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NETWORK INTERACTIONS OF MEDIAL PREFRONTAL CORTEX, HIPPOCAMPUS AND REUNIENS NUCLEUS OF THE MIDLINE THALAMUS

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Résumé

Le présent mémoire corrobore l'hypothèse selon laquelle l'hippocampe, le cortex préfrontal et le noyau reuniens du thalamus constituent un réseau fonctionnel dans lequel le noyau reuniens servirait d'interface entre l'hippocampe et le cortex préfrontal. Bien que la voie hippocampo-corticale de ce réseau ait été abondamment étudiée, cela n'est pas le cas pour la voie reuniens-préfrontale. Nous décrivons ici, pour la première fois, la réponse de neurones du cortex préfrontal médian aux stimulations du noyau reuniens. Chez des chats sous anesthésie (kétamine-xylazine), nous avons effectué simulatanément 1) des enregistrements intra- et extracellulaires dans le cortex préfrontal médian et 2) des stimulations du noyau reuniens ou de l'hippocampe à l'aide d'électrodes bipolaires. Nous avons ainsi démontré que la réponse de neurones du cortex préfrontal médian aux stimulations du noyau reuniens est distincte des réponses évoquées par des stimulations hippocampiques, que la voie reuniens-préfrontale est sujette à la plasticité à court terme et qu'une région restreinte du cortex préfrontal médian sert de relai à la voie hippocampo-cortico-thalamique.

Abstract

This thesis provides supporting evidence for the claim that the hippocampus, prefrontal cortex and reuniens nucleus of the thalamus together constitute a functional network. The reuniens nucleus has been proposed to serve as an interface between the hippocampus and prefrontal cortex. Implicit in this line of reasoning is the notion that the reuniens nucleus exerts physiological influences on the prefrontal cortex. Intracellular and local field potential recordings in the medial prefrontal cortex of anesthetized cats were performed and both the reuniens nucleus and hippocampus were stimulated with bipolar electrodes to further elucidate the nature of this influence. A physiological description of responses of medial prefrontal cortex neurons to stimulation of the reuniens nucleus is provided here for the first time. It is further demonstrated that the reuniens nucleus and hippocampus exert distinct actions on neurons of the medial prefrontal cortex, that this pathway is subject to short-term plasticity and that a restricted locus of the medial prefrontal cortex mediates the hippocampo-prefronto-thalamic relay.

Forward

"The stage has been set to attack the master unsolved problem of biology: how the hundred billion nerve cells of the brain work together to create consciousness".

-Edward O. Wilson

The work presented in this memoir was carried out in the laboratory of Dr. Igor Timofeev in the department of Anatomy and Physiology at Laval University, Québec, Canada and in the Centre de Recherche Université Laval Robert-Giffard, Québec, Canada from Fall 2005 to Winter 2007. It was funded in part by the National Science and Engineering Research Council, which provided a two year Postgraduate Research Award. Supplementary funding was provided by an operating grant to Dr. Timofeev from the Canadian Institutes of Health Research.

It is with much enthusiasm that I began my graduate studies at Laval University ready to join forces with those attacking this unsolved problem of the mind alluded to by Wilson. In particular, I find memory fascinating because of its apparent role in weaving the Self. Investigations of memory abound as does work on synaptic plasticity, its putative correlate at the molecular and cellular levels. What is lacking is work at the systems level: what are the networks of cognition?

Two years later my enthusiasm remains unaltered although I have come to realize that in my desire to answer such questions, I am in it for the long run. My experience here leaves me with a new and rich skill set as well as a deepened appreciation and understanding of neurophysiology research.

I take this opportunity to express my gratitude, first to my supervisor, Dr. Igor Timofeev, who welcomed me in his lab and who made available to me a wealth of resources and learning opportunities throughout my training. I wish also to express gratitude towards the late Professor Mircea Steriade for first inviting me to join the neurophysiology laboratory at Laval University. Many thanks go to my friends and colleagues for their general help and camaraderie. In particular I wish to thank Krisztina Kovács and Alex Ferecskó for their generosity, teachings and words of encouragement. I also thank Pierre Giguère and Sergiu Ftomov for technical

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À mes parents, Guy et Monique, qui on su cultiver en moi l'amour de la connaissance et à Vincent, qui a su agrémenter ces dernières années de bonheur et de complicité.

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AMPA	α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid
BDA	Biotinylated dextran-amine
CCK	Cholecystokinin
EEG	Electroencephalogram
EPSP	Excitatory postsynaptic potential
FA	Fast-adapting
FRB	Fast-rhythmic bursting
FS	Fast spiking
GABA	γ-aminobutyric acid
HP	Hippocampus
HRP	Horseradish peroxidase
IB	Intrinsically-bursting
IL	Infralimbic cortex
IPSP	Inhibitory postsynaptic potential
LTP	Long term potentiation
mPFC	Medial prefrontal cortex
NIB	Non-inactivating bursting
NMDA	N-methyl-D-aspartic acid
PHA-L	Phaseolus leucoagglutinin
PL	Prelimbic cortex
PV	Parvalbumin
REUN	Reuniens nucleus
RS	Regular spiking
Vglut2	Vesicular glutamate transporter 2
VIP	Vasoactive intestinal peptide
WGA	Wheat germ agglutinin

1 Introduction

Both the hippocampus and the prefrontal cortex play well recognized roles in cognitive processes and their interaction has been shown to be particularly critical to memory consolidation. The hippocampo-prefrontal pathway, however, is unidirectional; the prefrontal cortex does not return direct projections to the hippocampus. On the other hand, the reuniens nucleus of the midline thalamus is reciprocally connected to both the prefrontal cortex and the hippocampus and is thus well positioned to serve as an interface between these two structures. Implicit in this line of reasoning is the notion that the reuniens nucleus exerts physiological influences on the prefrontal cortex. A physiological description of medial prefrontal cortex intracellular responses to stimulation of the reuniens nucleus is provided here for the first time. It is further demonstrated that the reuniens nucleus and hippocampus exert distinct actions on neurons of the medial prefrontal cortex and that a restricted locus of the medial prefrontal cortex mediates the hippocampo-prefronto-thalamic relay. The introductory text that follows is made up of three sections. *Section 1.1.* provides a description of the anatomy and electrophysiology of the prefrontal cortex, hippocampus and reuniens nucleus. *Section 1.2.* reviews the body of work pertaining to interactions of these three structures. *Section 1.3.* provides a brief overview of spontaneous network activity in the brain with emphasis on oscillations of the thalamo-cortical and hippocampal systems. In *Section 2*, the specific aims of this study are enumerated. Materials, methods, results and discussion are presented in scientific article format in *Section 3* of this Master's dissertation. Finally, a general conclusion is found in *Section 4* and includes a summary of findings as well as a discussion of methodological considerations, of functional implications and of future directions. All references are appended at the end of the memoir.

1.1 The Prefrontal Cortex, Hippocampus and Reuniens Nucleus

The following section aims to provide an overview of the prefrontal cortex, hippocampus and reuniens nucleus of the thalamus, which together are believed to constitute a functional network. This will set the stage for the subsequent literature review of studies aiming to elucidate the nature of their interactions.

1.1.1 The Prefrontal Cortex

The prefrontal cortex has been shown to play a crucial role in higher cognitive functions such as memory and attention. It has been proposed, for example, to play a key role in incorporating newly acquired information in neocortical networks (Teixeira et al., 2006; Wiltgen et al., 2004; Fletcher and Henson, 2001). This part of the frontal lobe is defined as prefrontal by virtue of being densely and reciprocally connected with the mediodorsal nucleus of the thalamus (Musil and Olson, 1988; Thierry et al., 2000). It spans from the proreal gyrus to cortex immediately underlying the genu of the corpus callosum in cat and is similarly localized in rat and monkey (Musil and Olson, 1988). The prefrontal cortex is further subdivided into medial, lateral and ventral, or orbital, areas (Berendse and Groenewegen, 1991). The present memoir is concerned with the medial prefrontal cortex as it is reciprocally connected to the reuniens nucleus and a target of hippocampal projections.

The medial wall of the frontal lobes was initially segregated into two areas. The orbitofrontal cortex was that part which was innervated by the mediodorsal nucleus of the thalamus and the anterior limbic cortex was the one which was innervated by the anterior thalamic nucleus (Rose and Woolsey, 1948). It was later found, however, that the anterior limbic cortex also receives inputs from the mediodorsal nucleus (Leonard, 1969; Krettek and Price, 1977a). The anterior limbic area encompasses Brodmann's area 32 and 24, or prelimbic and anterior cingulate area, respectively. Caudal to the prelimbic area is area 25, or infralimbic cortex, which does not receive projections from the mediodorsal nucleus but is sometimes included in the prefrontal

cortex (Room et al., 1985; Beckstead, 1979; Groenewegen, 1988; Vertes, 2002). The prelimbic and infralimbic cortices are areas of particular interest to this work.

While the neocortex is conventionally subdivided into six layers, the organization of the prefrontal cortex is not distinctly laminar. Indeed, the prefrontal cortex is considered to be *agranular*—that is, it lacks much of the small densely packed neurons that are usually found in the middle layers. In particular, the anterior cingulate, prelimbic and infralimbic cortices lack a distinct layer IV (Jay and Witter, 1991). The prelimbic cortex, or area 32, is characterized by a relatively well-defined lamination. In cat prelimbic cortex, layer II is distinguished from layer III on the basis of higher cell density while layer III fuses with layer V, where the largest cells are found (Rose and Woolsey, 1948; Krettek and Price, 1977a; Room et al., 1985; Musil and Olson, 1988; Vertes, 2002; Jay and Witter, 1991). We will later see that the reuniens nucleus targets most prominently layers Ia, V and VI of the prelimbic and infralimbic cortices and that generally, the deep layers V/VI seem to be the preferential targets of hippocampo-prefrontal projection fibers.

Neurons of the neocortex are physiologically heterogenous. Their response to synaptic input is largely determined by intrinsic properties such as cell morphology, channel expression and channel distribution. Electrophysiological cell types constitute the basic functional unit of neural networks and are identified by the injection of current steps into the cell. Four basic electrophysiological cell types have been described in the neocortex based on the characteristics of individual action potentials such as spike width and afterpotential components, the response to threshold current pulses and the repetitive response pattern to prolonged current steps. These cell types are: regular-spiking (RS), intrinsically bursting (IB), fast-rhythmic bursting (FRB) and fast-spiking (FS). It is important to note, however, that subcategories exist and that while these cell categories are typical there exist a continuum of different cell types. In addition it should be noted that cells exhibit dynamic behavior such that neurons may change their firing pattern under different conditions, such as shifts in membrane potential (Connors and Gutnick, 1990; Gray

and Mccormick, 1996; Timofeev et al., 2000b; Steriade et al., 1998b; Steriade, 2004; Contreras, 2004).

Regular-spiking cells are the most common electrophysiological cell type in the neocortex. RS cells respond to current injection with sustained repetitive firing. The spike frequency of RS cells can reach 200-300 Hz and is linearly proportional to current intensity (Crill and Schwindt, 1984; Stafstrom et al., 1984). RS cells adapt to maintained stimuli. Fast-adapting cells will have their firing frequency decay to zero in just a few tens of milliseconds while slowly adapting cells will have their frequency decay to half of its initial value in a few hundred milliseconds (Agmon and Connors, 1992; Nowak et al., 2003; Nunez et al., 1993). Spike width in RS cells is about 1 ms at its base and both afterhyperpolarizations and afterdepoloarizations may be observed following action potentials (Connors et al., 1982; Mccormick et al., 1985; Nunez et al., 1993; Steriade et al., 2001).

Intrinsically-bursting cells are characterized by generating clusters of 3-5 spikes (bursts) at 100-200 Hz either singly or repetitively at interburst frequencies of 5-15 Hz (Connors and Gutnick, 1990; Contreras, 2004; Agmon and Connors, 1992; Nunez et al., 1993). Within bursts, successive spikes tend to decline in amplitude. IB cells were first described in layers IV and V of parietal and cingulate cortices of guinea pigs (Connors et al., 1982). Only 4% of cells are intrinsically bursting during waking (Steriade et al., 2001).

Fast-rhythmic-bursting cells are also characterized by spike bursts. The bursts of FRB cells contain 2-5 spikes with intraburst frequencies of 200-600 Hz and little spike inactivation. Spikes last approximately 0.6 ms and exhibit a clear albeit small afterhyperpolarization followed by an afterdepolarization. Interburst frequencies are of 20-80 Hz (Gray and Mccormick, 1996; Steriade et al., 1998b; Brumberg et al., 2000; Steriade et al., 2001; Steriade, 2004).

Fast-spiking neurons have very fast, thin spikes lasting less than 0.5 ms. They are characterized by highly repetitive firing patterns with firing frequencies reaching up to 800 Hz and undergo little to no accommodation. In addition, these cells have the

ability to generate membrane potential oscillations between 20 and 80 Hz (Connors and Gutnick, 1990; Llinas et al., 1991; Nunez et al., 1993; Contreras, 2004).

Anatomically, RS, IB and FRB are almost always pyramidal neurons (Contreras, 2004). RS cells, however, have also been correlated with spiny stellate and large GABAergic basket cell morphologies (Kawaguchi and Kubota, 1997). In addition, some double bouquet, bipolar and martinotti cells have been shown to exhibit RS electrophysiology. IB and FRB cells may be recorded from layers II-IV (Timofeev et al., 2000b). FS cells almost exclusively correspond to non-pyramidal aspiny or sparsely spiny morphologies. They release GABA (Ribak, 1978) and correspond to at least two types of interneurons: basket cells and chandelier cells. FS cells are almost always parvalbumin positive and are found in layers II-VI (Cauli et al., 1997; Kawaguchi and Kubota, 1998; Contreras, 2004).

Four main classes of pyramidal cells have been recorded in prefrontal cortex *in vivo* and characterized electrophysiologically as regular-spiking (RS), intrinsically-bursting (IB), non-inactivating bursting (NIB) and fast-adapting (FA) (Degenetais et al., 2003; Thierry et al., 2000). NIB cells are characterized by bursts of 3-8 spikes with average intraburst frequencies of approximately 90 Hz. The duration of successive spikes within a burst increases and the firing threshold also increases progressively within bursts. Intracellular recordings were performed in layers II-VI of the prelimbic and medial orbital cortices of anesthetized rats by Thierry and coworkers (2000). It was found that RS cells made up the majority of recorded cells (79%), while FA and IB cells were recorded in lesser and approximately equal proportions (10% and 11%, respectively) (Thierry et al., 2000). In the *Results* section of this thesis, fast-rhythmic bursting (FRB) cells are also reported to have been recorded in medial prefrontal cortex of anesthetized cats.

Interneurons have also been recorded in the prelimbic and medial orbital cortices. In a 2004 study by Tierney and colleagues, fifty-nine percent of recorded interneurons were found in layer V, 31% in layer VI and 10% in layer III. The majority were of stellate morphology (83%). Of these, most were aspiny (89%) and the remainder represented sparsely spiny cells. Seven percent of the recorded interneurons were

bitufted (Tierney et al., 2004). In an earlier ultrastructural study it was found that 6% of neurons in the rat prefrontal cortex are parvalbumin-containing (PV+) and of basket and chandelier cell morphology (Gabbott et al., 2002). In the prelimbic cortex, these local circuit PV+ neurons were most commonly found in layers III and V. Electrophysiologically, they were characterized as fast-spiking. Diaphorase reactive local circuit neurons were also found in area 32 but made up less than 1% of all cortical neurons in this area. Such cells have the ability to synthesize nitric oxide, which has been implicated in LTP, learning and memory (Gabbott et al., 2002; Gabbott et al., 1997).

While most cortical and subcortical connections with the prefrontal cortex are reciprocal, the prefrontal cortex does not receive afferents from the striatum nor send direct projections to the hippocampus (Thierry et al., 2000). Feline medial prefrontal cortex receives thalamic input from the mediodorsal, ventromedial, anteromedial, medial ventroanterior and medial lateroposterior nuclei. It also receives input from midline thalamic nuclei, namely from the paraventricular, parataenial and reuniens nuclei. Cortical input originates in the insular cortex, cingulate cortex, lateral frontal cortex, CA1 region of the hippocampus, parahippocampal cortex, entorhinal cortex, parasubiculum, subiculum and auditory association cortex. Hypothalamic input arises most importantly from the lateral hypothalamus but also from the ventromedial hypothalamus, dorsal hypothalamic area and supramammillary nucleus. Further afferents arise in the amygdala, diagonal band and claustrum (Musil and Olson, 1988; Jay and Witter, 1991; Gabbott et al., 2002). In terms of monoaminergic input, the prefrontal cortex is prominently innervated by dopaminergic fibers from the ventral tegmental area whose terminals mostly form symmetric synapses onto dendritic spines and shafts (Carr and Sesack, 1996). The anterior cingulate, prelimbic and infralimbic cortices project heavily and selectively to the midline thalamus, in particular to the paratenial, paraventricular, interanteromedial, anteromedial, intermediodorsal, mediodorsal, reuniens and the central medial nuclei. The medial agranular cortex, found dorsal to the prelimbic area, projects to the rostral intralaminar nuclei (central lateral, paracentral, central medial nuclei) as well as to the ventromedial and ventrolateral nuclei of the thalamus (Vertes, 2002). The anatomy of

the pathways linking the reuniens nucleus and hippocampus to the prefrontal cortex will be further described in *Section 1.2.*

1.1.2 The Hippocampus

The hippocampus is phylogenetically older than the neocortex. It is a very well studied structure in part due to the highly laminated arrangement of its cell bodies and connections but also because of its role in learning and memory. It is a region of polysensory convergence required for the processing of information from various sensory modalities and the encoding of memories (Squire, 1992; Johnston and Amaral, 2004).

There are different memory systems in the brain and the hippocampus is important for a specific type of memory known as *explicit* or *declarative* memory (Moscovitch, 1982; Schacter, 1985; Tulving, 1985; Wickelgren, 1979; Cohen, 1984; Squire, 1982; McDonald and White, 1993). This type of memory pertains to what philosopher Gilbert Ryle (1949) referred to as "knowing that" (e.g. knowing that Ottawa is the capital of Canada) and can be contrasted with *implicit* memory which corresponds to "knowing how" (e.g. knowing how to ride your bike). In particular, the hippocampus has been repeatedly shown to play a key role in declarative memory consolidation (Winocur, 1990; Zola-Morgan and Squire, 1990; Cho et al., 1993; Kim et al., 1995). This process allows newly acquired information to be transferred from short-term memory to long-term memory and to become permanently "stored", likely in neocortical sites (Squire, 1992; Frankland and Bontempi, 2005). Evidence for hippocampal involvement in memory consolidation comes from many clinical studies of amnesic patients (Scoville and Milner, 1957; Baddeley, 1982; Milner, 1972; Cummings et al., 1984; Zola-Morgan et al., 1986) as well as animal studies, notably in monkey (Zola-Morgan and Squire, 1990; Friedman and Goldman-Rakic, 1988; George et al., 1989) and rat (Auer et al., 1989; Davis et al., 1987; Winocur, 1990). The involvement of the hippocampus in memory consolidation, however, is thought to be time-limited (Zola-Morgan and Squire, 1990; Bontempi et al., 1999). The hippocampus has also been shown to exhibit several forms of activity-dependent

plasticity, which is widely considered as an important cellular mechanism for learning and memory (Bliss and Collingridge, 1993; Malenka and Nicoll, 1999; Bennett, 2000; Martin et al., 2000).

The hippocampal formation consists of three main regions: the dentage gyrus, Ammon's horn and the subicular complex. Ammon's horn is made up of subfields CA1, CA2 and CA3 and the subicular complex consists of the presubiculum, parasubiculum and subiculum. The proximal subiculum is located adjacent to CA1. The entorhinal cortex is sometimes included in the hippocampal formation and is itself further subdivided into ventromedial, ventral lateral and dorsal lateral areas (Krettek and Price, 1977a). The principal cells of the hippocampus are multipolar neurons called pyramidal cells. They are arranged in a single layer that is 3 to 6 cells deep. Above the pyramidal layer of the CA fields is the stratum oriens and below are the stratum radiatum and stratum lacunosum-moleculare. The hippocampus is also very rich in interneuron varieties (Freund and Buzsaki, 1996).

Anderson and colleagues coined the term trisynaptic circuit to describe the flow of information through the hippocampal formation (Anderson et al., 1971). The starting point of this circuit is the entorhinal cortex. The entorhinal cortex receives most of its input from the perirhinal, postrhinal and retrosplenial cortices (Van Groen and Wyss, 1992; Wyss and Van Groen, 1992). Most of the sensory information from various modalities converges in these cortical areas which, in turn, generally provide excitatory input to the entorhinal cortex (Martina et al., 2001). Layer II neurons of the entorhinal cortex then give rise to the perforant path which projects to the dentate gyrus and CA3 subfield; CA1 and subiculum receive input from layer III entorhinal cells. The dentate gyrus in turn gives rise to the mossy fibers which terminate on the proximal dendrites of CA3 pyramidal cells. These cells then project to other CA3 neurons and to CA1 pyramidal cells. This latter projection is called the Schaffer collateral. The CA1 pyramidals project to the subiculum and deep entorhinal layers. The principal cells of the subiculum also project to the deep layers of the entorhinal cortex. The deep layers of the entorhinal cortex reciprocate the majority of cortical projections and it is thought that the hippocampal processing of the initial inputs is

necessary for the formation of long-term memories. Anatomical studies in rat, cat and monkey have shown that the ventral CA1 and subiculum also project to the medial prefrontal cortex (Swanson, 1981; Ferino et al., 1987; Jay and Witter, 1991; Carr and Sesack, 1996; Irle and Markowitsch, 1982; Cavada et al., 1983; Goldman-Rakic et al., 1984). For this reason, the following section will describe these fields and their connectivity in greater detail.

The average CA1 pyramidal cell is smaller than the average CA2 and CA3 principal cell but can receive up to 30,000 excitatory inputs and 1,700 inhibitory inputs (Johnston and Amaral, 2004). It can fire up to several hundred Hertz but *in vivo,* some studies have reported firing rates of approximately 0.5 Hz (Schwartzkroin, 1975; Schwartzkroin, 1977; Xu and Pulsinelli, 1996). Their axons extend in the alveus or stratum oriens where they project to the subiculum; distal CA1 cells project to the proximal subiculum, middle CA1 cells to mid-subiculum and proximal CA1 cells to distal subiculum (Johnston and Amaral, 2004; Amaral et al., 1991). CA1 is the first hippocampal field to project back to the entorhinal cortex. More specifically, the CA1 projects to deep layers of the entorhinal cortex and predominantly to layer V of the medial entorhinal cortex (Finch and Babb, 1980; Finch and Babb, 1981;Van Groen and Wyss, 1990). A small number of CA1 neurons also project to the contralateral CA1(Van Groen and Wyss, 1990) while ventral CA1 further projects to the reuniens nucleus (McKenna and Vertes, 2004a). The CA1 receives input from the CA3, ipsilaterally via the Schaffer collateral and contralaterally via the commissural pathways. It also receives afferents from the septum and amygdaloid complex (Krettek and Price, 1977b; Pitkanen et al., 2000; Johnston and Amaral, 2004). The reuniens nucleus terminates on spines and dendrites of CA1 apical dendrites as well as interneurons in the stratum lacunosum-moleculare (Wouterlood et al., 1990; Dolleman-Van der Weel et al., 1997; Dolleman-Van der Weel and Witter, 2000).

