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Modulation of glutamatergic synaptic transmission and plasticity by sigma receptor type 1 in the CA1 region of the hippocampus

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Modulation of glutamatergic synaptic transmission and plasticity by sigma receptor type 1 in the CA1 region of the hippocampus.

By

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June 2006

Thesis submitted to the Faculty of Graduate and Postdoctoral Studies

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Abstract

The sigma receptor (σR), once considered a subtype of opioid receptor, is now described as a distinct pharmacological entity. σ1Rs are thought to be implicated in neuropsychiatric disorders such as schizophrenia, depression as well as in learning and memory. The modulation of *N*-methyl-D-aspartate receptor (NMDAR) by the σ1R has been extensively documented; however, the mechanism through which the σ1R modulates the NMDAR has been a mystery for almost two decades. Here, I report that σ1R activation increases NMDAR responses and long-term potentiation (LTP) by blocking a small conductance Ca^{2+}-activated K^+ current (SK channels) known to shunt NMDAR responses. Therefore, the blockage of SK channels and the resulting increased Ca^{2+} influx through the NMDAR enhances NMDAR responses and LTP. These results emphasize the importance of the σ1R as a post-synaptic regulator of synaptic transmission.

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Abbreviations

A_f	relative portion of fast decay time constant
A_s	relative portion of slow decay time constant
AC	associational commissural pathway
ACSF	artificial cerebrospinal fluid
AHP	afterhyperpolarization
AMPAR	alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptors
AP-5	D-2-amino-5-phosphonovaleric acid
BK channels	large conductance Ca^{2+}-activated K^+ channels
CaMKII	calmodulin kinase II
CGP 52432	3-[[[(3,4-dichlorophenyl)methyl]amino]propyl] diethoxymethyl)phosphinic acid
CA	Cornu Ammonis
CNS	central nervous system
CPA	cyclopiazonic acid
D2	dopamine type 2 receptor
DG	dentate gyrus
DTG	1,3 di-o-tolylguanidine
EC	enthorhinal cortex
EGTA	ethylene glycol bis(2-aminoethyl ether)-N,N,N'N'-tetracetic acid
EPSP	excitatory postsynaptic potential
EPSC	evoked postsynaptic current

ER	endoplasmic reticulum
fAHP	fast afterhyperpolarization
HEPES	N-2-hydroxy-ethylpiperazine-N'-2-ethanesulfonic acid
I_{AHP}	current underlining the medium AHP
IK	Voltage dependent Ca^{2+}-activated K^+ channels
IP_3	inosotol 1,4,5-triphosphate
KAR	kainate receptors
LTP	long term potentiation
mAHP	medium AHP
MF	mossy fibres
min	minutes
mV	millivolts
NBQX	1,2,3,4-tetrahydro-6-nitro-2,3-dioxobenzo[f]quinoxaline-7-sulfonamide
NMDA	N-methyl-D-aspartic acid
NMDAR	N-methyl-D-aspartate receptor
PCP	phenylcyclidine
PKA	protein kinase A
PKC	protein kinase C
PLC	phospholipase C
PP	perforant path
PPD	paired-pulse depression
PPF	paired-pulse facilitation
PSD	post-synaptic density

QX-314	lidocaine N-ethyl bromide
R_{in}	input resistance
sAHP	slow AHP
Sb	subiculum
SC	Schaffer collaterals
SK channels	small conductance Ca^{2+}-activated K^{+} current
sMRI	structural magnetic resonance imaging
TEA	tetraethylammonium
TTX	tetrodotoxin
V_m	membrane potential
σR	sigma receptor
σ1R	sigma receptor type 1
σ2R	sigma receptor type 2
τ_f	fast component of decay time constant
τ_s	slow component of decay time constant
τ_{mean}	weighted time constant

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Publications

1. Martina M., **B-Turcotte M.E.**, Halman S., Tsai G., Tiberi M., Coyle J.T. and Bergeron R. **(2005)** Reduced glycine transporter type 1 expression leads to major changes in glutamatergic neurotransmission of CA1 hippocampal neurons in mice. Journal of Physiology, 563: 777-793.
2. Martina M., **B-Turcotte M.E.**, Halman S. & Bergeron R. Sigma-1 receptor modulates NMDA receptor synaptic transmission and plasticity *via* SK channels. *Submitted to Journal of Physiology May 2006.*

Abstracts

1. Martina M., **B-Turcotte M.E.**, Halman S. and Bergeron R. **(2006)** Sigma-1 receptor modulates NMDA receptor synaptic transmission and plasticity *via* SK channels in rat hippocampus. FENS 2006.
2. Imamura Y., Martina M., **B-Turcotte M.E.**, Begovic D., Ma C. and Bergeron R. **(2005)** Glycine-induced NMDAR endocytosis is differently regulated at the synapse compared to the extra-synaptic area of CA1 pyramidal neurons. Society for Neuroscience.
3. Boyd J., Martina M., **B-Turcotte M.E.**, Tsai G., Coyle J.T. and Bergeron R. **(2004)** Altered layer distribution of NR2A and NR2B in the hippocampal CA1 region in mice in which the expression of glycine transporter type 1 is reduced. Society for Neuroscience.
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Chapter 1: Introduction

1. Preface

Schizophrenia is one of the most disabling and costly forms of mental illness, affecting 1% of the population. There are three major categories of symptoms which characterize schizophrenia: negative symptoms (social withdrawal, diminished motivation and blunted emotional expression), positive symptoms (delusions, hallucinations) and cognitive impairment (working memory). Although the exact cause of the illness is still unknown, there are significant evidences indicating that the hippocampus plays a major role in the pathophysiology of schizophrenia. Indeed, structural magnetic resonance imaging (sMRI) has shown that the volume of the hippocampal complex is significantly reduced in schizophrenic patients[1]. There are currently two leading hypothesis, both based on dysfunctions in neurotransmitters, put forward to explain schizophrenia: hyperactivity of the dopaminergic system[2] and hypofunction of glutamatergic transmission involving the *N*-methyl-D-aspartate receptor (NMDAR)[3]. It has long been recognized that NMDAR antagonists such as phencyclidine (PCP or "angel dust") produce a syndrome in normal individuals that closely resembles schizophrenia and exacerbate symptoms in patients with known chronic schizophrenia.

Recent studies have also showed some evidence for a potential link between sigma receptors (σRs) and the etiology of schizophrenia[4;5]. A major conclusion drawn from various studies is that many σR ligands have antipsychotic effects in animal models [4;5]. Indeed, it has been shown that haloperidol, a drug widely used in the treatment of schizophrenia, exhibits high affinity towards both dopamine type 2 receptors (D2) and σRs[6]. Moreover, post-mortem

studies have demonstrated that σRs localized in the brain are significantly decreased in schizophrenic patients [7].

σR type 1 (σ1R), a 25-30 kDa single polypeptide, is widespread in the central nervous system (CNS) and is present in high levels in the prefrontal cortex, hippocampus and striatum[8]. σ1Rs have been proposed to be involved in learning and memory as well as in neuropsychiatric disorders including depression[4;5;9]. It has been shown that several clinically used psychotropic drugs (such as haloperidol) can bind to the σ1Rs with high affinity [10]. Moreover, behavioral studies using animal models of amnesia have also shown that σ1R agonists improve learning, memory and cognition [11]. Although the precise mechanisms involved in the functional response of σ1Rs are still uncertain, σ1Rs have been implicated in the modulation of neuronal activity. In the hippocampus, σ1Rs are thought to play a role in modulation of the glutamatergic neurotransmission *via* modulation of the NMDAR [12-14].

2. *Hippocampal Formation*

The hippocampal formation is comprised of six cytoarchitechtonically distinct areas that are linked one to the next by largely unidirectional projections (**Fig. 1**)[15]. They include: the dentate gyrus (DG), hippocampus proper, subiculum (Sb), presubiculum, parasubiculum and entorhinal cortex (EC). The hippocampus proper (Cornu Ammonis, CA) is divided into three fields (CA1-CA3). A unique feature of the hippocampal circuitry is the largely unidirectional organization of the projections that interconnect the various hippocampal regions (for instance, CA3 projects to CA1). The principal cellular layer is the pyramidal cell layer in which the cell bodies of hippocampal pyramidal cells are densely packed[15]. The stratum

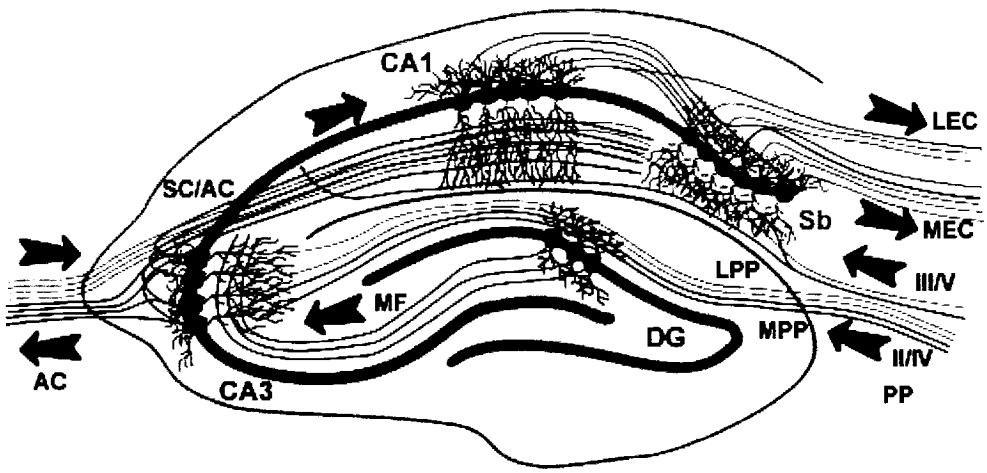

Figure 1 The hippocampal network

The hippocampus forms a principally unidirectional network, with input from the entorhinal cortex (EC) that forms connections with the dentate gyrus (DG) and CA3 pyramidal neurons *via* the perforant path (PP). CA3 neurons also receive input from the DG *via* the mossy fibres (MF). They send axons to CA1 pyramidal cells *via* the Schaffer collaterals Pathway (SC), as well as to CA1 cells in the contralateral hippocampus *via* the Associational Commissural pathway (AC). CA1 neurons also receive input directly from the PP and send axons to Subiculum (Sb). These neurons in turn send the main hippocampal output back to the EC, forming a loop. *This figure has been modified from ref. (Medical Research Council. 2003. Centre for synaptic plasticity.*

[Online] URL: http://www.bris.ac.uk/synaptic/info/pathway/hippocampal.htm#MF)

radiatum can be defined as the suprapyramidal region in which CA3 to CA3 associational connections and CA3 to CA1 Schaffer collaterals connections are located. Inputs terminating in the distal portion of the stratum radiatum are mainly glutamatergic[16]. The proportion of inhibitory inputs to distal stratum radiatum dendrites is small (~3%)[17].

3. *Glutamatergic Transmission*

Glutamate is the major excitatory neurotransmitter in the mammalian CNS. On the basis of pharmacological and biophysical characteristics, three subtypes of glutamate-gated ionotropic receptor channels have been identified: alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptors (AMPAR), kainate receptors (KAR) and NMDAR[18]. AMPAR and KAR mediate fast excitatory postsynaptic potentials (EPSPs) while NMDARs serve a modulatory function as they are blocked by Mg^{2+} in a voltage dependent manner (**Fig. 2**). Pre-synaptic activity causes the liberation of glutamate and consequently the post-synaptic activation of the KARs and/or AMPARs. The activation of these receptors causes a depolarization, which releases the Mg^{2+} block gating the NMDAR and induces the opening of the channel.

3.1 AMPARs

AMPARs are homomeric or heteromeric oligomers composed of four highly homologous subunits (GluR1-GluR4)[18]. The pharmacological and physiological properties of native AMPARs are consequences of different assemblies of these subunits. The binding of glutamate or AMPA to the AMPAR is associated with an inward depolarizing current primarily carried by Na^{+} ions. AMPAR in central neurons are also permeable to K^{+} but considered almost impermeable to Ca^{+2} [18].

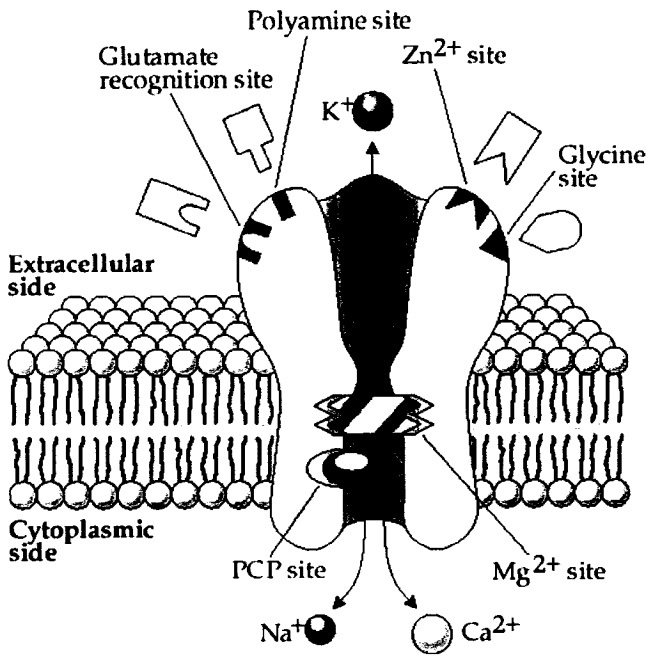

Figure 2 Model of NMDAR

Glutamate is the major excitatory neurotransmitter in CNS and it is known to bind to the NMDARs on a binding site located on NR2 subunit. NMDAR are also positively modulated by glutamate's necessary coagonist, glycine. Glycine binds to a specific, strychnine insensitive, $glycine_B$ site located on the NR1 subunit. Polyamines such as spermine and spermidine are positive modulators at low concentration but also block the channel at higher concentrations. At resting potential, the NMDAR is blocked by Mg^{2+} and may also be blocked uncompetitively by PCP whose binding site resides close to that of Mg^{2+}. $Zn^{2+}$is a potent, voltage-independent antagonist of the NR2A-containing NMDAR. In addition, most NMDARs are influenced by Zn^{2+} ions in a voltage-dependent manner, as well as by oxidation/reduction and pH. Importantly, all heteromeric NMDAR subtype complexes are permeant to Ca^{2+}, Na^+ and K^+.

[Online] URL: http://www.securitylab.ru/_Article_Images/2005/10/nmda_receptor.gif

3.2 NMDARs

NMDARs are heteromultimeric channels comprised of three different subunit families (NR1, NR2A-D, NR3A-B)[18]. NMDAR activation requires two co-agonists acting at the glutamate and glycine recognition sites located on the NR2 and NR1 subunits, respectively (for review [19]). The NR1 subunit, in combination with at least one type of NR2 subunit (NR2A-2D), gives rise to receptor subtypes with distinct pharmacological profiles, gating properties, glycine affinity and Mg^{2+} sensitivity [19]. The NMDAR is primarily permeable to Ca^{2+} and to a lesser extent, to Na^{+} and K^{+} (**Fig. 2**). NMDARs play a pivotal role in learning, memory, synaptic plasticity and neuropsychiatric disorders [20]. Indeed, Ca^{2+} influx through the NMDAR is necessary for the Long-Term Potentiation (LTP) of synaptic transmission and the persistent enhancement of synaptic efficiency[20]. NMDARs seem also to be involved in neuronal development and cell survival. They mediate the above functions by a combination of coincidence detection and signal transduction by elevating intracellular Ca^{2+} levels.

