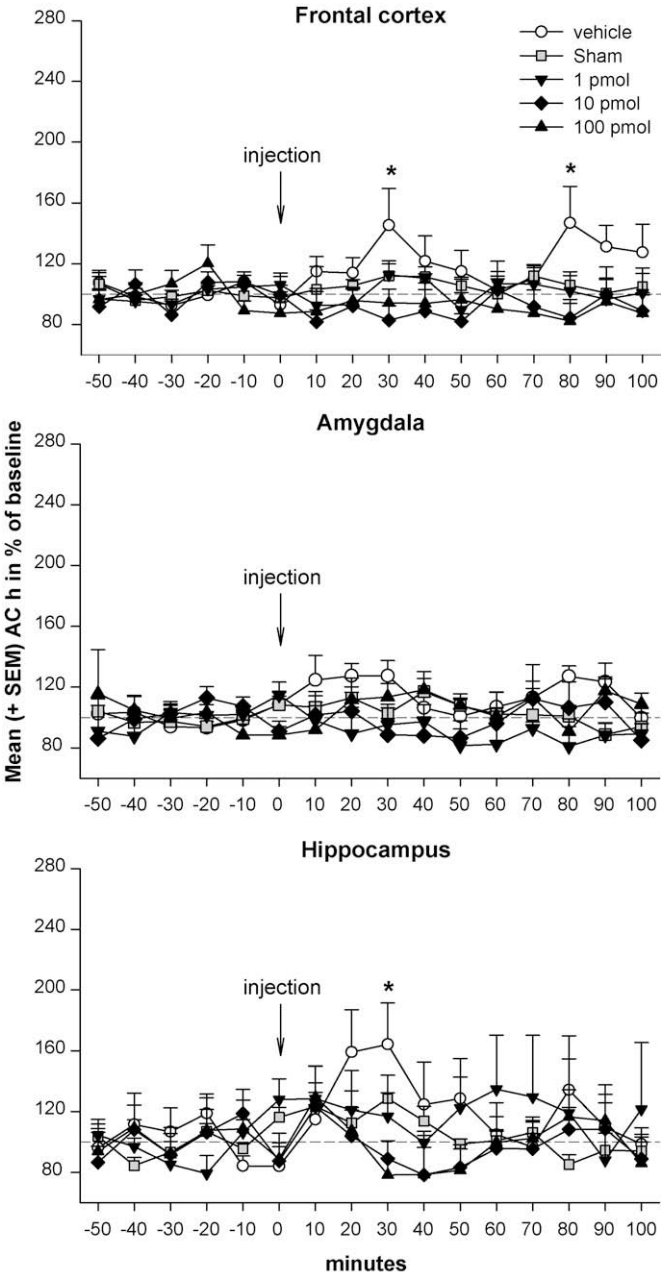

Fig. 3. Effect of local sham injection and injection of vehicle or 1, 10 and 100 pmol peptidic NK_2 receptor antagonist into the medial septum on extracellular ACh levels in the frontal cortex, amygdala and hippocampus in anesthetized Wistar rats. The values are given as mean (+S.E.M.) percent of baseline (average of the six baseline samples taken as 100%). Arrows indicate the time-point of the injection. ANOVA followed by post hoc Tukey HSD tests. $^*p < 0.05$.

antagonist was significantly higher than that of the vehicle treated group (Fig. 2A, Mann–Whitney *U*-test, $p = 0.001$), indicating again that memory for temporal order was re-established in this group.

3.3. Experiment 3: medial septal NK_2 receptor antagonism and ACh in hippocampus, frontal cortex and amygdala

After histological screening for correct probe- and injection-site placement and due to exclusion to leakage of probes during the experiment or HPLC analysis, a total of 36 animals were considered suitable for statistical analysis. The sample sizes were as follows: *frontal cortex*: sham, $n = 6$; vehicle, $n = 6$; 1 pmol, $n = 7$; 10 pmol, $n = 6$ and 100 pmol NK_2 receptor antagonist, $n = 5$; *amygdala*: sham, $n = 6$; vehicle, $n = 6$; 1 pmol, $n = 5$; 10 pmol, $n = 7$ and 100 pmol NK_2 receptor antagonist, $n = 5$; *hippocampus*: sham, $n = 6$; vehicle, $n = 5$; 1 pmol, $n = 5$; 10 pmol, $n = 5$ and 100 pmol NK_2 receptor antagonist, $n = 5$.

The mean ± S.E.M. basal levels of ACh in dialysates were: frontal cortex 0.298 ± 0.032 pmol/20 μl; amygdala 1.802 ± 0.253 pmol/20 μl; hippocampus 1.045 ± 0.150 pmol/20 μl. Levels of ACh (% of baseline, mean + S.E.M.) for the control groups (vehicle, sham) and the experimental groups (1, 10 and 100 pmol NK_2 receptor antagonist) in *frontal cortex*, *amygdala* and *hippocampus* are shown in Fig. 3. In the frontal cortex, one-way ANOVA between groups indicated a difference in the third ($F_{4,25} = 3.393$, $p = 0.025$) and eighth ($F_{4,25} = 3.008$, $p = 0.037$) samples collected after treatment. The level of ACh was higher in the vehicle treated group in comparison to the 10 pmol group 30 min after treatment administration (post hoc Tukey test, $p = 0.015$) and in comparison to the 10 and 100 pmol groups 80 min after treatment administration ($ps < 0.05$). In the *amygdala*, one-way ANOVA between groups did not indicate any differences. In the *hippocampus*, one-way ANOVA between groups revealed again a difference between groups in the third sample collected after treatment ($F_{4,22} = 3.623$, $p = 0.021$). Post hoc test indicated that the ACh level in the vehicle group was higher than in the 10 pmol group ($p = 0.009$).

4. Discussion

The aim of this study was to investigate the effect of local application of the peptidergic NK_2 receptor antagonist into the medial septum on learning and memory, as assessed by a paradigm which evaluates memory for objects, temporal order of encounter with objects and spatial location of encounter with objects. Furthermore, we investigated the effect of intra-septal NK_2 receptor antagonism on ACh levels in its main projection area, the hippocampus, and in two other projection areas of the cholinergic neurons of the basal forebrain, namely the frontal cortex and amygdala, in the anesthetized preparation. The main results are administration of the vehicle alone disrupted memory for temporal order and spatial location of objects. Application of 1 pmol of the NK_2 receptor antagonist partially reversed this deficit by re-establishing memory for temporal order. Injection of 10 pmol of the NK_2 receptor antagonist into the medial septum decreased ACh levels in the hippocampus at 30 min post-injection, as well as in the frontal cortex at 30 and 80 min post-injection, as compared to vehicle. However, the results suggest that this apparent decrease was due to the blockade of a vehicle-induced increase in ACh levels.

Object exploration in untreated animals: In line with other studies [11,26], the untreated animals spent more time exploring the "old" object relative to the "recent" object, suggesting that they remembered the object's order of appearance in the sample trials, reflecting memory for "what" and "when" (temporal order). Furthermore, they spent more time exploring the "spatial novel" object compared to the "spatial familiar" object, reflecting memory for "what" and "where" (spatial location).

Object exploration after NK_2 antagonism in medial septum: Pre-sample trial 1 injection of vehicle or the peptidergic NK_2 antagonist into the medial septum led to the following results: the rats which received the vehicle injection exhibited longer exploration times of the "recent" objects as compared to the "old" objects. Furthermore, in the vehicle group there was no significant difference in the duration of exploration of the "spatial familiar" and "spatial novel" objects in contrast to the performance of the untreated group above. This result suggests that vehicle infusion into the medial septum had a disruptive effect on memory for, both, temporal order and spatial location. The injection procedure, which involves some restraint of the animal, is likely to be stressful and, therefore, the impaired performance of the vehicle group might reflect stress-induced deficits in object recognition tasks, as has been reported in object recognition studies using systemic [25,26,48,49], as well as intracranial infusion of vehicle [46]. Similar to the present finding, it was found that, both, post-sample and 3 h pre-sample intra-perirhinal cortex infusion of saline, impaired object recognition performance in rats compared to a no-infusion group [46]. Disruptive effects of vehicle injection into the brain or applied systemically have also been reported in studies of delayed-non-matching-to-sample [6] and inhibitory avoidance learning [13,33]. It seems that particularly the basal forebrain cholinergic neurons are sensitive to disruptive effects of local vehicle infusion on performance in memory tasks [6,13,33].

The local injection of the peptidergic NK_2 receptor antagonist into the medial septum compensated, in part, for the disruptive effect of the vehicle injection. The group which received 1 pmol NK_2 receptor antagonist spent significantly more time exploring the "old" object relative to the "recent" object, indicating intact temporal-order discrimination. Thus, the NK_2 receptor antagonist reversed the detrimental effects of vehicle injection on object memory for temporal order. However, memory for spatial location was not reinstated by the NK_2 antagonist.

Disruptive effects of medial septal vehicle application: There are several possibilities to account for the disruptive effects of vehicle injection into the medial septum and nucleus basalis magnocellularis in learning tasks. For one, injection of the vehicle into brain tissue could have diverse physiological and non-physiological actions: (1) the most obvious, but generally ignored, effect is that mechanical pressure in various brain tissues can depolarize neurons leading: (a) to local epileptogenic-like activity with possible behavioral consequences and (b) to Leao's spreading depression, which can involve wide areas of the brain, particularly in neocortex, striatum and hippocampus, with diverse possibilities for behavioral disruption [4,23,35,41]. Such mechanically induced depolarization could account for the finding of intra-septal vehicle-induced disruption of object recognition in the present study, as well as for similar disruptive effects reported by others upon vehicle infusion into sites of cholinergic cell bodies, as mentioned above. (2) Potentially, other non-mechanical aspects of the vehicle infusion, such as fluid temperature, disruption of transport or concentration of substances, etc., could also activate or inhibit local neuronal activity. (3) An alternate explanation for behavioral effects of intracranial and also systemic vehicle injection relates to the likely stress induced by the injection procedure. Based on our neurochemical findings (discussed below) we consider it more likely that mechanical stimulation is responsible for the vehicle effects, since such injections led to slight increases in hippocampal and frontal cortical ACh levels, whereas sham injection failed to do so, and the NK_2 antagonist blocked this increase in ACh. However, due to the lack of sham controlled group in the behavioral study, a direct comparison cannot be made. Therefore, procedural stress must still be considered as a causal factor: stressors, such as fear

[1], restreint [24] and prolonged handling [38], strongly activate the septo-hippocampal cholinergic pathway. Restraint stress [24] and handling [34] increased ACh release in the hippocampus, which was abolished after disconnecting the hippocampus from its septal afferent pathway [1,34]. Systemic injection of vehicle also led to increased ACh levels in the nucleus accumbens [2]. (4) Another possibility to be considered is that the cannula implantation procedure by itself disrupted performance in this object recognition task.

Behavioral effects of medial septal NK_2 antagonism: In the case (a) that the vehicle effects are the result of mechanical stimulation, e.g. spreading depression or neuronal depolarization, we would have to interpret the protective effect of the NK_2 antagonist accordingly as counteracting such effects. In the case (b) that stress induced by the vehicle injection led to behavioral disruption, we would interpret the positive influence of the NK_2 antagonist in terms of counteracting such stress.

In fact, systemic NK_2 receptor antagonism by SR48968 reduced anxiety-like behavior associated to stress and to a threatening stimulus [19,20,27]. Furthermore, increased ACh release in the hippocampus, induced by central corticotrophin-releasing factor (CRF) administration or by stroking the back and neck of rats, were antagonized by systemic and intra-septal NK_2 receptor antagonism [12,44]. These findings suggest that NK_2 receptors may be involved in the stress-induced activation of the hippocampal cholinergic system. Thus, in our object memory study, the treatment with 1 pmol of the NK_2 receptor antagonist could have countered stress-induced release of ACh, and thereby, reinstated temporal-order memory. Therefore, based on our neurochemical results (see below), it seems that the vehicle injection, although it induces an increase in ACh levels in the frontal cortex and hippocampus, is detrimental to performance, possibly caused by the stress induced by the procedure of injection. The 1 pmol dose seems to establish the optimal condition for performance (counteract stress-induced effects), while, the higher doses seem to drive the neurochemical changes too strongly, leading again to detrimental effects on memory performance. Such results seem to indicate the existence of an inverted U-shaped dose–response curve. Recently it has been reported that the most fundamental shape of dose–response, is neither threshold, nor linear, but rather U-shaped or inverted U-shaped [5]. Neuropeptides, including neuropeptide Y, SP, and others, are known to have an inverted U dose–response curve [16,22]. The doses utilized in this study were apparently on the descending limb of an inverted U-shaped dose–response curve.

Neurochemical effects of intra-septal NK2 antagonism: For investigating the effects of NK_2 receptors antagonism on cholinergic activity we chose the anesthetized preparation rather than the freely moving one in order to avoid confounding the effects of behavioral activation and pharmacological manipulation [8,10].

Based on the findings of Steinberg et al. [44] that NK_2 agonism in the medial septum enhanced ACh in the hippocampus, we anticipated that intra-septal NK_2 antagonism would decrease hippocampal ACh. Such a decrease, relative to vehicle was in fact found in the hippocampus and frontal cortex. However, from inspection of Fig. 3, it seemed likely that this apparent decrease in ACh was, in fact, a result of a blockade of an increase in ACh levels induced by the injection of vehicle solution. This interpretation receives support by the finding of significantly higher ACh levels in the vehicle as compared to the sham treated group as well as the lack of difference between the sham group as compared to the NK_2 antagonist treated groups. The likelihood of a vehicle-induced increase in ACh is lent credence to by similar findings upon vehicle injection into the nucleus basalis magnocellularis, another major source of cholinergic cells of the basal forebrain [9]. The fact that vehicle injection also increased ACh level in the frontal cortex, which is not a primary projection area of the medial septum, would support an interpretation of the vehicle effects in terms of remote, mechanically (or physiologically) induced neuronal activity.

As argued above for the behavioral study, such a vehicle effect is most likely due to (a) mechanical stimulation of neuronal activity, which can result in significant local and remote spike-and-wave activity as well as spreading depression, or (b) to direct physiological effects of local dilution with vehicle. We are not aware of studies that could hint as to how NK_2 receptor antagonism could counter such mechanically induced depolarization of cholinergic neurons. That such a minor increase in hippocampal and cortical ACh should be detrimental to learning or memory is incongruent with expectations based on the link between forebrain ACh and performance in learning tasks. Such an incongruence, however, could be explained by our interpretation of the vehicle-induced ACh effects in terms of non-physiological mechanical (or physiological, see above) artifacts. However, one should also consider that in the behavioral experiment, the animals were awake while the injections were performed. In the neurochemical experiment, the animals were anesthetized. It is possible that the anesthesia might have attenuated the putative stress-induced increase in ACh levels.

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Neurokinin$_2$-R in Medial Septum Regulate Hippocampal and Amygdalar ACh Release Induced by Intraseptal Application of Neurokinins A and B

Sandra Schäble, Joseph P. Huston, and Maria A. de Souza Silva*

ABSTRACT: The neurokinin receptors (NK-R), NK$_2$- and NK$_3$-R, have been implicated in behavioral processes, but apparently in opposite ways: while NK$_2$-R agonism disrupts memory and has anxiogenic-like action, NK$_3$-R agonists facilitate memory and display anxiolytic-like effects. Systemic application of NK$_2$-R antagonists block the release of acetylcholine (ACh) in the hippocampus, which is induced by intraseptal administration of the NK$_2$-R ligand, neurokinin A (NKA). We investigated the effects of medial septal injection of NKA and a preferred ligand of NK$_3$-R, neurokinin B (NKB), on the activity of cholinergic neurons of the basal forebrain and assessed the role of the medial septal NK$_2$-R in the control of extracellular ACh levels in cholinergic projection areas. ACh was dialysed in the frontal cortex, amygdala and hippocampus of anesthetized animals and was analysed by HPLC-EC. ACh levels in hippocampus and amygdala, but not in frontal cortex were increased after intraseptal injection of either NKA or NKB (0.1, 1, 10 μM). Application of the nonpeptidic NK$_2$-R antagonist, saredutant SR48968 (1, 10, 100 pM), followed by NKA (1 μM) or NKB (10 μM) injection into the medial septum, blocked the ACh increase in hippocampus and amygdala. These results indicate that medial septal NK$_2$-R have an important role in mediating ACh release, for one, via the septal-hippocampal cholinergic projection and, secondly, via direct or indirect route to the amygdala, but not frontal cortex. They also support the hypothesis that hippocampal cholinergic neurotransmission controls amygdala function suggesting that this interaction is regulated via NK$_2$-R in the medial septum. © 2010 Wiley-Liss, Inc.

KEY WORDS: SR48968; in vivo microdialysis; rat; hippocampus; amygdala; frontal cortex; NK-receptors

INTRODUCTION

The mammalian brain's three neurokinin receptors (NK-R), namely the NK$_1$-, NK$_2$-, and NK$_3$-R, are just beginning to be functionally characterized. Their endogenous ligands, respectively, substance P (SP), neurokinin A (NKA) and neurokinin B (NKB), have a preferred, but unspecific binding profile to these three receptors. The recent development of selective nonpeptide NK-R antagonists provides an opportunity to advance our understanding of their functions.