As mentioned, the subiculum receives input from the CA1 and entorhinal cortex. The axons of pyramidal cells of the subiculum form dense collaterals and project, in addition to the entorhinal cortex, to several subcortical regions such as the nucleus accumbens, anterior thalamic nuclei, medial mammillary nucleus, lateral septal

nucleus and presubiculum (Johnston and Amaral, 2004). The subiculum and presubiculum project to the reuniens nucleus(McKenna and Vertes, 2004).

1.1.3 The Reuniens Nucleus

The reuniens nucleus is a small nucleus lying ventrally on the midline directly above the IIIrd ventricle and occupying the anterior third of the thalamus (Van der Werf et al., 2002). In cat, the reuniens nucleus has a size of approximately 2 mm^3. Like the prefrontal cortex and hippocampus, the reuniens nucleus of the thalamus has been implicated in memory function, albeit to a much lesser extent. Together with other midline and intralaminar nuclei it has also been implicated in awareness and vigilance (Mennemeier et al., 1992; Vann et al., 2000; Van der Werf et al., 2002).

The thalamus is divided into medial dorsal, anterior and lateral groups by the internal medullary lamina, a Y-shaped strip of white matter. The midline nuclei of the thalamus are found along the midline, as their name suggests, and consist of a thin conglomerate of cells spanning the anterior third of the thalamus. They include the paraventricular, parataenial, intermediodorsal, reuniens and rhomboid nuclei. The intralaminar nuclei are contained within the internal lamina, lateral to the mediodorsal nucleus. They include the central medial, paracentral, central lateral, centre médian and parafascicular nuclei (Van der Werf et al., 2002).

The midline and intralaminar nuclei have long been considered as non-specific nuclei of the thalamus. The midline and intralaminar nuclei are considered to be the rostral continuation of the reticular formation as they receive strong inputs from the mesencephalic, pontine and medullary reticular formation. These inputs were initially thought to be non-discriminatory with respect to individual nuclei. Further, the cortical projections of the midline and intralaminar nuclei were described as diffuse and non-specific. Lastly, electrical stimulation of these nuclei was reported to produce recruiting effects, where low frequency of stimulation induced both slow waves in the entirety of the cortical mantle and somnolence; high-frequency stimulation produced cortical desynchrony and arousal or epileptiform activity in case of intense stimulation. In short, pronounced yet putatively non-discriminatory inputs

from the ascending activating systems, diffuse cortical outputs and the ability to induce recruiting responses together account for the attribution of non-specificity to the midline and intralaminar nuclei (Van der Werf et al., 2002).

The view of non-specificity has been challenged in recent years (Van der Werf et al., 2002; Groenewegen and Berendse, 1994). Van der Werf and colleagues (2002) have proposed, based on similarity of connectivity, that these nuclei be subdivided into four "functionally homogeneous" groups: (1) the dorsal group includes the paraventricular, parataenial and intermediodorsal nuclei and is implicated in viscero-limbic functions; (2) the lateral group, made up of the central lateral, paracentral, and anterior central medial nuclei, is involved in cognitive function; (3) the posterior group is made up of the centre médian and parafascicular nuclei and is involved in limbic motor function, and (4) the ventral group, comprising the reuniens, rhomboid and posterior central medial nuclei is thought to modulate higher cognitive, affective and polysensory processing.

The ventral group is distinguished from other groups by its sparse innervation of the striatum. Its efferents project to superficial and deep layers of most cortical areas but especially to primary motor, sensory and associative cortices. The reuniens, and possibly also the rhomboid, also projects to the hippocampus proper. Because sensory input from different modalities converges in the hippocampus, it has been proposed that this nucleus may help modulate multimodal processing. Functional data on ventral group nuclei are scarce, however, in part because selective lesions of these nuclei in humans has not been reported and in part because very few animal studies have been carried out due to the small size of these nuclei (Van der Werf et al., 2002).

The nucleus reuniens is part of the ventral group of midline and intralaminar nuclei. Connections with cortical, thalamic, hypothalamic and brainstem structures have been reported (Herkenham, 1978; Robertson and Kaitz, 1981; Ohtake and Yamada, 1989; Azmitia and Segal, 1978). In essence, it appears to receive widespread afferents from limbic structures but to selectively send efferents to the hippocampus and medial prefrontal cortex (McKenna and Vertes, 2004). A description of inputs and outputs of the reuniens nucleus will now be provided.

According to McKenna and Vertes (2004), who carried out a detailed study of reuniens afferents, "no other nucleus of the thalamus, and none outside of the midline thalamus, receives a comparable degree and diversity of inputs". Inputs to the reuniens are very different from that of neighboring structures including the central medial, rhomboid, ventromedial and submedial nuclei of the thalamus and dorsomedial nucleus of the hypothalamus. Cortical inputs arise from the infralimbic, prelimbic, orbitomedial, insular, ectorhinal and perirhinal cortices, subiculum and CA1. In cats, other cortical inputs include those originating in the cingulate cortex, dorsal and ventral retrosplenial cortices and presubiculum. In contrast with many brain regions, the reuniens nucleus receives limited thalamic input. These inputs arise in the reticular nucleus, zona incerta, paraventricular and lateral geniculate nuclei. Following fluorogold tracer injections into the reuniens nucleus, however, the zona incerta was reported to be one of the most heavily labelled sites. Hypothalamic afferents are very pronounced and originate mainly from the anterior and ventromedial hypothalamic nuclei, premammillary and supramammillary nuclei. Brain stem inputs arise in the ventral tegmental area, dorsolateral tegmental nucleus, superior colliculus, central grey, dorsal, median and central raphé and parabrachial nucleus. Basal afferents originate in the nucleus of the diagonal band and bed nucleus of the stria terminalis. The reuniens nucleus also receives input from the medial nucleus of the amygdala. Other inputs include the claustrum, tania tecta, lateral septum, substantia innominata and lateral preoptic area of the basal forebrain, periaqueductal gray and pretectal nucleus (McKenna and Vertes, 2004; Van der Werf et al., 2002; Herkenham, 1978).

Some differences in the patterns of innervation of the rostral versus caudal reuniens nucleus have been reported. These differences pertained mostly to the densities of projections. For instance, the caudal part of the reuniens nucleus receives stronger inputs from the motor agranular, dorsal anterior cingulate, entorhinal and perirhinal cortices and interpeduncular nucleus. The ectorhinal, perirhinal, reticular and interpeduncular nuclei in fact do not project to the rostral end of the reuniens nucleus. On the other hand, the rostral reuniens nucleus receives more inputs from subcortical regions such as the lateral septum, anterior, ventromedial and posterior hypothalamic

nuclei, supramammilary nucleus, periaqueductal gray and CA1/subiculum (McKenna and Vertes, 2004).

The reuniens nucleus projects almost exclusively to the telencephalon. The densest sites of projection are to the medial and lateral entorhinal, medial frontal polar, infralimbic, medial and ventral orbital, perirhinal, anterior piriform, prelimbic and retrosplenial cortices, the claustrum, dorsal tania tecta, CA1 and subiculum. It was reported that injections of phaseolus vulgaris leuccoagglutinin into the reuniens proper led to bilateral labeling of these structures while injections into the perireuniens led to less dense, ipsilateral labeling (Vertes et al., 2006).

Most efferent fibers of the reuniens nucleus course ventrolaterally and pass through the ventromedial nucleus, zona incerta and dorsolateral hypothalamus. They course through the medial forebrain bundle. Some fibers then descend through the lateral hypothalamus and medial forebrain bundle to the hypothalamus and rostral midbrain. Other fibers pass through the lateral hypothalamus and head towards the amygdala and ventral regions of the perirhinal cortex. Some fibers of this tract also reach parts of the subiculum. Finally, most fibers will join the internal capsule, ascending through the lateral hypothalamus and medial forebrain bundle, to reach the ventromedial striatum and rostral forebrain They will either terminate in the frontal cortex or continue on to the hippocampus via the cingulate bundle (Vertes et al., 2006). Other efferent pathways head towards the medial fourth of the reticular nucleus and into the inferior thalamic peduncle where they continue to course rostrally before turning medialward and above the anterior commissure. Some of these fibers abut in the piriform cortex, claustrum and olfactory tubercle. Two distinct bundles terminate the rostral and caudal cortical regions. Reuniens efferents to the hippocampus reach this structure via the cingulate bundle, inferior thalamic peduncle or stria terminalis (Wouterlood et al., 1990).

The reuniens nucleus consists of a conglomerate of loosely packed cells comprised of dorsal mediocellular and ventral parvicellular parts (Yanagihara et al., 1987). At its rostral end, the reuniens nucleus is divided into two by the IIIrd ventricle and more caudally, these two structures fuse to become a single mass bordered by the

perireuniens nuclei (Van der Werf et al., 2002). Some studies suggest that the caudal reuniens innervates the rostral part of the reuniens nucleus (Wouterlood et al., 1990; Dolleman-Van der Weel et al., 1997).

Neurons in the reuniens nucleus are immunocytologically heterogeneous (Bokor et al., 2002). Both calretinin and calbindin immunoreactivity have been reported in the reuniens nucleus (Winsky et al., 1992; Arai et al., 1994). A significant proportion of cells co-express calbindin and calretinin (Bokor et al., 2002). While little is known about the functional significance of calbindin and calretinin, it has been reported that the reuniens of epilepsy-prone rats express lower levels of calbindin mRNA (Montpied et al., 1995). The majority of hippocampus-projecting cells are calbindin-positive (Bokor et al., 2002). Cortically projecting neurons have also been shown to be calbindin positive but not immunoreactive for parvalbumin (Lin et al., 1996; Bokor et al., 2002). Immunostaining for several neuropeptides such as somatostatin, cholecystokinin, neuropeptide Y, leucin-enkephalin, substance P, calcitonin gene-related peptide and vasoactive intestinal polypeptide was reported to be negative (Bokor et al., 2002) and in the rat thalamus, GABA-immunoreactive parvalbumin positive cells are strictly localized in the nucleus reticularis thalami and zona incerta, therefore absent in the reuniens nucleus (Houser et al., 1980; Ottersen and Storm-Mathisen, 1984; Lin et al., 1996). In contrast, dense immunolabeling for GABA has been reported in cat (Rinvik et al., 1987)

1.2 A Role for the Reuniens Nucleus of the Midline Thalamus in Hippocampo-Prefrontal Interactions

The hippocampo-prefrontal pathway is unidirectional as the hippocampus projects to the prefrontal cortex but not vice versa. Given that the reuniens nucleus is, on the other hand, reciprocally connected to both the hippocampus and prefrontal cortex, this thalamic nucleus has been proposed to serve as an interface between these two structures (Vertes, 2006). The microcircuits of the prefrontal cortex, hippocampus and reuniens nucleus have been discussed above and an overview of hippocampo-cortical, prefronto-thalamic and hippocampo-thalamic interactions will now follow.

1.2.1 Interactions of the hippocampus and prefrontal cortex

Interactions of the hippocampus and prefrontal cortex have been subjected to psychological, anatomical and electrophysiological investigation and have been shown to be necessary to learning and memory. For example, Floresco and colleagues (1997) have shown that blocking hippocampo-prefrontal communication by injecting lidocaine into the ventral CA1/subiculum, which projects profusely to the prefrontal cortex, produces the same effect as prefrontal lesions: it significantly impairs performance on memory tasks (Floresco et al., 1997; Floresco et al., 1999; Aujla and Beninger, 2001). It is also known that while the hippocampus is more involved in the early stages of learning, the prefrontal cortex appears to be more engaged as memory consolidates (Bontempi et al., 1999; Frankland et al., 2001; Maviel et al., 2004; Takehara et al., 2003). The mechanisms of hippocampo-prefrontal interaction, however, remain to be elucidated. Before discussing potential mechanisms or a role for the reuniens nucleus, however, the anatomy of this pathway will be described.

Anatomical studies in rat, cat and monkey have shown that the ventral hippocampus and subiculum project to the medial prefrontal cortex (Swanson, 1981; Ferino et al., 1987; Jay and Witter, 1991; Van Groen and Wyss, 1990; Carr and Sesack, 1996; Irle and Markowitsch, 1982; Cavada et al., 1983; Goldman-Rakic et al., 1984). This connectivity provides the structural basis for hippocampo-prefrontal interaction. In spite of pronounced projections from the hippocampus to the medial prefrontal cortex, however, there are no direct projections from the prefrontal cortex to the hippocampal formation (Beckstead, 1979; Goldman-Rakic et al., 1984; Room et al., 1985; Reep et al., 1987; Takagishi and Chiba, 1991). As such, the hippocampo-prefrontal pathway is, peculiarly, unidirectional. A detailed description pertaining to its anatomy will now follow.

Few studies have examined hippocampo-prefrontal projections in cat. One of the earliest was that reported by Irle and Markowitsch (1982). In this report, retrograde tracer horseradish peroxidase (HRP) was injected in various cortical sites. Dense labeling was found in the subiculum, indicating that this structure projects to the medial prefrontal cortex. Later, Musil and Olson (1988) demonstrated that ventral

deposits of retrograde tracers in the prefrontal cortex of cats also resulted in labeling of hippocampal cells in CA1.

Studies in rat have been far more numerous and have provided a more detailed account of hippocampo-prefrontal anatomy. It was reported that hippocampal CA1 and subicular afferents to the prefrontal cortex project heavily to the prelimbic, infralimbic and medial orbital cortices via the fimbria and precommissural fornix (Wyss et al., 1980; Swanson, 1981; Jay et al., 1992; Jay et al., 1989; Jay and Witter, 1991; Van Groen and Wyss, 1990). Jay and Witter (1991) further demonstrated that it is the ventral CA1, and not the dorsal CA1, which projects to the prefrontal cortex and, likewise, that only the proximal subiculum projects to the mPFC. In this study, anterograde tracer Phaseolus vulgaris-leucoagglutinin (PHA-L) was injected into the CA1 and subiculum. It was reported that following CA1 injections, afferents to the dorsal prelimbic cortex mainly targeted deep layers V and VI. On the other hand, afferents to ventral prelimbic cortex innervated both deep and superficial cortical layers. For its part, the medial orbital cortex was only sparsely labeled but labeling was seen across all layers, though preferentially in the deeper ones. Following subicular injections, varicose fibers and terminal plexuses were observed in all layers. Long, vertically-oriented fibers were seen in layers V and VI, short, ramified fibers with no preferred orientation were seen in layers II and III and, occasionally, long, vertically-oriented fibers were seen in layer I in the prelimbic cortex. In the medial orbital cortex, terminal labeling was diffuse and more intense than that observed following CA1 injections.

In a later study, Gabbott et al. (2002) found that CA1 injections of anterograde tracer biotininylated dextran amine (BDA) resulted in terminal labeling of hippocampal fibers in all layers of the infralimbic, prelimbic and to a lesser extent, anterior cingulate cortices of the medial prefrontal cortex. Terminal labeling was most dense in layers V and VI of infralimbic and prelimbic areas, in accordance with Jay and Witter's (1991) findings, while axonal varicosities where most prominent in layers III and V. Hippocampal afferents further gave rise to collaterals which ascended towards layer I. The CA1 projection fibers were found to preferentially contact dendritic

spines (95%); synapses were, on occasion, found to occur on dendritic shafts (4%) and very rarely, on cell bodies (1%). Boutons made one synaptic junction, on average. By combining anterograde tracing techniques with immunocytochemistry, Gabbott and coworkers (2002) were further able to demonstrate that CA1 afferents to the prelimbic and infralimbic cortices of rats innervate not only spiny neurons (putative pyramidal cells) but also two subclasses of local circuit interneurons, namely, parvalbumin positive cells and NADPH diaphorase reactive neurons. These findings built on previous ultrastructural studies that revealed that hippocampal afferents to the prefrontal cortex preferentially form asymmetrical axospinous synapses, implying that hippocampal fibers establish excitatory contacts on prefrontal pyramidal cells. Although far less frequent, however, axodendritic contacts were also observed suggesting that local circuit interneurons may also be contacted by hippocampofugal fibers(Carr and Sesack, 1996). Gabbott et al.'s study hence confirmed this (Gabbott et al., 2002).

Electrophysiological investigations have further contributed to characterizing the hippocampo-prefrontal pathway. Early reports have shown that conduction velocity in this pathway is slow. Indeed, by recording antidromic spikes in rat hippocampi following medial prefrontal stimulation, Ferino et al. (1987) were able to determine that the conduction delay of the hippocampo-prefrontal pathway is of approximately 15 ms and that therefore, the conduction velocity in these hippocampo-cortical fibers is of about 0.6 m/s. Other studies have demonstrated that hippocampal afferents to the medial prefrontal cortex exert excitatory actions (Degenetais et al., 2003; Tierney et al., 2004; Laroche et al., 2000; Ferino et al., 1987; Laroche et al., 1990; Jay et al., 1995). Indeed, evidence suggests that this pathway is glutamatergic. For instance, it was shown that injections of tritiated D-aspartate, in the prelimbic cortex produce (retrograde) labeling in the ventral hippocampus and ventral/intermediary subiculum. It was also reported that microiontophoretic application of AMPA and NMDA agonists in the prefrontal cortex activate cells responding to hippocampal stimulation (Jay et al., 1992). Tritiated D-aspartate is a compound used to identify excitatory connections; AMPA and NMDA receptors mediate the glutamatergic response.

Both pyramidal cells and inhibitory neurons were found to mediate the medial prefrontal response to hippocampal stimulation. Dégenètais et al. (2003) performed intracellular recordings in anesthetized rats. They observed that hippocampal stimulation induced, in the vast majority of recorded cells in the prelimbic and medial orbital cortices (91%), a response composed of an early, likely monosynaptic, EPSP thought to be mediated by AMPA receptors. This event was immediately followed by a prolonged IPSP which was found to be mediated, at least in part, by $GABA_A$ and $GABA_B$ conductances. The complexity of the response elicited by hippocampal stimulation indeed suggests that both pyramidal and non-pyramidal cells in the prefrontal cortex are involved in mediating responses to hippocampal stimulation. It was proposed, for example, that the late inhibitory component of the response could be due to feedforward inhibition and/or to feedback from interneurons activated by recurrent collaterals of the excited cell. A role for disfacilitation was not ruled out. The hyperpolarization phase was in turn often followed by a depolarizing rebound. Rebound activity has also been described in the thalamocortical system and reported to result from intrinsic properties of thalamocortical neurons (Grenier et al., 1998). A late EPSP occasionally followed the early excitatory event, perhaps due to recurrent excitation, reverberating activity within the hippocampus or activation of an indirect pathway (Degenetais et al., 2003). It is conceivable that this event could be due to the indirect activation of the reuniens nucleus.

In a study published the following year, Tierney and colleagues (2004) investigated the role of hippocampal stimulation on medial prefrontal cortex interneurons of anesthetized rats. In this study, extracellular recordings were performed, coupled with juxtacellular injections of neurobiotin or biotinylated dextran amine. Interneurons were identified morphologically and according to spike width. It was found that 70% of recorded interneurons responded to hippocampal stimulation. While interneuron response to hippocampal stimulation was similar to that observed in pyramidal cells, the excitatory component of the response was often crowned by bursts of action potentials. Some cells did not exhibit an early EPSP but only the long hyperpolarized component of the response. The response was also deemed monosynaptic and, notably, its latency was shorter that that observed in pyramidal cells. Indeed, when

pairs of pyramidal and interneuronal cells were recorded, the interneuron was found to always fire prior to its pyramidal counterpart. This was proposed to suggest that the hippocampus is able to exert feedforward inhibition in the medial prefrontal cortex (Tierney et al., 2004). Large variability in response latencies in this pathway (Degenetais et al., 2003) further emphasizes that both monosynaptic and polysynaptic mechanisms are involved in mediating the prefrontal response to hippocampal stimulation (Siapas et al., 2005).

The hippocampo-prefrontal pathway has, in addition, been reported to exhibit plasticity. Paired-pulse facilitation, paired-pulse depression, long-term potentiation, long-term depression and depotentiation have all been observed in this pathway (Degenetais et al., 2003; Jay et al., 1995; Laroche et al., 1990; Takita et al., 1999; Burette et al., 1997). The hippocampus, therefore, exerts powerful modulatory actions at the medial prefrontal cortex.

Lastly, spontaneous activity in the prefrontal cortex and hippocampus has been shown to exhibit strong synchrony. Siapas, Lubenov and Wilson (2005) demonstrated that a significant proportion (40%) of medial prefrontal cortex neurons, recorded with chronically-implanted multitetrodes in freely behaving rats, is phase-locked to hippocampal theta activity. This phase-locking was confirmed not to be due to intrinsic rhythmicity of neuronal firing; rather, correlative evidence indicates that this behavior is likely to be mediated by hippocampal input. It was shown, for instance, that prefrontal neurons spike preferably with a 50 ms delay with respect to hippocampal theta, suggesting hippocampo-prefrontal directionality, and that medial prefrontal cortex cells are phase locked if and only if they exhibit significant cross-covariance with hippocampal units (with lags up to 150 ms). It was further proposed that local circuit interneurons in the prefrontal cortex could play a role in this theta phase-locking. Other mechanisms proposed for mediating medial prefrontal neuronal phase locking to hippocampal theta include the involvement of subcortical structures. In an earlier study, it was found that during slow wave sleep, hippocampal ripples are coherent with spindle activity in the medial prefrontal cortex (Siapas and Wilson, 1998). Ripples have been proposed to play a role in mediating cortico-hippocampal

communication (Ylinen et al., 1995; Buzsaki, 1996). Consequently, the finding that hippocampal ripples and prefrontal spindles are co-occurrent led the authors of this study to postulate that co-activation of hippocampal and prefrontal pathways may contribute to the reorganizing and consolidating of memory traces (Siapas and Wilson, 1998). It is noteworthy to mention here that spindles are of thalamic origin (Contreras and Steriade, 1996). Spindles, ripples and theta oscillations will be described in *Section 1.3.*

To summarize, both the hippocampus and prefrontal cortex play well-recognized roles in memory function. In particular, these structures are known to interact in the process of memory consolidation. How they interact, however, remains unclear. Anatomical studies have revealed that ventral hippocampal and ventral/intermediary proximal subicular fibers project profusely to the infralimblic and prelimbic cortices via the fimbria/fornix where they establish excitatory contacts on pyramidal cells and, to a lesser extent, local circuit interneurons. Generally, the deep layers V/VI seem to be the preferential target of these contacts. In spite of marked hippocampal input to the medial prefrontal cortex, the latter does not reciprocate connectivity. Electrophysiological investigation has provided insight in the processing of hippocampal input in the medial prefrontal cortex. Most importantly, hippocampal stimulation produces complex responses that suggest both monosynaptic and polysynaptic mechanisms, the interplay of excitatory and inhibitory events and the involvement of pyramidal and non-pyramidal cells. Feedforward inhibition has been shown to be a viable mechanism in this pathway. The pathway has also been shown to exhibit various forms of synaptic plasticity. Coupled with the observed coordination of hippocampal ripples and prefrontal spindles during slow-wave sleep and medial prefrontal phase-locking to hippocampal theta, such findings start to narrow in on the physiological bases underlying memory consolidation.

1.2.2 Interactions of the prefrontal cortex and thalamus

The reuniens nucleus and prefrontal cortex are reciprocally connected. The anatomy and electrophysiology of the thalamocortical pathway will be considered first and will be followed by a discussion on cortico-thalamic connectivity.