4. Synaptic Plasticity

It is now widely accepted that synaptic plasticity is a critical component of the neural mechanisms underlying learning and memory. There are different forms of synaptic plasticity since synapses differ considerably in their response to repetitive activation, some showing synaptic depression and others showing facilitation. Moreover, depending on the stimulation frequency, short- or long-term plasticity can be induced. Short-term means having a time course of milliseconds to seconds, while long-term ranges from 30 minutes to hours or even days [21].

4.1 Short-term plasticity:

The short-term forms of synaptic plasticity are expressed in electrophysiological experiments as changes in the ratio of the amplitude of sequential evoked postsynaptic currents (EPSCs). The paired-pulse paradigm consists of two stimuli delivered at a fixed interval (50-100 ms). If the amplitude of the second EPSC is larger than the first one, we have facilitation (PPF). If the second EPSC is smaller than the first one, we have depression (PPD). The mechanisms by which two pulses given at a small interval lead to an enhancement of the second response can best be explained through Katz and Miledi's residual calcium hypothesis[22]. Facilitation is the result of a nonlinear vesicular release dependent upon intracellular Ca^{2+} concentrations. When an action potential occurs at the presynaptic terminal, there is influx of Ca^{2+} in the terminal, leading to docking and fusion of vesicles containing transmitters and subsequent release of the transmitter in the synaptic cleft. When two presynaptic events occur within a brief delay (such as in the paired-pulse paradigm), there is a high probability that some residual Ca^{2+} from the first event persists in the nerve terminal. Hence, more Ca^{2+} is available for vesicular exocytosis and more transmitters can be released, resulting in a facilitated response. If we assume that transmitter release is quantal in nature, the second response is greater because a larger amount of quanta have been released to elicit the response. Note that PPD can also be observed if the transmitter is inhibitory or if a substance acts to decrease intracellular Ca^{2+} influx. The paired-pulse paradigm relies on this form of short-term plasticity to determine if a substance alters pre-synaptic transmitter release.

The CA3 input to CA1 pyramidal cells, mediated by the Schaffer collaterals, is glutamatergic [16]. When the paired-pulse paradigm is applied in the hippocampus, one would expect to see facilitation of the response.

4.2 Long-term plasticity:

LTP refers to a long-lasting enhancement of synaptic transmission between the presynaptic and postsynaptic neurons. LTP is a phenomenon that occurs at different kinds of central and peripheral synapses but has been discovered and most thoroughly studied in the hippocampus. Activity-dependent changes in synaptic strength have been demonstrated at all three major excitatory pathways in the hippocampus: the perforant pathway to the dentate granular cells, the mossy fibers to CA3 pyramidal cells, and the Schaffer collaterals/commissural projections to CA1 pyramidal cells. LTP in the CA1 region of the hippocampus however, has generated more interest and more investigation than any other form of synaptic plasticity. It is well documented that LTP at the Schaffer collaterals/commissural-CA1 pyramidal cell synapse requires the activation of the NMDAR [23]. Indeed, the best understood form of LTP is induced by the activation of the NMDAR.

LTP properties (input-specificity, cooperativity, and associativity) can be easily explained with the special features of the NMDAR [23]. First, "Input-specificity" is explained by the need of sufficient concentration of glutamate at the synapse. Glutamate is usually provided by action potentials arriving at the presynaptic terminals. Second, "Cooperativity" reflects the voltage dependent block of its channel by Mg^{2+} that allows the NMDAR to behave as a molecular "coincidence" detector. Third, "Associativity" can also be explained by the need of sufficient postsynaptic depolarization. To trigger the induction of LTP, the membrane of the cells should be sufficiently depolarized to relieve the Mg^{2+} block from the NMDAR [23].

LTP of synaptic transmission in the CA1 region of the hippocampus is a useful model for studying the cellular and molecular mechanisms underlying cognitive processes such as learning and memory in vertebrates [20;23]. Pharmacological studies have demonstrated that the

hippocampal formation is involved in certain forms of long-term information storage. Moreover, LTP is consistent with the assumption that information is stored in the brain as changes in synaptic efficiency. Similarly to learning, LTP is induced rapidly and can last for many hours and days. Finally, LTP can be associative: temporally pairing "weak" with "strong" synaptic inputs leads to potentiation of the "weak"' inputs. The associativity feature of LTP resembles some simpler forms of learning such as classical conditioning and habituation, studied by psychologists and neurophysiologists at the behavioral level.

The expression of the synaptic potentiation involves mechanisms triggered by Ca^{2+} such as the increase in the number or the change of the properties of AMPARs and the increase of the transmitter release. LTP most likely also requires changes in protein synthesis [24] and alterations in dendritic morphology and synapse quantity. Ca^{2+} binds to calmodulin that activate calmodulin kinase II (CaMKII), which undergoes autophosphorylation and then phosphorylates the GluR1 subunit of AMPAR to increase their single channel conductance [20;25]. CaMKII can also contribute to LTP by participating in the delivery and insertion of additional AMPAR in the postsynaptic membrane [20;26]. Consequently, the postsynaptic expression of LTP most likely is due to both phosphorylation and rapid delivery and insertion of AMPARs within the postsynaptic membrane.

Since NMDARs are located at dendritic spines and have high permeability to Ca^{2+}, it is believed that NMDAR activation is necessary for LTP induction [27] and the persistent enhancement of synaptic efficiency at the Schaffer colateral/commissural-CA1 pyramidal cell synapse [23] (**Fig. 3**). The induction of LTP can be blocked by preventing the rise in

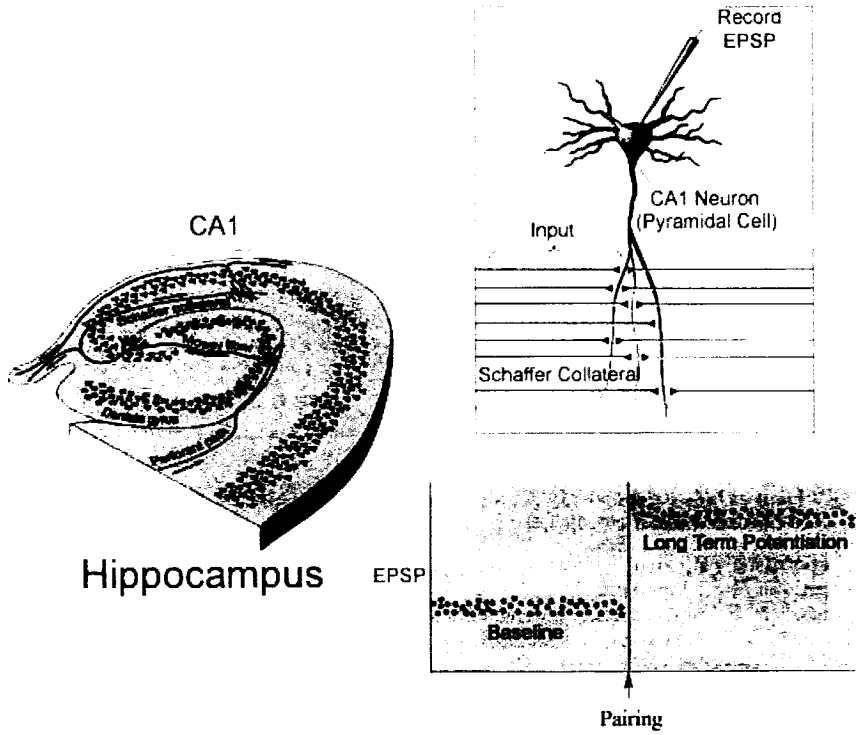

Figure 3 Pairing protocol to induce LTP in the CA1 region of the hippocampus

To induce LTP in the CA1 region of the hippocampus, post-synaptic currents are evoked by electrical stimulation of the Schaffer collaterals with a bipolar microelectrode positioned in the stratum radiatum. Post-synaptic currents are then evoked every 10 sec. Ten minutes of stable baseline is recorded in absence or presence of drugs followed by 3 brief high frequency tetani (50 pulses at 100 Hz, 4 s intervals) given at the end of a long depolarization (3 min at 0 mV) [28;29]. After the conditioning, post-synaptic currents are evoked every 10 sec during 40 min. This protocol induced an increase of the synaptic responses lasting for more than 40 min. The pairing protocol used here to induce LTP has been shown to be NMDAR dependent [30].

intracellular Ca^{2+} by loading postsynaptic cells with Ca^{2+} chelators [31]. Conversely, experimentally increasing the level of postsynaptic Ca^{2+} can mimic LTP [31].

5. *Ca^{2+} – activated K^+ channels*

Action potentials are usually followed by a rise in intracellular Ca^{2+} that leads to an afterhyperpolarization (AHP) of the membrane, which is an important determinant of neuronal excitability and firing patterns. In general, the AHP is comprised of three overlapping kinetic components: fast (fAHP) , medium (mAHP) and slow (sAHP), reflecting the activation of different families of Ca^{2+}-activated K^+ channels: large conductance (BK), small conductance (SK) and voltage independent (IK) [32]. The fAHP contributes to the action potential repolarization and might be responsible for the spike broadening during repetitive firing [32;33]. fAHP is activated immediately following the action potential and it typically lasts for < 20 ms and is due to the activation of BK channels [34;35]. Following the fAHP, cells may display a prolonged hyperpolarization phase lasting for hundreds of milliseconds, possibly seconds. These slower Ca^{2+}-activated K^+ currents may be separated into two distinct components, mAHP and the sAHP [32]. mAHP activates within 50 ms and lasts for more than 1 second (s) and it is underlined by the opening of SK channels [32]. SK channels are voltage-insensitive channels that have a widespread distribution throughout the nervous system [36;37]. These channels are potently and specifically blocked by apamin, a component of bee venom [38;39]. It has been suggested that in a number of cell types, including hippocampal and cortical pyramidal neurons, the mAHP underlined by SK channels controls action potential firing frequency [39]. The sAHP has a peak at ~ 500 ms and can last for several seconds. It is underlined by voltage independent K^+ channels (IK) whose activation requires

a rise in cytosolic Ca^{2+}, typically by Ca^{2+} influx through voltage-dependent Ca^{2+} channels [34]. IK channels are apamin and 4-aminopyridine insensitive but tetraethylammonium (TEA; low concentration) sensitive Ca^{2+}-activated K^+ channels [32;39]. IK channels have been poorly studied largely owing to their sparse distribution [40].

In CA1 pyramidal cells, synaptic activation may induce Ca^{2+} influx through NMDAR[41;42] as well as through voltage-gated Ca^{2+} channels[43]. Synaptic activation of a Ca^{+2}-activated K^+ current reduces postsynaptic excitability in response to high-frequency synaptic input[44]. This negative regulation occurs within individual spines, where SK channels respond to rapid increase in Ca^{2+} and reduce the amplitude of NMDAR-mediated Ca^{2+} transient[45]. The effects of different Ca^{2+} buffers show that SK channels are positioned within a synaptically activated Ca^{2+} activated signalling microdomain and act rapidly to influence the EPSP[45].

6. σRs

σR was first described as a subtype of opioid receptor[46]. Further studies using ligands with high affinity and selectivity have demonstrated that it is a distinct pharmacological entity[47;48]. σRs are localized in the CNS (brainstem areas, limbic structures and brain regions associated with endocrine function) as well as in peripheral organs (kidneys, heart, and, most of all, liver) [49;50]. Biochemical studies allowed the distinction of two classes of σRs: σR type 1 (σ1R) and σR type 2 (σ2R)[50]. These two types of receptors can be distinguished based on their different drug selectivity and molecular weights. The σ1R is a 25-30 kDa single polypeptide, and it was cloned in 1996, while the σ2R, a 18-21 kDa protein, has not yet been cloned[49;51;52]. The present study will focus on the σ1R, which is the major subtype in the

hippocampal formation and other limbic areas involved in cognition, emotion and depression.

6.1 Pharmacology of σ1Rs

Pharmacological studies have shown that σ1R can bind with high affinity many different drugs such as psychotomimetic drugs (benzomorphans, amphetamine, PCP and derivatives, cocaine and derivatives), endogenous neurosteroids (progesterone, pregnenolone sulphate and testosterone), neuroleptics and antipsychotic agents[50;53-55]. The classical neuroleptics used in the treatment of schizophrenia have important motor side effects. Because these undesired effects are not displayed by activation of σRs, there has been a growing interest in developing σR agonists and antagonists for the treatment of psychosis.

6.2 Roles of σ1Rs

The function of σ1R is not yet fully understood, however various recent studies have provided key elements to improve our understanding of the localization of these receptors. Indeed, it has been demonstrated that σ1Rs are mainly localized within post-synaptic densities (PSD), although they could also be observed at the pre-synaptic level in neurons. Interestingly, upon stimulation by psychoactive drugs, σ1R is translocated to the plasmatic and nuclear membranes [56;57]. Moreover, it has been suggested that σ1Rs are localized on smooth endoplasmic reticulum and form a dimeric complex with the cytoskeletal adaptor protein ankyrin-B [57]. σ1Rs have two hydrophobic stretches, and possess a double arginine endoplasmic reticulum retention signal at the N terminus (**Fig. 4**) [58;59]. These suggest that the σ1Rs are trafficking from the endoplasmatic reticulum to the membrane.

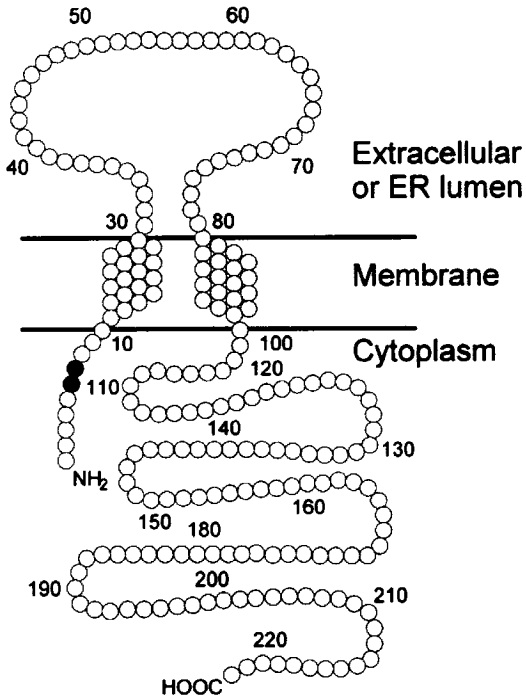

Figure 4 Putative structure of the σ1R

The most important structural aspects are that the protein is a single polypeptide comprising 223 amino acids and that the hydrophobicity plot predicts two hydrophobic stretches. The longer C-terminal end has almost complete homology with the sterol-binding fungal isomerase. There is also a typical arginine-arginine endoplasmic reticulum (ER) locating signal (filled circle) near the N terminal region. *This figure has been modified from ref*[60].