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The medial septum is known for its cholinergic projections to the hippocampus and is one of the brain regions with the highest levels of NK$_2$-R binding sites (Saffroy et al., 2003), and it also contains NK$_1$-R (Maeno et al., 1993) and NK$_3$-R (Shughrue et al., 1996). Substance P, the endogenous NK$_1$-R agonist, is known to influence frontal cortex ACh and behavior by injection into the nucleus basalis magnocellularis region (De Souza Silva et al., 2000). Anatomical and biochemical evidence suggests a role for all three NK-R also in septo-hippocampal cholinergic transmission: The NK$_1$- and NK$_3$-R are located on cholinergic neurons (Steinberg et al., 1998; Chen et al., 2001a,b). Cholinergic neurons of the septo-hippocampal pathway are activated by NK$_1$-, NK$_2$-, and NK$_3$-R agonists via postsynaptic mechanisms (Morozova et al., 2008). Injection of NK$_1$-, NK$_2$-, and NK$_3$-R agonists into the medial septum enhanced ACh levels in the hippocampus in anesthetized rats. In behaving animals intraperitoneal (i.p.) injection of the NK$_2$-R antagonist, saredutant SR48968, blocked the intraseptal NKA-induced increase in levels of ACh in the hippocampus (Steinberg et al., 1998). Stroking of the neck and back of the rat increased hippocampal Ach release. This effect was prevented by i.p. administration of the two nonpeptide NK$_2$-R antagonists, SR144190 (0.03–0.3 mg/kg, i.p.) and SR48968 (0.3 and 1 mg/kg, i.p.), and was reduced by intraseptal SR144190 (Steinberg et al., 1998). In rats and guinea pigs, the intracerebroventricular (i.c.v.) administration of corticotrophin releasing factor stimulated release of ACh in the hippocampus, and pretreatment with the NK$_2$-R antagonist SR48968 (i.p. 1 mg/kg), but not by NK$_1$- or NK$_3$-R antagonists, counteracted this effect (Desvignes et al., 2003).

The overall goal of the present study was (1) to investigate the effects of an intraseptal injection of the NK$_2$- and NK$_3$-R agonists, NKA and NKB, on extracellular ACh in its major cholinergic projection area, the hippocampus, as well as in the amygdala, and frontal cortex, and (2) to assess the influence of the nonpeptidic NK$_2$-R antagonist, SR48968, also applied to the medial septum, on the NKA- and NKB-induced ACh release in these cholinergic projection areas. Both, NK$_2$- and NK$_3$-Rs have been implicated in mnestic- and emotionally-relevant processes, but seemingly in contradictory directions: whereas NK$_2$-R agonism tends to disrupt memory and have anxiogenic-like action, agonists of NK$_3$-R are pro-

mnestic and display anxiolytic-like action (see discussion). A possible differential action on septo-hippocampal/amygdalar cholinergic activity was posited to possibly account for their opposite behavioral roles.

We first (a) replicated Steinberg et al.'s (1998) finding that medial septal application of NKA stimulates hippocampal release of ACh, as assessed by in vivo microdialysis and HPLC. Steinberg et al. (1998) reported that i.p. administration of SR48968 blocked this effect. Since this finding raises the question as to the site of action of the NK_2-R in the brain in mediating the septal NKA-induced ACh release, we then (b) determined whether the local injection of the nonpeptidergic NK_2-R antagonist SR48968 into the medial septum would also be able to block the hippocampal ACh release. Given the identification of NK_2-R in the septum, we hypothesized a blocking effect. Furthermore, since NK_3-R are also found in the medial septum (Maeno et al., 1993), we also examined whether (c) the intraseptal application of the endogenous NK_3-agonist NKB would have similar effects on hippocampal ACh release as NKA. Having found such an effect, we then examined (d) whether the intraseptal NK_2-R blockade would also influence this action. Furthermore, in addition to the hippocampus, (e) we measured the effects of intraseptal application of NKA and NKB on ACh in the amygdala, and frontal cortex, which has not as yet been investigated. Finally, having found positive effects also in the amygdala, but not frontal cortex, we (f) assessed the influence of intraseptal blockade of NK_2-R on this effect. On the basis of the differential binding affinity of the various neurokinins to the NK_2-R, with NKA being the preferred ligand (Almeida et al., 2004), we hypothesized that intraseptal injection of the NK_2-R antagonist SR48968 would block the release of ACh induced by intraseptal application of NKA, but not of NKB, in these two cholinergic projection sites.

MATERIALS AND METHODS

Subjects

Adult male Wistar rats obtained from the TVA (Tierversuchsanlage, University of Düsseldorf) were used for all experiments. The animals were kept under an artificial reversed 12:12 light-dark cycle (light off at 07:00 a.m.) with temperature controlled conditions (20 ± 2°C) and free access to food and water. They were housed in groups of five in translucent plastic cages (60.0 × 20.0 × 38.0 cm; length × depth × height) until the guide-cannulae were implanted; thereafter, they were housed individually. They were allowed to recover for one week postsurgery before being subjected to the microdialysis procedure. All experiments were performed in accordance with the German Animal Protection Law.

Drugs

The NK_2-R agonist NKA (Bachem, USA), NK_3-R agonist NKB (Bachem, USA) and the nonpeptidic NK_2-R antagonist saredutant SR48968 (Emonds-Alt et al., 1992) (Sanofi Recherche, France) were diluted in physiological saline (PBS). For intraseptal drug administration, an injection needle (17.1 mm long, 0.32 mm inner diameter) was inserted through a guide-cannula (16.3 mm long, 0.36 mm inner diameter) and 0.5 µl of the substance was applied using a microinfusion pump (CMA/100, Sweden) with a flow-rate of 1.0 µl/min. Following infusion the injection needle was left in place for another 30 s to allow diffusion.

Surgery

For surgery the animals were anesthetized with a mixture of ketamine hydrochloride (90.0 mg/kg; Pharmacia and Upjohn GmbH, Germany) and xylazine hydrochloride (8.0 mg/kg; Bayer, Germany). With the aid of a stereotaxic apparatus (David Kopf Instruments, USA) and according to a stereotaxic atlas (Paxinos and Watson, 1986), three stainless steel guide-cannulae for the microdialysis probes (15 mm long, 0.36 mm inner diameter) were implanted above the following brain areas: frontal cortex (AP: +3.7 mm, ML: ±3 mm, DV: −1.3 mm), amygdala (AP: −2.5 mm, ML: ±5.7 mm, DV: −7.8 mm, with 10° angle toward the midline) and hippocampus (AP: −6.0 mm , ML: ±4.8 mm , DV: −3.2 mm), coordinates were taken from bregma. The guide-cannula for microinjection was placed above the medial septum (AP: +8.2 mm; ML: ±1.6 mm; DV: −6.0 mm, with 15° angle toward the midline). All guide-cannulae were placed unilaterally and counterbalanced either in the right or left hemisphere. They were fixed with two stainless steel screws and dental cement. Animals were allowed to recover for five to seven days.

NEUROCHEMICAL MEASUREMENT

Microdialysis Procedure

The microdialysis probes were prepared according to the procedure described by (Boix et al., 1994). The experiments were conducted under urethane anesthesia (1.25 g/kg, Sigma Aldrich, USA). After the animals were anesthetized, the microdialysis probes were inserted. The probes had an active membrane length of 4.0 mm for the hippocampus and 2.0 mm for frontal cortex and amygdala. A catheter was placed into the intraperitoneal (i.p.) cavity to allow fluid supply without physical contact with the animal. The animal was placed on a heating pad in an acrylic box (45 cm × 25 cm × 22 cm) and its body temperature was maintained at 36.5 ± 0.5°C by a temperature controller (CMA/150, Sweden). The microdialysis probes were connected to a microinfusion pump (CMA/100, Sweden) and were perfused with Ringer's solution (Braun, Germany) containing neostigmine (10 µmol) at a flow rate of 2 µl/min. Samples were collected in a time-window of 10 min in vials containing 10 µl of ethylhomocholine (EHCh) solution which was used as internal standard (Potter et al., 1983). The experiments were carried out between 8:00 a.m. and 4:00 p.m.

Experiment 1: Effect of NKA and NKB Injection Into the Medial Septum on ACh in Frontal Cortex, Amygdala, and Hippocampus

Forty-one rats were randomly assigned to one of following groups: vehicle; 0.1, 1, or 10 μM NKA; 0.1, 1, or 10 μM NKB injections into the medial septum. After two hours of stabilization six baseline samples were collected at 10-min intervals. Afterwards, vehicle, NKA (0.1, 1, or 10 μM) or NKB (0.1, 1, or 10 μM) were injected into the medial septum, and another 10 samples were collected. After each sampling the animal received an injection of 0.1 ml Ringer's solution through the i.p. catheter and the depth of anesthesia was adjusted with urethane if necessary.

Experiment 2: Effects of Pretreatment With the NK_2-R Antagonist, SR48968, on ACh in Amygdala and Hippocampus Induced by Intraseptal Injection of NKA and NKB

Sixty-three rats were randomly assigned to one of following groups: vehicle + NKA (1 μM); vehicle + NKB (10 μM); 1, 10, or 100 pmol NK_2 receptor antagonist SR48968 + NKA (1 μM); 1, 10, or 100 pmol SR48968 + NKB (10 μM) injections into the medial septum.

After the stabilization period of two hours six baseline samples were collected. Afterwards, either vehicle or SR48968 (1, 10, and 100 pmol) followed by NKA (1 μM) or NKB (10 μM) were injected into the medial septum. Another 10 samples were collected in vials containing 10 μl of EHCh solution.

HPLC Analysis

After collection, the samples were analysed for the concentration of ACh by HPLC-EC according to the method of Damsma et al. (1987). EHCh was used as internal standard. Briefly, ACh separation was achieved using an analytical column (75 mm length, 2 mm inner diameter) filled with ChromSpher 5C18 (Merck, Germany) and loaded with sodiumdodecylsulfate (Sigma Aldrich, USA). The enzyme reactor was filled with LiChrosorb-NH_2 (Merck, Germany), activated by glutardialdehyde (Merck, Germany) and loaded with acetylcholinesterase (Sigma Aldrich, USA) and cholineoxidase (Sigma Aldrich, USA). The enzymes were covalently bound to the stationary phase. The enzyme reactor was attached to the end of the analytical column. On passage through the enzyme reactor, ACh was converted to hydrogenperoxide, which was electrochemically detected at a platinum electrode set at a potential of 0.350 mV vs. an Ag/AgCl reference electrode (Antec Leyden, Netherlands). The mobile phase was composed of 1 mM tetramethylammoniumchloride (Merck, Germany) and 0.18 M K_2HPO_4 (Merck, Germany), adjusted to pH 8.0 with KH_2PO_4 (Merck, Germany), and delivered by a HPLC pump (Merck, Germany) at a flow rate of 0.3 ml/min. The data were collected, processed and stored with the help of computer software (Chrom Perfect, Justice Laboratory Software).

Histology

After the microdialysis experiment the animals were deeply anesthetized with pentobarbital (Sanofi Ceva, France) and perfused with PBS, followed by 10% phosphate buffered formalin solution through the left heart ventricle. The brain was removed and stored in 10% formalin until histological verification. The brain was cross-sectioned in 60 μm thick slices with a cryostat (Leica, Germany), slices were stained with cresylviolet (Sigma Aldrich, USA) for verification of injection site and placement of microdialysis probes according to the atlas of Paxinos and Watson (1986). Only animals with correct injection site and microdialysis probe placement were considered for statistical analysis.

Statistical Analysis

The neurochemical data were analyzed for each brain area separately. ACh concentrations were converted into percentage of baseline (the mean of six baseline samples set as 100%). A two-way ANOVA with repeated measures was calculated with the factors "time" and "group." Post hoc Dunnett tests were applied for between-group comparisons. When appropriate, this was followed by a one-way ANOVA and post hoc Dunnett test to analyze group differences at individual time points. ANOVA was carried out for each brain region separately. The *P*-values given are two-tailed and were considered significant if $\alpha \leq 0.05$. The software SPSS 15.0 was used for all analyses.

RESULTS

Experiment 1: Effect of NKA and NKB Injection Into Medial Septum on ACh the Frontal Cortex, Amygdala, and Hippocampus

NKA injection into medial septum

In this experiment 24 animals were considered for statistical analysis. The sample sizes were as follows: frontal cortex: vehicle, $n = 6$; 0.1 μM NKA, $n = 6$; 1 μM NKA, $n = 6$; 10 μM NKA, $n = 6$; amygdala: vehicle, $n = 6$; 0.1 μM NKA, $n = 6$; 1 μM NKA, $n = 6$; 10 μM NKA, $n = 6$; hippocampus: vehicle, $n = 6$; 0.1 μM NKA, $n = 6$; 1 μM NKA, $n = 6$; 10 μM NKA, $n = 6$.

The mean ± S.E.M. basal levels of ACh in dialysates were: frontal cortex 0.713 ± 0.045 pmol/15 μl; amygdala 0.958 ± 0.071 pmol/15 μl; hippocampus 0.831 ± 0.069 pmol/15 μl.

The results for hippocampus and amygdala are summarized in Figure 1. In the frontal cortex the two-way repeated measures ANOVA revealed a main effect of time ($F_{15,30} = 3.791$, $P < 0.001$) but no main effect of group ($F_{3,20} = 1.194$, $P > 0.05$) nor an interaction ($F_{45,300} = 1.070$, $P > 0.05$) in ACh levels. In the amygdala two-way repeated measures ANOVA revealed a main effect of time ($F_{15,300} = 7.873$, $P < 0.001$)

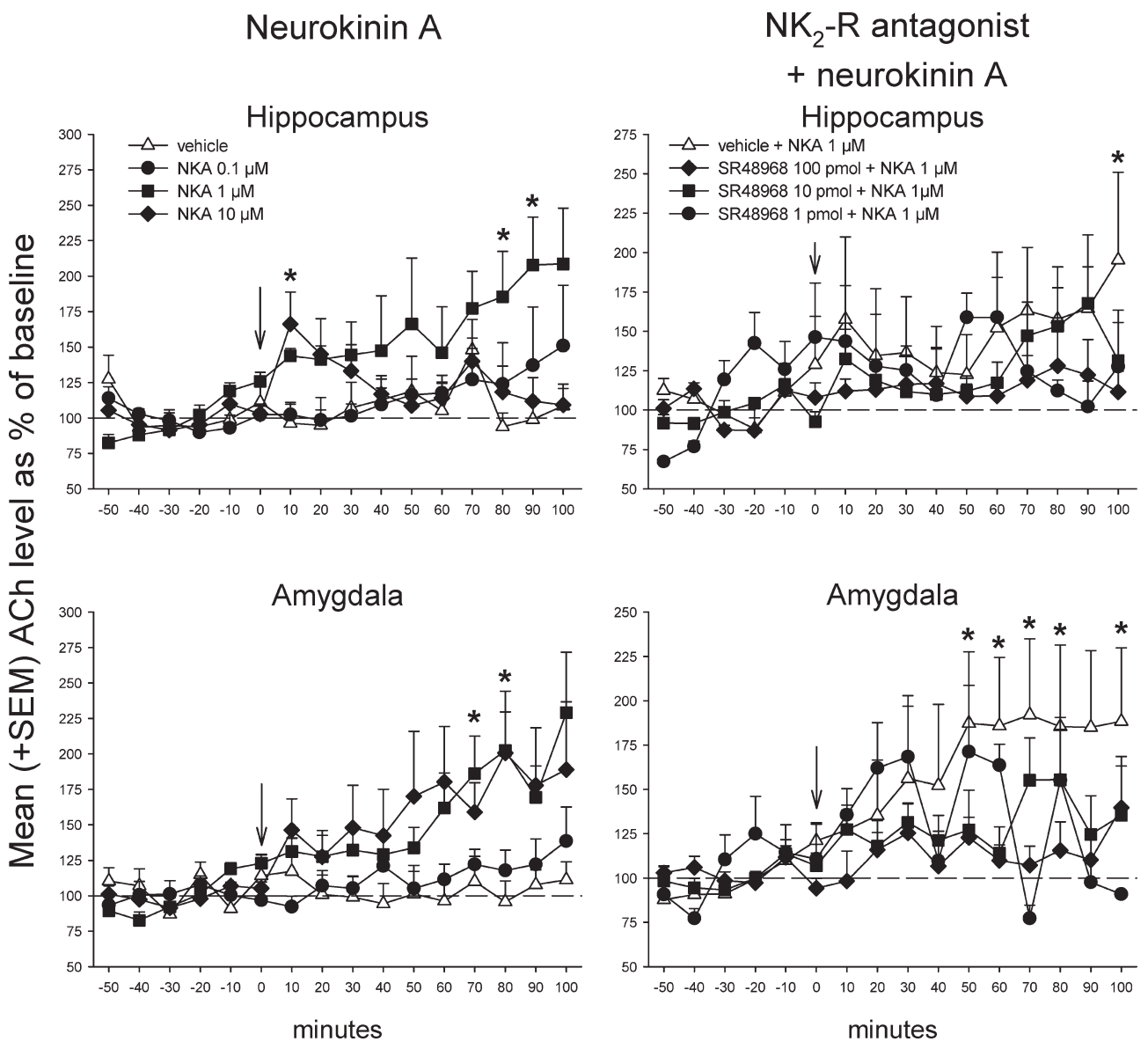

FIGURE 1. Left: Effect of injection of vehicle or 0.1, 1, and 10 µM NK_2-agonist NKA into the medial septum on extracellular ACh levels in the amygdala and hippocampus in anesthetized rats. Right: Effect of pretreatment with intraseptal injection of vehicle or SR48968 (SR 1, 10, and 100 pmol) on the effects of intraseptal NKA (1 µM). The values are given as mean + SEM percent of baseline (average of the six baseline samples taken as 100%). Arrows indicate the time-point of the injection. *$P \leq 0.05$.

and a time × group interaction ($F_{45,300} = 2.000$, $P < 0.001$) but no main effect of group ($F_{3,20} = 2.457$, $P > 0.05$) in ACh levels. Post hoc Dunnett test revealed no significant difference between vehicle and NKA treated animals. One-way ANOVA showed that the treatment with the NK_2-R agonist NKA significantly increased ACh release 70 min ($F_{3,23} = 3.123$, $P = 0.049$) and 80 min ($F_{3,23} = 4.028$, $P = 0.022$) after injection. Post hoc Dunnett test revealed a dose-dependent induced increase in ACh release (70 min: 1 µM NKA $P = 0.033$; 80 min: 1 µM NKA $P = 0.034$ and 10 µM NKA $P = 0.037$) compared to vehicle controls. In the *hippocampus* two-way repeated measures ANOVA revealed a main effect of time ($F_{15,300} = 4.374$, $P < 0.001$) and a time × group interaction ($F_{45,300} = 1.700$, $P = 0.005$) but no main effect of group ($F_{3,20} = 2.299$, $P > 0.05$) in ACh levels. Post hoc Dunnett test revealed no significant difference between vehicle and NKA treated animals. One-way ANOVA showed that the treatment with NKA significantly increased ACh release 10 min ($F_{3,23} = 5.653$, $P = 0.006$) after injection. Post hoc Dunnett test revealed a dose-dependent induced increase in ACh release (10 min: 10 µM NKA $P = 0.006$; 80 min: 1 µM NKA $P = 0.036$; 90 min: 1 µM NKA $P = 0.038$) compared with vehicle controls.