Several thalamic nuclei project to the medial prefrontal cortex. Musil and Olson (1988) performed injections of retrograde tracers nuclear yellow and bisbenzimide in the medial prefrontal cortex of cats and reported that cell labeling was observed most prominently in the mediodorsal nucleus and ventral complex, but also in the lateroposterior nucleus and in so-called limbic-associated thalamic nuclei, including the parataenial, paraventricular, basal ventromedial and reuniens nuclei (Musil and Olson, 1988). More specifically, Van der Werf and colleagues (2002) analyzed 40 injections of anterograde tracers PHA-L and BDA to identify the efferents of the reuniens nucleus. They reported that in the cerebral cortex, terminal labeling was pronounced in layers Ia, V and VI of the prelimbic and infralimbic cortices. Labeled fibers were also seen in layers Ia, III and V of the medial orbital cortex and agranular insular cortex layers I, III and V. Sparse labeling was seen in the anterior cingulated and motor cortex; cingular labeling was continuous with that seen in the dorsal retrosplenial cortex, preferentially targeting layer I (Van der Werf et al., 2002). A more recent study by Hur and Zaborsky (2005) has specified that glutamatergic cells from the reuniens nucleus project to the medial prefrontal cortex. By combining retrograde fluorescent tracing (Fluorogold) with in situ hybridization, these authors demonstrated that ~90% of Vglut2-containing cells projecting to the medial prefrontal cortex originate in the thalamus. Most of these cells were found in the midline and intralaminar nuclei, including the reuniens nucleus (Hur and Zaborszky, 2005). In addition, tritiated D-aspartate in prefrontal cortex had previously been shown to produce dense labeling in the reuniens nucleus (Pirot et al., 1994). The majority of efferents from the reuniens nucleus supply cortical regions via the inferior thalamic peduncle, where they course dorsal to the anterior commissure, first passing by the medial fourth of the reticular nucleus where they give off collaterals (Van der Werf et al., 2002).

Electrophysiological stimulation of the midline and intralaminar nuclei were shown in the 1940s to cause recruiting effects, where 5-12 Hz stimulation of the midline thalamus can induce spindle-like activity in the cortical mantle (Morison and Dempsey, 1942). Interestingly, the reuniens nucleus was specifically shown to produce such effects in the sigmoidal gyrus (Spencer and Brookhart, 1960). In contrast with the hippocampo-prefrontal pathway, however, the electrophysiological actions of the reuniens nucleus on the prefrontal cortex have received little attention. A recent study by Di Prisco and Vertes (2006) established that stimulation of the dorsal and ventral midline thalamus (but not of a null zone in between) resulted in short latency, large amplitude evoked potential in the medial prefrontal cortex. The most prominent responses were observed in layer V of prelimbic and infralimbic cortices following stimulation of the rhomboid/reuniens nucleus (Di Prisco and Vertes, 2006). Note that this is the layer where the hippocampus also preferentially projects. Evoked responses were characterized by an initial positive deflection followed by a negative wave which was in turn followed by a late positive component. The mean latency of the negative event in prelimbic cortex, approximately 4.5 ms, was deemed to be indicative of monosynaptic effects. It was also shown that paired-pulse stimulation of the reuniens nucleus could induce facilitation both in infralimbic and prelimbic cortex, with an 83% increase in evoked potential amplitude in the former and a 75% increase in the later. The results and interpretation of the findings of this study are addressed in greater detail in *Section 3* of this memoir. Medial prefrontal cortex intracellular responses to stimulation of the reuniens nucleus have not been previously described.

The medial prefrontal cortex, and especially the infralimbic and prelimbic cortices, project densely to the reuniens nucleus, thereby reciprocating the thalamo-cortical connectivity (McKenna and Vertes, 2004; Vertes, 2002; Di Prisco and Vertes, 2006). In 2002, Robert Vertes published a report on medial prefrontal cortex projections to the thalamus. Using anterograde tracer Phaseolus vulgaris-leucoagglutinin, it was found that all four divisions of the medial prefrontal cortex, namely the infralimbic, prelimbic, anterior cingulate and medial agranular cortices project heavily to the reuniens nucleus of the thalamus. In particular, the infralimbic, prelimbic and anterior

cinglulate fibers terminate predominantly in the lateral region of the reuniens nucleus. On the other hand, medial agranular fibers terminate mostly in the medial reuniens (Vertes, 2002). Injections of retrograde label Fluorogold in the reuniens nucleus have also confirmed the existence of dense projections from the medial prefrontal cortex. In particular, it was shown that labelling was heaviest in layers 5 and 6 of medial agranular, prelimbic and infralimbic cortices (McKenna and Vertes, 2004). Much earlier, Room et al. (1985) had reported, in cat, that the reuniens nucleus was the major termination field of fibers arising from the infralimbic cortex (Room et al., 1985). There are no known electrophysiological reports pertaining to this prefrontofugal pathway.

In short, layers Ia, V, and VI of the prelimbic and infralimbic cortices and layers Ia, III and V of the agranular insular and medial orbital cortices are the main recipients of reuniens afferents, which arrive to their destination via the inferior thalamic peduncle. These afferents are, at least in part, glutamatergic. In turn, layers V and VI of the medial agranular, prelimbic and infralimbic cortices project back to the reuniens nucleus. Very limited electrophysiological work has investigated interactions of the reuniens nucleus and prefrontal cortex.

1.2.3 Interactions of the thalamus and hippocampus

The reuniens nucleus provides the major, "virtually sole" thalamic input to the hippocampus and parahippocampal structures (McKenna and Vertes, 2004; Vertes et al., 2007; Herkenham, 1978; Su H.S. and Bentivoglio, 1990; Wouterlood et al., 1990). Evidence that the reuniens nucleus projects to the hippocampal formation comes from studies done in rat (Bertram and Zhang, 1999; DollemanVanderWeel and Witter, 1996), cat (Yanagihara et al., 1987) and monkey (Price and Amaral, 1981). Other thalamic nuclei such as the parataenial (Wyss et al., 1979; Riley and Moore, 1981), lateral dorsal and rhomboid nuclei (Yanagihara et al., 1987) have also been occasionally shown to project to the hippocampal formation but to a much lesser extent. Yanagihara et al. (1985, 1987) first investigated the nature of thalamic projections to the hippocampal formation, subiculum and entorhinal areas in cat.

Using WGA-HRP both as an anterograde and retrograde tracer, it was found that cells from the entire rostrocaudal extent of the reuniens nucleus projected to the superficial layers of the hippocampus, subiculum and entorhinal areas (Yanagihara et al., 1987; Yanagihara et al., 1985). In particular, terminal labeling of anterogradely labeled reuniens nucleus efferents to the hippocampus were found in the stratum lacunosum-moleculare (Yanagihara et al., 1985) and terminal labeling of reuniens efferents were present in both the first and deepest layers of the restrosplenial areas and in the superficial layers of the subiculum (Yanagihara et al., 1987). Further, Yanagihara and colleagues reported that cortical projections of the reuniens nucleus appeared to exhibit topographical organization. Pre- and parasubiculum projecting fibers originated medially within the reuniens nucleus. The dorsolateral entorhinal area also received input from the medial reuniens nucleus while cells projecting to the ventrolateral and medial entorhinal areas were found in the central part of the reuniens. Lastly, cells projecting to the ventromedial entorhinal areas were found to be distributed in the lateral regions of the reuniens nucleus.

In rat, the reuniens nucleus has been shown to project to layers I and III-IV of the entorhinal cortex (Herkenham, 1978; Wouterlood et al., 1990; DollemanVanderWeel and Witter, 1996), the stratum lacunosum moleculare of CA1 (heavily so), where the terminal fibers overlap with the perforant path fibers from the entorhinal cortex (Herkenham, 1978; Wouterlood et al., 1990), and the molecular layers of the subiculum, pre-subiculum and parasubiculum (Herkenham, 1978; Baisden et al., 1979; Su H.S. and Bentivoglio, 1990; Wouterlood et al., 1990; DollemanVanderWeel and Witter, 1996). In cat, hippocampus-projecting reuniens neurons were found throughout the antero-posterior extent of the reuniens nucleus. They were found to be widely scattered anteriorly yet confined to the ventral half of the nucleus caudally. In rat, on the other hand, hippocampus-projecting cells have been found to be located in clusters in the dorsolateral part of the reuniens nucleus(Bokor et al., 2002).

Hippocampus-projecting fibers originating in the reuniens nucleus, both in rat (Wouterlood et al., 1990) and cat (Yanagihara et al., 1987), reach the hippocampal formation via the cingulate bundle. In cat, the fibers will either course dorsolaterally

along the internal medullary lamina into the stria terminalis or course rostrolaterally towards the ventral pallidum, diagonal band or septum. Fibers from both trajectories will then converge in the cingulate fasciculus and fornix at the splenium of the corpus callosum (Yanagihara et al., 1987). In rat, these fibers course mainly within the inferior thalamic peduncle before curving dorsally around the genu of the corpus callosum where they eventually head in the caudal direction via the cingulated fasciculus (Wouterlood et al., 1990).

Hippocampus-projecting cells of the reuniens nucleus are glutamatergic and target both spines and dendrites. Tritiated D-aspartate is selectively taken up retrogradelly by cells that use glutamate as a neurotransmitter. As such, Bokor et al. (2002) observed that injections of this tracer into the stratum lacunosum moleculare of the CA1 subfield resulted in a significant amount of (retrogradely) labeled cells in the reuniens nucleus. Similar results were obtained by Herkenham (Herkenham, 1978), who also made use of tritiated amino acids to reveal the nature of reuniens input. In 1990, ultrastructural studies by Wouterlood et al. further revealed that reuniens nucleus axons form exclusively asymmetrical (i.e. excitatory) contacts on spines (50%) and dendritic shafts (50%) of hippocampal cells, thus further corroborating evidence for a glutamatergic, excitatory thalamo-hippocampal projection. Taken together with evidence that reuniens fibers terminate in the stratum lacunosum-moleculare of CA1, these data suggest that reuniens afferents to the hippocampus contact the spinous apical dendrites of pyramidal cells and dendritic shafts of local (aspinous) interneurons (Dolleman-Van der Weel et al., 1997).

The specific question of hippocampal interneuron innervation by reuniens afferents was ultrastructurally addressed by Dolleman-Van der Weel and Witter in 2000. Reuniens axons were anterogradely labeled with biotin-conjugated dextran amine (BDA) while CA1 neurons were pre-embeded with a GABA immunolabel. Approximately 16% of the observed thalamo-hippocampal synapses were found to occur on the dendrites of GABA-immunolabeled cells. Thalamic input to the hippocampus via the reuniens nucleus thus partially influences hippocampal transmission via activation of local inhibitory interneurons (Dolleman-Van der Weel

and Witter, 2000). Specifically, the authors propose that the following interneurons are likely subject to reuniens innervation: (1) interneurons of the lacunosum-moleculare, (2) VIP-containing interneurons located at the radiatum/lacunosum-moleculare border, (3) radiatum interneurons containing GABA and CCK and/or VIP, (4) vertical oriens/alveus cells, (5) basket cells and chandelier cells of the stratum pyramidale.

Few studies have examined the physiological effects of the reuniens nucleus on the hippocampus. Two will be discussed here. The first is by Dolleman-Van der Weel et al. (1997) and the second, by Bertram and Zhang (1999).

Findings that evoked potentials in CA1 following reuniens stimulation and of current source density analysis strongly suggest a stratum lacunosum-moleculare sink and radiatum source configuration. Dolleman-Van der Weel et al. (1997) indeed reported that CA1 field response polarity reversed (following reuniens stimulation) at the border of the stratum lacunosum-moleculare and stratum radiatum. More convincingly, current source density analysis revealed a well defined sink at the lacunosum-moleculare level and a source in the stratum radiatum. The authors also reported paired-pulse facilitation of reuniens-evoked CA1 field potentials. Population spikes were, however, not observed in the stratum pyramidale indicating that input from the reuniens nucleus was unable to discharge pyramidal cells. Extracellularly recorded unit activity in strata oriens/alveus and distal radiatum, on the other hand, revealed reuniens-evoked spiking. These data suggest that reuniens input to the hippocampus may elicit (subthreshold) EPSPs in apical dendrites of pyramidal cells in the lacunosum-moleculare layer while putatively activating local interneurons of the strata oriens/alveus and distal radiatum. As discussed above, later work by this team demonstrated that axons from the reuniens nucleus indeed contact dendrites of local CA1 interneurons.

In 1999, Bertram and Zhang demonstrated that the reuniens nucleus provides a potent direct excitatory input to the CA1 region of the hippocampus and that this input induces different physiological effects on CA1 pyramidal cells than that provided by CA3 innervation. It was found that electrical stimulation of the midline thalamus

induced similar responses to those induced by contralateral CA3 stimulation but in contrast to the findings reported by Dolleman-Van der Weel (1997), well developed field EPSPs and large population spikes with monosynaptic latency were observed following thalamic stimulation. This was attributed to the use of different stimulating electrodes. Although responses induced by contralateral CA3 and reuniens nucleus stimulation had similar morphology in the pyramidal layer, these inputs exhibited different laminar response profiles and were deemed to have disparate physiological effects on CA1 population responses. For instance, thalamic inputs were able to induce augmenting responses whereas CA3 stimulation was not. Further, paired pulse facilitation was observed following thalamic stimulation whereas paired pulse depression was observed in the case of CA3 stimulation. It was shown that inducing LTP in the CA3-CA1 pathway did not affect the response to thalamic stimulation and thus, that the two stimulated pathways were indeed distinct. The authors mention that the relative importance of thalamic and contralateral hippocampal influence may vary according to state of arousal.

The reuniens nucleus, therefore, influences hippocampal activity in a dual fashion. First, it may induce (subthreshold) depolarization of CA1 pyramidal cells and second, it may activate interneurons of the stratum oriens/oriens.

In turn, the hippocampus projects back to the thalamus. The deep layers of the dorsal subiculum have been known for some time to project to the anteromedial and anteroventral thalamus via the internal capsule and postcommissural fornix (Swanson and Cowan, 1975; Meibach and Siegel, 1977). Herkenham further reported that the reuniens nucleus also receives massive input from the subiclum (Herkenham, 1978). In this study, HRP was injected in the reuniens nucleus. As this retrograde label is taken up by cells and axons of passage alike, the afferents of the nucleus could not be precisely identified. More recently, McKenna and Vertes set out to clearly identify the afferents of the reuniens nucleus by performing injections of retrograde tracer Fluorogold into the reuniens nucleus of rats. They reported that this midline nucleus receives pronounced input from hippocampal structures, namely from Ammon's horn, the pre-and postsubiculum, both the dorsal and ventral subiculum, and the

perirhinal and lateral entorhinal areas (McKenna and Vertes, 2004). There are no known electrophysiological studies pertaining to the hippocampo-reuniens pathway.

1.3 Spontaneous Network Activity in the Brain

The brain generates various rhythms. Oscillatory behavior is thought to have important implications for brain function and notably contributes to synchronizing activity across neural networks. This section provides a brief overview of spontaneous network activity in the brain with emphasis on oscillations of the thalamo-cortical and hippocampal systems.

It has been postulated that synaptic plasticity associated with thalamocortical rhythms could contribute to memory formation (Steriade and Timofeev, 2003) and several studies provide evidence for the claim that sleep is necessary to learning and memory (Rasch et al., 2007; Marshall et al., 2006; Huber et al., 2004; Stickgold et al., 2000; Qin et al., 1997). In particular, a recent study by Rasch and coworkers (2007) provided compelling evidence for the hypothesis that

> memory consolidation evolves from repeated covert reactivation of newly encoded hippocampal representations during slow wave sleep, which takes place in a synchronized dialogue between thalamocortical and hippocampal circuitry and which eventually leads to the transfer of the representations to neocortical regions for long-term storage (Rasch et al., 2007).

As discussed in section 1.2., spontaneous activity in the prefrontal cortex and hippocampus exhibit strong synchrony, both in sleeping and behaving animals. To reiterate briefly, it has been shown that a significant proportion of medial prefrontal cortex neurons are phase-locked to the hippocampal theta rhythm of freely behaving rats (Siapas et al., 2005) and that during slow wave sleep, hippocampal ripples are co-occurent with medial prefrontal spindles (Siapas and Wilson, 1998). This co-activation of hippocampal and prefrontal activities during slow wave sleep has been postulated to contribute to the reorganization and consolidation of memory traces (Siapas and Wilson, 1998). A description of theta, ripple and spindle oscillations will shortly follow but first, a discussion on sleep and the slow oscillation.

During sleep, the brain is far from being devoid of activity. Indeed, sleep is characterized by distinct oscillations which have been taken to reflect the default state of the brain (Buzsaki, 2006). In the thalamocortical system, several sleep rhythms have been described including sleep spindles (7-14 Hz), ultrahigh-frequency oscillations (300-600 Hz), delta waves (1-4 Hz), and the slow oscillation (0.5-1 Hz) (Steriade et al., 1993a; Steriade, 1997; Steriade and Amzica, 1998; Steriade, 2003; Steriade, 2006; Buzsaki, 2006).

The slow oscillation has a frequency of less than 1 Hz (generally 0.5-1 Hz) and dominates cortical activity during natural sleep and certain types of anesthesia such as ketamine/xylazine and urethane (Steriade et al., 1993c; Contreras and Steriade, 1995; Amzica and Steriade, 1998; Timofeev et al., 2000b; Steriade et al., 2001).

The slow oscillation was first described by Mircea Steriade and colleagues in a series of papers published in the *Journal of Neuroscience* in August of 1993 (Steriade et al., 1993c; Steriade et al., 1993b; Steriade et al., 1993d). This oscillation is characterized by alternating membrane depolarizations and hyperpolarizations of neocortical neurons which give rise to depth-negative and depth-positive waves, respectively, in the electroencephalogram (EEG). While it has been shown that the slow oscillation is of cortical origin (Steriade et al., 1993d; Amzica and Steriade, 1995; Timofeev and Steriade, 1996b; Timofeev et al., 2000a; Sanchez-Vives and Mccormick, 2000), the thalamus has been shown to be entrained by the cortex (Contreras and Steriade, 1995; Steriade et al., 1993b; Timofeev and Steriade, 1996a). Indeed, nucleus reticularis thalami and thalamocortical neurons have been shown to exhibit behavior similar to cortical neurons—that is, of alternating patterns of membrane depolarization and hyperpolarization associated with EEG depth-negativity and positivity (Contreras et al., 1996; Timofeev and Bazhenov, 2005).

The slow oscillation has been reported to occur in the prefrontal cortex and indeed to have a greater tendency to originate in this region of the brain (Massimini et al., 2004; Gao et al., 2007).

The slow oscillation regroups other sleep rhythms such as spindles (Amzica and Steriade, 1998; Contreras and Steriade, 1995). The latter consist of waxing-and-waning field potentials of 7 to 14 Hz usually lasting 1-4 seconds and recurring at 5-15 second intervals (Contreras and Steriade, 1996; Timofeev and Bazhenov, 2005). Spindle oscillations are usually observed during early sleep and during the active (depolarized) phases of the slow oscillation. Studies have demonstrated that this rhythm is of thalamic origin (Contreras and Steriade, 1996; Timofeev and Steriade, 1996a) and more precisely, that it results from the interaction of the thalamic reticular nucleus and thalamocortical cells (Steriade et al., 1985; Deschenes et al., 1985; Steriade and Llinas, 1988; von Krosigk et al., 1993).

It has been proposed that spindles may contribute to memory consolidation in sleep (Steriade and Timofeev, 2003; Siapas and Wilson, 1998). Increases in spindle density following learning have been correlated with better recall on declarative memory tasks (Gais et al., 2002) and spindle oscillations have been shown to mediate short-term synaptic plasticity (Steriade and Timofeev, 2003). The augmenting response allows for the study of spindle-associated plasticity and consists in the increased magnitude of evoked potentials (or intracellular responses) to successive stimuli applied at 7-14 Hz (spindle frequency band) (Morison and Dempsey, 1943). There are thalamic and cortical components to the augmenting response (Steriade et al., 1998c; Timofeev and Steriade, 1998; Timofeev et al., 2002; Steriade and Timofeev, 2003; Timofeev and Bazhenov, 2005). In the *Results* section of this thesis, such responses are described in medial prefrontal neurons following stimulation of the reuniens nucleus of the thalamus.

Ripples are fast oscillations with frequencies exceeding 100 Hz and have been proposed to play a role in mediating cortico-hippocampal communication (Ylinen et al., 1995; Buzsaki, 1996). They were first described in the CA1 subfield of the hippocampus and in the perirhinal cortex under conditions of behavioral immobility, anesthesia or natural sleep (Ylinen et al., 1995; Chrobak and Buzsaki, 1996; Collins et al., 1999; Csicsvari et al., 1999). Subsequently, ripple activity has also been observed in the neocortex (Kandel and Buzsaki, 1997; Jones and Barth, 1999; Jones

et al., 2000; Grenier et al., 2001). Under pathological conditions, these oscillations are often correlated with seizure onset (Allen et al., 1992; Fisher et al., 1992; Steriade et al., 1998a; Grenier et al., 2003).

Finally, the theta oscillation is a local field potential oscillation of 4-10 Hz. The theta rhythm constitutes a "defining electrophysiological signature" of the hippocampus in exploratory behaviour and REM sleep (Buzsaki, 2006; Green and Arduini, 1954; Vanderwolf, 1969; Buzsaki, 2002). It is known to modulate activity in hippocampal (Buzsaki and Eidelberg, 1983; Fox et al., 1986), subcortical (Vinogradova and Brazhnik, 1977; Kocsis and Vertes, 1992; Pedemonte et al., 1996; Natsume et al., 1999; Gambini et al., 2002), limbic (Alonso and Garcia-Austt, 1987; Pare and Gaudreau, 1996), and cortical (Colom et al., 1988) structures. In turn, the supramammillary nucleus and posterior nucleus of the hypothalamus are known to modulate hippocampal theta and project heavily to the reuniens nucleus (Vertes, 1992; Vertes et al., 1995; McKenna and Vertes, 2004; Vertes and Kocsis, 1997). As such, it has been proposed that the reuniens may play a role in theta control at CA1 (McKenna and Vertes, 2004). Theta oscillations have also been recorded in the prefrontal cortex ("frontal theta") and have been correlated with attention (Delorme et al., 2007; Schutter and van Honk, 2006; Demiralp et al., 1994).

2 Specific Aims

Interactions of the hippocampus and prefrontal cortex have been shown to be important in mediating cognitive functions such as memory. How these two structures interact remains obscure, however, especially in light of the fact that the prefrontal cortex does not reciprocate hippocampo-prefrontal projections. The reuniens nucleus has been proposed to serve as an interface between the hippocampus and prefrontal cortex because it is reciprocally connected to both. If the reuniens nucleus serves as an interface between the hippocampus and prefrontal cortex, then it should exert physiological actions on these structures. At present, electrophysiological investigations pertaining to the reuniens-prefrontal pathway have strictly examined evoked potentials in medial prefrontal cortex fields following stimulation of the reuniens nucleus. No intracellular data is available. Thus:

- The ***first aim*** of this study was to provide electrophysiological evidence corroborating the claim that the hippocampus, prefrontal cortex and reuniens nucleus constitute a functional network by characterizing intracellular responses of medial prefrontal cortex neurons, *in vivo,* to stimulation of the reuniens nucleus.

- A ***second aim*** consisted in comparing this response with that elicited by hippocampal stimulation.

Intracellular recordings of responses *in vivo* allow for a holistic and highly physiological approach to the study of network interactions by taking into account the impact of synaptic bombardment and modulatory influences to which cells are subjected to in intact networks. The data should contribute to further elucidating mechanisms of hippocampo-prefrontal interactions and ultimately help to better understand the physiological bases of cognition.

