Generation and modulation of network oscillations in the rodent prefrontal cortex in vitro

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Thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

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ABSTRACT

Fast network oscillations (~12-80 Hz) are recorded extensively in the mammalian cerebral cortex *in vivo*. They are thought to subserve the neuronal mechanism by which local and distant neuronal populations orchestrate their firing activity to process cognitive-related information. The rat medial prefrontal cortex (mPFC) is considered to be functionally and anatomically homologous to the primate prefrontal cortex. Earlier *in vitro* studies have demonstrated that the mPFC can sustain carbachol-induced persistent beta1 or kainate-induced transient low gamma frequency oscillations.

We wished to establish an in vitro paradigm of carbachol (10 μM) / kainate (200 nM)-induced persistent fast network oscillations in the rat mPFC with the objective to investigate the distribution patterns and the mechanisms of these oscillations. Then we assessed the modulatory effects of the ascending catecholamine systems on fast network oscillations with exogenous application of dopamine and noradrenaline.

Persistent fast network oscillations in the ventral mPFC were stronger, more rhythmic but slower (~25 Hz) than oscillations in the dorsal mPFC (~28 Hz). The regional difference in the oscillation amplitude was correlated to the strong presence of bursting cells in the ventral but not the dorsal regions. Across all regions in the mPFC, oscillations were stronger in layer 5. Oscillations relied on $GABA_A$, kainate but not AMPA receptors. In the ventral mPFC, network oscillations were also dependent on NMDA receptor-mediated synaptic transmission.

Dopamine (100 μM) reduced the oscillation strength and rhythmicity in the ventral mPFC. Instead, dopamine increased the power and rhythmicity of network oscillations in the dorsal mPFC. The region-dependent dopamine effect was correlated to the induced effects on synaptic inhibition and neuronal firing. Noradrenaline (30 μM) reduced the oscillation strength in the ventral mPFC, but caused no effect on the dorsal mPFC.

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ABBREVIATIONS

AC	Autocorrelation
ACd	Dorsal anterior cingulate cortex
ACh	Acetylcholine
AHP	After-hyperpolarization
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole propionate
ANOVA	Analysis of Variance
AP	Action potential
cAMP	Cyclic adenosine monophosphate
Ch	Carbachol
DA	Dopamine
DAP	Depolarizing after-potential
DLPFC	Dorsolateral prefrontal cortex
DMSO	(s)-dimethylsulfoxide
DP	Dorsal peduncular cortex
EEG	Electroencephalography
EPSP	Excitatory post-synaptic potential
FFT	Fast Fourier transform
FIR	Finite impulse response
FS	Fast spiking
GABA	γ-Aminobutyric acid
IB	Intrinsically bursting cells
IL	Infralimbic cortex
ING	Interneuron network gamma
IPSC	Inhibitory post-synaptic current
IPSP	Inhibitory post-synaptic potential
IQR	Interquartile
ISI	Inter-spike interval
KA	Kainic acid; Kainate
LC	Locus coeruleus
LFP	Local field potential
LTP	Long term potentiation
LTS	Low threshold spike
MD	Mediodorsal nucles of the thalamus
mGluR	metabotropic gluatame receptor
mIPSC	miniature inhibitory post-synaptic current

mPFC	medial prefrontal cortex
NA	Noradrenaline
NMDA	N-methyl-D-aspartate
NRI	Neuronal rhythmicity index
PCA	Principal components analysis
PFA	Paraformaldehyde
PFC	Prefrontal cortex
PING	Pyramidal-interneuron network gamma
PrL	Prelimbic cortex
PSD	Power spectral density
PV	Parvalbumin
PV+	Parvalbumin-immunopositive
RI	Rhythmicity index
RM-ANOVA	Repeated Measures - Analysis of Variance
ROB	Repetitive oscillatory bursting cells
RS	Regular spiking cells
rHTS	regenerative high threshold spike
SD	Standard deviation
sIPSC	spontaneous inhibitory post-synaptic current
TTX	Tetrodotoxin
VTA	Ventral tegmental area
WM	Working memory

Chapter1

General Introduction

Chapter 1 – General Introduction

1.1. The mammalian prefrontal Cortex: anatomy and function

The inherent complexity of the mammalian brain anatomy, neurochemistry and connectivity, makes research regarding functional localization and subregional dissociation extremely difficult. Nevertheless, there is an increasing body of evidence highlighting the major contribution of the prefrontal cortex (PFC) in a dynamic neuronal network which controls intelligent, self-regulating behaviours (Fuster 2001; Goldman-Rakic 2011; Miller and Wallis 2008).

The primate PFC is the anterior-most cortical structure of the frontal lobe separated by the premotor and supplementary motor areas from the rest of the cerebral cortex (Fuster 2001; Goldman-Rakic 2011). It constitutes almost one third of the cortical surface and its expansion during primate evolution has been remarkable (Goldman-Rakic 2011). The primate PFC consists mainly of the medial, orbital and lateral areas. On the basis of functional and anatomical criteria it has been hypothesized that the orbital and medial cortical areas may influence emotional behaviour by processing the emotional quotient of sensory stimuli (Fuster 2001). The dorsolateral region of the primate PFC (DLPFC) has been functionally linked to the representation of mnemonic components of executive function, including working memory (Fuster 2001; Goldman-Rakic 2011).

The rat PFC, like the primate PFC, is organized in the medial, ventral/orbital and lateral areas. Functionally, the rat medial PFC (mPFC) presents many similarities with the primate PFC. Behavioural studies in rats have suggested that the mPFC, like the primate PFC, is involved in attentional processing, working memory, goal-directed behaviour and behavioural flexibility (Fujisawa et al. 2008; Gisquet-Verrier et al. 2000; Hoover and Vertes 2007; Killcross and Coutureau 2003; Totah et al. 2013; Vertes 2006).

Moreover, anatomical comparison studies have found similar connectivity patterns of thalamo-cortical, PFC-basal ganglia and cortico-cortical pathways between primates and rats. Strong projections originating in the mediodorsal (MD) nucleus of the thalamus have been reported in the primate PFC and the rat mPFC (Dalley et al. 2004; Goldman-Rakic 2011; Hoover and Vertes 2007; Kesner 2000;

Krettek and Price 1977; Uylings et al. 2003). Both the primate and rat PFC receive strong innervation from basal-ganglia structures and multimodal cortical sources (Uylings et al. 2003), as well as dopaminergic, noradrenergic and cholinergic nuclei (Arnsten 2011; Bentley et al. 2011; Sawaguchi and Goldman-Rakic 1994).

1.2. The rat medial Prefrontal cortex

The rat mPFC is a heterogeneous structure. The dorsal portion of the mPFC includes the rostral one third of the dorsal anterior cingulate (ACd) and the prelimbic (PrL) cortex (Figure 1.1A,B; Gabbott and Bacon 1995; Hoover and Vertes 2007; Jones et al. 2005; Kesner 2000; Krettek and Price 1977; van Eden and Uylings 1985). The ventral portion of the mPFC comprises the infralimbic (IL) cortex (Gabbott and Bacon 1995; Hoover and Vertes 2007; Jones et al. 2005; Kesner 2000; Krettek and Price 1977; van Eden and Uylings 1985) which is further subdivided into the IL (dorsal subdivision) and the dorsal peduncular cortex (DP; ventral subdivision; Gabbott et al. 2007; Jones et al. 2005; Paxinos 1998).

The delineation of the different subdivisions of the mPFC is predominantly based upon their distinctive laminar features (Figure 1.2 and 1.3; Krettek and Price 1977; van Eden and Uylings 1985). Both the ACd and the PrL regions have clear laminar hallmarks. Their cytoarchitecture is characterized by a clear transition from layer 1, which is devoid of cells, to a densely packed with cells layer 2. Layer 3 is broad and lightly stained with cells. Layer 5 in the ACd region is significantly broader compared to the PrL region. This sudden broadening of layer 5 is considered the transition point from the PrL to the ACd region (Krettek and Price 1977; van Eden and Uylings 1985).

In contrast the laminar hallmarks of the ventral portion of the mPFC are less clear. This is because layer 3 is thinner in the IL region and in the DP region it is almost indistinguishable from layer 2. The sudden narrowing of layer 3 is considered the transition point from the PrL to the IL region (Gabbott et al. 1997).

Unlike the primate PFC, the rat PFC lacks a granular layer 4 (Krettek and Price 1977; van Eden and Uylings 1985). In the majority of anatomical and behavioural

Figure 1.1. The rat mPFC

Figure 1.2. Cytoarchitecture of the rat mPFC

Figure 1.3. Cytoarchitecture of the rat anterior cingulate and the motor cortex

studies the division of the ventral mPFC into the IL and DP regions was not taken into account, therefore, in the Introduction chapter we will refer to the ventral mPFC as the IL.

1.2.1. *Cellular and chemical distribution in the rat medial PFC*

Most of the evidence for the laminar organization of pyramidal cells in the mPFC arises from retrograde/anterograde tracing studies and intracellular recordings coupled with biocytin injection. The pyramidal cell population is distributed into layers 2-6, with the highest densities in layers 3 and 5. The apical dendrites of neurons with cell bodies in layers 3-6 converge to layers 1 and 2 (Gabbott 2003; Gabbott et al. 2005; Jones et al. 2005; Yang et al. 1996a).

The rat mPFC contains a rich diversity of inhibitory interneurons that use γ-aminobutyric acid (GABA) as a neurotransmitter, including those expressing the calcium-binding protein parvalbumin (PV; Jones et al. 2005; Kawaguchi and Kondo 2002; Perez-Cruz et al. 2007). Staining mPFC sections with an antibody for PV revealed a heterogeneous distribution of this protein. Although the ACd, PrL and DP regions are densely stained for PV, the IL region expresses very lightly this protein (Figure 1.1.C; Jones et al. 2005). Moreover, layer 1 in the mPFC is devoid of PV-immunopositive (PV+) cells, whereas layer 5 displays the highest density (Jones et al. 2005).

1.2.2. *Cortico-cortical connectivity in the rat medial PFC*

Anterograde tracing techniques have been extensively applied to investigate the distribution of neural projections within the rat mPFC. They have shown that cortico-cortical connections within the mPFC are mostly shared within and between adjacent regions (Fisk and Wyss 1999; Jones et al. 2005). Therefore, anterograde tracer injections in the DP region labelled axons with synaptic terminals to the adjacent IL region. The IL region instead, was shown to send dense projections to the ventral region of the PrL but sparsely to the DP region. The PrL region was shown to project densely to the IL and the ACd but sparsely to the DP region. Finally, the ACd region was reported to share dense projections with the premotor cortex and the PrL region (especially the dorsal PrL). Cortico-cortical

connections in the mPFC were shared mostly within the deep layers (Fisk and Wyss 1999; Jones et al. 2005).

1.2.3. *The anatomical and functional dissociation of the rat medial PFC*

Substantial evidence from anatomical and behavioural studies suggest that the rat mPFC-mediated cognitive functions are sustained by its different but mutually interconnected subdivisions.

The ACd shares strong connections with sensorimotor systems via thalamic or cortico-cortical pathways indicating its contribution to sensorimotor control (Gisquet-Verrier et al. 2000; Kesner 2000). It has been shown that discrete lesions in the ACd reduce discriminative accuracy as a result of impairment in temporal processing of sensorimotor information (Chudasama et al. 2003; Gisquet-Verrier et al. 2000; Kesner et al. 1996).

In contrast, the PrL and IL regions receive less dense cortical innervation. The majority of the afferent projections to these areas originate in the limbic system including the amygdala, nucleus accumbens, hippocampus, entorhinal cortex, brainstem monoaminergic and basal forebrain nuclei (Gisquet-Verrier et al. 2000; Hoover and Vertes 2007; Kesner 2000; Vertes 2006).

Combined behavioural and lesion studies have further delineated the functional dissociation of the PrL and IL regions. Discrete lesions in the PrL have shown that this region sustains goal-directed behaviour during early stages of learning, when the causal relationship between the action and the goal are evaluated (Killcross and Coutureau 2003; Marquis et al. 2007). As training proceeds, goal-directed behaviour is progressively replaced by a habitual responding, when the stimulus presentation elicits a response irrespective of the goal value. This habitual behaviour is seriously impaired following IL lesions (Killcross and Coutureau 2003; Marquis et al. 2007). Moreover, other behavioural studies have shown that IL lesions elicit perseverative responses; a strong indication of impulsive behaviour (Chudasama et al. 2003; Murphy et al. 2005).

1.3. The functional importance of fast network oscillations

Rhythmic activity is ubiquitous in electroencephalography (EEG) recordings exhibiting synchronized patterns of electrical activity that range from 0.5 Hz up to 600 Hz, and are defined in distinct, partially overlapping, frequency bands that reflect different arousal and behavioural states (Buzsaki 2006; Whittington et al. 2000). The main spectral bands are delta (0.5-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), beta (12-30 Hz) and gamma (30-80 Hz). Beta frequency oscillations can be subdivided further into beta1 (12-20 Hz) and beta2 (20-30Hz) bands (Roopun et al. 2008b).

Oscillatory frequency of EEG activity is increased with increased levels of arousal. Delta frequency oscillations are prominent during periods of deep sleep and anaesthesia (Steriade et al. 1993). Alpha oscillations are present during relaxed wakefulness (Niedermeyer 1997; Uhlhaas et al. 2008) whereas theta oscillations are superimposed with gamma frequency oscillations in the hippocampal formation and the surrounding structures during locomotion and spatial navigation (Bragin et al. 1995; Buzsaki 2006).

Beta and gamma frequency oscillations are recorded *in vivo* either concurrently or separately. In EEG studies they are generated during different cognitive tasks and they can be classified as either *"evoked"* or *"induced"*, based on their temporal relation to the stimulus onset (Pantev 1995; Rodriguez et al. 1999; Tallon-Baudry et al. 1999). Evoked oscillations arise within the first ~100 msec after the stimulus onset and they are thought to reflect the synchronous activity of neural assemblies that are bound to the stimulus (Pantev 1995; Rodriguez et al. 1999). The so-called induced oscillatory activity arises later (~600-800 ms) and is not time-locked to the stimulus onset. These oscillations are thought to reflect the activation of an internal object representation (Tallon-Baudry et al. 1999).

1.3.1. *Gamma and beta oscillations in sensory processing*

Seminal multiunit and field recording studies in the visual cortex of anesthetized and awake cats, demonstrated that neuronal assemblies within or across spatially separate cortical columns, can synchronize their firing activity in a millisecond range in response to light stimuli (Engel et al. 1991; Gray et al. 1989;

Gray and Singer 1989). Synchronous activity was periodic, oscillating within the beta and gamma frequency bands (20-70 Hz), and was not driven by oscillatory thalamic input (Engel et al. 1991; Gray and Singer 1989). This finding gave rise to the "feature binding hypothesis", according to which the different features of sensory inputs are fragmented and processed individually by their dedicated cortical neuronal ensembles, the outcome of which is then integrated together to produce a unified perception of the presented stimulus. This neuronal process requires a fine temporal coordination (i.e. binding) of neural firing between different neuronal groups, and synchronous oscillatory activity is thought to provide the appropriate physiological framework for this process (Engel et al. 1991; Singer 1999).

Following the introduction of the feature binding hypothesis, physiological and neuronal communication theories were developed to emphasize the functional significance of neuronal oscillations in information processing. Initially, the concept of "feed forward coincidence detection" was introduced, according to which the excitatory influence of stimulus-activated groups of neurons is stronger and more likely to induce a post-synaptic response if their efferent outputs converge into the target groups of neurons within a small time window. This in turn postulates that neurons at the sending group should fire action potentials in synchrony, and this is exactly what happens when neurons are engaged in fast network oscillations and thereby undergo rhythmic fluctuations of membrane excitability, driven by rhythmic synaptic inhibition (See section 1.4; König et al. 1996; Singer 1999).

However, the efficacy of neuronal communication does not rely only on the synchronized firing activity of the sending group of neurons, but it also depends on the sensitivity of the receiving group to the incoming excitatory influence. The impact of converging synaptic excitation will be determined by the excitability state of the post-synaptic cells. Accordingly, converging excitatory inputs are likely to trigger a post-synaptic response if they coincide with the peak excitability of the target cells. Otherwise, excitation will be suppressed by the presence of strong synaptic inhibition and it will not elicit a response (Fries 2005, 2009). The concept of excitatory input gain modulation by synaptic inhibition has been encompassed in the "communication-through-coherence" hypothesis which suggests that two

neuronal groups can communicate effectively when they both oscillate phase-locked at the same frequency (Fries 2005). The coherence hypothesis provides also a functional link for flexible and selective neuronal communication between spatially distributed neuronal groups. It has been found experimentally in the visual cortex that neuronal groups activated by attended stimuli, synchronize their firing activity at the gamma-band more precisely than neuronal groups activated by unattended stimuli (Fries et al. 2001). In another study it was shown that neuronal groups in the higher visual areas bias their response to the attended stimulus, when a pair of stimuli is presented within their receptive field (Reynolds et al. 1999). In agreement with these results, the coherence hypothesis predicts that if both extrastriate neuronal groups provide converging and competing inputs to higher visual areas, then the receiving group will tend to phase-lock its response to the more synchronous attended input rather than the unattended one. Therefore, neuronal communication through coherent fast network oscillations could provide the appropriate framework of functional and selective neuronal communication which in turn may account for cognitive flexibility (Fries 2005, 2009).

An array of psycho-physiological experiments in human and non-human subjects suggests that fast network oscillations can coordinate complex behaviours that require the engagement of various cortical areas other than the extrastriate cortex. The predominance of beta frequency oscillations in visual, parietal and motor cortical areas in the cat brain was a characteristic feature of cortical activity in a visuomotor task which required sensory processing, attentive filtering and voluntary movement (Roelfsema et al. 1997). Strong, zero time-lag synchrony at the beta frequency range (20-25 Hz) was evident among these widely separated cortical areas, during the different behavioural phases of this visuomotor task. The strength of synchrony between the various cortical areas was altered in response to the functional demand of the different phases of the task. Therefore, during the attentive task phase when the animal anticipated the visual stimulus onset, synchrony was increased between the primary visual cortex and the parietal cortex. During the task phase when the visual stimulus changed direction, synchrony enhancement occurred only between the visual areas (Roelfsema et al. 1997).

Later human EEG studies made use of the cross frequency phase-synchrony method (Lachaux et al. 1999) to investigate the long range synchrony of induced oscillatory activity in a visual sensory task in which subjects were presented with Mooney faces (Rodriguez et al. 1999; Uhlhaas et al. 2006). Both studies confirmed that the synchrony of induced beta and gamma frequency oscillations (20-60 Hz) was significantly elevated in the “face perception” rather than in the “no face perception” trials. In one study the elevated synchrony was observed between the motor, parietal and occipital cortices (Rodriguez et al. 1999), whereas in the other synchrony was evident between the frontal, parietal and occipital cortices (Uhlhaas et al. 2006).

1.3.2. *Gamma and beta frequency oscillations in short-term memory*

A series of publication studies by Tallon-Baudry and colleagues (Tallon-Baudry et al. 1999; Tallon-Baudry et al. 2001; Tallon-Baudry et al. 2004) have highlighted the functional importance of gamma and beta frequency oscillations in visual short-term memory tasks. It has been consistently reported that during visual delayed match-to-sample tasks, gamma and beta frequency oscillations were induced and sustained during the delay period of the tasks in the occipital and frontal cortical areas of the human brain (Tallon-Baudry et al. 1999; Tallon-Baudry et al. 2001) and the posterior inferior temporal cortex of the non-human primate brain (Tallon-Baudry et al. 2004).

Moreover, the same visual short-term memory task studies showed that the synchrony strength of induced beta frequency oscillations is associated with the performance of the task (Tallon-Baudry et al. 2001; Tallon-Baudry et al. 2004). Therefore, when the monkey's response to the repetition of the same stimulus was correct, synchrony within distant sites of the posterior inferior temporal cortex was elevated. In contrast, synchrony was absent in error trials (Tallon-Baudry et al. 2004). In human EEG studies which used an equivalent visual short-term memory task (Tallon-Baudry et al. 1999; Tallon-Baudry et al. 2001), performance deteriorated, with increasing delay period (Tallon-Baudry et al. 1999). In parallel with that, the amplitude of induced gamma frequency oscillations in the occipital area, and the beta frequency oscillations in the frontal areas, slowly decreased with increasing delay period (Tallon-Baudry et al. 1999). Synchrony was abolished in

behavioural conditions with no memory retention requirements (Tallon-Baudry et al. 2001).

These findings suggest that gamma and beta frequency oscillations could provide the appropriate framework for the dynamic coordination of distributed neural activity, within and between sensory processing and frontal cortical areas that mediates sort-term memory retention of the object representation in the absence of the sensory input (Tallon-Baudry et al. 1999; Tallon-Baudry et al. 2001; Tallon-Baudry et al. 2004).

1.3.3. *Gamma frequency oscillations in working memory*

Working memory (WM) is a limited memory capacity system, triggered when the representation of previously encountered stimuli is required to be held "on-line" in order to organize and perform the appropriate motor response (Baddeley 2003; Goldman-Rakic 1995). According to Baddeley, this important cognitive process, "*supports human thought processes by providing an interface between perception, long-term memory and action*" (Baddeley 2003). Most WM theories engage attentional resources that filter out irrelevant information that could interfere with the individual's thought process (Baddeley 2003). Evidence from non-human primate anatomical and electrophysiological studies supports the theory that WM depends on the activity of different cortical areas including the prefrontal and parietal cortex (Goldman-Rakic 1995; Pesaran et al. 2002).

In two recent human EEG studies, it was shown that the amplitude of evoked and induced gamma frequency oscillations, gradually increased with increases in WM load in the occipital, parietal and frontal cortex of healthy subjects (Basar-Eroglu et al. 2007; Howard et al. 2003). Elevated oscillatory activity at gamma frequency range has also been recorded in dynamic memory fields of the parietal cortex in the macaque brain during WM performance (Pesaran et al. 2002).

1.3.4. *Beta frequency oscillations in sensorimotor processing*

Beta frequency oscillations are a prominent feature of the sensorimotor cortical activity in human and non-human primates and are functionally associated with voluntary motor behaviour (Brovelli et al. 2004; Murthy and Fetz 1992; Salenius and Hari 2003; Sanes and Donoghue 1993).

Beta frequency oscillations have been recorded at different sites in the sensorimotor cortex in non-human primates during motor tasks that required attentiveness, sensory integration, preparation and execution. During these tasks, oscillations appeared intermittently, in short epochs, lasting a few cycles (<1 sec) or several cycles (~10 sec; Murthy and Fetz 1992; Sanes and Donoghue 1993).

Beta frequency oscillations are suggested to support large-scale network synchrony between separate regions in the sensorimotor cortex. In one study, synchronous network activity between motor and sensory cortical areas in the primate brain was evident and decreased with distance. The longest distance between motor and sensory cortical sites that revealed synchronous field and unit activity was 20 mm (Murthy and Fetz 1992). Another EEG study, used Granger causality analysis to demonstrate that motor cortical oscillatory activity was modulated by input from the sensory cortex (Brovelli et al. 2004). Strong synchronous beta frequency oscillatory activity has also been observed between premotor and primary motor areas (Sanes and Donoghue 1993).

The sensorimotor cortex is composed of different cortical areas which play different roles in the analysis of sensorimotor information (Sanes and Donoghue 1993). The somatosensory cortex is important in sensory processing and somatosensory integration, the premotor cortex is engaged to motor planning and the primary motor cortex is related to the execution of voluntary movement (Brovelli et al. 2004; Sanes and Donoghue 1993). It has been hypothesized, in analogy to the feature binding process in the visual cortex (Engel et al. 1991; Singer 1999), that small-scale beta frequency oscillations could provide the neural substrate for the processing of different aspects of sensorimotor information at a local scale. According to the same scheme, large-scale synchrony at the beta frequency range could provide the temporal dynamic link where input from the sensory cortex could influence motor planning and execution to produce a unified motor behaviour (Sanes and Donoghue 1993).

1.4. *In vitro* models of fast network oscillations

Over the last two decades many *in vitro* models of fast network oscillations have been demonstrated in the hippocampal formation, entorhinal, sensorimotor and prefrontal cortex (Buhl et al. 1998; Cunningham et al. 2003; Fisahn et al. 1998; McNally et al. 2011; van Aerde et al. 2008; Whittington et al. 1995; Yamawaki et al. 2008).

The generation of *in vitro* fast network oscillations has been based on the pharmacological activation of cholinergic (Fisahn et al. 1998; van Aerde et al. 2008), metabotropic glutamate (Whittington et al. 1995) and ionotropic kainate receptors (Cunningham et al. 2003; McNally et al. 2011; Roopun et al. 2006), or the application of electrical/tetanic stimuli (Whittington et al. 1995). Oscillations can last for hundreds of milliseconds (transient; McNally et al. 2011; Whittington et al. 1995), minutes or even hours (persistent; Buhl et al. 1998; Cunningham et al. 2003; Fisahn et al. 1998; Roopun et al. 2006; van Aerde et al. 2008; Yamawaki et al. 2008). Although they cannot be directly associated with defined cognitive processes, *in vitro* models of network oscillations allow, by pharmacological and electrical means, the investigation of the neural mechanisms that generate and maintain population rhythms. Moreover, they prove that archicortical and neocortical neural circuits possess the properties that allow them to generate and sustain network oscillations without oscillatory input from subcortical structures.

1.4.1. *Gamma frequency oscillations in vitro*

The first study to generate *in vitro* gamma frequency oscillations in the CA1 area of the rat hippocampus and the superficial layers of the neocortex revealed the existence of a tonically activated small oscillating network of pyramidal cells and interneurons (Whittington et al. 1995). It was shown that administration of glutamate or metabotropic glutamate-receptor (mGluR) agonists could induce slow but long-lasting depolarizing effects on interneurons and pyramidal cells along with superimposed trains of inhibitory post-synaptic potentials (IPSPs) in both cell types. In the same study, rhythmic trains of IPSPs were unaffected by blockade of excitatory ionotropic glutamatergic transmission, suggesting that a network of tonically depolarized interconnected interneurons could sustain population oscillatory activity at the gamma frequency range (interneuron

network gamma; ING). The ING model was shown to depend on the $GABA_A$-receptor synaptic transmission, since blockade of this fast synaptic inhibition abolished network oscillations (Whittington et al. 1995).

Considerable evidence now suggests that a specific type of interneuron can support the generation of fast network oscillations; the PV+, fast spiking basket cells (Whittington and Traub 2003). Basket cells can exert strong synaptic inhibition, since they form inhibitory synapses with the proximal dendrites and the soma of pyramidal cells and other interneurons (Gonzalez-Burgos and Lewis 2008). Moreover, these cells have a peak impedance that favours inputs at the gamma frequency range (Whittington and Traub 2003). These interneurons are fast spiking cells, firing on average at a rate higher than gamma frequencies (>100 Hz; Kawaguchi and Kubota 1996; McCormick et al. 1985). Nevertheless, mutual inhibition shapes their firing rate to the gamma frequency range because when a neuron receives an IPSP, the highest probability of an action potential (AP) to occur is at the end of the IPSP decay phase (~25 ms). When enough interconnected interneurons are depolarized concurrently, then the mutual inhibition will force them progressively to fire successive APs at a frequency governed by the IPSP decay time, resulting in synchronous population firing activity (Whittington et al. 2000).

The frequency of oscillations relies on the IPSP kinetics and the excitation of interneurons. The first claim is based on evidence that an increase in the decay time of IPSPs by pharmacological means (i.e. barbiturates), leads to a decline in the frequency of oscillations (Whittington et al. 1995). The second claim is based on a network simulation study by Traub and colleagues (Traub et al. 1996) which showed that the level of excitation of the interneuron network can affect the frequency of oscillations. In principle, for interneurons to support fast network oscillations, they should be excited to the degree that when they are uncoupled from the network, they fire faster than the overall network frequency. As the excitation increases to the optimal level, then network oscillations could reach ~80 Hz. As the excitation decreases, then oscillations could become as slow as ~15 Hz. Excitation that could exceed those frequency limits cannot support network oscillations. Therefore, tonic excitation of interneurons can support beta and gamma frequency oscillations (Traub et al. 1996).

ING networks are considered an artificial condition since isolated oscillatory networks of interconnected interneurons do not exist *in vivo*. Instead, the interneuron network establishes reciprocal connections with excitatory pyramidal cells, leading to the so-called pyramidal-interneuron network gamma (PING) (Whittington et al. 2000). The PING model was initially demonstrated in hippocampal (Fisahn et al. 1998) and somatosensory cortical (Buhl et al. 1998) slice preparations via application of carbachol (Ch), or mixed application of carbachol/kainate, respectively. Carbachol is a cholinergic receptor agonist, whereas kainate (kainic acid; KA) binds and activates kainate and α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors (Seeburg 1993).

The PING model relies on the interplay of three types of phasic synaptic interaction: (i) interneuron-to-interneuron cell inhibition, (ii) interneuron-to-pyramidal cell inhibition and (iii) pyramidal-to-interneuron cell excitation (Whittington et al. 2000). According to the PING model, pyramidal cells establish excitatory synapses with other pyramidal cells and interneurons. Interneurons, in turn, establish inhibitory connections with other interneurons and pyramidal cells. Network oscillatory activity emerges from the interplay of periodically alternating inhibition and disinhibition phases. During the inhibition phase, interneuron firing prevails, suppressing pyramidal cells from reaching firing threshold. When interneuron firing activity is ceased and the inhibitory influence subsides, then pyramidal cells can fire action potentials. Due to the short duration of the disinhibition phase, the temporal coincidence of synaptic excitation reaching interneurons will drive them to firing threshold. That in turn, will trigger a new round of interneuron synchronous firing and therefore the initiation of a new oscillation cycle (Fisahn and Buhl 2001; Whittington et al. 2000).

The PING model has been shown to rely crucially on $GABA_A$ receptor-mediated fast synaptic inhibition, AMPA receptor-mediated fast synaptic excitation and kainate receptor-mediated tonic excitation (Buhl et al. 1998; Cunningham et al. 2003; Fisahn et al. 1998; Roopun et al. 2006). However, the contribution of N-methyl-D-aspartate (NMDA) receptor-mediated slow synaptic excitation is not essential for the generation of gamma frequency oscillations in the hippocampus and the somatosensory cortex (Buhl et al. 1998; Fisahn et al. 1998; Fisahn and Buhl 2001).

1.4.2. *Beta frequency oscillations in vitro*

The generation of *in vitro* beta frequency oscillations (beta2; 20-30 Hz) was initially demonstrated in layer 5 of horizontal somatosensory cortical slices of the rat brain (Roopun et al. 2006). Oscillations were induced with kainate (400 nM) application and persisted following AMPA, NMDA and $GABA_A$ receptor blockade due to an electrically coupled network of intrinsically bursting cells (Roopun et al. 2006).

The generation of beta frequency oscillations (beta2; ~27 Hz) has also been demonstrated in the rat motor cortex (Yamawaki et al. 2008). Oscillations were evoked by carbachol (50 μM) and kainate (400 nM) co-application and showed a strong dependence on $GABA_A$ but not on AMPA or NMDA receptor-mediated synaptic transmission (Yamawaki et al. 2008).

Beta frequency oscillations (beta1; ~12-15 Hz) have also been generated *in vitro* in the IL and PrL regions of submerged rat mPFC slices (van Aerde et al. 2008). Oscillations were evoked with carbachol (25 μM) application and showed strong dependence on $GABA_A$ and AMPA/kainate receptor-mediated synaptic transmission (van Aerde et al. 2008).

1.6. Modulation of PFC activity by subcortical ascending neurotransmitter systems

As discussed above the mammalian PFC receives strong innervation from subcortical cholinergic, dopaminergic and noradrenergic nuclei. Innervation from these neurotransmitter systems modulates cortical activity at a cellular, network and cognitive level. The modulatory effects of these neurotransmitter systems will be discussed briefly in this section and in greater detail in the following chapters.

1.6.1. *Modulation of PFC activity by the cholinergic system*

There is substantial evidence that PFC activity, along with the cognitive functions it subserves, are regulated by the subcortical ascending cholinergic system (Steriade et al. 1993). Cholinergic neurons originating from the basal forebrain innervate the mammalian PFC (Dalley et al. 2004; Dawson and Iversen 1993; Kozak et al. 2006). A combination of behavioural, microdialysis and lesion

studies have shown that behaviour-dependent PFC activity is correlated with cholinergic activity. Acetylcholine (ACh) concentration levels were shown to increase in the rat mPFC region during a sustained attention task (Kozak et al. 2006). In another rat study which engaged attentive processing (cue detection task), ACh concentration levels were correlated to the task performance in that increased cholinergic activity was evoked only by detected cues (Parikh et al. 2007).

WM performance was also shown to deteriorate in a rat test of delayed-matching problem by application of agents that suppress cholinergic activity. Deteriorating effects were partially reversed by agents that facilitate cholinergic activity (Dawson and Iversen 1993).

1.6.2. *Modulation of PFC activity by the dopaminergic system*

There is substantial evidence that the actions of dopamine (DA) can play an important modulatory role in cognitive processes mediated by the PFC (Brozoski et al. 1979; Sawaguchi and Goldman-Rakic 1994; Seamans et al. 1998). Converging evidence from monkey and rat behavioural/lesion studies indicate the importance of optimal cortical DA levels in the performance of delayed-response and attention-set-shifting tasks (Brozoski et al. 1979; Floresco et al. 2005; Ragozzino 2002; Sawaguchi and Goldman-Rakic 1994; Seamans et al. 1998; Zahrt et al. 1997).

The mammalian PFC receives strong innervation from the mesocorticolimbic DA pathway which originates in the midbrain ventral tegmental area (VTA; Divac et al. 1978; Grobin and Deutch 1998; Steketee 2003). The physiological actions of DA in the PFC are mediated by five structurally distinct dopamine receptors (D_1, D_2, D_3, D_4 and D_5; Beaulieu and Gainetdinov 2011; Missale et al. 1998).

At the cellular level, DA receptor activation can affect the excitability of both pyramidal cells and interneurons (Penit-Soria et al. 1987; Rubinstein et al. 2001; Seamans et al. 2001a; Yang and Seamans 1996b). At a network level, it has been shown that DA, by acting on D_1 and D_4 receptors can cause significant alterations in the strength of carbachol- and kainate-induced oscillations in the hippocampus (Andersson et al. 2012; Weiss et al. 2003; Wójtowicz et al. 2009).

1.6.3. *Modulation of PFC activity by the noradrenergic system*

Another neurotransmitter system which exerts a strong influence on cortical activity is the noradrenergic system. The mammalian neocortex, including the PFC, receives strong innervation from noradrenaline (NA)-containing cells originating in the brainstem locus coeruleus (Arnsten 1998; Kawaguchi and Shindou 1998; Steketee 2003).

NA produces modulatory effects on cortical activity by stimulating the three main subtypes of NA receptors (α1-, α2- and β-adrenoceptors; Arnsten 1998). There is converging evidence from monkey and rat behavioural studies suggesting that activation of NA receptors can support WM performance (Arnsten 1998; Brozoski et al. 1979; B.-M. Li et al. 1999; Ramos et al. 2008; Rossetti and Carboni 2005; Sawaguchi 1998).

Electrophysiological studies have shown that NA can cause alterations in the excitability of pyramidal cells (Malenka and Nicoll 1986; Stanton and Heinemann 1986) and interneurons (Kawaguchi and Shindou 1998) as well as the efficiency of synaptic inhibition by targeting both pre- and post-synaptic sites (Salgado et al. 2012). Moreover, NA was shown to alter the strength of gamma frequency oscillations in olfactory cortical and hippocampal slice preparations (Gire and Schoppa 2008; Wójtowicz et al. 2009).

1.7. Thesis objectives

Although there is strong evidence highlighting the behavioural significance of cortical fast network oscillations little is known about the mechanisms that underlie the generation of these rhythms in the mPFC. Moreover, behavioural and electrophysiological research has implicated the subcortical dopaminergic and noradrenergic activity to the modulation of cortical activity but again there are no studies to-date that have assessed the effects of these neurotransmitters on network activity in the mPFC.

Therefore the aims of this thesis are to:

- To develop an *in vitro* model of persistent fast network oscillations in the rat mPFC.

- Investigate the spectral and rhythmicity characteristics of network oscillations across the different regions and layers of the rat mPFC.
- Investigate the network interactions between oscillations recorded in different regions of the mPFC
- Probe the synaptic mechanisms implicated in the generation of fast network oscillations in the mPFC.
- Identify the neuronal populations engaged in the network oscillatory activity.
- Investigate the modulation of fast network oscillations in the mPFC by dopamine and noradrenaline.

Chapter 2

General Methods

Chapter 2 – General Methods

2.1. Animal Provision

Experiments presented in this thesis used *in vitro* slice preparations from young adult male Hooded Lister rats (Charles River laboratories, UK) aged between 58 and 70 days and weighing approximately 250 grams. Animals were housed at the Newcastle University animal facility in cages of 4 males in a room with a 12-hour light/dark cycle and free access to food and water (*ad libitum)*. Once transported into the building, animals were allowed to acclimatize for at least 48 hr before being used for experiments.

2.2. Animal procedures, slice preparation and maintenance

All surgical procedures were performed according to the requirements of the UK Animals (Scientific Procedures) Act, 1986.

Rats were placed in a 5-litre bell-jar and lightly anaesthetised with inhaled isoflurane (Abbott Laboratories LtD, Mainhead, UK). When the righting reflex was abolished, animals were given intramuscular injection of 0.35 ml 2% xylazine (10 $mg.kg^{-1}$; Animalcare LtD, York, UK) and 0.35 ml ketamine (100 $mg.kg^{-1}$; Fort Dodge Animal Health Ltd, Southampton, UK). When all responses to noxious stimuli (e.g. tail pinch, pedal withdrawal and corneal blink reflex) were completely abolished, intracardial perfusion was performed. Initially the abdominal cavity was opened and the ribcage was removed to expose the heart. An incision to the right atrium was made followed by the insertion of a needle to the left ventricle. The catheter was connected through a plastic tube to a syringe filled with chilled (~ $4^{o}C$), oxygenated, sucrose-containing artificial cerebrospinal fluid (sucrose-ACSF; Section 2.3). Approximately 60 ml of sucrose-ACSF were perfused manually at the rate of 1.0 to 2.0 $ml.sec^{-1}$. Intracardial perfusion with sucrose-ACSF was used as it has a protective effect on the viability of cells during *in vitro* slice preparations (Aghajanian and Rasmussen 1989).

Following perfusion the spinal cord was severed and a long incision along the midline of the head revealed the skull. The bone plates of the dorsal skull surface

along with the *dura mater* were detached and the brain was excised and immersed in a petri-dish filled with ice-cold oxygenated sucrose-ACSF. Using a razor blade the cerebellum, pons, medulla and part of the caudal cerebrum were removed. The remaining brain was lightly glued on its caudal surface to the disk plate of the Leica VT1000 vibratome (Leica Microsystems, Nussloch GmbH, Germany) and submerged in the cutting chamber filled with continuously oxygenated, ice-cold sucrose-ACSF. Serial sections of 450 μm-thick slices were cut in the coronal plane from the rostral to caudal direction. Slices containing the required cortical regions were immersed in a petri-dish of ice-cold oxygenated sucrose-ACSF and they were further trimmed to remove excess tissue.

To ensure that slices contained the cortical regions of interest, the diagrams of coronal planes from the "The Rat Brain in Stereotaxic Coordinates, 4th edition, (Paxinos 1998) were used. Slices of prefrontal cortex that contained the ACd, PrL, IL and DP cortices were cut between 12.20 mm to 11.20 mm anterior to the interaural line. Slices that contained the ACd and motor cortex (M1 and M2) were cut between 10.70 mm to 10.60 mm anterior to the interaural line (Figure 1.1B). The interaural line is a stereotaxic coordinate which represents the imaginary coronal plane situated across the ears (Paxinos 1998).

The overall process of animal anesthetization, perfusion and slice preparation lasted approximately 20 min to 25 min.

Slices were then relocated to a holding chamber where they were maintained for at least 60 min at room temperature in oxygenated normal-ACSF (Section 2.3) before being transferred to the recording chamber. It has been shown that isoflurane reduces the frequency of carbachol-induced network oscillations in the hippocampus *in vitro* (Dickinson et al. 2003). Ketamine, though, exerts no effect on either the power or frequency of them (Dickinson et al. 2003). However, these drugs along with xylazine (α_2-adrenoceptor agonist) are soluble to water. Therefore, it has been considered that slice maintenance for 60 min in the holding chamber at room temperature is a sufficient amount of time for the drugs to wash out from the tissue.

Slices were then transferred to the interface-type recording chamber where they were maintained at approximately 28^oC at the interface between continuous

flow (~1.2 ml.min^{-1}) of normal-ACSF and humidified carbogen gas (95% O_2 and 5% CO_2). Slices were allowed to recover for at least 30 min in the recording chamber before any drug was applied.

Slices remained viable in the holding and the interface-type recording chamber for approximately 8 hours.

2.3. Drug stocks and materials

The normal-ACSF used in the holding chamber and circulating in the interface-type recording chamber was composed of (units in mM) 126 NaCl, 3 KCl, 1.25 NaH_2PO4, 24 $NaHCO_3$, 1 $MgSO_4$, 1.2 $CaCl_2$ and 10 glucose. The sucrose-ACSF contained (units in mM) 252 sucrose, 3 KCl, 1.25 NaH_2PO_4, 24 $NaHCO_3$, 1 $MgSO_4$, 1.2 $CaCl_2$ and 10 glucose. The lower concentrations of Mg^{2+} and Ca^{2+} (in comparison to hippocampal ACSF) decrease the magnesium block on NMDA-receptors (Nowak et al, 1984) and reduce charge screening at the neuronal membrane increasing the excitability of cortical slices.

Drug solutions were prepared as concentrated stocks and stored as aqueous solutions at +4 ^oC or -20 ^oC until use. Most of the drugs were dissolved in de-ionized water, or if sparingly soluble in water, in dimethyl sulfoxide (DMSO). Dopamine, noradrenaline and isoprenaline were prepared fresh before each application. They were dissolved in ascorbic acid (10 mM) to prevent rapid oxidation. All drugs stocks were dissolved in normal-ACSF bath solution for dilution to the desired concentration during experiments. The dead volume of the tubing was ~3 ml, allowing complete exchange of the bath solution in ~2 min. Drugs and chemicals used in the present thesis are shown analytically in Table 2.1.

2.4. Recording techniques and Data acquisition

Three recording electrophysiological techniques were applied for the purpose of this thesis. These are (1) multi-electrode field potential and extracellular unit recordings, (2) extracellular single-electrode field potential and (3) intracellular recordings.

