Sensory Gating in the Hippocampus and the medial Prefrontal Cortex

by

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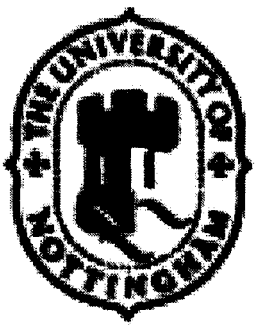

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Abstract

Sensory gating is a mechanism by which irrelevant sensory information is filtered in the brain, enabling efficient information processing. The auditory conditioning-test paradigm, an index of sensory gating, measures the reduction in the auditory-evoked response (AER) produced by a test stimulus following an initial conditioning stimulus. Schizophrenic patients demonstrate a lack of attenuation of the test response measured in the P50 component of the cortical auditory-evoked potential. The N2/N40 auditory-evoked potential recorded from rat hippocampus is considered homologous to the human P50 wave. Altered glutamatergic neurotransmission and the endocannabinoid system have been implicated in the pathogenesis of schizophrenia with structural and functional abnormalities in the hippocampus and the medial prefrontal cortex (mPFC).

The current study examined sensory gating using auditory conditioning-test paradigm in the dentate gyrus (DG) and the CA3 region of the hippocampus and in the medial prefrontal cortex (mPFC) before and after administration of N-Methyl-D-Aspartate (NMDA) receptor antagonist phencyclidine (PCP; 1mg/kg, i.p) or the cannabinoid agonist WIN55,212-2 (1.2mg/kg, i.p).

Electrophysiological recordings were conducted in Lister hooded rats, under isoflurane anaesthesia, during the presentation of paired auditory stimuli. Extracellular action potential spikes and local field potentials (LFPs) were recorded simultaneously using multi-electrode arrays and the effects of acute administration of PCP (1mg/kg, i.p) or WIN55,212-2 (1.2mg/kg, i.p) was determined. Gating of the N2 wave was assessed by measuring the ratio of the Test to Conditioning response amplitude (T/C ratio); T/C ratio ≤ 50% was indicative of gating.

Robust auditory-evoked potentials were recorded from the hippocampal CA3 and DG regions and the mPFC; some rats demonstrated auditory gating while others failed to. In rats that demonstrated gating of N2, mPFC showed higher

T/C ratios and shorter conditioning response latencies compared to DG and CA3.

PCP disrupted auditory gating in all three areas with a significant increase in test response amplitudes in the gating rats. PCP had no effect on T/C ratios in the non-gating rats. The atypical antipsychotic clozapine (5mg/kg, i.p) prevented PCP induced disruption of gating in the CA3, DG and mPFC. WIN55,212-2 disrupted auditory gating with a significant increase in test response amplitudes in the gating rats. WIN55,212-2 had no effect on T/C ratios in the non-gating rats. The cannabinoid receptor (CB1) antagonist SR141716A (1mg/kg, i.p) prevented WIN55,212-2 induced disruption of gating. Neither clozapine nor SR141716A had any effects on the non-gating rats. Both PCP and WIN55,212-2 disrupted gating of the single-unit responses in the CA3, DG and mPFC, effects which were prevented by the pre-administration of clozapine or SR141716A.

The non-gating rats may model some inhibitory deficits observed in schizophrenic patients. Administration of PCP disrupted auditory gating which was prevented by clozapine; similar deficits are observed in schizophrenic patients. Furthermore, cannabinoid receptor activation disrupted auditory gating which was prevented by CB1 receptor antagonism, suggesting the endocannabinoid system as a potential target for future clinical research in the treatment in schizophrenia.

Publications

Abstracts

- Dissanayake, W.D.N., Zachariou, M., Marsden, C.A., Mason, R., (2008) CB1 receptor mediated disruption of sensory gating in the rat hippocampus and medial prefrontal cortex, J. Psychopharmacol., 22 (5) MB 20.

- Dissanayake, W.D.N., Zachariou, M., Marsden, C.A., Mason, R., (2007) Effect of phencyclidine on auditory evoked potentials in the rat medial prefrontal cortex and hippocampus, J. Psychopharmacol., 21 (7) ME05.