Several studies have showed that σ1Rs can modulate several physiological and cellular events in the CNS. Behavior studies using animal models of amnesia have shown that σ1R agonists improve learning, memory and cognition [61]. σ1R agonists also exhibit antidepressant-like activities [62]. Although the precise mechanisms involved in the functional response of σRs are still uncertain, σ1Rs have been implicated in the modulation of neuronal activity. σ1R ligands have been described to regulate ion channels such as voltage-dependent Ca^{2+} channels [63], NMDAR[12-14;64], inosotol 1,4,5-triphosphate (IP3) receptors and Ca^{2+} signalling at the endoplasmic reticulum [56] as well as mobilization of cytoskeletal adaptor proteins (for review see [65]). Recently, Aydan and his co-workers [66] have also shown that σ1Rs modulate K^+ channel as a regulatory subunit by a direct interaction.

6.3 Regulation of NMDAR by σR ligands in " in vivo" models

In the hippocampus, σ1Rs are thought to play a role in the modulation of the glutamatergic neurotransmission *via* the modulation of the NMDAR [12-14;64]. Bergeron and Debonnel [12;67] showed that low doses of high affinity σ1R agonists (*1,3 di-o-tolylguanidine (*DTG), Igmesine and (+)pentazocine) potentiate the NMDAR response in CA3 granule cells of the hippocampus, while σ1R antagonists (haloperidol and NE-100) administered alone did not modify the NMDAR response. However, when the σ1R antagonists were applied following treatment with σ1R agonists, the potentiation observed through agonist interaction was suppressed.

7. Overall Rationale

Considering the role played by σ1Rs in the modulation of glutamatergic neurotransmission *via* NMDAR in the hippocampus [12-14;64;68] and the importance of NMDAR in schizophrenia and synaptic plasticity, I have studied the mechanism through which σ1Rs modulate the NMDAR response and LTP in rat hippocampal CA1 pyramidal cells.

Chapter 2: Materials and Methods

Preparation of hippocampal slices. Coronal brain slices containing the hippocampus were obtained from Sprague-Dawley rats (21 to 28 days old). Prior to decapitation, the animals were anaesthetized with isofluorane in agreement with the guidelines of the Canadian Council of Animal Care. The brain was removed and placed in an oxygenated (95% O_2/5% CO_2) physiological solution, artificial cerebrospinal fluid (ACSF) at 4°C, containing 126 mM NaCl, 2.5 mM KCl, 1 mM $MgCl_2$, 26 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, 2 mM $CaCl_2$ and 10 mM Glucose. The osmolarity of the ACSF was adjusted to 300 mOsm and the pH to 7.2. A block containing the region of interest was prepared, and sections (300 μm) were obtained with a vibrating microtome (Leica VT 1000S, Germany). The slices were stored for 1 hour in an oxygenated chamber at room temperature before they were used for the experiments.

Data recording and analysis. Voltage-clamp experiments were performed with borosilicate pipettes filled with a solution containing 130 mM K^+- gluconate, 10 mM N-2-hydroxy-ethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 10 mM KCl, 2 mM $MgCl_2$, 5 mM lidocaine N-ethyl bromide (QX-314), 2 mM ATP-Mg and 0.2 mM GTP-tris(hydroxy-methil) aminomethane (pH 7.2 with KOH). When indicated in the text 0.2 mM ethylene glycol bis(2-aminoethyl ether)-N,N,N'N'-tetracetic acid (EGTA) or 10 mM cesium-BAPTA were added to this solution or a cesium-based solution was used. The cesium-based solution contained 130 mM Cs^+-methanesulphonate, 10 mM HEPES, 10 mM CsCl, 2 mM $MgCl_2$, 5 mM QX-314, 2 mM ATP-Mg and 0.2 mM GTP (pH 7.2 with CsOH). The osmolarity of both the solutions was adjusted to 280-290 mOsm. With these solutions, the liquid junction potential was measured (~10 mV) and the membrane potential (V_m) was corrected accordingly. The pipettes had a resistance of 3-6 MΩ. Recordings with series resistance higher than 20 MΩ

were discarded. Bridge balance was monitored regularly during the recordings. To allow the drugs added in the pipette to induce their pharmacological action, a delay of 10-15 min was systematically observed prior to recording. Only one cell was recorded per slice.

Whole-cell patch-clamp recordings were obtained with a Multiclamp 700A amplifier (Axon Instruments, Foster City, CA) under visual control using differential interference contrast and infrared video microscopy (IR-DIC; Leica DMLFSA, Germany). Electroresponsive properties of neurons were studied by applying 500 ms current pulses from resting potential. The amplitude of current pulses was varied in fixed increments of 20 pA. The input resistance (R_{in}) was estimated in the linear portion of current-voltage plots. The recordings were performed at room temperature from individual pyramidal cells of the CA1 region of the hippocampus voltage-clamped at -65 mV.

EPSCs were evoked by electrical stimulation of the Schaffer collaterals with a bipolar microelectrode positioned in the stratum radiatum. The stimulation intensity consisted of 100 μs current pulses (10-200 μA) and was adjusted to evoke an EPSC amplitude in the range of 40-80 pA at V_m = -65 mV. Stimuli were delivered every 10 s.

To isolate the NMDAR-mediated component of evoked responses, I used ACSF containing a low concentration of $MgCl_2$ (0.1 mM) with osmolarity maintained by $CaCl_2$, and the AMPAR antagonist 1,2,3,4-tetrahydro-6-nitro-2,3-dioxobenzo[f]quinoxaline-7-sulfonamide (NBQX, 20 μM), the $GABA_A$ receptor antagonist picrotoxin (50 μM), the $GABA_B$ receptor antagonist 3-[[(3,4-dichlorophenyl)methyl]amino]propyl] diethoxymethyl)phosphinic acid (CGP 52432, 10 μM).

To isolate the AMPAR-mediated component of evoked responses, a normal ACSF solution with the addition of the NMDAR antagonist dl-2-amino-5-phosphonovaleric acid

(AP-5, 50 μM), the $GABA_A$ receptor antagonist picrotoxin (50 μM), the $GABA_B$ receptor antagonist CGP 52432 (10 μM) was used.

Local drug injections was performed applying air pressure pulses (3-10 ms) with a picospritzer (Parker Instruments) to a patch pipette containing 100 μM N-methyl-D-aspartic acid (NMDA). NMDA was dissolved in ACSF and applied every 30 s. The ejection pipette was positioned directly above the proximal dendrites. NMDA was then applied in presence of tetrodotoxin (TTX; 0.5 μM) to avoid polysynaptic phenomena.

Paired-pulse facilitation was used to determine whether σ1R agonist (+)pentazocine affects transmitter release. PPF is well described for Schaffer collaterals-CA1 synapses [69]. Paired pulses (100 μs duration, 50 ms intervals) were delivered to the Schaffer collaterals. The amplitude of the response as well as the peak-to-peak ratio in the absence (control) and in the presence of (+)pentazocine was compared.

Kinetic analysis was performed on averaged EPSCs (usually 20-25 consecutive traces). The rise times of NMDAR currents were measured at peak to the end. Their decays were fitted with the exponential functions: $y = A_f e^{-t/\tau_f} + A_s e^{-t/\tau_s}$ for double and $y = A_1 \exp^{-t/\tau}$ for single exponential decay, where A is the amplitude, τ is the decay time constant, and the subscript f and s denote fast and slow components, respectively. Weighted time constant (τ_{mean}) was calculated using the equation; $\tau_{mean} = [A_f/(A_f + A_s)]\tau_f + [A_s/(A_s + A_f)]\tau_s$ [70].

Action potentials are followed by a rise in intracellular Ca^{2+} that leads to an afterhyperpolarization (AHP) of the membrane. The AHP is comprised of 3 components, fAHP, mAHP and sAHP, reflecting the activation of different K^+ currents. The currents underlying the medium and slow AHPs were investigated in voltage clamp by giving a 100 ms, 50 mV step from holding potential of -50 mV in low Mg^{2+} ACSF. The medium AHP can

be distinguished from slow AHP by its fast time course and sensitivity to apamin [32]. The current underlining the medium AHP, I_{AHP}, blocked using apamin, was examined by subtracting current recorded in the presence of apamin from control currents.

In the slices used for LTP experiments, the CA3 region of the hippocampus was removed by a surgical cut. EPSCs were evoked by electrical stimulation of the Schaffer collaterals with a bipolar microelectrode positioned in the stratum radiatum.

Stimuli were delivered every 10 s. The recordings for the experiments using the pairing protocol to induce LTP were obtained in ACSF in the presence of picrotoxin (50 μM) at V_m = -65 mV. The pairing protocol used to induce LTP was composed of 3 brief high frequency tetani (50 pulses at 100 Hz, 4 s intervals) given at the end of a long depolarization (3 min at 0 mV) [28;29]. The pairing protocol was induced after 10-12 min of baseline in absence or presence of drugs. This protocol induced an increase of the synaptic responses lasting for more than 40 min. Stimuli were delivered every 10 s. The recordings for the experiments using the pairing protocol to induce LTP were obtained in ACSF in the presence of picrotoxin (50 μM) at V_m = -65 mV. The pairing protocol used to induce LTP was composed of 3 brief high frequency tetani (50 pulses at 100 Hz, 4 s intervals) given at the end of a long depolarization (3 min at 0 mV; **Fig. 3**)[28;29]. The pairing protocol was induced after 10-12 min of baseline in absence or presence of drugs. This protocol induced an increase of the synaptic responses lasting for more than 40 min.

Data were collected using software pClamp 9 (Axon Instrument, Foster City, CA). Analysis were performed off-line with the software IGOR (WaveMetrics Inc., Lake Oswego, OR). Statistical significance of the results was determined with paired *t* tests (two-tailed). All values are expressed as means ± SE.

All drugs are obtained from Sigma-Aldrich (St. Louis, MO, USA), with the exception of CGP 52432, NBQX and ryanodine (Tocris, Bristol, UK).

Chapter 3: Results

Effect of σ1R ligands on glutamatergic synaptic transmission and plasticity

σ1R activation does not affect NMDAR current

It has extensively been reported that in the hippocampus, σ1R may play a role in the modulation of glutamatergic neurotransmission *via* modulation of the NMDAR[11-14;71]. To investigate the mechanism through which σ1R modulates NMDAR at the cellular level, I recorded NMDAR-mediated EPSCs in the presence and in the absence of a high affinity and selective σ1R agonists (+)pentazocine [8] in rat hippocampal CA1 pyramidal cells using patch-clamp whole-cell recordings. To evoke glutamatergic EPSCs, the Schaffer collaterals were stimulated with a bipolar electrode. The NMDAR-mediated component of the EPSCs was pharmacologically isolated in a low Mg^{2+} ACSF containing NBQX, picrotoxin, CGP 52432 and strychnine to block AMPA-, $GABA_A$-, $GABA_B$- and glycine receptor-mediated responses, respectively. I recorded NMDAR-mediated currents using borosilicate electrodes filled with a cesium-based solution (intracellular solution; see Materials and Methods) containing QX-314 and 0.5 mM EGTA to block post-synaptic K^+ currents (known to shunt the NMDAR responses [72;73]), Na^+ currents and to chelate intracellular Ca^{2+}, respectively. The rise, decay, weighted time constants (τ_{mean}) as well as the relative portions (A_f and A_s) of decay time constants of NMDAR-mediated EPSCs were calculated (**Table 1**). Surprisingly, the application of (+)pentazocine (1 μM) did not significantly ($p > 0.05$) change the amplitude (from a meaning amplitude of 37.52 ± 7.02 pA in control to 34.90 ± 7.45 pA in (+)pentazocine; **Table 1**; **Fig. 5**; $n = 9$), while having no effect on their kinetic properties (**Table 1**). These results suggest that σ1R activation does

Intracellular solution: Cesium based solution + 0.5 mM EGTA

Control							(+)pentazocine 1μM						
		deactivation							deactivation				
Ampl (pA)	τ_{act} (ms)	τ_f (ms)	τ_s (ms)	τ_{mean} (ms)	A_f (%)	A_s (%)	Ampl (pA)	τ_{act} (ms)	τ_f (ms)	τ_s (ms)	τ_{mean} (ms)	A_f (%)	A_s (%)
37.52	7.49	75.4	494.4	166.0	79	21	34.90	7.51	74.4	424.0	158.3	77	23
±7.02	±0.57	±8.25	±73.0	±21.3	±1.46	±1.46	±7.45	±0.23	±9.10	±65.3	±38.1	±2.92	±2.92
$n = 9$	$n = 9$	$n = 9$	$n = 9$	$n = 9$	$n = 9$	$n = 9$	$n = 9$	$n = 9$	$n = 9$	$n = 9$	$n = 9$	$n = 9$	$n = 9$

Intracellular solution: Potassium based solution + 0.5 mM EGTA

Control							(+)pentazocine 1μM						
		deactivation							deactivation				
Ampl (pA)	τ_{act} (ms)	τ_f (ms)	τ_s (ms)	τ_{mean} (ms)	A_f (%)	A_s (%)	Ampl (pA)	τ_{act} (ms)	τ_f (ms)	τ_s (ms)	τ_{mean} (ms)	A_f (%)	A_s (%)
20.24	10.6	85.0	728.7	177.2	82	18	31.83 *	11.4	95.3	795.5	201.6	82	18
±2.07	±1.47	±3.93	±96.6	±15.3	±2.68	±2.68	±3.73	±1.26	±6.72	±115	±25.2	±2.52	±2.52
$n = 12$	$n = 12$	$n = 12$	$n = 12$	$n = 12$	$n = 12$	$n = 12$	$n = 12$	$n = 12$	$n = 12$	$n = 12$	$n = 12$	$n = 12$	$n = 12$

Intracellular solution: Potassium based solution + 10 mM BAPTA

Control							(+)pentazocine 1μM						
		deactivation							deactivation				
Ampl (pA)	τ_{act} (ms)	τ_f (ms)	τ_s (ms)	τ_{mean} (ms)	A_f (%)	A_s (%)	Ampl (pA)	τ_{act} (ms)	τ_f (ms)	τ_s (ms)	τ_{mean} (ms)	A_f (%)	A_s (%)
18.06	9.10	93.3	782.9	178.2	82	18	18.52	11.5	76.4	546.3	157.2	82	18
±3.12	±0.70	±13.4	±229	±31.2	±3.28	±3.28	±3.73	±2.55	±17.9	±115	±22.5	±3.29	±3.29
$n = 5$	$n = 5$	$n = 5$	$n = 5$	$n = 5$	$n = 5$	$n = 5$	$n = 5$	$n = 5$	$n = 5$	$n = 5$	$n = 5$	$n = 5$	$n = 5$

Table 1 Rise and decay time constants of NMDAR currents in CA1 pyramidal cells recorded in absence and presence of (+)pentazocine 1 μM at -65 mV.