Table 2.1. A list of drugs used in this thesis

2.4.1. *Multi-Electrode Activity Recordings*

Local field potential (LFP) and extracellular unit activity were recorded from multiple sites of the cortical slices with a multi-electrode Utah array (Alpha Omega GmbH, Germany; Figure 2.1A). The array was composed of 100 mono-crystalline silicon electrodes assembled in 10 rows and 10 columns. The inter-electrode space was 400 μm and the electrode impedance varied approximately at 500 kΩ. The array was attached to a multi-drive holder and advanced slowly until it reached the slice. The penetrating depth varied according to the desired level of detected activity. Once inserted into the slice, the array remained in the same position throughout the course of the experiment. Recording epochs lasted for 2 min. Analogue signals recorded from the electrodes were transferred through a flat cable to the Cerebus-128 Front-End Amplifier (I2S Micro Implantable Systems, LLC, Salt Lake City). Signals were amplified, filtered (0.3 Hz - 7.5 kHz) and digitized with a 16-bit resolution at a 30 kHz rate. Digital signals were transferred through a fiber optic link to the Cerebus-128 Neural Signal Processor. Further filtering separated the broadband digital signal into low (<250 Hz) and high (>250 Hz) frequency traces. The low frequency trace contained the LFP signal which was down-sampled to a 2 kHz rate. The high frequency trace contained the fast spiking events. The detection threshold of spikes was set manually. Once an extracellular spike exceeded the threshold, the waveform templates (reconstructed by 48 samples) and the exact firing times (i.e. timestamps) were extracted. LFP signals, waveform templates and timestamps recorded from every electrode, were transformed into a MATLAB compatible format and saved for further off-line analysis.

2.4.2. *Single-electrode recordings*

Microelectrodes used for extracellular LFP recordings were pulled from thin borosilicate glass tubing (1.2 mm O.D by 0.94 mm I.D.; Harvard Apparatus Ltd, Kent, UK) with a P-97 Flaming/Brown puller (Sutter Instruments Co., Novato, CA, USA). Field micro-electrodes were filled with normal-ACSF of the same composition as that circulating in the recording chamber, with impedances ranging between 2 – 5 MΩ.

Figure 2.1. Extracellular recordings at multiple sites with a Utah multi-electrode array

Sharp micropipettes employed for intracellular recordings were pulled by the aforementioned puller from thin borosilicate glass tubing (1.2 mm O.D. by 0.69 mm I.D.). Their impedance ranged between 100 – 130 MΩ and they were filled with potassium acetate (2 M).

Both electrode types were connected to amplifier headstages (Axon Instruments Inc., Union City, CA, USA) through a conductive electrode holder. Visual magnification with a microscope which supported the observation of the gross morphological characteristics of the slice along with a micro-manipulator, (Narishige, Tokyo, Japan) enabled us to advance electrodes in the desired position.

Field and intracellular signals were recorded in current-clamp mode and amplified (x10) by an Axoclamp-1A amplifier (Axon Instruments Inc.). 50 Hz main noise was eliminated from the raw signal with adaptive filtering by Humbugs (Quest Scientific Instruments Inc., North Vancouver, Canada). Extracellular signals were band-pass filtered in the range of 5-400 Hz and intracellular signals were low-pass filtered at 2 kHz with an 8-pole Bessel filter (Applegarth Electronics, Oxford, UK). Signals were subsequently digitized at 5 kHz by an Instrutech ITC-16 A/D board (Instrutech Corp., NY, USA). Digital data were stored on an Apple Mac computer using Axograph software. Stored data were transformed into a MATLAB compatible format for off-line analysis.

2.5. Experimental protocols

In extracellular experiments, single field electrodes were advanced in the cortical areas of interest and baseline activity was recorded prior to drug application. This was done in order to examine whether any oscillatory activity was present before the application of drugs used to evoke oscillations. Electrodes remained stationary throughout the rest of the experiment. Oscillations were generated with the bath application of carbachol (Ch; 10 μM) and kainate (kainic acid, KA; 200 nM). The different phases of oscillatory activity (i.e. emergence, build-up and stability) were observed and quantified with real-time analysis. Recording epochs of 60 sec were taken every 15 min and the power and frequency of the oscillations were extracted with power spectral analysis. When stability was reached (i.e. stability was confirmed when the area power of three successive recordings at 10 min intervals deviated by < ±10%), pharmacological agents were

bath applied. When feasible, drugs applied were washed-out in order to ensure that any manipulations of the oscillation characteristics were caused by the agents. In the wash-out phase the bath solution was replaced with a fresh normal-ACSF solution including carbachol (10 μM) and kainate (200 nM).

In multi-electrode experiments, oscillatory activity was initially observed with the use of single field electrodes (Section 2.4.2). When stability was reached, the Utah array was advanced into the slice. Stability was assessed for 45 min following application of DA (100 μM) into the bath solution. Recording epochs (120 sec) were taken from 0 to 16 min, as well as at 30, 45 and 60 min post-DA application. For the electrodes to cause more profound marks on the slice, when the experiment was completed the array was inserted deeper into the slice for ~15 min (Figure 2.1B). The laminar and regional identification of the recording electrodes was confirmed with a Nissl staining procedure (Section 2.8).

Intracellular recordings from mPFC neurons were performed in baseline conditions or in the presence of network oscillations. Once impaled, stability of the cell had to be confirmed (stable resting membrane potential, V_{rest}, over a 5 min period) before the membrane properties and the synaptic activity of the cell, were assessed. Only data recorded from cells with resting membrane potential of at least -50mV and APs exceeding 55 mV were used. Resting membrane potential was estimated by calculating the difference between the V_m reading when the electrode was inside the cell and the V_m reading when it was outside the cell at the end of the recording period (without DC current injection). IPSPs were recorded at holding potential of -30 mV (i.e. depolarization block).

2.6. Data Analysis of extracellular recordings

Off-line analysis of LFP traces as well as extracellular and intracellular signals was implemented with MATLAB (MathWorks Inc.,Natick, MA, USA) routines we developed for the purpose of the thesis. Before being analyzed, single electrode field and intracellular recordings were transformed from Axograph into a MATLAB compatible format.

2.6.1. *Power Spectral Density*

To identify the frequency component of the LFPs, power spectral density (PSD) estimates were computed via Welch's averaged modified periodogram method (Welch 1967). Briefly, LFP traces (60 or 120 sec) were divided into 8192-sample (~4 sec) windows with a 50% overlap and analyzed with an 8192-point fast Fourier transform (FFT). FFT decomposes time series into its constituent frequency components estimating the relative strength of every individual component (Proakis and Manolakis 1996). The segmented FFTs (periodograms) were averaged to form the spectrum estimate. Subsequently, the resulting spectrum estimate was scaled by frequency to produce the PSD. PSDs were calculated with a resolution of 0.25 Hz.

PSD analysis enabled us to quantify the oscillation power of the recorded field activity by calculating the area power within a frequency range. The area power of oscillations was calculated in the present thesis by integrating the area of the spectral amplitude between 15-45 Hz. The predominant oscillation frequency (i.e. peak frequency) was extracted from the point with maximum spectral amplitude (i.e. peak power).

2.6.2. *Signal filters*

The application of digital filters to the LFP traces was required in many of the analytical techniques included in this thesis. Filter choice was determined by two criteria: the filtered signal should experience the lowest (1) phase and (2) amplitude distortion. The first criterion was fulfilled by the application of linear-phase finite impulse response (FIR) filters. Phase linearity is a property where the phase response of the filter is directly proportional to frequency. This in turn means that the same delay in time is applied to each frequency component of the signal (Proakis and Manolakis 1996). Phase shift, although linear, could lead to false estimations of the instantaneous LFP phase. To eliminate possible phase shifts, signals were filtered forward and backward using the MATLAB "*filtfilt*" function. The second criterion was fulfilled with the application of equiripple FIR filters which minimize the ripple error in the passbands and stopbands (Proakis and Manolakis 1996).

2.6.3. ***Rhythmicity of network oscillations***

To investigate the rhythmicity of oscillating LFP traces we employed the useful properties of the crosscorrelation function (Proakis and Manolakis 1996). In a classical sense, the correlation between two signal sequences $\boldsymbol{x}(\boldsymbol{n})$ and $\boldsymbol{y}(\boldsymbol{n})$ measures the degree to which the variations in the amplitude of one signal resemble the variations of the other. Crosscorrelation is described by the equation

$$\boldsymbol{r_{xx}}(\boldsymbol{l}) = \sum_{n=-\infty}^{n=\infty} \boldsymbol{x}(\boldsymbol{n})\boldsymbol{y}(\boldsymbol{n}-\boldsymbol{l}) \qquad \text{Equation 2.1}$$

where ***n*** represents samples and ***l=0,±1,±2,...*** the number of shifts.

In the special case where $(\boldsymbol{n}) = \boldsymbol{y}(\boldsymbol{n})$, then Equation 2.1 produces the autocorrelation of $\boldsymbol{x}(\boldsymbol{n})$. If $\boldsymbol{x}(\boldsymbol{n})$ is a noisy periodic signal (e.g. bioelectric signal) then the graph of the sequences of $\boldsymbol{r_{xx}}$ against time will contain a sinusoidal waveform (damped with noise) with its maximum value at zero lag ($\boldsymbol{t} = \boldsymbol{0}$) and side peaks at the reciprocal of its predominant oscillation frequency ($\pm\boldsymbol{f_o}$) and its multiple values ($\pm\boldsymbol{2f_o}$, $\pm\boldsymbol{3f_o}$ ...).

The autocorrelation function was applied to 60 and 120 sec epochs of LFP traces. Initially, traces were band-pass filtered ($\mathrm{f_{pass1}} = 15$ Hz, $\mathrm{f_{pass2}} = 45$ Hz) to remove background noise that could cause amplitude perturbations. Subsequently, the autocorrelation function was applied. The autocorrelation sequences were normalized to the amplitude range [-1, 1]. The rhythmicity strength or rhythmicity index (RI) was measured by the amplitude of the first side peak which was detected manually (Figure 2.2).

2.6.4. ***Network synchrony of oscillatory activity***

Cross-frequency phase-synchrony analysis was introduced by Tass and colleagues (Tass et al. 1998) and since then it has found many applications in the research of network interactions. In theory, cross-frequency phase-synchrony (or phase-locking) examines the frequency adjustment of two periodic oscillating subsystems due to dynamic interactions. This in turn is reflected by the presence of a certain relation between their phases (Rosenblum et al. 2001).

Unlike the conventional frequency coherence (i.e. cross-correlation between narrow-band filtered signals) cross-frequency phase synchrony analysis

Figure 2.2. Autocorrelation function applied on oscillatory LFP traces

investigates the interaction of two oscillating subsystems by estimating their phase covariance regardless of their amplitude (Varela et al. 2001). Cross-frequency phase synchrony analysis has been employed so far in a variety of *in vivo* studies to investigate the emergence of stimulus-induced network interactions reflected by brief epochs of constant phase difference.

Phase-synchrony analysis was employed for the purpose of this thesis to measure the inter-laminar and inter-regional phase-difference. Subsequently, we wished to estimate the inter-laminar and inter-regional phase-synchrony of oscillatory network activity.

Two different analytical methods were developed and tested in simulated signals or real LFP traces. Both of these methods were inspired by the work of Tass and colleagues (Tass et al. 1998).

Initially, the spectral density of signals $\boldsymbol{x_1}$ and $\boldsymbol{x_2}$ were produced to extract the dominant oscillation frequencies (i.e. peak frequencies $\boldsymbol{f_1}$, $\boldsymbol{f_2}$; Figure 2.3A,B). Raw signals were band-pass filtered ±4 Hz around their peak frequencies to remove background noise that could cause phase perturbations (i.e. phase slips; Figure 2.3C). The instantaneous phase of the two filtered signals, $\boldsymbol{\varphi_1}$, $\boldsymbol{\varphi_2}$ were extracted by the means of the Hilbert transform and rescaled in the range of $[\mathbf{0}\,, \mathbf{2}\boldsymbol{\pi}]$ (Figure 2.3D). The "instantaneous phase-difference" or "relative phase-difference" (Figure 2.3E) was calculated by the following equation

$$\boldsymbol{\varphi_{m,n} = \varphi_1 - \frac{f_1}{f_2}\varphi_2} \qquad \text{Equation 2.2}$$

Subsequently, the instantaneous phase-difference was grouped in bins (bin size : 0.025 radians) and plotted in histograms ranging between $[\mathbf{0}\,, \mathbf{2}\boldsymbol{\pi}]$ or $[-\boldsymbol{\pi}\,, \boldsymbol{\pi}]$ (Figure 2.3F).

Phase-synchrony strength was evaluated in a statistical sense by estimating deviation of the cyclic relative phase distribution from a uniform one. For this purpose the *Shannon entropy,* $\boldsymbol{\rho}$, was employed.

$$\boldsymbol{\rho = \frac{(S_{max} - S)}{S_{max}}} \qquad \text{Equation 2.3}$$

where $\boldsymbol{S = -\sum_{k=1}^{N} p_k \ln p_k}$ and $\boldsymbol{S_{max} = \ln N}$, where $\boldsymbol{N}$ is the number of bins and $\boldsymbol{p_k}$ is the probability mass function of every bin.

Shannon entropy, $\boldsymbol{\rho}$, ranges between 0 and 1 and provides a good estimate of the phase covariance between two simultaneously recorded LFP traces. When $\boldsymbol{\rho}$ is small it indicates low levels of synchrony (i.e. uniform distribution) and when $\boldsymbol{\rho}$ is large it indicates the presence of strong synchrony (i.e. phase-difference varies slightly between a certain value and the phase histogram has a unimodal distribution).

The preferred phase difference, $\boldsymbol{\varphi_{dif}}$ between the two periodic signals was extracted from the phase bin with the maximum probability mass function of the smoothed (Savitzky-Golay filter) histogram (Figure 2.3F). The probability mass function is a dimensionless quantity produced by normalizing the number of observations in each bin by the total number of observations (Abeles 1982).

The analytical method we used for the extraction of phase-difference and synchrony was the result of a compromise between the inherent limitations of the tool itself and the high processing demands it required. The cross-frequency phase-synchrony tool employed for the purpose of this study was also tested on simulated periodical signals. In signal pairs with integer frequency ratios (1, 2, 3, ...N) it produced satisfactory results. However, when the frequency ratio deviated slightly from an integer number, then the adjustment of wrapped phases [0°, 360°] produced spurious results (e.g. the modulus of 2 times 360° is 0°, but the modulus of 1.3 times 360° is 108°). This inherent shortcoming was offset, though, by the non-stationary nature of the LFP signals. In all the LFP pairs tested in our study the frequency ratio was around 1 (~0.9 – 1.1). Any phase artefacts produced when the frequency ratio was below 1 were compensated when the frequency ratio was above 1. These ratio fluctuations alternated every few cycles and due to the low frequency ratio (~1) deviations from the real phase difference were small.

An alternative solution would be the frequency adjustment of unwrapped phases. This method was tested on simulated periodic signals and produced satisfactory results in pairs with integer or decimal frequency ratios. However, this method is insensitive to frequency fluctuations of LFP signals. To overcome this caveat, this method requires also the spectral analysis and prominent frequency extraction for sliding windows with duration of hundreds of milliseconds. This process requires large amounts of processing capacity and for this reason it was abandoned.

Figure 2.3. Network synchrony of oscillatory activity

2.6.5. *Spike sorting analysis*

Spiking activity, recorded with extracellular electrodes, may originate from one or more units. Therefore, separation and classification of multi-unit spiking activity is a prerequisite for single spike train analysis.

Spike classification was implemented with the K-means algorithm. K-means is an iterative algorithm which partitions **n**-dimensional observations (or points in the **n**-dimensional space) into **k** clusters by minimizing the within-cluster sums of the Euclidean point-to-cluster-centroid distances (Hartigan and Wong 1979).

Matrices (N-by-48 samples) containing spike waveforms recorded by individual electrodes were sorted into a number of, **k**, clusters defined by the user. The outcome of the spike sorting process was an N-by-1 vector (i.e. "*unitIDs*") which contained the indices (e.g. 1, 2, 3 …, k) assigned to every waveform based on the cluster it belongs to.

2.6.6. *Principal Components Analysis*

The decision to separate multi-unit spiking activity into a number of **k** clusters was reached following the visual inspection of the spike waveform features in a 2-dimensional principal components analysis (PCA) plot.

PCA simplifies complex datasets by reducing the dimensionality of them. Given a dataset of **N** observations and **n** variables (i.e. dimensions), PCA produces a new dataset with the same number of variables called the principal components (i.e. eigenvectors). The transformation is defined in such a way that principal components are sorted in a descending order of variance (i.e. eigenvalues). Principal components are uncorrelated to each other. A substantial amount of the variance that the original data exhibited is retained by the first eigenvalues. Therefore, the differences in the observations of the original data can be described with a smaller dataset of **N** observations and **r** variables, where **r < n** (Jolliffe 1986).

To produce the PCA of an N-by-48 matrix containing the spike waveforms recorded by individual electrodes, the eigenfactors and eigenvalues were extracted from the square covariance matrix. The new dataset was derived by projecting the original dataset into the first 2 eigenfactors. Finally, the derived data (N-by-2

Figure 2.4. Principal components analysis of spike waveforms and interspike interval analysis of spike trains

matrix) were presented as data points in the orthogonal axes of the first two principal components (Figure 2.4A).

2.6.7. *Inter-spike interval histogram analysis*

The excitability of a cell can be determined with the average rate of firing. This is simply calculated by dividing the total number of spikes by the recording period:

$$\boldsymbol{R_{avg} = n/T} \qquad \text{Equation 2.4}$$

where $\boldsymbol{n}$ is the number of spikes and $\boldsymbol{T}$ is the recording period (Abeles 1982).

However, the average rate of firing does not provide information about the temporal dynamics or rhythmicity of a neuron's firing activity. Statistical analysis of spike trains could provide that important information. A spike train contains the time of occurrence of a series of APs produced by a neuron observed over a period of time. Inter-spike interval (ISI) histogram analysis, proposed by Perkel and colleagues (Perkel et al. 1967a, 1967b), is a widely applied computational technique which calculates the firing discharge probabilities of neurons from the timing of spiking events. ISI histograms can provide us with important information about the different firing properties of distinct populations of neurons.

Single-unit spike trains were extracted from multi-unit spiking activity by assigning the unit number (from the N-by-1 *unitIDs* vector) to the timestamps (from the N-by-100 *timestamps* matrix) of the electrode they were recorded from.

To produce the ISI histogram from the spike trains of individual units, we first calculated the time difference of adjacent spikes (i.e. first order time interval, $\boldsymbol{\Delta_t = t_n - t_{n-1}}$; Figure 2.4B). Then time intervals were grouped into non-overlapping bins (bin size: 3 msec). Subsequently, the renewal density ($\boldsymbol{\lambda}$) was produced with the following equation

$$\boldsymbol{\lambda = \frac{n}{N \cdot \Delta_{bin}}} \qquad \text{Equation 2.4}$$

where, $\boldsymbol{n}$ is the number of events within every bin, $\boldsymbol{N}$ is the total number of spike events and $\boldsymbol{\Delta_{bin}}$ is the bin size. The renewal density is proportional to the pmf. However, unlike the pmf, it describes firing rates (spikes/sec) independent of the bin size (Abeles 1982).

The histogram of renewal densities was smoothed with a Savitzky-Golay filter. The preferred firing interval (T_n) was assigned to the interspike interval with the maximum renewal density (λ_{max}; Figure 2.4C). The preferred firing frequency (f_n) was calculated from the reciprocal of the preferred firing interval.

2.6.8. *Rhythmicity of neuronal firing activity*

If in the analytical process of the ISI histogram construction we include the 2nd, 3rd and Nth order time interval, then we produce the autocorrelation (AC) histogram of the spike train (Figure 2.5A). The autocorrelation analysis requires more processing capacity, and provides the same information about the preferred firing frequency and renewal density of the neuron's firing activity with the ISI analysis. More importantly, though, it provides us with a better estimate about the rhythmic properties of the firing activity (Perkel et al. 1967a, 1967b). The strength of the neuronal oscillation was quantified in the present thesis with a rhythmicity index (here we will refer to it as neuronal rhythmicity index, NRI, to differentiate from the field oscillation rhythmicity index, RI) calculated in a similar manner to that described previously by Lang and colleagues (Lang et al. 1997).

In brief, the first four peaks and valleys of the autocorrelation above or below 1 SD of the baseline were summed. The NRI was then defined by the following formula:

$$\boldsymbol{NRI} = \frac{\boldsymbol{p_1}}{\boldsymbol{z}} + \frac{\boldsymbol{v_1}}{\boldsymbol{z}} + \frac{\boldsymbol{p_2}}{\boldsymbol{z}} + \frac{\boldsymbol{v_2}}{\boldsymbol{z}} + \cdots \qquad \text{Equation 2.5}$$

where $\boldsymbol{z}$ is the total number of spikes, and $\boldsymbol{p_i}$, $\boldsymbol{v_i}$ ***(i=1,2,3,4)*** are the absolute differences between the individual peak and valley, respectively, with the baseline. Baseline activity was calculated by the mean value at time lags of 0.6-0.8 sec (Figure 2.5B). The greater the NRI is the great is the rhythmicity of neuronal firing.

Figure 2.5. Neuronal rhythmicity (NRI) index of neuronal firing activity

2.6.9. *Unit versus field phase-synchrony*

ISI and AC histogram analysis can provide us with important information about the firing frequency and rhythmicity of neuronal firing; however, they cannot fully determine the contribution of a cell to the network oscillatory activity. The latter information can be extracted by estimating the relationship between the phase of the oscillatory field potential and the timing of spike events. The computational model we employed to extract this information was inspired by the "*Phase correlations*" computational model introduced by Csicsvari and colleagues (Csicsvari et al. 1999). Initially, LFP traces were band-pass filtered ($f_{pass1} = 15$ Hz, $f_{pass2} = 45$ Hz) to remove background noise that could cause perturbations (i.e. phase slips; Figure 2.6A,B). The instantaneous phase $\boldsymbol{\varphi_n}$, of the filtered signals was extracted by the means of the Hilbert transform and rescaled in the range of $[0, 2\pi]$ radians (Figure 2.6C). Where field oscillations and spikes were recorded from the same electrode, the instantaneous phase corresponding to the timing of spike events was extracted and allocated to a phase bin (bin size: 5°) of the 0°-360° field cycle (Figure 2.6D). The number of events in each bin were divided by the total number of events to produce the probability mass function (Abeles 1982).

The distribution of the phase histogram was statistically described with the Shannon entropy, $\boldsymbol{\rho}$, (Section 2.6.4). The entropy, $\boldsymbol{\rho}$, is a normalized value ranging between 0 and 1. The higher the entropy is the more concentrated is the spike firing around a particular phase. To reduce bin-border variability a Savitzky-Golay filter was applied. The preferred firing phase (φ_{max}) was extracted from the phase-bin with the maximum probability of the smoothed histogram (Figure 2.6E).

Spike sorting, PCA, ISI, AC histogram and unit phase-synchrony analysis tools were integrated into a MATLAB graphic user interface (***MEAtoolbox***).

Figure 2.6. Phase-synchrony between neuronal firing activity and field potential activity

2.7. Data analysis of intracellular recordings

2.7.1. *Intrinsic membrane properties*

The amplitude of the APs was measured from the start of the rapid rising phase to the peak of the depolarization.

The input resistance of the membrane was calculated using the Ohm's law from injection of a hyperpolarizing current step which caused voltage deflection of 10mV.

The firing frequency and renewal density of cells was investigated with the ISI histogram analysis (Section 2.6.7). The time of occurrence of APs was extracted with the MATLAB function, *findpeaks*.

2.7.2. *Inhibitory Postsynaptic Potentials*

The computational analysis we implemented on IPSP trains extracted information regarding the (1) the frequency and rhythmicity of the periodic sequences of IPSPs, (2) the cross-correlation and cross-frequency phase-synchrony between IPSP trains and LFP traces and finally (3) the amplitude, rise time and decay time of IPSPs.

The frequency of periodic IPSP trains was extracted from power spectral analysis (Section 2.6.1).

Before any of the following analytical tools were applied, both LFP and IPSP traces were band-pass filtered $\left(\mathrm{f_{pass1}} = 15\ \mathrm{Hz}\,, \mathrm{f_{pass2}} = 45\ \mathrm{Hz};\ \text{Section 2.6.2}\right)$.

The rhythmicity of IPSP trains (60sec epochs) was calculated with the autocorrelation function (Section 2.6.3).

The cross-correlation function between IPSP trains and LFP traces was calculated with the Equation 2.1 (Section 2.6.3).

Phase-difference and phase-synchrony between IPSP trains and LFP traces was calculated with cross-frequency phase-synchrony analysis (Section 2.6.4).

Figure 2.7. Calculation of the IPSP kinetics

To calculate the IPSP kinetics, peaks and troughs of IPSPs were extracted from 60 sec epoch traces.

IPSP amplitude was determined by the absolute voltage difference between the trough and the average amplitude of its preceding and succeeding peaks. Troughs with amplitude less than 0.5 mV were not counted as IPSPs (Figure 2.7A).

Rise time was defined as the time taken between 10% to 90% of the peak-to-trough voltage difference (Figure 2.7B).

The decay phase resembled a simple exponential function, therefore it could be described appropriately by the exponential time constant, $\boldsymbol{\tau}$. The decay time was measured as the time taken for the IPSP to decay to 37% of the trough-to-peak voltage difference (Figure 2.7C).

Approximately 1500 IPSP events were extracted from every trace and pooled for statistical analysis.

Although, IPSPs were recorded at holding potential of -30 mV, APs were recorded occasionally. The presence of APs would contaminate the signal with non-biological ripples following the filtering process. To overcome that, events that exceeded the threshold limit of 2 SD above the average peak amplitude were detected and truncated. Troughs preceding and succeeding the APs were not included in the analysis.

Rhythmicity, correlation and IPSP kinetics analysis tools were integrated into a single MATLAB routine (***IPSPtoolbox***).

2.8. Histology

When multi-electrode recordings were completed, slices were placed in buffered (4%) paraformaldehyde (PFA) solution for a varied period of five to fifteen days. Slices were then removed from the PFA solution, mounted onto glass slides and dehydrated overnight at 4°C. Cortical tissue was Nissl stained with a Toluidine blue dye protocol. In brief the staining protocol included hydration by decreasing concentrations of ethanol (70%, 50%) for 1 min at each concentration, immersion in a Toluidine blue pH 0.5 dye solution for 7 min, dehydration by

Figure 2.8. Laminar and regional identification of electrode position

increasing concentrations of ethanol (50%, 70%, 90%, 100%) for 1 min and immersion in histoclear for 4min. Coverslips were mounted on slices with histomount before microscope viewing.

Images of the stained slices were taken from a microscope with magnification strength x4 and x10 (Figure 2.8) and the laminar and regional position of electrodes was identified.

2.9. Data Grouping and Statistical Analysis

Once electrode positions were identified, data extracted from multi-electrode, single-electrode field or intracellular recordings were categorized into different groups with respect to the mPFC region and layer they were obtained from (i.e. DP, IL, PrL and ACd). Data recorded from M1 and M2 regions were grouped together and assigned to the motor cortex group.

Statistical analysis of data was performed with SigmaStat (Systat Software Inc., San Jose, California, USA).

The normality of the data was assessed with the Kolmogorov-Smirnov normality test. When normality testing was accepted (parametric data), data were described with mean and standard deviation (SD) values. When normality testing failed (non-parametric data), data were described with median and 25%-75% quartile values (IQR).

To test the difference between two unrelated groups of measurements, the unpaired t-test, $t_{(\text{degrees of freedom})}$, was performed on parametric data and the Mann-Whitney U test on Ranks, $U_{(\text{degrees of freedom of small size sample})}$, on non-parametric data.

To examine the difference of more than two unrelated groups on a single measurement, the One Way Analysis of Variance (ANOVA), $F_{(\text{between groups degrees of freedom, residual degrees of freedom})}$, was performed on parametric data and the Kruskall-Wallis One Way ANOVA on Ranks, $H_{(\text{between groups degrees of freedom})}$, on non-parametric data.

To test the difference of more than two related groups of measurements, the Repeated-Measures ANOVA (RM-ANOVA), $F_{(\text{between treatments degrees of freedom, residual}}$

degrees of freedom), was performed on parametric data and the Friedman RM-ANOVA on Ranks, $\chi^2_{(\text{between groups degrees of freedom})}$, on non-parametric data.

The null hypothesis was rejected and the difference deemed statistically significant when $p<0.05$.

To isolate the group or groups of data that differed significantly from each other, multiple comparison procedures were performed.

With parametric data the Tukey test was employed for multiple comparisons (Tukey test: all pairwise comparisons) and the Dunnett's method was employed to examine the statistical difference between the control group versus the rest of the groups (Dunnett's method: ctrl vs. all comparison).

With non-parametric data the Tukey test was employed for multiple comparisons (Tukey test: all comparisons) and the Dunn's method was employed to examine the statistical difference between the control group versus the rest of groups (ctrl vs. all comparison).

Statistical analysis on phase results was performed on measurements taken from the modes of phase histogram distributions. The majority of the phase distributions were centred at approximately 180°, therefore, that phase results could be sufficiently described without the use of circular statistics.

Chapter 3

Generation of persistent cholinergic- and kainate-induced fast network oscillations in rat cortical and subcortical regions *in vitro*

Chapter 3 – Generation of persistent cholinergic- and kainate-induced fast network oscillations in rat cortical and subcortical regions *in vitro*

3.1. Introduction

3.1.1. *In vitro models of fast network oscillations in the rat medial PFC*

The generation of *in vitro* fast network oscillations has already been demonstrated in the rat mPFC. Fast network oscillations at the beta1-band (~12-15 Hz) were generated *in vitro* in the IL and PrL regions of submerged rat coronal mPFC slices, with carbachol (25 μM) application (van Aerde et al. 2008). Carbachol induced network oscillations by activating muscarinic acetylcholine receptors, since application of atropine, a non-selective muscarinic receptor antagonist, completely abolished them. Evidence that oscillations were sustained in the IL and PrL when those two regions were anatomically separated, as well as current-source density analysis of field recordings from intact slices, indicated that oscillations in these cortical regions were generated by separate neuronal networks (van Aerde et al. 2008).

Transient epochs of gamma frequency oscillations have also been produced in the PrL region of submerged mouse coronal mPFC slices, following brief focal application of kainate (1 mM; McNally et al. 2011). Oscillations lasted for 20-30 sec after cessation of kainate application and decreased in frequency over time from approximately 80 Hz to 30 Hz (McNally et al. 2011).

3.1.2. *Physiological function of cholinergic system*

Substantial evidence that implicated the contribution of cholinergic activity in the generation of cortical oscillations emerged from the seminal study by Steriade and colleagues (Steriade et al. 1991) which reported that stimulation of subcortical cholinergic nuclei elicits cortical gamma frequency oscillations in the anesthetized cat. In the same study application of muscarinic antagonists blocked the emergence of cortical oscillations. Since then, many *in vitro* models of network

oscillations have been introduced in the hippocampus (Fisahn et al. 1998) and the neocortex (Buhl et al. 1998; van Aerde et al. 2008; Yamawaki et al. 2008) that make use of the cholinergic receptor agonist, carbachol.

Muscarinic receptors are G-protein coupled receptors, which comprise with nicotinic receptors the two families of receptors that are activated by acetylcholine (Dencker et al. 2011). Five different muscarinic receptors have been genetically identified (M_1, M_2, M_3, M_4 and M_5), which are divided further into two major groups, based on their coupling to different intracellular signalling cascades; namely M_1-like family (M_1, M_3 and M_5 receptors) and M_2-like family (M_2 and M_4 receptors; Dencker et al. 2011; Gulledge et al. 2009).

Converging evidence from electrophysiological studies highlights the direct effect of muscarinic receptor activation in the excitability of cortical pyramidal cells and interneurons. It was shown in the guinea pig frontal cortex that ACh, via muscarinic receptor activation, induces a slow depolarization in cortical pyramidal cells by decreasing the voltage-dependent K^+ conductance (M-current; McCormick and Prince 1986). Muscarinic receptor activation was also shown to excite frontal cortical interneurons (Kawaguchi 1997; McCormick and Prince 1986).

Our knowledge about the roles of individual muscarinic receptors in modulating single-cell and network population activity has advanced over the last decade with the advent of knock-out mice lacking specific muscarinic receptors (Dencker et al. 2011; Gulledge et al. 2009). In wild type mice, tonic activation of muscarinic receptors with carbachol (10 μM) was shown to excite pyramidal cells localized in layer 5 of the mPFC (Gulledge et al. 2009). Excitation was the compound effect of cell depolarization from the resting membrane potential, reduction in the slow after-hyperpolarization (AHP) and the appearance of depolarizing after-potential (DAP) following AP generation (Gulledge et al. 2009). Experiments on knock-out mice showed that M_1 receptor tonic activation produced the carbachol-induced depolarization effect along with the DAP appearance. However, reduction in the AHP required the activation of M_1 receptors with at least one more member of the M_1-like family (Gulledge et al. 2009).

However, according to the same study, the excitability of cortical pyramidal cells depends on the duration of the exposure of muscarinic receptors to ACh (Gulledge

et al. 2009). In an earlier cue detection behavioural study it was shown that two different states of ACh activity can coexist in the rat PFC. A basal (tonic) state of ACh release which is present in awake animals and a transient (phasic) ACh release which takes place when cues are successfully detected (Parikh et al. 2007). Although, in the Gulledge and colleagues study (Gulledge et al. 2009) pyramidal cells were excited by carbachol application, which resembles the tonic state of ACh release, focal application of ACh in the mouse mPFC, which resembles the phasic ACh activity induced a marked hyperpolarization. Pyramidal cell hyperpolarization was shown to be the outcome of muscarinic receptor-activated Ca^{2+} release (Gulledge et al. 2009).

Another electrophysiological *in vitro* study highlighted the crucial importance of M_1 receptor activation for the generation of fast network oscillations in the hippocampus (Fisahn et al. 2002). Application of muscarine, a muscarinic selective agonist, produced oscillations in the CA3 area of the hippocampus of wild type mice. However, muscarine failed to produce oscillations in hippocampal slices obtained from knock-out mice lacking the M_1 receptors. Fast network oscillations were successfully produced in hippocampal slices obtained from both wild type and M_1 knock-out mice, with kainate application. The latter evidence indicates that despite the M_1 receptor expression deficiency, the hippocampus from M_1 knock-out mice could still sustain network oscillations (Fisahn et al. 2002).

3.1.3. *Physiological function of kainate receptors*

Kainate receptors form a group of ionotropic glutamate receptors (Huntley et al. 1994; Pinheiro and Mulle 2006). They are ligand-gated channels composed of the GluR5-7 and kainate-binding KA1 and KA2 subunits (Huntley et al. 1994). When activated by low concentrations of kainate (kainic acid), domoate (domoic acid) and glutamate, they become permeable to Na^+ and K^+ cations (Pinheiro and Mulle 2006).

Postsynaptic activation of kainate receptors was shown to trigger inward synaptic currents in CA3 pyramidal cells following tetanic stimulation of the mossy fibers in rat hippocampal slices (Vignes and Collingridge 1997). Moreover, in hippocampal slice preparations, kainate application at a concentration (100 nM)

which was adequate to induce gamma frequency oscillations, was shown to depolarize and increase the excitability of pyramidal cells and interneurons (Fisahn et al. 2004). The depolarizing effect of kainate on pyramidal cells was caused by a reduction in both the slow and medium AHP currents (Fisahn et al. 2005). Moreover, kainate receptors localized in the presynaptic terminals of GABAergic interneurons were shown to exert a suppressive effect on GABA release (Lerma 2006). The dual effect of kainate on GABAergic interneurons is somewhat contradictory, in that, on the one hand, it excites the cells potentiating GABAergic neurotransmission, but at the same time it suppresses GABA release. However, this compound effect may be beneficial for the sustainability of network oscillations preventing excessive GABA release (Fishan and Buhl 2001).

Other work in our lab suggests that fast network oscillations can be evoked in the mPFC with kainate application alone but only when using much higher (800 nM) concentrations (Gillougley et al unpublished observations). However, in this study we investigated the oscillogenic properties of combined application of carbachol and kainate.

3.1.4. *Objectives*

We wished to identify the types of oscillatory activity that could be generated in the mPFC *in vitro* and to determine whether different types of activity could be generated in different regions or different layers. This work would form the basis for further studies on neuronal activity during oscillations and catecholamine modulation of network activity.

3.2. Methods

Slices were prepared and maintained as outlined in section 2.2. Network oscillations were evoked with carbachol (10 μM) and kainate (200 nM) application. Multi-electrode field recordings were obtained from the mPFC with the recording equipment described in section 2.4. The preparation and application of drugs used in the experiments described in this chapter are outlined in section 2.3.

3.2.1. *Data analysis*

- Power spectral analysis performed on field traces, area power and peak frequency extraction techniques are described in section 2.6.1.
- Filter application on field traces is described in section 2.6.2.
- Rhythmicity index extraction from field traces is described in section 2.6.3.
- Cross-frequency phase-synchrony analysis applied between field traces is described in section 2.6.4.
- The histological procedures we used to identify the laminar and regional position of electrodes are outlined in section 2.8.
- Statistical analysis of grouped data is described in section 2.9.
- Descriptive statistics of spectral and rhythmicity characteristics of network oscillations were produced for every region by grouping the results extracted from recordings obtained from all the layers within this region.

3.3. Results

3.3.1. *Regional characterization of fast network oscillations in the rat medial PFC and the motor cortex*

Various *in vitro* models have been introduced over the two last decades, demonstrating the generation of network oscillations in the archicortex, entorhinal cortex and sensorimotor cortex (Buhl et al. 1998; Cunningham et al. 2003; Fisahn et al. 1998; Roopun et al. 2006; Whittington et al. 1995; Yamawaki et al. 2008). Recently, there have been attempts to generate *in vitro* fast network oscillations in regions of the prefrontal cortex employing AMPA/kainate or cholinergic-receptor activation (McNally et al. 2011; van Aerde et al. 2008). The main goal of the present study was the development of a novel *in vitro* model of persistent network oscillations elicited in regions of the rat mPFC by concurrent activation of cholinergic muscarinic and AMPA/kainate receptors. Although not the main focus of this study, in most cases the multi-electrode Utah array extended to the motor cortex (regions M1 and M2). Field recordings obtained from this cortical region were also analyzed and results of this analysis were grouped together (motor cortex) and presented in this thesis.

Our results demonstrate that concurrent application of carbachol (10 μM) and kainate (200 nM) can elicit persistent network oscillations in different regions of prefrontal cortical slice preparations at the temperature of ~28°C. Network oscillatory activity, in the range of 25-30 Hz, was recorded in the DP, IL, PrL and ACd subdivisions of the mPFC and the motor cortex (Figure 3.1-3.5). Oscillations emerged in all ventral regions at ~15-30 min and in the dorsal regions at ~60 min post-drug application and gradually increased in amplitude until they reached stability ~4 hours later. Once stable, network oscillations could persist for a period of up to 4 hours. The build-up phase of network oscillations was monitored with single field electrodes from emergence to stability in the DP and PrL regions. Grouped data from these recordings are included in Tables 3.1A,B and illustrated in Figures 3.6 and 3.7.

Figure 3.1. Fast network oscillations in the DP region

Figure 3.2. Fast network oscillations in the IL region

Figure 3.3. Fast network oscillations in the PrL region

Figure 3.4. Fast network oscillations in the ACd

Figure 3.5. Fast network oscillations in the motor cortex

Table 3.1. Generation and build-up of network oscillations in the mPFC

Figure 3.6. Generation and build-up of network oscillations in the DP region

Figure 3.7. Generation and build-up of network oscillations in the PrL region

There is increasing evidence that different subdivisions of the mPFC and the motor cortex receive different cortical and subcortical inputs and subserve different cognitive functions (Section 1.2.3). Therefore, we wished to examine whether there were any regional differences in the pattern of carbachol/kainate-evoked network oscillations between the four main subdivisions of the mPFC and the motor cortex. Initially, we assessed the regional differences between the area power, rhythmicity and peak frequency of network oscillations. For the sake of quality and in order to minimize interference by volume conduction, results presented in this chapter were derived from oscillations with area power greater than 100μV^2.Hz^{-1}. Field recordings that didn't fulfill this criterion were deemed non-oscillatory and for this reason they were not analyzed (discussed in section 7.2).

Stable network oscillatory activity in the DP region had a median area power of 569 μV^2.Hz^{-1} (IQR: 265-1396, n_{elec}=86, n_{slice}=22; Figure 3.8A). The median area power of network oscillations recorded in the IL region was 306 μV^2.Hz^{-1} (IQR: 186-590, n_{elec}=107, n_{slice}=25), in the PrL was 229 μV^2.Hz^{-1} (IQR: 141-429, n_{elec}=136, n_{slice}=21) and in the ACd was 266 μV^2.Hz^{-1} (IQR: 156-561, n_{elec}=106, n_{slice}=19). Finally, the motor cortex oscillated with a median area power of 247 μV^2.Hz^{-1} (IQR: 155-413, n_{elec}=157, n_{slice}=10). Multiple pairwise comparison analysis revealed that the median area power of network oscillations recorded in the DP was significantly greater than the median area power of network oscillations recorded in the IL, PrL, ACd and motor cortex. Moreover oscillations in the IL were significantly stronger to those recorded in the PrL region (Kruskall-Wallis One-Way ANOVA on Ranks: $H(4)=53.5$, $p<0.05$; Tukey test: all comparisons; Figure 3.8A). Overall, these data suggest that network oscillations elicited by simultaneous application of carbachol (10 μM) and kainate (200 nM) were stronger in the ventral mPFC (DP, IL) than in the dorsal mPFC (PrL, ACd) or the motor cortex.

A similar trend was also observed in the rhythmicity of network oscillations. Oscillations recorded in the DP had a rhythmicity index (RI) of 0.67 (IQR: 0.58-0.76, n_{elec}=86, n_{slice}=22; Figure 3.8B) whereas in the IL region they had a median RI of 0.61 (IQR: 0.50-0.71, n_{elec}=107, n_{slice}=25). Network activity in the PrL oscillated with a median RI of 0.57 (IQR: 0.44-0.66, n_{elec}=136, n_{slice}=21) and in the ACd with a

RI of 0.55 (IQR: 0.41-0.63, n_{elec}=106, n_{slice}=19). Rhythmicity levels in the motor cortex did not differ from those obtained from the dorsal regions of the mPFC, producing a median RI of 0.58 (IQR: 0.41-0.70, n_{elec}=157, n_{slice}=10). Statistical analysis revealed that oscillations recorded in the DP region had a significantly higher RI in comparison to the median RI values extracted from the rest of the regions. The second highest RI was observed in the IL which was statistically stronger to the one extracted from the ACd region (Kruskall-Wallis One-Way ANOVA on Ranks: $H(4)=43.1$, $p<0.05$; Tukey test: all comparisons; Figure 3.8B).

Although stronger and more rhythmic, oscillations recorded in the ventral mPFC were slower compared to the ones recorded in the PrL, ACd and motor cortex. Oscillations recorded in the DP region showed power spectra with a power peak at the frequency of 25.0 Hz (IQR: 23.3-27.0, n_{elec}=86, n_{slice}=22; Figure 3.8C). Network activity recorded in the IL oscillated at 24.0 Hz (IQR: 23-26, n_{elec}=107, n_{slice}=25), in the PrL region at 27.0 Hz (IQR: 24.0-30.0, n_{elec}=136, n_{slice}=21) and in the ACd at 28.0 Hz (IQR: 24.0-32.0, n_{elec}=106, n_{slice}=19). Multiple field recordings obtained from the motor cortex revealed oscillatory activity with a median frequency of 27.0 Hz (IQR: 24.0-29.0, n_{elec}=157, n_{slice}=10). Overall, network oscillations recorded in the PrL, ACd and motor cortex were significantly faster to the ones obtained from the DP and IL regions (Kruskall-Wallis One-Way ANOVA on Ranks: $H(4)=74.3$, $p<0.05$; Tukey test: all comparisons; Figure 3.8C).