Thus, the application of NKA into the medial septum did not affect ACh levels in the frontal cortex compared with vehicle controls. However, ACh levels in the amygdala and hippocampus were increased after NKA injection into the medial septum compared with vehicle controls.

NKB Injection Into Medial Septum

Twenty-four animals were considered for statistical analysis. The sample sizes were as follows: frontal cortex: vehicle, $n = 6$; 0.1 μM NKB, $n = 5$; 1 μM NKB, $n = 6$; 10 μM NKB, $n = 5$; amygdala: vehicle, $n = 6$; 0.1 μM NKB, $n = 5$; 1 μM NKB, $n = 5$; 10 μM NKB, $n = 6$; hippocampus: vehicle, $n = 6$; 0.1 μM NKB, $n = 5$; 1 μM NKB, $n = 6$; 10 μM NKB, $n = 6$.

In the frontal cortex and amygdala the two-way repeated measures ANOVA revealed main effects of time (frontal cortex: $F_{15,270} = 4.164$, $P < 0.001$; amygdala: $F_{15,270} = 5.072$, $P < 0.001$) but no main effects of group (frontal cortex: $F_{3,18} = 0.716$, $P > 0.05$; amygdala: $F_{3,18} = 1.223$, $P > 0.05$) nor an interaction (frontal cortex: $F_{45,270} = 0.541$, $P > 0.05$; amygdala: $F_{45,270} = 1.155$, $P > 0.05$) in ACh levels. In the hippocampus two-way repeated measures ANOVA revealed a main effect of time ($F_{15,285} = 4.433$, $P < 0.001$) and a time × group interaction ($F_{45,285} = 1.542$, $P = 0.020$) but no main effect of group ($F_{3,19} = 1.542$, $P > 0.05$) in ACh levels. Post hoc Dunnett test revealed no significant dose-dependent difference between vehicle and NKB treated animals. One-way ANOVA showed that the treatment with NKB significantly increased ACh release 80 min ($F_{3,22} = 3.924$, $P = 0.025$) after injection. Post hoc Dunnett test revealed a dose-dependent induced increase in ACh release (80 min: 0.1 μM NKB $P = 0.008$) compared with vehicle controls.

In summary, the application of NKB into the medial septum enhanced ACh levels in the hippocampus, but not in the frontal cortex and amygdala compared with vehicle controls.

Experiment 2: Effects of Pretreatment With the NK_2-R Antagonist SR48968 on ACh in Amygdala and Hippocampus Induced by Intraseptal NKA and NKB Injection

The mean ± SEM basal levels of ACh in dialysates were: amygdala 0.749 ± 0.044 pmol/15 μl; hippocampus 0.545 ± 0.036 pmol/15 μl.

SR48968 and NKA

Twenty-three animals were considered for statistical analysis. The sample sizes were as follows: amygdala: vehicle + NKA, $n = 5$; 1 pmol SR48968 + NKA, $n = 5$; 10 pmol SR48968 + NKA, $n = 7$ and 100 pmol SR48968 + NKA, $n = 6$; hippocampus: vehicle + NKA, $n = 5$; 1 pmol SR48968 + NKA, $n = 5$; 10 pmol SR48968 + NKA, $n = 7$ and 100 pmol SR48968 + NKA, $n = 6$.

The results are summarized in Figure 1. In the amygdala two-way repeated measures ANOVA revealed a main effect of time ($F_{15,285} = 4.763$, $P < 0.001$) and a time × group interaction ($F_{45,285} = 2.556$, $P < 0.001$) but no main effect of group ($F_{3,19} = 1.999$, $P > 0.05$) in ACh levels. Post hoc Dunnett test revealed no significant difference between the treatment vehicle + NKA and SR48968 + NKA. One-way ANOVA showed that the pre-treatment with the NK_2-R antagonist SR48968 significantly reduced the ACh release elicited by vehicle + NKA 60 min ($F_{3,22} = 3.342$, $P = 0.041$), 70 min ($F_{3,22} = 4.447$, $P = 0.016$), 80 min ($F_{3,22} = 3.588$, $P = 0.033$) and 100 min ($F_{3,22} = 4.528$, $P = 0.015$) after injection. Post hoc Dunnett test revealed a dose-dependent induced decrease in ACh release (50 min: 10 pmol SR48968 + NKA $P = 0.020$; 60 min: 100 pmol SR48968 + NKA $P = 0.045$; 70 min: 1 pmol SR48968 + NKA $P = 0.007$ and 100 pmol SR48968 + NKA $P = 0.038$; 80 min: 1 pmol SR48968 + NKA $P = 0.014$; 100 min: 1 pmol SR48968 + NKA $P = 0.016$ and 10 pmol SR48968 + NKA $P = 0.009$) compared with vehicle + NKA controls. In the hippocampus two-way repeated measures ANOVA revealed a main effect of time ($F_{15,285} = 3.559$, $P < 0.001$) and a time × group interaction ($F_{45,285} = 1.806$, $P = 0.002$) but no main effect of group ($F_{3,19} = 0.811$, $P > 0.05$) in ACh levels. Post hoc Dunnett test revealed no significant difference between the treatment vehicle + NKA and SR48968 + NKA. One-way ANOVA showed that the pre-treatment with SR48968 significantly reduced the increase in ACh release elicited by vehicle + NKA 100 min ($F_{3,22} = 3.177$, $P = 0.048$) after injection. Post hoc Dunnett test revealed a dose-dependent induced decrease in ACh release (100 min: 1 pmol SR48968 + NKA $P = 0.021$) compared with vehicle + NKA controls.

Accordingly, pretreatment with the NK_2-R antagonist SR48968 reduced ACh levels induced by intraseptal NKA administration in the amygdala and hippocampus.

SR48968 and NKB

Twenty-five animals were considered for statistical analysis. The sample sizes were as follows: amygdala: vehicle + NKB, $n = 5$; 1 pmol SR48968 + NKB, $n = 5$; 10 pmol SR48968 + NKB, $n = 7$ and 100 pmol SR48968 + NKB, $n = 7$; hippocampus: vehicle + NKB, $n = 5$; 1 pmol SR48968 + NKB, $n = 5$; 10 pmol SR48968 + NKB, $n = 8$ and 100 pmol SR48968 + NKB, $n = 7$.

The results are summarized in Figure 2. In the amygdala two-way repeated measures ANOVA revealed a main effect of time ($F_{15,300} = 7.503$, $P < 0.001$), group ($F_{3,20} = 3.830$, $P = 0.026$) and a time × group interaction ($F_{45,300} = 1.911$, $P = 0.001$) in ACh levels. Post hoc Dunnett test revealed a significant dose-dependent difference in 10 pmol SR48968 + NKB ($P = 0.039$) and 100 pmol SR48968 + NKB ($P = 0.017$) compared with vehicle + NKB. One-way ANOVA showed that the pretreatment with SR48968 significantly reduced the increase in ACh release elicited by vehicle + NKB 40 min ($F_{3,23} = 4.138$, $P = 0.020$), 60 min ($F_{3,23} = 3.412$, $P = 0.037$), 70 min ($F_{3,23} = 4.208$, $P = 0.018$) and 100 min ($F_{3,23} = 3.620$, $P = 0.031$) after injection. Post hoc Dunnett test revealed a dose-dependent induced decrease in ACh release (40 min: 10 pmol SR48968 + NKB $P = 0.015$ and 100 pmol SR48968 + NKB $P = 0.013$; 60 min: 100 pmol SR48968 + NKB $P = 0.017$; 70 min: 100 pmol SR48968 + NKB $P = 0.008$; 90 min: 100 pmol + NKB $P = 0.043$; 100 min: 100 pmol SR48968 + NKB $P = 0.015$) compared with

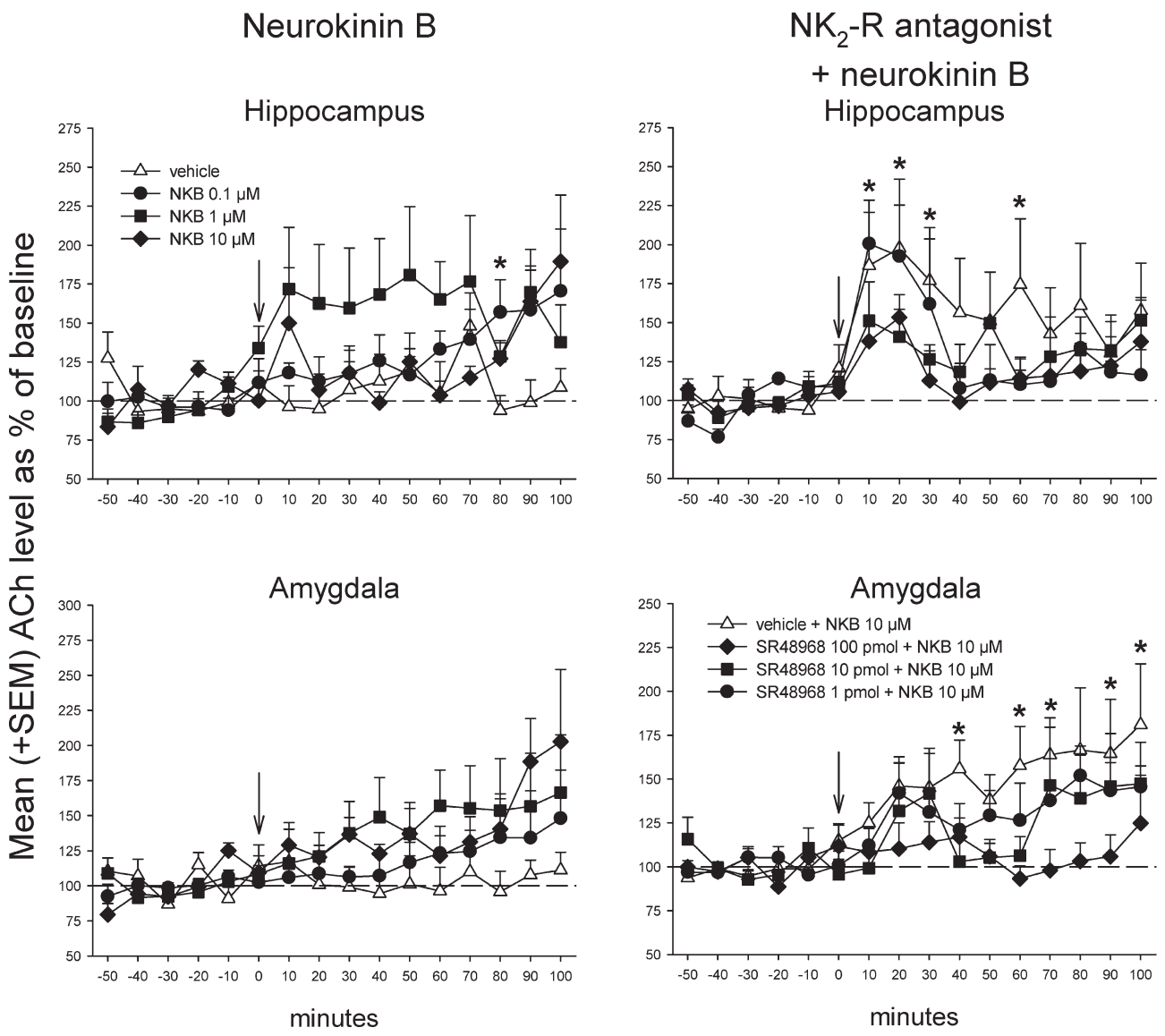

FIGURE 2. Left: Effect of injection of vehicle or 0.1, 1, and 10 µM NK_2-agonist NKB into the medial septum on extracellular ACh levels in the amygdala and hippocampus in anesthetized rats. Right: Effect of pretreatment with intraseptal injection of vehicle or SR48968 (SR 1, 10, and 100 pmol) on the effects of intraseptal NKB (10 µM). The values are given as mean + SEM percent of baseline (average of the six baseline samples taken as 100%). Arrows indicate the time-point of the injection. *$P \leq 0.05$.

vehicle + NKB controls. In the hippocampus two-way repeated measures ANOVA revealed a main effect of time ($F_{15,315} = 6.829$, $P < 0.001$) and a time × group interaction ($F_{45,315} = 1.746$, $P = 0.003$) but no main effect of group ($F_{3,21} = 2.768$, $P > 0.05$) in ACh levels. Post hoc Dunnett test revealed a significant dose-dependent difference between 10 pmol SR48968 + NKB ($P = 0.039$) and vehicle + NKB. One-way ANOVA showed that the pretreatment with SR48968 significantly reduced the increase in ACh release elicited by vehicle + NKB 10 min ($F_{3,24} = 5.143$, $P = 0.008$) and 20 min ($F_{3,24} = 3.217$, $P = 0.044$) after injection. Post hoc Dunnett test revealed a dose-dependent induced decrease in ACh release (10 min: 10 pmol SR48968 + NKB $P = 0.002$ and 100 pmol SR48968 + NKB $P = 0.031$; 20 min: 1 pmol SR48968 + NKB $P = 0.039$ and 10 pmol SR48968 + NKB $P = 0.034$; 30 min: 1 pmol SR48968 + NKB $P = 0.038$; 60 min: 10 pmol + NKB $P = 0.021$) compared with vehicle + NKB controls.

Thus, the NK_2-R antagonist SR48968 reduced ACh levels elicited by NKB in the amygdala and hippocampus. The results of Experiments 1 and 2 are summarized in Table 1.

DISCUSSION

The intent of this study was to investigate the effects of (a) applying the endogenous NK_2- and NK_3-R agonists, NKA and

TABLE 1.

Summarized Effects of Injection of the Endogenous NK_2- and NK_3-R Agonists, NKA and NKB, into the Medial Septum on ACh Levels in the Frontal Cortex, Amygdala and Hippocampus, and the Impact of Medial Septal Pretreatment with the NK_2-R Antagonist SR48968

ACh level in:	NK_2-R agonist NKA	NK_2-R agonist NKB	NK_2-R antagonist SR48968 + NKA	NK_2-R antagonist SR48968 + NKB
Frontal cortex	–	–		
Amygdala	↑	–	↓	↓
Hippocampus	↑	↑	↓	↓

NKB, into the medial septum on extracellular ACh neurotransmission in projection areas of the cholinergic neurons of the basal forebrain, namely the frontal cortex, amygdala and hippocampus, and (b) to examine the effects of blockade of NK_2-R by SR48968 in the medial septum on the NKA- and NKB-induced ACh release.

On the basis of identified NK_2- and NK_3-R in the medial septum (Shughrue et al., 1996; Saffroy et al., 2001) and on Steinberg et al.'s (1998) results, we hypothesized that injection of the endogenous NK_2- and NK_3-R agonists, respectively, NKA and NKB, into the medial septum would increase extracellular ACh in the hippocampus, as assessed by in vivo microdialysis and HPLC. In fact, we found that both neurokinins enhanced ACh release, not only in the hippocampus, but also in the amygdale, but not in the frontal cortex. We then found that injection of the nonpeptidergic NK_2-R antagonist SR48968 into the medial septum blocked the intraseptal NKA- and NKB-induced ACh release in both the hippocampus and amygdala.

NK_2-R Agonism in Medial Septum by NKA Application

In replication of Steinberg et al.'s (1998) results, we found that injection of the endogenous NK_2-R agonist NKA into the medial septum increased ACh levels in the hippocampus dose-dependently. Whereas the highest dose (10 μM) significantly increased ACh already within 10 min of application, the medium dose (1 μM) led to a gradual increase in ACh, which peaked at 80- and 90-min postinjection.

Application of NKA into the medial septum also increased ACh in the amygdala, with the strongest effect in the 10 μM treatment group, displaying a gradual increase in ACh, which peaked at 70- and 80-min after injection. No significant effects of intraseptal NKA were found on ACh in the frontal cortex.