Values are mean ± S.E.M. Electrically evoked NMDAR currents were recorded in a low Mg^{2+} ACSF (0.1 mM) in presence of NBQX (20 μM), picrotoxin (50 μM), CGP 52432 (10 μM) and strychnine (0.5 μM). The fast and slow decay components are designated τ_f and τ_s, the weighted time constant by τ_{mean}. The amplitude of the NMDAR currents is also shown. Electrically evoked NMDAR currents were recorded in absence (control) and presence of (+)pentazocine (1 μM). *Significant difference between two values ($p < 0.05$).

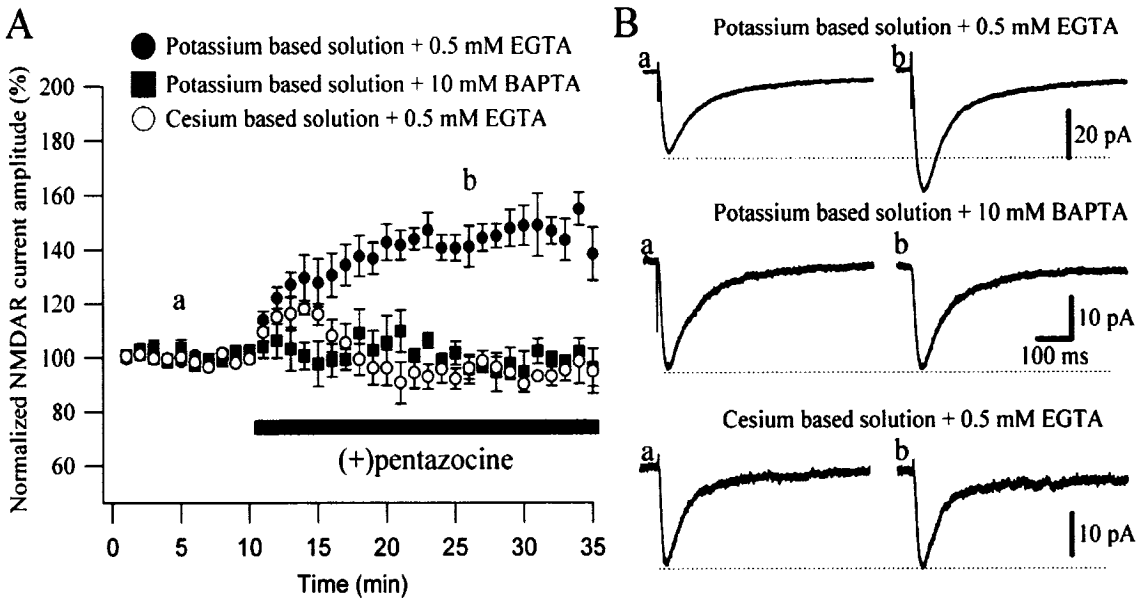

Figure 5 The effect of the σ1R agonist (+)pentazocine 1 μM on NMDAR currents is K^+ dependent

Responses were evoked by bipolar electrical stimuli at *V*m = -65 mV in CA1 pyramidal cells. The evoked NMDAR EPSCs were recorded in Low Mg^{+2} ACSF solution in the presence of NBQX (20 μM) picrotoxin (50 μM), CGP 52432 (5 μM) and strychnine (0.5 μM). (A) Normalized NMDAR current amplitudes (%) are plotted as a function of time. Each point (one every min; mean ± SEM) is the average of 6 points (stimulations every 10 s). The application of the σ1R agonist (+)pentazocine (1 μM) causes an increase in the amplitude of the NMDAR currents when the CA1 pyramidal cells were recorded with a K^+-based solution including 0.5 mM EGTA (•; *n* = 12). When a cesium-based solution including 0.5 mM EGTA (○; *n* = 9) or K^+-based solution including 10 mM BAPTA (■; *n* = 5) is used, (+)pentazocine has no effect on the NMDAR current amplitude. (B) Examples of traces of

NMDAR currents measured at the time points indicated in A (a and b) are shown. Each trace is an average of 20 traces.

not affect NMDAR EPSCs. Recordings were also performed at +50mV and similar results were obtained (data not shown).

Overall, these results suggest that the selective activation of σ1R does not modulate NMDAR-mediated EPSCs.

σ1R activation does not affect AMPAR-mediated EPSCs

To further investigate the involvement of σ1R in glutamatergic transmission in CA1 pyramidal cells, I studied the effect of pentazocine on the fast excitatory glutamatergic transmission, which involves AMPAR.

The recordings were performed using borosilicate electrodes filled with a cesium-based solution (intracellular solution; see Materials and Methods) containing QX-314 and 10mM BAPTA. To evoke EPSCs, the Schaffer collaterals were stimulated with a bipolar electrode. The AMPAR-mediated component of the EPSCs was pharmacologically isolated in a low Mg^{2+} ACSF containing AP-5, picrotoxin, CGP 52432 and strychnine in order to block NMDA-, $GABA_A$-, $GABA_B$- and glycine receptor-mediated responses, respectively. The rise and decay constant of AMPAR EPSCs were calculated. The application of (+)pentazocine (1 μM) did not significantly ($p > 0.05$) change the amplitude (average of the variation from control: -0.83% ± 2.50; $n = 6$; **Fig. 6**). The application of (+)pentazocine did not significantly ($p > 0.05$) change the rise constant of AMPAR EPSCs (Control: 3.77 ms ± 0.55; (+)pentazocine : 3.52 ms ± 0.57) or the decay kinetics of the AMPAR EPSCs (Control: 18.41 ms ± 2.34; (+)pentazocine : 19.25 ms ± 2.45), suggesting that σ1R activation does not affect AMPAR-mediated EPSCs.

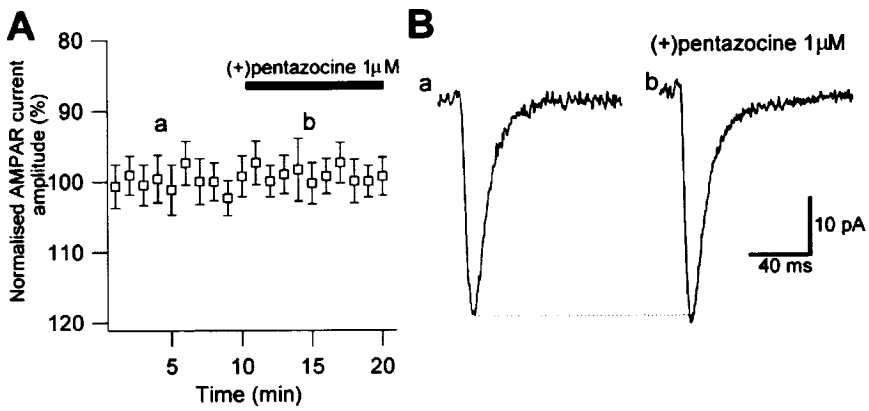

Figure 6 (+)pentazocine (1 μM) did not affect the amplitude of the evoked AMPAR currents in CA1 pyramidal cells.

Responses were evoked by bipolar electrical stimuli at *V*m = -65 mV in CA1 pyramidal cells. The evoked AMPAR EPSCs were recorded in normal ACSF solution in the presence of AP-5 (50 μM) picrotoxin (50 μM), CGP 52432 (5 μM) and strychnine (0.5 μM). (A) Normalized AMPAR current amplitudes (%) are plotted as a function of time. Each point (one every min; mean ± SEM) is the average of 6 points (stimulations every 10 s). The amplitude of the response was not changed significantly by the application of (+)pentazocine (control, 25.65 ± 5.91; (+)pentazocine, 25.88 ± , 5.64; n = 6; $p > 0.1$). (B) Examples of traces of NMDAR currents measured at the time points indicated in A (a and b) are shown. Each trace is an average of 20 traces.

Effect of pentazocine (1μM) on electrophysiological properties of CA1 pyramidal cells.

It has been suggested that σ1R ligands may interfere with channels that are permeable to Ca^{+2} , Na^{+} and K^{+} [63;66;72;74-77]. Indeed, it has been previously shown that σR ligands DTG and (+)pentazocine inhibited voltage-gated K^{+} channels[75-77]. Moreover, DTG and (+)pentazocine were able to affect some parameters of the action potential waveform such as amplitude and duration of both the action potential and the AHP[74]. Changes in channel activation can influence the resting potential, spontaneous activity, the response to other excitatory or inhibitory inputs and the amount of Ca^{+2} that enters during an action potential. Such effects clearly play a major role in signalling in the nervous system. Since it is known that σ1Rs activation modulates glutamatergic neurotransmission and since my data showed no effect of the σ1R activation on NMDAR and AMPAR EPSCs, to investigate the role played by σ1R in the modulation of synaptic transmission, I studied the effect of the σ1R activation on the electrophysiological properties of neurons.

In response to depolarizing current pulses CA1 pyramidal cells showed a repetitive-firing behavior with different degrees of adaptation. Spike frequency adaptation plays a key role in the transfer of integrated synaptic input to neuronal output. Spike frequency is controlled by Ca^{2+}-activated K^{+} channels (BK channels). Action potentials are followed by a hyperpolarization, which has several phases that are mediated through the activation of different channels. In addition to the hyperpolarization, neurons also undergo an AHP, which can be mAHP, occurring rapidly after the action potential (> 10 ms) and lasting between 50 and 100 ms[32], or sAHP which has a slow rising phase and can last several seconds[32]. The AHP phases were also analysed at each holding potential.

I investigated the effect of (+)pentazocine on CA1 pyramidal cell firing properties; I studied the electrophysiogical properties (membrane potential (V_m) at resting, spike amplitude, spike duration at half amplitude, internal resistance (R_{in}), mAHP and sAHP) and firing frequency of these cells.

Cells were recorded in current clamp at three different potentials (resting, -65 mV and –50 mV) and spike amplitude, spike duration at half amplitude, R_{in}, mAHP and sAHP as well as the firing frequency were measured. **Table 2** lists the physiological properties in the presence and in the absence of (+)pentazocine 1 μM. In all cases, the mAHP amplitude was reduced with application of (+)pentazocine (Vm = -70mV; control, 2.4 mV ± 0.2; (+)pentazocine 1.62 mV ± 0.2, *n* = 10; **Table 2**; **Fig. 7**) and the sAHP was also reduced by (+)pentazocine (Vm = -70mV; control, 3.3 mV ± 0.4; (+)pentazocine, 2.5 mV ± 0.3, *n* = 10; **Table 2; Fig. 7**). Spike interval analysis revealed that the first spike interval was reduced by (+)pentazocine (control, 79.0 mV ± 8.5; (+)pentazocine 40.6 mV ± 3.9, *n* = 10; **Table 2; Fig. 7**).

Since, mAHP and sAHP current amplitude are reduced as well as spike interval by σ1R agonists and because these currents are attributable to the activation of small conductance Ca^{+2}-activated K^+ channels [33;39;78;79] (SK channels), this suggests that σ1R activation might modulate Ca^{+2}-activated K^+ channel activity.

The effect of σ1R agonists on NMDAR currents is K^+-dependent

It has been shown that several σ1R ligands modulate and interact with Ca^{2+}-activated K^+ channels [66;80]. It has also been found that σ1Rs interact with Ca^{2+} activated K^+ channels and that ligands binding to the σ1R modulate channel activity through this interaction [66]. To test

Electrophysiologic Properties at Resting Potential										
	$V_{m(mV)}$	Sp.Amp(mV)	S.D.H.A.(ms)	τ(ms)	$R_{in(MΩ)}$	Thres.(mV)	mAHP(mV)	sAHP(mV)	1st int(ms)	2nd int(ms)
Control	-76.8 +/- 0.5	99.5 +/- 2.5	2.2 +/- 0.1	46.1 +/- 4.8	148.6 +/- 12.2	-59.4 +/- 1.0	2.3 +/- 0.3	2.9 +/- 0.3	62.9 +/- 5.4	70.6 +/- 3.4
Pentazocine	-77.7 +/- 1.1	96.7 +/- 2.5	2.4 +/- 0.1	60.5 +/- 5.9	148.7 +/- 15.5	-61.1 +/- 0.9	1.2* +/- 0.4	2.2* +/- 0.1	40.2* +/- 4.1	50.7* +/- 4.9

Electrophysiologic Properties at -70 mV										
	$V_{m(mV)}$	Sp.Amp(mV)	S.D.H.A.(ms)	τ(ms)	$R_{in(MΩ)}$	Thres.(mV)	mAHP(mV)	sAHP(mV)	1st int(ms)	2nd int(ms)
Control	-81.7 +/- 0.8	98.7 +/- 1.3	2.2 +/- 0.1	46.6 +/- 3.2	174.3 +/- 12.3	-61.5 +/- 1.1	2.4 +/- 0.2	3.3 +/- 0.4	79.0 +/- 8.5	110.9 +/- 16.7
Pentazocine	-80.8 +/- 0.4	95.1 +/- 1.6	2.3 +/- 0.2	51.3 +/- 4.4	184.1 +/- 10.0	-61.2 +/- 1.1	1.6* +/- 0.2	2.5* +/- 0.3	40.6* +/- 3.9	49.7* +/- 4.5

Electrophysiologic Properties at - 50 mV										
	$V_{m(mV)}$	Sp.Amp(mV)	S.D.H.A.(ms)	τ(ms)	$R_{in(MΩ)}$	Thres.(mV)	mAHP(mV)	sAHP(mV)	1st int(ms)	2nd int(ms)
Control	-60.1 +/- 0.5	79.9 +/- 2.3	2.4 +/- 0.1	62.0 +/- 6.4	197.5 +/- 19.3	-54.6 +/- 0.8	3.7 +/- 0.4	4.8 +/- 0.5	81.6 +/- 5.8	104.9 +/- 8.6
Pentazocine	-61.4 +/- 0.7	72.5 +/- 2.1	2.7 +/- 0.3	55.7 +/- 5.5	189.7 +/- 9.2	-56.6 +/- 0.8	1.7* +/- 0.6	2.7* +/- 0.5	48.4* +/- 6.5	75.4* +/- 7.4

Table 2 Effect of (+)pentazocine 1 μM on electrophysiological properties of CA1 Pyramidal cells.

Whole-cell current-clamp recordings were performed in normal ACSF solution before (control) and after (+)pentazocine application (*n* = 10*)* at -65 mV. Values are presented as means ± S.E.M. *Denotes significant difference for the slow and fast AHP values obtained during control and in presence of (+)pentazocine ($p < 0.05$). The same series of experiments was also performed at resting membrane potential as well as at -50mV and similar results were obtained (data not shown).