Together these results show that network oscillations recorded in the ventral mPFC (DP, IL) were stronger and more rhythmic, but slower in comparison to the ones recorded in the dorsal mPFC (PrL, ACd) and the motor cortex. The frequency range of network activity recorded in the DP and the IL regions was in the beta2-band (20-30 Hz), whereas network oscillatory activity recorded in the PrL, ACd and motor cortex was closer to the low gamma-band (30-80 Hz). This evidence could suggest that the conjoint effect of cholinergic muscarinic and AMPA/kainate receptor activation could elicit the emergence of two different mechanisms of network oscillations in distinct regions of the PFC. This hypothesis will be explored further in chapter 4 when we assess the neurotransmitter systems involved in generating this activity as well as the relation of the oscillation frequency with the recording temperature. Hereafter we will refer to this network activity as fast network oscillations.

Figure 3.8. Regional spectral and rhythmicity profile of fast network oscillations in the mPFC and the motor cortex

3.3.2. *Laminar characterization of fast network oscillations in the dorsal peduncular cortex*

Having identified the regional differences in the strength, rhythmicity and frequency of network oscillations in the distinct regions of the mPFC and the motor cortex, we then went on to explore in more detail the network properties of oscillations for each region individually.

The mPFC and the motor cortex are multilayer cortical regions composing primarily of five layers with their own subdivisions (e.g. layers 1, 2, 3, 5, 6a, 6b). Therefore, we wished to examine whether there were any laminar differences in the pattern of network oscillatory activity within each region.

A sufficient number of field recordings were obtained from layers 2 to 6b in the DP region. The number and quality (oscillations with area power >100 $\mu V^2.Hz^{-1}$) of field recordings recorded in layer 1 of the DP region was deemed insufficient. Therefore, data obtained from this layer were not included in the statistical analysis. The boundary limits between layers 2 and 3 in the DP region are not clear; therefore results recorded from these layers were grouped together and referred to in this thesis as layer 2/3.

Network oscillations recorded in layer 6b of the DP region had a median area power of 342 $\mu V^2.Hz^{-1}$ (IQR: 254-485, n_{elec}=17, n_{slice}=12; Figure 3.9A). Network activity in layer 6a produced oscillations with a median area power of 867 $\mu V^2.Hz^{-1}$ (IQR: 389-1710, n_{elec}=21, n_{slice}=14). Network oscillations had a median area power of 952 $\mu V^2.Hz^{-1}$ (IQR: 432-2038, n_{elec}=24, n_{slice}=16) in layer 5 and a median area power of 819 $\mu V^2.Hz^{-1}$ (IQR: 227-1261, n_{elec}=24, n_{slice}=15) in layer 2/3.

In summary, network oscillations recorded in layer 6b were the weakest among the different laminar groups. The rest of the layers (layers 2/3 to 6a) oscillated with a large magnitude. Among them, the strongest oscillations were recorded in layer 5 (Figure 3.9A). However, statistical analysis revealed that the difference between the area power values among the different laminar groups was not strong enough to reach statistical significance (Kruskall-Wallis One-Way ANOVA on Ranks: $H(3)=7.4$, $p>0.05$; Figure 3.9A).

We then went on to assess the laminar rhythmicity profile of network oscillations in the DP region. Network oscillations produced in layer 6b had a

Figure 3.9. Laminar spectral and rhythmicity profile of fast network oscillations in the dorsal peduncular cortex

median RI of 0.76 (IQR: 0.63-0.79, n_{elec}=17, n_{slice}=12; Figure 3.9B), whereas those in layer 6a had median RI of 0.71 (IQR: 0.66-0.76, n_{elec}=21, n_{slice}=14). Field activity recorded in layer 5 of the DP region oscillated with a RI of 0.63 (IQR: 0.56-0.71, n_{elec}=24, n_{slice}=16) and in layer 2/3 with a median RI of 0.63 (IQR: 0.58-0.73, n_{elec}=24, n_{slice}=15). Overall, rhythmicity did not vary across the layers. In agreement with that, statistical analysis showed that the difference in the RI values among the different laminar groups was not strong enough to reach significance (Kruskall-Wallis One-Way ANOVA on Ranks: H(3)=7.2, p>0.05; Figure 3.9B).

We also investigated the difference in the frequency of oscillations across the different layers of the DP region. Network activity in layer 6b oscillated at 25.0 Hz (IQR: 23.0-27.0, n_{elec}=17, n_{slice}=12; Figure 3.9C), whereas in layer 6a the median frequency was 24.0 Hz (IQR: 23.8-26.0, n_{elec}=21, n_{slice}=14). Network oscillations recorded in layer 5 showed spectra with a power peak at 26.0 Hz (IQR: 25.0-28.0, n_{elec}=24, n_{slice}=16). Finally, layer 2/3 oscillated with a median frequency of 25.0 Hz (IQR: 24.0-26.5, n_{elec}=24, n_{slice}=15). Analysis of variance failed to produce any statistical significance among the frequency values of the different laminar groups (Kruskall-Wallis One-Way ANOVA on Ranks: H(3)=4.3, p>0.05; Figure 3.9C).

In summary, network oscillations in layer 6b were the weakest in magnitude, whereas the rest of the layers produced strong oscillations. Although network activity recorded in layer 5 tended to oscillate with greater magnitude, the frequency and rhythmicity did not vary between layers in the DP region.

3.3.3. *Laminar characterization of fast network oscillations in the infralimbic cortex*

The laminar characterization of fast network oscillations was also performed in the IL region. Similar to DP, the number of field recordings obtained from layer 1 of the IL region was deemed insufficient. Therefore, data obtained from this layer were not included into the analysis and not presented in this thesis. The laminar hallmarks of layer 2 and 3 were clear in the IL region. Therefore, data produced from these groups were assessed separately.

Figure 3.10. Laminar spectral and rhythmicity profile of fast network oscillations in the infralimbic cortex.

We first explored the laminar area power profile of fast network oscillations. Network oscillations recorded in layer 6b had a median area power of 243μV^2.Hz^{-1} (IQR: 187-301, n_{elec}=17, n_{slice}=10; Figure 3.10A), while those recorded in layer 6a had a median area power of 379μV^2.Hz^{-1} (IQR: 179-738, n_{elec}=27, n_{slice}=14). The strongest network oscillations of the region were recorded in layer 5 with a median area power of 628μV^2.Hz^{-1} (445-880, n_{elec}=24, n_{slice}=14). Finally, network oscillations in layer 3 had an area power of 242μV^2.Hz^{-1} (IQR: 148-384, n_{elec}=11, n_{slice}=10), while those recorded in layer 2 showed spectra with a median area power of 239μV^2.Hz^{-1} (IQR: 181-418, n_{elec}=27, n_{slice}=18). These data suggest that network activity in the IL tended to oscillate with greater amplitude in layer 5 than in the rest of the layers. Analysis of variance confirmed that, there was a significant difference between the area power values of layer 5 and layers 2, 3 and 6b (Kruskall-Wallis One-Way ANOVA on Ranks: $H(4)=23.8$, $p<0.05$; Tukey test: all comparisons; Figure 3.10A). Similar to the DP region, the second strongest oscillations were recorded in layer 6a (Figure 3.10A). Moreover, very weak oscillations were recorded in layer 6b. However, in contrast to the DP region, oscillations recorded in the superficial layers (layer 2, 3) were significantly weaker to the ones recorded in layer 5.

Rhythmicity of network oscillations did not vary across the different layers of the IL region. Autocorrelation analysis of the field recordings produced a median RI of 0.66 (IQR: 0.53-0.73, n_{elec}=17, n_{slice}=10; Figure 3.10B) in layer 6b and a median RI of 0.65 (IQR: 0.51-0.72, n_{elec}=27, n_{slice}=14) in layer 6a. Network activity in layer 5 oscillated with an RI of 0.64 (IQR: 0.52-0.74) while in layer 3 median RI was 0.54 (IQR: 0.51-0.56, n_{elec}=11, n_{slice}=10). Finally, network oscillations recorded in layer 2 had a median RI of 0.60 (IQR: 0.49-0.67, n_{elec}=27, n_{slice}=18). No statistical significance was produced among the RI values of the different laminar groups (Kruskall Wallis One-Way ANOVA on Ranks: $H(4)=6.1$, $p>0.05$; Figure 3.10B).

We also assessed the laminar frequency profile of fast network oscillations in the IL region. Spectral analysis revealed that network activity oscillated at 24.0 Hz (IQR: 23.0-26.0, n_{elec}=17, n_{slice}=10; Figure 3.10C) in layer 6b and at 24.0 Hz (IQR: 23.0-26.0, n_{elec}=27, n_{slice}=14) in layer 6a. Network oscillations recorded in layer 5 showed spectra with a power peak at 26.0 Hz (IQR: 24.0-26.5, n_{elec}=24, n_{slice}=14), whereas those recorded in layer 3 had a prominent peak at 24.0 Hz (IQR: 22.5-

24.0, n_{elec}=11, n_{slice}=10). Finally, network oscillations in layer 2 had a median frequency of 25.0 Hz (IQR: 23.3-26.0, n_{elec}=27, n_{slice}=18). The difference in the peak frequency values among the laminar groups was not statistically significant (Kruskal Wallis One-Way ANOVA on Ranks: $H(4)=6.6$, $p>0.05$; Figure 3.10C).

These data demonstrate that, similar to the laminar profile of the DP region, network activity in the IL region tended to oscillate with a stronger amplitude in layer 5 and 6a (especially layer 5). Moreover, both the rhythmicity and the frequency of oscillations were similar between the different layers. Unlike the DP region though, oscillations in the superficial layers were significantly smaller in magnitude in comparison to oscillations recorded in layer 5.

3.3.4. *Laminar characterization of fast network oscillations in the prelimbic cortex*

Inter-regional characterization of network oscillations revealed significant differences in the strength, rhythmicity and frequency between network oscillations recorded in the ventral (DP, IL) and dorsal mPFC (PrL, ACd). Laminar characterization of network oscillations in the ventral mPFC showed that both DP and IL exhibit similar trends in the area power, rhythmicity and frequency laminar profile, with layer 5 producing the strongest oscillations. We then went on to assess whether network oscillations in the PrL and ACd regions exhibit similar laminar characteristics.

We first investigated the laminar characterization of fast network oscillations in the PrL region. Insufficient data were obtained from layer 1 of the PrL region and were not included in the data analysis.

The area power laminar profile of the PrL region showed many similarities to the laminar pattern produced in the IL region. Network oscillations recorded in layer 6b had a median area power of 145 $\mu V^2.Hz^{-1}$ (IQR: 117-350, n_{elec}=16, n_{slice}=11; Figure 3.11A), whereas those recorded in layer 6a were stronger with a median area power of 264 $\mu V^2.Hz^{-1}$ (IQR: 145-435, n_{elec}=38, n_{slice}=14). Network oscillations recorded in layer 5 were the strongest in magnitude with an area power of 355 $\mu V^2.Hz^{-1}$ (IQR: 233-585, n_{elec}=44, n_{slice}=17). Weaker oscillations were produced in the superficial layers with layer 3 oscillating with an area power of 196 $\mu V^2.Hz^{-1}$

Figure 3.11. Laminar spectral and rhythmicity profile of fast network oscillations in the prelimbic cortex

(IQR: 130-235, n_{elec}=19, n_{slice}=8) and layer 2 with an area power of 145μV^2.Hz^{-1} (IQR: 129-180, n_{elec}=19, n_{slice}=9). Analysis of variance showed that oscillations recorded in layer 5 of the PrL region were significantly stronger to those recorded in layers 2, 3 and 6b (Kruskall-Wallis One-Way ANOVA on Ranks: $H(4)=22.3$, $p<0.05$; Tukey test: all comparisons; Figure 3.11A).

The laminar rhythmicity profile of network oscillations was also assessed in the PrL region. Network oscillations in layer 6b had a median RI of 0.49 (IQR: 0.38-0.65, n_{elec}=16, n_{slice}=11; Figure 3.11B), whereas in layer 6a they had a median RI of 0.59 (IQR: 0.47-0.69, n_{elec}=38, n_{slice}=14). Network activity in layer 5 oscillated with an RI of 0.59 (IQR: 0.47-0.69, n_{elec}=44, n_{slice}=17) while in layer 3 oscillated with a low RI of 0.44 (IQR: 0.42-0.53, n_{elec}=19, n_{slice}=8). Finally, network oscillations recorded in layer 2 had a median RI of 0.56 (IQR: 0.52-0.60, n_{elec}=19, n_{slice}=9). Overall, the levels of rhythmicity between the different layers of the PrL region were similar. The only exception was in layer 3 which oscillated with a significantly lower rhythmicity to layer 5 (Kruskall-Wallis One-Way ANOVA on Ranks: $H(4)=10.6$, $p<0.05$; Tukey test: all comparisons; Figure 3.11B).

Finally, we assessed the laminar frequency pattern of network oscillations in the PrL region. Network activity oscillated at 24.5 Hz (IQR: 24.0-27.0, n_{elec}=16, n_{slice}=11; Figure 3.11C) in layer 6b and at 27.0 Hz (IQR: 24.0-30.0, n_{elec}=38, n_{slice}=14) in layer 6a. Field recordings showed power spectra with a power peak at 27.5 Hz (IQR: 24.0-30.0, n_{elec}=44, n_{slice}=17) in layer 5 and a power peak at 26.0 Hz (IQR: 24.0-28.0, n_{elec}=19, n_{slice}=8) in layer 3. Network oscillations in layer 2 had a median frequency of 28.0 Hz (IQR: 27.0-29.0, n_{elec}=19, n_{slice}=9). No statistical significance was reached between the frequency values among the different laminar groups (Kruskall-Wallis One-Way ANOVA on Ranks: $H(4)=3.6$, $p>0.05$; Figure 3.11C).

In summary, the laminar pattern of fast network oscillations in the PrL region did not vary from activity recorded in the ventral mPFC. The strongest oscillations in the region were produced in layer 5, while the second strongest oscillations were produced in layer 6a. Moreover, similar to the IL region, oscillations in the superficial layers and layer 6b of the PrL were significantly weaker to oscillations recorded in layer 5. Rhythmicity levels were similar between layers, except for

layer 3 which was significantly smaller, although rhythmicity in this layer was still evident. Finally the frequency of network oscillations did not vary between layers.

3.3.5. *Laminar characterization of fast network oscillations in the anterior cingulate cortex*

The generation of network oscillations in the ACd region was tested in cortical slices spanning between 12.20 mm and 10.60 mm anterior to the interaural line (see Figure 1.1). Among them, slices with stereotaxic coordinates between 10.70 and 10.60 mm did not include the DP, IL, PrL subdivisions of the mPFC. Despite this important anatomical difference no variation was observed in the laminar characteristics of ACd network oscillations (data not shown). Therefore recordings obtained from slices that contained the intact mPFC and slices that contained only the ACd were grouped and analyzed together.

Insufficient data were obtained from layer 1 of the ACd region and were not included in the data analysis.

We first explored the laminar area power profile of network oscillations. Network oscillations had a median area power of 158 $\mu V^2.Hz^{-1}$ (IQR: 141-272, n_{elec}=10, n_{slice}=6; Figure 3.12A) in layer 6b and a low median area power of 162 $\mu V^2.Hz^{-1}$ (IQR: 141-244, n_{elec}=17, n_{slice}=11) in layer 6a. By far the strongest oscillations of the region were recorded in layer 5 with a median area power of 447 $\mu V^2.Hz^{-1}$ (IQR: 229-690, n_{elec}=55, n_{slice}=16). Oscillations were weak in layer 3 with an area power of 224 $\mu V^2.Hz^{-2}$ (IQR: 161-338, n_{elec}=11, n_{slice}=6) as well as in layer 2 with a median area power of 176 $\mu V^2.Hz^{-1}$ (IQR: 130-269, n_{elec}=13, n_{slice}=5). Analysis of variance showed that the difference in the area power values between layer 5 and the rest of the layers was statistically significant (Kruskall-Wallis One-Way ANOVA on Ranks: $H(4)=26.8$, $p<0.05$; Tukey test: all comparisons; Figure 3.12A). These results are similar to the data obtained in the DP, IL and PrL regions, in that oscillations tended to be stronger in layer 5. However, in contrast to the DP, IL and PrL regions, oscillations recorded in layer 6a are equally weak to those recorded in layer 6b.

Despite the significant difference in the area power values, the rhythmicity of oscillations between layers was comparable. Therefore, network activity oscillated

with a RI of 0.51 (IQR: 0.38-0.57, n_{elec}=10, n_{slice}=6; Figure 3.12B) in layer 6b and 0.57 (IQR: 0.45-0.61, n_{elec}=17, n_{slice}=11) in layer 6a. Network oscillations in layer 5 had an RI of 0.56 (IQR: 0.41-0.65, n_{elec}=55, n_{slice}=16), whereas those recorded in layer 3 had a median RI of 0.53 (IQR: 0.35-0.59, n_{elec}=11, n_{slice}=6). Finally, autocorrelation analysis of field recordings obtained in layer 2 produced an RI value of 0.57 (IQR: 0.49-0.63, n_{elec}=13, n_{slice}=5). The difference in the rhythmicity of network oscillations among the different laminar groups was not statistically significant (Kruskall-Wallis One-Way ANOVA on Ranks: $H(4)=2.7$, $p>0.05$; Figure 3.12 B).

We then went on to analyze the laminar frequency profile of network oscillations in the ACd region. Network activity oscillated at 28.0 Hz (IQR: 27.0-32.0, n_{elec}=10, n_{slice}=6; Figure 3.12C) in layer 6b and at 27.0 Hz (IQR: 24.0-31.0, n_{elec}=17, n_{slice}=11) in layer 6a. Field recordings showed power spectra with a power peak at 29.0 Hz (IQR: 24.0-32.3, n_{elec}=55, n_{slice}=16) in layer 5 and a power peak at 24.0 Hz (IQR: 23.3-30.3, n_{elec}=11, n_{slice}=6) in layer 3. The fastest oscillations were recorded in layer 2 with a median frequency of 32.0 Hz (IQR: 27.0-33.0, n_{elec}=13, n_{slice}=5). Although variation between the frequency values of the different laminar groups was evident, it was not strong enough to reach statistical significance (Kruskall-Wallis One-Way ANOVA on Ranks: $H(4)=7.0$, $p>0.05$; Figure 3.12 C).

Overall, the laminar profile of fast network oscillations in the ACd presented many similarities to the laminar characteristics of network oscillations in the other subdivisions of the mPFC. Similar to the DP, IL and PrL regions, rhythmicity and frequency did not vary significantly between the different layers of the ACd region. Moreover, oscillations were significantly stronger in layer 5. However, the major difference was observed in layer 6a which produced remarkably weak oscillations.

3.3.6. *Laminar characterization of fast network oscillations in the motor cortex*

The motor cortex presents different connectivity patterns and subserves different cognitive functions to the mPFC (Gabbott et al. 2005). Therefore, it is possible that the laminar characterization of the fast network oscillations elicited

Figure 3.12. Laminar spectral and rhythmicity profile of fast network oscillations in the anterior cingulate cortex

in this region by carbachol (10 μM) and kainate (200 nM), would produce some unique qualities.

The multi-electrode array recorded oscillatory activity from a cortical area that covered layers 3 to 6b. No recordings were obtained from layers 1 and 2.

We first assessed the laminar area power profile of network oscillations in the motor cortex. Network oscillations recorded in layer 6b had a median area power of 132 $\mu V^2.Hz^{-1}$ (IQR: 108-149, n_{elec}=8, n_{slice}=4; Figure 3.13A) while those recorded in layer 6a had a median area power of 227 $\mu V^2.Hz^{-1}$ (IQR: 124-301, n_{elec}=30, n_{slice}=9). The strongest oscillations of the region were recorded in layer 5 with a median area power of 287 $\mu V^2.Hz^{-1}$ (IQR: 202-535, n_{elec}=95, n_{slice}=10), whereas oscillations recorded in layer 3 had an area power of 232 $\mu V^2.Hz^{-1}$ (IQR: 130-429, n_{elec}=24, n_{slice}=8). Statistical analysis revealed that the area power of oscillations recorded in layer 5 were significantly stronger to the area power values extracted from layer 6a and 6b (Kruskall-Wallis One-Way ANOVA on Ranks: H(3)=17.8, $p<0.05$; Tukey test: all comparisons; Figure 3.13A).

In contrast to the mPFC, strong variation was observed in the rhythmicity of network oscillations between the different layers of the motor cortex such that layer 6b oscillated with a high RI of 0.76 (IQR: 0.76-0.78, n_{elec}=8, n_{slice}=4; Figure 3.13B), and layer 6a oscillated with a rather similar RI of 0.68 (IQR: 0.59-0.76, n_{elec}=30, n_{slice}=9). In layer 5 the RI of network oscillations was decreased down to 0.53 (IQR: 0.39-0.67, n_{elec}=95, n_{slice}=10) and in layer 3 it was further reduced to 0.48 (IQR: 0.35-0.65, n_{elec}=24, n_{slice}=8). Analysis of variance showed that the rhythmicity of oscillations in layers 6a and 6b were significantly more rhythmic to the ones recorded in layers 3 and 5 (Kruskall-Wallis One-Way ANOVA on Ranks: H(3)=28.8, $p<0.05$; Tukey test: all comparisons; Figure 3.13B).

The laminar frequency profile of fast network oscillations in the motor cortex was also assessed. Network activity oscillated at 24.0 Hz (IQR: 24.0-29.0, n_{elec}=8, n_{slice}=4; Figure 3.13C) in layer 6b and at 27.0 Hz (IQR: 24.0-29.0, n_{elec}=30, n_{slice}=9) in layer 6a. Network oscillations recorded in layer 5 had a median frequency of 27.0 Hz (IQR: 24.0-30.0, n_{elec}=95, n_{slice}=10) whereas those recorded in layer 3 produced spectra with a power peak at 26.0 Hz (IQR: 24.0-28.0, n_{elec}=24, n_{slice}=8). No statistical difference was found between the frequency values of network

Figure 3.13. Laminar spectral and rhythmicity profile of fast network oscillations in the motor cortex

oscillations across the different layers (Kruskall-Wallis One-Way ANOVA on Ranks: $H(3)=3.6$, $p>0.05$; Figure 3.13C).

In summary, the laminar spectral and rhythmicity profile of network oscillations in the motor cortex presented some interesting properties. In contrast to the mPFC, oscillations in the deeper layers were significantly more rhythmic to the ones recorded in layers 3 and 5. Unlike any other region tested, layer 3 produced the second strongest oscillations in the region. However, similar to the mPFC, layer 5 produced the strongest oscillations in the region, whereas oscillations in layer 6b were remarkably weak. Finally, the laminar frequency profile showed no strong variation, which resembles the frequency profile observed in the mPFC.

3.3.7. *Inter-regional phase-synchrony and phase-difference characterization of fast network oscillations in the mPFC and the motor cortex*

Cross-frequency phase-synchrony analysis performed on simultaneously recorded field potentials provides us with the opportunity to investigate the network synchrony (indicated by the phase-synchrony Shannon entropy values) as well as the phase-difference between two spatially distributed oscillating recording sites. The presence of network synchrony is a strong indication of functional connectivity between two separate neuronal networks (Varela et al. 2001).

Initially, we investigated the phase-synchrony profile between the different PFC regions (sections 3.3.7.1-3.3.7.5). To do so we first calculated the Shannon entropy between field potentials recorded concurrently in layers 5 or 6a. The reason why we chose these layers was because they produced on average the strongest oscillations within their regions. Then we set the reference region and the reference location point within this region. In slices that contained the intact mPFC (12.20 mm – 11.20 mm Interaural; Figure 1.1B), electrodes positioned in the most ventral parts of the reference region were chosen as the reference location points of this grouping scheme. In slices that contained only the ACd and the motor cortex (10.70 mm – 10.60 mm Interaural; Figure 1.1B), electrodes positioned close to the boundary limit of these regions were chosen as the reference location points.

Figure 3.14. Inter-regional cross frequency phase-synchrony analysis

Shannon entropy values between the reference recording site and the secondary one were assigned to the distance between them as well as the region of the secondary electrode (Figure 3.14).

3.3.7.1. Inter-regional profile of network synchrony between the dorsal peduncular cortex and other mPFC subdivisions

To investigate the phase-synchrony profile of network oscillations in the mPFC with reference to the DP region, electrodes positioned at the most ventral parts of this region were chosen as the reference location points.

In direction dorsal to the reference location point at the distance of 400 μm (a distance extending within the DP limits; DP; Figure 3.15A) phase-synchrony was strong with a Shannon entropy of 0.29 (IQR: 0.26-0.31, n_{pair}=8, n_{slice}=8). 800 μm (IL) dorsal to the reference point, Shannon entropy declined down to 0.18 (IQR: 0.15-0.19, n_{pair}=6, n_{slice}=6). At 1200 μm (IL) Shannon entropy was further reduced to 0.13 (IQR: 0.12-0.14, n_{pair}=8, n_{slice}=8); however, phase-synchrony was still evident. At distances greater than 1200 μm phase histograms had a uniform distribution revealing no signs of phase-synchrony. 1600 μm (PrL) apart from the reference area, Shannon entropy had a median value of 0.11 (IQR: 0.11-0.12, n_{pair}=8, n_{slice}=8), at 2000 μm (PrL) it remained at 0.11 (IQR: 0.11-0.12, n_{pair}=8, n_{slice}=8) and at 2400 μm (PrL) it was 0.11 (IQR: 0.11-0.12, n_{pair}=6, n_{slice}=6).

3.3.7.2. Inter-regional profile of network synchrony between the infralimbic cortex and other mPFC subdivisions

To investigate the phase-synchrony profile of fast network oscillations in the mPFC with reference to the IL region, electrodes that were positioned at the most ventral parts of this region were chosen as the reference location points.

Phase-synchrony was evident between the reference electrodes and electrodes located 800 μm more ventrally (DP) with a Shannon entropy of 0.16 (IQR: 0.14-0.20, n_{pair}=4, n_{slice}=4; Figure 3.15B). 400 μm (DP) closer to the reference electrodes phase-synchrony became stronger with a Shannon entropy of 0.24 (IQR: 0.22-0.29, n_{pair}=8, n_{slice}=6). In direction dorsal to the reference area at the distance of 400 μm (IL) phase-synchrony remained strong with a Shannon entropy of 0.22 (IQR: 0.20-

Figure 3.15. Inter-regional phase-synchrony profile of fast network oscillations with reference to the dorsal peduncular cortex and the infralimbic cortex

0.25, n_{pair}=13, n_{slice}=11). Moving 800 μm (PrL) further away from the reference area Shannon entropy values declined to 0.15 (IQR: 0.14-0.17, n_{pair}=6, n_{slice}=6). 1200 μm further apart (PrL), Shannon entropy values became smaller at 0.12 (IQR: 0.11-0.13, n_{pair}=10, n_{slice}=9) and at the distance of 1600 μm (PrL) phase histograms with uniform distribution were evident producing Shannon entropy values of 0.11 (IQR: 0.11-0.12, n_{pair}=7, n_{slice}=6). At 2000 μm (ACd) dorsal to the reference area phase histograms had a uniform distribution with a median Shannon entropy of 0.11 (IQR: 0.11-0.13, n_{pair}=5, n_{slice}=5).

3.3.7.3. Inter-regional profile of network synchrony between the prelimbic cortex and other mPFC subdivisions

To investigate the phase-synchrony profile of network oscillations in the mPFC with reference to the PrL region, electrodes that were positioned at the most ventral parts of this region were chosen as the reference location points.

No signs of phase-synchrony were observed when we employed pair-wise phase comparisons between the reference electrodes and electrodes located 1600 μm (DP) more ventrally. The outcome of these comparisons were phase histograms with uniform distribution and a low median Shannon entropy value of 0.11 (IQR: 0.11-0.12, n_{pair}=5, n_{slice}=5; Figure 3.16A). Likewise, phase-synchrony was absent at the distance of 1200 μm ventral to the reference area (DP) producing Shannon entropy values of 0.12 (IQR: 0.12-0.12, n_{pair}=10, n_{slice}=10). At 800 μm (IL) ventral to the reference area, signs of weak phase-synchrony appeared producing Shannon entropy values of 0.13 (IQR: 0.13-0.14, n_{pair}=9, n_{slice}=9). At 400 μm (IL) closer to the reference area phase-synchrony became stronger producing Shannon entropy values of 0.23 (IQR: 0.20-0.23, n_{pair}=6, n_{slice}=5). In the direction dorsal to the reference area and at the distance of 400 μm (PrL), phase-synchrony remained strong with a median Shannon entropy of 0.23 (IQR: 0.21-0.25, n_{pair}=12, n_{slice}=12). Moving further away, at 800 μm (PrL) dorsal to the reference area, phase-synchrony declined again producing Shannon entropy values of 0.15 (IQR: 0.14-0.19, n_{pair}=8, n_{slice}=8). At even longer distances network synchrony was almost absent with only sparse cases of weak phase-synchrony. Therefore, at 1200 μm (PrL) dorsal to the reference area pair-wise phase comparisons produced Shannon entropy values of 0.13 (IQR: 0.12-0.14, n_{pair}=6,

n_{slice}=6) and at 1600 µm (ACd) they produced Shannon entropy values of 0.13 (IQR: 0.12-0.13, n_{pair}=4, n_{slice}=4).

3.3.7.4. Inter-regional profile of network synchrony between the anterior cingulate cortex and other mPFC subdivisions

To investigate the phase-synchrony profile of network oscillations in the mPFC with reference to the ACd, only slices that contained the intact mPFC were used (i.e. 12.20 mm - 11.20 mm Interaural; Figure 1.1B). Electrodes that were positioned at the most ventral parts of the ACd were chosen as the reference location points.

In the direction ventral to the reference area at a distance of 1200 µm (PrL) phase-synchrony was weak but still evident with a mean Shannon entropy of 0.14 (SD: ±0.03, n_{pair}=6, n_{slice}=4; Figure 3.16B). 800 µm ventral to the reference area (PrL) Shannon entropy values rose to 0.17 (SD: ±0.05, n_{pair}=7, n_{slice}=4) and at the distance of 400 µm (PrL) it increased further up to 0.20 (SD: ±0.05, n_{pair}=5, n_{slice}=3). In direction dorsal to the reference area, at a distance of 400 µm (ACd) phase-synchrony remained strong with Shannon entropy values of 0.22 (SD: ±0.06, n_{pair}=8, n_{slice}=4). 800 µm (ACd) dorsal to the reference area phase-synchrony was still evident with a mean Shannon entropy of 0.17 (SD: ±0.05, n_{pair}=5, n_{slice}=3).

In summary, data suggest that across the different subdivisions of the mPFC, network synchrony was strong between local network oscillations recorded within the inter-electrode space of 400 µm along the dorso-ventral axis. This trend did not differ when the electrodes localized either the same or different regions. In all cases tested, network synchrony declined progressively with the inter-electrode distance and was abolished at distances greater than 1200 µm. As a result, a substantial amount of synchrony was observed between oscillations recorded in the DP and the IL, the IL and the PrL, the PrL and the ACd. Instead, weak signs of synchrony were detected between oscillations recorded in the DP and the PrL, or the DP and the ACd, or the IL and the ACd. By far the stronger phase-synchrony was observed in network oscillations recorded within the DP region which sustained the strongest and most rhythmic oscillations in the mPFC.

Figure 3.16. Inter-regional phase-synchrony profile of fast network oscillations with reference to the prelimbic cortex and the anterior cingulate cortex

3.3.7.5. Inter-regional profile of network synchrony between the anterior cingulate and the motor cortex

We also investigated the phase-synchrony profile of network oscillations in the ACd and the motor cortex. To do so we included into the analysis only those slices that contained these two cortical regions (i.e. 10.70 mm – 10.60 mm anterior to the interaural line; Figure 1.1B).

Initially, the ACd was chosen as the reference region and the electrodes positioned within this region but close to the boundary limit of ACd and motor cortex where chosen as the reference electrodes (Figure 3.14B).

In the direction medial to the reference electrodes, at a distance of 1200 μm (ACd) network synchrony was absent with only sparse cases of weak phase-synchrony. Shannon entropy produced at this distance had a median value of 0.13 (IQR: 0.12-0.14, n_{pair}=4, n_{slice}=4; Figure 3.17A). 800 μm ventral to the reference area (ACd) phase-synchrony was evident with Shannon entropy values of 0.14 (IQR: 0.13-0.16, n_{pair}=6, n_{slice}=5). At the distance of 400 μm (ACd) phase-synchrony was strong producing Shannon entropy values of 0.21 (IQR: 0.17-0.24, n_{pair}=9, n_{slice}=5). In the direction lateral to the reference area, at a distance of 400 μm (motor) phase-synchrony remained at high levels with Shannon entropy values of 0.18 (IQR: 0.15-0.21, n_{pair}=9, n_{slice}=5). At 800 μm (motor) lateral to the reference electrodes phase-synchrony declined, however, the presence of network synchrony was still evident. At this distance, Shannon entropy had the median value of 0.15 (IQR: 0.13-0.16, n_{pair}=8, n_{slice}=4). 1200 μm lateral to the reference area (motor) phase-synchrony was almost absent with a median Shannon entropy of 0.13 (IQR: 0.12-0.15, n_{pair}=4, n_{slice}=4) and only sparse cases of weak phase-synchrony.

We then went on to analyze the phase-synchrony profile of network oscillations, choosing this time the motor cortex as the reference region. Electrodes that were positioned within this region and close to the boundary limit of ACd and motor cortex where chosen as the reference electrodes.

Figure 3.17. Inter-regional phase-synchrony profile of fast network oscillations with reference to the anterior cingulate and the motor cortex

In direction medial to the reference area, at a distance of 1200 μm (ACd), phase-synchrony was evident with Shannon entropy values of 0.14 (IQR: 0.13-0.15, n_{pair}=4, n_{slice}=3; Figure 3.17B). 800 μm medial to the reference area (ACd) Shannon entropy was 0.15 (IQR: 0.13-0.15, n_{pair}=7, n_{slice}=4), and at distance of 400 μm (ACd) it increased up to 0.17 (IQR: 0.16-0.18, n_{pair}=5, n_{slice}=4). In the lateral direction and 400 μm apart from the reference area (motor) phase-synchrony was strong producing Shannon entropy values of 0.20 (IQR: 0.18-0.26, n_{pair}=11, n_{slice}=9). 800 μm (motor) apart Shannon entropy declined down to 0.16 (IQR: 0.14-0.18, n_{pair}=10, n_{slice}=9), and at distance of 1200 μm (motor) network synchrony was still evident, although weaker with a median Shannon entropy of 0.14 (IQR: 0.13-0.16, n_{pair}=8, n_{slice}=7). Remarkably, at 1600 μm lateral to the reference electrodes network synchrony was still evident with Shannon entropy values of 0.14 (IQR: 0.13-0.16, n_{pair}=5, n_{slice}=4).

In summary, unlike the mPFC, Shannon entropy values produced at the inter-electrode space of 400 μm in slices that contained only the ACd and motor cortex were on average smaller than the ones that contained the intact mPFC. Another important difference was that in the motor cortex network synchrony was stronger at the inter-electrode distance of 1200 μm in comparison to the other regions. Remarkably, even at the distance of 1600 μm, substantial amounts of phase-synchrony were detected. However, similar to our conclusions in the subdivisions of the mPFC, the highest levels of phase-synchrony were detected between network oscillations recorded at the inter-electrode space of 400 μm across the medio-lateral axis. Equal levels of phase-synchrony were produced when the electrodes were both localized in the ACd or the motor cortex.

3.3.7.6. Inter-regional phase-difference profile of fast network oscillations in the rat medial PFC and the motor cortex

Having identified the inter-regional profile of network synchrony, we then proceeded to the investigation of the inter-regional phase-difference profile of network oscillations in the mPFC and the motor cortex. We determined that phase-difference could be reliably extracted from pair-wise phase comparisons with Shannon entropy values greater than 0.13. Smaller Shannon entropy values were

extracted from phase histograms with almost uniform distributions, indicating the absence of network interaction between the two oscillating sites.

Phase-difference was computed between field potentials recorded concurrently in layers 5 or 6a. We chose those layers because they produced on average the strongest oscillations within their regions.

The inter-regional phase-difference profile was first investigated in cortical slices that contained the intact mPFC (11.20 mm – 12.20 mm Interaural; Figure 1.1B). For these slices, the PrL was chosen as the reference region.

A small amount of variation was observed in the phase-difference values between the mPFC subdivisions at layer 6a. Phase-difference between LFP traces recorded in layer 6a within the PrL was only +0.1° (SD: ±12.2, n_{pair}=30, n_{slice}=6; Figure 3.18A). Network oscillations recorded in the PrL succeeded those recorded in the DP by -0.7° (SD: ±15.4, n_{pair}=8, n_{slice}=4) and those recorded in the ACd by -8.3° (SD: ±12.8, n_{pair}=18, n_{slice}=4). Finally, PrL oscillations were almost in phase with the IL oscillations, producing a mean phase difference of +0.7° (SD: ±11.5, n_{pair}=23, n_{slice}=6). No statistical significance was found in the phase difference values between any of the mPFC subdivisions (One-Way ANOVA: $F(3,75)=2.2$, $p>0.05$; Figure 3.18A).

Greater variation was observed in the phase-difference values between the mPFC subdivisions in layer 5. The phase-difference between LFP traces recorded in layer 5 within the PrL was -11.6° (SD: ±15.7, n_{pair}=19, n_{slice}=8; Figure 3.18B) and the phase-difference between oscillations recorded in the PrL and DP regions was +0.7° (SD: ±19.9, n_{pair}=8, n_{slice}=4). Greater differences were detected in the other two subdivisions of the mPFC. Hence, PrL oscillations preceded the IL ones by +18.2° (SD: ±13.8, n_{pair}=12, n_{slice}=6) and succeeded the ACd ones by -18.0° (SD: ±23.1, n_{pair}=15, n_{slice}=5). The latter two differences, although small, were statistically significant (One-Way ANOVA: $F(3,50)=9.9$, $p<0.05$; Tukey test: all comparisons; Figure 3.18B).

Figure 3.18. Inter-regional phase-difference profile of fast network oscillations in the mPFC.

For the estimation of the regional difference between the ACd and the motor cortex we analyzed LFP traces recorded from slices that contained only those two regions (i.e. 10.70 mm – 10.60 mm Interaural; Figure 1.1B). Moreover, the ACd was chosen as the reference region.

Phase-difference was small between LFP traces recorded in layer 6a. Therefore, the phase-difference between LFP traces recorded within ACd layer 6a was -7.3^o (SD: ±8.9, n_{pair}=14, n_{slice}=3; Figure 3.19A), and between ACd and motor cortex was -5.8^o (SD: ±6.3, n_{pair}=8, n_{slice}=3). Statistical comparison of the two different groups did not reach significance (unpaired t-test: t(20)=-0.4, p>0.05; Figure 3.19A).

Similar to layer 6a, the phase-difference between the two regions in layer 5 did not vary significantly. Therefore, the phase-difference between LFP traces recorded within ACd layer 5 was +4.2^o (SD: ±26.8, n_{pair}=46, n_{slice}=5; Figure 3.19B) and between ACd and motor cortex was -10.3^o (SD: ±22.5, n_{pair}=49, n_{slice}=5). Again, the difference between the phase values of the two groups was not great enough to reach significance (Mann-Whitney U test on Ranks: U(46)=106, p>0.05; t-test; Figure 3.19B).

3.3.8. *Inter-laminar phase-synchrony and phase-difference characterization of fast network oscillations in the mPFC and the motor cortex*

Cross-frequency phase coherence analysis was also employed for the investigation of phase-synchrony and phase-difference between network oscillations recorded simultaneously in different layers of the same region. Results from this analysis can give us an insight about the involvement of different layers in the generation of network oscillations. The synchrony strength was quantified with Shannon entropy values. The laminar profile was extracted by grouping the outcomes of pair-wise phase comparisons between layer 5 oscillations and the rest of the layers. For the sake of simplicity when we describe the phase-synchrony or phase-difference between the reference layer (i.e. layer 5) and the secondary layer (e.g. layer 3), it should be understood that these results will be attributed to the secondary layer (i.e. layer 3). Moreover, phase difference results are described with signs.

Figure 3.19. Inter-regional phase-difference profile of fast network oscillations in slices containing the ACd and the motor cortex

When the sign is positive, it means that the phase variations of layer 5 oscillations precede the phase variations of the oscillations recorded in the second layers. When the sign is negative, it means that the phase variations of oscillations in layer 5 succeed the phase variations of the oscillations recorded by the second layer.

3.3.8.1. Inter-laminar phase-synchrony and phase-difference profile of fast network oscillations in the dorsal peduncular cortex

In the DP region, phase-synchrony was evident between all layers. Shannon entropy between LFP traces recorded from different locations within layer 5 was 0.27 (SD: ±0.06, n_{pair}=4, n_{slice}=3; Figure 3.20A). Shannon entropy in layer 6b was 0.27 (SD: ±0.07, n_{pair}=15, n_{slice}=7), whereas in layer 6a it was 0.25 (SD: ±0.06, n_{pair}=5, n_{slice}=5). Phase synchrony in layer 2/3 remained strong with a mean Shannon entropy of 0.24 (SD: ±0.08, n_{pair}=11, n_{slice}=5).

Phase-difference between LFP traces recorded within layer 5 had the median value of +9.8^{o} (SD: ±13.9, n_{pair}=4, n_{slice}=3; Figure 3.20B). Oscillations in layer 5 co-varied with oscillations in layer 6b at -5.9^{o} (SD: ±22.2, n_{pair}=15, n_{slice}=7) and with layer 6a around -19.4^{o} (SD: ±20.1, n_{pair}=5, n_{slice}=5). Oscillations in layer 5 preceded oscillations in layer 2/3 by +99.0^{o} (SD: ±56.8, n_{pair}=11, n_{slice}=5). The latter difference was statistically significant (Kruskall-Wallis One-Way ANOVA on Ranks: $H(3)=27.0$, $p<0.05$; Dunn's method: layer 5 vs. all; Figure 3.20B).

3.3.8.2. Inter-laminar phase-synchrony and phase-difference profile of fast network oscillations in the infralimbic cortex

Strong inter-laminar phase-synchrony was observed in the IL region. Pairwise phase comparisons between traces recorded in layer 5 produced Shannon entropy values of 0.22 (SD: ±0.03, n_{pair}=7, n_{slice}=5; Figure 3.21A). Shannon entropy in layer 6b had the mean value of 0.23 (SD: ±0.04, n_{pair}=8, n_{slice}=4) and in layer 6a the mean value of 0.22 (SD: ±0.05, n_{pair}=8, n_{slice}=5). Phase-synchrony was slightly weaker in layer 3 producing Shannon entropy values of 0.19 (SD: ±0.04, n_{pair}=7, n_{slice}=5) but it recovered in layer 2 where Shannon entropy had the mean values of 0.23 (SD: ±0.05, n_{pair}=19, n_{slice}=9).