NKA is the preferred endogenous ligand for the NK_2-R, which are predominantly expressed in the hippocampus, thalamus, septum, and frontal cortex (Hagan et al., 1993; Saffroy et al., 2001, 2003). They mediate excitatory responses in septo-hippocampal cholinergic neurons (Morozova et al., 2008). Steinberg et al. (1998) showed that NK_2-R activation by NKA facilitated hippocampal ACh release induced also by sensory stimulation. This finding is consistent with evidence indicating that ACh release in the hippocampus is activated by behaviorally relevant stimuli (Nilsson et al., 1990; Imperato et al., 1991; Moore et al., 1992; Rosenblad and Nilsson, 1993; Acquas et al., 1996). In vivo microdialysis studies have demonstrated that sensory stimulation (Nilsson et al., 1990; Inglis and Fibiger, 1995), a novel environment (Inglis and Fibiger, 1995; Aloisi et al., 1997; Giovannini et al., 1998; Ceccarelli et al., 1999) and arousal (Inglis and Fibiger, 1995) increase ACh levels in cerebral cortex and hippocampus. The distribution of NK_2-R within "limbic" sites suggests an involvement in emotional processes, and several studies have demonstrated effects of NK_2-R agonists and antagonists on anxiety. For example, i.c.v. application of the endogenous NK_2-R agonist NKA had anxiogenic-like effects in mice (Teixeira et al., 1996), as did the NK_2-R agonist GR64349 in rats (Stratton et al., 1993). The central injection of NKA had anxiogenic- and depressive-like effects that were diminished by NK_2-R antagonists (Griebel, 1999; Holmes et al., 2003). Accordingly, the NK_2-R antagonist, saredutant SR48968, given i.p., had anxiolytic- and antidepressant-like activity (Micale et al., 2008). Fear, presumably associated with arousal, strongly activates the septo-hippocampal pathway (Acquas et al., 1996; Ceccarelli et al., 1999), and this action may be mediated by medial septum NK_2-R.

Our finding that intraseptal NKA activates cholinergic transmission in the amygdala is novel. It remains to be determined whether this effect is due to direct or indirect pathways from septum to the amygdala. Our results support the hypothesis that hippocampal cholinergic neurotransmission controls amygdala function (Calandreau et al., 2006) and suggest that this interaction could be regulated via NK_2-R activation in the medial septum. Such an interaction could also provide a functional link to the anxiogenic effects attributed to centrally applied NK_2-R agonists.

Blockade of NKA-Induced ACh Release by Medial Septal NK_2-R Antagonism

Steinberg et al. (1998) had found that i.p. administration of the NK_2-R antagonist SR48968 blocked the cholinergic stimulating effect of intraseptal NKA in the hippocampus. Since this finding raises the question as to the site of action of the NK_2-R in the brain in mediating the NKA-induced ACh release, we asked whether the local injection of SR48968 into the medial septum would also be able to block the hippocampal ACh release induced by septal NKA application. Given the identification of NK_2-R in the septum, we hypothesized that a blockade of septal NK_2-R would counteract the effects of local NKA on hippocampal ACh release. As predicted, ACh release caused by application of NKA into the medial septum was reduced by the NK_2-R antagonist SR48968 in the amygdala and hippocampus.

The hippocampus, together with the septum, contains high labeling of NK_2-sensitive NKA (Saffroy et al., 2001), and thus can account for the effects of i.p. and i.c.v. administered NK_2-R antagonists on septal NKA-induced hippocampal ACh release. However, the hippocampal NK_2-R cannot account for

the inhibitory effects of intraseptal injection of the NK_2-R blocker on septal NKA-induced hippocampal ACh release. This suggests that NK_2-R in the medial septum directly influence the action of the septal-hippocampal cholinergic pathway.

Our finding that blockade of NK_2-R in the medial septum is effective in modulating septal NKA-induced cholinergic responses, is particularly important, since NK_2-R have very low levels in the brain and are mostly found in the periphery, such as the gastrointestinal tract, where they are involved in contraction of smooth muscles. Our results, thus, strengthen the evidence for significant central action of these receptors and their role in mediating effects of the endogenous NKA (and NKB).

NK_2-R antagonist have consistently been found to attenuate NK-induced release of ACh (Hagan et al., 1993): e.g., ACh release in the hippocampus, induced by central corticotrophin-releasing factor (CRF) administration or by stroking the back and neck of rats, were antagonized by systemic NK_2-R antagonism; Morozova et al. (2008) demonstrated electrophysiologically, that septo-hippocampal cholinergic activation by a NK_2-R agonist was blocked by NK_2-R antagonism. Our results indicate that the medial septal NK_2-R may be critically involved in regulating amygdala and hippocampal ACh release. The inhibition of ACh release under stimulated conditions by SR48968 suggests that cholinergic projections from the medial septum may be regulated by the NK_2-R impinging on the cholinergic cell bodies where the NK_2-R antagonist acts.

Our finding that antagonism of medial septum NK_2-R blocked the ACh release in the amygdala induced by intraseptal administration of the NKA has behavioral implications: NK_2-R antagonism has been shown to play a role in the modulation of stress-related behaviors: e.g., i.c.v. injection of CRF also induced hippocampal ACh release. Since this effect, as well as the stroking-induced release of hippocampal ACh were attenuated by antagonism of NK_2-R and CRF_1-R, it was suggested that stress-induced hippocampal ACh release is controlled by the NK_2-R agonist NKA (Desvignes et al., 2003). Systemic NK_2-R antagonism by SR48968 reduced anxiety-like behavior associated to stress and to a threatening stimulus (Griebel et al., 2001a,b; Salome et al., 2006; Louis et al., 2008; Micale et al., 2008). The blockade of NKA-induced release of ACh in the amygdala by the NK_2-R antagonist may relate to the known antianxiety activity of NK_2-R antagonists (Stratton et al., 1993; Walsh et al., 1995; Teixeira et al., 1996). Given the link also between NK_2-R and anxiety and depressive-like behavior discussed above, our finding that medial septum NK_2-R antagonized NKA-induced ACh release, also suggests an involvement of these receptors in the processing of emotionally relevant behaviors via septo-hippocampal and/or septo-amygdalar networks.

NK_3-R Agonism in Medial Septum by NKB Application

Others have shown that injection of the NK_3-R agonist, senktide, into the medial septum increases ACh level in the hippocampus (Steinberg et al., 1995; Marco et al., 1998). In support of these results, we here show that the endogenous NK_3-R agonist ligand, NKB, also increased ACh levels in the hippocampus, with the strongest effect in the 1-μM treatment group. There were no significant effects in the frontal cortex. In the amygdala there is a nonsignificant increase in ACh (inspection of Fig. 2 suggests that a higher *n* might have given a positive result). However, intraseptal administration of 10 μM of NKB given alone induced a significantly increase in ACh higher than when given together with the NK_2-R antagonist (see below and Fig. 2), indicating a facilitative action on ACh release in the amygdala.

NKB is very selective toward the NK_3-R, but has some affinity for the NK_2-R (Alvaro and Di Fabio, 2007; Sarau et al., 1997). NK_3-R are expressed throughout the brain, including the hippocampus, frontal cortex, medial septum, hypothalamus, and amygdala (Ding et al., 1996; Shughrue et al., 1996; Mileusnic et al., 1999; Duarte et al., 2006), and, both, NKB and NK_3-R, are present in the septum (Dam et al., 1990; Ding et al., 1996). At least 66% of the cholinergic neurons in the medial septum express NK_3-R, suggesting a close link between NKB and cholinergic neurons (Chen et al., 2001a). The NK_3-R agonist senktide was found to activate cholinergic neurons in the septo-hippocampal pathway (Morozova et al., 2008), to increase hippocampal ACh release following intraseptal application (Marco et al., 1998) and to increase striatal ACh release (Steinberg et al., 1995); besides, NKB induced ACh release in striatal slices (Arenas et al., 1991).

Senktide has a number of behavioral effects, including memory-facilitating action (Kameyama et al., 1998; Ukai et al., 1998; Zlomuzica et al., 2008). In aged rats, subcutaneously (s.c.) administered senktide increased ACh in hippocampus, amygdala and frontal cortex and had promnestc effects, along with anxiolytic and antidepressant-like action (Schäble et al., 2010 in preparation). I.c.v. application of senktide was also found to induce anxiolytic-like activity in mice (Teixeira et al., 1996; Ribeiro et al., 1999). Thus, based on the central and systemic behavioral effects of senktide, there is compelling evidence that central NK_3-R have a modulatory role in anxiety and learning processes, and it seems likely that the forebrain cholinergic systems participate in these actions. However, studies on the behavioral and neurochemical actions of NKB are still sparse.

Blockade of NKB-Induced ACh Release by Medial Septal NK_2-R Antagonism

We found that the release of ACh in the amygdala and hippocampus in response to application of NKB into the medial septum was decreased by intraseptal injection of the NK_2-R antagonist SR48968, as was shown with NKA. Application of the NK_3-R agonist senktide into the septal area stimulated hippocampal ACh release, but this was not blocked by the NK_2-R antagonist SR144190 given i.p. (Steinberg et al., 1998). And, senktide-induced stimulation of ACh in the striatum also was not reduced by i.p. SR48968 (Steinberg et al., 1995). In this study blockade of the NKB-induced ACh was found by SR48968 applied locally into the medial septum, suggesting a NK_2-R-

mediated effect of NKB in the medial septum. NKB could act on cholinergic neurons containing both NK_2- and NK_3-R in its effects on ACh release in the septo-hippocampal pathway.

Our results suggest a cross-activity of the NK_2-R antagonist SR48968 on NK_2- and NK_3-R, with no clear differential effects on the activated cholinergic septo-hippocampal pathway engendered by the endogenous agonists NKA and NKB. The present study provide additional evidence that ligand cross-interaction occurs between neurokinins and their receptors (Liu et al., 1994).

Behavioral Implications

We posited that a differential action of the NKA and NKB neurokinins on the medial septal-hippocampal/amygdalar/frontal cortex cholinergic systems with respect to NK_2-R antagonism could account for their apparently opposite behavioral roles. NK_3-R and NK_2-R seem to have opposite effects in relation to memory- and fear-related behaviors: Their roles in stress-and fear-like behaviors are discussed above. With respect to memory tasks, for example, whereas the NK_3-R agonist senktide (i.p.) had memory-promoting effects (Zlomuzica et al., 2008), the NK_2-R seems disruptive to memory-related performance, since NK_2-R antagonism in the medial septum improved object memory (Schäble et al., 2010). The attenuating effects of NK_2-R antagonism on NKA-induced ACh release in cholinergic projection areas may be relevant to the finding that injection of an NK_2-R antagonist into the medial septum unexpectedly partly reversed a saline-evoked decrement in temporal order memory. Furthermore, injection of the NK_2-R antagonist into the medial septum also decreased levels of ACh in the hippocampus and frontal cortex (although, this decrease was likely the result of the blockade of a saline-induced increase in ACh levels) (Schäble et al., 2010). Although this attenuation of hippocampal ACh is consistent with the findings of NK_2-R agonist induced increases in hippocampal ACh, and the blockade of this by intraseptal NK_2-R antagonism, a promnestic effect seems at odds with the neurochemical results. It is not known whether intraseptal NKA (NK_2-R agonism) would accordingly disrupt memory performance, although such an action could be surmised from the consensus that such agonism is related to stress-, anxiogenic-, and pro-depression-like action. In any case, contrary to expectations, the results of this study do not indicate an explanation of the differential behavioral actions of the NK_2- and NK_3-R in terms of differential medial septal modulation of hippocampal, amygdalar, and frontal cortex cholinergic activity.

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Neurokinin$_3$-R agonism in aged rats has anxiolytic-, antidepressant-, and promnestic-like effects and stimulates ACh release in frontal cortex, amygdala and hippocampus

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Abstract

Neurokinin-3 receptors (NK_3-R) are localized in brain regions which have been implicated in processes governing learning and memory as well as emotionality. The effects of acute subcutaneous (s.c.) senktide (0.2 and 0.4 mg/kg), a NK_3-R agonist, were tested in aged (23–25 month old) Wistar rats: (a) in an episodic-like memory test, using an object discrimination task (this is the first study to test for deficits in episodic-like memory in aged rats, since appropriate tests have only recently became available); (b) on parameters of anxiety in an open field test, (c) on indices of depression in the forced swimming test and (d) on the activity of cholinergic neurons of the basal forebrain, using *in vivo* microdialysis and HPLC. Neither the saline-, nor senktide-treated aged animals, exhibited episodic-like memory. However, the senktide-, but not the vehicle-treated group, exhibited object memory for spatial displacement, a component of episodic memory. Senktide injection also had anxiolytic- and antidepressant-like effects. Furthermore, the active doses of senktide on behavior increased ACh levels in the frontal cortex, amygdala and hippocampus, suggesting a relationship between its cholinergic and behavioral actions. The results indicate cholinergic modulation by the NK_3-R in conjunction with a role in the processing of memory and emotional responses in the aged rat.

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1. Introduction

Neurokinins belong to the neuropeptide family of tachykinins, which include substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) as the preferred endogenous ligands of three distinct neurokinin receptors: the NK_1-, NK_2- and NK_3-R. These receptors have been identified in the brain, with widespread distribution of NK_1- and NK_3-R, whereas the NK_2-R distribution is restricted to a few brain areas (Chen et al., 2001a,b; Saffroy et al., 2003).

Although relatively little is known about the behavioral and pharmacological mechanisms mediated by the NK_3-R, recent results suggest an involvement in processes underlying learning and memory (Siuciak et al., 2007; Zlomuzica et al., 2008) as well as emotionality (Ribeiro et al., 1999; Salome et al., 2006). For instance, promnestic effects of NK_3-R agonism were described in spatial memory tasks in mice (Kameyama et al., 1998; Ukai et al., 1998; Zlomuzica et al., 2008), and disruption of NK_3-R resulted in opposite effects in spatial memory and in avoidance tasks (Siuciak et al., 2007). Moreover, it was shown, that NK_3-R activation has reinforcing properties in a conditioned place preference paradigm (Ciccocioppo et al., 1998). On the other hand, with respect to its involvement in emotionality, current available studies provide contradictory results: In gerbils blockade of the NK_3-R had anxiolytic-like action (Salome et al., 2006), while in mice such an effect was found with NK_3-R agonism (Ribeiro et al., 1999). Similarly, antidepressant-like activity was found with NK_3-R agonists in mice (Panocka et al., 2001), but with NK_3-R antagonists in rats (Dableh et al., 2005).

With age, humans show a decline in memory functions, which can vary from mild impairment to debilitating cases of Alzheimer's disease. Individuals with mild cognitive impairment (MCI) are at risk of developing Alzheimer's disease (Arnaiz and Almkvist, 2003). The aged rat (18–24 months old rats) has shown to be a very useful model of the aged human (Ingram et al., 1994; Gallagher and Pelleymounter, 1988; Gallagher et al., 2003; LaSarge et al., 2007). In aged rats, degeneration of cholinergic cells of the basal forebrain has been associated with learning/memory impairments (Fischer et al., 1989; Hellweg et al., 1990; Martinez-Serrano et al., 1995). A similar relationship between cholinergic degeneration and cognitive impairment is also seen in patients affected with Alzheimer's disease (Patel and Tariot, 1991; Van den Berg et al., 2000).

Acute local application of NKB into the nucleus basalis magnocellulatis (NBM) prevented the decline in cortical ChAT activity associated with injection of NMDA into the NBM, attenuated a reference memory deficit in the radial maze produced by entorhinal cortex lesions (Wenk et al., 1997), and reversed effects of Aβ toxicity, which appears to be the main constituent of amyloid plaques, a hallmark of Alzheimer's disease (Mantha et al., 2006). These results support the hypothesis of potential beneficial function of the NK_3-R in the aging brain. Besides, NK_3-R are localized on basal forebrain cholinergic neurons (Chen et al., 2001a). Senktide, which was shown to be a highly potent NK_3-R agonist in rats, mice, gerbils and guinea pigs (Massi et al., 2000), increased striatal ACh when infused into the striatum (Steinberg et al., 1995), and hippocampal ACh when applied into the medial septum (Marco et al., 1998).

The present study aimed to assess the effects of the NK_3-R agonist senktide on learning and memory as well as emotionality and cholinergic neurotransmission in the aged rat. Since episodic memory loss is the main clinical characteristic of cognitive decline in MCI, dementia and Alzheimer's disease (Belleville et al., 2008; Sperling et al., 2010), aged rats were submitted to an episodic-like memory task, the first test of its kind in aged rats, since appropriate tasks have only recently been developed (Dere et al., 2007; Kart-Teke et al., 2006). The open-field and forced-swimming tests were used to ascertain effects on anxiety- and antidepressant-like behaviors, respectively. Changes in cholinergic neurotransmission upon s.c. senktide administration were assessed in the hippocampus, amygdala and frontal cortex – brain regions which are implicated in emotionality and processes of learning and memory – by *in vivo* microdialysis with HPLC-ECD.

2. Experimental procedure

2.1. Subjects

Experimentally naïve aged male Wistar rats at 23–25 month of age (weight 470–800 g) from the breeding colony of the University of Düsseldorf were used for all experiments. Animals were housed in translucent plastic cages (60.0×20.0×38.0 cm in height) under controlled laboratory conditions (temperature: 20±2 °C) with free access to food and water under an artificial reversed 12:12 light–dark cycle (light off at 07:00 a.m.). Experiments were performed during the animal's active period between 08.00 a.m. and 5.00 p.m. Thirty-nine animals were used for the open-field and episodic-like memory test, 46 animals for the forced swimming test, and 17 animals for the neurochemical study. They were housed in groups of two or three animals per cage. Animals for the neurochemical study were housed individually after surgery. The animals were allowed to adjust to the housing conditions for 2 weeks and were handled daily for 5 days preceding the experiments. All rats were weighed once a week and health status (food and drinking behavior, coat condition, and body's orifices) was controlled daily. All experiments were carried out according to the German Law of Animal Protection of 1998.