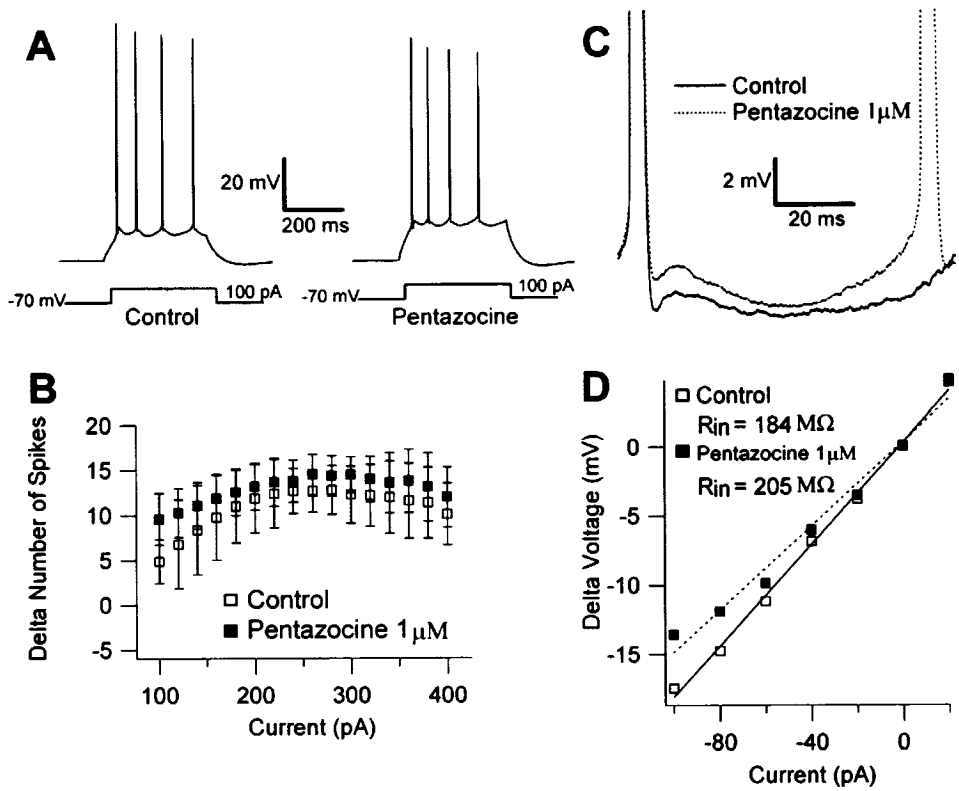

Figure 7 Effect of (+)pentazocine (1 μM) on electrophysiological properties of CA1 Pyramidal cells recorded at – 70 mV

Whole-cell current-clamp recordings were performed in normal ACSF at -70mV. Voltage response of a pyramidal cell to a series of intracellular pulses (A; 600 ms in length, 20 pA step depolarization from 100 pA to 300 pA) in absence and in presence of (+)pentazocine. The afterhyperpolarization (AHP) with and without (B; control, full line) (+)pentazocine (B; dashed line) are superimposed. (+)pentazocine significantly reduced the medium and slow AHP ($p < 0.05$). The analysis of the voltage response to a graded series of intracellular current pulses reveals that pattern of firing frequency did not significantly change by application of (+)pentazocine (C, control □, (+)pentazocine ■; $n = 11$; $p > 0.1$). Each marker represents the difference between the number of spikes during the (+)pentazocine application and control. The internal resistance (R_{in}; D) of pyramidal cells was not modified by (+)pentazocine application. The same series of experiments were performed at resting membrane potential as well as at -50mV and similar results were obtained (data not shown).

the implication of a K^+ conductance in the potentiation of the NMDAR responses by σ1R agonists, I recorded NMDAR currents from CA1 pyramidal cells with a K^+-based solution in the recording pipette and applied (+)pentazocine (1 μM). The NMDAR currents recorded with this solution showed no significant difference in the kinetic properties compared to those recorded with the cesium-based solution (**Table 1**). In 12 out of 18 CA1 pyramidal cells the application of (+)pentazocine (1 μM) significantly increased the amplitude of the NMDAR current by 56.50% ± 4.87 (from a mean amplitude of 20.24 pA ± 2.07 in control to 31.83 pA ± 3.73 in (+)pentazocine; $p < 0.05$; **Fig. 5; Table 1**), while application of (+)pentazocine had no effect on the kinetic properties (**Table 1**). This finding strongly suggests the involvement of a K^+ conductance in the potentiating effect observed on NMDAR currents following the application of (+)pentazocine.

To verify the specificity of the σ1R activation, I observed the effect of haloperidol, a well-known and potent σ1R antagonist, when applied with (+)pentazocine. In 3 CA1 pyramidal cells where the amplitude of the NMDAR currents was significantly enhanced by (+)pentazocine, I added haloperidol (1 μM). The application of (+)pentazocine (1 μM) significantly ($p < 0.05$) increased the NMDAR current by 47.9 % ± 3.66 (from a meaning amplitude of 21.54 pA ± 6.46 in control to 32.15 pA ± 10.14 in (+)pentazocine; $n = 3$; **Fig. 8**), the subsequent addition of haloperidol (1 μM) completely reversed the effect of (+)-pentazocine (1 μM), returning the NMDAR currents to 98.4% ± 3.7 of its initial amplitude ($n = 3$; **Fig. 8**). To rule out any effect of haloperidol on the NMDAR currents, I recorded NMDAR currents and applied haloperidol (1 μM) alone. Haloperidol reduced the NMDAR current by 15.85% ± 5.43 ($n = 3$; **Fig. 9**). This value is significantly smaller ($p < 0.05$) than

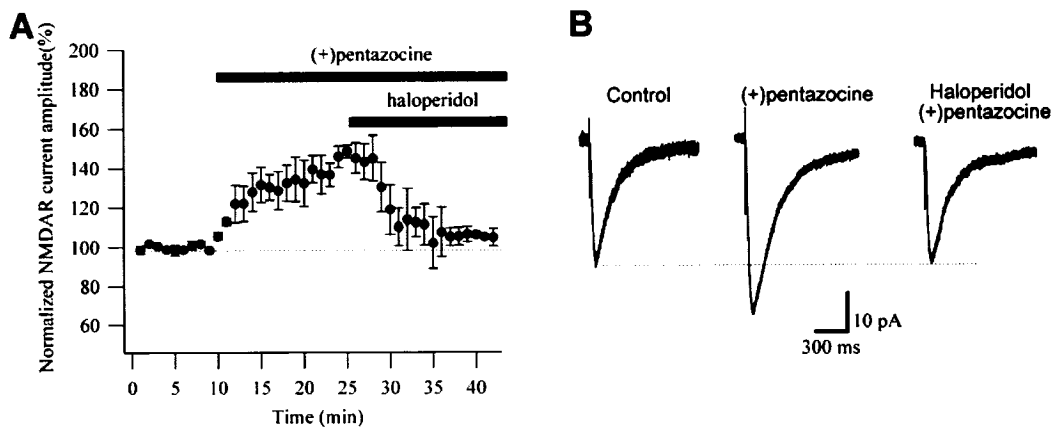

Figure 8 Haloperidol reverses the effect of (+)pentazocine on NMDAR currents

Normalized NMDAR current amplitudes (%) are plotted as a function of time (A). Each point (one every min; mean ± SEM) is the average of 6 points (stimulations every 10 sec). The application of the σR1 agonist (+)pentazocine (1 μM) causes an increase in the amplitude of the NMDAR currents (n = 3). The subsequent addition of haloperidol (1 μM) completely reversed the effect of (+)pentazocine, returning the NMDAR currents to its initial amplitude (n = 3). (B) Examples of traces of the NMDAR currents measured in absence (right) and presence of (+)pentazocine (center) and (+)pentazocine plus haloperidol (left), respectively. Each trace is an average of 20 traces.

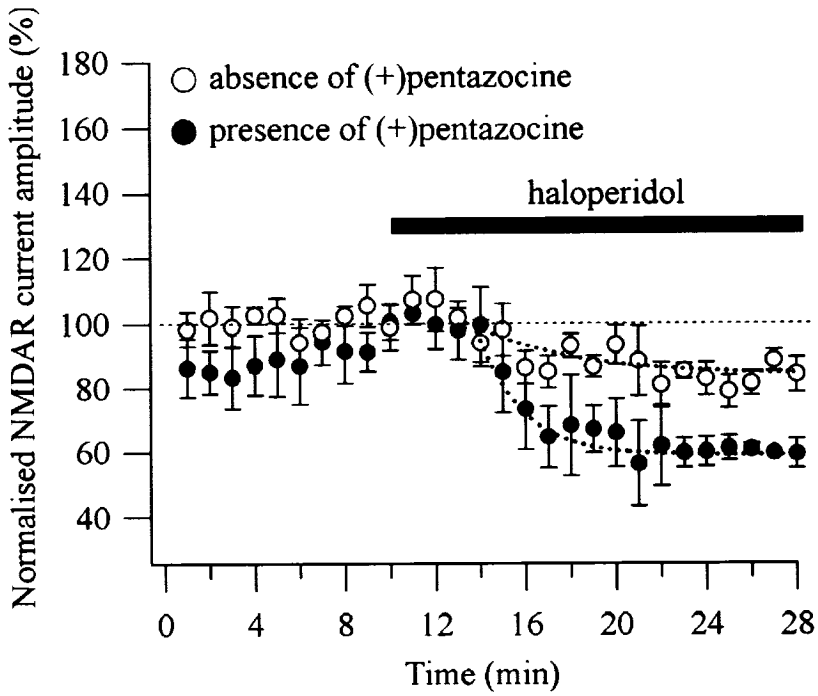

Figure 9 Effect of haloperidol on NMDAR currents

Normalized NMDAR current amplitudes (%) are plotted as a function of time in presence (○) and absence (●) of (+)pentazocine. Each point (one every min; mean ± s.e.m.) is the average of 6 points (stimulations every 10 sec). The time course of NMDAR currents recorded in presence of (+)pentazocine is the same as in Figure 2. An offset has been added to the curve (presence of (+)pentazocine) to be able to superimposed this one to that obtained in absence of (+)pentazocine. Note that the application haloperidol (1 μM) causes a significantly different reduction in the amplitude of the NMDAR currents in absence (○; n = 3) and presence (●; n = 3) of (+)pentazocine.

that obtained in the presence of (+)pentazocine. Overall these results demonstrate the specificity of the σ1R activation in enhancing the amplitude of NMDAR currents. This clearly demonstrates the specificity of the σ1R activation on the enhancing effect of NMDAR currents.

σ1R agonist needs a rise in intracellular Ca^{2+} to exercise its modulation on the NMDAR current

It has been shown that Ca^{2+} that enters the cell through NMDARs activates SK channels and shunts the resultant synaptic potential [72;73]. It has also been described that blocking SK channels in CA1 pyramidal cells enhances NMDAR-dependent Ca^{2+} signal within the dendritic spines [45]. To evaluate whether a rise in the intracellular Ca^{2+} concentration is necessary for the σ1R agonist to exercise its action on the NMDAR currents, I recorded CA1 pyramidal cells with a K^+-based solution with the addition of 10 mM BAPTA in the recording pipette to buffer cytosolic Ca^{2+}. The NMDAR currents recorded with this solution showed no significant difference in kinetic properties than those recorded with the cesium based solution (**Table 1**). I found that the application of (+)pentazocine (1 μM) did not significantly increase the amplitude (average of the variation from control 2.66% ± 1.85; n = 5; $p > 0.05$; **Fig 5; Table 1**) nor change the kinetics of the NMDAR currents (**Table 1**), suggesting that a rise in the intracellular Ca^{2+} concentration is necessary for the σ1R agonist to exert its effect on the NMDAR currents. Previous experiments using a cesium based intracellular solution including 0.5 mM EGTA instead of 10 mM BAPTA in the recording pipette, ruled out any effect of the σ1R agonist on the rise of Ca^{2+} alone in its action on the NMDAR currents.

NMDARs are highly permeable to Ca^{2+} and are blocked by Mg^{2+} at resting membrane potential in a voltage-dependent manner [81]. NMDAR activation requires binding of glutamate and its coagonist, glycine, as well as membrane depolarization [19]. If the σ1R activation potentiates the NMDAR currents through its action on K^+ channels (Ca^{2+} activated K^+ channels) and/or by Ca^{2+} entering the cells through the NMDARs, then the effect of (+)pentazocine will be greater at more depolarized potential where the Mg^{2+} block is relieved and the driving force for the K^+ ion larger. To test this hypothesis I recorded CA1 pyramidal cells with a K^+-based solution in the recording pipette (plus 0.5 mM EGTA). The NMDAR currents were pharmacologically isolated using a normal ACSF containing NBQX, picrotoxin, CGP 52432 and strychnine. The NMDAR currents were recorded and the effect of (+)pentazocine (1 μM) was observed at different potentials (-30, -50 and -70 mV). The NMDAR currents were pharmacologically isolated in normal ACSF. The amplitude of the NMDAR currents was significantly increased ($p < 0.05$) by (+)pentazocine (1 μM) at a holding potential of -30 mV (131.6% ± 25.3; $n = 6$) compared to -70 mV (64.3% ± 33.06; $n = 6$; **Fig. 10**). Overall, these findings strengthen the relationship between NMDAR-mediated rise in the Ca^{2+} concentration and K^+ current, underlining the involvement of a Ca^{2+} activated K^+ current in the NMDAR current modulation by the σ1R agonist.