Figure 3.20. Phase-synchrony and phase-difference profile of fast network oscillations in the dorsal peduncular cortex

Phase-difference within oscillations recorded in layer 5 was -12.5° (SD: ±9.9, n_{pair}=7, n_{slice}= 5; Figure 3.21B). Phase-difference between LFP traces recorded in layer 5 and layer 6b was -12.3° (SD: ±14.7, n_{pair}=8, n_{slice}= 4) and between layer 5 and layer 6a it was -1.8° (SD: ±8.2, n_{pair}=8, n_{slice}= 5). Layer 5 oscillations preceded layer 3 oscillations by +67.9° (SD: ±17.6, n_{pair}=7, n_{slice}= 5). Phase-difference in layer 2 increased further up to +121.9° (SD: ±20.8, n_{pair}=19, n_{slice}= 9). The difference in the mean phase values between layer 5 and layers 2 and 3 was statistically significant (One-Way ANOVA: $F(4,40)=162.5$, $p<0.05$; Dunnett's method: layer 5 vs. all; Figure 3.21B).

3.3.8.3. Inter-laminar phase-synchrony and phase-difference profile of fast network oscillations in the prelimbic cortex

Shannon entropy between oscillations recorded in layer 5 of the PrL had the mean value of 0.19 (SD: ±0.06, n_{pair}=21, n_{slice}=8; Figure 3.22A). Phase-synchrony was also strong in deeper layers producing Shannon entropy values of 0.22 (SD: ±0.03, n_{pair}=4, n_{slice}=4) in layer 6b and 0.18 (SD: ±0.04, n_{pair}=34, n_{slice}=6) in layer 6a. Strong phase-synchrony was present in superficial layers as well. In layer 3 Shannon entropy was 0.23 (SD: ±0.04, n_{pair}=11, n_{slice}=4), while in layer 2 it was 0.18 (SD: ±0.05, n_{pair}=13, n_{slice}=6).

Phase-difference between LFP traces recorded within layer 5 was -10.3° (SD: ±15.4, n_{pair}=21, n_{slice}=8; Figure 3.22B). Phase-difference between layer 5 and layer 6b was -16.2° (SD: ±51.5, n_{pair}=4, n_{slice}=4), and in layer 6a it had the median value of -15.9° (SD: ±24.5, n_{pair}=34, n_{slice}=6). Phase-difference in layer 3 increased to +92.4° (SD: ±28.4, n_{pair}=11, n_{slice}=4) and in layer 2 it further increased to +108.1° (SD: ±39.6, n_{pair}=13, n_{slice}=6). The phase-difference between layer 5 and layers 2 and 3 was statistically significant (Kruskall-Wallis One-Way ANOVA on Ranks: $H(4)=47.9$, $p<0.05$; Dunn's method: layer 5 vs. all, Figure 3.22B).

Figure 3.21. Phase-synchrony and phase-difference profile of fast network oscillations in the infralimbic cortex

Figure 3.22. Phase-synchrony and phase-difference profile of fast network oscillations in the prelimbic cortex

3.3.8.4. Inter-laminar phase-synchrony and phase-difference profile of fast network oscillations in the anterior cingulate cortex

Inter-laminar phase-synchrony and phase-difference analysis was carried out in the ACd with pairwise phase comparisons obtained from slices spanning between 12.20mm and 10.60mm anterior to the interaural line.

Shannon entropy values between LFP traces recorded within layer 5 had a median value of 0.19 (IQR: 0.16-0.22, n_{pair}=48, n_{slice}=10; Figure 3.23A). Shannon entropy in layer 6b was 0.15 (IQR: 0.13-0.19, n_{pair}=24, n_{slice}=5), in layer 6a was 0.21 (IQR: 0.16-0.23, n_{pair}=43, n_{slice}=7). Phase-synchrony remained strong in the superficial layers as well, producing Shannon entropy results with a median value of 0.20 (IQR: 0.16-0.24, n_{pair}=21, n_{slice}=4) in layer 3 and a median value of 0.15 (IQR: 0.13-0.20, n_{pair}=42, n_{slice}=4) in layer 2.

Phase-difference between network oscillations recorded within layer 5 was +1.4^{o} (IQR: -7.0/+19.7, n_{pair}=48, n_{slice}=10; Figure 3.23B). Phase-difference between LFP traces recorded between layer 5 and layer 6b was -7.7^{o} (IQR: -20.4/+11.9, n_{pair}=24, n_{slice}= 5), and in layer 6a was -1.4^{o} (IQR: -14.8/+4.2, n_{pair}=43, n_{slice}=7). Phase-difference in layer 3 increased to +90.0^{o} (IQR: +65.4/+140.9, n_{pair}=21, n_{slice}=4), and in layer 2 it increased further up to +106.8^{o} (IQR: +81.6/+122.3, n_{pair}=42, n_{slice}=4). The phase-difference between layer 5 and layers 2 and 3 was statistically significant (Kruskall-Wallis One-Way ANOVA on Ranks: $H(4)=105.8$, $p<0.05$; Dunn's method: layer 5 vs. all; Figure 3.23B).

3.3.8.5. Inter-laminar phase-synchrony and phase-difference profile of fast network oscillations in the motor cortex

In the motor cortex the Shannon entropy between traces recorded at layer 5 was 0.17 (IQR: 0.14-0.22, n_{pair}=305, n_{slice}=10; Figure 3.24A). Shannon entropy in layer 6b was 0.17 (IQR: 0.14-0.22, n_{pair}=54, n_{slice}=4), in layer 6a it was 0.18 (IQR: 0.15-0.23, n_{pair}=242, n_{slice}=9) and in layer 3 it had the median value of 0.18 (IQR: 0.14-0.23, n_{pair}=218, n_{slice}=8).

Figure 3.23. Inter-laminar phase-synchrony and phase-difference profile of fast network oscillations in the anterior cingulate cortex

Figure 3.24. Phase-synchrony and phase-difference profile of fast network oscillations in the motor cortex.

In the motor cortex phase-difference within layer 5 was +14.3° (IQR: 0.0/+32.3, n_{pair}=305, n_{slice}=10; Figure 3.24B), in layer 6b it was -16.2° (IQR: -29.5/+1.4, n_{pair}=54, n_{slice}=4), and in layer 6a it was -8.4° (IQR: -30.9/+2.8, n_{pair}=242, n_{slice}=9). In layer 3 phase-difference increased up to +90.7° (IQR: +60.5/+122.3, n_{pair}=218, n_{slice}=8). Phase-difference between layer 5 and the rest of the layers was significant (Kruskall-Wallis One-Way ANOVA on Ranks: $H(3)=475.2$, $p<0.05$; Dunn's method: all comparisons; Figure 3.24B).

In summary, the inter-laminar phase-synchrony results demonstrate that within the mPFC region and the motor cortex synchrony was strong and uniform between the different layers. However, similar to results of the area power and rhythmicity, phase-synchrony was stronger in the ventral mPFC compared to the dorsal mPFC and the motor cortex.

Interesting results were also extracted from the inter-laminar phase-difference analysis. In all regions, phase variations of oscillations recorded in layers 6a and 6b preceded slightly the phase variations in layer 5 oscillations. Phase variations in layer 5 oscillations preceded the phase variations of oscillations in layers 2 and 3 by more than 70°.

3.4. Discussion

3.4.1. *Summary*

Results from this chapter demonstrate that persistent fast network oscillations can be evoked by concurrent application of carbachol (10 µM) and kainate (200 nM) in cortical slice preparations containing the mPFC and the motor cortex. Spectral, rhythmicity and phase-synchrony analysis of the multichannel Utah array recordings revealed:

- Fast network oscillations (~24-30 Hz) were generated at the DP, IL, PrL, ACd subdivisions of the mPFC as well as the motor cortex.

- Following application of carbachol (10 µM) and kainate (200 nM) in the bath solution, oscillations emerged at ~15-30 min in the DP region and at ~60 min in the PrL region and reached stability ~4 hours later. Oscillation frequency did not change once the oscillatory activity had first emerged.

- Oscillations in the ventral mPFC (especially the DP region) were significantly stronger and more rhythmic compared to the oscillations recorded in the dorsal mPFC and the motor cortex.

- Although weaker and less rhythmic, oscillations in the PrL, ACd and motor cortex were significantly faster at ~27-28 Hz compared to those recorded in the ventral mPFC which were ~24-25 Hz.

- The strongest oscillations within each subdivision of the mPFC were recorded in layer 5, and the second strongest oscillations were recorded in layer 6a. Layer 6b, 2 and 3 produced comparably weak oscillations. No significant variation was observed regarding the rhythmicity and laminar frequency profile of network oscillations within each region.

- Similar to the mPFC, oscillations in layer 5 of the motor cortex were the strongest in this region. The weakest oscillations were recorded in layer 6

(especially layer 6b). Although the weakest, oscillations in layer 6 were significantly more rhythmic to oscillations recorded in layers 5 and 3. Oscillation frequency was similar between the different layers.

- Intra-laminar (within layer 5 and 6a) phase-synchrony of cortical oscillations depended more on the distance of the oscillating sites from each other rather than the regions they were recorded from. Therefore, throughout the mPFC and the motor cortex, maximum synchrony was obtained when the recording sites were 400 μm apart (minimum inter-electrode distance) and progressively declined with longer distances. In the mPFC, intra-laminar phase synchrony was abolished at distances longer than 1200 μm. However, in the motor cortex a substantial amount of network synchrony was still present at distances of up to 1600 μm.

- Intra-laminar (within layer 5 and 6a) phase-difference between the different mPFC regions was small ranging between -20° and 20° degrees.

- Inter-laminar phase-synchrony was equally strong between all layers within each mPFC region.

- Within all cortical regions, oscillations recorded in deep layers (layer 5 and 6) preceded those in layer 3 by ~70°-90° and those in layer 2 by ~100°-120°.

3.4.2. *The build-up of fast network oscillations in the rat PFC*

It has been reported for gamma frequency oscillations in the hippocampus that oscillations emerge within the first 15-30 min and once stable they can persist for hours (Weiss et al. 2003; Wójtowicz et al. 2009). In our *in vitro* model, fast network oscillations emerged at ~15-30 min in the DP region and ~60 min in the PrL region, following carbachol and kainate application in the normal-ACSF solution. Oscillations increased gradually and reached stability ~4 hours later. This build up of activity suggests some form of synaptic activity is occurring in the network

(Whittington et al. 1997a), but we did not investigate this further. The oscillation frequency remained stable from the point at which measureable activity first appeared, suggesting that we did not see any clear shift in the type of activity being evoked (i.e. the oscillations do not start at gamma frequency and subsequently slow over time to beta2 frequency).

3.4.3. *Regional differences of fast network oscillations in the rat PFC*

There is increasing evidence that the different subdivisions of the mPFC and the motor cortex receive different cortical and subcortical inputs and subserve different cognitive functions (Section 1.2.3). Therefore, we wished to examine whether there were any regional differences in the pattern of network activity evoked by the conjoint effect of carbachol (10 μM) and kainate (200 nM) between the four main subdivisions of the mPFC and the motor cortex. The results of this analysis support the idea that different types of oscillatory activity can be generated in each region that may therefore subserve different cognitive functions. Fast network oscillations in the ventral regions of the mPFC were significantly stronger and more rhythmic than the oscillations elicited in the dorsal regions. In addition, the dorsal regions oscillated at a significantly faster frequency than the ventral regions.

These results are somewhat different from the data obtained in an earlier study which reported carbachol-induced generation of fast network oscillations in the mPFC (van Aerde et al. 2008). In agreement with our results, Van Aerde and colleagues found that oscillations recorded in the IL region where significantly stronger than the oscillations in PrL region. However, unlike our results they reported that oscillations in the IL region occurred at higher frequencies than oscillations in the PrL region. However, it should be taken into account that in the van Aerde and colleagues study, oscillations were induced by carbachol (25 μM) alone at a lower temperature (~25°C) than the one we used in the present study (~28°C). Moreover, they reported oscillations that lie within the beta1-band (~12-17 Hz) in comparison to our oscillations that lie within the beta2-band (~24-30 Hz).

Another finding from the current study which suggests that fast network oscillations in the ventral and dorsal regions of the mPFC are generated by separate neuronal networks comes from the phase-synchrony analysis. We found that along the dorso-ventral axis of the mPFC, phase-synchrony in the deep layers declines with distance and therefore is restricted only between adjacent regions. This is similar to what van Aerde and colleagues (van Aerde et al. 2008) found in the carbachol model of fast network oscillations in the mPFC. Current source density analysis of multi-electrode field recordings revealed the presence of alternating current sink-source dipoles between the deep and superficial layers. These dipoles were restricted within the PrL or the IL region, depending on which region was chosen as the reference one (van Aerde et al. 2008). In the same study, when PrL and IL regions were anatomically separated, it was found that both regions could sustain network oscillations independently (van Aerde et al. 2008). Our phase-synchrony results are also in agreement with anatomical studies which showed that the majority of cortico-cortical connections in the mPFC are shared within or between adjacent regions (Section 1.2.2).

3.4.4. *Layer 5 drives fast network oscillatory activity*

In the present thesis we report that across the different subdivisions of the mPFC and the motor cortex, the strongest oscillations were recorded in layer 5, and oscillations in the deep layers preceded those of the superficial layers. These findings suggest that the oscillatory activity we recorded in mPFC is mainly generated in layer 5 and is then projected to more superficial layers.

This hypothesis is supported by evidence from other electrophysiological studies in the rodent neocortex. It has been found that carbachol- (50 μM)/kainate- (400 nM) induced beta frequency oscillations in coronal sections of the motor cortex are stronger in layer 5 and precede those of the superficial layers (Yamawaki et al. 2008). In the same study local application of tetrodotoxin (a neurotoxin which blocks APs, by binding and blocking voltage-gated Na^+ channels; TTX) in the deep layers abolished oscillations across all layers, whereas local application of TTX in the superficial layers failed to block oscillations in the deep layers.

Moreover, in the mouse mPFC (Gulledge et al. 2009), the muscarinic receptor-mediated hyperpolarizing effect evoked by focal ACh application was observed in pyramidal cells localizing in layer 5 but not in layer 2/3. The same study did not investigate the laminar effect of carbachol application in pyramidal cell excitability. However, results from phasic ACh release, could suggest in our model a stronger, tonic oscillogenic effect of carbachol in layer 5 than in the superficial layers.

However, we should also take into account evidence from the van Aerde and colleagues study (van Aerde et al. 2008), which demonstrated that anatomically separated mPFC mini-slices containing only superficial layers (2 and 3) can sustain carbachol-induced beta1 frequency oscillations.

An earlier study reported that kainate (400 nM) application in the somatosensory cortex could elicit beta2 frequency oscillations in the deep layers which coexisted with gamma frequency oscillations in the superficial layers (Roopun et al. 2006). Unlike these findings, we did not observe the coexistence of oscillatory activity in different bands in any of our experiments. This difference could be due to the different laminar structure of the somatosensory cortex (granular vs. agranular cortex), or between the different network structures of our coronal slices compared to the horizontal ones used by Roopun and colleagues (Roopun et al. 2006).

Chapter 4

Receptor contributions underlying carbachol- and kainate-induced fast network oscillations in the rodent medial PFC

Chapter 4 - Receptor contributions underlying carbachol- and kainate-induced fast network oscillations in the rodent medial PFC

4.1. Introduction

4.1.1. *Synaptic excitation in the generation of fast network oscillations*

As described previously (section 1.4.1) the PING model of network oscillations relies on pyramidal-to-pyramidal and pyramidal-to-interneuron excitation (Fisahn and Buhl 2001; Whittington et al. 2000). The primary excitatory neurotransmitter in the cortical circuit is glutamate (L-Glutamate; Seeburg 1993). Glutamate binds to and activates a broad family of metabotropic and ionotropic receptors (Ottersen and Landsend 1997; Seeburg 1993). Ionotropic glutamate receptors are hetero-multimers, the properties of which depend on the subunit stoichiometry (Ottersen and Landsend 1997). Apart from glutamate, selective receptor ligands bind to them and alter their activity state. Ionotropic glutamate receptors are subclassified into three groups according to their binding affinity to AMPA, NMDA and kainate (Ottersen and Landsend 1997; Seeburg 1993). The contribution of kainate receptors to the excitability of cortical cells, synaptic transmission and network oscillations have been discussed and demonstrated in chapter 3. The following paragraphs are dedicated to the functional properties of AMPA and NMDA receptors.

AMPA receptors are composed of four types of subunits designated as GluR1, GluR2, GluR3 and GluR4 (Huntley et al. 1994; Song and Huganir 2002). They have fast kinetics (Seeburg 1993) and when activated, they become permeable to Na+ (Malenka and Nicoll 1999) and Ca^{2+}, although their permeability to the latter cation is low in comparison to the NMDA receptors (Ottersen and Landsend 1997; Seeburg 1993). Their fast kinetics and low permeability to Ca^{2+} are attributed to the GluR3 and GluR2 subunits, respectively (G. Li et al. 2005; Ottersen and Landsend 1997). The expression of GluR subunits varies between different cell types. In the hippocampus PV+ cells express high levels of the GluR1, GluR4 subunits, moderate levels of the GluR3 and low levels of the GluR2 subunits

(Catania et al. 1998). Instead high expression of GluR2 subunits has been found in pyramidal cells in the hippocampus (Racca et al. 1996). The fast kinetics of AMPA receptors renders them ideal for the periodic phasic excitation which is a prerequisite in the PING model (Fisahn and Buhl 2001; Whittington et al. 2000).

Selective blockade of AMPA receptors was shown to cause a major suppressive effect on gamma frequency oscillations in the somatosensory (Roopun et al. 2006), PrL (McNally et al. 2011) and entorhinal cortex (Cunningham et al. 2003). Instead, beta2 frequency oscillations in the somatosensory (Roopun et al. 2006) and motor cortex (Yamawaki et al. 2008) were insensitive to selective AMPA receptor antagonists.

NMDA receptors along with AMPA receptors contribute to the induction of LTP and LTD in the hippocampus and in many brain regions (Malenka and Nicoll 1999). NMDA receptors are expressed in high densities in the rat hippocampus and PFC (Monaghan and Cotman 1985). They are composed of the NR1 and NR2A-D subunits (Huntley et al. 1994) and they have binding sites for glutamate and glycine molecules (Millan 2005). They are ligand- and voltage-gated channels with slow kinetics (Millan 2005; Seeburg 1993). To become activated both glutamate and glycine sites need to be occupied. However, their permeability to Ca^{2+} depends on a voltage-dependent Mg^{2+} block which prevails at resting membrane potential and is released when the membrane is depolarized (Millan 2005; Seeburg 1993). The NMDA receptor-mediated depolarizing effect was demonstrated in frontal cortical slice preparations bathed in a solution with minimal Mg^{2+} concentrations. Free from the Mg^{2+} blockade NMDA receptors induced spontaneous brief and long lasting synchronized depolarizations among cortical pyramidal cells and interneurons (Kawaguchi 2001).

The contribution of NMDA receptors to the generation of gamma frequency oscillations is highly variable depending upon region. It has been shown that NMDA receptor blockade does not affect persistent gamma frequency oscillations induced by carbachol in the hippocampus (Fisahn et al. 1998; Whittington et al. 1997a) and carbachol/kainate in the somatosensory cortex (Buhl et al. 1998). However NMDA receptor blockade caused a suppressive effect on kainate-induced persistent gamma frequency oscillations in the somatosensory (Roopun et al. 2006) and entorhinal cortex (Cunningham et al. 2003). Moreover, in the PrL cortex

application of the NMDA receptor antagonist, ketamine was shown to increase the power of transient gamma-frequency oscillations (McNally et al. 2011). Finally, the contribution of NMDA receptor-mediated synaptic excitation was not essential in the generation of beta2 frequency oscillations in the somatosensory (Roopun et al. 2006) and the motor cortex (Yamawaki et al. 2008).

4.1.2. *Synaptic inhibition in the generation of network oscillations*

It is generally agreed that synaptic inhibition is crucially involved in the induction of network oscillations (discussed in chapter 1, section 1.4). Synaptic inhibition in the central nervous system is primarily mediated by GABA receptors (Bormann 2000). Three different types of GABA receptors have been classified. These are $GABA_A$, $GABA_B$ and $GABA_C$ receptors (Bormann 2000).

$GABA_A$ receptors are ligand-gated ionotropic receptors with fast kinetics. When the GABA binding site is occupied, they become activated allowing Cl^- influx which hyperpolarizes the cell (Bormann 2000; Macdonald and Olsen 1994). They also have modulatory binding sites including those for benzodiazepines and barbiturates (Bormann 2000; Macdonald and Olsen 1994). $GABA_B$ receptors are metabotropic receptors with slow kinetics. They exert a hyperpolarizing effect by coupling to K+ channels via G-proteins (Bormann 2000; Bowery et al. 1980; Sodickson and Bean 1996). $GABA_C$ receptors are ionotropic Cl^- permeable channels, insensitive to ligands that modulate the activity state of $GABA_A$ and $GABA_B$ receptors, and are densely localized in the vertebrate retina (Bormann 2000). Moreover they have slower kinetics than the $GABA_A$ receptors (Bormann 2000).

The importance of $GABA_A$ receptor-mediated synaptic inhibition in the generation of fast network oscillations has been highlighted by evidence from pharmacological manipulations that targeted $GABA_A$-receptors. Therefore, $GABA_A$-receptor blockade abolished gamma frequency oscillations in the hippocampus (Fisahn et al. 1998; Whittington et al. 1995), entorhinal cortex (Cunningham et al. 2003) and somatosensory cortex (Buhl et al. 1998), as well as beta frequency oscillations in the motor (Yamawaki et al. 2008) and mPFC (Van Aerde et al. 2009).

Administration of barbiturates that prolongs the decay time of $GABA_A$-receptor mediated IPSPs (Nicoll et al. 1975), decreased the frequency of population field activity (Buhl et al. 1998; Cunningham et al. 2003; Fisahn et al. 1998; Yamawaki et al. 2008).

Moreover, recording temperature which is known to reduce the decay constant of spontaneous bicuculline-sensitive receptor-mediated IPSPs (Collingridge et al. 1984) was shown to increase the peak frequency of gamma frequency oscillations in the hippocampus (Dickinson et al. 2003).

In contrast, blockade of $GABA_B$ receptors had no effect on either gamma frequency oscillations in the hippocampus (Whittington et al. 1995) or gamma and beta2 frequency oscillations in the somatosensory cortex (Roopun et al. 2006).

4.1.3. *Objectives*

Having established an *in vitro* model which generates persistent fast network oscillations in the mPFC with the coapplication of carbachol and kainate, we wished to investigate the synaptic mechanisms that underlie this population network activity. In view of results described above we examined the pharmacological profile of fast network oscillations by targeting the different types of ionotropic glutamate and GABA receptors. Moreover, we wished to assess the effects of recording temperature on the spectral characteristics of network oscillations. Because of the significant differences observed between the DP (ventral mPFC) and PrL (dorsal mPFC) regions, future data presented in this thesis mainly focus on these two regions.

4.2. Methods

Slices were prepared and maintained as outlined in section 2.2. Network oscillations were evoked with carbachol (10 μM) and kainate (200 nM) application. Field recordings were obtained from the mPFC with the recording equipment described in section 2.4.2. The preparation and application of drugs used in the experiments described in this chapter are outlined in section 2.3.

4.2.1. *Data analysis*

- Power spectral analysis performed on field traces, area power and peak frequency extraction techniques are described in section 2.6.1.
- Filter application on field traces is described in section 2.6.2.
- Rhythmicity index extraction from field traces is described in section 2.6.3.
- The histological procedures we used to identify the laminar and regional position of electrodes are outlined in section 2.8.
- Statistical analysis of grouped data is described in section 2.9.
- Descriptive statistics of spectral and rhythmicity characteristics of network oscillations were produced for every region by grouping the results extracted from recordings obtained from all the layers within this region.

4.3. Results

4.3.1. *Evaluation of the characteristics of fast network oscillations in the medial PFC by single-electrode field recordings*

Most of the results presented in this chapter, as well as the following ones, were obtained with single-electrode field recordings from the DP and PrL subdivisions of the mPFC. In chapter 3 we reported the significantly different spectral and rhythmicity characteristics between DP and PrL oscillations. We first wished to demonstrate that we could replicate these results with a different recording technique.

As shown with the data obtained with multi-electrode field recordings, oscillations recorded with single field electrodes in the DP region were significantly stronger than the PrL oscillations (DP: 1932 $\mu V^2.Hz^{-1}$ IQR: 1058-3275, n_{DP}=27; PrL: 242 $\mu V^2.Hz^{-1}$ IQR: 152-416, n_{PrL}=20, Mann-Whitney U test on Ranks: U(20)=496, $p<0.05$, Figure 4.1A,B,D). Moreover, similar to multi-electrode field recordings, oscillations recorded with single electrodes in the DP region were significantly more rhythmic than the network activity recorded in the PrL region (DP: 0.61 ± 0.22, n_{DP}=13; PrL: 0.34 ± 0.17, n_{PrL}=9, unpaired t-test: t(45)=3.0, $p<0.05$, Figure 4.1C,E). However, although weaker and less rhythmic, oscillations recorded in the PrL region were significantly faster than oscillations recorded in the DP region (DP: 25.2 ± 1.9, n_{DP}=27; PrL: 27.3 ± 2.4, n_{PrL}=20, unpaired t-test: t(45)=-3.3, $p<0.05$, Figure 4.1B,F).

In summary, results obtained with single electrodes verified those obtained with multi-electrodes in that, fast network oscillations in the DP region are significantly stronger, more rhythmic and slower than the oscillations in the PrL.

4.3.2. *Contribution of AMPA and kainate receptor-mediated synaptic transmission in the generation of fast network oscillations in the medial PFC*

The contribution of distinct types of excitatory synaptic transmission to fast network oscillations in the DP and PrL regions was evaluated in a series of experiments where AMPA, kainate and NMDA-receptor antagonists were bath-applied once stable oscillations had been attained.

Figure 4.1. Regional profile of fast network oscillations in the mPFC

We first investigated the contribution of AMPA receptors to the generation of persistent carbachol/kainate-evoked fast network oscillations. To do so the potent, non-competitive AMPA receptor antagonist, SYM2206 (20 μM; SYM) was applied to the normal-ACSF.

We first assessed the effect of AMPA receptor blockade on fast network oscillations in the DP region. Application of SYM2206 (20 μM) did not exert any significant effect on the oscillation area power (ctrl: 1748 $\mu V^2.Hz^{-1}$ IQR: 977-5967; SYM-5min: 1977 $\mu V^2.Hz^{-1}$ IQR: 776-6302; SYM-10min: 1988 $\mu V^2.Hz^{-1}$ IQR: 1275-6198; SYM-15min: 2000 $\mu V^2.Hz^{-1}$ IQR: 1170-6223; SYM-30min: 2144 $\mu V^2.Hz^{-1}$ IQR: 685-6533; SYM-45min: 2334 $\mu V^2.Hz^{-1}$ IQR: 1150-6222; SYM-60min: 2309 $\mu V^2.Hz^{-1}$ IQR: 1139-5103, n_{slice}=5; Friedman One-Way RM-ANOVA on Ranks: $\chi^2(6)=3.5$, $p>0.05$; Figure 4.2A,B,C,D). Moreover, no significant change was elicited by SYM2206 (20 μM) on the peak frequency (ctrl: 24.6 Hz IQR: 23.8-25.1; SYM-5min: 24.6 Hz IQR: 23.2-25.2; SYM-10min: 24.5 Hz IQR: 22.9-24.7; SYM-15min: 24 Hz IQR: 22.9-24.7; SYM-30min: 23.9 Hz IQR: 22.9-24.4; SYM-45min: 23.8 Hz IQR: 22.3-24.9; SYM-60min: 23.9 Hz IQR: 23.2-25.2, n_{slice}=5; Friedman One-Way RM-ANOVA on Ranks: $\chi^2(6)=9.3$, $p>0.05$; Figure 4.2A,B,C,E) of network oscillations.

In four (n=4) of the slices being treated with SYM2206, the potent AMPA and kainate receptor antagonist, NBQX (20 μM) was added to the ACSF solution. Simultaneous blockade of the AMPA and kainate receptors abolished network oscillations within the course of 45 min (SYM-60min: 3677 ± 2304 $\mu V^2.Hz^{-1}$; NBQX-5min: 3116 ± 2012 $\mu V^2.Hz^{-1}$; NBQX-10min: 2875 ± 2284 $\mu V^2.Hz^{-1}$; NBQX-15min: 1827 ± 1365 $\mu V^2.Hz^{-1}$; NBQX-30min: 832 ± 676 $\mu V^2.Hz^{-1}$; NBQX-45min: 64 ± 126 $\mu V^2.Hz^{-1}$; NBQX-60min: 10 ± 0.1 $\mu V^2.Hz^{-1}$, n_{slice}=4; One-way RM-ANOVA: $F(3,7)=6.1$, $p<0.05$; Dunnett's method: SYM-60min vs. all; Figure 4.3A,B,C,D) revealing the strong dependence of network activity in the DP region on the kainate receptor-mediated excitation. The effect of AMPA and kainate receptor blockade on frequency was also significant (SYM-60min: 23.7 Hz IQR: 23.0-24.3; NBQX-5min: 24.1 Hz IQR: 23.4-24.4; NBQX-10min: 23.7 Hz IQR: 23.0-24.6; NBQX-15min: 24.0 Hz IQR: 23.4-24.7; NBQX-30min: 17.5 Hz IQR: 8.3-23.7; NBQX-45min: 17.8 Hz IQR: 8.1-24.6; NBQX-60min: 5.5 Hz IQR: 0.0-11.4, n_{slice}=4; Friedman One-Way RM-ANOVA on Ranks: $\chi^2(7)=14.6$, $p<0.05$; Dunn's method: SYM-60min vs. all; Figure 4.3A,B,C,E).

Figure 4.2. AMPA receptor blockade has no effect on fast network oscillations in the DP region

Figure 4.3. Combined AMPA and kainate receptor blockade abolishes fast network oscillations in the DP region

The effects of AMPA receptor blockade on the generation of fast network oscillations in the PrL region were tested in seven (n=7) oscillating mPFC slices. Application of SYM2206 (20 μM) increased the area power of network oscillations in 3/7 slices (Figure 4.4C). However, the overall effect of AMPA receptor-blockade was not significant (ctrl: 377 $\mu V^2.Hz^{-1}$ IQR: 265-639; SYM-15min: 426 $\mu V^2.Hz^{-1}$ IQR: 278-622; SYM-30min: 432 $\mu V^2.Hz^{-1}$ IQR: 282-656; SYM-45min: 450 $\mu V^2.Hz^{-1}$ IQR: 299-691; SYM-60min: 455 $\mu V^2.Hz^{-1}$ IQR: 284-803, n_{slice}=7; Friedman One-Way RM-ANOVA on Ranks: $\chi^2(6)=8.2$, $p>0.05$; Figure 4.4A,B,C,D). Moreover, SYM2206 (20 μM) application did not elicit any significant change in the peak frequency of fast network oscillations (ctrl: 26.3 Hz IQR: 25.2-28.7; SYM-15min: 25.7 Hz IQR: 24.1-30.5; SYM-30min: 25.6 Hz IQR: 24.0-29.3, n_{slice}=10; SYM-45min: 25.2 Hz IQR: 23.9-27.1, SYM-60min: 25.4 Hz IQR: 24.0-26.4, n_{slice}=7, Friedman One-Way RM-ANOVA on Ranks: $\chi^2(6)=10.6$, $p>0.05$; Figure 4.4A,B,C,E).

In six (n=6) slices being treated with SYM2206, the potent AMPA and kainate receptor antagonist, NBQX (20 μM) was added to the normal-ACSF. Similar to results in the DP region, the AMPA and kainate receptor antagonist abolished network oscillations in all slices within the first 45 min of application (SYM-60min: 430 $\mu V^2.Hz^{-1}$ IQR: 244-702; NBQX-5min: 420$\mu V^2.Hz^{-1}$ IQR: 212-618; NBQX-10min: 400$\mu V^2.Hz^{-1}$ IQR: 236-682; NBQX-15min: 408 $\mu V^2.Hz^{-1}$ 205-660; NBQX-30min: 22 $\mu V^2.Hz^{-1}$ IQR: 0-372; NBQX-45min: 13 $\mu V^2.Hz^{-1}$ IQR: 2-20; NBQX-60min: 10 $\mu V^2.Hz^{-1}$ IQR: 5-12, n_{slice}=6; Friedman One-way RM-ANOVA on Ranks: $\chi^2(6)=29.5$,$p<0.05$; Dunn's method: SYM-60min vs. all; Figure 4.5 A,B,C,D). The effect of AMPA and kainate receptor block on the peak frequency of network oscillations in the PrL region was also significant (SYM-60min: 24.8 Hz IQR: 24.0-26.6; NBQX-5min: 24.1 Hz IQR: 22.8-25.3; NBQX-10min: 25.1 Hz IQR: 24.0-26.4; NBQX-15min: 25.1 Hz IQR: 23.3-26.5; NBQX-30min: 23.2 Hz IQR: 21.2-25.5; NBQX-45min: 23.2 Hz IQR: 12.8-24.6; NBQX-60min: 12.7 Hz IQR: 5.0-17.8, n_{slice}=6; Friedman One-Way RM-ANOVA on Ranks: $\chi^2(6)=17.3$, $p<0.05$; Dunn's method: SYM-60min vs. all; Figure 4.5A,B,C,E).

These results demonstrate that oscillations in both the DP and PrL regions did not depend on fast AMPA receptor-mediated synaptic transmission, but were dependent upon kainate receptor activation.

Figure 4.4. AMPA receptor blockade has no effect on fast network oscillations in the PrL region

Figure 4.5. Combined AMPA and kainate receptor blockade abolishes fast network oscillations in the PrL region

4.3.3. *Contribution of NMDA receptor-mediated synaptic transmission in the generation of fast network oscillations in the medial PFC*

To evaluate the importance of NMDA receptor-mediated synaptic transmission to the generation of fast network oscillations in the mPFC, the competitive NMDA receptor antagonist, DAP5 was used. Bath-application with DAP5 (100 μM) produced a significant reduction in the area power of network oscillations in the DP region. The decrease became significant 15 min post-drug application and remained decreased for the duration of the application (ctrl: 2147 $\mu V^2.Hz^{-1}$ IQR: 1517-2688; DAP5-15min: 1793 $\mu V^2.Hz^{-1}$ IQR: 1367-2341; DAP5-30min: 1908 $\mu V^2.Hz^{-1}$ IQR: 1311-2432; DAP5-45min: 1694 $\mu V^2.Hz^{-1}$ IQR: 1307-2451; DAP5-60min: 1688 $\mu V^2.Hz^{-1}$ IQR: 1272-2288; wash: 1440 $\mu V^2.Hz^{-1}$ IQR: 1108-2422, n_{slice}=8, Friedman One-Way RM-ANOVA on Ranks: $\chi^2(5)=36.2$, $p<0.05$; Dunn's method: ctrl vs. all; Figure 4.6A,B,C). However, oscillations were not fully abolished in any of the tested slices. At 60 min post-drug application the area power was reduced by -16.3% (IQR: -16.3/-18.3, n_{slice}=8) compared to control conditions. Changes in the peak frequency elicited by NMDA receptor block were not strong enough to reach statistical significance (ctrl: 24.8 Hz ± 1.9; DAP5-15min: 23.7 Hz ± 1.8; DAP5-30min: 24.3 Hz ± 1.7; DAP5-45min: 24.2 Hz ± 1.6; DAP5-60min: 24.1 Hz ± 1.9; wash: 24.5 Hz ± 1.8, n_{slice}=8; One-Way RM-ANOVA: $F(5,35)=2.5$, $p>0.05$; Figure 4.6A,B,D).

We also investigated the effect of NMDA receptor blockade on fast network oscillations in the PrL region. In contrast to the DP region, bath application of DAP5 (100 μM) had no effect on both the area power (ctrl: 985 ± 236 $\mu V^2.Hz^{-1}$; DAP5-15min: 871 ± 257 $\mu V^2.Hz^{-1}$; DAP5-30min: 940 ± 258 $\mu V^2.Hz^{-1}$; DAP5-45min: 869 ± 271 $\mu V^2.Hz^{-1}$; DAP5-60min: 813 ± 271 $\mu V^2.Hz^{-1}$; wash: 929 ± 209 $\mu V^2.Hz^{-1}$, n_{slice}=5; One-way RM-ANOVA: $F(5,20)=1.4$, $p>0.05$; Figure 4.7A,B,C) or the peak frequency (ctrl: 24.6 ± 2.2 Hz; DAP5-15min: 23.5 ± 2.6 Hz; DAP5-30min: 23.7 ± 2.1 Hz; DAP5-45min: 23.4 ± 2.4 Hz; DAP5-60min: 23.7 ± 1.8 Hz; wash: 23.7 ± 2.9 Hz, n_{slice}=5; One-Way RM-ANOVA: $F(5,20)=1.9$, $p>0.05$; Figure 4.7A,B,D) of fast network oscillations.

Figure 4.6. NMDA receptor blockade reduces fast network oscillations in the DP region

Figure 4.7. NMDA receptor blockade has no effect on fast network oscillations in the PrL region

4.3.4. *Contribution of $GABA_A$ receptor-mediated synaptic transmission in the generation of fast network oscillations in the medial PFC*

The contribution of fast synaptic inhibition to the generation of fast network oscillations in the mPFC was assessed with application of the $GABA_A$ receptor antagonist, gabazine (GBZ). Gabazine was administered at a 1 μM concentration for 30 min. Following this, the concentration of gabazine was increased to 10 μM and recordings were taken for a further 15 min. Then, gabazine was washed out of the ACSF for 30 min.

Gabazine (1 μM) abolished fast network oscillations in the DP region within the first 30 min (ctrl: 332 $\mu V^2.Hz^{-1}$ IQR: 208-788; GBZ-1μM/30min: 11 $\mu V^2.Hz^{-1}$ IQR: 0-29; GBZ-10μM/15min: 3 $\mu V^2.Hz^{-1}$ IQR: 0-11; wash: 16 $\mu V^2.Hz^{-1}$ IQR: 0-33, n_{slice}=8; Friedman One-Way RM-ANOVA on Ranks: $\chi^2(3)=12.3$, $p<0.05$; Dunn's method: ctrl vs. all; Figure 4.8A,B,C). This strong decrease coincided with the emergence of epileptiform burst discharges in 3/8 slices. Epileptiform activity intensified (incidence at GBZ-10μM/15min: 0.5 ± 0.06 Hz) with the higher concentration of gabazine (Figure 4.8A). Oscillations never recovered in the wash-out. In the three slices where epileptiform activity emerged, burst discharges were still present, although, weaker and more scarce in the wash-out (Figure 4.8A). As a result of the complete abolition of network oscillations, gabazine application produced a significant decrease on the peak frequency of network oscillations in the DP region (ctrl: 22.0 Hz IQR: 21.5-24.0; GBZ-1μM/30min: 0.0 Hz IQR: 0.0-0.0; GBZ-10μM/15min: 0.0 Hz IQR: 0.0-0.0; wash: 0.0 Hz IQR: 0.0-0.0, n_{slice}=8; Friedman One-Way RM-ANOVA on Ranks: $\chi^2(3)=18.0$, $p<0.05$; Dunn's method: ctrl vs. all; Figure 4.8A,B,D).

At 1μM, gabazine also produced a significant reduction in the oscillation power in the PrL region. At the higher concentration (gabazine; 10 μM) synchronous oscillatory activity was completely abolished. Oscillations did not recover in the wash-out (ctrl: 223 ± 67 $\mu V^2.Hz^{-1}$; GBZ-1μM/30min: 118 ± 35 $\mu V^2.Hz^{-1}$; GBZ-10μM/15min: 5 ± 9 $\mu V^2.Hz^{-1}$; wash: 10 ± 10 $\mu V^2.Hz^{-1}$, n_{slice}=5; One-Way RM-ANOVA: $F(3,8)=28.8$, $p<0.05$; Dunnett's method: ctrl vs. all; Figure 4.9A,B,C). In 4/5 slices, gabazine precipitated epileptiform activity which intensified with the higher concentration (incidence at GBZ-10μM/15min: 0.5 ± 0.09 Hz).

Figure 4.8. GABA$_A$ receptor blockade abolishes fast network oscillations in the DP region

Figure 4.9. $GABA_A$ receptor blockade abolishes fast network oscillations in the PrL region

In these slices, weak epileptiform burst discharges were also present in the wash-out. A significant reduction in frequency was produced by gabazine in the peak frequency of network oscillations in the PrL region (ctrl: 26.0 Hz IQR: 25.8-27.3; GBZ-1μM/30min: 21.0 Hz IQR: 20.3-23.3; GBZ-10μM/15min: 0.0 Hz IQR: 0.0-0.0; wash: 0.0 Hz IQR: 0.0-0.0, n_{slice}=5; One-Way RM-ANOVA: $F(3,8)=565.7$, $p<0.05$; Dunn's method: ctrl vs. all; Figure 4.9A,B,D).

4.3.5. *Contribution of $GABA_B$ receptor-mediated synaptic transmission in the generation of fast network oscillations in the medial PFC*

The contribution of $GABA_B$ receptor-mediated synaptic inhibition was assessed with the application of the $GABA_B$ receptor antagonist, CGP55845 (10 μM; CGP). Application of the $GABA_B$ receptor antagonist for 60 min had no effect on either the area power (ctrl: 821 $\mu V^2.Hz^{-1}$ IQR: 611-1304; CGP-15min: 900 $\mu V^2.Hz^{-1}$ IQR: 698-1465; CGP-30min: 929 $\mu V^2.Hz^{-1}$ IQR: 785-1644; CGP-45min: 1004 $\mu V^2.Hz^{-1}$ IQR: 742-1576; CGP-60min: 911 $\mu V^2.Hz^{-1}$ IQR: 737-1551; wash: 1000 $\mu V^2.Hz^{-1}$ IQR: 645-1588, n_{slice}=7; Friedman One-Way RM-ANOVA on Ranks: $\chi^2(5)=9.1$, $p>0.05$; Figure 4.10A,B,C) or the peak frequency (ctrl: 23.4 ± 3.1 Hz; CGP-15min: 23.1 ± 3.2 Hz; CGP-30min: 22.7 ± 2.3 Hz; CGP-45min: 23.3 ± 2.5 Hz; CGP-60min: 22.9 ± 3.1 Hz; wash: 22.7 ± 2.8 Hz, n_{slice}=7; One-Way RM-ANOVA: $F(5,30)=0.95$, $p>0.05$; Figure 4.10A,B,D) of fast network oscillations in the DP region.

Similar to the DP region, application of CGP55845 (10 μM) had no effect on either the area power (ctrl: 537 ± 413 $\mu V^2.Hz^{-1}$; CGP-15min: 627 ± 507 $\mu V^2.Hz^{-1}$; CGP-30min: 626 ± 520 $\mu V^2.Hz^{-1}$; CGP-45min: 674 ± 538 $\mu V^2.Hz^{-1}$; CGP-60min: 655 ± 515 $\mu V^2.Hz^{-1}$; wash: 635 ± 471 $\mu V^2.Hz^{-1}$, n_{slice}=4; One-Way RM-ANOVA: $F(5,15)=0.9$, $p>0.05$; Figure 4.11A,B,C) or the peak frequency (ctrl: 23.5 Hz IQR: 20.5-27.0; CGP-15min: 23.5 Hz IQR: 20.5-27.0; CGP-30min: 24.0 Hz IQR: 21.5-26.5; CGP-45min: 24.5 Hz IQR: 22.0-27.5; CGP-60min: 23.5 Hz IQR: 21.0-26.0; wash: 24.0 Hz IQR: 21.5-27.0 , n_{slice}=7, $p>0.05$; Friedman One-way RM-ANOVA on Ranks: $\chi^2(5)=9.9$,$p>0.05$; Figure 4.11A,B,C) of network oscillations in the PrL region.