2.2. Drug administration

The NK_3-R agonist, senktide ([succinyl-Asp^6-Me-Phe^8]SP$_{6-11}$; Bachem, USA), was diluted with 5% dimethylsulphoxide in phosphate-buffered saline. The vehicle, 5% dimethylsulphoxide in phosphate-buffered saline, served as control. Animals were randomly assigned to either vehicle, 0.2 mg/kg or 0.4 mg/kg senktide (De Souza Silva et al., 2006; Jocham et al., 2007; Zlomuzica et al., 2008) and injected subcutaneously (s.c.) in the back of the neck 30 min prior to behavioral testing. The injection volume was 1 ml/kg of body weight.

2.3. Open field test

The open field used was a square arena (60×60 cm) with grey acrylic walls (30 cm high) and open roof, located in a sound-attenuating room with masking noise (60 dB). The arena was illuminated by four 40 W light bulbs that provided a light density of approximately 13 lx at the centre and 6 lx in the corners of the field. A video camera (SSC-M388CE; Sony), was mounted 1.6 m above the arena to record the experiment on video tapes for post-hoc analysis.

Animals were individually placed into the center of the open field and allowed to explore it for 15 min. The arena was cleaned with 60%

ethanol solution before each animal was exposed. The frequency of line crossings was used to assess total general motor activity. For this purpose, the floor was divided into 9 virtual quadrants of equal size. A line crossing was considered when the animal entered into another virtual quadrant with all four paws. In order to derive measures of emotionality (Prut and Belzung, 2003), the number of entries and time spent in the center (30×30 cm) were scored. Data were analyzed for the whole 15-min exposure as well as in 5-min intervals to check for potential time dependent behavioral changes.

2.4. Episodic-like memory task

The episodic-like memory task (Dere et al., 2007; Kart-Teke et al., 2006) employed an open-field arena as described above with 9 virtual quadrants of equal size on the floor, in this case indicating the possible positions of object placement, except for the central square, which was not used for object placement. Two different objects (in quadruplicate), without ethological significance to the rats, differing in material (glass or ceramic), shape (rectangle, circular), surface texture (plain, grooved), color (green, white) and height (22 cm, 28 cm) were used. The objects had a sufficient weight (1500 g) to ensure that the rats could not displace them. Pilot studies ensured that rats could discriminate the two objects, and that there was no *per se* preference for any of the objects.

After a washout period of 10 days, the same animals used for the open-field test were re-exposed to the open-field on three consecutive days (habituation). They were placed into the central part of the open-field arena and allowed to explore for 10 min. One day after the last habituation trial, the episodic-like memory task was conducted. The animals were assigned to the same groups as in the open-field test and were exposed to the episodic-like memory task receiving two sample trials followed by the test trial (Fig. 1). They were always placed into the central part of the open-field, all facing in the same direction. Each trial lasted 5 min with an inter-trial interval of 25 min. For each animal, 4 out of 8 squares were randomly chosen to position the 4 copies of the "old familiar" object in the first sample trial. During the second sample trial, 4 copies of another object ("recent familiar") were presented. Two copies of the "recent familiar" object were randomly placed onto positions that had been occupied in the first sample trial and 2 copies were placed in new positions, which were randomly chosen from the remaining 4 positions. In the test trial, 2 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) which were not occupied during the sample trials. All 4 objects were placed onto positions previously encountered in the sample trials (Kart-Teke et al., 2006). The experiments were carried out between 8:00 a.m. and 4:00 p.m. After each trial, the objects and the open-field were cleaned with 70% ethanol in order to remove odour cues.

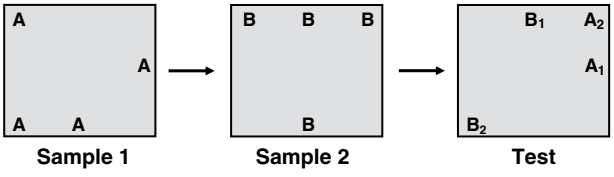

Figure 1 Episodic-like memory design: This schematic picture shows a possible object placement in the episodic-like memory task. The animals received three trials with 5 min duration and an inter-trial-interval (ITI) of 25 min in each case. During the test trial, two "old familiar" and two "recent familiar" 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".

The exploration time of the objects in the test trial was analyzed. Object exploration was defined as a physical contact with the object with nose, vibrissae or forepaws. The exploration time was used for calculating preference ratios (PR): PR_1, exploration time *old familiar stationary*/(exploration time *old familiar stationary*+*recent familiar stationary*), indicates whether the temporal order in which the objects were presented can be remembered; PR_2, exploration time *recent familiar displaced*/(exploration time *recent familiar stationary*+*recent familiar displaced)*, indicates whether the place in which the objects were presented can be remembered; PR_3, exploration time *old familiar stationary*/(exploration time *old familiar stationary*+*old familiar displaced)*, indicates an inverse relationship based on the place in which the old object is present. A performance suggesting episodic-like memory is characterized by a significant difference in all three PRs concurrently.

2.5. Forced swimming test

We examined the effect of senktide for the screening of an antidepressant-like effect on the forced swimming test with a new set of aged animals.

The forced swimming test (FST) apparatus consisted of a Plexiglas cylinder (46 cm height, 20 cm diameter) containing 30 cm of water (24±1 °C). A video camera (SSC-M388CE; Sony), connected to a video recorder, was located next to the cylinder providing a front view to record the experiment on video tapes for post-hoc analysis.

On the first day (pretest session), animals were exposed to the cylinder for 15 min without prior drug administration. At the end of this pretest session, each rat was removed from the water, and placed in a plastic cage under a red light heating lamp for 15 min to allow drying before returning to the home cage. Twenty-four hours later, the animals were exposed to the same experimental conditions for only 5 min (test session) and 30 min following drug treatment.

The duration of *immobility* (defined as a lack of motion of the animal, except for movements required to keep its head above the water), *swimming* and *climbing* (vigorous movements with forepaws in and out of the water, usually in contact with the walls) was analyzed semi-automatically by a trained blind observer.

2.6. *In vivo* microdialysis and ACh measurement

2.6.1. Surgery

Five to seven days prior to the beginning of the *in vivo* microdialysis, the animals were anaesthetized with a mixture of ketamine hydrochloride (90.0 mg/kg; Pharmacia & Upjohn GmbH, Germany) and xylazine hydrochloride (8.0 mg/kg; Bayer, Germany). With the help of a stereotaxic apparatus (David Kopf Instruments, USA) and according to a stereotaxic atlas (Paxinos and Watson, 1986) three stainless steel guide-cannulae for the microdialysis probes (15 mm long, 0.22 gauge) were implanted above the following brain areas: frontal cortex (AP: +3.7 mm, ML: ±3.0 mm, DV: −1.3 mm), amygdala (AP: −2.5 mm, ML: ±5.7 mm, DV: −7.8 mm, with 10° angle toward the midline) and hippocampus (AP: −6.0 mm, ML: ±4.8 mm, DV: −3.2 mm), coordinates were taken from bregma. All guide-cannulae were implanted unilaterally, alternately into the right or left hemisphere.

2.6.2. Microdialysis procedure

The experiments were carried out between 8:00 a.m. and 4:00 p.m. Seventeen aged Wistar rats from the forced swimming test were assigned to the microdialysis experiment. The microdialysis probes were prepared according to the procedure described elsewhere (Boix et al., 1994). Five to seven days after guide-cannulae implantation, the animals were anaesthetized with urethane (1.25 g/kg, Sigma Aldrich, USA). We chose an anaesthetized preparation because one cannot rule out that motor activity, behavioral changes and the procedure of handling in the freely moving animals led to cholinergic activation and that this could obscure the physiological effects of drug treatment on ACh, or interact with the neurochemical effects (De Souza Silva et al., 2007). Microdialysis probes were inserted with the help of the guide-cannulae. The microdialysis probes had an active membrane length of 4.0 mm for the hippocampus and 2.0 mm for frontal cortex and amygdala. A catheter was placed into the intraperitoneal (i.p.) cavity to allow fluid supply without physical contact with the animal. The animal was placed on a heating pad in an acrylic box (45 cm × 25 cm × 22 cm) and its body temperature was maintained at 37.5 ± 0.5 °C by a temperature controller (CMA/150, Sweden). The probes were perfused at a flow rate of 2 μl/min with Ringer's solution (Braun, Germany) containing neostigmine (10 μmol), by a microinfusion pump (CMA/100, Sweden). Samples were collected in cups containing 10 μl of ethylhomocholine (EHCh) solution, used as the internal standard, kept ice-cold during collection and until being analyzed.

After a stabilization period of two hours, six baseline samples, each corresponding to a time-window of 10 min, were collected. Afterwards, vehicle, 0.2 mg/kg or 0.4 mg/kg senktide was injected. Another 10 samples (10 min per sample) were collected. After each sampling the animal received an injection of 0.1 ml Ringer's solution through the i.p. catheter and the depth of anesthesia was adjusted with urethane if necessary.

2.6.3. HPLC analysis

After collection, the samples were analyzed for the concentration of acetylcholine (ACh) by HPLC-EC according to standard method (Damsma et al., 1987). EHCh was used as internal standard (Potter et al., 1983). Briefly, ACh separation was achieved using an analytical column (75 mm length, 2 mm inner diameter) filled with ChromSpher 5 C18 (Merck, Germany) and loaded with sodium dodecylsulfate (Sigma Aldrich, USA). The enzyme reactor was filled with LiChrosorb-NH_2 (Merck, Germany), activated by glutaraldehyde (Merck, Germany) and loaded with acetylcholine-esterase (Sigma Aldrich, USA) and cholineoxidase (Sigma Aldrich, USA), which were covalently bound to the stationary phase and attached to the end of the analytical column for enzymatic cleavage of ACh. On passage through the enzyme reactor ACh was converted to hydrogen peroxide, which is electrochemically detected by a platinum electrode set at a potential of 350 mV versus an Ag/AgCl reference electrode (Antec Leyden, Netherlands). The mobile phase, composed of 1 mM tetramethylammoniumchloride (Merck, Germany), 0.18 M K_2HPO_4 (Merck, Germany) and adjusted to pH 8.0 with KH_2PO_4 (Merck, Germany), was delivered by a HPLC pump (Merck, Germany) at a flow rate of 0.3 ml/min. The data were collected, processed and stored with the help of computer software (Chrom Perfect, Justice Laboratory Software).

2.6.4. Histology

After the microdialysis procedure, the animals were deeply anaesthetized with pentobarbital (Sanofi Ceva, France), and perfused with PBS and 10% formalin through the left heart ventricle. The brain was removed and stored in 10% formalin until histological verification. After slicing the brain with a cryostat (Leica, Germany), slices were stained with cresyl violet (Sigma Aldrich, USA). The accuracy of placement of the microdialysis probes was verified, according to the atlas of Paxinos and Watson (1986).

2.7. Statistical analysis

Since most of the behavioral data failed to reach the criterion of normality of distribution and homogeneity of variance, non-parametric statistics were used. Behavioral data are expressed as medians and interquartile range. The Mann–Whitney U-test was used for pair-wise between-group comparisons against the vehicle group. For the data obtained from the episodic-like memory experiment, wherein components of the episodic-like memory, namely object memory for temporal order and object memory for spatial displacement, were assessed, two-tailed Wilcoxon tests for within-group comparisons were calculated. All p-values given for the behavioral data are two-tailed. Due to the large number of comparisons made in the tests for episodic-like memory and forced swimming the p values are presented as "measures of effect".

The neurochemical data are expressed as means and standard error of the mean (SEM) and were analyzed for each brain area separately. ACh concentrations were converted into percentage of baseline (the mean of six baseline samples were set as 100%). A two-way ANOVA with repeated measures was calculated with the factors "time" and "group". *Post hoc* Dunnett tests were applied for between-group comparisons. When appropriate, this was followed by a one-way ANOVA and *post hoc* Dunnett tests to analyze group differences at individual time points. The software SPSS 15.0 was used for the analysis.

3. Results

3.1. Open field test

The analysis of the open field data did not reveal significant changes in general motor activity between groups (Table 1). However, the animals exhibited significant differences in the time spent in the center upon senktide administration. Mann–Whitney U-test revealed that rats treated either with 0.2 mg/kg or 0.4 mg/kg senktide spent significantly more time in the center compared to vehicle treated controls during the entire 15 min of exposure ($p<0.001$, and $p=0.002$, respectively, Fig. 2A). A more detailed analysis on the different 5-min intervals revealed that this difference was also evident during the first 5 min of open field exposure ($p=0.001$ and $p<0.001$, respectively, Fig. 2B), but not for the second and third intervals ($ps>0.05$).

3.2. Episodic-like memory

Data on preference ratios in the episodic-like memory paradigm are shown in Fig. 3. The Mann–Whitney U-test revealed a difference between vehicle and 0.2 mg/kg senktide ($p=0.010$) for the preference ratio PR_2, suggesting that senktide improved

Table 1 General motor activity in the open field.

Behavior	vehicle		0.2 mg/kg senktide		0.4 mg/kg senktide	
	Mean	SEM	Mean	SEM	Mean	SEM
Line crossings Total	42.11	5.54	31.57	5.96	41.20	5.43
0–5 min interval	13.22	2.73	10.71	2.20	16.13	2.67
5–10 min interval	12.80	2.56	10.21	2.08	12.60	1.86
10–15 min interval	13.60	2.49	10.64	2.84	12.47	2.39

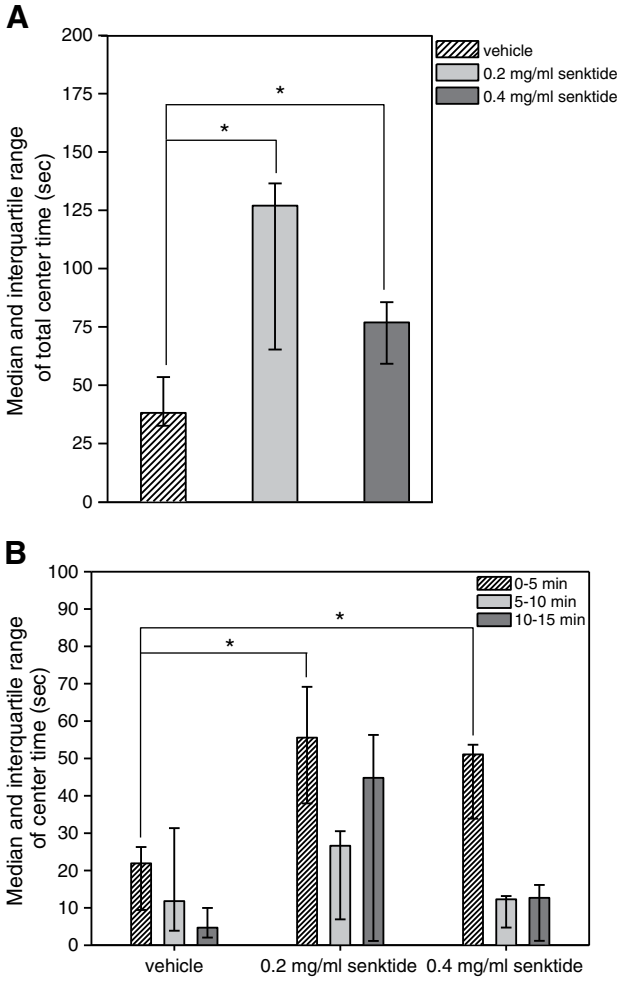

Figure 2 Effect of subcutaneous administration of vehicle, 0.2 mg/kg and 0.4 mg/kg NK_3 receptor agonist senktide on anxiety-related parameters in the open field test in aged Wistar rats. The values are given as median and 75% and 25% interquartile range of total centre time (A) and center time in 1–5 min, 6–10 min, 11–15 min intervals (B). *$p \leq 0.05$.

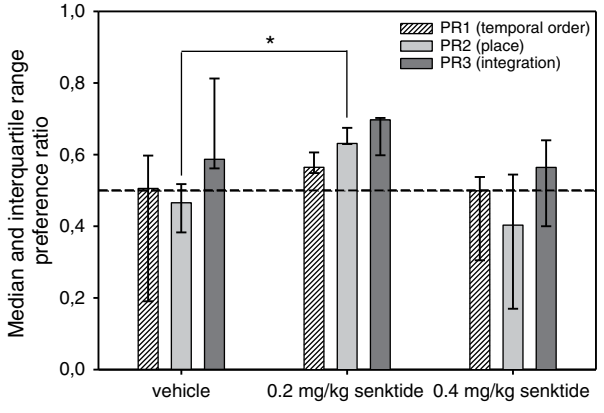

Figure 3 Effect of s.c. administration of vehicle, 0.2 mg/kg and 0.4 mg/kg NK_3 receptor agonist senktide on episodic-like memory in aged Wistar rats. The values are given as median and 75% and 25% interquartile range of preference ratios (PR_1, PR_2, and PR_3) during the test trial. *$p \leq 0.05$.

object memory for place order. For the preference ratio PR_1 there was no difference between 0.2 mg/kg senktide and vehicle ($p = 0.070$). However, 0.4 mg/kg senktide had no effect on PR_1 and PR_2 compared to vehicle ($ps > 0.05$). There were also no differences for PR_3 between vehicle and 0.2 mg/kg as well as 0.4 mg/kg senktide ($ps > 0.05$). Taken together, neither the senktide- nor vehicle-groups showed episodic-like memory.