To rule out a pre-synaptic effect of (+)pentazocine, I performed experiments using paired pulse paradigms. Paired pulses were delivered with an inter-pulse interval of 50 ms. The second response showed facilitation in CA1 pyramidal cells. Stimuli-induced NMDAR currents with similar ratios (peak2/peak1; $p > 0.05$) in absence (control, 2.74 ± 0.34; $n = 6$; **Fig. 11** and presence of (+)pentazocine (2.93 ± 0.46; $n = 6$; **Fig. 11**), which result is consistent with a post-synaptic effect of (+)pentazocine. These data were also supported by

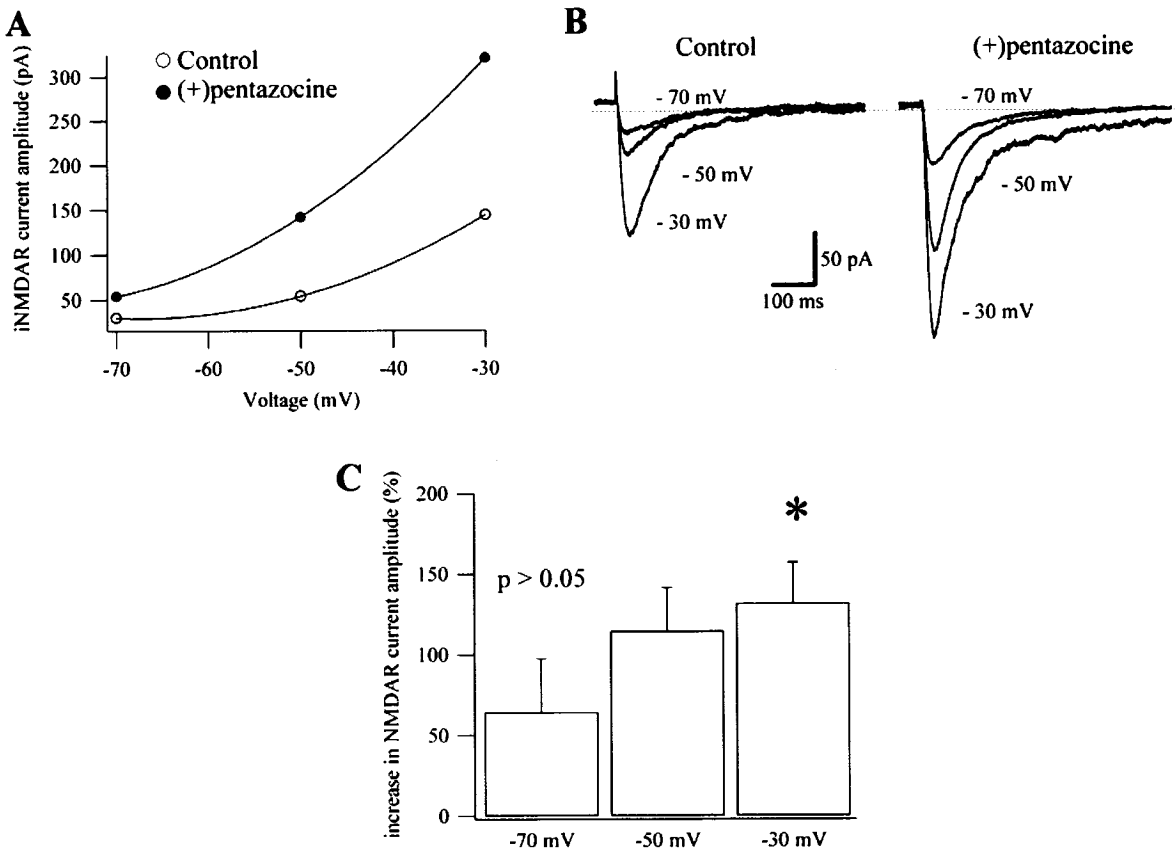

Figure 10 Voltage-dependent effect of (+)pentazocine on NMDAR currents

(A) Current-voltage relationship for the NMDAR currents shown in B. The application of (+)pentazocine (1 μM) causes a voltage-dependent enhancement of the NMDAR currents. (B) Examples of traces of NMDAR currents recorded in voltage clamp obtained from CA1 pyramidal cells held at -70, -50 and -30 mV. Each trace is an average of 6 traces. (C) Histogram showing the average of the enhancing effect of (+)pentazocine application on NMDAR currents at different holding potential in 6 CA1 pyramidal cells (mean ± SEM). *Significant difference between the percentages of augmentation of the NMDAR currents recorded at -70 and -30 mV.

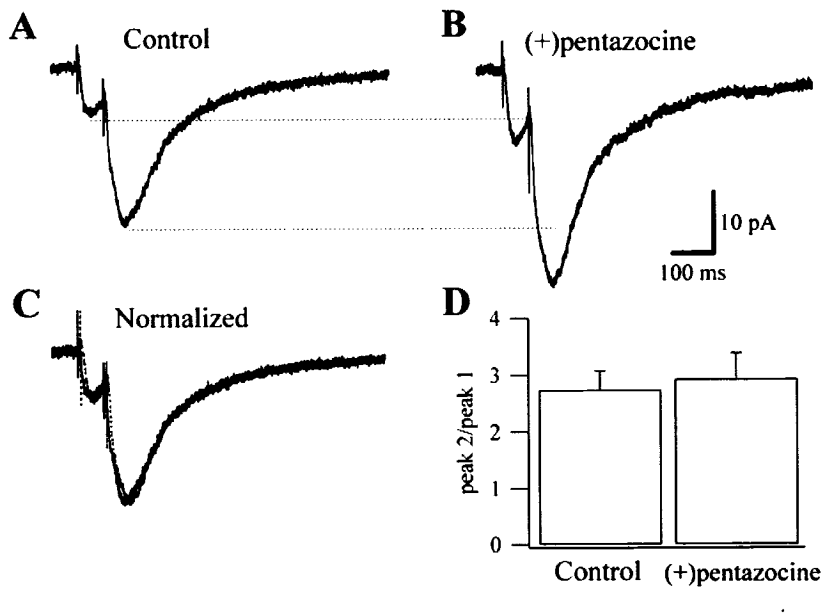

Figure 11 Effect of (+)pentazocine on Paired-Pulse facilitation

The application of (+)pentazocine (1 μM) does not increase the release probability. (A & B) Typical traces of postsynaptic responses in the rat CA1 region of the hippocampus after stimulation of Schaffer collaterals with two consecutive synaptic stimulations (100 μs duration and 50 ms interval) in control (A) and after the application of (+)pentazocine (B). The second response showed facilitation under control conditions (A), as well as during application of (+)pentazocine (B). Note that the application of (+)pentazocine causes an increase in the amplitude of the response but not in the paired-pulse facilitation. This is shown by superimposing the normalized traces (C). (D) Histogram showing the average ratio between the second and the first peak measured in 6 neurons (mean ± SEM).

previous experiments showing that the effect of (+)pentazocine was abolished by loading the post-synaptic cells with 10 mM BAPTA or cesium (**Fig. 5, Table 1**).

Only NMDAR-mediated rise in the Ca^{2+} concentration is needed for σ1R agonist modulation of NMDAR currents

A rise in the intracellular Ca^{2+} concentration can originate from the influx of Ca^{2+} *via* NMDARs, voltage-gated Ca^{2+} channels and/or from its release from intracellular Ca^{2+} stores. To further verify that Ca^{2+} influx through post-synaptic NMDAR is necessary for (+)pentazocine to exert the enhancement of the NMDAR currents, I measured the effects of (+)pentazocine (1 μM) on the response to local application of NMDA (100 μM). NMDA (100 μM) was applied in a low Mg^{2+} ACSF (0.1 mM) in presence of TTX (0.5 μM). The amplitude of the currents in response to NMDA application was 22.29 pA ± 3.78 (n = 4). Application of (+)pentazocine (1 μM) induced a significant ($p < 0.05$) enhancement of 76.21% ± 10.53 of the evoked-NMDA responses in 3 out of 4 CA1 pyramidal cells (**Fig. 12**). Application of AP-5 (50 μM) completely abolished the responses (n = 4; data not shown).

It has been found that σ1R regulates Ca^{2+} release from intracellular Ca^{2+} storage sites *via* inositol 1,4,5-triphosphate (IP3) receptors located on the endoplasmic reticulum [56] as well as voltage-dependent Ca^{2+} channels [63]. To study the possibility that the rising of Ca^{2+}, responsible for the σ1R action on NMDAR currents, originates from sources other than NMDARs (such as voltage-sensitive Ca^{2+} channels or intracellular Ca^{2+} stores), I examined the enhancing effect of (+)pentazocine (1 μM) on NMDAR currents in presence of an L-type Ca^{2+} channel blocker (nicardipine) and two Ca^{2+} ATPase inhibitors that cause the depletion of intracellular Ca^{2+} stores (cyclopiazonic acid (CPA), and ryanodine).When (+)pentazocine

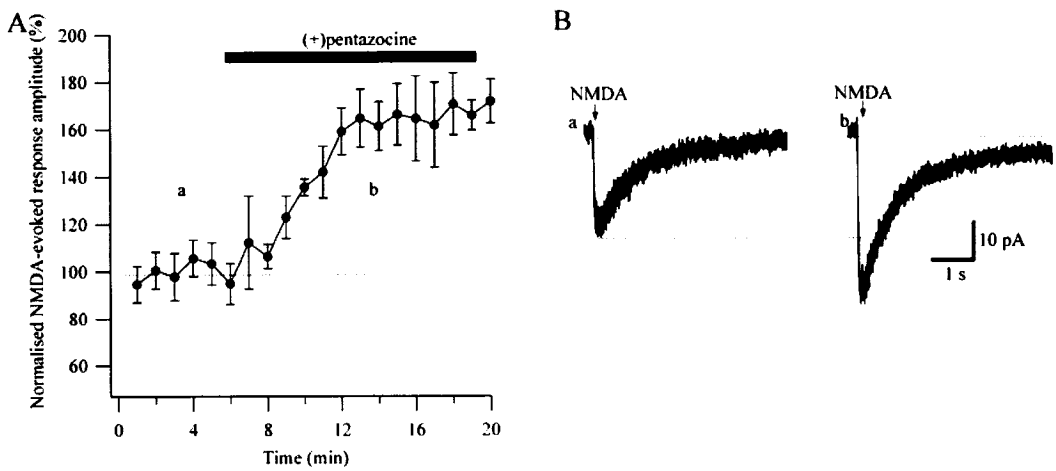

Figure 12 Effect of (+)pentazocine on the responses evoked by local pressure application of NMDA

The NMDA (100 μM) was applied through a patch pipette positioned directly above the proximal dendrites. TTX (0.5 mM) was present throughout the experiments. The cells were voltage-clamped at *V*m = -65 mV. (A) Normalized NMDA-evoked response amplitudes (%) are plotted as a function of time. Each point (one every min; mean ± SEM) is the average of 2 points (pressure application every 30 s). The application of the σ1R agonist (+)pentazocine (1 μM) causes an increase in the amplitude of the NMDA-evoked responses (n = 3). (B) Examples of NMDA-evoked responses observed in low-Mg^{2+} ACSF (a) and during application of (+)pentazocine (b). Each trace is an average of 10 traces.

(1 μM) was applied after nicardipine (5 μM), the amplitude of the NMDAR currents was significantly increased by 68.15% ± 3.46 ($n = 4$; $p < 0.05$; from 18.68 pA ± 5.89 in control to 30.97 pA ± 9.18; **Fig. 13**), similar to when (+)pentazocine (1 μM) was applied alone (56.5% ± 4.87, $n = 12$; **Fig. 13**). This ruled out a role of the Ca^{2+} entering through L-type Ca^{2+} channels in the enhancing effect of the σ1R agonist on NMDAR currents.

When (+)pentazocine (1 μM) was applied after CPA (30 μM) and ryanodine (10 μM), the amplitude of the NMDAR currents was significantly increased by 66.36% ± 6.01 ($n = 4$; $p < 0.05$; from 15.26 pA ± 2.37 in control to 25.14 pA ± 3.58; **Fig. 13**) and 63.03% ± 17.21 ($n = 4$; $p < 0.05$; from 19.10 pA ± 2.29 in control to 29.99 pA ± 2.64; **Fig. 13**), respectively. These data were similar ($p > 0.05$) to that observed with (+)pentazocine (56.5% ± 4.87, $n = 12$; **Fig. 13**), ruling out a role of the Ca^{2+} freed by the intracellular Ca^{2+} stores in the potentiating effect of the σ1R agonist on NMDAR currents. Experiments with CPA and ryanodine ruled out also the involvement of the σ1R action on the Ca^{2+} store in the regulation of NMDAR currents.

Overall, these results suggest that the augmentation of the NMDAR current amplitude observed following the application of (+)pentazocine depends on NMDAR-mediated rise in the Ca^{2+} concentration, strengthening the hypothesis that a Ca^{2+}-activated K^+ current underlies the NMDAR current modulation by the σ1R agonist.

(+)pentazocine modulation of the NMDAR current is similar to that of apamin

Ca^{2+} influx through NMDARs can open Ca^{2+}-activated K^+ channels in hippocampal slices and cultured neurons [72;82;83]. In CA1 pyramidal cells, these channels are small conductance voltage insensitive Ca^{2+}-activated K^+ channels (SK channels), apamin-sensitive and

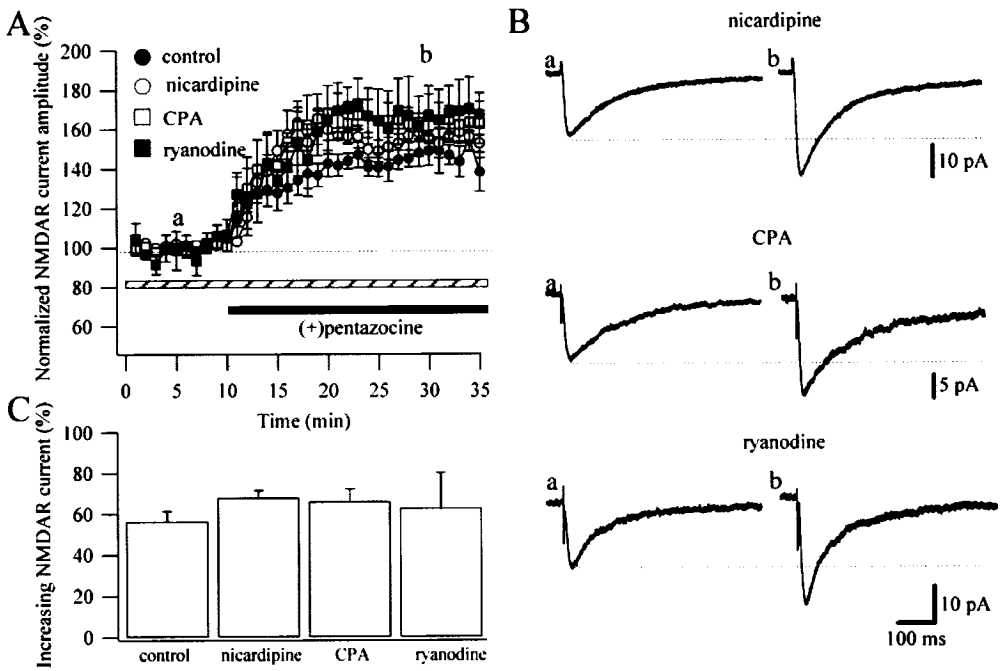

Figure 13 σ1R agonist modulation of NMDAR currents depends on NMDAR-mediated rise in the Ca^{2+} concentration

(A) Normalized NMDAR current amplitudes (%) are plotted as a function of time. Each point (one every min; mean ± SEM) is the average of 6 points (stimulations every 10 sec). The application of (+)pentazocine (1 μM; black bar) causes an increase in the amplitude of the NMDAR currents when the CA1 pyramidal cells were recorded in presence of nicardipine (5 μM; ○; *n* = 4; mean ± SEM), CPA (30 μM; □; *n* = 4; mean ± SEM) and ryanodine (10 μM; ■; *n* = 4; mean ± SEM), dotted bar. (B) Examples of traces of the NMDAR currents measured at the time points indicated in A (a and b) are shown for nicardipine, CPA and ryanodine. Each trace is an average of 20 traces. (C) Histogram showing the average of the enhancing effect of (+)pentazocine on NMDAR currents when the CA1 pyramidal cells are recorded in control (no additional drug; *n* = 12), in presence of

nicardipine (○; n = 4), CPA (□; n = 4) and ryanodine (■; n = 4). All the values are means ± SEM.

responsible for the mAHP [72;84]. They are also involved in the control of repetitive firing [39]. Moreover, apamin-sensitive SK channels respond to rapid increase in NMDAR-mediated rise in the Ca^{2+} concentration and reduce the amplitude of NMDAR currents [45;72].