Figure 4.10. $GABA_B$ receptor blockade has no effect on fast network oscillations in the DP region

Figure 4.11. $GABA_B$ receptor blockade has no effect on fast network oscillations in the PrL region

4.3.6. *The effect of temperature on carbachol/kainate-induced oscillations in the rat medial PFC*

We also examined the effect of temperature on the spectral characteristics of fast network oscillations in the DP and PrL subdivisions of the mPFC. To do so we placed the thermo-conductive probe of a digital thermometer in contact with the ACSF solution, a few millimetres away from the recordings sites (the probe was positioned approximately opposite the IL region). The probe thermometer monitored the temperature of the ACSF solution during the course of the experiment. We were careful so that the probe did not disturb the flow of the solution adjacent to the slice.

To estimate the relationship of the oscillation frequency with temperature we grouped temperature readings and the peak frequencies of stable oscillations recorded from the two regions in different experiments. Grouped data of temperature vs. frequency were plotted into a scatterplot along with lines of least squares regression fit. As figure 4.12 shows the frequency of oscillations was dependent on the recording temperature in the range of 27-31 ^{o}C with a linear increase in frequency from 24-32 Hz. From the line of best fit we calculated that a 1°C increase in temperature increased the frequency of oscillations in the DP (n=27) by 2.5 Hz and in the PrL (n=16) by 1.9 Hz. When we grouped the data from both regions together (n=43) we found that a 1°C increase in temperature increased the frequency of oscillations by 2.3 Hz.

We also monitored in two slices (n=2), the temperature along with the spectral characteristics of oscillations, while recording temperature was progressively increased. We observed that increasing temperature (27-32°C) increased the oscillation frequency but dramatically decreased the oscillation power. At higher temperatures the oscillation frequency could no longer be determined as oscillations had collapsed (Data not shown).

Figure 4.12. Recording temperature has an effect on the frequency of fast network oscillations in the mPFC

4.4. Discussion

4.4.1. *Summary*

Assessing the contribution of different types of synaptic transmission as well as the recording temperature in the generation of carbachol (10 μM) and kainate (200 nM) induced fast network oscillations in the DP and the PrL subdivisions of the mPFC has revealed the following:

- Single extracellular field recordings replicated the results obtained from multi-electrode Utah array recordings, in that oscillations in the DP region were significantly stronger, more rhythmic but slower than oscillations evoked in the PrL region.

- Blockade of AMPA receptor-mediated synaptic transmission did not affect the area power or frequency of fast network oscillations in either the DP or the PrL subdivisions of the mPFC.

- Concurrent blockade of AMPA and kainate receptors abolished all fast network oscillations in the DP and PrL subdivisions of the mPFC.

- Blockade of NMDA receptor-mediated synaptic transmission significantly reduced but did not abolish the area power of network oscillations in the DP region. No significant effect of NMDA receptor block was observed in the area power of network oscillations in the PrL region. Peak frequency of network oscillations remained unaffected by the NMDA receptor antagonist in both regions.

- Blockade of $GABA_A$ receptor-mediated synaptic inhibition by a high concentration of gabazine (10 μM) abolished oscillatory activity in both regions. However, the PrL region showed a lower sensitivity to $GABA_A$ receptor blockade than the DP region, since at 1 μM of gabazine, oscillations were reduced but still present in the PrL region, but abolished in the DP region.

- Blockade of $GABA_B$ receptor-mediated synaptic inhibition did not elicit any effect on the power and frequency of fast network oscillations in both the DP and PrL region.

- Oscillations in mPFC were dependent on the recording temperature in that an increase in temperature produced an increase in the oscillation frequency along with a reduction in the oscillations power.

4.4.2. *Single-electrode field recordings replicate results obtained from the multi-electrode field recordings*

The characteristics of fast network oscillations in the mPFC, presented in Chapter 3, were produced from field recordings obtained by a multi-electrode Utah array. However, the pharmacological manipulations presented in this chapter were tested with recordings obtained by single field electrodes. Therefore, before we proceeded to any pharmacological manipulations, we first wished to investigate if there were any discrepancies between the spectral and rhythmicity properties of fast network oscillations obtained by the multi-electrode and single-electrode field recordings.

Single-electrode extracellular field recordings were made in the DP and the PrL subdivisions of the mPFC. We chose these two regions for three reasons: (1) the DP belongs to the ventral portion, whereas the PrL belongs to the dorsal portion of the mPFC; (2) these two regions presented significant spectral and rhythmicity differences; (3) phase-synchrony analysis showed weak signs of network interaction between them.

Our results from single extracellular field electrode recordings replicated those from multi-field electrode recordings as again it was found that oscillations in the DP region were significantly stronger, more rhythmic but slower than oscillations in the PrL region. Having demonstrated that both recording techniques, resulted in similar observations we then went on to assess with single-electrode recordings the contribution of different neurotransmitters to the generation of network oscillations in dorsal versus ventral mPFC.

4.4.3. *AMPA receptors do not contribute to the generation of fast network oscillations in the rat mPFC*

Our results demonstrated that in both the PrL and DP regions of the mPFC, AMPA receptor activation was not required for the generation of fast network oscillations evoked with carbachol/kainate. The contribution of AMPA receptor-mediated excitation to the generation of fast network oscillations is a major differentiating factor in the mechanisms underlying gamma and beta frequency oscillations. The different mechanisms for the generation of beta2 and gamma frequency oscillations were clearly demonstrated in the somatosensory cortex, where application of kainate (400 nM) elicited gamma frequency oscillations in the superficial layers which co-existed with beta2 frequency oscillations in the deep layers (Roopun et al. 2006). In the same study application of the selective AMPA receptor antagonist, SYM2206 (20 μM) which preserves the kainate receptor-mediated component of the kainate drive, abolished gamma frequency oscillations but did not affect the beta2 ones. This result showed that unlike gamma frequency oscillations, oscillatory activity in the beta2 frequency range does not depend on fast excitatory AMPA receptor-mediated synaptic transmission. In agreement with this, it was subsequently shown that AMPA receptor blockade did not affect beta2 frequency oscillations evoked by carbachol/kainate application in the motor cortex (Yamawaki et al. 2008) but completely abolished transient gamma frequency oscillations (30-80 Hz) in the PrL region (McNally et al. 2011).

4.4.4. *NMDA receptor blockade does not block fast network oscillations in the rat medial PFC*

It is possible that during network activity, endogenous glutamate release could activate NMDA receptors (Buhl et al. 1998; Millan 2005) which are highly expressed in the rat mPFC (Millan 2005; Monaghan and Cotman 1985). We therefore assessed whether there was any contribution of NMDA receptors to the generation of fast network oscillations evoked with carbachol/kainate application in either the PrL or DP regions of the mPFC.

Previous studies have shown that the contribution of NMDA receptors to fast network oscillations varies between different *in vitro* models. In the hippocampus the contribution of NMDA receptors is not essential for the generation of gamma

frequency oscillations *in vitro*. Tetanic stimulation in the hippocampus could elicit a slow depolarization superimposed with firing activity at the gamma frequency range in both pyramidal cells and interneurons (Whittington et al. 1997b). Pharmacological manipulations revealed a noticeable but not essential component of NMDA receptor synaptic transmission in this post-tetanic depolarizing effect (Whittington et al. 1997b). Transient oscillations evoked by focal application of K^+ were also unaffected by NMDA receptor blockade in the rat hippocampus (LeBeau et al. 2002). In addition, the role of NMDA receptors was not essential to maintain gamma frequency oscillations on either the persistent carbachol model in the hippocampus (Fisahn et al. 1998) or the carbachol/kainate model in the somatosensory cortex (Buhl et al. 1998).

In contrast, in the entorhinal cortex NMDA receptor blockade with ketamine reduced the power of the gamma frequency oscillations in the superficial layers, probably by affecting the NMDA receptor-mediated excitatory drive on interneurons localized in these layers, but it did not affect the gamma frequency oscillations in the deep layers of the same cortical area (Cunningham et al. 2006).

NMDA receptor blockade caused a selective effect on fast network oscillations in the somatosensory cortex. Application of DAP5 abolished gamma frequency oscillations in the superficial layers of the somatosensory cortex, but increased beta2 frequency oscillations in the deep layers of the same cortical area (Roopun et al. 2006). In the motor cortex NMDA receptor blockade failed to elicit any change on the area power of beta2 frequency oscillations (Yamawaki et al. 2008).

Only one study has assessed the role of NMDA receptors in the generation of network oscillations in the mPFC where it was found that in the PrL region different NMDA receptor antagonists (ketamine, MK-801, D-AP5) all produced a consistent increase in the area power of transient, kainate-evoked gamma frequency oscillations (McNally et al. 2011).

In our carbachol/kainate model, NMDA receptor blockade revealed a significant NMDA receptor component in the generation of fast network oscillations in the DP region. However, this component was not essential for the sustainability of network oscillations in this area, since DAP5 (100 μM) application caused a significant reduction but not abolition of network oscillations in this region. In

contrast, results obtained from the PrL region indicate that the NMDA receptor mediated excitatory synaptic drive is not necessary for the induction of network oscillations.

The regional difference in the contribution of NMDA receptors to network oscillations between DP and PrL regions could be possibly correlated to the area power difference between them. This is because a significant difference in the oscillation strength could reflect a difference in the level of pyramidal cell firing activity. This in turn would result to different levels of glutamate release and NMDA receptor stimulation (Buhl et al. 1998; Millan 2005). As a result, the NMDA receptor component in the generation of network oscillations would be stronger in the DP region than the PrL region.

McNally and colleagues (McNally et al. 2011) reported that ketamine but not MK-801 or DAP5 application caused a significant reduction in the peak frequency of kainate-induced transient gamma frequency oscillations in the PrL region. In our *in vitro* carbachol/kainate model, application of DAP5 did not cause any significant alterations in the frequency of persistent fast network oscillations.

4.4.5. *AMPA and kainate receptor blockade abolishes fast network oscillations in the rat medial PFC*

We found that AMPA receptor-mediated synaptic transmission does not contribute to the generation of fast network oscillations in the mPFC. Moreover, we showed that NMDA receptor-mediated synaptic transmission contributes only to network oscillations in the DP region. We then assessed the significance of the tonic kainate-induced drive to the generation of network oscillations. To do so we applied the potent AMPA/kainate receptor antagonist, NBQX, in the bath solution already containing the selective AMPA receptor antagonist, SYM2206. Similar to the findings of the carbachol/kainate model of beta2 frequency oscillations in the motor cortex (Yamawaki et al. 2008), we found that blockade of the kainate receptors completely abolished fast network oscillations in the DP and PrL regions. The abolition of network oscillations by kainate receptor blockade is not surprising, in view of earlier evidence that kainate receptors regulate the excitability of pyramidal cells and interneurons, as well as control synaptic

inhibition (Section 3.1.3; Fisahn and Buhl 2001; Fisahn et al. 2004; Fisahn et al. 2005; Lerma 2006).

Overall, results from pharmacological manipulations targeting the excitatory synaptic transmission, suggest that apart from the kainate-induced tonic excitatory drive, excitatory synaptic transmission is not crucial for the generation of fast network oscillations in the mPFC. The peak frequency of network oscillations was not affected by any of the drugs tested. This evidence suggests that blockade of excitatory synaptic transmission did not cause any alterations on kinetics of synaptic inhibition.

4.4.6. *$GABA_A$ but not $GABA_B$ receptor blockade abolishes fast network oscillations in the rat medial PFC*

We showed that application of gabazine abolished fast network oscillations in both the PrL and DP regions. Evidence from different induction models of *in vitro* fast network oscillations in various cortical regions has already demonstrated the contribution of $GABA_A$ receptors in the generation of these rhythms (section 4.1.2.). However, different levels of sensitivity to $GABA_A$ receptor blockade were revealed between oscillations recorded in the DP and PrL regions. Although, oscillations were completely blocked within the first 30min of the low gabazine concentration (1 μM) in the DP region, oscillations in the PrL were reduced but still present. A further increase of the gabazine concentration to 10 μM abolished oscillations in the PrL region.

In the kainate-model of fast network oscillations in the somatosensory cortex (Roopun et al. 2006) it was shown that 200 nM of gabazine abolished gamma frequency oscillations in the supeficial layers but increased the power of concurrently recorded beta2 frequency oscillations in layer 5. This result suggested the contribution of a synaptic mechanism which together with the perisomatic $GABA_A$ receptors it also involved the contribution axon initial segment $GABA_A$ receptors (Roopun et al. 2006; Szabadics et al. 2006). We did not investigate the effects of such low concentrations of gabazine on the fast network oscillations in the mPFC.

Reduction in the network oscillatory activity following gabazine application was shown to coincide with burst discharges. The emergence of epileptiform activity as a consequence of severe disruption of synaptic inhibition has been reported in the kainate-model of persistent gamma frequency oscillations in the hippocampus (Pais et al. 2003) and somatosensory cortex (Roopun et al. 2006) as well as the kainate-model of transient gamma frequency oscillations in the mPFC (McNally et al. 2011).

We also showed that CGP55845 (10 μM) application did not affect the spectral characteristics of fast network oscillations in the mPFC. These findings are in agreement with earlier evidence which showed that $GABA_B$ receptor blockade did not block the tetanic induction of gamma frequency oscillations in the hippocampus (Whittington et al. 1995) or kainate-induced gamma and beta frequency oscillations in the somatosensory cortex (Roopun et al. 2006).

4.4.7. *Recording temperature affects the frequency and power of fast network oscillations in the rat medial PFC*

The temperature dependence of network oscillatory activity has been previously reported for the beta1 frequency oscillations in the mPFC (Van Aerde et al. 2009) and hippocampal gamma frequency oscillations (Dickinson et al. 2003). It has been shown that this dependence relies on the direct effect of temperature on the kinetics of fast IPSPs (Antkowiak and Heck 1997; Banks et al. 1998; Collingridge et al. 1984). All our experiments were carried out at ~28°C. This temperature was chosen as we found that with the higher temperatures of 32°C generally used in hippocampal in vitro oscillation studies (Dickinson et al. 2003; Fisahn et al. 1998) the oscillations in the PFC declined after 2-3 hours and we could not reach stability. Reducing the temperature to ~28°C improved stability and viability of the slices. However, our oscillations were temperature dependent. By grouping temperature readings and spectral analysis results from different experiments, we found that in both the DP and PrL regions, oscillation frequency increases but oscillation power decreases with recording temperature.

Chapter 5

Electrophysiological and firing characteristics of different neuronal populations in the medial PFC in the absence and presence of *in vitro* fast network oscillations

Chapter 5 - Electrophysiological and firing characteristics of different neuronal populations in the medial PFC in the absence and presence of *in vitro* fast network oscillations

5.1. Introduction

Cortical oscillations emerge from the orchestrated activity of populations of pyramidal cells and interneurons (Whittington et al. 2000; Whittington and Traub 2003). The neocortical circuit is composed of a heterogeneous population of neurons with different morphological and firing properties (Amitai 1994; Kawaguchi and Kubota 1997; McCormick et al. 1985; Yang et al. 1996a). The majority of the reported electrophysiological properties of these neurons have been examined in the absence of network oscillations. Therefore, little is known about the individual cell types and the synaptic mechanisms that favour the generation of neocortical network oscillations. In the following sections we will discuss some of the electrophysiological and morphological properties of the neocortical cell types.

5.1.1. *Pyramidal cells in the rodent neocortex*

The majority of neurons in the neocortex are pyramidal cells (60% - 70%) (Connors and Gutnick 1990). The electrophysiological and morphological properties of pyramidal cells have been investigated in the rodent neocortex (Agmon and Connors 1992; Connors et al. 1982; McCormick et al. 1985; Yang et al. 1996a). Two major pyramidal cell types have been identified and classified as regular spiking (RS) and bursting cells, which are further separated in subtypes.

RS cells fire single APs followed by a prominent after-hyperpolarizing potential (AHP) in response to threshold depolarizing current pulses (Amitai 1994; Connors et al. 1982; McCormick et al. 1985; Yang et al. 1996a). Their firing activity is also characterized by strong frequency adaptation (i.e. APs with an increasing interspike interval; Amitai 1994; Connors et al. 1982; McCormick et al. 1985; Yang et al. 1996a).

Bursting cells are characterized by their intrinsic capacity to generate bursts of APs (Agmon and Connors 1992; Chagnac-Amitai and Connors 1989; Connors et al. 1982; Connors and Gutnick 1990; Gray and McCormick 1996; McCormick et al. 1985). It has been reported that during prolonged depolarizing current pulses bursting activity occurs in regular inter-burst intervals (Agmon and Connors 1992; Chagnac-Amitai and Connors 1989; Connors and Gutnick 1990; McCormick et al. 1985). Prolonged depolarizing pulses were also reported to yield single APs followed by a prominent depolarizing after-potential (DAP; Chagnac-Amitai and Connors 1989). The DAP is Ca^{2+}-dependent and very often triggers a second AP (Connors et al. 1982; Yang et al. 1996a).

An earlier electrophysiological *in vitro* study in layers 5 and 6 of the rat mPFC subdivided further bursting neurons into intrinsically bursting (IB) and repetitive oscillatory bursting (ROB) cells (Yang et al. 1996a).

Intrinsically bursting (IB) cells fire mostly doublets of APs intermingled with single APs with fast repolarization, followed by a prominent DAP. At suprathreshold levels they tend to fire trains of single APs with increasing inter-spike intervals (Yang et al. 1996a). ROB cells fire spontaneously bursts of two to three APs with sustained rhythmicity. Each burst is followed by a prominent AHP. When depolarized by prolonged current pulses they fire single, non-adapting APs followed by a prominent DAP (Yang et al. 1996a).

Morphological analysis of the pyramidal cells in the rat mPFC showed that RS cells have large pyramidal somata with a single axon and several axon collaterals (Yang et al. 1996a). The proximal dendrites of RS cells branch within ~400 μm around the soma, whereas the apical dendrite ascends to superficial layers (Yang et al. 1996a). Both IB and ROB cells have pyramidal cell bodies with proximal dendrites that bifurcate profusely and apical dendrites that reach the superficial layers (Yang et al. 1996a). The projection of their main axon reaches the white matter, whereas axon collaterals project in the horizontal and perpendicular direction (Yang et al. 1996a).

5.1.2. *Interneurons in the rodent neocortex*

Among the different types of GABAergic interneurons localized within the mPFC, fast spiking (FS) interneurons present intrinsic firing and immunohistochemical characteristics that suggest they could contribute to the generation of fast network oscillations. Those GABAergic interneurons elicit episodes of fast (>100 Hz) and non-adapting repetitive discharges in response to depolarizing current pulses (Kawaguchi and Kubota 1996; McCormick et al. 1985). FS cells are immunopositive for PV (Kawaguchi and Kubota 1996) and they possess the morphological characteristics of basket cells (Kawaguchi and Kubota 1997). In the rat neocortex they localize within layers 2 to 6 (McCormick et al. 1985).

5.1.3. *Objectives*

We wished to investigate with intracellular and extracellular recordings the distribution of different neuronal populations localized within the mPFC, as well as the intrinsic and firing properties of different cell types and understand how they contributed to the generation of network oscillations in both the DP and PrL regions. Moreover, we wished to assess the contribution of different synaptic mechanisms to the generation of network oscillations.

5.2. Methods

Slices were prepared and maintained as outlined in section 2.2. Network oscillations were evoked with carbachol (10 μM) and kainate (200 nM) application. Multi-electrode and single-electrode field recordings as well extra- and intracellular recordings were obtained from the mPFC with the recording equipment described in section 2.4. The preparation and application of drugs used in the experiments described in this chapter are outlined in section 2.3.

5.2.1. *Data analysis*

- Power spectral analysis performed on field and IPSP traces, area power and peak frequency extraction techniques are described in section 2.6.1.
- Filter application on field and IPSP traces is described in section 2.6.2.
- Rhythmicity index extraction from IPSP traces is described in section 2.6.3.
- Cross-frequency phase-synchrony analysis applied between field and IPSP traces is described in section 2.6.4.
- Spike sorting analysis applied to extracellular unit recordings is described in section 2.6.5.
- Average firing rate, firing frequency and renewal density extraction from the timestamps of extracellularly recorded units was performed with computational techniques described in section 2.6.7.
- Rhythmicity of neuronal firing activity was estimated from autocorrelation analysis described in section 2.6.8.
- The preference of firing of extracellularly recorded units in relation to the field phase angle was estimated with the unit vs. field phase-synchrony analysis described in section 2.6.9.
- Extraction of the amplitude, rise time and decay time of IPSPs was performed with a computational analysis described in section 2.7.2.
- The histological procedures we used to identify the laminar and regional position of electrodes are outlined in section 2.8.
- Statistical analysis of grouped data is described in section 2.9.

5.3. Results

Several different electrophysiological properties of neurons have been identified in diverse cortical regions including the PFC. These include regular spiking (RS), intrinsically bursting (IB), repetitive oscillatory bursting (ROB) pyramidal cells as well as fast-spiking (FS) and non fast-spiking interneurons (Connors et al. 1982; Kawaguchi and Kubota 1997; McCormick et al. 1985; Yang et al. 1996a). There is little information on whether the proportion of these cell types differs between regions of the PFC. In view of our findings that the major characteristics of network oscillations (i.e. area power, rhythmicity and frequency) between dorsal and ventral regions of the mPFC are significantly different, we first characterized the proportion of cell types and their electrophysiological properties observed in the DP and compared this with the PrL. In order to compare our data with that previously reported on electrophysiological properties of cortical cells we first carried out experiments in the absence of any oscillation in normal ACSF.

Stable intracellular recordings were obtained from DP and PrL neurons located in layers 5 and 6. We chose these layers because results in Chapter 3 suggested that oscillations are generated by activity in these layers. Only neurons with resting membrane potentials greater than -50mV and APs that overshoot (> 0mV) were included in the data analysis (apart from two exceptions in section 5.3.2.3).

5.3.1. *Electrophysiological properties of mPFC cells in the absence of network oscillations*

In total we recorded from eighteen (n=18) cells in the DP region. These have been classified as outlined below, into RS, IB and ROB cells based on the classification scheme of (Yang et al. 1996a). In these experiments we did not record from any fast spiking interneurons.

5.3.1.1. Regular spiking cells in the dorsal peduncular cortex in the absence of network oscillations

Eleven (n=11/18, 61%) RS cells were recorded in the DP. They were classified as RS cells because they elicited trains of single APs, followed by prominent AHPs, when held at threshold with a sustained DC current injection (Figure 5.1A).

Table 5.1. Electrophysiological properties of different types of cells in the DP and PrL regions of the mPFC

Figure 5.1. Firing activity of RS cells in the DP region in the absence of network oscillations

Figure 5.2. Intrinsic properties of RS cells in the DP region in the absence of network oscillations

Moreover, when injected with a depolarizing current pulse that held their membrane potential transiently (300 msec) near firing threshold, they elicited single APs (Figure 5.1Bii). Injection of stronger depolarizing currents that raised their membrane potential above threshold elicited a firing response composed of trains of single APs with strong adaptation of firing frequency (Figure 5.1.Biv). Under no condition did these cells fire bursts of APs. Spontaneous firing occurred very rarely during stable impalements.

RS cells (n=11) had a mean resting membrane potential of -71 ± 5.1 mV with neuronal input resistance of 55.7 ± 22.5 MΩ. When depolarized, the mean firing threshold was -59.3 ± 4.9 mV and they fired APs with mean amplitude of 70.8 ± 6.9 mV (Table 5.1).

In addition, RS cells in the DP region exhibited some interesting intrinsic membrane properties. First, when hyperpolarizing current pulses were injected, 10/11 (91%) RS cells elicited a hyperpolarizing "sag" at the onset and a rebound after-depolarization at the end of every pulse (Figure 5.2A), revealing the presence of a presumed I_h-current that tended to rectify their membrane towards resting values. Moreover, when held at subthreshold potentials, RS cells (3/11) displayed rhythmic membrane voltage oscillations ranging between 2-5 Hz (Figure 5.2B). The latter evidence could indicate intrinsic oscillatory properties of RS cells in the deep layers of the DP region.

5.3.1.2. Intrinsically bursting cells in the dorsal peduncular cortex in the absence of network oscillations

Two (n=2/18, 11%) intrinsically bursting (IB) cells were recorded in the DP region in the absence of network oscillations. Both neurons were categorized as IB cells because when held at threshold with a sustained DC current injection they fired doublets of APs intermingled with single APs with fast repolarization and strong after-depolarization potentials (DAP; Figure 5.3A). Spontaneous firing occurred quite often in these cells, consisting mostly of spike doublets. At suprathreshold levels they tended to fire only single APs followed by a prominent DAP (Figure 5.3B).

Figure 5.3. Electrophysiological properties of IB cells in the DP region in the absence of network oscillations

Figure 5.4. Firing activity of IB cells in the DP region in the absence of network oscillations

Figure 5.5. Intrinsic properties of IB cells in the DP region in the absence of network oscillations

When at resting membrane potential, injection of depolarizing current pulses (300 msec) that increased the membrane potential to firing threshold elicited single APs followed immediately by a prominent DAP (Figure 5.4A). In response to suprathreshold depolarizing current pulses, IB cells elicited a doublet of fast APs on the depolarizing envelope which was followed by single APs with progressive frequency adaptation (Figure 5.4B,C,D).

Similar to RS cells, IB cells responded to the onset of a hyperpolarizing pulse with a hyperpolarizing "sag" (Figure 5.5), suggesting that they possess a presumed I_h-current. At the end of this pulse a rebound depolarizing envelope occurred, which often triggered spike doublets.

The first cell had a resting membrane potential of -63mV with an input resistance of 37 MΩ and fired APs of 73 mV amplitude at the firing threshold of -59 mV. The second IB cell had a resting potential at -66 mV and an input resistance of 50 MΩ. At the firing threshold of -61 mV it fired APs with 69.5 mV amplitude (Table 5.1).

5.3.1.3. Repetitive oscillatory bursting cells in the dorsal peduncular cortex in the absence of network oscillations

Five (n=5/18, 28%) repetitive oscillatory bursting (ROB) cells were recorded in the DP region in the absence of network oscillations. These cells displayed a great variety of firing activity. When held at firing threshold with sustained current injection, they fired spike complexes composed of two or three APs. Each burst was terminated by prolonged AHP (Figure 5.6A). Quite often bursts were intermingled with single APs followed immediately by a prominent DAP (Figure 5.6B). When depolarized even further at suprathreshold levels, firing activity elicited by these cells became highly rhythmic (Figure 5.7A) with interspike intervals at 2-13 Hz (Figure 5.7B). This rhythmicity distinguished them from IB cells. At this depolarized potential, both bursts and single APs had a rapid onset (Figure 5.7A). Moreover, single APs were not followed by a DAP (Figure 5.7A). Spontaneous firing occurred in these cells, consisted mostly of clusters of two to three APs.

Figure 5.6. Electrophysiological properties of ROB cells in the DP region in the absence of network oscillations

Figure 5.7. Firing activity of ROB cells in the DP region in the absence of network oscillations

Figure 5.8. Firing properties of ROB cells in the DP region in the absence of network oscillations

Figure 5.9. Intrinsic properties of ROB cells in the DP region in the absence of network oscillations

When held at resting potential, injection of depolarizing current pulses (300 msec) elicited a burst of three APs followed by a train of non-adapting single APs (Figure 5.8A,B,C). The absence of firing frequency adaptation in ROB cells proved to be the most reliable criterion to distinguish this type of neuron from the IB cells (Yang et al. 1996a).

Similar to the RS and IB cells in the DP region, ROB cells responded to the onset of hyperpolarizing current pulses (300 msec) with a hyperpolarizing "sag" and with a rebound depolarization along with bursts of APs at the end of it (Figure 5.9). This response suggests that ROB cells possess an I_h-current.

ROB cells (n=5) had a mean resting potential of -67.2 ± 2.6 mV and a mean input resistance of 49.1 ± 10.3 MΩ. Firing activity of these cells was triggered at the firing threshold of -63.4 ± 6.1 mV and APs had a mean amplitude of 70.5 ± 4.1 mV (Table 5.1).

5.3.1.4. Regular spiking cells in the prelimbic cortex in the absence of network oscillations

In total five cells were recorded in the PrL region in baseline conditions and all of them were classified as RS (n=5; 100%) cells. When these cells were held at threshold potential with a sustained DC current injection, they elicited trains of APs followed by a prominent AHP (Figure 5.10A). At resting potential, injection of short duration (300 ms), near-threshold depolarizing current pulses elicited single APs (Figure 5.10Bii). Further transient depolarization of RS cells to suprathreshold potentials, elicited trains of APs with progressively increasing inter-spike intervals (i.e. frequency adaptation; Figure 5.10Biv). Under no condition did these cells fire bursts of APs.

When held at resting membrane potential, injection of hyperpolarizing current pulses did not elicit a hyperpolarizing "sag" or rebound after-depolarization (Figure 5.11A). These data suggest that unlike RS cells in the DP region, these cells do not possess an inward I_h-current.

However, similar to RS cells in the DP region, when the membrane potential was held between resting potential and firing threshold via continuous DC current

Figure 5.10. Firing activity of RS cells in the PrL region in the absence of network oscillations

Figure 5.11. Intrinsic properties of RS cells in the PrL region in the absence of network oscillation

injection, RS cells (3/5) displayed rhythmic membrane voltage oscillations at the frequency range of 2-15 Hz (Figure 5.11B). RS cells (n=5) in the PrL region had a mean resting membrane potential of -72.8 ± 2.7 mV and a mean neuronal input resistance of 47.3 ± 13.4 MΩ. When depolarized to the mean firing threshold of -55.8 ± 3.1 mV they fired APs with mean amplitude of 69.9 ± 5.0 mV (Table 5.1).

Although we recorded fewer cells in the PrL region, these results suggest that a greater diversity of cell types might be present in the DP region. Comparing the data obtained from RS cells recorded in the DP and PrL regions (sections 5.3.1.1 and 5.3.1.4 respectively), we may conclude that these two different cell groups present many similar electrophysiological properties and two major differences (Table 5.1). Both groups of RS cells have a similar resting membrane potential and fire single APs with similar amplitude. In response to transient suprathreshold depolarization they both fire single APs with frequency adaptation. APs elicited by RS cells in both regions are followed by prominent AHPs and under no condition did these cells fire bursts of APs. Moreover, both groups of cells produced rhythmic membrane oscillations when held at subthreshold potentials. However, the firing threshold of RS cells in the PrL was more positive than the firing threshold of RS cells in the DP region. Another major difference between them is that only RS cells in the DP possessed an I_h-current.

5.3.2. *Electrophysiological properties of mPFC cells in the presence of network oscillations*

Having identified the cell types localized within the DP and PrL regions and studied their electrophysiological properties in the absence of network oscillations, we then wished to record from the same cells once stable network oscillations had been evoked. Such recordings could give us an insight into the role of different cell types in the generation of fast network oscillations. Moreover, by investigating the synaptic input of these cells, we could identify the type of synaptic drive that sustains network oscillations.

5.3.2.1. Regular spiking cells in the dorsal peduncular cortex during network oscillations

Twenty four (n=24) regular spiking cells were recorded in the DP region during stable network oscillations. In the presence of carbachol (10 μM) and kainate (200 nM) the resting membrane potential of these cells was significantly depolarized ($V_{rest/ctrl}$: -71.0 ± 5.1mV, n_{cells}=11; $V_{rest/osc}$: -59.2 ± 4.3mV, n_{cells}=24, unpaired t-test: t(33)=-7.1, p<0.05; Table 5.1). Input resistance ($R_{in/ctrl}$: 55.7 ± 22.5MΩ, n_{cells}=11; $R_{in/osc}$: 53.1 ± 10.4MΩ, n_{cells}=24, Mann-Whitney U test on Ranks: U(11)=42.5, p>0.05) and firing threshold ($V_{thr/ctrl}$: -59.3 ± 4.9mV, n_{cells}=11, $V_{thr/osc}$: -59.9 ± 2.6mV, n_{cells}=24, unpaired t-test: t(33)=0.4, p>0.05) were not altered during network oscillations. However, APs had a significantly smaller amplitude ($AP_{amp/ctrl}$: 70.8 ± 6.9mV, n_{cells}=11, $AP_{amp/osc}$: 65.6 ± 4.3mV, n_{cells}=24, unpaired t-test: t(33)=2.5, p<0.05).

Strong, rhythmic trains of IPSPs were present at the firing threshold (Figure 5.12A) exerting strong control over the neuronal firing activity. Cells tended to fire single APs only on the decaying phase of the IPSPs. As a result, depolarizing pulses that exceeded membrane potential over threshold, elicited trains of non-adapting single APs (Figure 5.12B). In contrast to non-oscillatory conditions, no sign of inward rectification (i.e. hyperpolarizing "sag", rebound depolarization) was observed in response to hyperpolarizing current pulses (Figure 5.12C).

10/24 RS cells had a resting potential equal or greater to their firing threshold. These cells fired single APs at multiple intervals to the field oscillation period (Figure 5.13A). They fired APs with the average rate of 7.4 ± 6.7 spikes.sec^{-1} at the firing frequency of 11.0 Hz (IQR: 6.0-14.0, n=10) with a renewal density of 8.8 spikes.sec^{-1} (IQR: 5.6-13.7, n=10; Figure 5.13B).

When held at a membrane potential of -30 mV (depolarization block), the IPSPs had a mean amplitude of 9.1 ± 4.9 mV (n=23; Figure 5.14A,B). IPSP trains oscillated at 25.1 ± 2.1 Hz (n=23; Figure 5.14Ci) with an RI of 0.59 ± 0.26 (n=23; Figure 5.14Ciii). Network activity recorded concurrently with the IPSP trains from the same layer, oscillated at the mean frequency of 25.6 ± 2.5 Hz (n=23; Figure 5.14Cii) with a high RI of 0.55 ± 0.22 (n=23). Both IPSP trains and field traces were in anti-phase (Figure 5.14Ciii) with a high Shannon entropy of 0.32 ± 0.10 (n=23).

Figure 5.12. Electrophysiological properties of RS cells in the DP region in the presence of fast network oscillations

Figure 5.13. Firing activity of RS cells in the DP region in the presence of fast network oscillations

Figure 5.14. RS cells in the DP region receive strong synaptic inhibition in the presence of network oscillations

5.3.2.2. Intrinsically bursting cells in the dorsal peduncular cortex during network oscillations

Four (n=4) IB cells were recorded in the DP region during network oscillations. These cells fired at subthreshold or threshold potentials either spike doublets or single APs followed immediately by a prominent DAP (Figure 5.15A,B). Depolarizing pulses that exceeded firing threshold elicited trains of non-adapting single APs (Figure 5.15B).

When injected with hyperpolarising or depolarizing current pulses (300 ms) at resting potential, IB cells could become very depolarized, firing bursts of APs. Depolarization was spontaneous with a duration (~1 sec) that exceeded the current step (Figure 5.15C).

In the presence of carbachol (10 μM) and kainate (200 nM), and in comparison to non-oscillatory conditions, IB cells (n=4) were depolarized with a mean resting potential of -55 ± 4.2 mV (when in the absence of oscillations the resting potential was -63mV and -66mV; Table 5.1). They had an input resistance of 49.7 ± 15.0MΩ (when in the absence of oscillations the input resistance was 37MΩ and 50MΩ) and they fired APs with a smaller amplitude of 66.0 ± 2.6 mV (when in the absence of oscillations they fired APs with 73.0mV and 69.5mV amplitude) at a similar firing threshold of -58.3 ± 4.1 mV (when in the absence of oscillations they fired APs at -59mV and -61mV).

Firing activity was controlled by strong, rhythmic IPSP trains (Figure 5.16A). At resting potential bursting activity was abolished. They instead fired single APs time-locked to the decay phase of IPSPs and at multiple intervals to the network oscillation period. IB cells fired APs with the average rate of 3.6 ± 2.5 spikes.sec^{-1} (n=3) at the firing frequency of 10.3 ± 1.5 Hz (n=3) with a renewal density 7.9 ± 0.3 spikes.sec^{-1} (n=3; Figure 5.16B).

In contrast to non-oscillatory conditions, when IB cells were injected with hyperpolarizing current pulses, they did not elicit a hyperpolarizing "sag" or rebound depolarization (Figure 5.16C).

Synaptic inhibition was strong and highly rhythmic in IB cells (Figure 5.17A). At -30 mV the amplitude of the estimated IPSPs was 7.4 ± 4.9 mV (n=4; Figure 5.17B). IPSPs trains oscillated at the mean frequency of 22.6 ± 1.8 Hz (n=4; Figure 5.17Ci)

Figure 5.15. Electrophysiological properties of IB cells in the DP region in the presence of fast network oscillations

Figure 5.16. Firing activity and intrinsic properties of IB cells in the DP region in the presence of fast network oscillations

Figure 5.17. IB cells in the DP region receive strong synaptic inhibition in the presence of fast network oscillations

and RI of 0.624 ± 0.303 (n=4; Figure 5.17Ciii). Simultaneously recorded network activity oscillated at 22.7 ± 1.8 Hz (n=4; Figure 5.17Cii) with the mean RI of 0.642 ± 0.317. IPSP trains and field traces were in anti-phase (n=4; Figure 5.17Civ) but strongly phase-locked with a mean Shannon entropy of 0.356 ± 0.127.

5.3.2.3. Repetitive oscillatory bursting cells in the dorsal peduncular cortex during network oscillations

Two (n=2) ROB cells were recorded in the DP region during network oscillations. They were grouped as ROB cells because they fired bursts of three APs at subthreshold and threshold potentials (Figure 5.18A).

In the presence of carbachol (10 μM) and kainate (200 nM) ROB cells became very depolarized. They had a resting potential at -48 mV and -47 mV (when in the absence of network oscillations it was -67.2 ± 2.6 mV; Table 5.1). Moreover, they fired APs with lower amplitude of 63 mV and 52 mV (when in the absence of network oscillations they fired APs of 70.5 ± 4.1 mV). They had an input resistance of 60 MΩ and 58 MΩ (when in the absence of oscillations the input resistance was 49.1 ± 10.3 MΩ) and a firing threshold of -57 mV and -58 mV (when in the absence of oscillations they fired APs at -63.4 ± 6.1 mV).

Depolarizing pulses that reached, or exceeded, the firing threshold of these cells elicited trains of non-adapting APs (Figure 5.18B). Similar to IB cells in the same region, ROB cells quite often elicited transient (0.5 sec maximum) epochs of large membrane depolarization, superimposed with bursts of APs, when injected with hyperpolarized or depolarized current pulses (Figure 5.18C). Any signs of inward rectification (I_h-current) that were observed on baseline conditions were absent in the presence of network oscillations (Figure 5.18D).

Bursting activity was abolished at resting membrane potential. ROB cells fired only single APs the onset of which coincided with the decay phase of the rhythmic IPSP trains (Figure 5.19A). They fired APs with an average rate of 16.4 and 9.7 spikes.sec^{-1} at the firing frequency of 24 and 27 Hz with a renewal density of 29.1 and 53.4 spikes.sec^{-1}, respectively (Figure 5.19B).

Depolarization block revealed strong rhythmic, inhibitory synaptic drive to those cells (Figure 5.20A).

Figure 5.18. Electrophysiological properties of ROB cells in the DP region in the presence of fast network oscillations

Figure 5.19. Firing activity of ROB cells in the DP region in the presence of fast network oscillations

Figure 5.20. ROB cells in the DP region receive strong synaptic inhibition in the presence of fast network oscillations

The first cell received IPSPs with an amplitude of 6.2 mV (Figure 5.20B), an RI of 0.609 (Figure 5.20Ciii) and an oscillation frequency at 26.86 Hz (Figure 5.20Ci). Field activity oscillated at 26.86 Hz (Figure 5.20Cii) with an RI of 0.703 (Figure 5.20B). The second ROB cell received IPSP trains with an amplitude of 5.6 mV and an RI of 0.325 at the frequency of 27.0 Hz. At the same time, network oscillations had a peak frequency of 27.47 Hz with an RI of 0.477. In both cells, IPSP trains and field traces were in anti-phase (Figure 5.20Civ) and phase-locked with a high Shannon entropy of 0.314 and 0.281 for the first and the second cell, respectively.

5.3.2.4. Regular spiking cells in the prelimbic cortex during network oscillations

Nine (n=9) cells were recorded in the PrL region during stable network oscillations all of which were categorized as RS cells. When held at threshold, RS cells fired trains of single APs followed by prominent AHPs (Figure 5.21A). Injection of depolarizing pulses (300 msec) that reached or exceeded the firing threshold elicited trains of APs with increased interspike intervals and prominent AHPs that did not differ from those in non-oscillatory conditions (Figure 5.21B). Under no condition did these cells fire bursts of APs. Similar to non-oscillatory conditions, no sign of inward rectification was observed when hyperpolarizing pulses (300 ms) were injected at resting potential (Figure 5.21C).

Following carbachol (10 μM) and kainate (200 nM) application, the resting membrane potential of RS cells was significantly depolarized ($V_{rest/ctrl}$: -72.8 ± 2.7 mV, n_{cells}=5, $V_{rest/osc}$: -62.7 ± 5.9 mV, n_{cells}=9, unpaired t-test: $t(12)=-4.3$, $p<0.05$; Table 5.1). However, mean input resistance ($R_{in/ctrl}$: 47.3 ± 13.4 MΩ, n_{cells}=5, $R_{in/osc}$: 51.2 ± 7.8 MΩ, n_{cells}=9, unpaired t-test: $t(12)=-0.7$, $p>0.05$), firing threshold ($V_{thr/ctrl}$: -55.8 ± 3.1 mV, n_{cells}=5, $V_{thr/osc}$: -54.6 ± 4.7 mV, n_{cells}=9, unpaired t-test: $t(12)=-0.6$, $p>0.05$) and AP amplitude ($AP_{amp/ctrl}$: 69.8 ± 5.0 mV, n_{cells}=5, $AP_{amp/osc}$: 68.9 ± 4.3 mV, n_{cells}=9, unpaired t-test: $t(12)=0.4$, $p>0.05$) were not significantly altered during network oscillations.

In three out of nine cells the resting membrane potential was equal to or just above the firing threshold (Figure 5.22A). RS cells fired APs with the average rate of 3.5 ± 2.6 spikes.sec^{-1} (n=3) at the firing frequency of 3.0 ± 2.3 Hz (n=3) with a median renewal density of 5.7 spikes.sec^{-1} (IQR: 5.5-7.9, n=3; Figure 5.22B).

At depolarization block, IPSPs were small (Figure 5.23A) with a mean amplitude of 2.9 ± 1.9 mV (n=9; Figure 5.23B). Power spectral analysis revealed that IPSP trains oscillated weakly at the mean frequency of 14.2 ± 9.9 Hz (n=9; Figure 5.23Ci) when network activity oscillated at 25.3 ± 2.1 Hz (n=9; Figure 5.23Cii). Moreover they had a mean RI of 0.279 ± 0.222 (n=9; Figure 5.23Ciii) when network oscillations had a mean RI of 0.428 ± 0.170 (n=9; Figure 5.23Civ). IPSP trains were in anti-phase with the simultaneously recorded (from the same layer) population field activity (Figure 5.23Civ). However, phase-locking was rather weak with a mean Shannon entropy of 0.140 ± 0.082 (n=9).