Based on the result that senktide treatment did not induce episodic-like memory in the aged rats, we analyzed the components of the episodic-like memory, namely object memory for temporal order and object memory for spatial displacement, separately. The exploration time during the test trial for the object types "old" and "recent", representing object memory for temporal order, and the object types "stationary" and "displaced", representing object memory for spatial displacement, is summarized in Fig. 4. The vehicle treated rats showed no difference in object exploration between "old" and "recent" objects ($p > 0.05$, Wilcoxon test) as well as between "stationary" and "displaced" objects ($p > 0.05$). The animals which received 0.2 mg/kg senktide spent more time exploring the "displaced" object compared to the "stationary" object ($p = 0.035$), reflecting memory for "what" and "where" and indicating intact object memory for spatial displacement in this group. There was no difference in the time exploring the "old" object relative to the "recent" object ($p = 0.061$). The treatment with 0.4 mg/kg senktide had no effect on object exploration between "old" and "recent" objects ($p > 0.05$) as well as between "stationary" and "displaced" objects ($ps > 0.05$). In summary, 0.2 mg/kg senktide led to the appearance of one component of episodic-like memory, namely, memory for spatial displacement.

3.3. Forced swimming test

Data of the forced swimming test session are shown in Fig. 5. Mann–Whitney U-test revealed an effect of 0.2 mg/kg senktide

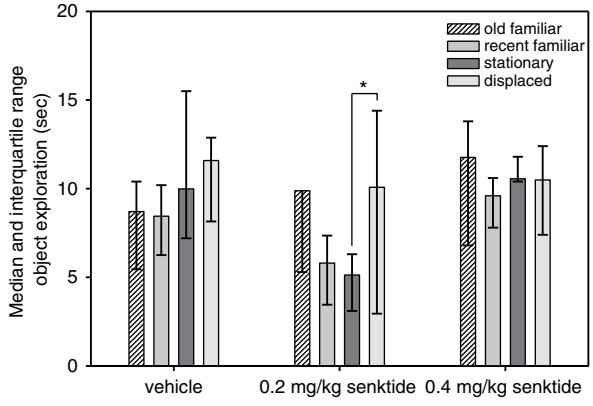

Figure 4 Effect of s.c. administration of vehicle, 0.2 mg/kg and 0.4 mg/kg NK_3 receptor agonist senktide on episodic-like memory in aged Wistar rats. The values are given as median and 75% and 25% interquartile range of the exploration time of episodic-like memory components. The object types "old" and "recent" representing object memory for temporal order ("what" and "when") and the object types "stationary" and "displaced" representing object memory for spatial displacement ("what" and "where") during the test trial. *$p \leq 0.05$.

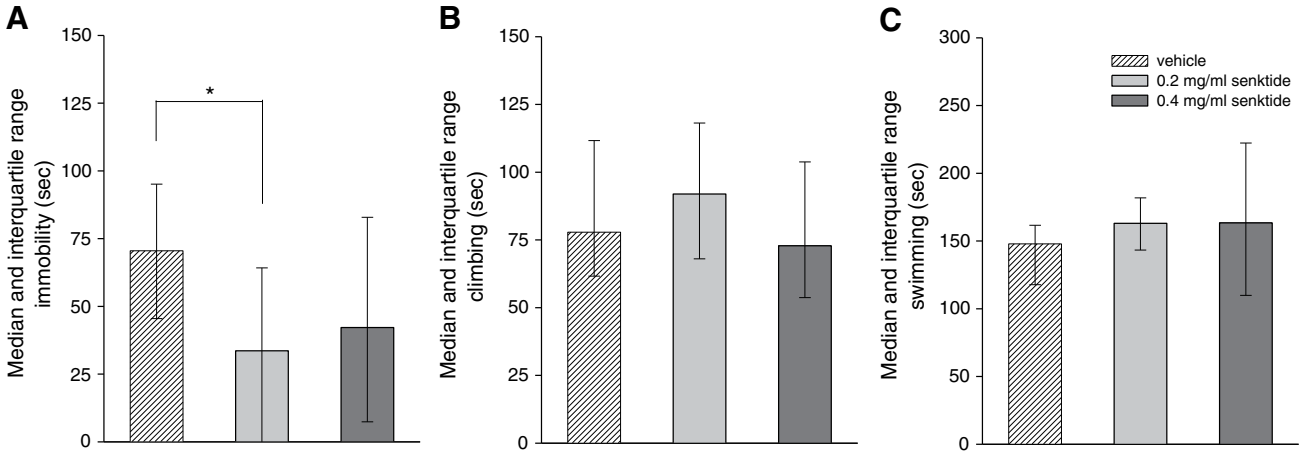

Figure 5 Effect of s.c. administration of vehicle, 0.2 mg/kg and 0.4 mg/kg NK_3 receptor agonist senktide in the forced swimming test in aged Wistar rats. The values are given as median and 75% and 25% interquartile range of immobility (A), climbing (B) and swimming (C) behavior during the 5 min test session. *$p \leq 0.05$.

compared to vehicle for the duration of immobility in the forced swimming test session ($p=0.049$). Animals treated with 0.2 mg/kg senktide exhibited a reduction in the duration of immobility. Group comparisons failed to reveal an effect between senktide and vehicle for the duration of climbing as well as swimming in the forced swimming test session (Mann–Whitney U-test, $p>0.05$). The 0.4 mg/kg senktide treated group showed no differences in the duration of immobility, climbing and swimming compared to vehicle controls.

3.4. *In vivo* microdialysis and ACh measurement

After histological screening for correct probe placement, all 17 animals were considered for statistical analysis. For all three regions analyzed the sample sizes were as follows: vehicle, $n=6$; 0.2 mg/kg, $n=6$ and 0.4 mg/kg senktide, $n=5$. The mean ± SEM basal levels of ACh in dialysates were: frontal cortex 1.174 ± 0.436 pmol/15 μl; amygdala 0.878 ± 0.248 pmol/15 μl; hippocampus 1.866 ± 0.747 pmol/15 μl. ANOVAs were carried out for each brain region separately. Levels of ACh (% of baseline, mean ± SEM) for control group (vehicle) and the experimental groups (0.2 mg/kg and 0.4 mg/kg senktide) in the *frontal cortex*, *amygdala* and *hippocampus* are shown in Fig. 6.

Significant main effects of time (two-way repeated measures ANOVA, $F_{15,210}=6.161$, $p<0.001$), group ($F_{2,14}=5.169$, $p=0.021$) and a time × group interaction ($F_{30,210}=2.663$, $p<0.001$) in ACh levels were seen in the *frontal cortex*. *Post hoc* Dunnett test revealed a significant difference (0.2 mg/kg senktide, $p=0.012$) compared to vehicle. One-way ANOVA showed that senktide treatment significantly increased ACh release 40 min ($F_{2,16}=4.102$, $p=0.040$), 60 min ($F_{2,16}=4.470$, $p=0.032$), 90 min ($F_{2,16}=8.092$, $p=0.005$) and 100 min ($F_{2,16}=7.498$, $p=0.006$) after injection. *Post hoc* Dunnett test revealed significant increases in ACh release by 0.2 mg/kg senktide 40 min ($p=0.031$), 60 min ($p=0.018$), 90 min ($p=0.003$) and 100 min ($p=0.004$) after injection compared to vehicle controls.

In the *amygdala* there was a significant main effect of time (two-way repeated measures ANOVA, $F_{15,210}=3.133$, $p<0.001$) and a time × group interaction ($F_{30,210}=2.043$, $p=0.002$), but only a tendency in the main effect of group ($F_{2,14}=3.498$, $p=0.059$) in ACh levels. *Post hoc* Dunnett test revealed a significant difference (0.2 mg/kg senktide, $p=0.049$) compared to vehicle. One-way ANOVA showed that senktide significantly increased ACh release 10 min ($F_{2,16}=11.144$, $p=0.001$) and 80 min ($F_{2,16}=4.177$, $p=0.038$) after injection. *Post hoc* Dunnett test revealed significant increases in ACh release by 0.2 mg/kg senktide 10 min ($p=0.001$) and 80 min ($p=0.049$) after injection compared to vehicle controls.

In the *hippocampus* there were significant main effects of time (two-way repeated measures ANOVA, $F_{15,210}=18.543$, $p<0.001$) and time × group interaction ($F_{30,210}=2.342$, $p<0.001$), but only a tendency in a main effect of group ($F_{2,14}=3.318$, $p=0.066$) in ACh levels. *Post hoc* test revealed a significant difference (Dunnett, 0.4 mg/kg senktide, $p=0.042$) compared to vehicle. Senktide significantly increased ACh release 60 min (one-way ANOVA, $F_{2,16}=4.693$, $p=0.028$), 70 min ($F_{2,16}=5.960$, $p=0.013$), 80 min ($F_{2,16}=5.910$, $p=0.014$) and 100 min ($F_{2,16}=6.257$, $p=0.011$) after injection. There were significant increases in ACh release by 0.4 mg/kg senktide at 60 min (*post hoc* Dunnett test, $p=0.016$), 70 min ($p=0.008$), 80 min ($p=0.008$) and 100 min ($p=0.007$) after injection compared to vehicle controls.

4. Discussion

The aim of the present study was to ascertain the effects of acute systemic administration of the selective NK_3-R agonist senktide in aged Wistar rats on performance in an episodic-like memory test, on parameters of emotionality in the open-field and forced-swimming tests and on ACh neurotransmission in the frontal cortex, amygdala and hippocampus, using *in vivo* microdialysis with HPLC. The results indicate that NK_3-R agonism had anxiolytic- and antidepressant-like effects as assessed by the open-field and the forced-swimming tests, respectively. Senktide had no beneficial effects on episodic-like memory; however, it led to the improvement of a component of episodic-like memory, namely, object memory for spatial displacement. Furthermore, *in vivo* microdialysis data revealed that ACh levels in the frontal cortex, amygdala and hippocampus of aged rats

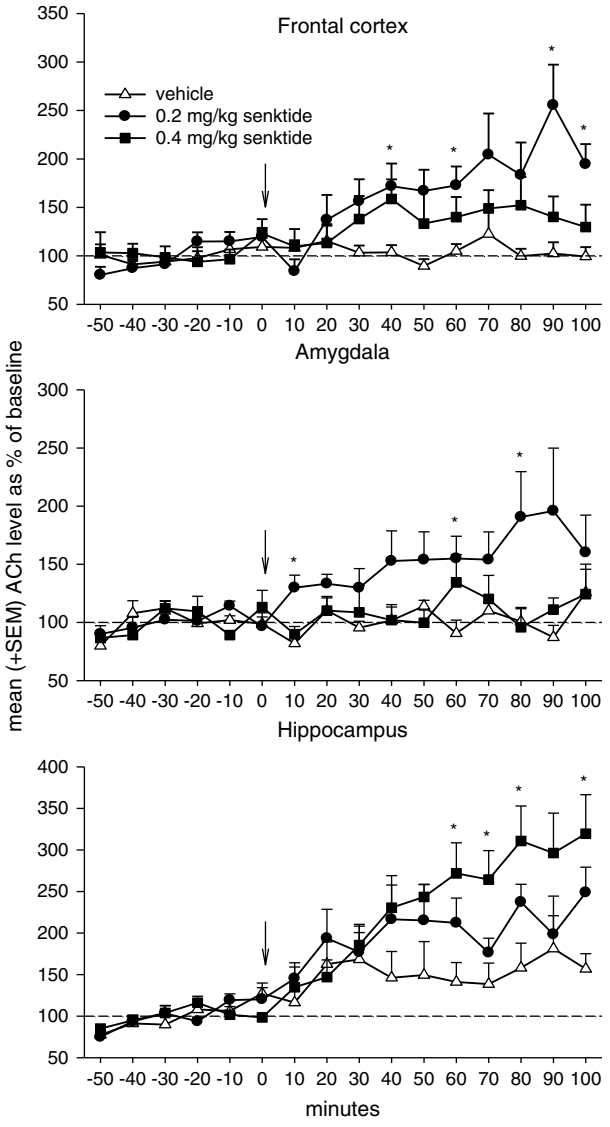

Figure 6 Effect of s.c. administration of vehicle, 0.2 mg/kg and 0.4 mg/kg NK_3 receptor agonist senktide on extracellular ACh levels in the frontal cortex, amygdala and hippocampus of anaesthetized aged Wistar rats. The values are given as mean + SEM percent of baseline (average of the six baseline samples taken as 100%). Arrows indicate the time-point of the injection. *$p \leq 0.05$.

were increased after senktide administration, raising the possibility of a causal link between the increase in cholinergic activity and the various behavioral manifestations of NK_3-R agonism.

The dose–response relationships found in this study may indicate inverted U-shaped dose–response curves. Recently it has been reported that the most fundamental shape of dose–response, is neither threshold, nor linear, but rather U-shaped or inverted U-shaped (Calabrese and Blain, 2005). Neuropeptides, including neuropeptide Y, SP, and others, are known to have an inverted U dose–response curve (Huston and Hasenohrl, 1995; Kastin and Pan, 2010). The doses utilized in this study were possibly on the descending limb of an inverted U-shaped dose–response curve. Both, in the episodic-like memory and forced-swimming tasks, the effective doses were the lower one (0.2 mg/kg). In the open-field, both were active, whereby the lowest was effective in increasing ACh levels in the frontal cortex and amygdala, and the highest (0.4 mg/kg) in increasing ACh in the hippocampus.

Improvement of memory for spatial displacement: This is the first study to test for deficits in episodic-like memory in aged rats, since appropriate tests have only recently became available (Kart-Teke et al., 2006). This episodic-like memory paradigm combines different versions of the novelty preference paradigm into one task, including the assessment of object recognition memory, memory for locations in which objects are presented and temporal order memory for objects presented at distinct time points (Dere et al., 2007). Adult Wistar rats displayed episodic-like memory in this task (Kart-Teke et al., 2006). In the present study, the vehicle treated rats did not exhibit episodic-like memory, suggesting that the aged animals are deficient in this task (in a previous study, it was shown that untreated aged rats do not display episodic-like memory on the task used in this study, unpublished data). Treatment with senktide did not lead to the appearance of episodic-like memory, since animals failed to show an interaction between the temporal order and spatial displacement (PR_3). However, administration of 0.2 mg/kg senktide led to the appearance of memory for spatial displacement ("what" and "where", PR_2), while the memory for temporal order ("what" and "when", PR_1) was tendentially enhanced. These results are in agreement with another recent study in adult mice, where the 0.2 mg/kg dose of senktide facilitated components of episodic-like memory; however, contrary to the present study in aged rats, the higher dose of 4 mg/kg established episodic-like memory in adult C57BL/6 mice (Zlomuzica et al., 2008). Since the NK_3-R agonism is considered to have anxiolytic effects (as also shown in this paper), one could surmise that the apparent facilitation of memory for spatial displacement was simply a result of an alleviation of stress-induced deficits in performance (as proposed previously for neurokinin-induced memory enhancement (Kart-Teke et al., 2006)). However, if that were the case, we might have expected the higher dose of senktide, which we showed to be anxiolytic-like, to have also reinstated spatial displacement memory, which it did not.

Episodic-like memory is defined as an integrated memory for the object that was experienced (what), the time it was experienced (when) and the place it was experienced in (where) (Kart-Teke et al., 2006; Tulving, 2002). This integrated memory was exhibited only in a sub-set of untreated naïve aged animals (data not shown). Such an overall deficit in episodic-like memory in the aged rat is in accordance with the well-known deficits in episodic memory found in the aged human (Gallagher et al., 2003; Lasarge et al., 2007). Age-related structural differences, potentially in the hippocampus which is compromised in the aged (Lister and Barnes, 2009), seem to be a likely factor; hippocampal lesion in rats has, in fact, been shown to disrupt performance in the episodic-like memory task used in the present study (Li and Chao, 2008). However, it is also possible that the injection procedure may have induced or contributed to the disruption in memory performance shown by the vehicle treated group. In this case, senktide would have promoted memory by reducing stress induced by the injection procedure (see Kart-Teke et al., 2006).

In NK_3-R knockout mice, contradictory findings have been reported: Siuciak et al. (2007) found deficits in conditioned avoidance and the Morris water maze task. Nordquist et al. (2008) reported an improvement in performance of operant tasks and in spatial learning. Such a difference in direction of effect could be due to the use of distinct genetic backgrounds used for generating the knockouts (see Nordquist et al. (2008) for discussion). Besides, since they were not conditional knockouts, they may have compensated the lack of NK_3-Rs in different ways during ontogeny. Our results are in agreement with the findings by Siuciak et al. (2007). Senktide was also found to attenuate scopolamine-induced impairment in a spatial working memory task (Kameyama et al., 1998; Ukai et al., 1998).

Age-related deficits have also been found in recognition memory paradigms (Eichenbaum et al., 2005; Ennaceur and Delacour, 1988; de Lima et al., 2005; Robitsek et al., 2008), whereby the hippocampus (Mumby et al., 2002), perirhinal cortex (Winters and Bussey, 2005) and prefrontal cortex (Hannesson et al., 2004) may play a major role in object recognition (Dere et al., 2007). Given that the integrity of the hippocampus is necessary for spatial memory (Eichenbaum et al., 1999; Martin and Clark, 2007) and, thus, memory for spatial displacement, it is possible that the memory-promoting effect of senktide on this task was mediated by an action of NK_3-R activation on the hippocampus. Here it may be of relevance that NK_3-R agonism in the medial septum, which is the source of hippocampal ACh, leads to extracellular ACh release in the hippocampus (Steinberg et al., 1995; Schäble et al., 2010).