3 Reuniens nucleus and hippocampus exert distinct synaptic actions on medial prefrontal cortex neurons: An in vivo study in the cat

3.1 Abstract

Interactions of the hippocampus and prefrontal cortex are vital to cognition. The reuniens nucleus of the midline nucleus has been proposed to serve as an interface between these two structures. We provide an intracellular description of the synaptic influence of the reuniens nucleus on medial prefrontal cortex neurons of cats under ketamine/xylazine anesthesia and contrast this influence with that of the hippocampus. Thalamic and hippocampal stimulation evoked complex responses consisting of an initial excitatory component followed by a long lasting hyperpolarization. Reuniens stimulation elicited responses with significantly shorter latencies than hippocampal stimulation. We found antidromic responses to thalamic stimulation to occur in a restricted locus of the medial prefrontal cortex that overlaps with the site of hippocampal projections, providing electrophysiological evidence for a hippocampo-prefronto-thalamic relay. We further report that the pathway linking the reuniens nucleus to the prefrontal cortex is subject to short-term plasticity.

3.2 Introduction

Interactions of the hippocampus and prefrontal cortex are vital to cognition. In particular, their involvement in memory function has been well documented (Squire, 1992; Kim et al., 1995; Floresco et al., 1997; Fletcher and Henson, 2001; Wiltgen et al., 2004; Frankland and Bontempi, 2005; Teixeira et al., 2006). Much remains to be elucidated with respect to *how* these two structures interact.

The reuniens nucleus has been proposed to serve as an interface between the hippocampus and prefrontal cortex because it is reciprocally connected to both of these structures (Vertes et al., 2007; Vertes et al., 2006; Hur and Zaborszky, 2005; McKenna and Vertes, 2004; Van der Werf et al., 2002; Bokor et al., 2002; Wouterlood et al., 1990; Musil and Olson, 1988). This is of particular interest because

hippocampo-prefrontal connectivity is unidirectional, with the prefrontal cortex failing to return projections to the hippocampus (Beckstead, 1979; Goldman-Rakic et al., 1984; Room et al., 1985; Reep et al., 1987; Takagishi and Chiba, 1991).

Implicit to the claim that the reuniens nucleus may serve as an interface between the hippocampus and prefrontal cortex is that it exerts physiological influences on both of these structures. Electrophysiological investigation of these interactions remains sparse. Previous studies have shown that the reuniens nucleus exerts excitatory actions at the CA1 (Bertram and Zhang, 1999; Dolleman-Van der Weel et al., 1997; Dolleman-Van der Weel and Witter, 2000). In the medial prefrontal cortex, influences of the reuniens nucleus remain poorly characterized as only field potential data is available (Di Prisco and Vertes, 2006). The present paper aims to further elucidate the nature of this influence by providing evidence from intracellular recordings, *in vivo*, of medial prefrontal cortex neurons of anesthetized cats.

We have found that: 1) responses to reuniens stimulation consist in a depolarizing event followed by a long hyperpolarization; 2) a significant proportion of medial prefrontal cortex neurons respond to stimulation of the reuniens nucleus orthodromically, antidromically or orthodromically and antidromically; 3) response latency to reuniens stimulation is significantly shorter than that to hippocampal stimulation; 4) a restricted locus of medial prefrontal cortex mediates the hippocampo-prefronto-reuniens relay and 5) the reuniens-prefrontal pathway is subject to short-term plasticity. We conclude that the reuniens nucleus exerts powerful actions on medial prefrontal cortex neurons.

3.3 Materials and Methods

All experimental procedures used in this study were performed in accordance with the Canadian guidelines for animal care and were approved by the committee for animal care of Laval University.

3.3.1 Preparation

Adult cats (n=20) were anesthetized with ketamine and xylazine (10-15 mg/kg and 2-3 mg/kg IM). Pressure points were infiltrated with lidocaine (2%). When the electroencephalogram (EEG) showed low-frequency, high-amplitude oscillations as in resting sleep, the animals were paralyzed with gallamine triethiodide and artificially ventilated by maintaining the end-tidal CO_2 concentration at 3.5-3.8%. The EEG was monitored continuously and additional doses of anesthetics were administered at the slightest tendency toward an activated pattern. Body temperature was maintained at 37-39 °C.

3.3.2 Recording and Stimulation

Intracellular recording of medial prefrontal cortex neurons were performed with micropipettes filled with a solution of 3 M potassium acetate (DC resistances of 30-60 MΩ). Recordings of mPFC were performed anterior, or slightly posterior, to the cruciate sulcus. The stability of intracellular recordings was ensured by cisternal drainage, hip suspension, and bilateral pneumothorax and by covering the decorticated hemisphere with a warm agar solution (4% in 0.9% saline). A high-impedance amplifier with active bridge circuitry was used to record from and inject current into neurons. Thalamic (H+1 L0 A+12) and hippocampal (H-4.5 L10.5 A+7.2) field potentials were recorded through bipolar (coaxial) electrodes, also used for stimulation. Stimuli (0.2 ms, 0.3-1.5 mA) were delivered at 1 Hz and in trains of 5 pulses delivered every 2 seconds at 5 Hz, 10 Hz, 20 Hz and 100 Hz. Signals were recorded with a bandpass of 0.1 Hz-10 KHz, and digitized at 20 KHz for offline computer analysis. A total of 50 neurons were recorded in 11 animals. Neurons that were held long enough—that is, beyond the duration of the stimulation protocol—were given current pulses (300 ms, 0.5-1.25 nA) in order to characterize their firing discharge pattern.

Arrays of 4 tungsten microelectrodes (9-12 MΩ) were positioned in the medial prefrontal cortex of 9 animals to study the antero-posterior and dorso-ventral response profiles to thalamic and/or hippocampal stimulation. These arrays were positioned as

close to the midline as possible in pericruciate gyrus sigmoidus and lowered manually in 1 mm steps. The stimulation protocol was the same as that applied during intracellular recordings.

Antidromic events were tested for in accordance with previously described methods (Lipski, 1981).

3.3.3 Histology

At the end of experiments, electrolytic lesions were performed by delivering 0.75 mA, 1 s current pulses every 3 s for 5 minutes in order to confirm stimulation sites. Animals were subsequently perfused intracardially, first with physiological saline (0.9%), followed by 4% paraformaldehyde solution for fixation and 10% sucrose dissolved in 4% paraformaldehyde for cryoprotection. Brains were then placed in 20% and 30% sucrose solutions over a total of approximately one week, for further cryoprotective purposes. Following this procedure, brains were sectioned (100 μm thickness) by use of a freezing microtome. Sections were mounted on gelatinized glass slides, dried, and subsequently stained for Nissl bodies with Cresyl Violet according to standard staining procedures.

3.3.4 Analysis

Analysis was performed offline with Igor Pro 4.0. software by WaveMetrics (Lake Oswego, OR). Stimuli were detected manually and minimal binomial smoothing was performed to diminish noise on occasion. The latency of orthodromic responses was calculated as the time when the response reached 10% of the maximal amplitude of the sigmoidal fit of the rise of the EPSP. The amplitude of the EPSP was calculated as the maximum of this sigmoidal fit, and the slope of the EPSP calculated as the maximum of the differentiation of this fit. A horizontal line was drawn across the response from the base of the early EPSP and the interception of this line by the hyperpolarizing potential was used to calculate the duration of this latter component. Responses to sweeps of five pulse stimulation trains were averaged with an IgorPro algorithm (N≈30 per stimulation protocol). The EPSP response magnitude elicited by

the stimulation of the reuniens nucleus was computed by computing the area below the curve. Average response areas evoked by pulses 2 through 5 of the train were subsequently normalized by dividing by the response evoked by the first pulse. Data were collected in 7 cells (5 Hz, 10 Hz) and 4 cells (20 Hz). Responses to hippocampal trains of stimulation were not quantified because stimuli did not evoke responses reliably within trains (figure 3).

3.3.5 Statistics

Statistical analyses were performed with JMP 5.01 (a SAS software, Cary, NC). The experimental design was that of a mixed model with repeated measures. We replicated measurements in all recorded cells, which by virtue of the methodology were randomly sampled. To assess whether reuniens stimulation and hippocampal stimulation evoked distinct responses in neurons of the medial prefrontal cortex, we performed *t*-tests for each of the following parameters: latency of response, slope of EPSP, amplitude of EPSP and duration of hyperpolarization. For each group (hippocampal stimulation and reuniens stimulation), repeated measurements were pooled from 6 cells, with 8 to 10 replications per cell. Within cell analysis was carried out for one cell that responded to both hippocampal and thalamic stimulation. Outliers were excluded in calculation of the means when indicated. They were defined as values exceeding the first and third quartiles by 1.5 times the interquartile difference. Normality was assessed by normal quantile plots and Shapiro-Wilk tests. Homogeneity of variance was assessed by Levene's test. Confidence intervals were set at 95% ($p<0.05$).

The effect of trains of stimulation delivered in the reuniens nucleus was assessed with a standard least squares model ($R=0.82$, $N=60$). We quantified the difference in amplitude of response elicited by different pulse number and frequencies of stimulation, and the interaction between these two parameters. ANOVA was used when data was normally distributed and variance homogeneous, Welch ANOVA when variance was non-homogeneous. Failing normality, such differences were assessed by non-parametric testing. Post-hoc analyses were used to identify

differences between groups. The Tukey HSD test was used where the data were normally distributed. When the data was non-normally distributed, we used the ranked scores. Two-tailed *t*-tests or Wilcoxon signed rank tests were used to compare group means with null hypothesis of 1 (because responses were normalized).

3.4 Results

3.4.1 Electrophysiological Cell Types

The data set used to describe the recorded cells represents neurons of the pericruciate medial prefrontal cortex. The slow oscillation was observed in all recorded neurons. A total of 33 cells from 11 animals were characterized in terms of their discharge pattern and classified according to electrophysiological criteria (Figure 1A), which are well documented in the literature (Connors and Gutnick, 1990; Gray and Mccormick, 1996; Steriade et al., 2001; Contreras, 2004). They were also retained for analysis due to the stability and quality of recording; in particular, they possessed action potentials overshooting 0 mV, at minimum, and in most cases, 15 mV. Twenty-two of these were regular-spiking (RS, 67%), of which 5 were fast-adapting (FA). The remaining cells displayed intrinsically bursting (IB, 21%) and fast-rhythmic bursting (FRB, 12%) behavior. We did not record any fast-spiking cells. The distribution of cell types with respect to depth of recording (from the cortical surface) is shown in figure 1B.

3.4.2 Intracellular Responses to Single Pulse Stimulation of the Reuniens Nucleus and Hippocampus

Intracellular recordings of 29 neurons in the medial prefrontal cortex of 10 cats were obtained whilst delivering single pulse stimulation to the reuniens nucleus. Of these, 38% (11/29) responded to the stimulation either orthodromically (8/11, or 73%, figure 2A) antidromically (2/11, figure 2B) or both orthodromically and antidromically (1/11, figure 2C). Orthodromic responses were characterized by an early depolarizing event followed by a long-lasting hyperpolarizing component, the latter often followed by a rebound that was in turn occasionally crowned by spikes

(figure 2). The early depolarization was sometimes followed by a second (data not shown). Of the 8 responding with a strictly orthodromic response, 6 were analyzed. We quantified the latencies of the early and late EPSPs, the amplitude and slope of the former and the duration of the hyperpolarizing event. Intracellular recordings of 15 neurons in the mPFC were obtained whilst delivering single pulse stimulation to the ventral CA1/subiculum region of the hippocampus. Six of these elicited responses, or 40%. The morphology of responses elicited by hippocampal stimulation was similar to that elicited by thalamic stimulation, allowing us to quantify the similarities and differences between the two responses. One notable finding was that stimulation of the ventral hippocampus led to a high variability of responses. In a single responding cell, some stimuli elicited no response at all while others evoked the above described EPSP-IPSP sequence; still others elicited both early and late EPSPs followed by a long-lasting hyperpolarization. The evoked patterns of responses were therefore variable yet repeatable. This was also observed during trains of stimulation (Figure 3). We also observed a reversal of the response around resting membrane potential with a hyperpolarization of the response at more depolarized membrane potentials and a depolarization of the response at more hyperpolarized membrane potentials (Figure 3A, right).

Response latency to stimulation of the reuniens nucleus was significantly shorter than that to stimulation of the hippocampus ($p<0.0001$) in neurons of the medial prefrontal cortex. The average response latency (± standard deviation) to thalamic stimulation was 6.05±3.6 ms (exluding 3 outliers), the median latency 5.3 ms, the minimum latency 1.8 ms and the maximum latency 36 ms (N=57). Mean response latency to single pulse stimulation of the hippocampus was 9.95±4.6 ms, median latency, 9.1 ms, maximum latency 20 ms and minimum latency 1.2 ms. Response amplitude in medial prefrontal cortex neurons was significantly larger following stimulation of the hippocampus (8.4±4.1 mV, N=60) than it is following stimulation of the reuniens nucleus (3.1±2.0 mV, N=55) ($p<0.0001$). The slopes of the rising EPSP were significantly different following hippocampal and reuniens stimulation ($p<0.0001$). The rise of the EPSP due to thalamic stimulation was of 0.56±0.27 V/s (N=55) and that due to hippocampal stimulation was of 1.9±0.9 V/s (N=60). The means of the

duration of hyperpolarization events evoked by reuniens and hippocampal stimulation were not significantly different ($p>0.2$). Responses to thalamic stimulation were normally distributed with a mean and standard deviation of 254±108 ms (n=57). Responses to hippocampal stimulation were also normally distributed with mean and standard deviation of 278±94 ms (n=60). Response histograms are shown in figure 4.

One cell out of 19 (5.3%) responded to both hippocampal and thalamic stimulation. This cell was recorded approximately 5200 μm below the cortical surface. Within cell analysis revealed that response latency, EPSP amplitude, EPSP slope and hyperpolarization duration of responses were significantly different when evoked by reuniens stimulation compared to those evoked by hippocampal stimulation. See Figure 5.

Antidromic responses were recorded 6050-6600 microns below the surface in three different animals; the cell responding to reuniens stimulation both antidromically and orthodromically was recorded 6050 μm below the surface (figure 6B). The nature of antidromic responses was confirmed with collision tests (e.g., figure 6C). The average latency of antidromic spikes was 2.9±0.25 ms (mean±standard error). Within cells the mean antidromic latency and standard deviation were 4.5±0.51 ms, 3.7±0.14 ms and 0.90±0.07 ms. We further observed putative antidromic events in field recordings of the medial prefrontal cortex following stimulation of the reuniens nucleus. These events were depth negative and had both a short latency and a fast rise. They were found to occur in a restricted locus of the prefrontal cortex (figure 6A) where ventral hippocampus stimulation induced large amplitude responses (Figure 6A).

3.4.3 Intracellular Responses to Trains of Stimulation

Nine neurons recorded in 6 cats were used to investigate prefrontal cortex intracellular response to 5 Hz stimulation of the reuniens nucleus, 9 neurons recorded in 6 cats to investigate response to 10 Hz stimulation and 4 neurons in 3 cats to investigate responses to 20 Hz stimulation. Fifteen neurons recorded in 7 animals were used to investigate prefrontal cortex intracellular response to single pulse

stimulation of the hippocampus and 4 cells in 2 animals to investigate prefrontal response to hippocampal trains of stimulation.

There was a significant difference in the average normalized response area (V·s) elicited by different frequencies of stimulation ($p<0.0001$). The least square mean of normalized average responses elicited by 5 Hz stimulation was 1.48 V·s (N=6), that of responses elicited by 10 Hz stimulation was 1.89 V*s (N=6) and that of responses elicited by 20 Hz stimulation was 0.97 V·s (N=4). Within any frequency level, there were no differences in response amplitude between responses elicited by pulses 2 through 5 of the stimulation train although there appeared to be an incremental trend within 10 Hz stimulation trains. Responses to pulses 2 through 5 of 10 Hz stimulation trains were significantly greater than those elicited by pulse 1 by up to 120%. There was also a slight decreasing trend in response magnitude at 20 Hz (figure 7).

In certain cells we observed augmenting responses following 10 Hz stimulation. An example of such a response is shown in figure 8. Two components of the depolarizing event could be distinguished. The primary component decreased in amplitude from pulse 1 to pulse 2 while the magnitude of the secondary component of the depolarization underwent a marked increase from pulses 1 through 4. Such phenomena have been previously described in other thalamocortical systems (Steriade et al., 1998c). Note that at 20 Hz, there was synaptic depression of the response and that at 5 Hz, only stimuli falling during the hyperpolarizing phase could elicit increases in response magnitude.

Four cells produced clear responses to 5 Hz and 10 Hz stimulation of the ventral hippocampus. Responses were not as reliable as those evoked by stimulation of the reuniens nucleus. Some trains produced no responses at all while in other trains, only 1 response was evoked. A few trains elicited 2 or more responses, sometimes consecutively. Similar patterns of evoked responses within the trains were reproducible. See figure 3.

3.5 Discussion

We have provided a description of intracellular responses to reuniens stimulation in neurons of the medial prefrontal cortex of ketamine/xylazine anesthetized cats and have found that the reuniens nucleus and the ventral hippocampus exert distinct actions on neurons of the medial prefrontal cortex. We found antidromic responses to thalamic stimulation to occur in a restricted locus of the medial prefrontal cortex that overlaps with the site of hippocampal projections. Finally, we reported that the pathway linking the reuniens nucleus to the prefrontal cortex is subject to short-term plasticity.

With the exception of one study by Di Prisco and Vertes (2006), who examined the extracellular effects of midline thalamus stimulation on medial prefrontal cortex, no previous study has examined the physiological influence of the reuniens nucleus on prefrontal cortex. Thus, intracellular medial prefrontal cortex responses to reuniens stimulation were described here for the first time and as recordings were carried out *in vivo,* these neuronal responses reflect the impact of synaptic bombardment and modulatory influences to which cells are subjected to in intact networks (Steriade et al., 2001).

Our intracellular data reflects the behavior of medial prefrontal cortex neurons responding to stimulation of the reuniens and/or hippocampus. We recorded regular-spiking (RS), fast-adapting (FA), intrinsically bursting (IB) and fast-rhythmic bursting (FRB) cells (Figure 1). Twenty-one percent of the characterized cells displayed IB firing patterns and made up the greatest proportion of cells recorded in the depth of the mPFC (Figure 1). A further 12% of recorded cells exhibited FRB behavior. To our knowledge, this cell type has not previously been reported to occur in prefrontal cortex. We encountered fewer RS cells than reported in previous studies (Degenetais et al., 2002; Nunez et al., 1993) but a high population of bursting cells. This could be of particular interest in light of the findings that the slow oscillation most often originates in the frontal lobes (Massimini et al., 2004) and that intrinsically bursting cells tend to fire at the onset of the active state of the slow oscillation (Volgushev et al., 2006). It is also significant in light of reports asserting

the importance of bursting activity for information processing in the brain reward circuit, including the prefrontal cortex (Cooper, 2002). The recorded electrophysiological cell types generally correspond to pyramidal cell morphology although exceptions have been known to occur (Kawaguchi and Kubota, 1997; Kawaguchi and Kubota, 1998; Steriade, 2004).

We did not record any fast-spiking cells, which correspond almost exclusively to interneuron morphology (Cauli et al., 1997; Kawaguchi and Kubota, 1998; Contreras, 2004). To our knowledge, intracellular recordings of prefrontal cortex interneurons have not been carried out *in vivo*. Extracellular recordings have however been performed and most electrophysiological studies of prefrontal cells have been carried out in the slice preparation (Tierney et al., 2004; Krimer and Goldman-Rakic, 2001; Yang et al., 1996; de la Pena and Geijo-Barrientos, 1996). Parvalbumin positive interneurons of basket and chandelier morphology only make up approximately 6% of neurons of the prefrontal cortex (Gabbott et al., 1997) and are typically smaller than pyramidals. They are therefore arguably more difficult to encounter by blind intracellular impalements but have been recorded in this laboratory in associative cortical areas (Volgushev et al., 2006).

With respect to intracellular responses elicited by hippocampal stimulation, our observations are, overall, in line with previous studies (Degenetais et al., 2003; Thierry et al., 2000; Tierney et al., 2004; Laroche et al., 2000; Gigg et al., 1994; Laroche et al., 1990; Ferino et al., 1987). We observed a response rate of 40%, which corroborates previous findings where a 42% response rate in the prelimbic cortex of rats was reported in one study (Laroche et al., 1990) and where a 32% response rate was observed in the prefrontal cortex of rats in yet another study (Gigg et al., 1994). These results are to be contrasted, however, with those of Dégenètais and colleagues (2002) who reported that 91% of intracellularly recorded cells in the prelimbic and medial orbital cortices of rats were responsive to hippocampal stimulation. This difference is probably due to the fact that we did not limit our sampling to the prelimbic and medial orbital cortices, nor did Laroche et al. (1990) or Gigg and colleagues (1994).

The latency of responses to hippocampal stimulation were shorter than previously reported (Ferino et al., 1987; Degenetais et al., 2003; Thierry et al., 2000). This likely reflects species specificity. The mean latency of responses to hippocampal stimulation was of approximately 9 ms while in rat it has been reported that the conduction delay of the hippocampo-prefrontal pathway is slow, resulting in response latencies of 18 ms (Ferino et al., 1987; Laroche et al., 1990; Degenetais et al., 2003). The conduction velocity of the hippocampo-prefrontal pathway in cat remains to be tested.

Evidence suggests that both pyramidal cells and inhibitory neurons mediate the hippocampo-prefrontal response. In cells of the prelimbic and medial orbital cortices, the early component of the response is thought to be monosynaptic and primarily mediated by AMPA receptors (Degenetais et al., 2003; Jay et al., 1992). The subsequent prolonged IPSP is mediated, at least in part, by $GABA_A$ and $GABA_B$ conductances (Degenetais et al., 2003). A role for disfacilitation has not been ruled out. Interneurons have been shown to respond to hippocampal stimulation similarly to pyramidal cells, although response latency in interneurons is shorter than that observed in pyramidal cells, which suggests that the hippocampus is able to exert feedforward inhibition in the medial prefrontal cortex (Tierney et al., 2004). Responses in the mediodorsal thalamo-prefrontal pathway have been shown to be mediated by similar mechanisms (Gigg et al., 1992). Projections from the reuniens nucleus are glutamatergic (Hur and Zaborszky, 2005; Pirot et al., 1995). The primary response to reuniens stimulation in medial prefrontal cells is likely also mediated by AMPA receptors and the hyperpolarizing component by GABA inhibition but this remains to be tested.