Although we cannot draw definitive conclusions due to the small number of samples, these data suggest that the different types of cells localized in the DP region (e.g. RS, IB and ROB) became significantly depolarized by concurrent activation of the cholinergic and AMPA/kainate-receptors. Firing threshold and input resistance were not affected though. More importantly during network oscillations these cells received strong and rhythmic inhibition which shaped their firing activity into a periodic pattern coinciding with the decay phase of IPSPs. Bursting cells, when reached firing threshold, fired only trains of single APs. Moreover, the presumed inward I_h-current which was recorded from all the DP cells in non-oscillatory conditions, was absent in the presence of network oscillations.

RS cells in the PrL region were also significantly depolarized by concurrent activation of the cholinergic and AMPA/kainate receptors. However, their intrinsic properties were not altered significantly in the presence of network oscillations. The difference in the firing threshold between RS cells in the PrL and the DP region became significant in the presence of network oscillations, with PrL cells firing APs at more positive potentials. However, in contrast to DP cells, RS cells in the PrL region received a weaker, slower and less rhythmic inhibitory synaptic drive.

Moreover, intracellular recordings from the mPFC in the presence of network oscillations confirm our suggestion that the DP region contains a greater diversity of cells than the PrL region. In total we recorded from 48 cells in the DP region. 35/48 cells were RS cells, 6/48 cells were IB cells and 7/48 cells were ROB cells. In contrast, in the PrL region we recorded from 14 cells all of which were RS cells.

Figure 5.21. Electrophysiological properties of RS cells in the PrL region in the presence of fast network oscillations

Figure 5.22. Firing activity of RS cells in the PrL region in the presence of fast network oscillations

Figure 5.23. RS cells in the PrL region receive weak synaptic inhibition in the presence of fast network oscillations

5.3.3. *Firing activity of different neuronal populations in the presence of fast network oscillations in the medial PFC – Extracellular recordings with the Utah array.*

In total we recorded 247 units in the mPFC and the motor cortex in the presence of network oscillations. According to their firing characteristics, these units were grouped into five (5) different types. These are the fast-firing rhythmic (FR), medium-firing rhythmic (MR), fast spiking (FS), fast-firing non-rhythmic (FNR) and slow-firing non-rhythmic (SNR) units (Table 5.2).

5.3.3.1. Fast-firing rhythmic (FR) units

The most frequently recorded type of units was the FR units (n=100; 40%; Figure 5.26; Table 5.2). In the mPFC and the motor cortex, they fired trains of spikes with the average rate of 6.4 spikes.sec^{-1} (IQR: 4.8-9.3, n_{units}=100, n_{slice}=23; Figure 5.24A) at the firing frequency of 24.0 Hz (IQR: 10.0-30.0, n_{units}=100, n_{slice}=23; Figure 5.24B) with a median renewal density of 8.8 spikes.sec^{-1} (6.7-11.7, n_{units}=100, n_{slice}=23; Figure 5.24C). Firing activity occurred at 185° (IQR: 175-205, n_{units}=100, n_{slice}=23; Figure 5.25A) of the field potential phase with a high Shannon entropy of 0.11 (0.08-0.13, n_{units}=100, n_{slice}=23; Figure 5.25B). Strong rhythmic firing activity was the most distinctive hallmark in the spiking activity of the FR units (Figure 5.26B,C). Interspike and autocorrelation histograms revealed numerous peaks centred at multiples of the field main oscillatory period. Evidence that FR units fired spikes with strong rhythmicity (an indication of oscillatory activity) and phase-synchrony along with a firing frequency which was similar to the field oscillation frequency could indicate their strong engagement in the generation of network oscillations.

We therefore, wished to investigate the presence of any regional differences on the firing activity of the FR units. Grouped results are presented in Table 5.3 and Figure 5.27 and 5.28.

Table 5.2. Firing properties of different types of units in the PFC recorded with the UTAH array in the presence of network oscillations

Figure 5.24. Distinct firing properties of the different types of units recorded with the Utah array

Figure 5.25. Distinct phase-synchrony properties of the different types of units recorded with the Utah array

Figure 5.26. Firing activity of FR units is highly rhythmic and synchronous to the field phase in the presence of network oscillations

Table 5.3. Regional distribution of FR units in the PFC

Figure 5.27. Regional differences in the firing characteristics of FR units

Figure 5.28. Regional differences in the phase-synchrony of FR units

5.3.3.2. Medium-firing rhythmic (MR) units

Eighteen (n=18; 7%) MR units were recorded in the different subdivisions of the mPFC and the motor cortex (Figure 5.29, Table 5.2). These units fired spikes with the mean average rate of 6.7 spikes.sec^{-1} (IQR: 5.0-7.6, n_{units}=18, n_{slice}=8; Figure 5.24A). They displayed inter-spike interval histograms with a prominent narrow peak centred at 8.0 Hz (IQR: 8.0-11.0, n_{units}=18, n_{slice}=8; Figure 5.24B) with a renewal density of 12.6 spikes.sec^{-1} (IQR: 10.0-14.5, n_{units}=18, n_{slice}=8; Figure 5.24C). Spike timing coincided with 185° (IQR: 174-216, n_{units}=18, n_{slice}=8; Figure 5.25A) of the field potential phase, however, phase-synchrony was rather weak with a Shannon entropy of 0.03 (IQR: 0.02-0.05, n_{units}=18, n_{slice}=8; Figure 5.25B).

The majority of these units were recorded in layers 5 and 6 of the motor cortex (n=11; 61%; multi-unit activity from layers 2 and 3 was not recorded). Among the rest of the MR units (n=7; 39%), two (n=2) were recorded in layers 2 and 6b of the DP, one (n=1) was recorded in layer 3 of the IL, two (n=2) were recorded in layers 2 and 6b of the PrL and two (n=2) in layers 5 and 6b of the ACd region.

Although firing activity elicited by MR units was highly regular, evidence of weak phase-synchrony indicates that spike timing was uncorrelated to population activity (Figure 5.29). Therefore the engagement of MR units to the network oscillatory activity is considered unlikely.

5.3.3.3. Fast spiking (FS) units

Fourteen (n=14; 6%) FS units were recorded at the mPFC and the motor cortex (Figure 5.30, Table 5.2). FS units fired spikes with the high average rate of 15.1 spikes.sec^{-1} (IQR: 13.5-18.7, n_{units}=14, n_{slice}=10; Figure 5.24A). Inter-spike interval analysis produced histograms with a single peak at 31.5 Hz (IQR: 28.5-42.0, n_{units}=14, n_{slice}=10; Figure 5.24B) with a renewal density of 15.9 spikes.sec^{-1} (IQR: 12.4-20.3, n_{units}=14, n_{slice}=10; Figure 5.24C). The onset of this peak was rapid followed immediately by an inverse exponential decay. No signs of rhythmic firing activity were revealed by autocorrelation analysis. Phase-locking at 220° (IQR: 167-240, n_{units}=14, n_{slice}=10; Figure 5.25A) of the field phase was weak with a Shannon entropy of 0.02 (IQR: 0.01-0.04, n_{units}=14, n_{slice}=10; Figure 5.25B).

Figure 5.29. Firing activity of MR units is highly rhythmic but weakly locked to the field phase in the presence of network oscillations

Figure 5.30. Firing activity of FS units is intense but weakly locked to the field phase in the presence of network oscillations

Five (n=5; 36%) FS units were recorded at layers 5 and 6 of the motor cortex. Among the rest of the units (n=9; 64%), two (n=2) were recorded in layers 5 and 6a of the DP, one (n=1) was recorded in layer 5 of the IL, three (n=3) were recorded in layers 2, 3 and 6a of the PrL and three (n=3) were recorded in layers 3, 5 and 6a of the ACd region.

The firing activity of FS units was intense; however, evidence of weak phase-synchrony indicates that spike timing was uncorrelated to population activity (Figure 5.30). Therefore the engagement of FS units to the network oscillatory activity is considered unlikely.

5.3.3.4. Fast-firing non-rhythmic (FNR) units

Thirty-nine (n=39; 16%) FNR units were recorded in the mPFC and the motor cortex (Figure 5.31, Table 5.2). These units fired spikes with the average rate of 5.7 spikes.sec^{-1} (IQR: 4.1-7.0, n_{units}=39, n_{slice}=15; Figure 5.24A). Interspike interval and autocorrelation analysis revealed a non-rhythmic firing activity elicited by these units. Spikes occurred at the median frequency of 14.0 Hz (IQR: 10.0-24.5, n_{units}=39, n_{slice}=15; Figure 5.24B) with a median renewal density of 6.2 spikes.sec^{-1} (IQR: 5.3-7.9, n_{units}=39, n_{slice}=15; Figure 5.24C). Moreover, spike timing was weakly locked at 195° (IQR: 176-225, n_{units}=39, n_{slice}=15; Figure 5.25A) of the field phase with a Shannon entropy of 0.04 (IQR: 0.03-0.05, n_{units}=39, n_{slice}=15; Figure 5.25B).

Many of the FNR units were recorded at layers 3, 5 and 6a of the motor cortex (n=17; 44%). The remainder were recorded at layers 2, 5 and 6b of the DP region (n=3), layers 2 and 6 of the IL region (n=5), layers 3, 5 and 6a of the PrL region (n=10) and layers 2, 5 and 6a of the ACd region (n=4).

5.3.3.5. Slow-firing non-rhythmic (SNR) units

Seventy-five (n=75; 31%) SNR units were recorded in the mPFC and the motor cortex (Figure 5.32, Table 5.2). These units fired spikes at the low average rate of 3.5 spikes.sec^{-1} (IQR: 2.8-4.1, n_{units}=75, n_{slice}=19; Figure 5.24A). Firing activity was non-rhythmic, and interspike interval analysis revealed histograms with obscure peaks at 4.0 Hz (IQR: 4.0-6.0, n_{units}=75, n_{slice}=19; Figure 5.24B) with a median renewal density of 5.2 spikes.sec^{-1} (IQR: 4.2-7.0, n_{units}=75, n_{slice}=19; Figure 5.24C).

Figure 5.31. FNR units exhibit variable firing properties in the presence of fast network oscillations

Spikes occurred at 190° (IQR: 175-225, n_{units}=75, n_{slice}=19; Figure 5.25A) of the field potential phase. The mean Shannon entropy of 0.04 (IQR: 0.03-0.07, n_{units}=75, n_{slice}=19; Figure 5.25B) revealed a weak but evident phase-synchrony.

Twenty-five (n=25; 33%) of SNR units were recorded in layers 3, 5 and 6a of the motor cortex. Nine (n=9; 12%) of them were recorded in layers 2, 5 and 6a of the DP region, thirteen (n=13; 17%) units were recorded in layers 2, 3, 5 and 6b of the IL region and thirteen (n=13; 17%) units were located in layers 3, 5 and 6 of the PrL. Finally fifteen (n=15; 20%) SNR units were recorded in layers 2, 3, 5 and 6 of the ACd region.

Both FNR and SNR units presented obscure firing properties. Firing activity was not rhythmic and network-synchrony analysis revealed a weak phase-synchrony at the trough of the field potential phase. Therefore the engagement of these units to the network oscillatory activity is considered unlikely.

Overall, FR, MR and FS units displayed explicit firing properties. All three cell types elicited trains of spikes at distinct frequencies, with a high average rate and high renewal density. The firing activity of FS unit was intense but non-rhythmic. FR and MR units were less excitable but they fired at regular intervals. However, among them only FR units fired spikes phase-locked to the negative peaks of the field cycles. In contrast, both MR and FS units elicited firing activity with weak phase-synchrony. Although the identity of the extracellularly recorded units is unknown we can argue, based on their clear firing discrepancies that FR, MR and FS units correspond to distinct cell populations. Moreover, among them only the FR units exhibited firing characteristics (e.g. strong phase-synchrony, high firing frequency, rhythmicity) that could indicate the contribution of these units to the generation of network oscillations.

FNR and SNR units displayed obscure firing properties. Interspike interval analysis revealed that these units elicited an irregular firing activity at different frequency bands. Phase-synchrony was also weak.

Neuronal firing in the presence network oscillations depends on many parameters including synaptic activity, intrinsic excitability, and the strength of network synchrony. The first two parameters cannot be addressed by extracellular recording techniques. Network synchrony though could be estimated by spectral

Figure 5.32. SNR units exhibit variable firing properties in the presence of fast network oscillations

means and area power extraction from the field potentials. Therefore we grouped and statistically analyzed the area power of oscillations recorded by the same electrodes which detected units. Analysis of variance revealed that FR units belonged to neuronal populations that oscillated with a significantly stronger area power in comparison to the other types of units (FR: 621 $\mu V^2.Hz^{-1}$ IQR: 335-1009, n=100; MR: 231 $\mu V^2.Hz^{-1}$ IQR: 115-261, n=18; FS: 216 $\mu V^2.Hz^{-1}$ IQR: 124-460, n=14; FNR: 242 $\mu V^2.Hz^{-1}$ IQR: 188-412, n=39; SNR: 238 $\mu V^2.Hz^{-1}$ IQR: 149-352, n=75; Kruskall-Wallis One-Way ANOVA on Ranks: $H(4)=73.6$, $p<0.05$; Tukey test: all comparisons; Figure 5.25C).

5.4. Discussion

5.4.1. *Summary*

In this chapter, we wished to characterize the proportion of different neuronal populations within the DP and PrL subdivisions of the mPFC. Previous studies have already reported the rich neuronal diversity of the cellular populations localizing the mPFC (Kawaguchi and Kubota 1997; Van Aerde et al. 2009; Yang et al. 1996a). However, none of them recorded single cell activity from the DP region. Moreover, they did not compare cell activity before and after the emergence of network oscillations.

For the purpose of this thesis we investigated by means of intracellular and extracellular recordings the electrophysiological properties and firing activities of different neuronal populations within these cortical areas, in the absence and presence of carbachol/kainate-induced fast network oscillations.

Results obtained from intracellular recordings in the absence of network oscillations are:

- Three distinct classes of cells were recorded in the DP region. Based on electrophysiological criteria these were termed RS, IB and ROB cells.

- RS cells which constituted 61% of cells in the DP region fired single APs followed by prominent AHPs with frequency adaptation in response to depolarizing pulses. These cells also possessed an inward presumed I_h-current.

- IB cells (11%) in the DP region fired either spike doublets or single APs followed by a prominent DAP. When injected with depolarizing pulses, IB cells elicited firing activity with marked frequency adaptation. These cells also possessed a presumed inward I_h-current and a low threshold Ca^{2+}-conductance.

- ROB cells (28%) in the DP region fired either bursts of two-to-three APs or single APs followed by a prominent DAP. When injected with depolarizing

pulses, ROB cells elicited firing activity without frequency adaptation. These cells also possessed an inward presumed I_h-current and a low threshold Ca^{2+}-conductance.

- In contrast to the diversity of response properties in the DP region only RS cells were recorded in the PrL region (100%). RS cells in the PrL region fired single APs followed by prominent AHPs with spike frequency adaptation in response to depolarizing pulses. These cells did not possess an inward I_h-current.

- RS cells in both regions displayed subthreshold transient 2-15 Hz intrinsic oscillations.

Results obtained from intracellular recordings in the presence of network oscillations are:

- We recorded intracellular activity from RS (80%), IB (13%) and ROB (7%) cells in the DP region. All cells were significantly depolarized with respect to non-oscillatory conditions and fired APs at a frequency range of 1-28 Hz. IB and ROB cells were more excitable than the RS cells.

- In the DP region both IB and ROB cells stopped burst firing activity during the on-going oscillation and instead fired only single APs.

- All the three cell types in the DP region received strong and highly rhythmic IPSPs, tightly phase-locked and in anti-phase to the field oscillations. The strong presence of synaptic inhibition was apparent at resting potential in all cells, shaping their firing activity at intervals which coincided with the decay phase of IPSPs. As a result of that, frequency adaptation was abolished in RS and IB cells.

- The presumed I_h-current current was absent in the presence of network oscillations in all the types of cells in the DP region.

- We recorded intracellular activity only from RS cells in the PrL region (100%). Apart from membrane depolarization, the physiological properties of these cells were not altered significantly by the presence of network oscillations.

- Firing threshold (~ -54.6 mV) of RS cells in the PrL region was significantly more positive to the firing threshold (~ -59.9 mV) of RS cells in the DP region.

- RS cells in the PrL region fired single APs with frequency adaptation when injected with suprathreshold depolarizing current pulses in the presence of network oscillations.

- RS cells in the PrL region received smaller and less rhythmic IPSPs in the presence of network oscillations.

- During network oscillations, RS cells in the PrL region fired irregular APs with a low frequency (2-6 Hz).

Results obtained from extracellular Utah array recordings in the presence of network oscillations are:

- Five distinct classes of firing behaviour were observed in the mPFC and the motor cortex. These were termed as (1) FR, (2) MR, (3) FS, (4) FNR and (5) SNR units.

- FR (40%) units exhibited high frequency (~24 Hz), highly rhythmic firing activity. FR units fired APs at times that tightly coincided with the negative peak of the field potential cycle.

- MR (7%) units fired spikes with an intermediate frequency (~8 Hz) and a highly rhythmic pattern. Firing activity was weakly locked to the negative peak of the field potential cycle.

- FS (6%) units fired spikes with the highest frequency (~31.5 Hz) among the different groups of units. Interspike interval analysis of spike trains elicited from these cells had a prominent peak with a rapid onset and an inverse exponential decay. Firing activity was weakly locked to the negative peak of the field potential cycle.

- FNR (16%) and SNR (31%) units were characterized by their irregular firing activity. Both types of units fired spikes that were weakly locked to the negative peak of the field potential cycle. Firing frequency was the major difference between these units. FNR units fired spikes at a frequency of ~14 Hz, whereas SNR fired at a slower frequency of ~4 Hz.

- FR units were primarily recorded in the deep layers of the PFC, at location sites with strong oscillations.

5.4.2. *Different neuronal populations are localized within the DP and PrL regions in the absence of network oscillations.*

Initially, we performed intracellular recordings in the deep layers 5 and 6 of the DP and PrL subdivisions of the mPFC in the absence of network oscillations. Cell identification of was based on electrophysiological criteria.

The most frequently recorded cells in the DP region were RS cells. These cells exhibited all the typical electrophysiological properties that have already been reported in the guinea pig sensorimotor and anterior cingulate cortex (Connors et al. 1982; McCormick et al. 1985), the mouse barrel cortex (Agmon and Connors 1992) and the rodent mPFC (Yang et al. 1996a). Firing activity of RS cells was characterized by single APs followed by a prominent AHP and marked adaptation of firing frequency during prolonged injection of suprathreshold current pulses. Moreover, RS cells within the DP region responded to hyperpolarization current pulses with a hyperpolarizing "sag" and a rebound depolarization. This response suggests that these cells possess an I_h-current. The I_h-current is an inwardly rectifying cation current mediated by both Na^+ and K^+ conductances which contributes to the resting membrane potential and to rhythmic burst firing of thalamocortical cells (McCormick and Pape 1990).

We also recorded from RS cells in the PrL region. These cells exhibited similar electrophysiological properties (i.e. single APs, prominent AHPs and frequency adaptation) to the RS cells in the DP region, but differed from DP cells due to the absence of the I_h-current. Moreover, their firing threshold was more positive to the firing threshold of pyramidal cells in the DP region.

RS cells in both regions of the mPFC displayed subthreshold transient membrane oscillations that ranged between 2 - 15 Hz. Cortical pyramidal cells with similar intrinsic properties have already been reported in the rat sensorimotor (Amitai 1994; Silva et al. 1991) and mPFC (Yang et al. 1996a) cortex. Assuming that cortical cells fire APs near the peak of membrane fluctuations, intrinsic oscillations are thought to facilitate the engagement of neurons to network oscillations, shaping their firing activity towards certain frequencies (Amitai 1994). Membrane oscillations are believed to be the compound result of interplay between inward NA^+ channels (rise phase) and outward K^+ channels (repolarizing phase; Amitai 1994; Silva et al. 1991). Ca^{2+}-depended conductances are not considered essential for the generation of these oscillations, but they rather influence the amplitude and rhythmicity of them (Amitai 1994; Silva et al. 1991). Investigation of the membrane oscillations of mPFC cells was beyond the scope of this study. Therefore, the frequency and amplitude range of membrane oscillations at different potentials in the RS cells as well as the existence of membrane oscillations in bursting cells was not assessed.

Although RS cells were the most common (61%) in the DP region, we also readily recorded from both IB (11%) and ROB (28%) cells. IB cells have been previously reported in the deep layers of the mPFC (Yang et al. 1996a). These cells fire either spike doublets or single APs, followed immediately by prominent DAPs and they respond to suprathreshold depolarizing current injections with firing frequency adaptation.

The electrophysiological properties we found in the ROB cells in the DP subdivision of the mPFC were identical to those described in the guinea pig sensorimotor and anterior cingulate cortex (Connors et al. 1982; McCormick et al. 1985) and the rat neocortex (Chagnac-Amitai and Connors 1989; Yang et al. 1996a). ROB cells fired either bursts of two or three APs, or single APs with prominent DAPs. The absence of firing frequency adaptation was a defining

characteristic of ROB cells (Yang et al. 1996a). When ROB cells were depolarized at suprathreshold levels, they elicited a highly rhythmic firing activity at 2-13 Hz which was similar to the one described in layer 5 bursting neurons in the rat somatosensory cortex (Silva et al. 1991).

Finally, both IB and ROB cells recorded in the DP region responded to the onset of a hyperpolarizing current pulse with a hyperpolarizing "sag", indicating that these cells possess an I_h-current. Recently, electrophysiological recordings from layer 5 cells in the mouse mPFC combined with retrograde tracing, demonstrated the presence of a correlation between the I_h-current level these cells exhibit and their main projection targets (Gee et al. 2012). By measuring the sum of the voltage sag and rebound depolarization in response to hyperpolarizing current pulses they quantified the level of I_h-current pyramidal cells possess. They found that pyramidal cells that project to the ipsilateral thalamus or brainstem present high levels of I_h-current in comparison to pyramidal cells that send axon collaterals to the contralateral cortex which do not possess an I_h-current (Gee et al. 2012). In view of these findings, evidence from our study could indicate a strong tendency for DP cells to project to thalamic and brainstem regions and pyramidal cells in the PrL region to target contralateral cortical regions.

At the end of the hyperpolarising step a rebound depolarizing envelope was evident which was often sufficient to trigger bursting activity. It has been shown that this intrinsic inward depolarization is mediated by the so-called low threshold Ca^{2+}-conductance which remains inactivated at resting potentials and becomes de-inactivated by membrane hyperpolarization (Llinás and Yarom 1981; Yang et al. 1996a).

5.4.3. *Neuronal response properties within the DP and PrL in the presence of fast network oscillations*

In the presence of carbachol/kainate-induced fast network oscillations, we observed significant changes in the membrane properties of neurons localized within the DP and PrL regions. All cells were significantly depolarized. In the presence of network oscillations, the resting membrane potential of RS cells in both regions and IB cells in the DP region was depolarized by ~10 mV, whereas in

the ROB cells it became more positive by ~20 mV. The depolarizing effects of carbachol and kainate on pyramidal cells have already been discussed in chapter 3 (section 3.1.2 and 3.1.3).

The difference in the firing threshold potentials of RS cells between PrL and DP regions was slightly increased in the presence of network oscillations and became significant. This difference shows that RS cells in the PrL region require stronger excitatory drive than the DP cells to reach threshold. In support of that, RS cells in the DP region fired more intensely than RS cells in the PrL region. Therefore, the difference in firing threshold potentials could possibly account to the difference in oscillation power between the two regions.

All cells in the DP region received strong and highly rhythmic IPSPs, tightly phase-locked and in anti-phase to the field oscillations. The presence of strong synaptic inhibition was apparent at resting membrane potential in all cells which resulted in rhythmic firing activity occurring at regular interspike intervals, preferably at the decay phase of IPSPs. In contrast, synaptic inhibition in the PrL region was weak and non-rhythmic. These findings show that network oscillations in the DP region depend on GABAergic inhibition more than oscillations in the PrL region and fit with evidence from chapter 4 (section 4.3.4) which showed that oscillations in the DP region were more sensitive to $GABA_A$-receptor blockade than oscillations in the PrL region.

In both IB and ROB cells, bursting activity at subthreshold potentials was replaced by single AP activity at the depolarized resting potential. Bursting activity has been shown to rely on the low threshold Ca^{2+}-conductance that these cells possess (McCormick et al. 1985). This current remains inactivated at resting potential and membrane hyperpolarization removes this inactivation allowing intrinsic burst generation (McCormick et al. 1985). It is possible that the strong membrane depolarization evoked by carbachol and kainate inactivated this Ca^{2+}-dependent current.

5.4.4. *Extracellular recordings revealed neuronal populations with different firing activities in the presence of fast network oscillations in the rat PFC*

We also investigated the firing pattern of extracellularly recorded units in the presence of fast network oscillations with the use of a multi-electrode Utah array. Based on their firing pattern, units recorded at the mPFC and the motor cortex, were categorized into five distinct groups. Among them, FR, MR and FS units elicited trains of spikes with clear and distinctive firing patterns. However, only the firing properties of FR units appeared to contribute to the network oscillatory activity. (1) FR units exhibited an intense and rhythmic firing activity. Rhythmicity (reflected by the interspike interval separation) was similar to the field oscillation period. More importantly, (2) only FR units fired spike trains predominantly concentrated at the negative peak of the field potential cycle. Finally, (3) the majority of FR units (92%) were recorded in the deep layers (i.e. layers 5 and 6), at cortical sites that produced the strongest oscillations. This evidence fits with our hypothesis that the deep layers are the major driving force for fast network oscillations in the mPFC.

The rest of the units (e.g. FNR and SNR units) exhibited irregular firing properties and were recorded in cortical sites that produced weak oscillations.

By combining results obtained from intracellular with extracellular experiments, we observed that the firing activity of extracellularly recorded FR units across the different subdivisions of the PFC was similar to the firing activity of intracellularly recorded pyramidal cells in the DP region. In the presence of network oscillations, FR units fired spikes with strong rhythmicity and with a high preference to the negative peak of the field potential cycle. Intracellularly recorded pyramidal cells (e.g. RS, IB and ROB cells) in the DP region elicited a firing activity with similar rhythmic properties (compare Figure 5.13, 5.16, 5.19 with 5.26).

Moreover, APs occurred at the positive peak of IPSP trains (decaying phase of IPSPs) which in turn were shown to be in anti-phase with the field cycle. Evidence that in the presence of fast network oscillations PFC pyramidal cells fired APs preferably at the negative peak of the field potential have already been reported in the *in vitro* models of beta1- and gamma-frequency oscillations in the mPFC (Van Aerde et al. 2009) and the somatosensory cortex (Buhl et al. 1998), respectively, as well as in the oscillating somatosensory cortex *in vivo* (Murthy and Fetz 1992).

5.4.5. *The contribution of cellular organization and neuronal markers in the regional differences of fast network oscillations in the rat mPFC*

The spectral and rhythmicity characteristics of network oscillations could be correlated to the underlying cellular organization. The dependence of fast network oscillations on fast synaptic inhibition is well documented (Buhl et al. 1998; Dickinson et al. 2003; Whittington and Traub 2003) and there is evidence that is mediated by PV+ fast spiking interneurons (Gonzalez-Burgos and Lewis 2008). Evidence presented in this chapter show stronger synaptic inhibition in the DP than the PrL region. Therefore, we could hypothesize that significant differences in the area power and rhythmicity of oscillations could be attributed to a difference in the density of PV+ fast spiking GABAergic cells across the different subdivisions of the mPFC.

Fast spiking cells have been physiologically, morphologically and chemically identified in layers 2, 3 and 5 of the frontal cortex, targeting the somata and axon initial segments of other cells (Kawaguchi and Kubota 1997; Kawaguchi and Kondo 2002). Immunocytochemistry studies have shown that layer 5 in the mPFC expresses the highest density of PV+ interneurons (Gabbott et al. 1997) and that except for the IL region, the rest of the mPFC is heavily stained for PV (Figure 1.1 B; Jones et al. 2005).

In view of our results and evidence from the immunohistochemistry studies we can infer that fast synaptic inhibition is important for the generation and maintenance of fast network oscillations in the mPFC. However, its influence is not the only determinant factor of the oscillation strength. This is because, although the area power laminar profile is consistent with the laminar distribution of PV+ cells (i.e. layer 5 of all regions produced the strongest oscillations and expresses the highest density of PV+ cells), on the other hand the area power regional profile does not fit with the regional distribution of PV+ cells. According to our results the IL region oscillated with stronger amplitude than the ACd, however, the latter region has the highest density of PV+ cells in the mPFC and the IL the lowest (Jones et al. 2005). It is possible though, that the IL neuronal network receives strong oscillatory drive from the adjacent DP region, in that a strong interaction between those regions was revealed by our phase-synchrony analysis and also reported by anatomical studies (Fisk and Wyss 1999; Jones et al. 2005).

The amplitude of the oscillations could also be affected by the density and distribution of neuronal populations. During neuronal activity, ionic current flows through the cell membrane forming sinks (inward current) or sources (outward current). At a macroscopic scale current sink and source dipoles are the key components of field potentials (Mitzdorf 1985). Fast network oscillations rely on the synchronous activity of cortical cells. Therefore, we could assume that the higher the number of cortical cells firing in synchrony in a unit volume, the stronger the sink/source dipoles would be, which in turn would enhance the amplitude of the field potential. Since the DP is a thinner cortical region than the PrL, we would expect it to have higher neuronal density and therefore produce stronger oscillations. This hypothesis partially fits with evidence from a light microscopical study which showed that both regions have the same neuronal density; however layer 5 in the DP region has a 20% higher neuronal density than the layer 5 in the PrL (Gabbott et al. 1997).

The intrinsic membrane properties and connection patterns of the different cell types localized in the PFC could contribute to the generation of synchronous activity. It has been reported that in the somatosensory cortex, bursting cells initiate synchronous neuronal population activity (Chagnac-Amitai and Connors 1989). This function is supported by their intrinsic property to elicit synchronous trains of APs at modest depolarizing events as well as their strong mutually excitatory interconnections (Chagnac-Amitai and Connors 1989). Consistent with that, it has been reported that in the deep layers of the somatosensory cortex, kainate-induced beta2-frequency oscillations were dependent on the activity of a network of bursting cells interconnected by axo-axonic gap junctions (Roopun et al. 2006).

We recorded from 48 pyramidal cells in the DP region, 11 (23%) of which were bursting cells. In contrast, we recorded from 14 pyramidal cells in the PrL region, all of which were RS cells. Although the sample size is small, it indicates a smaller presence of bursting cells in the PrL region. That in turn could suggest the presence of a smaller amount of intrinsic excitation within the PrL region, which could account for the significant difference in the oscillation power between the PrL and DP regions.

In agreement with results from the present study that oscillations were stronger in layer 5, there is evidence that the somata of bursting cells localize predominantly within layer 5 of the neocortex (Agmon and Connors 1992; Amitai 1994; Chagnac-Amitai and Connors 1989; Connors et al. 1982; Connors and Gutnick 1990; McCormick et al. 1985; Yang et al. 1996a). The same studies have reported that bursting cells send their axon collaterals in both a perpendicular and horizontal direction. This evidence fit to our hypothesis that oscillations emerge from layer 5 and propagate to the superficial layers. Moreover, the horizontal arborization of the axon collaterals of bursting cells provides more support to our hypothesis that oscillations in the IL region are driven by network activity in the DP region.

Chapter 6

The effects of Catecholamines on fast network oscillations in the mPFC

Chapter 6 – The effects of Catecholamines on fast network oscillations in the mPFC

6.1. Introduction

The mammalian PFC is a major cortical target of catecholamine influence, including dopamine (DA) and noradrenaline (NA) (Arnsten 1998, 2011; Steketee 2003).

6.1.1. *The mesocorticolimbic dopaminergic system and prefrontal cortical activity*

The mammalian PFC receives strong innervation from the mesocorticolimbic DA pathway (the A10 pathway of DA-containing cells) which originates in the midbrain VTA (Divac et al. 1978; Grobin and Deutch 1998; Steketee 2003). In the rat brain DA terminals innervate densely the PrL and IL subdivisions of the mPFC (Grobin and Deutch 1998).

The physiological actions of DA are mediated by five G-protein coupled receptors with distinct genetic structure (D_1, D_2, D_3, D_4 and D_5; Beaulieu and Gainetdinov 2011). The five receptors are divided into two major groups, namely, D_1-like (D_1 and D_5) and D_2-like (D_2, D_3 and D_4) receptor subtypes (Beaulieu and Gainetdinov 2011; Missale et al. 1998; Vallone et al. 2000). The D_1-like receptors are coupled to the G protein, Gs, which subsequently activates adenylyl cyclase increasing the concentration of cAMP. Instead, the D_2-like receptors are coupled to the G protein, Gi, which inhibits the formation of cAMP by inhibiting the enzyme adenylyl cyclase (Missale et al. 1998).

Converging evidence from behavioural and lesion studies in the monkey and rat brain, have highlighted the crucial importance of DA receptor activation in the cognitive functions mediated by the PFC. A seminal study by Brozoski and colleagues (Brozoski et al. 1979) reported that DA depletion in the monkey DLPFC produced significant deficits in a spatial delayed alteration performance task. Impairment was reversed by local application of DA precursor L-dopa (Brozoski et al. 1979). Subsequent studies in the monkey brain revealed that deficits in the

mnemonic processes engaged in delayed alteration tasks can be evoked by local application of D_1-receptor antagonists (Sawaguchi and Goldman-Rakic 1991).

In the rat brain, it has been shown using a delayed radial arm maze task that optimal activation of D_1 receptors localized in the rat mPFC support WM performance by improving the efficiency of long-term memory retrieval from the hippocampus (Seamans et al. 1998).

Moreover, in a visual-cue discrimination task that required the animal to shift attention from one stimulus dimension to the other, alterations in the activity of D_1, D_2 and D_4 receptors in the rat mPFC, impaired set shifting performance and resulted in perseverative responses (Floresco and Magyar 2006; Ragozzino 2002). The latter evidence highlighted the involvement of DA in behavioural flexibility (Floresco and Magyar 2006; Ragozzino 2002).

However, like DA depletion, excessive DA activity can also cause deficits in PFC-engaging cognitive functions in both monkeys and rats (Arnsten 1998, 2011; Zahrt et al. 1997).

Based on anatomical criteria, DA actions in the rat mPFC cortex are thought to be predominantly mediated by D_1 and D_2 receptors, because these receptors have the highest levels of expression, especially in the deep layers (Yang et al. 1999). Instead, low density expression levels have been reported for D_3, D_4 and D_5 receptors (Yang et al. 1999).

DA was shown to modulate the excitability of cortical cells, either directly or indirectly by affecting the interneuron excitability or inhibitory synaptic efficiency (Grobin and Deutch 1998; Penit-Soria et al. 1987; Rubinstein et al. 2001; Seamans et al. 2001a; Sesack and Bunney 1989; X. Wang et al. 2002; Yang and Seamans 1996b). Moreover, significant modulatory effects of DA have also been reported in carbachol and kainate-evoked models of gamma frequency oscillations in hippocampal slice preparations (Andersson et al. 2012; Weiss et al. 2003; Wójtowicz et al. 2009).

6.1.2. *The locus coeruleus noradrenergic system and prefrontal cortical activity*

There is evidence that activation of the mesocorticolimbic DAergic system is not sufficient alone to support mnemonic processes, including WM. Instead, DA and NA systems act synergistically to support WM performance (Arnsten 1998; Brozoski et al. 1979; Rossetti and Carboni 2005). In the Brozoski and colleagues study (Brozoski et al. 1979), depletions of NA terminals in the DLPFC elicited modest behavioural deficits in the spatial delayed alteration performance task (Brozoski et al. 1979). There is also evidence of increased DA and NA concentrations in the rat mPFC during a delayed (T-maze) alteration task (T-maze; Rossetti and Carboni 2005). DA elevation was associated with reward expectancy, whereas, NA elevation was correlated to the attentional demand of goal-directed behaviour (Rossetti and Carboni 2005).

NA exerts a strong influence on cortical activity by stimulating a family of NA receptors. NA receptors are grouped in three main families: alpha1-, alpha2- and beta-adrenergic receptors (α1-, α2-, β-adrenoceptors; Arnsten 1998).

It has been shown in the monkey DLPFC that during the delay period of spatial WM tasks neurons exhibit a sustained firing activity, the magnitude of which is correlated to the direction of the visual object. Sustained activity is thought to represent a "memory field" at the neuronal level (Sawaguchi and Goldman-Rakic 1991; Sawaguchi 1998). Blockade of α_2-adrenoceptors was shown to attenuate the firing activity of these neurons during the delay period of spatial WM tasks, suggesting the involvement of α_2-adrenoceptors in the modulation of cortical activity engaged in spatial WM (B.-M. Li et al. 1999; Sawaguchi 1998).

Selective blockade of β_1-adrenoceptors by local infusion of the antagonist betaxolol in the rat PrL and systemic administration of the same drug in the monkey, was shown to produce significant improvement in the WM performance in both species (Ramos et al. 2005). This evidence suggests that endogenous activation of β_1-adrenoceptors impairs PFC-mediated cognition (Ramos et al. 2005). In contrast, selective activation of β_2-adrenoceptors improved spatial WM performance in animals with WM impairments (Ramos et al. 2008).

At the cellular level, it has been shown that adrenoceptor activation alters the excitability of pyramidal cells in the hippocampus (Malenka and Nicoll 1986; Stanton and Heinemann 1986) and interneurons in the rat frontal cortex (Kawaguchi and Shindou 1998), and moreover, modulates the efficiency of synaptic inhibition (Salgado et al. 2012). Moreover, NA was shown to affect the area power of stimulus-induced gamma frequency oscillations in the olfactory bulb (Gire and Schoppa 2008) and kainate-induced gamma frequency oscillations in the hippocampus (Wójtowicz et al. 2009).

6.1.3. *Objectives*

In view of the important role DA and NA have in modulating cognitive performance we wished to investigate how these catecholamines could modulate the network activity that might underlie these cognitive functions. We have therefore assessed the effects of DA and NA on network oscillations and single cell activity in the DP and PrL regions of the mPFC.

6.2. Methods

Slices were prepared and maintained as outlined in section 2.2. Network oscillations were evoked with carbachol (10 μM) and kainate (200 nM) application. Multi-electrode and single-electrode field recordings as well extra- and intracellular recordings were obtained from the mPFC with the recording equipment described in section 2.4. The preparation and application of drugs used in the experiments described in this chapter are outlined in section 2.3.

6.2.1. *Data analysis*

- Power spectral analysis performed on field traces, area power and peak frequency extraction techniques are described in section 2.6.1.
- Filter application on field and IPSP traces is described in section 2.6.2.
- Rhythmicity index extraction from field and IPSP traces is described in section 2.6.3.
- Cross-frequency phase-synchrony analysis applied between field and IPSP traces is described in section 2.6.4.
- Spike sorting analysis applied to extracellular unit recordings is described in section 2.6.5.
- Average firing rate, firing frequency and renewal density extraction from the timestamps of extracellularly recorded units was performed with computational techniques described in section 2.6.7.
- Rhythmicity of neuronal firing activity was estimated from autocorrelation analysis described in section 2.6.8.
- The preference of firing of extracellularly recorded units in relation to the field phase angle was estimated with the unit vs. field phase-synchrony analysis described in section 2.6.9.
- Extraction of the amplitude, rise time and decay time of IPSPs was performed with a computational analysis described in section 2.7.2.
- The histological procedures we used to identify the laminar and regional position of electrodes are outlined in section 2.8.
- Statistical analysis of grouped data is described in section 2.9.

6.3. Results

6.3.1. *Regional effect of dopamine on the characteristics of fast network oscillations in the rat medial PFC assessed by multi-electrode recordings*

To investigate the regional effects of DA on fast network oscillations in the mPFC, multi-electrode recordings were obtained with a Utah array. Field activity from the cortical area of interest (i.e. DP, IL and PrL) was recorded by approximately 20 electrodes in every experiment. This allowed us to record simultaneously network oscillatory activity from various sites of the different mPFC subdivisions.

The effect of DA on network oscillations was quantified by means of rhythmicity and power spectral analysis. Area power, peak frequency and rhythmicity index values were grouped with respect to the region they were recorded from. Network oscillations in the dorsal and ventral parts of the PrL (e.g. dPrL and vPrL) and IL (e.g. dIL and vIL) cortices were modulated differently by DA, therefore data produced from these regions were grouped and analysed individually. Laminar variability within each region was negligible therefore the laminar characterization of the DA effect on network oscillations was not included in our analysis and not presented in this thesis. The number and quality of network oscillations recorded from layer 1 was small (Section 3.3.1) therefore, data obtained from this layer were not included in the analysis.

The effects of DA were tested when network oscillatory activity had reached stability. Application of DA (100 μM) to the bath solution produced a rapid and strong reduction in the area power of network oscillations in the DP region. This reduction became significant at 6 min post-DA application and remained significant until 60 min with DA. Oscillations partially recovered after 30 min in the wash-out, but overall they remained significantly reduced (Friedman One-Way RM-ANOVA on Ranks: $\chi^2(12)=118.6$, $p<0.05$; Dunn's method: ctrl vs. all; $n_{elec}=22$, $n_{slice}=7$; Figure 6.1, 6.2A,Bi; Table 6.1A). At the maximum of its suppressive effect (8 min) DA caused an area power reduction in the range of 41.4% (IQR: 52.9-26.1, $n_{elec}=22$, $n_{slice}=7$).

In the vIL region, DA (100 μM) caused a small almost linear decrease in the area power, which became significant at 60 min (DA60: 36.1% IQR: 53.5-11.2; Friedman One-Way RM-ANOVA on Ranks: $\chi^2(12)=21.3$, $p<0.05$; Dunn's method: ctrl vs. all; $n_{elec}=15$, $n_{slice}=7$; Figure 6.1, Table 6.1B). Recovery in the wash-out was partial and variable.

In the dIL region, the DA effect on network oscillations was altered. Application of DA (100 μM) initially produced an increase which reached significance at 6 min and became maximum at 12 min (DA12: 27.8% IQR: 10.3-50.3; Friedman One-Way RM-ANOVA on Ranks: $\chi^2(12)=60.8$, $p<0.05$; Dunn's method: ctrl vs. DA groups; $n_{elec}=15$, $n_{slice}=7$; Figure 6.1, Table 6.2A). Following this point, the area power gradually declined until the end of the DA application. Recovery was partial and variable in the wash-out.

One of the strongest DA effects was observed in the vPrL region. Application of DA (100 μM) induced a marked increase in the area power which reached significance at 6 min. At 8 min post-DA application the area power increase reached its maximum value (DA8: 44.5% IQR: 14.4-58.3; Friedman One-Way RM-ANOVA on Ranks: $\chi^2(12)=171.1$, $p<0.05$; Dunn's method: ctrl vs. all; $n_{elec}=26$, $n_{slice}=6$; Figure 6.1, 6.2A,Bii; Table 6.2B). From 8 min and for the rest of the DA application, area power increase was replaced by a gradual reduction. Recovery was partial and highly variable in the wash-out.