Anxiolytic and antidepressant-like effects: In the aged human, neurodegenerative disorders are often co-morbid with changes in emotionality (Teri et al., 1999; Vloeberghs et al., 2007). Also, neurochemical, anatomical, genetic and pharmacological studies point to a relationship between emotionality and learning and memory (Kalueff, 2007). Therefore, an intricate link between age-related decline in learning and memory and higher levels of anxiety has been suggested (Rowe et al., 1998; Schulz et al., 2007). NK_3-R are widely distributed throughout the brain, the amygdala, hippocampus and medial septum, which are known to be involved in mediating fear and anxiety (Davis, 1992; Pesold and Treit, 1995). In the present study 0.2 and 0.4 mg/kg systemic senktide administration had an anxiolytic-like effect in the open-field test. Although it would be rash to base a conclusion of anxiolytic action solely on the open-field test, this finding is in agreement with a previous study in adult mice, where senktide had an anxiolytic-like effect in the elevated plus-maze, which was abolished by NK_3-R antagonism (Ribeiro et al., 1999). On the other hand, in gerbils the blockade of NK_3-R had anxiolytic-like effects (Salome et al., 2006). The reason for these contradictory findings could be differences in brain localization of NK_3-R between species (Rigby et al., 2005). Interestingly, the role of NK_3-R in anxiety seems to be opposite to that found for the NK_1- and NK_2-R. Behavioral studies in adult animals point to an anxiolytic-like effect for agonists of NK_1-R (File, 2000; Kramer et al., 1998; Steinberg et al., 2002) and antagonists of NK_2-R (Griebel et al., 2001; Salome et al., 2006).

In the forced swimming test, application of 0.2 mg/kg senktide decreased the duration of immobility in aged rats when compared to control animals, indicating that NK_3-R activation induces antidepressant-like activity. The effect of senktide on immobility could be mediated through an interaction with noradrenaline, serotonin, and/or other neurotransmitters in the brain classically involved in depression. For example, in the raphe nuclei NK_3 binding sites have been shown (Dam et al., 1990), and these were decreased in the 5,7-dihydroxytryptamine-treated median raphe nucleus (Stoessl and Hill, 1990), thus, providing a possibility of neurokinin and serotonergic interactions. Moreover, senktide has been shown to activate local glutamatergic inputs to serotonergic neurons of the dorsal raphe nucleus (Liu et al., 2002). Intracisternal (i.c.v.) application of senktide in mice induced head twitches and forepaw treading similar to that observed after stimulating the serotonergic system, and this behavior was attenuated by 5-HT_1 and 5-HT_2 receptor antagonists (Stoessl et al., 1987). In rats, senktide induced similar responses, including wet dog shakes and forepaw treading, and these effects were abolished by 5-HT receptor antagonists and p-chlorophenylalanine-induced depletion of 5-HT (Stoessl et al., 1990). The noradrenergic system could also play a role: senktide increased firing of noradrenergic neurons when applied onto guinea pig locus coeruleus slices, and increased noradrenaline release in the medial prefrontal cortex of guinea pigs when given i.c.v., an effect that was blocked by the NK_3-R antagonist, SR142801 (Jung et al., 1996).

Our results are in line with those found for the NK_3-R agonist aminosenktide in an adult mice line that express enhanced activity of the opioid system (Panocka et al., 2001). However, in the adult rat, antagonism of the NK_3-R, as well as of NK_1- and NK_2-R, had antidepressant-like action (Dableh et al., 2005). In the gerbil, both, NK_2- and NK_3-R antagonism had antidepressant-like and anxiolytic effects (Salome et al., 2006).

Increase in ACh in frontal cortex, hippocampus and amygdala: In the present study, systemic senktide increased ACh levels in the frontal cortex, amygdala and hippocampus. In the hippocampus only the higher dose of 0.4 mg/kg senktide increased ACh levels; in the amygdala and frontal cortex only the lower dose of 0.2 mg/kg had such effects. The latter areas receive dense cholinergic innervations from the cholinergic neurons within the NBM, whereas the hippocampus receives cholinergic input from the medial septum (Mesulam et al., 1983). In the basal forebrain, choline acetyltransferase containing neurons were shown to be co localized with NK_3R (Chen et al., 2001a). An increase on ACh neurotransmission in the hippocampus was also found after administration of senktide into the medial septum in the guinea pig (Marco et al., 1998). This indicates that the effects of senktide on cholinergic activity in the hippocampus, amygdala and frontal cortex were probably due to a direct action of the agonist on cholinergic cell bodies.

A decline in cholinergic activity in the brain has been observed during aging (Araujo et al., 1990; Takei et al., 1989). Also, cholinergic projection neurons in the basal forebrain undergo significant atrophy in the aged rodent (Cooper and Sofroniew, 1996; De Lacalle et al., 1996). The degree of atrophy is highly correlated with the cognitive impairment (Armstrong et al., 1988; Gage et al., 1988). Previous studies revealed that increased hippocampal ACh release is associated with novel object exploration (Degroot et al., 2005) and correlates with an improvement in spatial

learning and reference memory (Fadda et al., 2000; Kopf et al., 2001). Furthermore, it was shown, that drugs which increase cholinergic activity in the hippocampus, like AChE inhibitors, improved episodic memory in humans (Gron et al., 2005). Conversely, reduced hippocampal ACh neurotransmission has been associated with deficits in the Morris water-maze, T-maze, radial arm maze and passive avoidance, among others (Chang and Gold, 2004; Lehmann et al., 2000). The amygdala plays a major role in emotional memory (McGaugh, 2004) and it has been suggested that ACh within the amygdala modulates the activity of amygdaloid neurons and, thus, is involved in processes underlying emotional memory (Del Arco and Mora, 2009). Acetylcholinergic activity in the frontal cortex has been implicated in attentional processes (Levin and Simon, 1998; McGaughy et al., 2002). Therefore, the increase in ACh in all three brain structures could potentially be involved in improving object memory for spatial displacement observed in the episodic-like memory task. However, given the difference in the preparations used for the behavioral and microdialysis study, a direct comparison of dose–response is not possible, since anesthesia can influence the response of a neurotransmitter system to pharmacological challenges, while ACh measurements in the freely moving animal are confounded by movement (de Souza Silva et al., 2007).

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The founding source did not participate in the development of this study.

Contributors

All authors contributed for this study.

Conflict of interest

No conflict of interest.

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The NK_3 receptor agonist senktide ameliorates scopolamine-induced deficits in memory for object, place and temporal order

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ABSTRACT

Senktide, a potent neurokinin-3 receptor (NK_3-R) agonist, increases acetylcholine (ACh) release in the striatum, the prefrontal cortex (Schäble et al., 2011), the amygdala and hippocampus, presumably via postsynaptic mechanisms. A promnestic action of NK_3-R agonists has been described in a variety of learning/memory tasks. The memory-enhancing effects of NK_3-R agonists and their activating influence on ACh suggest a possible role of the NK_3-R in learning and memory via cholinergic modulation. Deterioration of the cholinergic system in the basal forebrain has been associated with learning and memory deficits and cholinergic agents have promnestic effects in a variety of learning paradigms. The anticholinergic drug, scopolamine, a muscarinic ACh receptor antagonist, incurs deficits in a variety of learning tasks and provides a useful tool to investigate the role of the cholinergic systems in mechanisms underlying learning and memory. The aim of this study was to ascertain the effect of the NK_3-R agonist, senktide, in the scopolamine-induced deficit model. We hypothesized that senktide treatment would attenuate scopolamine-induced (subcutaneous – s.c. 0.75 mg/kg) memory impairment in three novelty preference paradigms based on spontaneous object exploration: namely object recognition, object–place recognition and object recognition for temporal order. Administration of senktide reversed the scopolamine-induced memory deficits by re-establishing object recognition (s.c. 0.2 mg/kg), object–place recognition (0.2 and 0.4 mg/kg), as well as object recognition for temporal order (0.4 mg/kg) in adult Wistar rats. These results indicate memory enhancing effects of senktide in animals subjected to scopolamine-induced memory impairments and indicate that the promnestic action of NK_3-R agonists is mediated by muscarinic cholinergic mechanisms.

1. Introduction

The neurokinins are neuropeptides of the tachykinin family that include substance P (SP), neurokinin A (NKA) and neurokinin B (NKB). All have been identified in the brain, along with their respective neurokinin receptors (NK-R): the NK_1-, NK_2- and NK_3-R (Chen, Wei, Liu, Ding, et al., 2001; Chen, Wei, Liu, Qiu, & Chan, 2001; Gerfen, 1991; Saffroy, Torrens, Glowinski, & Beaujouan, 2001, 2003).

NK_3-R are widespread throughout the brain including the frontal cortex, amygdala, medial septum and hippocampus (Ding et al., 1996; Duarte, Schutz, & Zimmer, 2006; Shughrue, Lane, & Merchenthaler, 1996), regions of high relevance for processes underlying learning and memory. It was shown, that NK_3-R are expressed on cholinergic neurons (Chen, Wei, Liu, Ding, et al., 2001) and that such neurons within the septo-hippocampal cholinergic system can be activated by NK_3-R agonists (Morozova, Wu, Dumalska, & Alreja, 2008). Furthermore, NK_3-R agonists led to ACh release (Arenas, Alberch, Perez-Navarro, Solsona, & Marsal, 1991) in slices and protected cholinergic neurons within the basal forebrain from neurotoxicity (Wenk, Zajaczkowski, & Danysz, 1997). Senktide, a potent NK_3-R agonist, has high affinity to the NK_3 receptors in rats, mice, gerbils and guinea pigs (Massi, Panocka, & de Caro, 2000). Senktide application was found to increase ACh release in the striatum (Steinberg et al., 1995), the prefrontal cortex (Schäble et al., 2011), the amygdala (Schäble et al., 2011) and hippocampus (Marco et al., 1998; Schäble et al., 2011). This action is presumably mediated by postsynaptic mechanisms, since NK_3-R agonism induced excitatory responses within the septo-hippocampal cholinergic pathway, which was blocked by an NK_3-R antagonist (Morozova et al., 2008).

Few studies have examined the effects of NK_3-R agonists in learning and memory paradigms. A promnestic action of NK_3-R agonism was described in a spontaneous alternation task in mice (Kameyama, Ukai, & Shinkai, 1998; Ukai, Shinkai, & Kameyama, 1996, 1998) and in an episodic-like memory paradigm in mice (Zlomuzica, Dere, Huston, & De Souza Silva, 2008) and rats (Schäble et al., 2011).

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Furthermore, NK_3-R knockout mice displayed deficits in conditioned avoidance learning as well as in water maze escape learning (Siuciak et al., 2007). The promnestic effects of NK_3-R agonism and its activating influence on ACh suggest a possible role of the NK_3-R in learning and memory via cholinergic modulation (Schäble et al., 2011).

ACh is widely acknowledged to have a prominent role in learning and memory processes (Bruno, Sarter, Moore, & Himmelheber, 1999; Durkin & Toumane, 1992; Gold, 2003a, 2003b; Hasselmo, 2006; Parent & Baxter, 2004; van der Staay, Raaijmakers, Lammers, & Tonnaer, 1989). Deterioration of the cholinergic system in the basal forebrain has been associated with learning and memory deficits (Chang & Gold, 2003; Janis, Glasier, Fulop, & Stein, 1998; Lamprea, Cardenas, Silveira, Morato, & Walsh, 2000; Leanza, Nilsson, Wiley, & Bjorklund, 1995; LeBlanc et al., 1999; Lehmann et al., 2002; Walsh, Herzog, Gandhi, Stackman, & Wiley, 1996). Cholinergic agents have been shown to have promnestic effects in a variety of learning paradigms (Gold, 2003a; M'Harzi, Palou, Oberlander, & Barzaghi, 1995; Masuoka & Kamei, 2009; Murray, Cross, & Green, 1991; Puma, Deschaux, Molimard, & Bizot, 1999).

A well characterized pharmacological model used to induce cognitive impairment in experimental animals is based on cholinergic blockade by anticholinergic drugs, e.g. by scopolamine (Klinkenberg & Blokland, 2010). This muscarinic ACh receptor antagonist has been found to incur deficits in a variety of cognitive tests such as inhibitory avoidance (Botton et al., 2010), radial maze (Ennaceur & Meliani, 1992), water maze (Hodges, Lindner, Hogan, Jones, & Markus, 2009) and object recognition tasks (Dodart, Mathis, & Ungerer, 1997; Ennaceur & Meliani, 1992; Sambeth, Riedel, Smits, & Blokland, 2007). Hence, scopolamine has become a useful tool to investigate the contribution of the cholinergic system in mechanism underlying learning and memory.

The present study aimed to assess the effects of the NK_3-R agonist senktide on learning and memory in the scopolamine-induced deficit model. We hypothesized that senktide treatment would attenuate scopolamine-induced memory impairment in three novelty preference paradigms based on spontaneous object exploration: namely object recognition, object–place recognition and object recognition for temporal order.

2. Material and methods

2.1. Subjects

Sixty male Wistar rats, weighing between 280 g and 350 g, bred in the Tierversuchsanlage, University of Düsseldorf, were used in this study. They were housed in groups of five animals in standard translucent plastic cages (60 cm × 20 cm × 38 cm; length × depth × height) under standard laboratory conditions with a reversed 12:12 h light–dark cycle (light on from 07:00 p.m. to 07:00 a.m.) with access to food and water *ad libitum*. After arrival the animals were allowed to adapt to the housing conditions for 2 weeks preceding the experiments. All behavioral testing was performed during the animal's active period, i.e. the artificial dark period between 07.00 a.m. and 07.00 p.m. This study was carried out in accordance with the German Animal Protection Law.

2.2. Drugs

The NK_3-R agonist, senktide ([succinyl-Asp^6-Me-Phe^8]SP_{6-11}; Bachem, USA), was diluted with 5% dimethylsulfoxide in phosphate-buffered saline (PBS) and administered in doses of 0.2 mg/kg or 0.4 mg/kg. Scopolamine (Sigma–Aldrich, Germany) was dissolved in PBS to achieve the 0.75 mg/kg dose. Animals received subcutaneous (s.c.) injections in a volume of 1 ml/kg of body weight. Scopolamine injection was administered 1 h and senktide 30 min prior to the start of sample trial object exposure in each of the three experiments with a 7-days interval between experiments.

The animals were randomly assigned to the following groups: vehicle + vehicle (n = 10), vehicle + 0.2 mg/kg senktide (n = 10), vehicle + 0.4 mg/kg senktide (n = 10), scopolamine + vehicle (n = 10), scopolamine + 0.2 mg/kg senktide (n = 10) and scopolamine + 0.4 mg/kg senktide (n = 10). The rats received the identical treatment within the different behavioral tests.

2.3. Behavioral testing

The behavioral tasks were conducted in an open-field arena (60 cm × 60 cm × 30 cm) with gray walls and open roof, located in a sound-attenuating room with masking noise at 60 dB. The open-field was dimly lit by four LED lights, providing a light density of approximately 7 lx at the center of the arena.

Three novelty preference paradigms which rely on spontaneous object exploration: object recognition, object–place recognition and object recognition for temporal order were employed. Object exploration was defined as the time spent exploring the objects by physical contact either with the nose, vibrissae or forepaws.

Two different objects made of various materials (glass, ceramic), shape (rectangle, circular), surface texture (plain, grooved), color (blue, white) and height (22 cm, 28 cm) without ethological significance to the rats were used. The weight of the objects (1500 g) was chosen to ensure that the animals are not able to displace them. In pilot works it was shown that these objects are distinguishable by the rats and that they had no per se preference for any of the objects.

All animals were habituated to the open-field devoid of any objects for 10 min one day before the beginning of the behavioral testing.

2.3.1. Object recognition

The object recognition memory task involved a sample trial followed by a test trial after an inter trial interval of 25 min. In the sample trial two copies of a novel object were placed in the open-field and the animals were allowed to explore the arena for 5 min. In the test trial two objects were presented in the same position – one object used in the sample trial (familiar object) and the other was a novel object. Object recognition was defined as more time engaged in exploring the novel object as compared to the familiar object (Ennaceur & Delacour, 1988). After each trial, the objects and the open field were cleaned with a 70% ethanol solution in order to remove odor cues.

2.3.2. Object–place recognition

One week later, the object–place recognition memory task was administered. The animals were allowed to explore two copies of an object in the open field during the sample trial of 5 min. After an inter-trial interval of 25 min they received a 5 min lasting test trial identical to the sample trial except that two copies of the original object were present, one in the same position it had occupied in the sample trial (spatial stationary) and one in a novel position (spatial displaced). Object–place recognition memory was defined as an increase in time engaged in exploring the spatially displaced object in favor of the spatially stationary one (Dere, Huston, & Souza Silva, 2007; Ennaceur, Neave, & Aggleton, 1997).

2.3.3. Object recognition for temporal order

After a washout period of 1 week the animals were exposed to the object recognition for temporal order task. The object recognition task for temporal order comprised two sample trials and one test trial with an inter-trial interval of 25 min between each trial. In each sample trial the animals were allowed to explore two

copies of the same object for 5 min. Different objects were used for the two sample trials. During the test trial one object from sample trial 1 (old familiar) and one object from sample trial 2 (recent familiar) were presented and the animals were allowed to explore the open-field for 5 min. The positions of objects within the two sample trials and the test trial were identical. Intact object recognition memory for temporal order was defined if the animals spent more time exploring the old familiar object compared with the recent familiar object (Mitchell & Laiacona, 1998).

2.4. Statistical analysis

Two-way repeated measures ANOVA were conducted for statistical analysis of the object exploration times during the test trial of the object recognition, object–place recognition as well as object recognition for temporal order tasks. Additional analyses examined whether the treatment groups had discriminated between the two objects during the test trials, using the paired samples *t*-test.

The data obtained on the exploration times are expressed as means (and standard error). The *p*-values given are two-tailed and were considered significant if $\alpha \leqslant 0.05$. The software PASW Statistic 18.0 was used for all analyses.