- Dissanayake, W.D.N., Marsden, C.A., Mason, R., (2006) Effects of phencyclidine on hippocampal sensory gating under isoflurane anaesthesia in the rat, Proc Brit J Pharmacol. www.pA2online.org.

- Dissanayake, W.D.N., Zachariou, M., Marsden, C.A., Mason, R., (2006) Abolition of sensory gating by the cannabinoid WIN55,212-2 in the rat hippocampus, J. Psychopharmacol., 20 (5) A45.

Oral presentations

- Dissanayake, W.D.N (2008) CB1 receptor mediated disruption of sensory gating in the rat hippocampus and medial prefrontal cortex, Lilly Fellowship Oral Presentation – British Association of Psychopharmacology meeting.

Manuscripts

- **Dissanayake, W.N.D.,** Zachariou, M., Marsden, C.A., Mason, R., (2008) Effects of phencyclidine on auditory gating in the rat hippocampus and the medial prefrontal cortex, submitted to Brain Research.

- **Dissanayake, W.N.D.,** Zachariou, M., Marsden, C.A., Mason, R., (2008). Auditory gating in rat hippocampus and medial prefrontal cortex: Effect of the cannabinoid agonist WIN55,212-2, Neuropharmacology, Article in Press, doi:10.1016/j.neuropharm.2008.08.039.

- Zachariou, M., **Dissanayake, W.N.D.**, Owen, M.R., Mason, R., Coombes, S., (2008) Sensory gating and its modulation by cannabinoids: electrophysiological, computational and mathematical analysis. Cognitive Neurodynamics, 2: 159–170.

- Zachariou, M., **Dissanayake, W.N.D.**, Owen, M.R., Mason, R., Coombes, S., (2007) The Role of Cannabinoids in the Neurobiology of Sensory Gating: A firing rate model study Neurocomputing 70: 1902-1906.

Dedication

To Dheera, Isuri and Dilini for their love and enduring patience

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Abbreviations

AER	Auditory-evoked response
AERs	Auditory-evoked responses
AMPA	amino-3-hydroxy-5-methyl-isoxazole-4-proprionic acid
ANOVA	Analysis of variance
CT	Computer axial tomography
CAMP	Conditioning response amplitude
CB	Cannabinoid
CLAT	Conditioning response latency
CLOZ	Clozapine
Cs	Conditioning stimulus
DG	Dentate Gyrus
EEG	Electroencephalography
fMRI	functional magnetic resonance imaging
GABA	Gamma-aminobutyric acid
LFP	Local Field Potential
mPFC	medial prefrontal cortex
NMDA	*N*-methyl *D*-aspartate
PCP	Phencyclidine
PET	positron emission tomography
PFC	Prefrontal cortex
PPI	Prepulse inhibition
SR	SR141716A
TAMP	Test response amplitude
Δ^9–THC	Δ^9 tetrahydrocannnabinol
TLAT	Test response latency
Ts	Test stimulus
WIN	WIN55,212-2

Chapter One

General Introduction

1. General Introduction

This PhD study focuses on sensory gating, a process by which the central nervous system filters incoming sensory stimuli and prevents 'flooding' of the brain with irrelevant or repetitive information. Filtering of unnecessary information is crucial for efficient information processing. Sensory gating can be measured reliably by examining the stimuli-evoked brain responses using electrophysiology. Studies on sensory gating have attracted both psychiatrists and neuroscientists as this process is altered in several psychiatric disorders, the most well known of which is schizophrenia. Studying sensory gating may lead to a better understanding of the mechanisms by which the brain filters, prioritises and processes relentless information received via all the senses. Moreover, it is possible that sensory gating may eventually be used as a diagnostic tool by psychiatrists and assist in new discoveries relating to the pathogenesis and treatment of schizophrenia. To examine the mechanisms of sensory gating and to test possible treatment regimens, it would be desirable to reproduce the phenomenon in laboratory animal models which will assist in examining neural substrates of these mechanisms even at cellular level. In this study we sought to reproduce the sensory gating phenomenon in the rat by examining this process at the level of both local field potentials and single-units in two brain areas (i.e. hippocampus and prefrontal cortex) implicated in the pathogenesis of schizophrenia. We also sought to determine if sensory gating could be disrupted via pharmacological interventions in order to model the general deficits in information processing and the specific deficits in sensory gating observed in schizophrenia.