In the dPrL region, DA (100 μM) caused a small and nonsignificant area power reduction for the first 12 min post-DA application. From 12 min and for the rest of the DA application, the area power steadily decreased reaching statistical significance (Friedman One-Way RM-ANOVA on Ranks: $\chi^2(12)=154.1$, $p<0.05$; Dunn's method: ctrl vs. all; $n_{elec}=17$, $n_{slice}=5$; Figure 6.1; Table 6.3).

Overall, the effect of DA on the area power of network oscillations in the mPFC was region-dependent. In the ventral mPFC a suppressive effect was caused by DA which was more profound and consistent in the DP region. In the dorsal mPFC though, network oscillations responded to DA with an area power increase which was most consistent in the vPrL. Data analysis of the area power results suggest that the transition line between DA causing either a decrease or in increase in the power of network oscillations lies between ventral and dorsal IL.

Figure 6.1. Regional effect of DA (100 μM) on the area power of fast network oscillations in the mPFC

Table 6.1. The effect of DA (100 μM) on the area power of fast network oscillations in the DP and ventral IL regions

Table 6.2. The effect of DA (100 μM) on the area power of fast network oscillations in the dorsal IL and ventral PrL

Table 6.3. The effect of DA (100 μM) on the area power of fast network oscillations in the dorsal PrL region

Figure 6.2. Regional effect of DA (100 μM) on the area power of fast network oscillations in the DP and PrL regions

Network oscillations rely on the rhythmic entrainment of neuronal firing (Whittington et al. 1995). Therefore we wished to test whether DA exerts its significant effects on the power of network oscillations by altering the strength of network rhythmicity. To do so, we focused our analysis on the two areas with the strongest and most consistent DA effect (i.e. DP and vPrL).

In agreement with our prediction, DA (100 μM) decreased the rhythmicity of network oscillations recorded in the DP region which coincided with the area power reduction. The reduction in rhythmicity started at 4 min reaching significance at 14 min and remained significantly decreased for the rest of the DA application (Friedman One-Way RM-ANOVA on Ranks: $\chi^2(12)=61.0$, $p<0.05$; Dunn's method: ctrl vs. all; $n_{elec}=22$, $n_{slice}=7$; Figure 6.3; Table 6.4A). At 8 min post-DA application, when DA produced the maximum reduction in the area power, the rhythmicity index declined by 8.1% (IQR: 13.7-1.2%, $n_{elec}=22$, $n_{slice}=7$) in comparison to control conditions. Recovery was partial and variable in the wash-out.

In the vPrL region, DA (100 μM) produced an increase in the rhythmicity of network oscillations which became significant at 4 min and remained significant until 60 min in the presence of DA in the bath solution (Friedman One-Way RM-ANOVA on Ranks: $\chi^2(12)=116.6$, $p<0.05$; Dunn's method: ctrl vs. all; $n_{elec}=26$, $n_{slice}=6$; Figure 6.3; Table 6.4B). At 8 min with DA, when the area power increase in the network oscillations became maximal, the rhythmicity index increased by 18.7% (IQR: 11.2-30.7, $n_{elec}=26$, $n_{slice}=6$). Recovery was full in the wash-out in 3/6 slices, but was partial and highly variable in the remainder.

The effects of DA (100 μM) on the peak frequency of network oscillations were assessed in the DP and vPrL regions. Statistical analysis revealed a small but significant reduction in the peak frequency of fast network oscillations in the DP region, taking place between 4 min and 8 min post-DA application (Friedman One-Way RM-ANOVA on Ranks: $\chi^2(12)=49.7$, $p<0.05$; Dunn's method: ctrl vs. all; $n_{elec}=22$, $n_{slice}=7$; Figure 6.4A, Table 6.5A). Likewise, there was a small but significant reduction in the peak frequency of network oscillations recorded in the vPrL between 6 min and 10 min as well as at 60 min post DA-application and at 30 min in the wash-out (Friedman One-Way RM-ANOVA on Ranks: $\chi^2(12)=111.9$, $p<0.05$; Dunn's method: ctrl vs. all; $n_{elec}=$, $n_{slice}=8$; Figure 6.4B, Table 6.5B).

Figure 6.3. Regional effects of DA (100 μM) on the rhythmicity of network oscillations in the DP and PrL regions

Table 6.4. Regional effect of DA (100 µM) on the rhythmicity of fast network oscillations in the DP and vPrL regions

Figure 6.4. Regional effects of DA on the peak frequency of network oscillations in the DP and vPrL regions

Table 6.5. Regional effect of DA (100 μM) on the peak frequency of fast network oscillations in the DP and vPrL regions

Although significant, changes in the peak frequency of network oscillations in both regions were often small (in the range of 1Hz) and the functional significance of these changes is unclear.

6.3.2. *Regional effect of dopamine on the characteristics of fast network oscillations in the rat medial PFC assessed by single-electrode field recordings*

We tested the effects of DA (100 μM) on fast network oscillations recorded with single field electrodes. Similar to the multi-electrode recordings, DA application produced in the DP region an area power reduction which became significant at 6 min and remained significant until 14 min post-DA application. For the rest of the DA application the area power increased and recovered in the wash-out (Friedman One-Way RM-ANOVA on Ranks: $\chi^2(12)=65.5$, $p<0.05$; Dunn's method: ctrl vs. all; $n_{slice}=13$; Figure 6.5A; Table 6.6A). At 8 min into DA application the area power decreased by 24.2% (IQR: 28.7-20.4, $n_{slice}=13$) in comparison to control conditions.

Similar to the area power, rhythmicity of network oscillations in the DP decreased by DA (100 μM). The reduction in rhythmicity became significant at 6 min and remained reduced throughout the DA application and at 30min in the wash-out (Friedman One-Way RM-ANOVA on Ranks: $\chi^2(12)=24.8$, $p<0.05$; Dunn's method: ctrl vs. all; $n_{slice}=13$; Figure 6.5B; Table 6.7A). At 8 min post-DA application rhythmicity was reduced by 12.1% (IQR: 20.8-3.1, $n_{slice}=13$) compared to control oscillations.

Application of DA (100 μM) exerted no effect on the peak frequency of fast network oscillations (Friedman One-Way RM-ANOVA on Ranks: $\chi^2(12)=22.0$, $p<0.05$; Dunn's method: ctrl vs. all; $n_{slice}=13$; Figure 6.5C; Table 6.8A).

We next tested the effects of DA (100 μM) application on fast network oscillations in the vPrL region using single electrodes. DA was bath applied for 15 min (this was adequate time for DA to alter the oscillation characteristics) followed by the wash-out (30 min). Network oscillations in the vPrL responded to DA with an area power increase which became significant at 4 min to 8 min post-drug application (Friedman One-Way RM-ANOVA on Ranks: $\chi^2(9)=37.7$, $p<0.05$; Dunn's method: ctrl vs. all; $n_{slice}=10$; Figure 6.6A; Table 6.6B). At 8 min with DA there was a 24.4% (IQR: 15.1-36.4,) area power increase in comparison to control conditions.

Figure 6.5. The effect of DA (100 μM) on the spectral and rhythmicity characteristics of fast network oscillations in the DP region recorded with single field electrodes

Figure 6.6. The effect of DA (100 μM) on the spectral and rhythmicity characteristics of fast network oscillations in the vPrL region recorded with single field electrodes

Table 6.6. The effect of DA (100 μM) on the area power of fast network oscillations in the DP and vPrL regions recorded with single field electrodes

Table 6.7. The effect of DA (100 μM) on the rhythmicity of fast network oscillations in the DP and vPrL regions recorded with single field electrodes

Table 6.8. The effect of DA (100 μM) on the frequency of fast network oscillations in the DP and vPrL regions recorded with single field electrodes

As expected, DA (100 μM) produced a significant increase in the rhythmicity of fast network oscillations (Friedman One-Way RM-ANOVA on Ranks: $\chi^2(9)=19.3$, $p<0.05$; Dunn's method: ctrl vs. all; $n_{slice}=10$; Figure 6.6B; Table 6.7B). At 8 min post-DA application the rhythmicity index increased by 11.6% (IQR: 4.7-20.4, $n_{slice}=10$) compared to control conditions.

Peak frequency declined slightly but significantly at 6 min and 10 min in the presence of DA (One-Way RM-ANOVA: $F(9,79)=5.1$, $p<0.05$; Dunn's method: ctrl vs. all; $n_{slice}=10$; Figure 6.6C; Table 6.8B).

Together, results presented in sections 6.3.1 and 6.3.2 show that both multi- and single-electrode field recordings confirm that DA (100 μM) produces two opposing effects on fast network oscillations in the DP and vPrL subdivisions of the mPFC. Overall, DA decreases the area power and rhythmicity of network oscillations in the DP region. Instead oscillations in the vPrL responded to DA application with a significant increase in area power and rhythmicity. In both regions, peak frequency remains largely unchanged.

6.3.2.1. Concentration-dependent effects of DA on network oscillations in the mPFC

There are numerous published *in vitro* studies investigating the modulation of neuronal and network activity by DA in which a wide variety of DA concentrations have been used. To investigate whether lower concentrations of DA application could elicit alterations in fast network oscillations recorded in the mPFC we carried out a series of single-electrode field experiments applying 10 μM and 50 μM concentrations of DA. DA application lasted for 15 min followed by 30 min of wash-out.

Application of DA (10 μM) failed to produce any significant effect on either the area power (ctrl: 1756 $\mu V^2.Hz^{-1}$ IQR: 1063-1918; DA-10min: 1565 $\mu V^2.Hz^{-1}$ IQR: 1053-1877; DA-15min: 1643 $\mu V^2.Hz^{-1}$ IQR: 1061-1962; wash: 1748 $\mu V^2.Hz^{-1}$ IQR: 1317-2025, $n_{slice}=7$, Friedman One-Way RM-ANOVA on Ranks: $\chi^2(3)=5.3$, $p>0.05$) or the peak frequency (ctrl: 25.5 Hz IQR: 24.7-27.2; DA-10min: 25.6 Hz IQR: 24.2-26.9; DA-15min: 25.1 Hz IQR: 24.2-26.2; wash: 24.7 HZ IQR: 24.3-26.1, $n_{slice}=7$,

Friedman One-Way RM-ANOVA on Ranks: $\chi^2(3)=6.1$, $p>0.05$) of network oscillations in the DP region.

Likewise, both area power (ctrl: 321.5 ± 97.4 $\mu V^2.Hz^{-1}$; DA-10min: 310.0 ± 41.3 $\mu V^2.Hz^{-1}$; DA-15min: 299.5 ± 39.4 $\mu V^2.Hz^{-1}$; wash: 361.3 ± 69.7 $\mu V^2.Hz^{-1}$, $n_{slice}=4$, One-Way RM-ANOVA: $F(3,9)=0.8$, $p>0.05$) and peak frequency (ctrl: 27.2 Hz IQR: 25.6-29.1; DA-10min: 28.4 Hz IQR: 25.4-30.6; DA-15min: 25.7 Hz IQR: 22.7-28.8; wash: 27.9 Hz IQR: 25.4-29.3, $n_{slice}=4$, Friedman One-Way RM-ANOVA on Ranks: $\chi^2(3)=0.6$, $p>0.05$) of fast network oscillations in the vPrL region remained unaffected by DA (10 µM) application.

Bath application with DA (50 µM) reduced significantly by 21.3% (±21.2, $n_{slice}=7$) the area power of network oscillations in the DP region (ctrl: 1041$\mu V^2.Hz^{-1}$ IQR: 315-2743; DA-10min: 459 $\mu V^2.Hz^{-1}$ IQR: 255-2395; DA-15min: 530 $\mu V^2.Hz^{-1}$ IQR: 298-2649; wash: 1055 $\mu V^2.Hz^{-1}$ IQR: 368-3278, $n_{slice}=7$, Friedman One-Way RM-ANOVA on Ranks: $\chi^2(3)=35.2$, $p<0.05$; Dunn's method: ctrl vs. all). There was no effect on the peak frequency though (ctrl: 26.3 ± 1.2 Hz; DA-10min: 26.3 ± 1.1 Hz; DA-15min: 25.6 ± 0.5 Hz; wash: 27.0 ± 1.6 Hz, $n_{slice}=7$, One-Way RM-ANOVA: $F(3,18)=1.5$, $p>0.05$).

In the vPrL region, DA (50 µM) failed to produce any significant effect on either the area power (ctrl: 190 ± 122 $\mu V^2.Hz^{-1}$; DA-10min: 191 ± 170 $\mu V^2.Hz^{-1}$; DA-15min: 155 ± 140 $\mu V^2.Hz^{-1}$; wash: 232 ± 104 $\mu V^2.Hz^{-1}$, $n_{slice}=6$, One-Way RM-ANOVA: $F(3,15)=0.6$, $p>0.05$) or the peak frequency (ctrl: 26.3 ± 1.3 Hz; DA-10min: 25.7 ± 1.5 Hz; DA-15min: 25.5 ± 1.3 Hz; wash: 26.9 ± 1.1 Hz, $n_{slice}=6$, One-Way RM-ANOVA: $F(3,15)=1.3$, $p>0.05$) of fast network oscillations.

Overall, these results demonstrate that the significant regional effects of DA on the characteristics of fast network oscillations in the mPFC are concentration-dependent. Moreover, oscillations recorded in the DP region are more sensitive to DA than oscillations recorded in the vPrL region, as 50 µM caused a significant effect only in the DP region.

6.3.3. *The effect of dopamine on the firing activity of extracellularly recorded units in the medial PFC*

Having shown the regional differences in the effects of DA (100 μM) on the spectral, rhythmicity and synchrony profile of fast network oscillations in the rat mPFC, we wished to investigate whether DA mediated different effects on the firing activity of distinct neuronal populations in the DP and vPrL subdivisions. The results obtained from this analysis could provide us with an important insight into the neuronal populations being targeted by DA. Due to the large number of samples, along with their clear, distinctive firing properties which resemble those of the RS cells, our analysis was mainly focused on the FR units.

6.3.3.1. The effect of dopamine on the firing activity of FR units in the DP region

Application of DA (100 μM) produced a strong reduction in the average firing rate of FR units in the DP region, which partially recovered at 30 min in the washout. However, the difference between the values of the different groups was not strong enough to reach statistical significance (ctrl: 11.0 ± 4.8 spikes.sec^{-1}, DA-8min: 7.5 ± 2.8 spikes.sec^{-1}, wash: 8.9 ± 6.1 spikes.sec^{-1}, n=8, One-Way RM-ANOVA: $F(2,14)=2.1$, $p>0.05$; Figure 6.7, 6.8A).

Application of DA (100 μM) caused a reduction in the firing frequency of FR units. Recovery was full in the wash-out however significance was not reached between any of the different groups (ctrl: 27.0 ± 6.2 Hz, DA-8min: 23.4 ± 7.5 Hz, wash: 26.0 ± 11.9 Hz, n=8, One-Way RM-ANOVA: $F(2,14)=0.3$, $p>0.05$; Figure 6.7, 6.8B).

However, a significant reduction was caused by DA (100 μM) in the renewal density values. Recovery in the wash-out was partial (ctrl: 11.8 ± 10.1 spikes.sec^{-1}, DA-8min: 8.1 ± 7.1 spikes.sec^{-1}, wash: 8.6 ± 5.6 spikes.sec^{-1}, n=8, One-Way RM-ANOVA on Ranks: $F(2,14)=9.8$, $p<0.05$; Tukey test: all comparisons; Figure 6.7, 6.8C).

Figure 6.7. Application of DA (100 μM) decreases the excitability of FR units in the DP region

Figure 6.8. Application of DA (100 μM) decreases the firing rate, frequency and renewal density of FR units in the DP region

Figure 6.9. Application of DA (100 μM) decreases the firing rhythmicity of FR units in the DP region

Figure 6.10. Application of DA (100 μM) reduces unit vs. field phase-synchrony of FR units in the DP region

Figure 6.11. Application of DA (100 μM) reduces the firing rhythmicity and unit vs. field phase-synchrony of FR units in the DP region

A strong and significant reduction was produced by DA (100 μM) on the firing rhythmicity of FR units in the DP region. There was a partial recovery in the wash-out (ctrl: 0.14 ± 0.06, DA-8min: 0.08 ± 0.75, wash: 0.09 ± 0.07, n=8, Friedman One-Way RM-ANOVA: $F(2,14)=3.5, p<0.05$; Tukey test: all comparisons; Figure 6.9, 6.11A).

No significant effect was caused by DA (100 μM) on the spike timing of these units with respect to the field potential phase (ctrl: 210.0 ± 34.5°, DA-8min: 204.4 ± 39.5°, wash: 211.9 ± 44.2°, n=8, One-Way RM-ANOVA: $F(2,14)=0.7$, $p>0.05$; Figure 6.10, 6.11B). However, DA exerted a significant but reversible reduction on the phase-synchrony (ctrl: 0.12 ± 0.06, DA-8min: 0.07 ± 0.06, wash: 0.10 ± 0.05, n=8, $p<0.05$, One-Way RM-ANOVA: $F(2,14)=8,4$, $p<0.05$, Tukey test: all comparisons; Figure 6.10, 6.11C).

6.3.3.2. The effect of dopamine on the firing activity of FR units in the ventral PrL region

A strong but nonsignificant increase was produced by DA (100 μM) in the average firing rate of FR units in the vPrL region. However, this increase was followed by a marked reduction in the wash-out. As a result, the difference in the average rate values between the DA and wash-out treatment groups was significant (ctrl: 6.1 ± 2.3 spikes.sec^{-1}, DA-8min: 7.5 ± 3.5 spikes.sec^{-1}, wash: 4.8 ± 2.9 spikes.sec^{-1}, n=10, One-Way RM-ANOVA: $F(2,18)=4.2$, $p<0.05$, Tukey test: all comparisons; Figure 6.12, 6.13A).

DA also caused a strong increase in the firing frequency, which became reduced in the wash-out. Due to the high variability in the wash-out values, statistical significance was not reached (ctrl: 13.0 IQR: 9.0-24.0 Hz, DA-8min: 18.0 IQR: 13.0-24.0 Hz, wash: 7.0 IQR: 5.0-24.0 Hz, n=10, Friedman One-Way RM-ANOVA on Ranks: $\chi^2(2)=2.7$, $p>0.05$; Figure 6.12, 6.13B).

A significant increase in the renewal density of the firing activity of FR units, was replaced by a significant reduction in the wash-out (ctrl: 7.0 ± 2.8 spikes.sec^{-1}, DA-8min: 10.6 ± 4.4 spikes.sec^{-1}, wash: 5.4 ± 2.9 spikes.sec^{-1}, n=10, One-Way RM-ANOVA: $F(2,18)=8.8$, $p<0.05$; Tukey test: all comparisons; Figure 6.12, 6.13C).

Similar to the renewal density a very strong and significant increase was induced by DA in the rhythmicity of firing activity. The increase was fully recovered in the wash-out (ctrl: 0.08 ± 0.05, DA-8min: 0.21 ± 0.06, wash: 0.04 ± 0.07, n=10, One-Way RM-ANOVA: $F(2,18)=17.9$, $p<0.05$; Tukey test: all comparisons; Figure 6.14, 6.16A).

DA did not produce any significant change in the spike timing with respect to the field potential phase. However, a significant shift towards larger degrees was observed in the wash-out (ctrl: 178.5 ± 13.9°, DA-8min: 170.5 ± 7.9°, wash: 199.0 ± 16.9°, n=10, One-Way RM-ANOVA: $F(2,18)=13.4$, $p<0.05$, Tukey test: all comparisons; Figure 6.15, 6.16B).

Finally, phase-synchrony was slightly increased by DA but significantly decreased in the wash-out (ctrl: 0.01 ± 0.03, DA-8min: 0.12 ± 0.03, wash: 0.08 ± 0.04, n=10, One-Way RM-ANOVA: $F(2,18)=8.2$, $p<0.05$, Tukey test: all comparisons; Figure 6.15, 6.16C).

In summary, a DA-induced significant reduction in the area power, rhythmicity and synchrony of network oscillations in the DP region coincided with a marked reduction in the excitability (i.e. reduction in the average firing rate, interspike interval frequency, renewal density and firing rhythmicity) and phase-synchrony of the FR units. In contrast, a DA-induced significant increase in the area power, rhythmicity and synchrony of fast network oscillations in the vPrL region, coincided with a marked increase in the excitability and phase-synchrony of the FR units.

Figure 6.12. Application of DA (100 µM) increases the excitability of FR units in the vPrL region

Figure 6.13. Application of DA (100 μM) increases the firing rate, frequency and renewal density of FR units in the vPrL region

Figure 6.14. Application of DA (100 μM) increases the firing rhythmicity of FR units in the vPrL region

Figure 6.15. Application of DA (100 µM) increases the unit vs. field phase-synchrony of FR units in the vPrL region

Figure 6.16. Application of DA (100 μM) increases the firing rhythmicity and unit vs. field phase-synchrony of FR units in the vPrL region

6.3.3.3. The effect of dopamine on the firing activity of the other types of units

Unfortunately, it proved difficult to assess the effects of DA on the firing properties of the other types of units. This was mainly due to the small number of recorded units in the DP and vPrL regions. We may conclude though from the small number of samples we studied, that the effect of DA (100 μM) on any of the SR (n_{DP}=1), SNR (n_{DP}=2, n_{vPrL}=4), FNR (n_{DP}=2, n_{vPrL}=1), and FS (n_{vPrL}=2) unit types was rather variable producing either increases or decreases or no effects at all on the excitability and rhythmicity of their firing activity. We have however isolated two interesting cases of SNR units recorded in the vPrL region. During control oscillations these units displayed the firing properties of typical SNR units. However, in the presence of DA (100 μM / 8 min), their firing activity became similar to the one of FR units, in that excitability, rhythmicity and phase-synchrony were increased (Figure 6.17, 6.18, 6.19). Transformation in the firing properties of these cells coincided with an increase in the oscillations strength.

6.3.4. *The effects of dopamine on synaptic inhibition in the presence of network oscillations in the DP region*

We recorded the IPSP trains from RS cells (n=7) in the DP region (layers 5 and 6a) in order to assess the effects of DA application on synaptic inhibition.

Similar to its effect on the amplitude of network oscillations, application of DA (100 μM) produced a significant reduction in the amplitude of IPSP trains recorded at 10 min, which recovered partially in the wash-out (ctrl: 9.5 ± 3.8 mV, DA-8min: 6.7 ± 2.0 mV, wash: 7.6 ± 3.1 mV, n=7, One-Way RM-ANOVA: $F(2,16)=12.1$ $p<0.05$; Tukey test: all comparisons; Figure 6.20, 6.21).

The rise-time of IPSPs did not vary significantly following DA (100 μM) application (ctrl: 6.4 IQR: 5.8-6.6 msec, DA-8min: 6.2 IQR: 5.6-6.7 msec, wash: 6.60 IQR: 5.4-6.6 msec, n=7, Friedman One-Way RM-ANOVA on Ranks: $\chi^2(2)=0.8$, $p>0.05$; Figure 6.22).

However, DA (100 μM) caused a significant reduction on the decay-time of IPSP trains which did not recover in the wash-out (ctrl: 10.6 IQR: 9.2-11.7 msec, DA-8min: 9.0 IQR: 8.0-10.4 msec, wash: 9.2 IQR: 8.4-10.3 msec, n=7, Friedman One-Way RM-ANOVA on Ranks: $\chi^2(2)=10.7$, $p<0.05$; Tukey test: all comparisons; Figure 6.23).

We also tested the effect of DA on the rhythmicity of IPSP trains. Application of DA (100 μM) produced a significant but reversible reduction in the rhythmicity of IPSPs (ctrl: 0.59 ± 0.17, DA-8min: 0.44 ± 0.19, wash: 0.54 ± 0.16, n=7, One-Way RM-ANOVA: $F(2,12)=5.6$, $p<0.05$; Tukey test: all comparisons; Figure 6.24). We expected the rhythmicity of network oscillations to be strongly correlated to the rhythmicity of IPSP trains. Therefore, we examined the effect of DA on the rhythmicity of the network oscillations recorded simultaneously with the IPSP trains. Similar to its effect on IPSP trains, DA (100μM) caused a significant reduction in the rhythmicity of field oscillations, which recovered in the wash-out (ctrl: 0.61 ± 0.16, DA-8min: 0.50 ± 0.17, wash: 0.58 ± 0.17, n=7, One-Way RM-ANOVA: $F(2,12)=7.1$, $p<0.05$; Tukey test: all comparisons).

We then went on to examine the effect of DA (100 μM) on the phase-synchrony between IPSP trains and network oscillations recorded simultaneously from the same layer 5 or 6a. Shannon-entropy values revealed a significant phase-synchrony reduction in the presence of DA which recovered in the wash-out (ctrl: 0.31 ± 0.05, DA-8min: 0.25 ± 0.05, wash: 0.30 ± 0.06, n=7, One-Way RM-ANOVA: $F(2,12)=7.1$, $p<0.05$; Tukey test: all comparisons; Figure 6.25).

In summary, a DA-mediated reduction in the strength and rhythmicity of fast network oscillations in the DP region coincides with a reduction in the amplitude, decay time, rhythmicity and phase-synchrony of the trains of IPSPs.

Figure 6.17. Application of DA (100 μM) increases the excitability of SNR units in the vPrL region

Figure 6.18. Application of DA (100 μM) increases the firing rhythmicity of SNR units in the vPrL region

Figure 6.19. Application of DA (100 μM) increases the unit vs. field phase-synchrony of SNR units in the vPrL region

Figure 6.20. The effect of DA (100 μM) on the amplitude of IPSPs recorded from pyramidal cells in the DP region

Figure 6.21. Application of DA (100 μM) decreases the amplitude of IPSPs recorded from pyramidal cells in the DP region

Figure 6.22. Application of DA (100 μM) does not affect the rise-time of IPSP trains recorded from pyramidal cells in the DP region

Figure 6.23. Application of DA (100 μM) decreases the decay-time of IPSP trains recorded from pyramidal cells in the DP region

Figure 6.24. Application of DA (100 μM) reduces the rhythmicity of IPSP trains recorded from pyramidal cells in the DP region

Figure 6.25. Application of DA (100 μM) reduces the IPSP vs. LFP phase-synchrony in the DP region

6.3.5. *Contribution of the D_1 receptor subtype to the dopamine-mediated effect on fast network oscillations in the mPFC*

Having identified the regional effect of DA on the spectral and rhythmicity characteristics of network oscillations in the mPFC and assessed the effects of this catecholamine on the neuronal firing and synaptic inhibition, we then proceeded to investigate the contribution of specific DA receptors to the DA-mediated effect. The effects of selective activation or blockade of DA receptors were assessed in the DP and vPrL, because the DA effect in these regions was the strongest and most consistent.

Application of the D_1 receptor agonist SKF38393 (10 µM; SKF) failed to induce any significant effects in the DP region on either the area power (ctrl: 803 $\mu V^2.Hz^{-1}$ IQR: 426-992; SKF-5min: 836 $\mu V^2.Hz^{-1}$ IQR: 395-999; SKF-10min: 834 $\mu V^2.Hz^{-1}$ IQR: 406-986; SKF-15min: 778 $\mu V^2.Hz^{-1}$ IQR: 395-983, n_{slice}=8, Friedman One-Way RM-ANOVA on Ranks: $\chi^2(3)=1.9$, $p>0.05$; Figure 6.26A,B,C) or the peak frequency (ctrl: 25.8 Hz IQR: 25.1-27.7; SKF-5min: 26.3 Hz IQR: 25.4-27.5; SKF-10min: 25.8 Hz IQR: 25.4-27.5; SKF-15min: 25.5 Hz IQR: 22.4-26.9, n_{slice}=8, Friedman One-Way RM-ANOVA on Ranks: $\chi^2(3)=1.4$, $p>0.05$; Figure 6.26A,B,D) of fast network oscillations. We also assessed the effect of SKF38393 (20 µM) application for 45 min (n=4) in the DP region but we did not observe any significant effect on either area power or frequency of network oscillations (data not shown).

Although SKF38393 (10 µM) failed to mimic the DA-mediated reduction on network oscillations recorded in the DP region, we wished to further investigate the contribution of D_1 receptor subtypes employing the D_1 receptor antagonist SCH23390. In the presence of SCH23390 (10 µM; SCH) for 30 min, network oscillations in the DP region remained stable suggesting that that endogenous activation of D1 receptors does not modulate oscillatory activity. However, in the presence of the D1 antagonist, DA (100 µM) evoked a significant reduction in the area power of network oscillations by 76.3% (± 22.1, n_{slice}=4). Reduction was almost three times greater (24.4% IQR: 15.1-36.4, n_{slice}=10) in comparison to the area power reduction observed with DA (100 µM) application (ctrl: 1055 ± 496 $\mu V^2.Hz^{-1}$, SCH=30min: SCH-30min: 1106 ± 681 $\mu V^2.Hz^{-1}$; DA-10min: 200 ± 100 $\mu V^2.Hz^{-1}$, n_{slice}=4, One-Way RM-ANOVA: $F(2,6)=14.9$, $p<0.05$; Tukey test: all comparisons; Figure 6.27A,B,C).

Figure 6.26. D_1 receptor activation failed to mimic the DA-mediated reduction in the area power of fast network oscillations in the DP region

Figure 6.27. D_1 receptor blockade failed to prevent the DA-mediated reduction in the area power of fast network oscillations in the DP region

Figure 6.28. D_1 receptor activation failed to mimic the DA-mediated increase in the area power of fast network oscillations in the vPrL region

No significant change was observed in the peak frequency during the course of both drug treatments (ctrl: 24.3 ± 1.7 Hz; SCH-30min: 25.1 ± 0.7 Hz; DA-10min: 26.3 ± 1.8 Hz, n_{slice}=4, One-Way RM-ANOVA: $F(2,6)=1.5$, $p>0.05$; Figure 6.27A,B,D). These results suggest DA may be exerting opposing effects by activating different receptors such that activation of D1 receptors alone does not enhance the oscillation, but when blocked the reduction evoked by DA is now significantly larger.

We also assessed the contribution of D_1 receptors to the DA-mediated area power increase in oscillations in the vPrL region. Application of the D_1 agonist, SKF38393 (10 µM; SKF) did not produce any significant effect on either the area power (ctrl: 215 $\mu V^2.Hz^{-1}$ IQR: 125-609; SKF-5min: 236 $\mu V^2.Hz^{-1}$ IQR: 119-660; SKF-10min: 231 $\mu V^2.Hz^{-1}$ IQR: 134-607; SKF-15min: 238 $\mu V^2.Hz^{-1}$ IQR: 127-688, n_{slice}=4, Friedman One-Way RM-ANOVA on Ranks: $\chi^2(3)=1.7$, $p>0.05$; Figure 6.28A,B,C) or the peak frequency (ctrl: 28.9 ± 2.5 Hz; SKF-5min: 28.7 ± 2.4 Hz; SKF-10min: 29.5 ± 3.4 Hz; SKF-15min: 29.0 ± 2.5 Hz, n_{slice}=4, One-Way RM-ANOVA: $F(3,9)=1.4$, $p>0.05$; Figure 6.28A,B,D) of fast network oscillations in the vPrL region. Together, results indicate that D_1 receptors did not contribute to the DA-mediated effect on fast network oscillations in the vPrL.

6.3.6. *Contribution of the D_2 receptor subtype to the DA-mediated effect on fast network oscillations in the mPFC*

We investigated the contribution of the D_2 receptors on the DA-mediated reduction in the area power of network oscillations recorded in the DP region. Application of the D_2 receptor agonist quinpirole (10 µM; Quin) failed to mimic the DA-mediated area power reduction. Both the area power (ctrl: 1190$\mu V^2.Hz^{-1}$ IQR: 889-2461; Quin-5min: 1239$\mu V^2.Hz^{-1}$ IQR: 885-2774; Quin-10min: 1308$\mu V^2.Hz^{-1}$ IQR: 864-2805; Quin-15min: 1338$\mu V^2.Hz^{-1}$ IQR: 816-2842, n_{slice}=5; Friedman One-Way RM-ANOVA on Ranks: $\chi^2(3)=1.1$, $p>0.05$; Figure 6.29A,B,C) and peak frequency (ctrl: 26.3 ± 2.1 Hz; Quin-5min: 25.9 ± 2.1 Hz; Quin-10min: 25.7 ± 2.4 Hz; Quin-15min: 25.9 ± 2.1 Hz, n_{slice}=5; One-Way RM-ANOVA: $F(3,12)=1.2$, $p>0.05$; Figure 6.29A,B,D) remained stable during the course of the quinpirole application.

Figure 6.29. D_2 receptor activation fails to mimic the DA-mediated reduction in the area power of fast network oscillations in the DP region

Figure 6.30. D_2 receptor activation fails to mimic the DA-mediated increase in the area power of fast network oscillations in the vPrL region

Similar results were obtained in the vPrL region where quinpirole (10 μM) application failed to produce any significant effects on either the area power (ctrl: 932 ± 695 $\mu V^2.Hz^{-1}$; Quin-5min: 945 ± 739 $\mu V^2.Hz^{-1}$; Quin-10min: 985 ± 691 $\mu V^2.Hz^{-1}$; Quin-15min: 1025 ± 725 $\mu V^2.Hz^{-1}$, n_{slice}=4; One-Way RM-ANOVA: $F(3,9)=3.0$, $p>0.05$; Figure 6.30A,B,C) or peak frequency (ctrl: 25.2 Hz ±2.4; Quin-5min: 24.9 Hz ±2.9; Quin-10min: 25.3 Hz ±2.4; Quin-15min: 24.7 Hz ±2.9, n_{slice}=4; One-Way RM-ANOVA: $F(3,9)=0.5$, $p>0.05$; Figure 6.30A,B,D) of fast network oscillations.

We also investigated the effect of quinpirole (10-20 μM) application for 45 min in both cortical regions, but we did not observe any significant effect on either the area power or frequency of fast network oscillations (data not shown).

Together, these results indicate that activation of D_2 receptors alone did not mimic the DA-mediated regional effect on fast network oscillations in the mPFC.

6.3.7. *Contribution of the D_4 receptor subtype to the DA-mediated effect on fast network oscillations in the mPFC*

We also assessed the contribution of D_4 receptors to the DA-mediated reduction of the oscillation strength in the DP region. Bath application with the D_4 receptor agonist, PD168077 (10 μM; PD) did not elicit any significant change on the area power (ctrl: 2154 IQR: 611-3984 $\mu V^2.Hz^{-1}$; PD-5min: 2314 IQR: 656-3329 $\mu V^2.Hz^{-1}$; PD-10min: 2312 IQR: 810-3484 $\mu V^2.Hz^{-1}$; PD-15min: 2408 IQR: 876-3379 $\mu V^2.Hz^{-1}$, n_{slice}=6; Friedman One-Way RM-ANOVA on Ranks: $\chi^2(3)=5.2$, $p>0.05$; Figure 6.31A,B,C) or peak frequency (ctrl: 27.5Hz ±3.7; PD-5min: 27.4Hz ±3.7; PD-10min: 27.2Hz ±3.5; PD-15min: 27.3Hz ±2.9, n_{slice}=6; One-Way RM-ANOVA: $F(3,15)=0.3$, $p>0.05$; Figure 6.31A,B,D) of network oscillations in the DP region.

Likewise both the area power (ctrl: 698 ± 267 $\mu V^2.Hz^{-1}$; PD-5min: 676 ± 213 $\mu V^2.Hz^{-1}$; PD-10min: 699 ± 162 $\mu V^2.Hz^{-1}$; PD-15min: 724 ± 94 $\mu V^2.Hz^{-1}$, n_{slice}=5; One-Way RM-ANOVA: $F(3,12)=0.2$, $p>0.05$; Figure 6.32A,B,C) and peak frequency (ctrl: 26.5 ± 2.4 Hz; PD-5min: 26.1 ± 2.5 Hz; PD-10min: 25.7 ± 1.9 Hz; PD-15min: 26.2 ± 2.7 Hz, n_{slice}=5; One-Way RM-ANOVA: $F(3,12)=1.7$, $p>0.05$; Figure 6.32A,B,D) of network oscillations in the vPrL region remained stable in the presence of PD168077 (10 μM).

Figure 6.31. D_4 receptor activation fails to mimic the DA-mediated reduction in the area power of fast network oscillations in the DP region

Figure 6.32. D_4 receptor activation fails to mimic the DA-mediated increase in the area power of fast network oscillations in the vPrL region

Results from the application of the D_4 receptor agonist, PD168077 (10 μM) suggest that activation of D4 receptors alone does not mimic the DA-mediated regional effect on fast network oscillations in the mPFC. Together, these results show that activation of D_1, D_2 and D_4 alone could not reproduce the effects seen with DA on either the DP or PrL regions. The possible reasons for this will be considered in section 6.4.

6.3.8. *The effects of noradrenaline on fast network oscillations in the mPFC*

We subsequently went on to assess whether NA could also modulate network oscillations in the mPFC and whether this modulation differed from that described above for DA. Application of NA (30 μM) caused a reduction in the oscillation strength in the DP region which became significant at 10 min and remained decreased throughout the 20 min course of the NA application. Oscillations recovered after 30 min in the washout (ctrl: 1567 $\mu V^2.Hz^{-1}$ IQR: 952-3232; NA-5min: 1323 $\mu V^2.Hz^{-1}$ IQR: 735-1587; NA-10min: 970 $\mu V^2.Hz^{-1}$ IQR: 489-1498; NA-15min: 745 $\mu V^2.Hz^{-1}$ IQR: 476-1140; NA-20min: 768 $\mu V^2.Hz^{-1}$ IQR: 447-1307; wash: 1375 $\mu V^2.Hz^{-1}$ IQR: 935-2269, n_{slice}=13; Friedman One-Way RM-ANOVA: $\chi^2(5)=34.9$, $p<0.05$; Dunn's method: ctrl vs. all; Figure 6.33A,B,C). These results show that, similar to DA, NA also caused a reduction in oscillations in the DP region. At 10 min post-NA application the area power decreased by 37.8% (IQR: 61.4-6.8, n_{slice}=13) compared to control oscillations. Peak frequency was not significantly affected by NA application (ctrl: 25.8 ± 2.0 Hz; NA-5min: 27.7 ± 2.0 Hz; NA-10min: 26.7 ± 2.1 Hz; NA-15min: 27.1 ± 2.5 Hz; NA-20min: 27.1 ± 2.7 Hz; wash: 25.7 ± 2.2 Hz, n_{slice}=13; One-way RM-ANOVA: $F(5,60)=2.7$, $p>0.05$; Figure 6.33A,B,D).

In contrast to the effects of DA, application of NA (30 μM) in the vPrL did not produce any significant effect on the area power of network oscillations (ctrl: 293 $\mu V^2.Hz^{-1}$ IQR: 227-555; NA-5min: 408 $\mu V^2.Hz^{-1}$ IQR: 247-498; NA-10min: 314 $\mu V^2.Hz^{-1}$ IQR: 235-572; NA-15min: 269 $\mu V^2.Hz^{-1}$ IQR: 197-580; NA-20min: 244 $\mu V^2.Hz^{-1}$ IQR: 149-634; wash: 304 $\mu V^2.Hz^{-1}$ IQR: 278-479, n_{slice}=10; Friedman One-Way RM-ANOVA: $\chi^2(5)=3.8$, $p>0.05$; Figure 6.34A,B,C).

Figure 6.33. Noradrenaline reduces the area power of network oscillations in the DP region

Figure 6.34. Noradrenaline has no effect on fast network oscillations in the vPrL region

Likewise, peak frequency remained unchanged in the presence of NA (ctrl: 27.2 ± 2.5 Hz; NA-5min: 27.4 ± 2.5 Hz; NA-10min: 27.7 ± 2.8 Hz; NA-15min: 28.1 ± 2.3 Hz; NA-20min: 28.1 ± 2.1 Hz; wash: 27.2 ± 2.6 Hz, n_{slice}=10; One-Way RM-ANOVA: $F(5,43)=1.5$, $p>0.05$; Figure 6.34A,B,D).

Results obtained from the NA (30 μM) application indicate that although this catecholamine produces a strong but reversible reduction in the area power of network oscillations in the DP region, however, it does not exert any effect on the oscillation strength of network activity in the vPrL region. In both regions NA application did not elicit any effect on the peak frequency of oscillations.

6.3.9. *Contribution of the β-adrenoceptor to the noradrenaline-mediated effects on network oscillations in the mPFC*

The NA-mediated reduction in the strength of network oscillations in the DP region could be functionally correlated to a reduction in the efficiency of information processing (Section 1.3). Activation of β-adrenoceptors has been implicated in WM impairment (Ramos et al. 2005). Therefore, we tested whether the NA-mediated suppressive effect in the DP region is mediated by β-adrenoceptors. Isoprenaline (1 μM; ISO), a non-selective β-adrenoceptor agonist was applied to the normal-ACSF solution, but failed to produce any significant effect on either the area power (ctrl: 1602 $\mu V^2.Hz^{-1}$ IQR: 925-5204; ISO-5min: 2256 $\mu V^2.Hz^{-1}$ IQR: 1227-5906; ISO-10min: 1841 $\mu V^2.Hz^{-1}$ IQR: 1119-4758; ISO-15min: 1574 $\mu V^2.Hz^{-1}$ IQR: 1231-3654; wash: 2802 $\mu V^2.Hz^{-1}$ IQR: 1726-3487, n_{slice}=7; Friedman One-Way RM-ANOVA on Ranks: $\chi^2(4)=5.6$, $p>0.05$; Figure 6.35A,B,C) or the peak frequency (ctrl: 25.3 ± 3.1 Hz; ISO-5min: 25.6 ±3.3 Hz; ISO-10min: 25.7 ± 3.8 Hz; ISO-15min: 26.3 ± 2.9 Hz; wash: 25.7 ± 3.5 Hz, n_{slice}=10; One-Way RM-ANOVA: $F(4,24)=1.0$, $p>0.05$; Figure 6.35A,B,D) of network oscillations in this region.

We then assessed the effects of β_1-adrenoceptor blockade on the NA-mediated suppressive effect in the DP region. Application of betaxolol (30 μM; BTX) for 30 min failed to elicit any significant change in the area power of network oscillations suggesting that activation of the β_1-adrenoceptor by endogenous NA release does not contribute to the modulation of network oscillations in this mPFC area.

However, when betaxolol was co-applied with NA (30 μM) a fast and strong reduction was observed which became significant at 10 min post-NA application and remained reduced for the rest of the 20 min-course application. Oscillations were fully recovered at 30 min in the wash-out (ctrl: 3651 ± 2299 $\mu V^2.Hz^{-1}$; BTX-30min: 3653 ± 2141 $\mu V^2.Hz^{-1}$; NA-5min: 2031 ± 1326 $\mu V^2.Hz^{-1}$; NA-10min: 1360 ± 753 $\mu V^2.Hz^{-1}$; NA-15min: 1419 ± 716 $\mu V^2.Hz^{-1}$; NA-20min: 1347 ± 683 $\mu V^2.Hz^{-1}$; wash: 3929 ± 2606 $\mu V^2.Hz^{-1}$, n_{slice}=6,One-Way RM-ANOVA: $F(6,30)=7.8$, $p<0.05$; Tukey test: all comparisons; Figure 6.36A,B,C). Similar to the effects of D_1 receptor blockade by SCH23390, the presence of the β_1-adrenoceptor antagonist resulted in a larger decrease in the area power of network oscillations. At 10min of the co-application, the area power of oscillations decreased by 66.7% (IQR: 71.8-52.1, n_{slice}=6). This is almost a two-fold reduction in comparison to the area power reduction observed with NA (30 μM) application alone which was 37.8% (IQR: 61.4-6.8, n_{slice}=13).