It has been reported that there is a large variability in response latency in the hippocampo-prefrontal pathway (Degenetais et al., 2003) which has been taken to emphasize that both monosynaptic and polysynaptic mechanisms are involved in mediating the prefrontal response to hippocampal stimulation (Siapas et al., 2005). In the present study we further observed variability in response patterns. Patterns were however repeatable, suggesting that different yet recurring network states may

modulate prefrontal responses to hippocampal stimulation. For example, we observed that responding cells may not respond at all within a stimulation train that otherwise evokes responses (Figures 3 and 5). This may reflect the role of shunting/feedforward inhibition via the activation of local inhibitory cells, or of cholinergic, monoaminergic or thalamic gating mechanisms (Gigg et al., 1994; Williams and Goldman-Rakic, 1995; Floresco and Grace, 2003; Gioanni et al., 1999; Robbins, 2005; Mansvelder et al., 2006). The precise positioning of the stimulation electrode along the antero-posterior axis of the hippocampus may also play a decisive role in shaping responses seen in medial prefrontal neurons. In sum, hippocampal stimulation produces complex responses that suggest both monosynaptic and polysynaptic mechanisms, the interplay of excitatory and inhibitory events and the involvement of pyramidal and non-pyramidal cells

In contrast with the hippocampo-prefrontal pathway the electrophysiological actions of the reuniens nucleus on the prefrontal cortex have received little attention. The mean response latency in prefrontal neurons to reuniens stimulation was 6 ms (median 5 ms). The latency of antidromic spikes was approximately 3 ms. Taken together, these data suggest that the early excitatory potentials observed following reuniens stimulation were monosynaptic. These findings confirm those of Di Prisco and Vertes (2006) who observed prominent evoked potentials in prelimbic and infralimbic cortices with putatively monosynaptic latencies of 4.5 ms following stimulation of the reuniens/rhomboid nucleus (Di Prisco and Vertes, 2006).

We also observed synaptic plasticity of responses following stimulation of the reuniens nucleus. The most significant change was observed for interstimulus intervals of 100 ms, or 10 Hz trains. Paired-pulse facilitation of medial prefrontal evoked potentials following reuniens nucleus stimulation has previously been observed with this same interstimulus interval. Prior work has shown that paired-pulse stimulation of the reuniens nucleus induces 83% and 75% increases in response magnitude in the infralimbic and prelimbic cortices, respectively. In contrast, changes in the medial orbital cortex are less, at 22% (Di Prisco and Vertes, 2006). In line with these previously published observations, we observed at 10 Hz stimulation a 66%

increase, on average, from pulse 1 to pulse 2 (N=6). Our slightly lower increase in response magnitude from pulse 1 to pulse 2 may thus be due to the inclusion of cells recorded dorsal to the prelimbic cortex. Paired-pulse facilitation has also been observed in medial prefrontal cortex following the stimulation of other afferents such as the hippocampus and anterior and intralaminar thalamic nuclei (Christoffersen et al., 2003; Izaki et al., 2003; Jay et al., 1995; Kung and Shyu, 2002).

We observed augmenting responses in certain cells following 10 Hz stimulation. In contrast, 20 Hertz stimulation of the same pathway led to synaptic depression and 5 Hz stimulation was only efficient in increasing responses when stimuli were coincident with non-activated epochs. We hypothesize that, in line with previous reports of augmenting responses in thalamocortical systems (Steriade et al., 1998c), the observed augmenting response is due to intrinsic properties of thalamocortical cells. Further studies are required to investigate this possibility.

There was also an incremental trend in response magnitude elicited by subsequent pulses, suggesting that the reuniens-prefrontal pathway is not only subject to paired-pulse facilitation but also to augmenting, in like manner to other thalamo-cortical pathways (Timofeev and Steriade, 1998; Bazhenov et al., 1998). Augmenting responses are physiologically relevant manifestations of short-term plasticity which ultimately could contribute to the consolidation of memory traces during sleep (Timofeev et al., 2002; Steriade and Timofeev, 2003).

Due to its reciprocal connections with the prefrontal cortex and hippocampus, the reuniens nucleus has been proposed to serve as an interface between these two structures, whose interactions are known to play a key role in memory processing (Vertes, 2002; Vertes, 2006; Vertes et al., 2007; Vertes et al., 2006; McKenna and Vertes, 2004; Bontempi et al., 1999; Frankland et al., 2001; Maviel et al., 2004; Takehara et al., 2003; Floresco et al., 1997; Van der Werf et al., 2002; Musil and Olson, 1988; Hur and Zaborszky, 2005; Wouterlood et al., 1990). As stated by Di Prisco and Vertes, the reuniens nucleus is "pivotally positioned to synchronize the activity of these two important forebrain structures (Di Prisco and Vertes, 2006)". Demonstrations that the nucleus reuniens exerts excitatory actions at the CA1 and

also at the prefrontal cortex (intracellularly confirmed and described in this study) provides supporting evidence for this claim (Bertram and Zhang, 1999; Dolleman-Van der Weel et al., 1997; Di Prisco and Vertes, 2006). We have also found that strong responses to hippocampal stimulation were evoked in cortical areas coincident with the recording site of antidromic events following reuniens stimulation. Such findings provide electrophysiological evidence for a prefrontal locus mediating a hippocampo-prefronto-thalamic relay and, together with recent ultrastructural evidence demonstrating that prefrontal cortex cells project onto hippocampus-projecting cells of the reuniens nucleus (Vertes et al., 2007) supports the calim that the reuniens nucleus, hippocampus and prefrontal cortex together constitute a functional loop.

We have further shown that at least three populations of putatively pyramidal cells of the medial prefrontal cortex mediate responses to hippocampal and reuniens stimulation: (1) cells which strictly respond to hippocampal stimulation; (2) cells which respond to reuniens stimulation either orthodromically, antidromically or both orthodromically and antidromically and (3) cells that respond to both hippocampal and reuniens stimulation. The latter subpopulation is presumably the smallest, with only 1 cell out of 19 (5.3%) exhibited this behavior. Such cells could have considerable importance in integrating limbic information from the reuniens nucleus with hippocampal outputs to the prefrontal cortex which is of particular interest in light of the fact that memory consolidation is strongly influenced by emotion (McGaugh, 2000).

In conclusion, the reuniens nucleus elicits complex synaptic responses in neurons of the medial prefrontal cortex. This provides corroborating evidence for the claim that the reuniens may serve as an interface between the hippocampus and prefrontal cortex. Further, some cells would appear to respond both to hippocampal and reuniens stimulation. Responses to reuniens stimulation are however distinct from those elicited by hippocampal stimulation and the underlying mechanisms mediating the former remain to be elucidated. The reuniens-prefrontal pathway is subject to short-term plasticity.

Figures

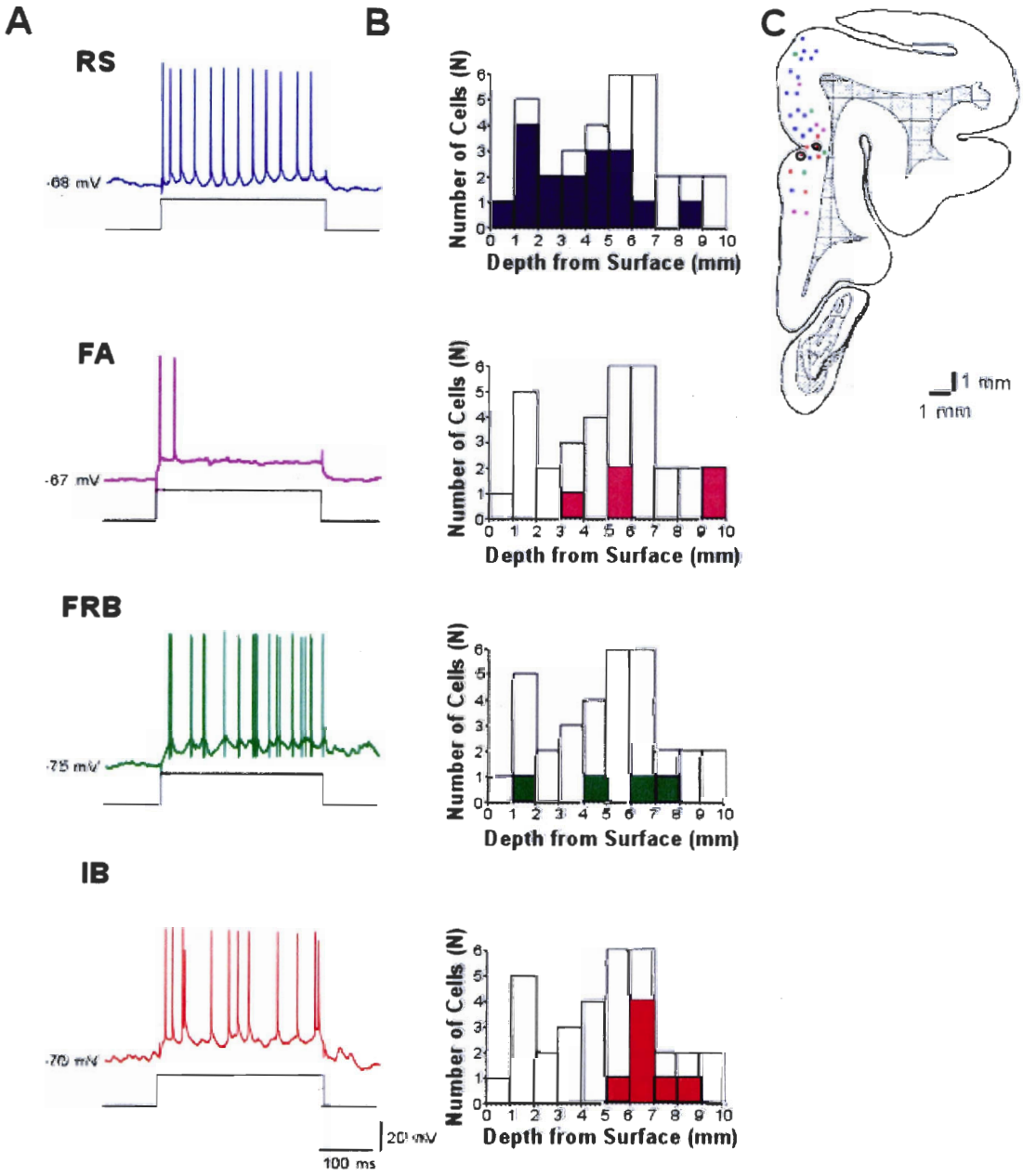

Figure 1

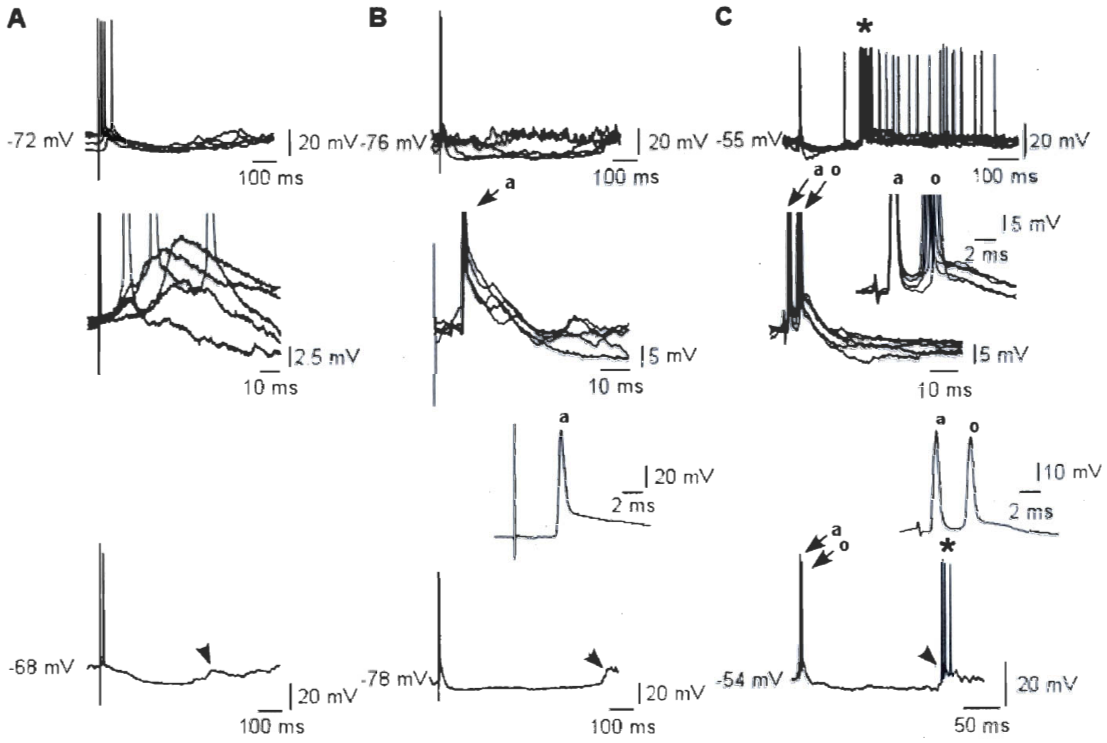
A
B
C
-72 mV
20 mV
100 ms
2.5 mV
10 ms
-68 mV
20 mV
100 ms
-76 mV
20 mV
100 ms
a
5 mV
10 ms
a
20 mV
2 ms
-78 mV
20 mV
100 ms
-55 mV
20 mV
100 ms
a o
a o
15 mV
2 ms
5 mV
10 ms
a o
10 mV
2 ms
a o
-54 mV
20 mV
50 ms

Figure 2.

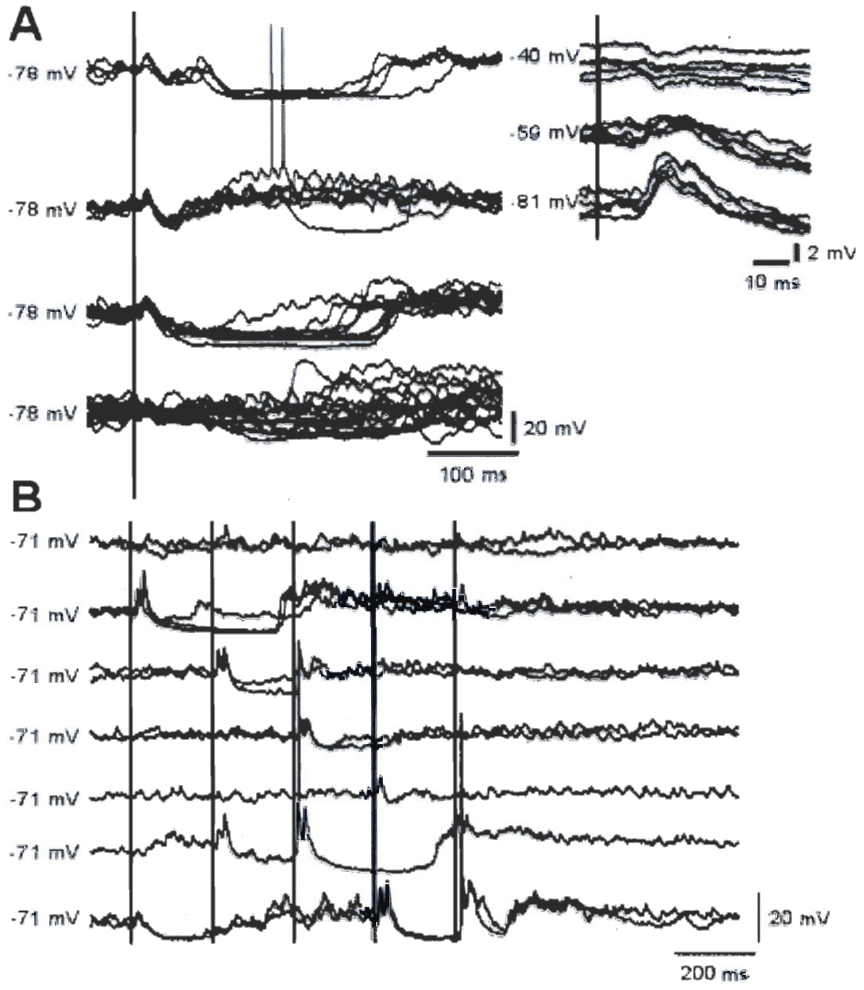
A
-78 mV
-78 mV
-78 mV
-78 mV
-40 mV
-59 mV
-81 mV
2 mV
10 ms
20 mV
100 ms
B
-71 mV
-71 mV
-71 mV
-71 mV
-71 mV
-71 mV
-71 mV
20 mV
200 ms

Figure 3

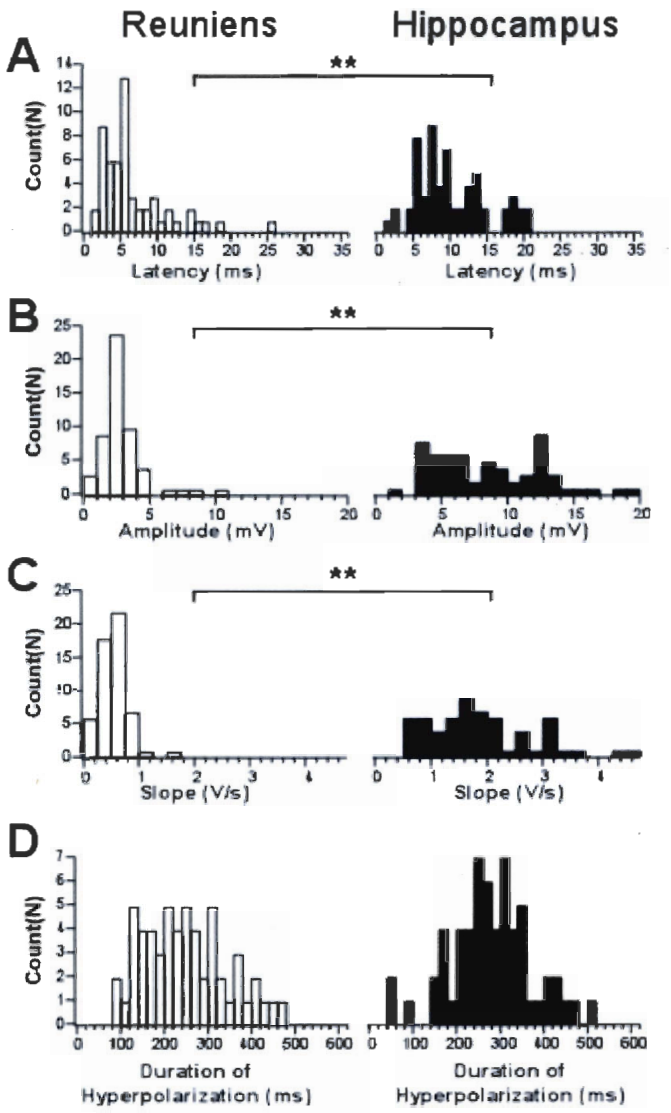
Reuniens
Hippocampus
A
B
C
D
**
Count(N)
Latency (ms)
Amplitude (mV)
Slope (V/s)
Duration of Hyperpolarization (ms)

Figure 4

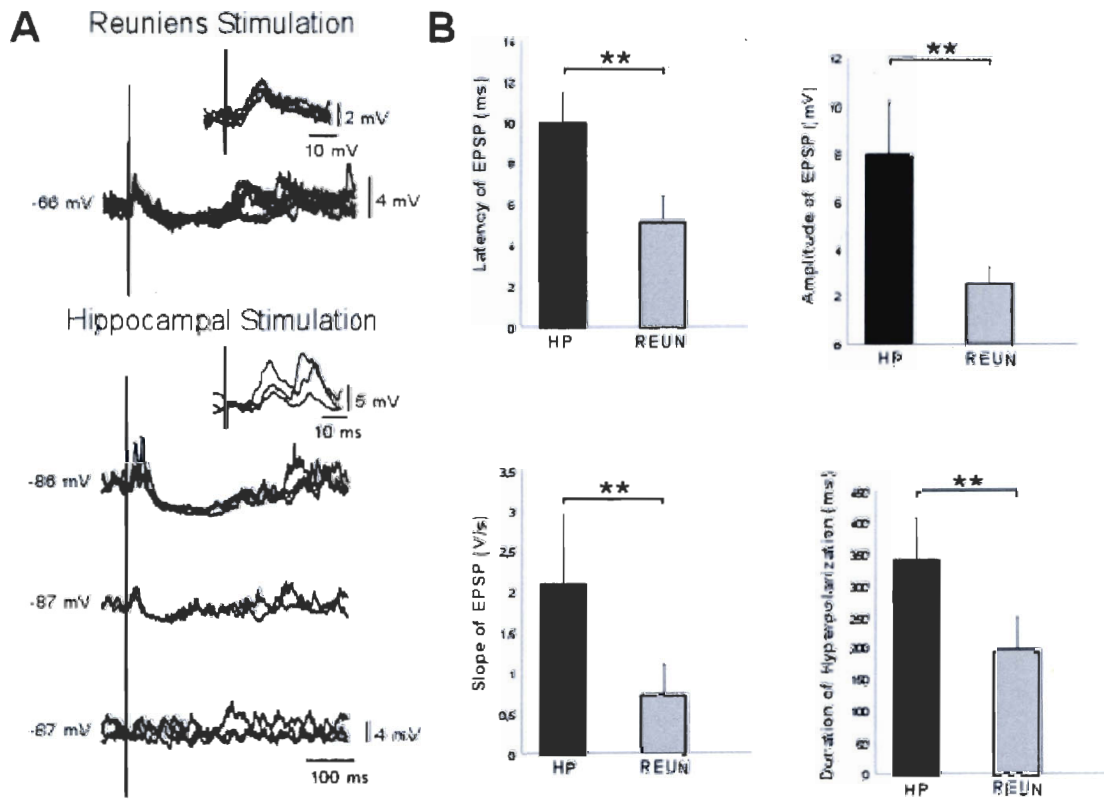
A
Reuniens Stimulation
2 mV
10 mV
-66 mV
4 mV
Hippocampal Stimulation
5 mV
10 ms
-86 mV
-87 mV
-87 mV
4 mV
100 ms
B
Latency of EPSP (ms)
**
HP
REUN
Amplitude of EPSP (mV)
**
HP
REUN
Slope of EPSP (V/s)
**
HP
REUN
Duration of Hyperpolarization (ms)
**
HP
REUN