3. Results

3.1. Object recognition

Data on object exploration time in the object recognition task are presented in Fig. 1. The two-way repeated measures ANOVA revealed a main effect of objects ($F_{1,54} = 31.605$, $p < 0.001$) and treatment ($F_{5,54} = 2.678$, $p = 0.031$) but no interaction ($F_{5,54} = 0.782$, $p > 0.05$). Paired samples *t*-test revealed that rats treated with vehicle–vehicle ($p < 0.001$), vehicle–0.2 mg/kg senktide ($p = 0.003$), vehicle–0.4 mg/kg senktide ($p = 0.006$) spent significantly more time exploring the novel than the familiar object, suggesting intact object recognition in these groups. However, scopolamine–vehicle and scopolamine–0.4 mg/kg senktide treatment caused deficits in object recognition ($p > 0.05$) compared to vehicle–vehicle controls. The scopolamine–0.2 mg/kg senktide treated animals ($p = 0.013$) were not impaired in object recognition, indicating that 0.2 mg/kg senktide prevented the scopolamine induced object recognition deficit.

3.2. Object–place recognition

The exploration time of the spatial stationary and spatial displaced object during the test trial during the object–place recognition task is presented in Fig. 2. The two-way repeated measures ANOVA revealed a main effect of objects ($F_{1,54} = 80.015$, $p < 0.001$), treatment ($F_{5,54} = 4.283$, $p = 0.002$) and an interaction ($F_{5,54} = 3.458$, $p = 0.009$). Paired samples *t*-test showed that the vehicle–vehicle group ($p = 0.013$), vehicle–0.2 mg/kg senktide ($p < 0.001$), vehicle–0.4 mg/kg senktide ($p = 0.006$) spent more time exploring the spatial displaced object relative to the spatial stationary object, indicating that this group had an intact object–place recognition memory. The scopolamine–vehicle treated animals could not distinguish the spatial position of objects ($p > 0.05$) during the test trial. The scopolamine–0.2 mg/kg senktide ($p = 0.009$) as well as the scopolamine–0.4 mg/kg senktide groups ($p = 0.001$) showed significantly more exploration of the spatial displaced object compared to the spatial stationary object, indicating reversal of the scopolamine-induced deficit.

3.3. Object recognition for temporal order

Data on object exploration time in the object recognition task are presented in Fig. 3. The two-way repeated measures ANOVA revealed a main effect of objects ($F_{1,54} = 43.728$, $p < 0.001$), treatment ($F_{5,54} = 3.265$, $p = 0.012$) and an interaction ($F_{5,54} = 6.867$, $p < 0.001$). Paired samples *t*-test revealed that the vehicle–vehicle ($p < 0.001$), vehicle–0.2 mg/kg senktide ($p < 0.001$), vehicle–0.4 mg/kg senktide ($p < 0.001$) groups spent significantly more time exploring the old familiar object than the recent familiar object. Treatment with scopolamine–vehicle caused a deficit in the temporal order memory ($p > 0.05$) which was reversed by 0.4 mg/kg senktide ($p < 0.001$) but not by the lower dose 0.2 mg/kg senktide ($p > 0.05$).

4. Discussion

The aim of this study was to ascertain the effect of the NK_3-R agonist senktide in combination with the muscarinic antagonist

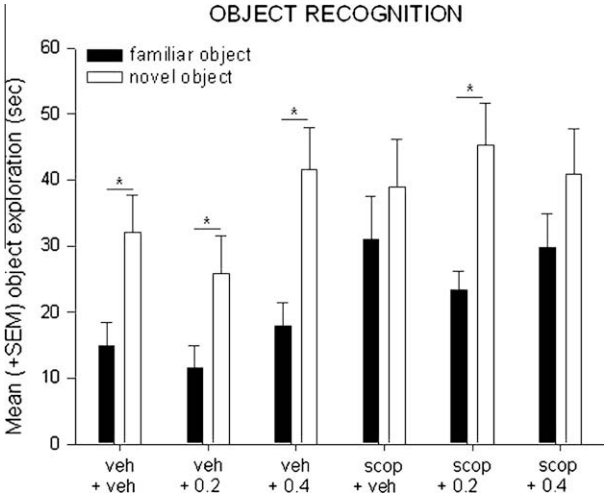

Fig. 1. Object recognition performance of rats treated with vehicle + vehicle (veh + veh), vehicle + 0.2 mg/kg senktide (veh + 0.2), vehicle + 0.4 mg/kg senktide (veh + 0.4), scopolamine + vehicle (scop + veh), scopolamine + 0.2 mg/kg senktide (scop + 0.2) and scopolamine + 0.4 mg/kg senktide (scop + 0.4). The data are represented as mean and standard error of object exploration time (s) of familiar and novel objects in the test trial. $^*p < 0.05$.

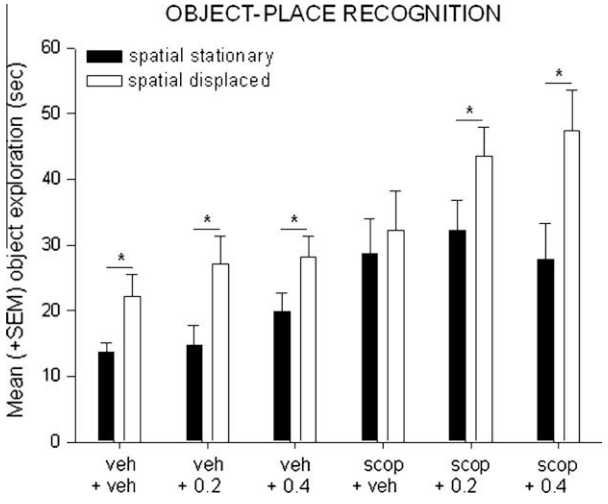

Fig. 2. Object–place recognition of rats treated with vehicle + vehicle (veh + veh), vehicle + 0.2 mg/kg senktide (veh + 0.2), vehicle + 0.4 mg/kg senktide (veh + 0.4), scopolamine + vehicle (scop + veh), scopolamine + 0.2 mg/kg senktide (scop + 0.2) and scopolamine + 0.4 mg/kg senktide (scop + 0.4). The data are represented as mean and standard error of object exploration time (s) of spatial stationary and spatial displaced objects in the test trial. $^*p < 0.05$.

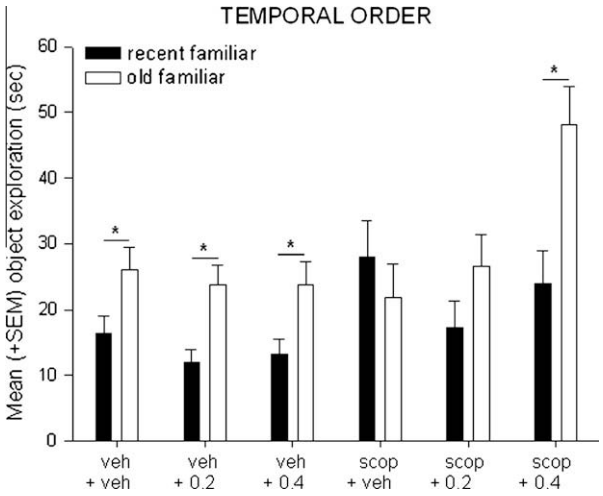

Fig. 3. Object recognition for temporal order performance of rats which received injections of vehicle + vehicle (veh + veh), vehicle + 0.2 mg/kg senktide (veh + 0.2), vehicle + 0.4 mg/kg senktide (veh + 0.4), scopolamine + vehicle (scop + veh), scopolamine + 0.2 mg/kg senktide (scop + 0.2) and scopolamine + 0.4 mg/kg senktide (scop + 0.4). The data are represented as mean and standard error of object exploration time (s) of recent familiar and old familiar objects during the test trial. $^{*}p < 0.05$.

scopolamine on object recognition, object–place recognition and object recognition for temporal order. The results indicate that the selective NK_3–R agonist senktide reversed the scopolamine-induced memory deficits by re-establishing object recognition, object–place recognition as well as object recognition for temporal order. These results indicate memory enhancing effects of senktide in animals subjected to scopolamine-induced memory impairments. In the present study, the rats that received scopolamine (0.75 mg/kg; scop + veh) displayed a disruption of *recognition memory* for the object recognition task. These animals were unable to discriminate between novel and familiar objects. Treatment with senktide was effective in preventing memory impairment by scopolamine for the object recognition, since animals receiving 0.2 mg/kg senktide (scop + 0.2) spent more time exploring the novel relative to the familiar object. In addition, scopolamine impaired the performance in *object–place recognition* whereby the animals failed to discriminate between spatial stationary and spatial displaced objects in terms of exploration time. Senktide at the doses 0.2 mg/kg and 0.4 mg/kg (scop + 0.2 and scop + 0.4) counteracted the scopolamine-induced performance deficits in the object–place recognition task. In the *object recognition for temporal order* task we found that administration of scopolamine impaired discrimination between old familiar and recent familiar objects. Senktide ameliorated the scopolamine-induced deficits by re-establishing the discrimination between old familiar and recent familiar objects, i.e. the rats receiving 0.4 mg/kg senktide (scop + 0.4) spent more time exploring the old familiar compared to the recent familiar object, thus, indicating intact object recognition for temporal order.

When administered alone, senktide (veh + 0.2 and veh + 0.4) had no effect on performance in the three recognition tasks and was comparable to that of the vehicle controls (veh + veh) with intact recognition for object, object–place as well as temporal order.

Evidence for a role of NK_3-R in learning and memory has been found previously in rats (Schäble et al., 2011) and mice (Kameyama et al., 1998; Ukai et al., 1996; Zlomuzica et al., 2008). For instance, beneficial effects of NK_3-R agonism were described in a spontaneous alternation (Kameyama et al., 1998; Ukai et al., 1996) and episodic-like memory task (Zlomuzica et al., 2008) in mice. Moreover, deficits in passive avoidance, conditioned avoidance and the Morris water maze task were found after NK_3-R knockout (Siuciak et al., 2007). Our results presented here are in line with previous studies, showing that central application of senktide alleviated scopolamine-induced impairment in a spontaneous alternation task in mice (Kameyama et al., 1998; Ukai et al., 1998), suggesting a possible role of the NK_3-R in learning and memory via cholinergic modulation.

Using in vivo microdialysis, the NK_3-R agonist senktide has been reported to increase extracellular ACh levels in the frontal cortex, amygdala and hippocampus (Schäble et al., 2011), brain regions which have been implicated in learning and memory processes. Accordingly, earlier studies demonstrated that the activation of NK_3-R modulated the release of acetylcholine (Marco et al., 1998; Steinberg et al., 1995). Senktide caused increases in striatal ACh when applied into the striatum (Steinberg et al., 1995). An increase of ACh neurotransmission in the hippocampus was also obtained after senktide administration into the medial septum (Marco et al., 1998). NK_3-R have been shown to be localized on cholinergic neurons (Chen, Wei, Liu, Ding, et al., 2001; Chen, Wei, Liu, Qiu, et al., 2001). Moreover, it was shown that drugs that increase cholinergic activity in the hippocampus, such as acetylcholine esterase (AChE) inhibitors, improved episodic memory in humans (Gron, Kirstein, Thielscher, Riepe, & Spitzer, 2005). Increased ACh release in the hippocampus was reported to be associated with novel object exploration (Degroot, Wolff, & Nomikos, 2005). Furthermore, treatment with the ACh agonist nicotine improved (Puma et al., 1999), whereas the ACh antagonist scopolamine impaired memory for object recognition (Bartolini, Casamenti, & Pepeu, 1996; Ennaceur & Meliani, 1992).

A promnestic effect of senktide seems to become evident only in a deficit model. Accordingly, it was shown that senktide significantly improved a component of episodic-like memory in age-associated memory impaired rats (Schäble et al., 2011), promoted episodic-like memory in a time-delay deficit model in mice (Zlomuzica et al., 2008) and attenuated scopolamine-induced impairment in a spatial working memory task in mice (Kameyama et al., 1998; Ukai et al., 1996). Interestingly, it was shown that the NK_3-R ligand Neurokinin B (NKB) had a stimulatory effect on AChE and reversed the toxic effects Aβ (Mantha, Moorthy, Cowsik, & Baquer, 2006). In addition, infusion of NKB into the nucleus basalis magnocellularis (NBM) prevented a decrease in choline acetyltransferase (ChAT) activity, a specific marker for the loss of cholinergic neurons, and enhanced a reference memory deficit in the radial maze (Wenk et al., 1997). These results, together with the present data, strongly indicate beneficial functions of NK_3-R agonism in memory-deficit models linked to cholinergic dysfunction.

Scopolamine has been found to impair performance in object recognition (Bartolini et al., 1996; Botton et al., 2010; Ennaceur & Meliani, 1992), object–place recognition (Barker & Warburton, 2009; Pitsikas, 2007) and object recognition for temporal order tests (Barker & Warburton, 2011a) and our data support these findings. The scopolamine and senktide used in this set of experiments were applied systemically, therefore it cannot be fully ruled out that attentional or sensorimotor factors had influenced the animals' performance. Previous studies reported that scopolamine administration caused hyperlocomotion in rodents (Bertrand et al., 2001; Morita et al., 1995; Mueller & Peel, 1990). However, we found no evidence of a drug-associated change in the animals' general locomotor activity, since line crossings were not significantly altered between groups and over trials (data not shown). Moreover, scopolamine (scop + veh) as well as senktide (veh + 0.2 and veh + 0.4), when administered alone, had no effect on total object exploration, which suggests that performance was unrelated to locomotion and exploratory behavior. By contrast, total object exploration was increased in the scop + 0.2 and scop + 0.4

groups in the object–place recognition test and in the scop + 0.4 group in the test for object recognition for temporal order.

Several studies have highlighted an important role for the medial prefrontal cortex (Barker, Bird, Alexander, & Warburton, 2007; Kesner, Hunt, Williams, & Long, 1996), hippocampus (Barker & Warburton, 2011b; Mumby, Gaskin, Glenn, Schramek, & Lehmann, 2002) and perirhinal cortex (Aggleton, Keen, Warburton, & Bussey, 1997; Barker et al., 2007; Bussey, Duck, Muir, & Aggleton, 2000) in recognition memory involving relative familiarity of objects i.e. object recognition (Ennaceur & Delacour, 1988), location of preceding encountered objects, i.e. object–place recognition (Dix & Aggleton, 1999) or the temporal order in which objects were presented previously (Mitchell & Laiacona, 1998). Winters and colleagues (2004) reported that the hippocampus is functionally crucial for object–place, but not object recognition and the perirhinal cortex is critical for object recognition, but not object–place recognition. Bilateral hippocampal lesions caused deficits in object–place and object recognition for temporal order, whereas no impairments in object recognition were obtained (Barker & Warburton, 2011b). Disconnection studies revealed that interactions between the medial prefrontal cortex, hippocampus and perirhinal cortex are necessary for the temporal order recognition memory (Warburton & Brown, 2010), while object as well as object–place recognition are not contingent upon the integrity of a medial prefrontal cortex–perirhinal cortex–hippocampus circuit (Barker & Warburton, 2011b).

NK_3-R expression have been identified in brain regions related to learning and memory processes, including the frontal cortex and hippocampus (Ding et al., 1996; Duarte et al., 2006; Shughrue et al., 1996). Given that various studies have suggested an involvement of the perirhinal cortex in object information processing (Aggleton et al., 1997; Barker & Warburton, 2011b), the medial prefrontal cortex is being critical to forming an association between object and spatial information (Barker et al., 2007) and the hippocampus as necessary for spatial information processing (Barker & Warburton, 2011b), it might be proposed that the memory promoting effect of the NK_3-R agonist senktide on object, object–place as well as object recognition for temporal order was mediated by an action of NK_3-R activation in these brain areas. In the present study, the lower dose of 0.2 mg/kg senktide re-established object recognition, indicating that the behavioral effect of senktide may be mediated by the medial frontal cortex and perirhinal cortex. The higher dose of 0.4 mg/kg senktide was effective to reinstate the scopolamine-induced memory deficits in the object–place and object recognition for temporal order task, which may be mediated by the hippocampus. Our behavioral data are in agreement with a previous study showing that 0.2 mg/kg senktide increased ACh level in the frontal cortex, whereas in the hippocampus 0.4 mg/kg senktide increased ACh level (Schäble et al., 2011). This suggests that the effects of senktide were likely due to changes in the cholinergic neurotransmission. The frontal cortex receives dense cholinergic innervations from the cholinergic neurons within the NBM in which NK_3-R were localized (Chen, Wei, Liu, Ding, et al., 2001), whereas the hippocampus receives its input from the medial septum (Mesulam, Mufson, Wainer, & Levey, 1983).

Considering the current findings, it is likely that senktide modulates the activity of muscarinic ACh receptors, given that senktide counteracted the impairments induced by scopolamine, a muscarinergic receptor antagonist. The known cholinergic activating action of senktide in hippocampus and frontal cortex indicates these structures as potential loci of action for its promnestic effects. This suggests a possible relationship between activation of the septo-hippocampal/frontal cortex cholinergic pathways and the promnestic effects of NK_3-R agonism by senktide and its endogenous ligands, such as Neurokinin B and Substance P, the latter having minor receptor affinity and long been implicated in memory- and ACh-enhancing action (De Souza Silva, Hasenohrl, Tomaz, Schwarting, & Huston, 2000; Hasenöhrl et al., 2000).

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8 Erklärung

Die hier vorgelegte Dissertation habe ich selbständig und nur unter Verwendung der angegebenen Literaturquellen angefertigt. Diese Arbeit wurde in der vorgelegten oder ähnlichen Form bei keiner anderen Institution eingereicht. Zudem erkläre ich, dass ich bisher keine erfolglosen Promotionsversuche unternommen habe.

Düsseldorf, den 27.02.2012

Sandra Schäble