There was no significant effect on the peak frequency of network oscillations during application of either betaxolol or betaxolol/NA (ctrl: 24.7 Hz IQR: 23.9-27.8; BTX-30min: 24.7 Hz IQR: 23.2-28.5; NA-5min: 24.1 Hz IQR: 22.3-28.8; NA-10min: 24.4 Hz IQR: 22.6-28.4; NA-15min: 24.4 Hz IQR: 22.8-30.2; NA-20min: 25.6 Hz IQR: 23.0-30.2; wash: 25.2 Hz IQR: 22.6-27.7, n_{slice}=6; Friedman One-Way RM-ANOVA on Ranks: $\chi^2(6)=6.7$, $p>0.05$; Figure 6.36A,B,D).

We also assessed the effects of the β-adrenoceptor agonist on fast network oscillations in the vPrL region. Previously we found that NA (30 μM) exerted no effect on the spectral characteristics of network oscillations in this region. Similar to the effects of β-adrenoceptor activation in the DP region, application of isoprenaline (1 μM) for 15 min again failed to induce any significant effect on either area power (ctrl: 557 $\mu V^2.Hz^{-1}$ IQR: 324-1005; ISO-5min: 569 $\mu V^2.Hz^{-1}$ IQR: 332-1158; ISO-10min: 637 $\mu V^2.Hz^{-1}$ IQR: 303-1165; ISO-15min: 477 $\mu V^2.Hz^{-1}$ IQR: 359-1227; wash: 513 $\mu V^2.Hz^{-1}$ IQR: 398-1649, n_{slice}=5; Friedman One-Way RM-ANOVA on Ranks: $\chi^2(4)=6.4$, $p>0.05$; Figure 6.37A,B,C) or peak frequency (ctrl: 25.6 Hz IQR: 24.9-27.8; ISO-5min: 25.0 Hz IQR: 24.6-27.5; ISO-10min: 26.9 Hz IQR: 25.3-28.1; ISO-15min: 25.0 Hz IQR: 24.7-27.8; wash: 26.3 Hz IQR: 23.4-26.4, n_{slice}=5; Friedman One-way RM-ANOVA on Ranks: $\chi^2(4)=7.9$, $p>0.05$; Figure 6.37A,B,D) of network oscillations.

Figure 6.35. β-adrenoceptor activation fails to mimic the NA-mediated reduction in the area power of fast network oscillations in the DP region

Figure 6.36. β_1-adrenoceptor blockade fails to prevent the NA-mediated area power reduction of fast network oscillations in the DP region

We also tested the effect of NA (30 μM) on fast network oscillations in the vPrL region in slices pre-incubated with the β_1-adrenoceptor antagonist. Pre-treatment with betaxolol (30 μM) failed to elicit any change in the area power of network oscillations, suggesting that activation of the β_1-adrenoceptor by endogenous NA release does not contribute to the modulation of network oscillations in this mPFC subdivision. However, when NA (30 μM) was co-applied with betaxolol, we observed a significant reduction in the area power that reached significance at 20 min (ctrl: 812 ± 175 $\mu V^2.Hz^{-1}$; BTX-30min: 834 ± 258 $\mu V^2.Hz^{-1}$; NA-5min: 763 ± 254 $\mu V^2.Hz^{-1}$; NA-10min: 698 ± 351 $\mu V^2.Hz^{-1}$; NA-15min: 631 ± 235 $\mu V^2.Hz^{-1}$; NA-20min: 463 ± 120 $\mu V^2.Hz^{-1}$; wash: 748 ± 298 $\mu V^2.Hz^{-1}$, n_{slice}=5; One-Way RM-ANOVA: $F(6,24)=3.2$, $p<0.05$; Tukey test: all comparisons; Figure 6.38A,B,C). Peak frequency of network oscillations remained unaffected by NA (ctrl: 27.7 ± 2.9 Hz; BTX-30min: 28.6 ± 3.9 Hz; NA-5min: 28.6 ± 3.9 Hz; NA-10min: 28.3 ± 4.8 Hz; NA-15min: 28.9 ± 4.5 Hz; NA-20min: 28.9 ± 4.5 Hz; wash: 27.9 ± 4.1 Hz, n_{slice}=5; One-Way RM-ANOVA: $F(6,24)=0.6$, $p>0.05$; Figure 6.38A,B,D).

These data are similar to that seen with DA in the presence of the D_1 receptor antagonist. In the presence of the β_1-adrenoceptor antagonist, NA now produces a larger effect than it did when applied alone. Again this suggests opposing effects such that activation of β_1-adrenoceptor alone with isoprenaline did not increase the oscillations (as the D_1 agonist did not) but when blocked the reduction seen with NA is now larger.

Figure 6.37. β-adrenoceptor activation has no effect on fast network oscillations in the vPrL region

Figure 6.38. Noradrenaline reduces the area power of fast network oscillations in the vPrL in the presence of a β_1-adrenoceptor antagonist

6.4. Discussion

6.4.1. *Summary*

In this chapter we assessed the involvement of DA and NA receptors in the modulation of carbachol/kainate-induced fast network oscillations in the DP and PrL subdivisions of the mPFC.

Initially, we assessed the effect of DA (100 μM) on the spectral and rhythmicity characteristics of fast network oscillations in the different mPFC subdivisions. The results obtained from extracellular single-electrode and multi-electrode field recordings are:

- Application of DA (100 μM) produced a region-specific effect on network oscillations in the mPFC. In the ventral mPFC, DA reduced oscillation strength. The reduction in oscillations was more profound and consistent in the DP region. In contrast, DA caused a significant increase in the area power of network oscillations recorded in the dorsal mPFC. The increase in oscillations was most consistent in the vPrL region. The transition line between the decrease and increase in oscillation power caused by DA was between ventral and dorsal IL.

- The effect of DA on rhythmicity of network oscillations was regional and correlated with the changes in area power. Therefore, DA (100 μM) reduced significantly the RI of network oscillations in the DP region, while at the same time increased significantly the RI of network oscillations in the vPrL region.

- A small but significant reduction was observed in the peak frequency of fast network oscillations, following DA (100 μM) application, in both DP and vPrL regions.

Analysis of extracellular unit recordings revealed:

- DA (100 μM) reduced the average rate, frequency, renewal density, rhythmicity and phase-synchrony of firing activity of FR units localized in the DP region.

- DA (100 μM) increased the average rate, frequency, renewal density, rhythmicity and phase-synchrony of firing activity of FR units localized in the vPrL region.

- We also observed two cases of SNR units in the vPrL region which elicited the firing properties of FR units following DA (100 μM) application.

Analysis of intracellular recordings revealed:

- DA (100 μM) produced a significant reduction in the amplitude, decay time, rhythmicity and phase synchrony of IPSPs recorded from RS cells in the DP region.

We then assessed the contribution of different DA receptors to the modulation of spectral characteristics of fast network oscillations in the mPFC subdivisions. Analysis of single-electrode field recordings revealed:

- Application of the D_1 receptor agonist, SKF38393 (10 μM), failed to mimic the DA-mediated decrease in the area power of network oscillations in the DP region. In fact, SKF38393 (10 μM) did not produce any significant effect on either the area power or peak frequency of fast network oscillations in the DP region.

- Application of the D_1 receptor antagonist, SCH23390 (10 μM) alone, did not produce any significant effect on either the area power or peak frequency of network oscillations in the DP region. However, co-application of SCH23390

(10 μM) with DA (100 μM) produced a marked area power reduction. Area power reduction was three-times greater than the decrease observed with DA (100 μM) application alone. No significant effect was observed in the peak frequency of fast network oscillations during drug co-application.

- Application of the D_1 receptor agonist, SKF38393 (10 μM), failed to mimic the DA-mediated increase in the area power of network oscillations in the vPrL region. Application of SKF38393 (10 μM) did not produce any significant effect on either the area power or peak frequency of fast network oscillations in the vPrL region.

- We tested the contribution of D_2 receptor activation to the DA-mediated area power reduction in the DP region. We found that application of quinpirole (10 μM) failed to produce any significant effect on either the area power or peak frequency of network oscillations.

- Application of quinpirole (10 μM) failed to mimic the DA-mediated increase in fast network oscillations recorded in the vPrL region, as it produced no significant effect on either the area power or peak frequency.

- Activation of D_4 receptors with PD168077 (10 μM) failed to elicit any significant effect on either the area power or peak frequency of fast network oscillations in the DP region.

- No significant effect was produced on either the area power or peak frequency of fast network oscillations in the vPrL region following application of the D4 agonist PD168077 (10 μM).

We also assessed the effect of NA (30 μM) and β-adrenoceptors on the spectral characteristics of fast network oscillations in the mPFC subdivisions. Analysis of single-electrode field recordings revealed:

- Application of NA (30 μM) produced a significant but fully reversible reduction in the area power of network oscillations in the DP region. There was no significant effect on the peak frequency of network oscillations.

- No significant effect was produced on either the area power or peak frequency of fast network oscillations in the vPrL region following NA (30 μM) application.

- We tested the contribution of β-adrenoceptors on the NA-mediated reduction in the area power of network oscillations in the DP region. Application of isoprenaline (1 μM) failed to produce any significant effect on either the area power or peak frequency of network oscillations.

- We also tested whether β_1-adrenoceptor blockade could block the NA-mediated decrease in the area power of network oscillations in the DP region. Application of betaxolol (30 μM) alone did not produce any significant effect on either the area power or peak frequency. Coapplication of betaxolol (30 μM) and NA (30 μM) produced a larger decrease in the area power of network oscillations than NA (30 μM) alone. There was no significant effect on the peak frequency of fast network oscillations by drug coapplication.

- β-adrenoceptor activation with isoprenaline (1 μM) did not produce any significant effect on either the area power or peak frequency of network oscillations in the vPrL region.

- Application of betaxolol (30 μM) alone did not produce any significant effect on either the area power or peak frequency of fast network oscillations in the vPrL region. However, coapplication of betaxolol (30 μM) and NA (30 μM) elicited a significant reduction in the area power of network oscillations. No significant effect was produced by drug coapplication in the peak frequency of network oscillations.

6.4.2. *Regional effect of dopamine on fast network oscillations in the rat medial PFC*

A seminal *in vitro* study in the rat mPFC, showed that exogenous DA (400 μM; interfaced recording chamber) application produced a small excitatory effect on both pyramidal cells and GABAergic interneurons (Penit-Soria et al. 1987). This evidence gave rise to the hypothesis that the DA-mediated modulation of cortical activity could be the outcome of an interplay of DA's direct effects on both excitatory and inhibitory cells (Penit-Soria et al. 1987).

In principle, the oscillatory behaviour of cortical networks emerges from an interplay of a rhythmic alteration between brief inhibitory and excitatory synaptic events (Buhl et al. 1998; Whittington et al. 2000). Results from Chapter 4 showed that in our *in vitro* model, phasic AMPA receptor-mediated excitation is not required, but a tonic KA receptor-mediated excitatory drive is important for the generation and maintenance of carbachol/kainate-induced fast network oscillations in the rat mPFC. In addition, fast network oscillations were shown to rely on $GABA_A$ receptor-mediated synaptic inhibition. The rhythmic pattern of synaptic inhibition, we reported in Chapter 5, is the outcome of periodic interneuron firing activity (Andersson et al. 2012; Whittington and Traub 2003). Any change in the temporal pattern of interneuron firing, could cause significant alterations in the rhythmicity and strength of synaptic inhibition. By taking into account that inhibitory synaptic currents have a substantial impact on the power of fast network oscillations (Oren and Paulsen 2010), changes in the strength and rhythmicity of periodic synaptic inhibition would be tightly correlated to the area power and rhythmicity of field oscillations.

DA (100 μM) application produced region-dependent effects on the area power of fast network oscillations in the mPFC. DA produced a significant reduction in rhythmicity and area power of fast network oscillations in the ventral portion of the mPFC which coincided with a significant reduction in the amplitude and rhythmicity of IPSPs. Moreover, a reduction in the phase-synchrony between field traces and IPSP trains provides more evidence that DA reduced the efficiency of the interneuron network to control the population activity in the ventral mPFC.

In contrast, DA increased the strength and rhythmicity of fast network oscillations recorded in the dorsal mPFC. Unfortunately we did not record inhibitory synaptic activity from pyramidal cells in the vPrL region during DA

application. Although, in Chapter 5 we showed that pyramidal cells in the vPrL region receive weak and non-rhythmic IPSP trains in the presence of network oscillations, evidence from FR units indicate that DA improved the rhythmicity of inhibitory synaptic activity in this cortical region. During the inhibition phase of an oscillation cycle interneuron firing prevails, but as the inhibitory currents are reduced, the pyramidal cells can fire APs. Overall, the excitability and rhythmicity of pyramidal cell firing in our *in vitro* model would therefore be correlated to the excitability and rhythmicity of interneuron firing. Indeed, the average firing rate, firing frequency and renewal density of FR units decreased in the DP region, suggesting a marked reduction in the excitability of these units. A decrease in the excitability coincided with a significant reduction in the rhythmicity of firing as well as the unit vs. field phase-synchrony. In contrast, in the vPrL region a significant increase in the area power and rhythmicity of network oscillations coincided with a marked increase in the excitability (e.g. average firing rate, firing frequency and renewal density), rhythmicity and unit vs. field phase-synchrony of FR units. In both regions the preferred field phase of spike firing by FR units was not markedly affected by DA.

Results presented in this chapter are in agreement with evidence from a recent *in vitro* study (Andersson et al. 2012) which showed that an increase in the strength and rhythmicity of kainate-induced gamma frequency oscillations in the hippocampus coincided with an improvement in the firing preference of FS interneurons in relation to the field phase, as well as an increase in the IPSP vs. field phase-synchrony (Andersson et al. 2012).

In the present thesis we did not assess the DA effects on the membrane properties of interneurons and pyramidal cells, however, an increase in the excitability and rhythmicity of two SNR units in the vPrL region, could suggest that DA may exert a depolarizing effect in a population of cells localized to this region. Moreover, this evidence could indicate that SNR units belong to the same group of cellular population with FR units; however due to low depolarization or weak engagement to the network oscillatory activity, SNR units do not exhibit the same firing properties as FR units prior to DA application.

A small but significant reduction in the oscillation frequency in the DP region could be attributed to a significant reduction in the decay time of IPSPs. The DA-induced decrease in the decay time of IPSPs could result from an increase in the

rate of closing of a population of open $GABA_A$ channels, or a fast removal from the postsynaptic site by GABA uptake (Nicoll et al. 1975).

The effects of DA application on the characteristics of fast network oscillations have already been assessed in the hippocampus *in vitro*. DA (30-200 μM; interface recording type) was shown to reduce the strength of both kainate- and carbachol-induced gamma frequency oscillations (Weiss et al. 2003; Wójtowicz et al. 2009). The influence of the DA system on cortical activity has an inverted-U shape function (Arnsten 2011). Therefore, although an optimal concentration of DA can improve PFC physiology and cognition, lower or higher concentrations of DA can cause significant impairments (Arnsten 2011). To investigate the concentration-dependence of DA effect on network oscillations in the mPFC we performed a concentration response profile. DA (10 μM) had no effect on the spectral characteristics of network oscillations in the DP or vPrL regions. When the concentration of DA was increased to 50 μM it produced a significant reduction in the area power of the DP region but no effect on the vPrL region. Overall, these results demonstrate that the effect of DA on fast network oscillations in the mPFC is concentration-dependent and that oscillations in the DP region are more sensitive to DA than oscillations in the vPrL region.

6.4.2. *The contribution of D_1 receptors to the dopamine-mediated effect on fast network oscillations in the rat medial PFC*

As outlined in section 6.1.1 the physiological actions of DA are mediated by five G-protein coupled receptors D_1, D_2, D_3, D_4 and D_5, which are classified into two major groups, namely, D_1-like (D_1 and D_5) and D_2-like (D_2, D_3 and D_4) receptor subtypes, based on their coupling relationship with adenylyl cyclase (Beaulieu and Gainetdinov 2011; Missale et al. 1998; Vallone et al. 2000). We first assessed the contribution of D_1 receptors in the DA-induced effect on network oscillations in the DP and the vPrL regions.

D_1 receptor activation has been shown to increase the excitability of both pyramidal cells and interneurons in the mPFC (Seamans et al. 2001a; Yang and Seamans 1996b) and suppress gamma frequency oscillations in the hippocampus (Weiss et al. 2003; Wójtowicz et al. 2009).

It has been shown that D_1 receptor activation increases the excitability of pyramidal cells (including RS, IB and ROB cells) in the rat mPFC, by modulating the efficiency of two opposing voltage-dependent ionic conductances that regulate their firing threshold (Yang and Seamans 1996b). The first current is the tetrodotoxin (TTX)-sensitive slowly inactivating ("persistent") Na^+ current, which is responsible for inward membrane rectification. When activated at subthreshold potentials this ionic conductance mediates Na^+ plateau potentials with overlying voltage oscillations in response to depolarizing pulses (Yang et al. 1996a). DA, as well as D_1 receptor antagonists were shown to increase the duration of Na+ plateau potentials and shift their activation threshold to more negative potentials (Yang and Seamans 1996b). The second ionic current is the slowly inactivating K^+ outwardly rectifying conductance. This conductance, when activated at subthreshold potentials, prevents further membrane depolarization (Yang et al. 1996a). It was demonstrated that DA via D_1 receptor activation removes this outward rectification (Yang and Seamans 1996b). Together these results show that a reduction in the efficiency of outward rectification by D_1 receptor activation in favour of inward rectification will enable pyramidal cells to be more responsive to synaptic excitatory input (Yang and Seamans 1996b).

Spontaneous IPSCs (sIPSCs) are predominantly caused by AP-dependent GABA release. An *in vitro* whole-cell patch-clamp recording study in the rat mPFC (Seamans et al. 2001a) showed that D_1 receptor activation increased the frequency but not the amplitude of sIPSCs, suggesting a D_1 receptor-mediated increase in the intrinsic excitability of interneurons, but not in GABA release (Seamans et al. 2001a).

D_1 receptor activation was shown to suppress gamma frequency oscillations in two different *in vitro* models. In the carbachol model of persistent gamma frequency oscillations in the hippocampus, selective stimulation of D_1 receptors (SKF383393; 20 μM; interface-type recording chamber) could mimic the DA-induced decrease in area power (Weiss et al. 2003). Moreover, D_1 receptor activation (SKF38393; 100μM; interface-type recording chamber) was shown to suppress the DA-induced facilitation of stimulus-induced gamma frequency oscillations in the same area (Wójtowicz et al. 2009).

In our *in vitro* model of fast network oscillations in the mPFC we assessed the effect of selective D_1 receptor activation. SKF38393 (10 μM) application for 15 min did not produce any significant effect on either the area power or frequency of network oscillations in the DP and vPrL subdivisions of the mPFC. We also investigated the effect of SKF38393 (10-20 μM) application for 45 min, but we did not observe any significant effect (data not shown). We then went on to examine the effect of D_1 receptor blockade on fast network oscillations in the DP region. Application of SCH23390 (10 μM) alone for 30 min did not cause any significant alterations in the spectral characteristics of network oscillations, suggesting that activation of D_1 receptors by endogenous DA release do not exert a significant influence. However, when DA (100 μM) was co-applied with the D_1 receptor antagonist, it produced a marked area power reduction in the DP region. Area power reduction was three-times greater than the one observed with DA (100 μM) application alone. These results suggest that although selective activation of D_1 receptors did not decrease the area power of oscillations, however, D_1 receptors play a modest supportive role on the generation of network oscillations in this cortical area.

6.4.3. *The contribution of D_2 receptors to the dopamine-mediated effect on fast network oscillations in the rat medial PFC*

A suppressive role of D_2 receptors on the excitability of mPFC cells has been reported *in vivo* in the rat brain (Sesack and Bunney 1989). It was shown that iontophoretic application of DA inhibited the spontaneous activity of deep layer pyramidal cells. In the same study, blockade of D_2 receptors with sulpiride but not D_1 receptors, prevented the DA-induced suppression of firing. The firing rate of pyramidal cells was also shown to be increased by iontophoretic application of sulpiride (a D_2 antagonist) alone, suggesting that endogenous DA release exerts a tonic inhibitory influence via D_2 receptor stimulation (Sesack and Bunney 1989).

Another *in vivo* microdialysis study showed that local administration of the D_2 receptor agonist, quinpirole, elicited a significant increase in action potential-induced GABA release. The increase was successfully blocked by the D_2 receptor antagonist, sulpiride, but not by of the D_1 receptor antagonist, SCH23390 (Grobin and Deutch 1998).

Subsequent evidence from whole-cell patch-clamp recordings in the mPFC (Seamans et al. 2001a) revealed that D_2 receptor activation reduces the amplitude of TTX-insensitive mIPSCs, indicating that D_2 receptors reduce the post synaptic sensitivity of GABA receptors. Application of $GABA_B$ receptor blockers did not mimic the D_2 receptor-mediated effect, reflecting a $GABA_A$ receptor component to the miniature IPSCs (mIPSCs) amplitude reduction (Seamans et al. 2001a).

In the present study application of quinpirole (10 μM) for 15 min did not produce any significant change on either the area power or frequency of carbachol/kainate-induced oscillations in the DP and vPrL subdivisions of the mPFC. We also investigated the effect of quinpirole (10-20 μM) application for 45 min in both cortical regions, but we did not observe any significant effect (data not shown). These results indicate that D_2 receptors activated alone do not contribute to the DA-mediated regional effects on the fast network oscillations in the mPFC.

6.4.4. The contribution of D_4 receptors to the dopamine-mediated effect on fast network oscillations in the rat medial PFC

Converging evidence from electrophysiological studies have demonstrated the involvement of D_4 receptors in the modulation of cortical excitability. One study reported that complete deficiency of D_4 receptors in mutant mice results in an increased excitability of prefrontal cortical pyramidal cells without affecting their resting membrane potential (Rubinstein et al. 2001). The same effect was replicated by pharmacological blockade of D_4 receptors in wild type animals suggesting that D_4 receptors exert an inhibitory influence on the neuronal activity of PFC cells (Rubinstein et al. 2001; Seamans et al. 2001a).

An electrophysiological study in cultured rat PFC neurons showed that activation of D_4 receptors produces a reduction in postsynaptic $GABA_A$ receptor currents (X. Wang et al. 2002). By blocking action potential-evoked synaptic activity with TTX application, the same study showed that activation of D_4 receptors reduced the amplitude but not the frequency of mIPSCs, suggesting that D_4 receptors modulate the postsynaptic response to GABA neurotransmission (X. Wang et al. 2002). Consistent with this evidence, an anatomical study showed that D_4 receptors are localized predominantly on the cell body of PFC pyramidal cells (Wedzony et al. 2000), in the vicinity of $GABA_A$ receptor-mediated synaptic inhibition occurs (Gonzalez-Burgos and Lewis 2008). Together, these findings

suggest that D_4 receptors may play an important role in the efficiency of fast synaptic inhibition by exerting a modulatory effect on the postsynaptic site.

In view of this evidence we wished to investigate the contribution of D_4 receptors in the DA-induced regional effect of fast network oscillations in the rat mPFC. A recently published study reported that application of the D_4 receptor agonist, PD168077 increased the area power of *in vitro* kainate-induced gamma frequency oscillations in the CA3 area of the hippocampus (Andersson et al. 2012). Intracellular recording techniques revealed that the increase in area power was a result of an increase in the unit vs. field phase-synchrony strength of fast spiking interneuron firing. The increase in area power could be prevented by D_4 receptor or NMDA-receptor blockade, suggesting that D_4 receptors modulate network oscillations by controlling the temporal dynamics of FS interneurons via NMDA-receptor mediated currents (Andersson et al. 2012).

Our results show that selective activation of D_4 receptors, by PD168077 (10 μM) failed to mimic any of the regional DA-mediated effects on network oscillations. In fact D_4 receptor blockade did not elicit any significant effect on either the amplitude or frequency of network oscillations in any of the DP and PrL regions. The complete absence of effect could be explained by a recent immunochemistry study which found that although D_4 receptors are densely expressed in the rat PFC, however the PrL has very little expression while the IL is almost devoid of D4 receptors (Rivera et al. 2008).

Together, these data demonstrate that activation of different DA receptors alone does not mimic the effects of DA itself. This could be due to the fact that DA will activate, in a complex combination, all five receptor subtypes with different at times opposing effects on interneurons and pyramidal cells. It is not possible to predict from the individual effects of different DA receptors on pyramidal cells and interneurons what the overall change in network activity would be.

6.4.5. The contribution of noradrenaline receptors to the dopamine-induced effect on fast network oscillations in the rat medial PFC

There is strong evidence to show that the NA system can modulate cortical cell and network activity. In the rat frontal cortex it was shown that NA excites interneurons and increases the frequency and amplitude of spontaneous $GABA_A$

receptor-mediated IPSCs via α-adrenoceptor stimulation (Kawaguchi and Shindou 1998).

In the rat auditory cortex a large proportion of PV+ cells are colocalized with α_2- and β-adrenoceptors (Salgado et al. 2012). Patch-clamp recordings in this cortical area showed that the NA system can modulate the efficiency of $GABA_A$ receptor-mediated synaptic inhibition by targeting both pre- and post-synaptic sites. Although, activation of α_2- and β-adrenoceptors in the presynaptic site were shown to increase GABA release probability, at the same time α_1-adrenoceptor stimulation at the postsynaptic site decreased the amplitude of $GABA_A$ receptor-mediated IPSCs (Salgado et al. 2012).

It was demonstrated that NA increased the excitability of cells in the hippocampal dentate gyrus by increasing the Ca^{2+} influx and K^+ outflow following electrical stimulation (Stanton and Heinemann 1986). The same study replicated the ionic efflux increase with selective β-adrenoceptor activation (Stanton and Heinemann 1986).

At a network level, it has been reported that NA increased the strength of stimulus-induced gamma frequency oscillations in the olfactory bulb via α- and β-adrenoceptor stimulation (Gire and Schoppa 2008).

In the hippocampus, NA (30-100 μM; interface-type recording chamber) suppressed kainate-induced gamma frequency oscillations in a dose-dependent manner (Wójtowicz et al. 2009). Oscillation frequency was slightly but significantly increased by NA and area power reduction was correlated with a rhythmicity reduction. The NA-induced suppression of gamma activity was mimicked by the β-adrenoceptor agonist, isoprenaline (2 μM; Wójtowicz et al. 2009). A similar suppression of gamma activity evoked by NA (30-100 μM) application was also observed for carbachol-induced gamma frequency oscillations in the hippocampus (Wójtowicz et al. 2009). However, in contrast to pharmacologically activated persisted gamma frequency oscillations, stimulus-induced transient gamma frequency oscillations were enhanced by NA (100 μM) in both duration, amplitude and oscillation frequency (Wójtowicz et al. 2009). NA-mediated increase was prevented by selective β-adrenoceptor activation (isoprenaline; 2μM). Therefore, although NA exerts opposing effects on transient and persistent fast network

oscillations; however β-adrenoceptors can cause a suppressive effect on both them.

We initially examined the effect of NA (30 μM) application on fast network oscillations in the DP region. Results indicate that NA caused a strong and significant reduction in the area power of network oscillations. Decrease was actually stronger than the one we observed with DA. We assessed the contribution of β-adrenoceptors to the NA-induced area power reduction. The β-adrenoceptor agonist, isoprenaline (1 μM) application did not cause any significant effect on the spectral characteristics of network oscillations in the DP region. Blockade of β_1-adrenoceptors with betaxolol (30 μM) also had no significant effect on either the area power or frequency, suggesting that β_1-adrenoceptor activation by endogenous NA does not contribute to the modulation of network oscillations. However, coapplication of betaxolol (30 μM) and NA (30 μM) elicited an almost two-fold greater area power reduction compared to NA (30 μM) application alone, suggesting the β_1-adrenoceptors may play a modest supportive role in the generation and maintenance of fast network oscillations in the DP region.

Application of NA (30 μM), or the selective β-adrenoceptor agonist, isoprenaline (1 μM) did not elicit any significant effect on either the area power or peak frequency of fast network oscillations in the vPrL region. However coapplication of betaxolol (30 μM) and NA (30 μM) produced a significant reduction in the area power of fast network oscillations in this cortical area. The latter evidence suggests that similar to the DP region, β_1-adrenoceptors localizing the vPrL region play a modest supportive role on the generation and maintenance of fast network oscillations.

Overall, in this chapter we showed that DA (100 μM) exerted a region-dependent effect on the area power of carbachol/kainate-induced fast network oscillations in the mPFC. DA decreased the area power of network oscillations in the ventral mPFC. This effect correlated with a significant reduction in the amplitude and rhythmicity of synaptic inhibition, probably due to alterations in the firing rhythmicity of the interneuron network. D_1 receptors were shown to play a

modest supportive role in the generation of network oscillations. NA (30 μM) application caused an area power reduction; however β-adrenoceptors did not contribute to this decreasing effect. They were instead shown to play a modest supportive role on network oscillations.

DA (100 μM) increased the strength and rhythmicity of fast network oscillations in the dorsal portion of the mPFC. Evidence from extracellular unit recordings could suggest that the DA-mediated effect on fast network oscillations in this cortical area is correlated to an increase in the firing rhythmicity of the interneuron network firing activity. Application of DA receptor ligands showed that selective activation or blockade of D_1, D_2 and D_4 receptors does not mimic the DA-mediated effect. Moreover, NA application was shown to exert no modulatory effect on fast network oscillations in the vPrL region. However, results from betaxolol application, revealed that β_1-adrenoceptors play a modest supportive role in the generation network oscillations in this cortical area.

Chapter 7

General discussion

Chapter 7 - General discussion

7.1. Overview

In this thesis we have demonstrated that fast network oscillations (~25-30 Hz) can be induced and persist for hours across the different subdivisions of the mPFC and the motor cortex, with the coapplication of the oscillogenic compounds of carbachol and kainate. We investigated the spectral and rhythmicity characteristics of network oscillations for every region and layer they were recorded from. We found that fast network oscillations recorded in the ventral regions of the mPFC were stronger, more rhythmic but slower than oscillations recorded in the dorsal regions of this agranular cortical area. Within each subdivision of the mPFC and the motor cortex, layer 5 produced the strongest oscillations. The amplitude laminar profile and the laminar phase-difference of oscillations suggest that oscillatory activity is mainly generated in layer 5 and is then projected to the superficial layers.

We also assessed the phase-synchrony and phase-difference between field oscillations recorded within the deep layers. We found that phase-difference was small, ranging between -20° to +20° and that phase-synchrony decreased with increasing inter-electrode distance. Phase-synchrony analysis was applied in the present thesis to investigate network interactions between distant neuronal populations. Results from this analysis suggest that network interactions exist only between adjacent regions.

We investigated the different neuronal types localizing in the mPFC in the absence of network oscillations. We found that more bursting cells were present in the ventral than in the dorsal mPFC. RS cells in the ventral mPFC showed signs of inward rectification and rebound depolarization. Moreover, their firing threshold was significantly more negative to the firing threshold of RS cells in the dorsal mPFC. These important differences in the population diversity and excitability of cells could account for the significant difference in the amplitude of network oscillations recorded in these regions. We also found that in the presence of network oscillations, cells from both regions were equally depolarized; however,

only pyramidal cells in the ventral mPFC received strong and highly rhythmic synaptic inhibition.

We assessed the contribution of different types of inhibitory and excitatory synaptic transmission. We found that oscillations in both the ventral and dorsal regions of the mPFC relied on a tonic kainate receptor-mediated excitation and fast synaptic inhibition, although the ventral mPFC was more sensitive to $GABA_A$ receptor blockade. Fast, AMPA receptor-mediated synaptic excitation was not important for the generation of fast network oscillations in either the dorsal or ventral regions of the mPFC. In the ventral mPFC, an NMDA receptor-mediated contribution to the generation of the network oscillations was also observed.

We investigated the modulatory effects of DA and NA on the network oscillatory activity. Application of DA at a concentration which has been shown to affect excitability and synaptic efficiency of cortical cells caused region-dependent effects on the fast network oscillations in the mPFC. The area power and rhythmicity of network oscillations were markedly decreased in the ventral mPFC, but significantly increased in the dorsal mPFC. The regional DA-mediated effect was correlated with significant changes in the rhythmicity of synaptic inhibition and neuronal firing. We also found that although D_1 receptors do not contribute to the DA-mediated decrease in the power and rhythmicity of network oscillations in the DP region; however, play a modest contributory role in the generation of fast network oscillations.

NA produced a significant area power reduction in the ventral mPFC, but did not elicit any effect on network oscillations in the dorsal mPFC. Pharmacological manipulations in the activity state of β-adrenoceptors revealed a supportive role of these receptors to the generation of network oscillations in both ventral and dorsal regions of the mPFC.

In the following paragraphs we discuss some of the technical considerations we took into account before analyzing field recordings that helped us improve the quality of our results. We also discuss the functional relevance of our results, along with issues that need to be addressed with further research.

7.2. Functional relevance of the catecholamine effects on fast network oscillations in the rat medial PFC

It is well accepted that fast network oscillations provide the neuronal substrate by which local and distant neuronal populations orchestrate their firing activity to process cognitive-related information (Engel et al. 1991; Singer 1999). Such neuronal mechanism could support information processing in the mPFC regions, which guide behaviour by integrating converging information from various cortical and subcortical areas (Hoover and Vertes 2007; Kesner 2000; Killcross and Coutureau 2003; Rossetti and Carboni 2005; Uylings et al. 2003; Vertes 2006).

Our results revealed the DA elicited two opposing effects on the characteristics of fast network oscillations in the ventral and dorsal regions of the mPFC. The regional dissociation of the DA-induced effect is correlated to the functional dissociation of these two agranular cortical regions which support different aspects of cognitive behaviour.

The functional dissociation between ventral and dorsal mPFC regions was demonstrated explicitly in an instrumental learning behavioural task (Killcross and Coutureau 2003). This study showed that sham-lesioned rats which received small amounts of instrumental training (lever pressing - reward) demonstrated reduced responding behaviour when reward was devalued. This evidence indicated that at this early stage of training, response initiation was associated with the reward value (goal-directed behaviour). With longer periods of training, response sensitivity to reward devaluation was decreased, suggesting that at this protracted stage of training, instrumental response was not associated to the reward value, but instead, it was bound to the stimulus (habitual response; Killcross and Coutureau 2003). Goal-directed behaviour can support behavioural flexibility with a cost of increased capacity for information processing. This could be balanced with the development of habits (Killcross and Coutureau 2003).

The same study showed that in animals with PrL excitotoxic lesions, response sensitivity was not altered by reward devaluation in the early stages of training. Instead, in animals with IL lesions, response sensitivity to devaluation persisted even after extensive overtraining (Killcross and Coutureau 2003). Therefore, this evidence indicates that the PrL region is responsible for establishing the response-reward value association which supports goal-directed behaviour, whereas the IL

region is responsible for establishing the stimulus-response association which leads to habit formation (Killcross and Coutureau 2003).

In our model the DA-mediated increase in the amplitude and rhythmicity of network oscillations in the dorsal mPFC, could improve the efficiency of information processing that would support goal-directed behaviour. At the same time, the DA-mediated suppression of fast network oscillations in the ventral mPFC could reduce the efficiency of information processing dedicated to habit formation.

It has been shown in the monkey brain that the firing activity of DAergic neurons is inversely proportional to reward prediction (Fiorillo et al. 2003). Therefore, the DA-induced opposing effect on the characteristics of fast network oscillations in the mPFC could improve behavioural flexibility in conditions with increased prediction uncertainty, by favouring goal-directed behaviour over habit formation.

There is also evidence that the NA system is engaged in goal-directed behaviour (Arnsten 1998; Rossetti and Carboni 2005). It has been shown in a recent behaviour/microdialysis study that during the delayed alteration task (T-maze), that NA levels increased significantly in the mPFC of alteration-trained rats compared to the alteration-untrained ones (Rossetti and Carboni 2005).

In our *in vitro* model, exogenous NA application suppressed fast network oscillations in the ventral but not the dorsal mPFC. This evidence could suggest that DA and NA systems act synergistically in the ventral mPFC to reduce the efficiency of information processing that mediates habit formation in an environment which demands the continuous adaptation to alternating conditioning rules in order to achieve reward.

7.3 The regional effect of DA: Experimental considerations

The effects of DA on the electrophysiological properties of different neuronal populations in the rodent mPFC have been investigated extensively over the last decades. Despite the significant effects of this catecholamine on the cell excitability

or efficiency of excitatory and inhibitory synaptic transmission, results from these studies were characterized by increased variability (Gorelova et al. 2002; Penit-Soria et al. 1987; Yang and Seamans 1996b). To our knowledge the present study is the first one to demonstrate the region-dependent effects of DA on the characteristics of fast network oscillations in the mPFC. We showed that although DA facilitated network oscillations in the dorsal mPFC, it suppressed network oscillations in the ventral mPFC. Moreover, we showed that the transition line of these opposite effects lied vaguely between the dorsal and ventral IL. It remains to be investigated whether this region-dependent effect is accounted to significant regional differences in the expression of DA receptors or to the differences in the expression of cellular types and the mechanisms of network oscillations. However, in view of these results, the increased variability in the DA effect reported by the aforementioned studies could be accounted to the lack of regional specificity.

7.4. Interference of volume conduction in multi-electrode field recordings

Volume conduction is an inherent caveat of multi-electrode recordings which occurs when the volumes recorded by two or more electrodes overlap (Lachaux et al. 1999). Volume conduction could influence the quality and spatial accuracy of the spectral and rhythmicity characterization analysis we performed, in that signals produced by local networks could be picked up by distant electrodes. That in turn could lead to false conclusions about the scale of this network activity. Moreover, and more importantly volume conduction could create spurious phase-synchrony between field traces.

Quite often, when the Utah array was employed to record multi-electrode field activity, strong oscillations in the mPFC coincided with recordings of very weak oscillations (<100 $\mu V^2.Hz^{-1}$), from electrodes positioned in areas other than the mPFC. The magnitude of these oscillations was remarkably similar, regardless of the electrode position (e.g. white matter, lateral ventricle or subcortical region), or the electrode distance from the oscillating mPFC. It is possible that recordings of this very weak oscillation did not reflect local network oscillatory activity but rather interference from volume conduction. Therefore, we analyzed only those signals that contained oscillatory activity with an area power greater than 100 $\mu V^2.Hz^{-1}$. Field traces with lower area power than the threshold were considered to

be recording oscillatory activity through volume conduction and were not included in any further analysis.

Evaluation of the phase-difference results from the remaining field traces (>100 $\mu V^2.Hz^{-1}$) does not indicate the interference of volume conduction. If signals travel through volume conduction with a certain speed, that would be the same in every direction (Lachaux et al. 1999). Therefore, we would expect that it would take the same time for a signal to travel a certain distance along the dorso-ventral or the medio-lateral axis. This difference in time would correspond to a certain amount of phase-difference (through the equation: $\varphi = 360^{\circ} \cdot f \cdot \Delta t$). However, this hypothesis is not confirmed by our results, since the phase-difference between intra-laminar field recordings was substantially smaller than the phase-difference of equally spaced inter-laminar field recordings. For example, the phase-difference between PrL and IL electrodes in layer 5 was ~20°, whereas the difference between layer 5 and layer 2 electrodes in the PrL was ~100°. In both pair-wise comparisons, the inter-electrode space was 400 μm. The same phase-difference analogy existed for every region tested.

7.5. The DA and NMDA synergistic effect

Earlier electrophysiological studies in the mPFC have revealed an interesting synergistic effect of DA and NMDA receptors on the excitability of cortical and subcortical cells. It has been shown that selective activation of D_1 receptors increases post-synaptic NMDA EPSCs although at the same time it reduces Glutamate release by almost 15% in the mPFC (Seamans et al. 2001b). Selective activation of D_1 receptors has been reported to suppress the regenerative high threshold spikes (rHTS) in layer 5 bursting cells in the mPFC (Yang and Seamans 1996b). These Ca^{2+} spikes have dendritic origin and they are believed to increase the dendritic excitability of cells by releasing NMDA receptors from the Mg^{+} block (Yang et al. 1996a). Co-application of NMDA and a selective D_1 receptor agonist has been shown to elicit a significant increase in the excitability of layer 5 mPFC pyramidal cells. Increase was more pronounced than the sum of the independent actions of these ligands (J. Wang and O'Donnell 2001). Finally a recent study reported that selective activation of D_2 receptors which are solely expressed in Ih-

current possessing cells, enhanced NMDA-dependent EPSPs in the mPFC (Gee et al. 2012).

In the present study we investigated the synaptic mechanisms sustaining fast network oscillations in the mPFC. We showed that the major difference between the ventral and dorsal mPFC networks lies on the NMDA receptor component. Accordingly, fast network oscillations in the ventral mPFC rely significantly on NMDA receptor activation, whereas network oscillations in the dorsal mPFC are independent. Therefore, we cannot rule out the possibility that DA's regional effect could be linked to the NMDA-dependent component difference. This hypothesis could be tested with the selective blockade of NMDA receptors and the concurrent activation of DA receptors.

7.6. Evidence that the dorsal peduncular cortex drives oscillations in the infralimbic cortex

Until the mid-80s, the agranular cortical area lying dorsal to the trilaminar tenia tecta and ventral to the PrL region was defined as the IL cortex (Figure 1.1; Krettek and Price 1977; van Eden and Uylings 1985). However, more recently a new delineation was adopted which divided the ventral mPFC into the IL region (dorsal portion) and the DP region (ventral portion). A search in the *PubMed* website gave us 586 results for the "infralimbic cortex" and only 50 results for the "dorsal peduncular cortex". Among the studies that came up in the "dorsal peduncular cortex" search, the majority of them simply referred to the DP in the figure legends. It is therefore apparent that the DP region has not attracted much scientific interest in comparison to the adjacent IL region which along with the PrL region, has been the main focus of behavioural and electrophysiological research, over the last decades.

Results from the present study reveal that neuronal populations localized in the DP region sustain the strongest and most rhythmic network oscillations of any region in the mPFC. Moreover we demonstrated that the IL produces the second strongest oscillations. By taking into account the importance of the PV expressing basket cell interneurons in the generation of fast network oscillations (Sohal et al. 2009), one would expect that these two regions would express the highest density

of PV+ cells. Surprisingly though, the IL presents the lowest expression of the PV protein in the mPFC (Figure 1.1C; Jones et al. 2005). Moreover, phase-synchrony analysis revealed evidence of strong network interactions between the two regions, which is in agreement with anatomical evidence of strong afferent projections arriving at the IL from the DP region (Fisk and Wyss 1999; Jones et al. 2005). Overall the evidence reported in this thesis, could suggest that oscillations recorded in the IL region were driven by network oscillatory activity in the adjacent DP region. This theory could be tested with parallel recordings from the DP and IL regions, following either an anatomical separation between them, or focal application of TTX on the DP region.

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