Figure 5

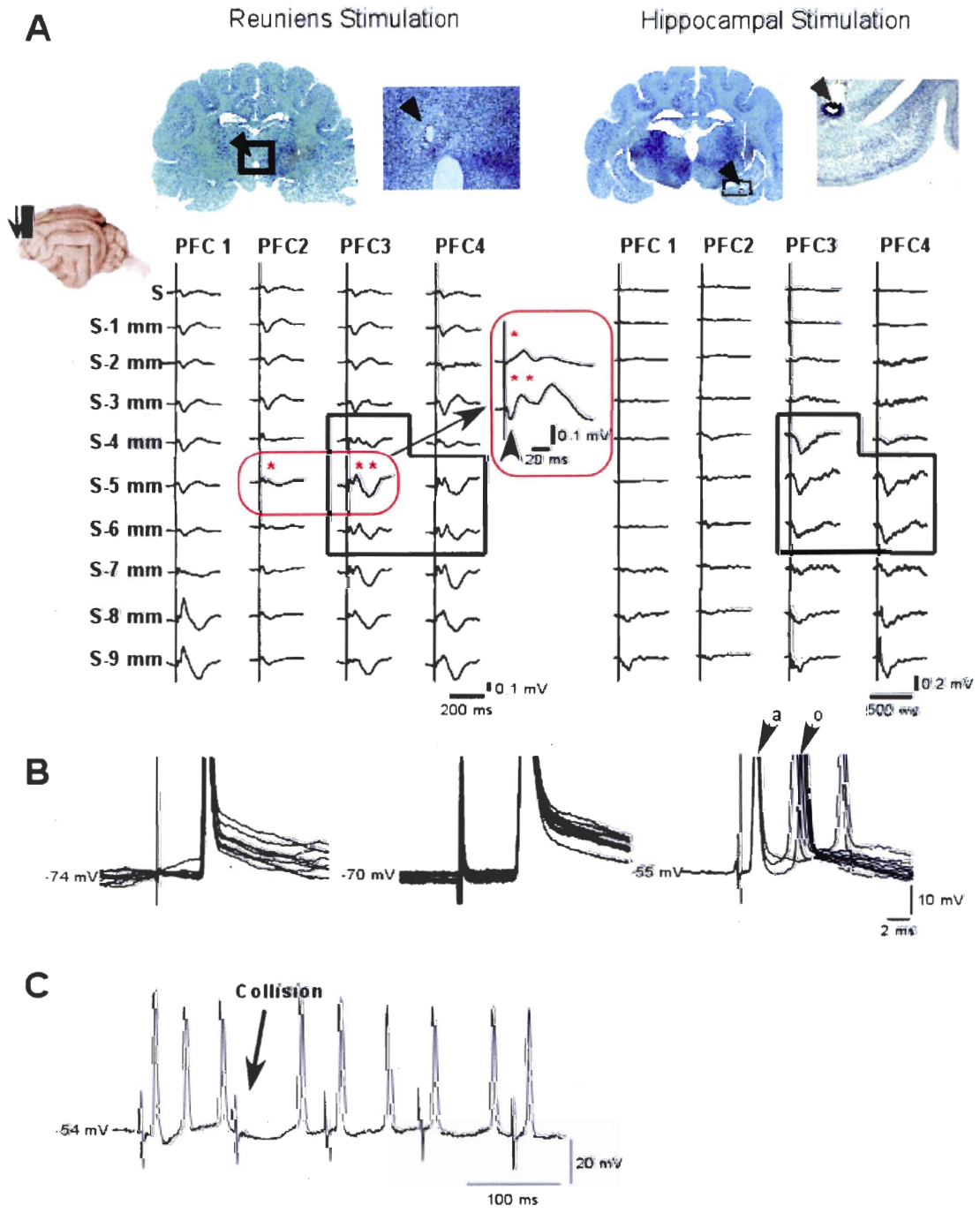
A
Reuniens Stimulation
Hippocampal Stimulation
PFC 1
PFC2
PFC3
PFC4
S
S-1 mm
S-2 mm
S-3 mm
S-4 mm
S-5 mm
S-6 mm
S-7 mm
S-8 mm
S-9 mm
0.1 mV
20 ms
0.1 mV
200 ms
0.2 mV
500 ms
B
a
o
-74 mV
-70 mV
-55 mV
10 mV
2 ms
C
Collision
-54 mV
20 mV
100 ms

Figure 6

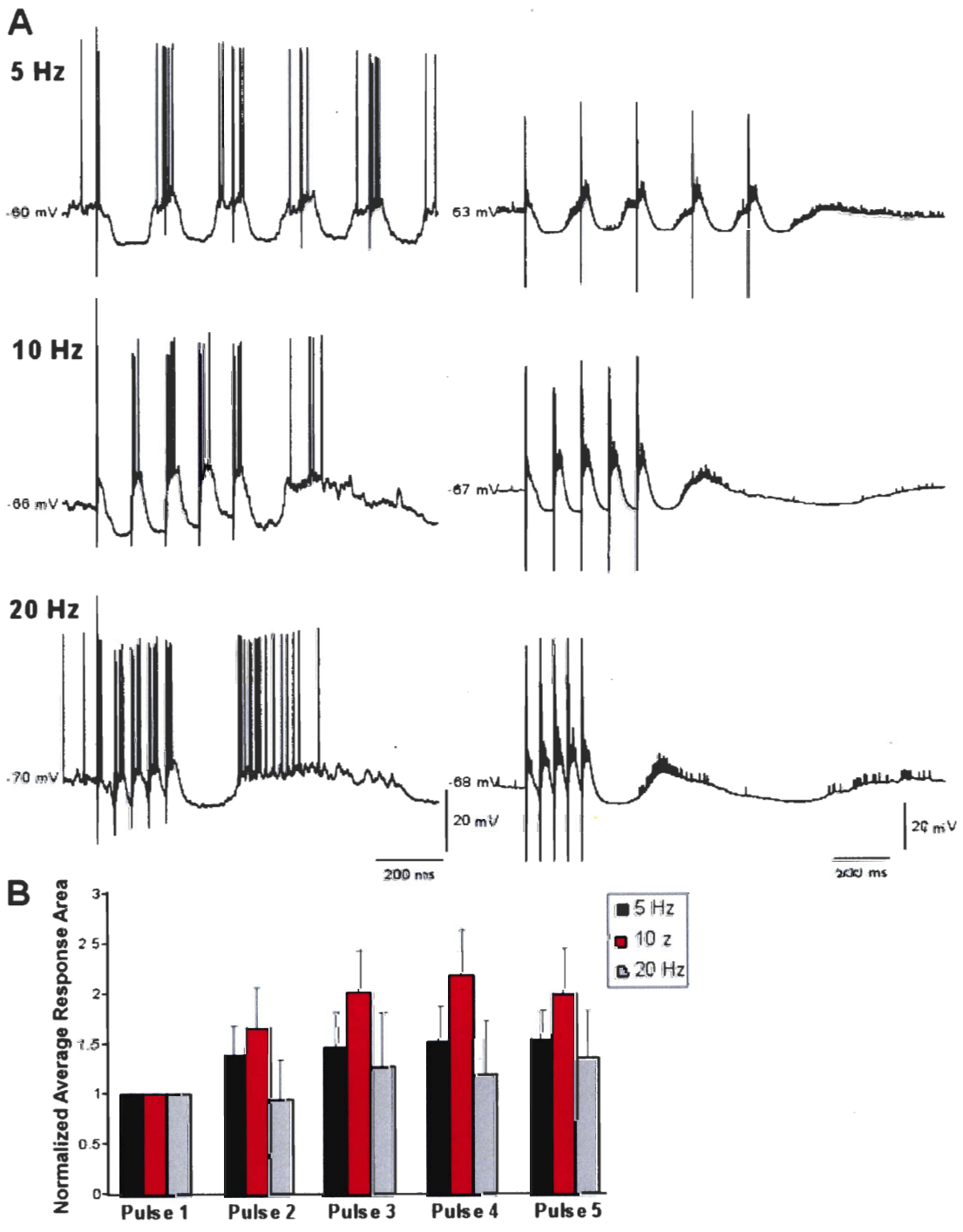
A
5 Hz
-60 mV
-63 mV
10 Hz
-66 mV
-67 mV
20 Hz
-70 mV
-68 mV
20 mV
20 mV
200 ms
B
Normalized Average Response Area
3
2.5
2
1.5
1
0.5
0
5 Hz
10 z
20 Hz
Pulse 1
Pulse 2
Pulse 3
Pulse 4
Pulse 5

Figure 7

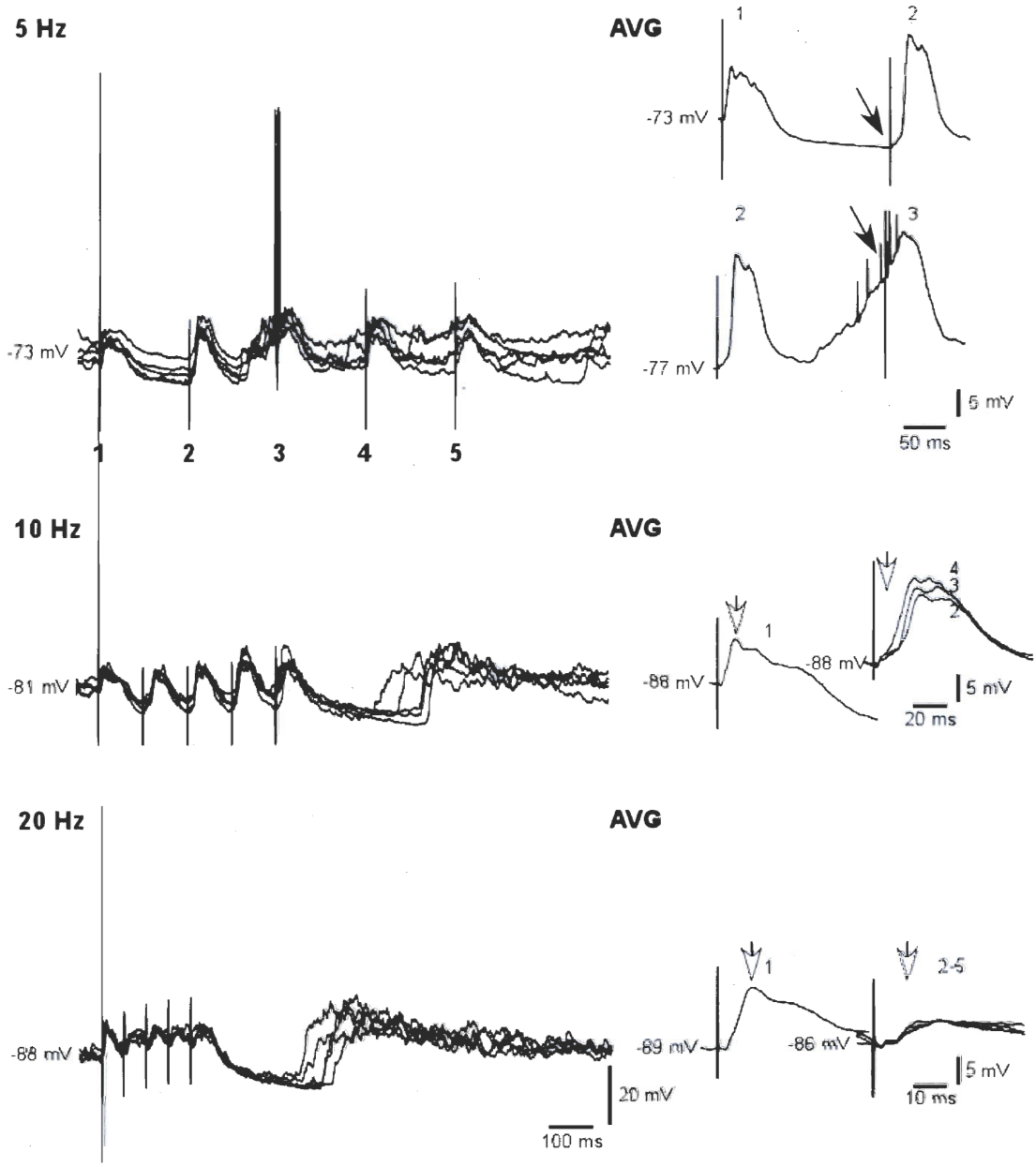
5 Hz
AVG
-73 mV
1
2
3
4
5
-77 mV
5 mV
50 ms
10 Hz
AVG
-81 mV
-88 mV
20 ms
20 Hz
AVG
-88 mV
-89 mV
-86 mV
20 mV
100 ms
10 ms
2-5

Figure 8

3.6 Figure Legends

Figure 1. Proportion of electrophysiological cell types recorded from various depths along the medial surface of the pericruciate prefrontal cortex. ***A*** Characteristic discharge patterns in response to current pulse injection of the four electrophysiological cell types that were recorded in medial prefrontal cortex of 11 cats. Regular spiking (RS, blue), fast-adapting (FA, pink), fast-rhythmic bursting (FRB, green) and intrinsically bursting (IB, red). ***B*** Histograms depicting the number of cells recorded with respect to depth from the cortical surface (bins, 1 mm). Following the color scheme in ***A***, RS cells are plotted in blue, FA cells in pink, FRB cells in green and IB cells in red. The unfilled bars on each graph represent the total number of characterized cells (n=33) recorded at each respective depth, allowing for the visualization of the proportion of sampled electrophysiological cell types. ***C*** Schema of pericruciate cross-section of medial prefrontal cortex. Dots depict recorded cell. Circled dots are cells that responded antidromically to reuniens stimulation.

Figure 2. Intracellular responses to single pulse stimulation of the reuniens nucleus. ***A*** Example of an orthodromic response to single pulse stimulation of the reuniens nucleus in a medial prefrontal cortex cell recorded 8500 μm below the cortical surface. ***B*** A strictly antidromic response to single pulse reuniens stimulation, recorded in a neuron lying 6050 μm below the cortical surface. ***C*** One neuron was found to exhibit both orthodromic and antidromic spiking following thalamic stimulation. This cell was also found 6050 μm below the cortical surface, but in a different animal. In all three cases, the top panel depicts a superposition of 5 responses; the middle panel overlaps and expands these traces. Note the jitter in the timing of orthodromic spikes, in contrast with that of antidromic spikes, in panel *C*. The bottom traces are single examples, with an expansion of the early response action potentials in inset. Arrowheads indicate putative rebounds, ***a***, antidromic spikes, **o**, orthodromic spikes and *, increased spiking following rebound activity.

Figure 3. Intracellular response to hippocampal stimulation. *A* Response to hippocampal stimulation in a single cell. *Top right,* Reversal of response with depolarization. ***B*** Responses to 5 Hz stimulation. In both cases, note the variability but repeatability of the response patterns.

Figure 4. Reuniens nucleus and hippocampus exert distinct responses on medial prefrontal cortex neurons. Histograms of response latency ***(A)***, EPSP amplitude ***(B),*** EPSP slope ***(C)*** and duration of the hyperpolarizing component of the response ***(D)*** following stimulation of the reuniens nucleus (left panel) and of the hippocampus. **$p<0.0001$.

Figure 5. Single cell response to stimulation of both the reuniens nucleus and the hippocampus. ***A,*** Representative example of responses to reuniens stimulation, *top*, and to hippocampal stimulation, *bottom*. Note the variability in responses to hippocampal stimulation, as in figure 3. ***B,*** Mean and standard deviation of the duration of the hyperpolarizing component, amplitude of EPSP, latency of response and slope of EPSP. ** $p<0.01$.

Figure 6. Extracellular and intracellular evidence for a hippocampo-prefronto-thalamic relay in medial prefrontal cortex. 4 microelectrode recording array was positioned along the antero-posterior axis of the medial prefrontal cortex and lowered from the cortical surface (S) in 1 mm steps. ***A,***Threshold intensity stimuli were delivered at 1 Hz to the REUN ***(left panel)***, and to the HP (*right panel*). All traces represent average responses. Electrolytic lesions confirmed the stimulation site in both cases (top). Thalamic responses were evoked across a much wider territory than were hippocampal responses. Interestingly, large hippocampal responses were observed in areas coinciding with regions where antidromic responses to REUN stimulation were elicited (highlighted in black). Evidence for antidromic events lie in the observation of short latency depth negative events (see red inset (**), and compare with response lacking this event (*), also in inset). ***B*** Three cells in 3 different animals responded antidromically to REUN stimulation. They were all recorded between 6 and 6.5 mm below the cortical surface. One cell responded both antidromically and orthodromically. ***C*** The nature of intracellularly recorded

antidromic spikes was confirmed with collision tests. *a,* antidromic spike; *o*, orthodromic spike.

Figure 7. Responses of medial prefrontal cortex neurons to trains of stimulation applied to the reuniens nucleus. ***A***, *left panel.* Representative intracellular responses to a single train of stimulation following 5 Hz (*top*), 10 Hz (*middle*) and 20 Hz (*bottom*) stimulation. *Right panel,* sweep averages. ***B,*** Normalized average response area by pulse number and frequency. Bars represent mean and standard error (N=6 for 5 and 10 Hz stimulation, N=4 for 20 Hz stimulation

Figure 8. Augmenting responses in neurons of the medial prefrontal cortex following stimulation of the reuniens nucleus. ***Left,*** medial prefrontal cortex responses to 5 Hz (top), 10 Hz (middle) and 20 Hz (bottom) stimulation. Five traces are overlapped in all cases. ***Right***, average responses. At 5 Hz stimulation, there is an increase in response magnitude from pulse 1 to pulse 2, where the second stimulation falls when the cell is hyperpolarized (arrow). Pulse 3 coincides with cell activation (arrow) and results in a marked decrease in response magnitude. At 10 Hz, there is a clear decrease in the primary response from pulse 1 to pulses 2 (unfilled arrows). The response area of the secondary response increases from pulse 1 to pulse 4. At 20 Hz, there is a marked depression of the response. *Avg,* average.

4 Conclusion

An overview of the principal findings and a discussion of methodological considerations, functional significance and potential future directions will now follow and conclude this Master's dissertation.

4.1 Summary

In this memoir, corroborating evidence in support of the claim that the reuniens nucleus serves as an interface between the hippocampus and prefrontal cortex was presented. This claim makes the tacit assumption that the reuniens nucleus exerts physiological actions on the prefrontal cortex. These actions, however, remained poorly characterized with no intracellular data available. Such data was provided here for the first time. It was shown that the reuniens nucleus elicits complex synaptic responses in a significant proportion (38%) of neurons of the medial prefrontal cortex. The responses consisted of a depolarizing event followed by a long hyperpolarization with response latencies significantly shorter than those of responses to hippocampal stimulation. Furthermore, the reuniens-prefrontal pathway was found to be subject to short-term plasticity. Finally it was shown that a restricted locus of medial prefrontal cortex mediates the hippocampo-prefronto-reuniens relay.

It was further shown that at least three populations of putatively pyramidal cells of the medial prefrontal cortex mediate responses to hippocampal and reuniens stimulation: (1) cells which strictly respond to hippocampal stimulation; (2) cells which respond to reuniens stimulation either orthodromically, antidromically or both orthodromically and antidromically and (3) cells that respond to both hippocampal and reuniens stimulation. The latter subpopulation is presumably the smallest, with only 1 cell out of 19 (5.3%) exhibiting this behavior. A role for local circuit interneurons is also posited based on the observed reversal of some hippocampal responses near resting membrane potential, suggesting a chloride conductance putatively mediated by $GABA_A$, and based on previous studies (using barbiturate anesthesia) which have: a) implicated both $GABA_A$ and $GABA_B$ conductances in the

mediation of the hyperpolarizing component of prefrontal responses to hippocampal stimulation, b) provided evidence for feedforward inhibition in the hippocampo-prefrontal pathway and c) presented anatomical evidence for hippocampo-prefrontal synapses onto interneurons. Responses to reuniens stimulation are hypothesized to be mediated, at least in part, by similar mechanisms. A conceptual scheme of microcircuits of the medial prefrontal cortex involved in mediating interactions with the reuniens and hippocampus is shown in figure 9.

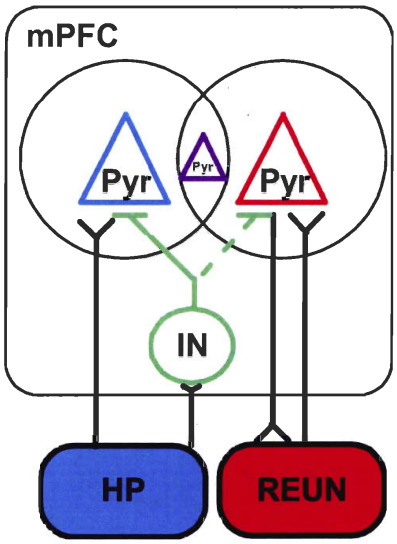

Figure 9. Conceptual scheme of medial prefrontal cortex microcircuits mediating interactions with the reuniens nucleus and hippocampus. At least three populations of putatively pyramidal cells of the medial prefrontal cortex mediate responses to hippocampal and reuniens stimulation: (1) cells which strictly respond to hippocampal stimulation; (2) cells which respond to reuniens stimulation either orthodromically, antidromically or both orthodromically and antidromically and (3) cells that respond to both hippocampal and reuniens stimulation. A role for interneurons is also posited (see text).

4.2 Methodological Considerations

Very few studies have investigated the electrophysiology of pathways liking the reuniens with the prefrontal cortex and hippocampus. In this study, intracellular recordings were performed in the anesthetized cat preparation. Methodological considerations are discussed below.

One of the greatest strengths of this study is that it provided the first description of intracellular responses of medial prefrontal cortex neurons to stimulation of the reuniens nucleus, data that was not previously available in this pathway. This data was however awaited:

> Unlike the well organized laminar structure of the hippocampus, the cellular organization of the medial prefrontal cortex is not distinctly laminar but contains multiple cell layers with apical dendrites of pyramidal cells extending across all layers. As such, the analysis of the properties of the field potentials at the medial prefrontal cortex is not as straightforward as for the hippocampus, and seemingly requires combined cellular (intracellular) and field analyses to resolve characteristics of evoked potentials at the medial prefrontal cortex (Di Prisco and Vertes, 2006).

Intracellular recordings indeed provide high temporal resolution as well as a valuable window into single cell physiology, providing insight into the microcircuits of the prefrontal cortex and possible response mechanisms.

As in all *in vivo* electrophysiological experiments, however, a major question that arises is what structures are stimulated. This is particularly true in this case because high intensity of stimulation was used (0.3-1.5 mA) and because the reuniens nucleus is such a small nucleus. Indeed, very few animal studies have been carried out on the reuniens nucleus due to its small size (Van der Werf et al., 2002). To ensure that the reuniens nucleus was the site of stimulation, the reuniens and hippocampus were stimulated and recorded from in turn at the start of each experiment to ensure that each structure could evoke responses in the other. In addition, at the end of experiments electrolytic lesions were performed in most cats. In cases where stimulation sites were not histologically confirmed, there remains the possibility that stimulation of structures lying slightly outside the reuniens nucleus evoked responses in both the hippocampus and prefrontal cortex. In such cases, response morphology was corroborated, as much as possible, with those evoked by stimulation of histologically confirmed sites. As the reuniens is practically the only thalamic nucleus projecting to the hippocampus, however, such responses to stimulation of sites outside of the reuniens could be caused by the recruiting of the latter by current spread. That other midline structures were likewise recruited by reuniens stimulation

is also a possibility but this is a methodological pitfall which can only be partially circumvented by use of minimal stimulation intensities.

It may be objected that intensity of stimulation was too high and non-physiological. The use of high intensity of stimulation could however mimic the effects of synchronization of inputs/outputs in physiological conditions. High intensity stimulation may also help overcome local inhibitory influences, as the cat reuniens nucleus contains many GABAergic interneurons. It should also be noted that equal and even higher stimulation intensities, up to 1.8 mA, have been used in previous studies, for example to stimulate the hippocampo-prefrontal pathway (Floresco and Grace, 2003).

In this study we do not specify the layer of the recorded cells. It has been shown, however, that the reuniens nucleus projects to layers Ia, V and VI of the prelimbic and infralimbic cortices as well as to layers I, III and V of the medial orbital and agranular insular cortices(Van der Werf et al., 2002). The ventral hippocampus projects most profusely to layers V and VI of the infralimbic and prelimbic cortices with collaterals ascending towards layer I (Jay and Witter, 1991; Gabbott et al., 2002).

Another question that may arise is why the cat preparation was chosen when most studies pertaining to the reuniens nucleus have been carried out in rat. First, relatively few studies have been carried out on this midline nucleus because its small size makes it difficult to investigate (Van der Werf et al., 2002). In cat, this nucleus spans approximately 2 mm^3 and allows for the simultaneous stimulation and recording of the reuniens nucleus, hippocampus and prefrontal cortex (intracellularly and extracellularly). In addition, the mechanisms of sleep have been extensively studied in cat (Steriade et al., 1993c; Timofeev et al., 1996; Amzica and Steriade, 1998; Timofeev et al., 2000a; Steriade et al., 2001). Sleep plays crucial roles in learning and memory and in particular, slow wave sleep has been shown to mediate hippocampo-prefrontal communication (Rasch et al., 2007; Marshall et al., 2006; Sirota et al., 2003; Maquet, 2001; Siapas and Wilson, 1998; Steriade, 1999; Qin et al., 1997). If the reuniens nucleus plays a role in mediating these interactions, the study of its

influence on medial prefrontal cortex during slow oscillations, here induced by ketamine/xylazine anesthesia, is of high physiological relevance. Lastly, cats can be trained to sleep in a stereotaxic frame (Steriade et al., 2001; Nita et al., 2007). The present study was carried out in an acute preparation but sets the stage for future studies in chronic animals where responses in natural sleep could be investigated, possibly following learning, with the aim of helping to elucidate the role of the reuniens nucleus in slow wave sleep-mediated memory consolidation.