To verify if the effect of σ1R agonists was mimicked by the blockage of SK channels, I recorded CA1 pyramidal cells with a K^+-based solution and compared the effect of SK channel blockers (apamine and bicuculline methiodide) to that of (+)pentazocine on NMDAR current. The percentage of increment of the NMDAR current amplitude caused by the application of (+)pentazocine (1 μM; 56.5% ± 4.87; n = 12; **Fig. 14**) was similar ($p > 0.05$) to that induced by apamin (100 nM; 47.8% ± 8.12, n = 4; **Fig. 14**) and bicuculline methiodide (10 μM; 52.5% ± 12.94, n = 5; **Fig. 14**). Contrary, the application of a low concentration of a BK channel blocker, tetraethylammonium (TEA; 1 mM; 0.05% ± 2.43, n = 3; **Fig. 14**) did not increase the amplitude of NMDAR currents (0.05% ± 2.43, n = 3; **Fig. 14**).

These results strongly suggest that (+)pentazocine and apamin may act by blocking the same current, implicating that σ1R may modulate NMDAR currents through its action on SK channels.

σ1R activation blocks SK channels

To test the effect of σ1R activation on SK channel, I recorded the Ca^{2+} activated K^+ current (I_{AHP}) that underlies the m_{AHP} in voltage-clamp (see Materials and Methods). 19 out of 27 CA1 pyramidal cells (~70%) showed I_{AHP}. In these cells the SK channel blocker apamin (100

nM) abolished the I_{AHP} (**Fig. 15**) [79;85]. Similarly to apamin, the σ1R agonist (+)pentazocine (1 μM) also blocked the I_{AHP} (**Fig. 15**). The blocked currents were extracted by subtraction of

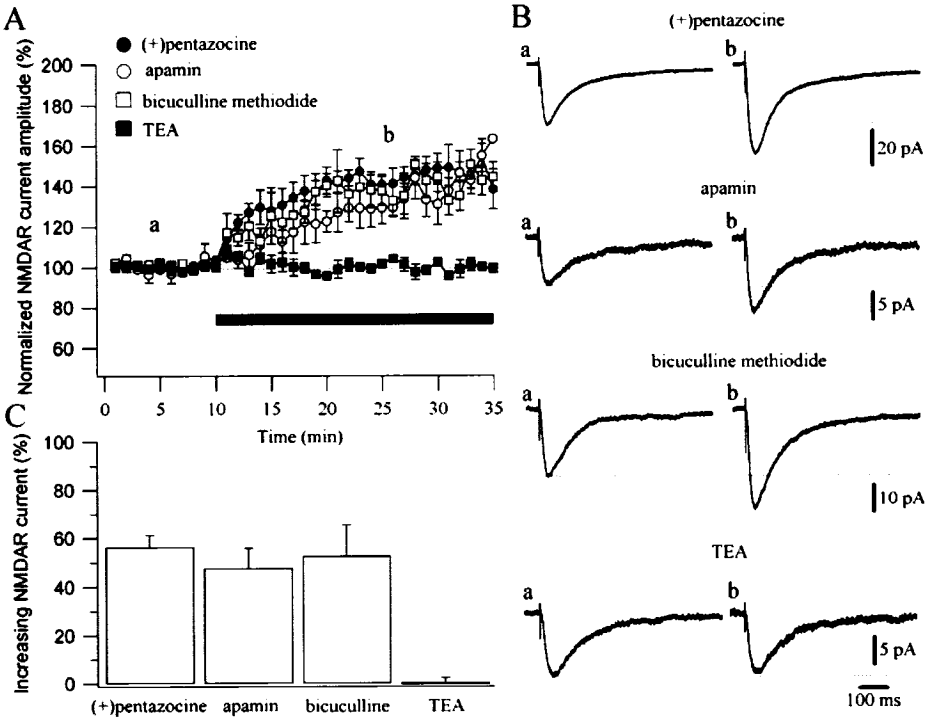

Figure 14 (+)pentazocine modulation of the NMDAR current is similar to that cause by blockers of SK channels

(A) Normalized NMDAR current amplitudes (%) are plotted as a function of time. Each point (one every min; mean ± SEM) is the average of 6 points (stimulations every 10 s). The application of drugs (black bar) causes an increase in the amplitude of the NMDAR currents when the CA1 pyramidal cells were recorded in presence of (+)pentazocine (1 μM; •; n = 12; mean ± SEM), apamin (100 nM; ○; n = 4; mean ± SEM) and bicuculline methiodide (10 μM; □; n = 5; mean ± SEM). The application of TEA (1 mM; ■; n = 3; mean ± SEM) does not increase the amplitude of the NMDAR currents. (B) Examples of traces of the NMDAR currents measured at the time points indicated in A (a and b) are shown for (+)pentazocine,

apamin, bicuculline methiodide and TEA. Each trace is an average of 20 traces. (C) Histogram showing the average of the enhancing effect of (+)pentazocine (n = 12; mean ± SEM), apamin (n = 4; mean ± SEM), bicuculline methiodide (n = 5, mean ± SEM) and TEA (n = 3; mean ± SEM) application on NMDAR currents.

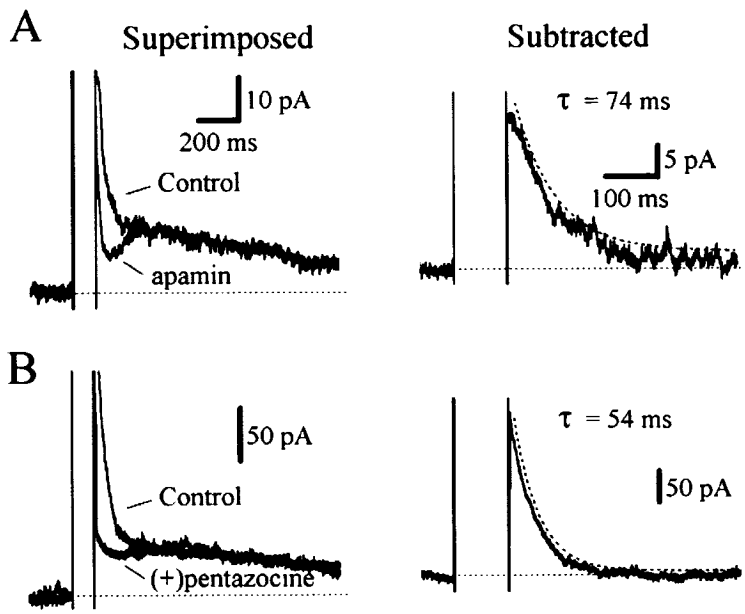

Figure 15 (+)pentazocine blocks SK channels

The currents generating the AHP, recorded in voltage clamp in response to a 100 ms step from a holding potential of -50 mV, are shown. The currents recorded in absence (control) and presence of apamin (100 nM; A) or (+)pentazocine (1 μM; B) are superimposed. The SK mediated I_{AHP} obtained by subtraction is shown on the right for apamin (A) and (+)pentazocine (B).

the current before and after application of apamin or (+)pentazocine (**Fig. 15**). These currents decayed with similar ($p > 0.05$) time constants: 132.7 ms ± 42.5 ($n = 8$) for apamin and 130.1 ms ± 29.2 ($n = 11$) for (+)pentazocine. The currents had a peak amplitude of 101.0 pA ± 35.0 ($n = 8$) and 114.1 pA ± 21.3 ($n = 11$) for apamin and (+)pentazocine, respectively. These values were not significantly different ($p > 0.05$). In 3 CA1 pyramidal cells, apamin (100 nM) was applied after (+)pentazocine (1 μM) to verify if apamin blocked an additional current. The currents were extracted by subtraction of the current before the application of the drugs and after application of apamin and (+)pentazocine. The currents blocked by (+)pentazocine decayed with a time constant of 100.9 ms ± 49.7 ($n = 3$). The additional application of apamin did not significantly change the decay of the blocked current (87.7 ms ± 32.6; $n = 3$, $p > 0.05$; data not shown). These data strongly suggest that the current blocked by apamin and (+)pentazocine was the same. The I_{AHP} peaks rapidly following Ca^{2+} influx and decays with a time constant of 50 to several hundred ms [39;85]. The fitting values of the decays were similar to that previously described in the CA1 region of the hippocampus [86], suggesting that σ1R agonist (+)pentazocine blocks SK channels.

It has been described in hippocampal CA1 pyramidal cells and in the principal cells of the lateral amygdala that the block of SK channels by apamin increases the NMDAR response [45;73]. As I showed above, (+)pentazocine had an effect on ~67% of the recorded cells. Since not all CA1 pyramidal cells expressed the mAHP and its corresponding I_{AHP}, I applied apamin in neurons where (+)pentazocine did not enhanced the amplitude of the NMDAR currents to verify if the lack of SK channels was responsible for this absence of effect. In 3 CA1 pyramidal cells where the amplitude of the NMDAR currents was not significantly ($p > 0.05$) increased by (+)pentazocine (from a mean amplitude of 29.84 pA ± 6.78 in control to 31.52 pA ± 6.9 in (+)pentazocine; $n = 3$), the subsequent addition of

apamin (100 nM) did not change the amplitude of the NMDAR currents (30.11 pA ± 6.51; n = 3; **Fig. 16**). This lack of effect by apamin suggests that in neurons where the enhancing effect of (+)pentazocine is not observed, the SK channels were distant from the NMDARs, not enough to have a visible effect or not expressed.

Overall my results suggest that the potentiation of the NMDAR currents observed following the application of σ1R agonists is due to the block of SK channels operated by the σ1R agonist.

σ1R activation enhances LTP

Synaptic plasticity is the cellular mechanism proposed to underlie learning and memory. LTP of the Schaffer collateral synapses in the CA1 region of the hippocampus is a well-known model system for the study of the associative synaptic modification [23;73]. Since in CA1 pyramidal cells, application of apamin enhances LTP [36;87] and blockade of SK channels facilitates the induction of LTP by enhancing NMDAR-dependent Ca^{2+} signals within dendritic spines [45], I observed the effect of (+)pentazocine on LTP to study the effect of σ1R activation on synaptic plasticity [84].

I recorded CA1 pyramidal cells and induced LTP with a pairing protocol made of 3 brief high frequency tetani (50 pulses at 100 Hz, 4 s intervals) given at the end of a 3 min long depolarization at 0 mV. Cells were held at - 65 mV and stimuli given every 10 s. I recorded 10 min of stable baseline of synaptic responses, followed by the pairing. This protocol induced a 90.9% ± 11.9 (control, n = 13; $p < 0.005$) increase above baseline of the synaptic responses lasting for more than 40 min (**Fig. 17**). This LTP was NMDAR-

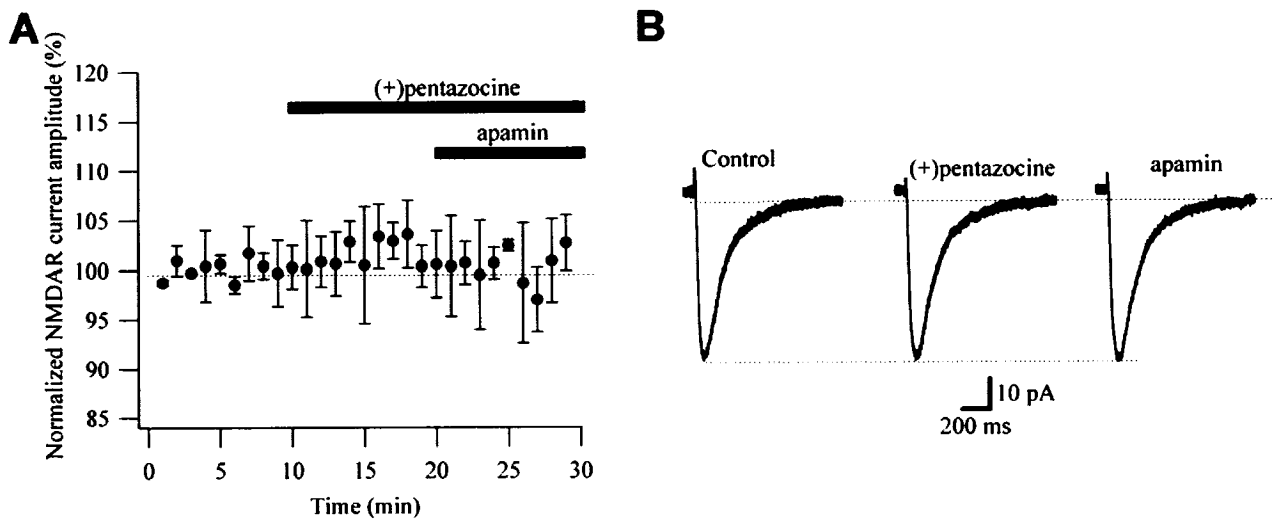

Figure 16 Apamin does not affect the amplitude of NMDAR currents in the CA1 pyramidal cells in which (+)pentazocine has no effect

(A) Normalized NMDAR current amplitudes (%) are plotted as a function of time. Each point (one every min; mean ± SEM) is the average of 6 points (stimulations every 10 s). In CA1 pyramidal cells where the application of the σ1R agonist (+)pentazocine (1 μM) does not increase the amplitude of the NMDAR currents (n = 3; mean ± SEM), the subsequent addition of apamin (100 nM) has no effect (n = 3; mean ± SEM). (B) Examples of traces of the NMDAR currents measured in control (absence of drugs), in (+)pentazocine and in (+)pentazocine plus apamin, respectively. Each trace is an average of 20 traces.

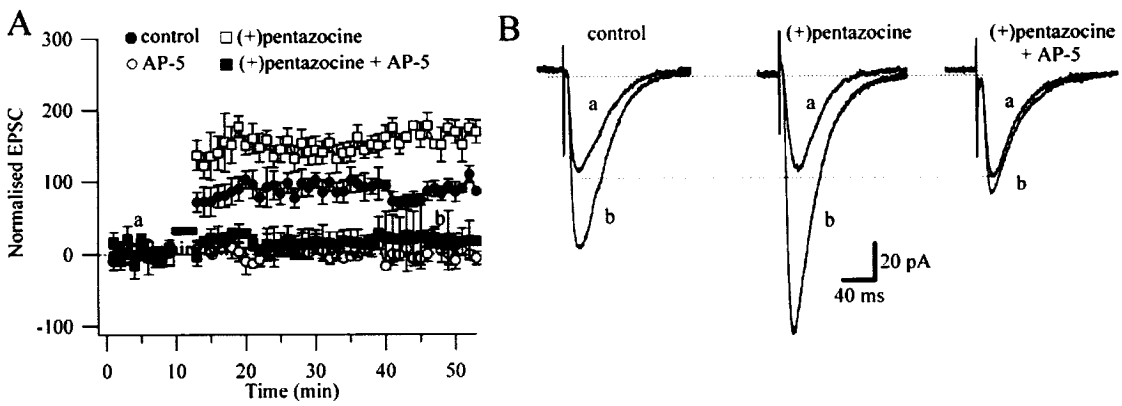

Figure 17 Effect of (+)pentazocine on LTP

(A) Pooled data of the effect of AP-5 (50 μM; ○; *n* = 4; mean ± SEM), (+)pentazocine (1 μM; □; *n* = 5; mean ± SEM) and (+)pentazocine (1 μM) plus AP-5 (50 μM; ■; *n* = 5; mean ± SEM) on the LTP, compared to the LTP in absence of drugs (control; ●; n = 13; mean ± SEM). (B) Examples of traces of EPSCs measured at the time points indicated in A (a and b) are shown for control, (+)pentazocine and (+)pentazocine plus AP-5. Each trace is an average of 20 traces.

dependent since it was prevented by application of AP-5 (50 μM; -9.50% ± 8.77 above baseline; $n = 3$; $p > 0.05$; **Fig. 17**). LTP in presence of (+)pentazocine (1 μM) was significantly enhanced (154.26% ± 17.4 above baseline; $n = 5$; $p < 0.05$; **Fig. 17**) compared to control condition, and was abolished by the concomitant application of (+)pentazocine (1 μM) and AP-5 (50 μM; 8.82% ±15.6 above baseline; $n = 5$; **Fig. 17**). The enhanced LTP observed in presence of (+)pentazocine was likely the result of the enhanced Ca^{2+} influx through the NMDAR due to the block of SK channels.