Lastly, an ambiguous point in this study was the observation of a clear augmenting trend following 10 Hz stimulation that nonetheless lacks statistical significance. Increasing the sample number may resolve this issue but care must be taken not to bias the experimental design by accumulating data until the desired outcome is achieved. Also, while increasing the sample number is always desirable in conjuring more convincing generalizations it invariably begs the question: how much is enough? One way around this problem will be to compute an *N* value in accordance with the desired power of the statistical tests.

4.3 Future Directions

The underlying mechanisms mediating medial prefrontal responses to reuniens stimulation remain to be elucidated. Reuniens afferents to prefrontal cortex are glutamatergic and likely exert their actions via AMPA and/or NMDA receptors. As discussed previously, it is hypothesized that the mechanisms mediating the subsequent hyperpolarization component of the response is similar to those mediating that of the hippocampal response. These mechanisms probably involve GABA-mediated inhibition, either feedforward or feedback. A role for disfacilitation or subcortical gating is also possible.

Future investigations delving into the mechanisms mediating medial prefrontal responses to reuniens stimulation are thus a logical next step. Specifically, the following hypotheses could be tested:

1. The depolarization component of the response is mediated by AMPA and/or NMDA receptors.

2. The hyperpolarization component of the response is mediated by $GABA_A$ and $GABA_B$.

3. The hyperpolarization component of the response is mediated in part by disfacilitation.

These hypotheses are non-exhaustive. Metabotropic glutamate receptors may for instance also be involved in prefrontal responses to reuniens stimulation.

Other interesting avenues of research include the ultrastructural characterization of reuniens afferents to medial prefrontal cortex: what proportion of pyramidal cells are targeted? Of interneurons? Are the preferred synaptic targets proximal dendrites, distal dendrites or the perisomatic region? What proportion of cells receives both hippocampal and reuniens input and what cellular compartment do each target? Also of interest would be to tease apart the microcircuits of the medial prefrontal cortex mediating interactions with the hippocampus and reuniens nucleus. A simplified scheme of these microcircuits was proposed above and represented in figure 8. At best, however, this conceptualization still represents a gross approximation.

Future studies elucidating the nature of interneuron response to reuniens stimulation promise to be of value in elucidating the prefrontal microcircuits mediating interactions of the reuniens nucleus and hippocampus, especially in the light of evidence supporting their role in regulating the activity of PFC pyramidal cells involved in working memory (Lewis et al., 2002).

Intracellular staining of recorded neurons, increasing of the sample number and testing of the conduction velocity in the hippocampo-prefrontal pathway of cats will be undertaken in the near future.

4.4 Functional Implication

The reuniens nucleus appears ideally positioned to modulate hippocampo-prefrontal interactions. It is reciprocally connected to and exerts physiological actions on both of these structures. Why the prefrontal cortex does not reciprocate connections with the hippocampus remains puzzling. One may conjecture that the involvement of the reuniens nucleus ensures that feedback from the prefrontal cortex is integrated with limbic information before modulating hippocampal processing. It is known that memory consolidation is strongly influenced by emotion (McGaugh, 2000) and it was proposed by Llinas and coworkers (1998) that the non-specific thalamus may mediate communication between the cortex and other parts of the brain (Llinas et al., 1998). In addition, Robert Vertes and colleagues have also proposed that the "reuniens serves to gate the flow of information between the medial prefrontal cortex and hippocampus thereby controlling the type of prefrontal information that gains access to the hippocampus for its long term storage (Vertes et al., 2007)". At this time, these claims remain hypotheses.

Finally, a deeper understanding of reuniens nucleus physiology promises to be of clinical value. For instance, damage to the midline nuclei, including the reuniens nucleus, has been implicated in memory impairments (Mennemeier et al., 1992). The reuniens nucleus has also been implicated in Alzheimer's disease (Braak and Braak, 1991; Braak and Braak, 1998) and in epilepsy (Montpied et al., 1995).

5 Bibliographie

Agmon A, Connors BW (1992) Correlation between intrinsic firing patterns and thalamocortical synaptic responses of neurons in mouse barrel cortex. J Neurosci 12: 319-329.

Allen PJ, Fish DR, Smith SJ (1992) Very high-frequency rhythmic activity during SEEG suppression in frontal lobe epilepsy. Electroencephalogr Clin Neurophysiol 82: 155-159.

Alonso A, Garcia-Austt E (1987) Neuronal sources of theta rhythm in the entorhinal cortex of the rat. II. Phase relations between unit discharges and theta field potentials. Exp Brain Res 67: 502-509.

Amaral DG, Dolorfo C, Alvarez-Royo P (1991) Organization of CA1 projections to the subiculum: a PHA-L analysis in the rat. Hippocampus 1: 415-435.

Amzica F, Steriade M (1995) Disconnection of intracortical synaptic linkages disrupts synchronization of a slow oscillation. J Neurosci 15: 4658-4677.

Amzica F, Steriade M (1998) Electrophysiological correlates of sleep delta waves. Electroencephalogr Clin Neurophysiol 107: 69-83.

Anderson P, Bliss TV, Skrede KK (1971) Lamellar organization of hippocampal pathways. Exp Brain Res 13: 222-238.

Arai R, Jacobowitz DM, Deura S (1994) Distribution of calretinin, calbindin-D28k, and parvalbumin in the rat thalamus. Brain Res Bull 33: 595-614.

Auer RN, Jensen ML, Whishaw IQ (1989) Neurobehavioral deficit due to ischemic brain damage limited to half of the CA1 sector of the hippocampus. J Neurosci 9: 1641-1647.

Aujla H, Beninger RJ (2001) Hippocampal-prefrontocortical circuits: PKA inhibition in the prefrontal cortex impairs delayed nonmatching in the radial maze in rats. Behav Neurosci 115: 1204-1211.

Azmitia EC, Segal M (1978) An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. J Comp Neurol 179: 641-667.

Baddeley AD (1982) Implications of neuropsychological evidence for theories of normal memory. Philos Trans R Soc Lond B Biol Sci 298: 59-72.

Baisden RH, Hoover DB, Cowie RJ (1979) Retrograde demonstration of hippocampal afferents from the interpeduncular and reuniens nuclei. Neurosci Lett 13: 105-109.

Bazhenov M, Timofeev I, Steriade M, Sejnowski TJ (1998) Cellular and network models for intrathalamic augmenting responses during 10-Hz stimulation. J Neurophysiol 79: 2730-2748.

Beckstead RM (1979) Autoradiographic Examination of Cortico-Cortical and Sub-Cortical Projections of the Mediodorsal-Projection (Prefrontal) Cortex in the Rat. J Comp Neurol 184: 43-62.

Bennett MR (2000) The concept of long term potentiation of transmission at synapses. Prog Neurobiol 60: 109-137.

Berendse HW, Groenewegen HJ (1991) Restricted cortical termination fields of the midline and intralaminar thalamic nuclei in the rat. Neuroscience 42: 73-102.

Bertram EH, Zhang DX (1999) Thalamic excitation of hippocampal CA1 neurons: a comparison with the effects of CA3 stimulation. Neuroscience 92: 15-26.

Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361: 31-39.

Bokor H, Csaki A, Kocsis K, Kiss J (2002) Cellular architecture of the nucleus reuniens thalami and its putative aspartatergic/glutamatergic projection to the hippocampus and medial septum in the rat. Eur J Neurosci 16: 1227-1239.

Bontempi B, Laurent-Demir C, Destrade C, Jaffard R (1999) Time-dependent reorganization of brain circuitry underlying long-term memory storage. Nature 400: 671-675.

Braak H, Braak E (1991) Alzheimer's disease affects limbic nuclei of the thalamus. Acta Neuropathol 81: 261-268.

Braak H, Braak E (1998) Evolution of neuronal changes in the course of Alzheimer's disease. J Neural Transm Suppl 53: 127-140.

Brumberg JC, Nowak LG, Mccormick DA (2000) Ionic mechanisms underlying repetitive high-frequency burst firing in supragranular cortical neurons. J Neurosci 20: 4829-4843.

Burette F, Jay TM, Laroche S (1997) Reversal of LTP in the hippocampal afferent fiber system to the prefrontal cortex in vivo with low-frequency patterns of stimulation that do not produce LTD. J Neurophysiol 78: 1155-1160.

Buzsaki G (1996) The hippocampo-neocortical dialogue. Cereb Cortex 6: 81-92.

Buzsaki G (2002) Theta oscillations in the hippocampus. Neuron 33: 325-340.

Buzsaki G (2006) The Brain's Default State: Self-Organized Oscillations in Rest and Sleep. In: Rhythms of the Brain pp 175-205. New York: Oxford University Press.

Buzsaki G, Eidelberg E (1983) Phase relations of hippocampal projection cells and interneurons to theta activity in the anesthetized rat. Brain Res 266: 334-339.

Carr DB, Sesack SR (1996) Hippocampal afferents to the rat prefrontal cortex: Synaptic targets and relation to dopamine terminals. J Comp Neurol 369: 1-15.

Cauli B, Audinat E, Lambolez B, Angulo MC, Ropert N, Tsuzuki K, Hestrin S, Rossier J (1997) Molecular and physiological diversity of cortical nonpyramidal cells. J Neurosci 17: 3894-3906.

Cavada C, Llamas A, Reinososuarez F (1983) Allocortical Afferent Connections of the Prefrontal Cortex in the Cat. Brain Res 260: 117-120.

Cho YH, Beracochea D, Jaffard R (1993) Extended temporal gradient for the retrograde and anterograde amnesia produced by ibotenate entorhinal cortex lesions in mice. J Neurosci 13: 1759-1766.

Christoffersen GR, Petersen S, daCosta NM (2003) Potentiation of prelimbic field potentials during and seconds after trains of excitations in the rat hippocampo-prefrontal pathway. Neurosci Lett 341: 143-146.

Chrobak JJ, Buzsaki G (1996) High-frequency oscillations in the output networks of the hippocampal-entorhinal axis of the freely behaving rat. J Neurosci 16: 3056-3066.

Cohen RL (1984) Individual differences in event memory: a case for nonstrategic factors. Mem Cognit 12: 633-641.

Collins DR, Lang EJ, Pare D (1999) Spontaneous activity of the perirhinal cortex in behaving cats. Neuroscience 89: 1025-1039.

Colom LV, Christie BR, Bland BH (1988) Cingulate cell discharge patterns related to hippocampal EEG and their modulation by muscarinic and nicotinic agents. Brain Res 460: 329-338.

Connors BW, Gutnick MJ (1990) Intrinsic firing patterns of diverse neocortical neurons. Trends Neurosci 13: 99-104.

Connors BW, Gutnick MJ, Prince DA (1982) Electrophysiological properties of neocortical neurons in vitro. J Neurophysiol 48: 1302-1320.

Contreras D (2004) Electrophysiological classes of neocortical neurons. Neural Netw 17: 633-646.

Contreras D, Steriade M (1995) Cellular basis of EEG slow rhythms: a study of dynamic corticothalamic relationships. J Neurosci 15: 604-622.

Contreras D, Steriade M (1996) Spindle oscillation in cats: the role of corticothalamic feedback in a thalamically generated rhythm. J Physiol 490 (Pt 1): 159-179.

Contreras D, Timofeev I, Steriade M (1996) Mechanisms of long-lasting hyperpolarizations underlying slow sleep oscillations in cat corticothalamic networks. J Physiol 494 (Pt 1): 251-264.

Cooper DC (2002) The significance of action potential bursting in the brain reward circuit. Neurochem Int 41: 333-340.

Crill WE, Schwindt P (1984) Ionic mechanisms underlying excitation-to-frequency transduction: studies by voltage clamp methods. Arch Ital Biol 122: 31-41.

Csicsvari J, Hirase H, Czurko A, Mamiya A, Buzsaki G (1999) Fast network oscillations in the hippocampal CA1 region of the behaving rat. J Neurosci 19: RC20.

Cummings JL, Tomiyasu U, Read S, Benson DF (1984) Amnesia with hippocampal lesions after cardiopulmonary arrest. Neurology 34: 679-681.

Davis HP, Baranowski JR, Pulsinelli WA, Volpe BT (1987) Retention of reference memory following ischemic hippocampal damage. Physiol Behav 39: 783-786.

de la Pena, Geijo-Barrientos E (1996) Laminar localization, morphology, and physiological properties of pyramidal neurons that have the low-threshold calcium current in the guinea-pig medial frontal cortex. J Neurosci 16: 5301-5311.

Degenetais E, Thierry AM, Glowinski J, Gioanni Y (2002) Electrophysiological properties of pyramidal neurons in the rat prefrontal cortex: An in vivo intracellular recording study. Cerebral Cortex 12: 1-16.

Degenetais E, Thierry AM, Glowinski J, Gioanni Y (2003) Synaptic influence of hippocampus on pyramidal cells of the rat prefrontal cortex: an in vivo intracellular recording study. Cereb Cortex 13: 782-792.

Delorme A, Westerfield M, Makeig S (2007) Medial prefrontal theta bursts precede rapid motor responses during visual selective attention. J Neurosci 27: 11949-11959.

Demiralp T, Basareroglu C, Rahn E, Basar E (1994) Event-Related Theta-Rhythms in Cat Hippocampus and Prefrontal Cortex During An Omitted Stimulus Paradigm. Int J of Psychophysiol 18: 35-48.

Deschenes M, Madariaga-Domich A, Steriade M (1985) Dendrodendritic synapses in the cat reticularis thalami nucleus: a structural basis for thalamic spindle synchronization. Brain Res 334: 165-168.

Di Prisco GV, Vertes RP (2006) Excitatory actions of the ventral midline thalamus (rhomboid/reuniens) on the medial prefrontal cortex in the rat. Synapse 60: 45-55.

Dolleman-Van der Weel MJ, Lopes da Silva FH, Witter MP (1997) Nucleus reuniens thalami modulates activity in hippocampal field CA1 through excitatory and inhibitory mechanisms. J Neurosci 17: 5640-5650.

Dolleman-Van der Weel MJ, Witter MP (2000) Nucleus reuniens thalami innervates gamma aminobutyric acid positive cells in hippocampal field CA1 of the rat. Neurosci Letters 278: 145-148.

DollemanVanderWeel MJ, Witter MP (1996) Projections from the nucleus reuniens thalami to the entorhinal cortex, hippocampal field CA1, and the subiculum in the rat arise from different populations of neurons. J Comp Neurol 364: 637-650.

Ferino F, Thierry AM, Glowinski J (1987) Anatomical and electrophysiological evidence for a direct projection from Ammon's horn to the medial prefrontal cortex in the rat. Exp Brain Res 65: 421-426.

Finch DM, Babb TL (1980) Neurophysiology of the caudally directed hippocampal efferent system in the rat: projections to the subicular complex. Brain Res 197: 11-26.

Finch DM, Babb TL (1981) Demonstration of caudally directed hippocampal efferents in the rat by intracellular injection of horseradish peroxidase. Brain Res 214: 405-410.

Fisher RS, Webber WR, Lesser RP, Arroyo S, Uematsu S (1992) High-frequency EEG activity at the start of seizures. J Clin Neurophysiol 9: 441-448.

Fletcher PC, Henson RN (2001) Frontal lobes and human memory: insights from functional neuroimaging. Brain 124: 849-881.

Floresco SB, Braaksma DN, Phillips AG (1999) Thalamic-cortical-striatal circuitry subserves working memory during delayed responding on a radial arm maze. J Neurosci 19: 11061-11071.

Floresco SB, Grace AA (2003) Gating of hippocampal-evoked activity in prefrontal cortical neurons by inputs from the mediodorsal thalamus and ventral tegmental area. J Neurosci 23: 3930-3943.

Floresco SB, Seamans JK, Phillips AG (1997) Selective roles for hippocampal, prefrontal cortical, and ventral striatal circuits in radial-arm maze tasks with or without a delay. J Neurosci 17: 1880-1890.

Fox SE, Wolfson S, Ranck JB, Jr. (1986) Hippocampal theta rhythm and the firing of neurons in walking and urethane anesthetized rats. Exp Brain Res 62: 495-508.

Frankland PW, Bontempi B (2005) The organization of recent and remote memories. Nat Rev Neurosci 6: 119-130.

Frankland PW, O'Brien C, Ohno M, Kirkwood A, Silva AJ (2001) Alpha-CaMKII-dependent plasticity in the cortex is required for permanent memory. Nature 411: 309-313.

Freund TF, Buzsaki G (1996) Interneurons of the hippocampus. Hippocampus 6: 347-470.

Friedman HR, Goldman-Rakic PS (1988) Activation of the hippocampus and dentate gyrus by working-memory: a 2-deoxyglucose study of behaving rhesus monkeys. J Neurosci 8: 4693-4706.

Gabbott P, Headlam A, Busby S (2002) Morphological evidence that CA1 hippocampal afferents monosynaptically innervate PV-containing neurons and NADPH-diaphorase reactive cells in the medial prefrontal cortex (Areas 25/32) of the rat. Brain Res 946: 314-322.

Gabbott PL, Dickie BG, Vaid RR, Headlam AJ, Bacon SJ (1997) Local-circuit neurones in the medial prefrontal cortex (areas 25, 32 and 24b) in the rat: morphology and quantitative distribution. J Comp Neurol 377: 465-499.

Gais S, Molle M, Helms K, Born J (2002) Learning-dependent increases in sleep spindle density. J Neurosci 22: 6830-6834.

Gambini JP, Velluti RA, Pedemonte M (2002) Hippocampal theta rhythm synchronizes visual neurons in sleep and waking. Brain Res 926: 137-141.

Gao M, Liu CL, Yang S, Jin GZ, Bunney BS, Shi WX (2007) Functional coupling between the prefrontal cortex and dopamine neurons in the ventral tegmental area. J Neurosci 27: 5414-5421.

George PJ, Horel JA, Cirillo RA (1989) Reversible cold lesions of the parahippocampal gyrus in monkeys result in deficits on the delayed match-to-sample and other visual tasks. Behav Brain Res 34: 163-178.

Gigg J, Tan AM, Finch DM (1992) Glutamatergic excitatory responses of anterior cingulate neurons to stimulation of the mediodorsal thalamus and their regulation by GABA: an in vivo iontophoretic study. Cereb Cortex 2: 477-484.

Gigg J, Tan AM, Finch DM (1994) Glutamatergic hippocampal formation projections to prefrontal cortex in the rat are regulated by GABAergic inhibition and show convergence with glutamatergic projections from the limbic thalamus. Hippocampus 4: 189-198.

Gioanni Y, Rougeot C, Clarke PB, Lepouse C, Thierry AM, Vidal C (1999) Nicotinic receptors in the rat prefrontal cortex: increase in glutamate release and facilitation of mediodorsal thalamo-cortical transmission. Eur J Neurosci 11: 18-30.

Goldman-Rakic PS, Selemon LD, Schwartz ML (1984) Dual pathways connecting the dorsolateral prefrontal cortex with the hippocampal formation and parahippocampal cortex in the rhesus monkey. Neuroscience 12: 719-743.

Gray CM, Mccormick DA (1996) Chattering cells: superficial pyramidal neurons contributing to the generation of synchronous oscillations in the visual cortex. Science 274: 109-113.

Green JD, Arduini AA (1954) Hippocampal electrical activity in arousal. J Neurophysiol 17: 533-557.

Grenier F, Timofeev I, Steriade M (1998) Leading role of thalamic over cortical neurons during postinhibitory rebound excitation. Proc Natl Acad Sci U S A 95: 13929-13934.

Grenier F, Timofeev I, Steriade M (2001) Focal synchronization of ripples (80-200 Hz) in neocortex and their neuronal correlates. J Neurophysiol 86: 1884-1898.

Grenier F, Timofeev I, Steriade M (2003) Neocortical very fast oscillations (ripples, 80-200 Hz) during seizures: intracellular correlates. J Neurophysiol 89: 841-852.

Groenewegen HJ (1988) Organization of the Afferent Connections of the Mediodorsal Thalamic Nucleus in the Rat, Related to the Mediodorsal Prefrontal Topography. Neuroscience 24: 379-431.

Groenewegen HJ, Berendse HW (1994) The specificity of the "nonspecific" midline and intralaminar thalamic nuclei. Trends Neurosci 17: 52-57.

Herkenham M (1978) The connections of the nucleus reuniens thalami: evidence for a direct thalamo-hippocampal pathway in the rat. J Comp Neurol 177: 589-610.

Houser CR, Vaughn JE, Barber RP, Roberts E (1980) GABA neurons are the major cell type of the nucleus reticularis thalami. Brain Res 200: 341-354.

Huber R, Ghilardi MF, Massimini M, Tononi G (2004) Local sleep and learning. Nature 430: 78-81.

Hur EE, Zaborszky L (2005) Vglut2 afferents to the medial prefrontal and primary somatosensory cortices: a combined retrograde tracing in situ hybridization. J Comp Neurol 483: 351-373.

Irle E, Markowitsch HJ (1982) Widespread cortical projections of the hippocampal formation in the cat. Neuroscience 7: 2637-2647.

Izaki Y, Takita M, Nomura M, Akema T (2003) Differences between paired-pulse facilitation and long-term potentiation in the dorsal and ventral hippocampal CA1-prefrontal pathways of rats. Brain Res 992: 142-145.

Jay TM, Burette F, Laroche S (1995) NMDA receptor-dependent long-term potentiation in the hippocampal afferent fibre system to the prefrontal cortex in the rat. Eur J Neurosci 7: 247-250.

Jay TM, Glowinski J, Thierry AM (1989) Selectivity of the hippocampal projection to the prelimbic area of the prefrontal cortex in the rat. Brain Res 505: 337-340.

Jay TM, Thierry AM, Wiklund L, Glowinski J (1992) Excitatory Amino Acid Pathway from the Hippocampus to the Prefrontal Cortex. Contribution of AMPA Receptors in Hippocampo-prefrontal Cortex Transmission. Eur J Neurosci 4: 1285-1295.

Jay TM, Witter MP (1991) Distribution of hippocampal CA1 and subicular efferents in the prefrontal cortex of the rat studied by means of anterograde transport of Phaseolus vulgaris-leucoagglutinin. J Comp Neurol 313: 574-586.

Johnston D, Amaral DG (2004) Hippocampus. In: The Synaptic Organization of the Brain (Shepherd GM, ed), pp 455-498. New York: Oxford University Press.

Jones MS, Barth DS (1999) Spatiotemporal organization of fast (>200 Hz) electrical oscillations in rat Vibrissa/Barrel cortex. J Neurophysiol 82: 1599-1609.

Jones MS, MacDonald KD, Choi B, Dudek FE, Barth DS (2000) Intracellular correlates of fast (>200 Hz) electrical oscillations in rat somatosensory cortex. J Neurophysiol 84: 1505-1518.

Kandel A, Buzsaki G (1997) Cellular-synaptic generation of sleep spindles, spike-and-wave discharges, and evoked thalamocortical responses in the neocortex of the rat. J Neurosci 17: 6783-6797.

Kawaguchi Y, Kubota Y (1997) GABAergic cell subtypes and their synaptic connections in rat frontal cortex. Cereb Cortex 7: 476-486.

Kawaguchi Y, Kubota Y (1998) Neurochemical features and synaptic connections of large physiologically-identified GABAergic cells in the rat frontal cortex. Neuroscience 85: 677-701.

Kim JJ, Clark RE, Thompson RF (1995) Hippocampectomy impairs the memory of recently, but not remotely, acquired trace eyeblink conditioned responses. Behav Neurosci 109: 195-203.

Kocsis B, Vertes RP (1992) Dorsal raphe neurons: synchronous discharge with the theta rhythm of the hippocampus in the freely behaving rat. J Neurophysiol 68: 1463-1467.

Krettek JE, Price JL (1977a) Cortical Projections of Mediodorsal Nucleus and Adjacent Thalamic Nuclei in Rat. J Comp Neurol 171: 157-191.

Krettek JE, Price JL (1977b) Projections from the amygdaloid complex and adjacent olfactory structures to the entorhinal cortex and to the subiculum in the rat and cat. J Comp Neurol 172: 723-752.

Krimer LS, Goldman-Rakic PS (2001) Prefrontal microcircuits: membrane properties and excitatory input of local, medium, and wide arbor interneurons. J Neurosci 21: 3788-3796.

Kung JC, Shyu BC (2002) Potentiation of local field potentials in the anterior cingulate cortex evoked by the stimulation of the medial thalamic nuclei in rats. Brain Res 953: 37-44.

Laroche S, Davis S, Jay TM (2000) Plasticity at hippocampal to prefrontal cortex synapses: dual roles in working memory and consolidation. Hippocampus 10: 438-446.

Laroche S, Jay TM, Thierry AM (1990) Long-Term Potentiation in the Prefrontal Cortex Following Stimulation of the Hippocampal Ca1/Subicular Region. Neurosci Letters 114: 184-190.

Leonard CM (1969) Prefrontal Cortex of Rat .I. Cortical Projection of Mediodorsal Nucleus .2. Efferent Connections. Brain Res 12: 321-&.

Lewis DA, Melchitzky DS, Burgos GG (2002) Specificity in the functional architecture of primate prefrontal cortex. J Neurocytol 31: 265-276.

Lin RC, Nicolelis MA, Zhou HL, Chapin JK (1996) Calbindin-containing non-specific thalamocortical projecting neurons in the rat. Brain Res 711: 50-55.

Lipski J (1981) Antidromic activation of neurones as an analytic tool in the study of the central nervous system. J Neurosci Methods 4: 1-32.

Llinas R, Ribary U, Contreras D, Pedroarena C (1998) The neuronal basis for consciousness. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 353: 1841-1849.

Llinas RR, Grace AA, Yarom Y (1991) In vitro neurons in mammalian cortical layer 4 exhibit intrinsic oscillatory activity in the 10- to 50-Hz frequency range. Proc Natl Acad Sci U S A 88: 897-901.

Malenka RC, Nicoll RA (1999) Neuroscience - Long-term potentiation - A decade of progress? Science 285: 1870-1874.

Mansvelder HD, van Aerde KI, Couey JJ, Brussaard AB (2006) Nicotinic modulation of neuronal networks: from receptors to cognition. Psychopharmacol (Berl) 184: 292-305.

Maquet P (2001) The role of sleep in learning and memory. Science 294: 1048-1052.

Marshall L, Helgadottir H, Molle M, Born J (2006) Boosting slow oscillations during sleep potentiates memory. Nature 444: 610-613.

Martin SJ, Grimwood PD, Morris RG (2000) Synaptic plasticity and memory: an evaluation of the hypothesis. Annu Rev Neurosci 23: 649-711.

Martina M, Royer S, Pare D (2001) Propagation of neocortical inputs in the perirhinal cortex. J Neurosci 21: 2878-2888.

Massimini M, Huber R, Ferrarelli F, Hill S, Tononi G (2004) The sleep slow oscillation as a traveling wave. J Neurosci 24: 6862-6870.

Maviel T, Durkin TP, Menzaghi F, Bontempi B (2004) Sites of neocortical reorganization critical for remote spatial memory. Science 305: 96-99.

Mccormick DA, Connors BW, Lighthall JW, Prince DA (1985) Comparative electrophysiology of pyramidal and sparsely spiny stellate neurons of the neocortex. J Neurophysiol 54: 782-806.

McDonald RJ, White NM (1993) A triple dissociation of memory systems: hippocampus, amygdala, and dorsal striatum. Behav Neurosci 107: 3-22.

McGaugh JL (2000) Memory--a century of consolidation. Science 287: 248-251.

McKenna JT, Vertes RP (2004) Afferent projections to nucleus reuniens of the thalamus. J Comp Neurol 480: 115-142.

Meibach RC, Siegel A (1977) Thalamic projections of the hippocampal formation: evidence for an alternate pathway involving the internal capsule. Brain Res 134: 1-12.

Mennemeier M, Fennell E, Valenstein E, Heilman KM (1992) Contributions of the left intralaminar and medial thalamic nuclei to memory. Comparisons and report of a case. Arch Neurol 49: 1050-1058.

Milner B (1972) Disorders of learning and memory after temporal lobe lesions in man. Clin Neurosurg 19: 421-446.

Montpied P, Winsky L, Dailey JW, Jobe PC, Jacobowitz DM (1995) Alteration in levels of expression of brain calbindin D-28k and calretinin mRNA in genetically epilepsy-prone rats. Epilepsia 36: 911-921.

Morison RG, Dempsey CW (1943) Mechanisms of thalamocortical augmentation and repetition. Am J Physiol 138: 297-308.

Moscovitch M (1982) Multiple dissociations of function in amnesia. In: Human Memory and Amnesia (Cermak L, ed), pp 337-370. Hillsdale: Erlbaum.

Musil SY, Olson CR (1988) Organization of cortical and subcortical projections to medial prefrontal cortex in the cat. J Comp Neurol 272: 219-241.

Natsume K, Hallworth NE, Szgatti TL, Bland BH (1999) Hippocampal theta-related cellular activity in the superior colliculus of the urethane-anesthetized rat. Hippocampus 9: 500-509.

Nita DA, Cisse Y, Timofeev I, Steriade M (2007) Waking-sleep modulation of paroxysmal activities induced by partial cortical deafferentation. Cereb Cortex 17: 272-283.

Nowak LG, Azouz R, Sanchez-Vives MV, Gray CM, Mccormick DA (2003) Electrophysiological classes of cat primary visual cortical neurons in vivo as revealed by quantitative analyses. J Neurophysiol 89: 1541-1566.

Nunez A, Amzica F, Steriade M (1993) Electrophysiology of cat association cortical cells in vivo: intrinsic properties and synaptic responses. J Neurophysiol 70: 418-430.

Ohtake T, Yamada H (1989) Efferent Connections of the Nucleus Reuniens and the Rhomboid Nucleus in the Rat - An Anterograde Pha-l Tracing Study. Neurosci Res 6: 556-568.

Ottersen OP, Storm-Mathisen J (1984) GABA-containing neurons in the thalamus and pretectum of the rodent. An immunocytochemical study. Anat Embryol (Berl) 170: 197-207.

Pare D, Gaudreau H (1996) Projection cells and interneurons of the lateral and basolateral amygdala: distinct firing patterns and differential relation to theta and delta rhythms in conscious cats. J Neurosci 16: 3334-3350.

Pedemonte M, Pena JL, Velluti RA (1996) Firing of inferior colliculus auditory neurons is phase-locked to the hippocampus theta rhythm during paradoxical sleep and waking. Exp Brain Res 112: 41-46.

Pirot S, Glowinski J, Thierry AM (1995) Excitatory responses evoked in prefrontal cortex by mediodorsal thalamic nucleus stimulation: influence of anaesthesia. Eur J Pharmacol 285: 45-54.

Pitkanen A, Pikkarainen M, Nurminen N, Ylinen A (2000) Reciprocal connections between the amygdala and the hippocampal formation, perirhinal cortex, and postrhinal cortex in rat. A review. Ann N Y Acad Sci 911: 369-391.

Price JL, Amaral DG (1981) An autoradiographic study of the projections of the central nucleus of the monkey amygdala. J Neurosci 1: 1242-1259.

Qin YL, McNaughton BL, Skaggs WE, Barnes CA (1997) Memory reprocessing in corticocortical and hippocampocortical neuronal ensembles. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 352: 1525-1533.

Rasch B, Buchel C, Gais S, Born J (2007) Odor cues during slow-wave sleep prompt declarative memory consolidation. Science 315: 1426-1429.

Reep RL, Corwin JV, Hashimoto A, Watson RT (1987) Efferent connections of the rostral portion of medial agranular cortex in rats. Brain Res Bull 19: 203-221.

Ribak CE (1978) Aspinous and sparsely-spinous stellate neurons in the visual cortex of rats contain glutamic acid decarboxylase. J Neurocytol 7: 461-478.

Riley JN, Moore RY (1981) Diencephalic and brainstem afferents to the hippocampal formation of the rat. Brain Res Bull 6: 437-444.

Rinvik E, Ottersen OP, Storm-Mathisen J (1987) Gamma-aminobutyrate-like immunoreactivity in the thalamus of the cat. Neuroscience 21: 781-805.

Robbins TW (2005) Chemistry of the mind: neurochemical modulation of prefrontal cortical function. J Comp Neurol 493: 140-146.

Robertson RT, Kaitz SS (1981) Thalamic connections with limbic cortex. I. Thalamocortical projections. J Comp Neurol 195: 501-525.

Room P, Russchen FT, Groenewegen HJ (1985) Efferent connections of the prelimbic (area 32) and the infralimbic (area 25) cortices: An anterograde tracing study in the cat. J Comp Neurol 242: 40-55.

Rose JE, Woolsey CN (1948) Structure and Relations of Limbic Cortex and Anterior Thalamic Nuclei in Rabbit and Cat. J Comp Neurol 89: 279-347.

Sanchez-Vives MV, Mccormick DA (2000) Cellular and network mechanisms of rhythmic recurrent activity in neocortex. Nat Neurosci 3: 1027-1034.

Schacter DL (1985) Priming of old and new knowledge in amnesic patients and normal subjects. Ann N Y Acad Sci 444: 41-53.

Schutter DJ, van Honk J (2006) An electrophysiological link between the cerebellum, cognition and emotion: frontal theta EEG activity to single-pulse cerebellar TMS. Neuroimage 33: 1227-1231.

Schwartzkroin PA (1975) Characteristics of CA1 neurons recorded intracellularly in the hippocampal in vitro slice preparation. Brain Res 85: 423-436.

Schwartzkroin PA (1977) Further characteristics of hippocampal CA1 cells in vitro. Brain Res 128: 53-68.

Scoville WB, Milner B (1957) Loss of recent memory after bilateral hippocampal lesions. J Neurol Neurosurg Psychiatry 20: 11-21.

Siapas AG, Lubenov EV, Wilson MA (2005) Prefrontal phase locking to hippocampal theta oscillations. Neuron 46: 141-151.

Siapas AG, Wilson MA (1998) Coordinated interactions between hippocampal ripples and cortical spindles during slow-wave sleep. Neuron 21: 1123-1128.

Sirota A, Csicsvari J, Buhl D, Buzsaki G (2003) Communication between neocortex and hippocampus during sleep in rodents. Proc Natl Acad Sci U S A 100: 2065-2069.

Squire LR (1982) The neuropsychology of human memory. Annu Rev Neurosci 5: 241-273.

Squire LR (1992) Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. Psychol Rev 99: 195-231.

Stafstrom CE, Schwindt PC, Crill WE (1984) Repetitive firing in layer V neurons from cat neocortex in vitro. J Neurophysiol 52: 264-277.

Steriade M (1997) Synchronized activities of coupled oscillators in the cerebral cortex and thalamus at different levels of vigilance. Cereb Cortex 7: 583-604.

Steriade M (1999) Coherent oscillations and short-term plasticity in corticothalamic networks. Trends Neurosci 22: 337-345.

Steriade M (2003) The corticothalamic system in sleep. Front Biosci 8: d878-d899.

Steriade M (2004) Neocortical cell classes are flexible entities. Nat Rev Neurosci 5: 121-134.

Steriade M (2006) Grouping of brain rhythms in corticothalamic systems. Neuroscience 137: 1087-1106.

Steriade M, Amzica F (1998) Coalescence of sleep rhythms and their chronology in corticothalamic networks. Sleep Res Online 1: 1-10.

Steriade M, Amzica F, Neckelmann D, Timofeev I (1998a) Spike-wave complexes and fast components of cortically generated seizures. II. Extra- and intracellular patterns. J Neurophysiol 80: 1456-1479.

Steriade M, Contreras D, Curro DR, Nunez A (1993a) The slow (< 1 Hz) oscillation in reticular thalamic and thalamocortical neurons: scenario of sleep rhythm generation in interacting thalamic and neocortical networks. J Neurosci 13: 3284-3299.

Steriade M, Deschenes M, Domich L, Mulle C (1985) Abolition of spindle oscillations in thalamic neurons disconnected from nucleus reticularis thalami. J Neurophysiol 54: 1473-1497.

Steriade M, Llinas RR (1988) The functional states of the thalamus and the associated neuronal interplay. Physiol Rev 68: 649-742.

Steriade M, Mccormick DA, Sejnowski TJ (1993b) Thalamocortical oscillations in the sleeping and aroused brain. Science 262: 679-685.

Steriade M, Nunez A, Amzica F (1993c) A novel slow (< 1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. J Neurosci 13: 3252-3265.

Steriade M, Nunez A, Amzica F (1993d) Intracellular analysis of relations between the slow (< 1 Hz) neocortical oscillation and other sleep rhythms of the electroencephalogram. J Neurosci 13: 3266-3283.

Steriade M, Timofeev I (2003) Neuronal plasticity in thalamocortical networks during sleep and waking oscillations. Neuron 37: 563-576.

Steriade M, Timofeev I, Durmuller N, Grenier F (1998b) Dynamic properties of corticothalamic neurons and local cortical interneurons generating fast rhythmic (30-40 Hz) spike bursts. J Neurophysiol 79: 483-490.

Steriade M, Timofeev I, Grenier F (2001) Natural waking and sleep states: a view from inside neocortical neurons. J Neurophysiol 85: 1969-1985.

Steriade M, Timofeev I, Grenier F, Durmuller N (1998c) Role of thalamic and cortical neurons in augmenting responses and self-sustained activity: Dual intracellular recordings in vivo. J Neurosci 18: 6425-6443.

Stickgold R, James L, Hobson JA (2000) Visual discrimination learning requires sleep after training. Nat Neurosci 3: 1237-1238.

Su H.S., Bentivoglio M (1990) Thalamic mdline cell populations projecting to the nucleus accumbens, amygdala, and hippocampus in the rat. J Comp Neurol 297: 582-593.

Swanson LW (1981) A direct projection from Ammon's horn to prefrontal cortex in the rat. Brain Res 217: 150-154.

Swanson LW, Cowan WM (1975) Hippocampo-hypothalamic connections: origin in subicular cortex, not ammon's horn. Science 189: 303-304.

Takagishi M, Chiba T (1991) Efferent projections of the infralimbic (area 25) region of the medial prefrontal cortex in the rat: an anterograde tracer PHA-L study. Brain Res 566: 26-39.

Takehara K, Kawahara S, Kirino Y (2003) Time-dependent reorganization of the brain components underlying memory retention in trace eyeblink conditioning. J Neurosci 23: 9897-9905.

Takita M, Izaki Y, Jay TM, Kaneko H, Suzuki SS (1999) Induction of stable long-term depression in vivo in the hippocampal-prefrontal cortex pathway. Eur J Neurosci 11: 4145-4148.

Teixeira CM, Pomedli SR, Maei HR, Kee N, Frankland PW (2006) Involvement of the anterior cingulate cortex in the expression of remote spatial memory. J Neurosci 26: 7555-7564.

Thierry AM, Gioanni Y, Degenetais E, Glowinski J (2000) Hippocampo-prefrontal cortex pathway: anatomical and electrophysiological characteristics. Hippocampus 10: 411-419.

Tierney PL, Degenetais E, Thierry AM, Glowinski J, Gioanni Y (2004) Influence of the hippocampus on interneurons of the rat prefrontal cortex. Eur J Neurosci 20: 514-524.

Timofeev I, Bazhenov M (2005) Mechanisms and Biological Role of Thalamocortical Oscillations. In: Trends in Chronobiology Research (Columbus F, ed), pp 1-47. Nova Science Publishers.

Timofeev I, Contreras D, Steriade M (1996) Synaptic responsiveness of cortical and thalamic neurones during various phases of slow sleep oscillation in cat. J Physiol 494 (Pt 1): 265-278.

Timofeev I, Grenier F, Bazhenov M, Houweling AR, Sejnowski TJ, Steriade M (2002) Short- and medium-term plasticity associated with augmenting responses in cortical slabs and spindles in intact cortex of cats in vivo. Journal of Physiology-London 542: 583-598.

Timofeev I, Grenier F, Bazhenov M, Sejnowski TJ, Steriade M (2000a) Origin of slow cortical oscillations in deafferented cortical slabs. Cereb Cortex 10: 1185-1199.

Timofeev I, Grenier F, Steriade M (2000b) Impact of intrinsic properties and synaptic factors on the activity of neocortical networks in vivo. J Physiol Paris 94: 343-355.

Timofeev I, Steriade M (1996b) Low-frequency rhythms in the thalamus of intact-cortex and decorticated cats. J Neurophysiol 76: 4152-4168.

Timofeev I, Steriade M (1998) Cellular mechanisms underlying intrathalamic augmenting responses of reticular and relay neurons. J Neurophysiol 79: 2716-2729.

Tulving, E. How many memory systems are there? American Psychologist 40, 385-398. 1985.

Van der Werf YD, Witter MP, Groenewegen HJ (2002) The intralaminar and midline nuclei of the thalamus. Anatomical and functional evidence for participation in processes of arousal and awareness. Brain Res Rev 39: 107-140.

Van Groen T, Wyss JM (1990) Extrinsic projections from area CA1 of the rat hippocampus: olfactory, cortical, subcortical, and bilateral hippocampal formation projections. J Comp Neurol 302: 515-528.

Van Groen T, Wyss JM (1992) Connections of the retrosplenial dysgranular cortex in the rat. J Comp Neurol 315: 200-216.

Vanderwolf CH (1969) Hippocampal electrical activity and voluntary movement in the rat. Electroencephalogr Clin Neurophysiol 26: 407-418.

Vann SD, Brown MW, Aggleton JP (2000) Fos expression in the rostral thalamic nuclei and associated cortical regions in response to different spatial memory tests. Neuroscience 101: 983-991.

Vertes RP (1992) PHA-L analysis of projections from the supramammillary nucleus in the rat. J Comp Neurol 326: 595-622.

Vertes RP (2002) Analysis of projections from the medial prefrontal cortex to the thalamus in the rat, with emphasis on nucleus reuniens. J Comp Neurol 448: 53-101.

Vertes RP (2006) Interactions among the medial prefrontal cortex, hippocampus and midline thalamus in emotional and cognitive processing in the rat. Neuroscience 142: 1-20.

Vertes RP, Crane AM, Colom LV, Bland BH (1995) Ascending projections of the posterior nucleus of the hypothalamus: PHA-L analysis in the rat. J Comp Neurol 359: 90-116.

Vertes RP, Hoover WB, Do Valle AC, Sherman A, Rodriguez JJ (2006) Efferent projections of reuniens and rhomboid nuclei of the thalamus in the rat. J Comp Neurol 499: 768-796.

Vertes RP, Hoover WB, Szigeti-Buck K, Leranth C (2007) Nucleus reuniens of the midline thalamus: link between the medial prefrontal cortex and the hippocampus. Brain Res Bull 71: 601-609.

Vertes RP, Kocsis B (1997) Brainstem-diencephalo-septohippocampal systems controlling the theta rhythm of the hippocampus. Neuroscience 81: 893-926.

Vinogradova OS, Brazhnik ES (1977) Neuronal aspects of septo-hippocampal relations. Ciba Found Symp 145-177.

Volgushev M, Chauvette S, Mukovski M, Timofeev I (2006) Precise long-range synchronization of activity and silence in neocortical neurons during slow-wave oscillations [corrected]. J Neurosci 26: 5665-5672.

von Krosigk M, Bal T, Mccormick DA (1993) Cellular mechanisms of a synchronized oscillation in the thalamus. Science 261: 361-364.

Wickelgren WA (1979) Chunking and consolidation: a theoretical synthesis of semantic networks, configuring in conditioning, S--R versus congenitive learning, normal forgetting, the amnesic syndrome, and the hippocampal arousal system. Psychol Rev 86: 44-60.

Williams GV, Goldman-Rakic PS (1995) Modulation of memory fields by dopamine D1 receptors in prefrontal cortex. Nature 376: 572-575.

Wiltgen BJ, Brown RA, Talton LE, Silva AJ (2004) New circuits for old memories: the role of the neocortex in consolidation. Neuron 44: 101-108.

Winocur G (1990) Anterograde and retrograde amnesia in rats with dorsal hippocampal or dorsomedial thalamic lesions. Behav Brain Res 38: 145-154.

Winsky L, Montpied P, Arai R, Martin BM, Jacobowitz DM (1992) Calretinin distribution in the thalamus of the rat: immunohistochemical and in situ hybridization histochemical analyses. Neuroscience 50: 181-196.

Wouterlood FG, Saldana E, Witter MP (1990) Projection from the nucleus reuniens thalami to the hippocampal region: light and electron microscopic tracing study in the rat with the anterograde tracer Phaseolus vulgaris-leucoagglutinin. J Comp Neurol 296: 179-203.

Wyss JM, Swanson LW, Cowan WM (1979) A study of subcortical afferents to the hippocampal formation in the rat. Neuroscience 4: 463-476.

Wyss JM, Swanson LW, Cowan WM (1980) The organization of the fimbria, dorsal fornix and ventral hippocampal commissure in the rat. Anat Embryol (Berl) 158: 303-316.

Wyss JM, Van Groen T (1992) Connections between the retrosplenial cortex and the hippocampal formation in the rat: a review. Hippocampus 2: 1-11.

Xu ZC, Pulsinelli WA (1996) Electrophysiological changes of CA1 pyramidal neurons following transient forebrain ischemia: an in vivo intracellular recording and staining study. J Neurophysiol 76: 1689-1697.

Yanagihara M, Niimi K, Ono K (1987) Thalamic projections to the hippocampal and entorhinal areas in the cat. J Comp Neurol 266: 122-141.

Yanagihara M, Ono K, Niimi K (1985) Thalamic projections to the hippocampal formation in the cat. Neurosci Letters 61: 35.

Yang CR, Seamans JK, Gorelova N (1996) Electrophysiological and morphological properties of layers V-VI principal pyramidal cells in rat prefrontal cortex in vitro. J Neurosci 16: 1904-1921.

Ylinen A, Bragin A, Nadasdy Z, Jando G, Szabo I, Sik A, Buzsaki G (1995) Sharp wave-associated high-frequency oscillation (200 Hz) in the intact hippocampus: network and intracellular mechanisms. J Neurosci 15: 30-46.

Zola-Morgan S, Squire LR (1990) The neuropsychology of memory. Parallel findings in humans and nonhuman primates. Ann N Y Acad Sci 608: 434-450.

Zola-Morgan S, Squire LR, Amaral DG (1986) Human amnesia and the medial temporal region: enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus. J Neurosci 6: 2950-2967.