In summary, the potentiation of the NMDAR current observed following the activation of σ1R by (+)pentazocine is due to the blockage of SK channels. In general, Ca^{2+} entering the cells through the NMDAR activates a Ca^{2+}-activated K^+ current, underlined by SK channels, which in turn shunts the NMDAR responses. Consequently, the block of SK channels by σ1R activation increases the NMDAR response and LTP.

Chapter 4: Discussion

My data show that the potentiation of the NMDAR current observed following the activation of σ1R by (+)pentazocine is indirect and is due to the blockage of SK channels. In general, Ca^{2+} entering the cells through the NMDAR activates a Ca^{2+}-activated K^+ current, underlined by SK channels, which in turn shunts the NMDAR response. Hence, the blockade of SK channels by σ1R activation increases the NMDAR response. As a consequence, the σ1R activation increases LTP, suggesting a role for σ1R as a post-synaptic regulator in the neurotransmission and plasticity.

σ1R has been suggested to be implicated in the modulation of synaptic transmission. In the hippocampus, σRs are thought to play a role in the modulation of the glutamatergic neurotransmission *via* indirect modulation of the NMDAR [12-14;71]. The existence of a functional interaction between σRs and NMDARs has been proposed by several studies using biochemical, neuroendocrinological and behavioural models (for review see [8;65;88]). It has been previously shown that, in the hippocampus, σ1R modulates NMDAR function *in vivo* and *in vitro* preparations using extracellular recordings [8;14;67]. Moreover, several σ1R ligands such as DTG and (+)pentazocine have also been shown to increase the NMDAR response [12;68;89-92]. My results suggest that σ1R modulates NMDARs through an indirect mechanism. Indeed, σ1R agonist (+)pentazocine failed to potentiate the NMDAR currents (**Table 1, Fig. 5**) when they were isolated using a cesium based intracellular solution, which abolishes all the K^+ conductance. However, when a K^+-based solution was used, (+)pentazocine induced a potentiation of the NMDAR current, suggesting that the implication of a K^+ conductance is required in order for this modulation to occur.

Consequently, this result suggests that σ1R and NMDARs functionally interact but they are not coupled by a direct mechanism. Experiments using haloperidol, a σR antagonist, which reversed the potentiating effect on NMDAR currents, clearly demonstrated that this effect is specifically mediated by σ1Rs. In addition, the lack of effect of the activation of σ1R in changing the properties of the AMPAR EPSCs, suggests that, the activation of σ1R does not affect the fast excitatory glutamatergic transmission in CA1 pyramidal cells.

In summary, the activation of σ1R by its agonist (+)pentazocine does not affect glutamatergic neurotransmission by a direct mechanism in CA1 pyramidal cells.

It has been suggested that σ1R ligands may interfere with channels that are involved in synaptic transmission such as Ca^{2+} channels (changes duration of action potential), Na^{+} channels (changes amplitude of action potential) and K^{+} channels (changes duration of action potential and AHP) [63;66;72;74-77]. It has been suggested that the activation of σ1R by a variety of ligands reduces current flow through two distinct K^{+} channel types, the A current channel and the Ca^{2+} activated K^{+} channel [80]. Indeed, σ1Rs have been described to modulate the excitability of peptidergic nerve terminals in the neurohypophysis by inhibiting voltage dependent K^{+} channels [76;80]. In the hippocampus, σR ligands DTG and (+)pentazocine inhibit voltage-gated K^{+} channels [75-77]. Moreover, DTG and (+)pentazocine can affect some parameters of the action potential waveform such as amplitude and duration of both the action potential and the AHP [74]. Changes in channel activation can influence the resting potential, spontaneous activity, the response to other excitatory or inhibitory inputs and the amount of Ca^{2+} that enters during an action potential. Such effects clearly play a major role in signalling throughout the nervous system. Assessment of physiological properties of CA1 pyramidal cells in both the presence and absence of (+)pentazocine revealed that σ1R

modulates electrophysiological properties of neurons, namely by significantly reducing the amplitude of both mAHP and sAHP as well as reducing the first spike interval **(Table 2; Fig.7).** Since both the afterhyperpolarizing currents are reduced by (+)pentazocine, I hypothesize that this compound may act to inhibit I_{AHP} and sI_{AHP}. Indeed, it has been shown that the currents that mediate the AHP are Ca^{2+}-activated K^+ currents and they control spike frequency adaptation, which has been shown to play a key role in the transfer of integrated synaptic input to neuronal output. Indeed, I_{AHP} controls the interspike interval during repetitive activity, while activation of sI_{AHP} is responsible for spike frequency adaptation [93]. Hence, pharmacological alteration of Ca^{2+}-activated K^+ currents has important implications regarding the modulation of various neuronal networks.

The K^+ and rise of intracellular Ca^{2+} concentration dependence of the effect of (+)pentazocine on the NMDAR currents together with the blockade of I_{AHP} and sI_{AHP} point to the involvement of a Ca^{2+} activated K^+ conductance. Evidence of an interaction between NMDAR and Ca^{2+} activated K^+ channel has been demonstrated by Isaacson and Murphy in olfactory bulb granule cells [94]. The authors showed that NMDAR mediated Ca^{2+} influx was coupled to large conductance (BK) Ca^{2+} activated K^+ channels [94]. However, in cultured hippocampal neurons, NMDA application was observed to activate SK channels, but not BK channels [72]. Indeed, the NMDAR mediated rise in Ca^{2+} concentration results in the activation of apamin sensitive SK channels that underlines the slow AHP, which in turn acts to limit the activation of NMDARs and reduce Ca^{2+} influx [72;77].

It has also been established that Ca^{2+} influx through NMDARs can open Ca^{2+} activated K^+ channels in several systems. It has been demonstrated that in hippocampal slices, glutamate-evoked membrane depolarization could be followed by a Ca^{2+}-dependent and K^+-mediated AHP[82]. Similarly, NMDA application evoked a Ca^{2+}-dependent K^+ current

in cultured hippocampal neurons [83]. These findings are in agreement with the K^+ dependence of the increasing effect of (+)pentazocine on the NMDAR currents observed in my experiments with cesium (no change in the response amplitude; **Table 1, Fig 5**) and K^+-(increase of the response amplitude; **Table 1, Fig 5**) based intracellular solution, respectively. The lack of effect of (+)pentazocine on the NMDAR currents in presence of 10 mM BAPTA (**Table 1, Fig 5**) underlines the dependence of the rise in intracellular Ca^{2+} concentration of the effect. Moreover, it is also supported by the fact that (+)pentazocine abolishes a current similar to the one abolished by apamine, a SK channels blocker, suggesting the modulation of SK channels by the activation of σ1R by (+)pentazocine. Furthermore, the voltage dependence of the potentiating effect of (+)pentazocine on the NMDAR currents strengthens the relationship between NMDAR-mediated rise in the Ca^{2+} concentration and K^+ current. In addition, the experiments using local application of NMDA confirmed that the Ca^{2+} influx through post-synaptic NMDARs is necessary to activate Ca^{2+} activated K^+ channels. Finally, these data were further supported by my experiments demonstrating the enhancing effect of (+)pentazocine on NMDAR currents was mimicked by apamin and bicuculline methiodide, two SK channel blockers, but not by low concentration of TEA, a BK channels blocker. This strongly suggests an implication of the SK channel in the modulation of the NMDAR currents.

SK channels are activated by submicromolar concentration of intracellular Ca^{2+} and behave as high affinity Ca^{2+} sensors that convert fluctuation of intracellular Ca^{2+} concentrations into changes in membrane potential [95]. All the SK channel subtypes exhibit a similar Ca^{2+} dose-response relationship with Ca^{2+} concentration required for half maximal activation ($K_{0.5}$) of ~0.3 μM and an onset of the currents within 1 ms with a time constant of 5-12 ms [95]. The intracellular concentration of Ca^{2+} is maintained very low (10-100 nM) by

channels, pumps and exchangers, allowing rapid metabolic response to Ca^{2+} changes. It has been shown that in the spines of CA1 pyramidal cells, the synaptic Ca^{2+} signals are primarily caused by Ca^{2+} influx through NMDARs [42], with a rise time of the Ca^{2+} transient of 13 ± 2 ms [45]. Since the activation kinetics of the NMDAR currents recorded in my experiments is ~7-11 ms (**Table 1**) and a concentration of 0.7 μM could be reached in the spine of CA1 pyramidal cells just after subthreshold NMDAR activation and/or single spontaneous synaptic vesicle release [42], the Ca^{2+} influx to the spine can easily activate SK channels (from the first ms) and affect the peak amplitude of the NMDAR currents (7-11 ms). I suggest that both subthreshold-activated NMDAR channels and evoked NMDAR currents may provide the source of Ca^{2+} that rapidly activates the SK channels and consequently influences the peak amplitude of NMDAR currents.

It has been shown that σ1Rs can modulate several physiological and cellular events such as Ca^{2+} release and signalling from intracellular Ca^{2+} storage sites *via* inositol 1,4,5-triphosphate (IP3) receptor on the endoplasmatic reticulum (ER) [56]. Indeed, the σ1Rs have been described to be localized on the ER [56]. However, the inability of CPA and ryanodine, two Ca^{2+} ATPase inhibitors that cause the depletion of intracellular Ca^{2+} stores, to reverse the effect of (+)pentazocine on NMDAR currents rules out the possibility that this effect is mediated through an action on the Ca^{2+} store in the regulation of NMDAR currents. σ1Rs have also been described to regulate voltage-dependent Ca^{2+} channels [63]. Nicardipine, an L-type Ca^{2+} channel blocker, was unable to block the increasing effect cause by (+)pentazocine on NMDAR current, excluding the implication of L-type voltage gated Ca^{2+} channels. I cannot use other Ca^{2+} channel blockers such as nifedipine, because of the presence of pre-synaptic Ca^{2+} channels that are involved in the release of neurotransmitters.

For the last three decades, it has been established that glutamatergic neurotransmission, especially *via* NMDARs, plays a role in learning and memory. LTP of the Schaffer collateral synapses is NMDAR-dependent and requires pre-synaptic activity and post-synaptic depolarization. The post-synaptic depolarization is necessary due to the properties of the NMDAR, which require the relief of the Mg^{2+}-block to open [81]. NMDAR activation also requires the binding of glutamate (pre-synaptic activity). Once NMDARs are open, Ca^{2+} influx triggers synaptic plasticity [23]. Since (+)pentazocine enhances NMDAR current through the block of SK channels, the enhanced LTP observed in presence of the σ1R agonist was likely the result of the enhanced Ca^{2+} influx through the NMDAR. The abolishment of the effect on LTP by AP-5, an NMDAR antagonist, further supports this notion. My hypothesis was also supported by previous publications which described that, in CA1 pyramidal cells, application of apamin enhances LTP [36;87] and that blocking SK channels facilitates the induction of LTP by enhancing NMDAR-dependent Ca^{2+} signals within dendritic spines [45]. The influence of σ1Rs on SK channels can play an important role in modulating synaptic transmission. Indeed, apamin has been described to attenuate the memory deficit of scopolamine, a cholinergic antagonist that induces amnesia [96;97]. In the same way, selective σ1R agonists have been shown to reverse pharmacologically induced amnesia [98]. Consequently, the influence of σ1Rs on SK channels can play an important role in modulating synaptic transmission.

Future developments

The results presented in this study are innovative since they clarify the role played by σ1Rs in NMDAR-mediated glutamatergic neurotransmission. However, more questions need to be answered and one of them could be to determine whether σ1R activation can change the efficacy of synaptic transmission through the modulation of other sources of Ca^{2+} than NMDARs in the CA1 region of the hippocampus.

Increase in intracellular Ca^{2+} concentration is necessary for the LTP induction and the persistent enhancement of synaptic efficiency [23]. There are many sources of Ca^{2+}, other than through NMDARs, which are also known to be involved in the induction of LTP at CA1 pyramidal cell synapses. Indeed, there are three major sources of Ca^{+2} in the pyramidal cells of the CA1 region of the hippocampus: influx of Ca^{2+} through voltage-gated Ca^{2+}channels and NMDARs and liberation of Ca^{2+} from the intracellular stores [56] (ryanodine and inosotol 1,4,5-triphosphate receptors, IP3R; **for review see** [65]) [23].

Since σ1R ligands have been described to regulate voltage-dependent Ca^{2+} channels [63], NMDAR and IP3R causing an increase in cytosolic Ca^{2+} concentration, it would be interesting to investigate the effect of σ1R modulation on Ca^{2+} channels and intracellular stores on LTP in rat hippocampal CA1 pyramidal cells using patch-clamp whole-cell techniques. Firstly, to determine whether σ1R activation can change the efficacy of synaptic transmission through the modulation of voltage-gated Ca^{2+} channels, rat CA1 pyramidal cells could be recorded using a cesium-based solution containing D890 (3 mM) in order to block the voltage-gated Ca^{2+} channels[42] and the LTP will be induced in control and in presence of σ1R agonist (+)pentazocine (1 μM). Secondly, to determine whether σ1R activation can change the efficacy of synaptic transmission through the modulation of Ca^{2+}

release from the intracellular stores, CA1 pyramidal cells could be recorded using a cesium-based solution containing Ca2+ ATPase inhibitors CPA (30 μM) or ryanodine (10 μM) in order to cause the depletion of intracellular Ca2+ stores and the LTP will be induced in control and in presence of σ1R agonist (+)-pentazocine (1 μM).

Chapter 5: Conclusion

In conclusion, this study sheds light on the long-time unanswered question regarding the mechanism underlining the modulation of NMDAR function by the σ1Rs. These results elucidate the role played by σ1R ligands in higher order brain functions such as memory, cognition and mood as well as in the pathophysiology of certain neuropsychiatric disorders, such as drug dependence and depression, in which the σ1R has been involved. Furthermore, a better understanding of the implication of the σ1R through NMDAR activity in the etiology of mood disorders such as schizophrenia will provide us with a direction to follow in the attempt to explain the symptoms and may help to develop innovative drugs to treat this disease.

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