1.1 Sensory gating

Sensory gating has been conceptualized as a continuously active process, contributing to an individual's ability to modulate a continuous stream of sensory and cognitive information (Adler et al., 1982; Light & Braff, 2003). This process allows the central nervous system to selectively attend to important stimuli while ignoring redundant, repetitive and trivial stimuli. Sensory gating allows for the identification of the occurrence of a stimulus, the assessment of its importance and the elicitation of a response. Response generation occurs to novel or changing stimuli ("gating-in") whereas suppression of responding ("gating-out") occurs when a stimulus is redundant or repetitive (Boutros et al., 1999; Cromwell et al., 2005). Sensory gating has been observed with different sensory modalities, including olfactory (Best et al., 2005; Murakami et al., 2005), somatosensory (Thoma et al., 2007), Visual (Bless et al., 2007) and auditory (Adler et al., 1982; Freedman et al., 1996; Boutros et al., 1999; Cadenhead et al., 2000,2005). The gating process is most commonly assessed in both humans and animals by examining the brain responses to auditory stimuli using the auditory conditioning-test paradigm. The experiments discussed in this thesis utilized the auditory-conditioning-test paradigm to assess sensory (i.e. auditory) gating in the rat brain.

1.1.1 Auditory conditioning-test paradigm

A widely used quantitative measure of a subject's ability to filter sensory information is the amplitude ratio of the auditory-evoked responses, recorded

electrophysiologically, to two click stimuli presented 500 ms apart (Fig. 1.1). Sensory gating is operationally defined as the ratio of the amplitudes of the responses to the second (Test: T) stimulus to the first (conditioning: C) stimulus (T/C x 100%). A ratio <100% indicates suppression of the second response and a ratio of >100% indicates enhancement of the second response. Normal subjects have a smaller response to the second, or test click, than to the first, or conditioning click. Lower ratios reflect stronger attenuation of irrelevant input and thus better sensory gating.

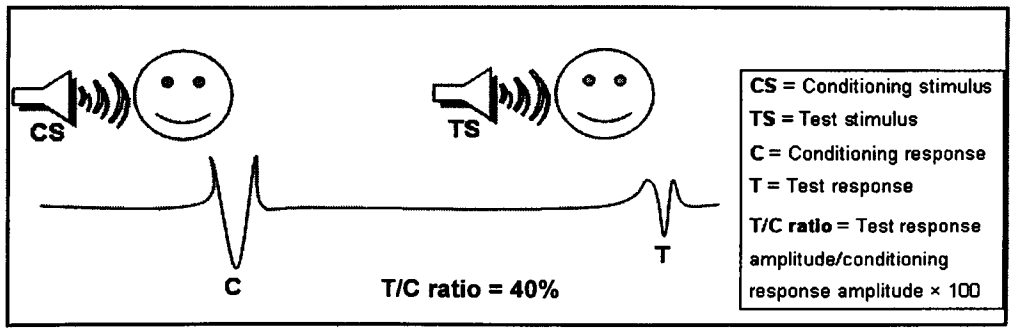


Fig 1.1 Illustration of the auditory conditioning-test paradigm demonstrating the reduction of the response to the test stimulus (T) compared to the response to the identical conditioning stimulus (C) resulting in a T/C ratio <100%

Auditory-evoked responses in the brain

The auditory-evoked potential responses (AER) are recorded in humans using an electroencephalogram (EEG), the recording of electrical activity in the brain obtained from electrodes placed on the scalp. AERs consist of a series of peaks and troughs in the EEG, reflecting the response to an auditory stimulus such as a tone or a click (Fig 1.2). Most evoked potentials are generally of low amplitude relative to background brain activity and are not clearly evident in the EEG

record. One strategy employed to overcome this problem is to present the subject with a series of identical stimuli and use the average evoked activity. This method removes random background activity, resulting in the clear visualization of evoked potentials (de Bruin et al., 2003). AERs can be used to examine successive stages of information processing in the presence of an external stimulus. AERs occurring within 200 ms after the onset of stimulus are called exogenous or stimulus-driven responses, as they represent early stages of information processing and are mainly driven by the physical characteristics of the stimulus. Responses' occurring after 200 ms are called endogenous or concept-driven, as the latter part of the process is based on the cognitive and emotional importance of the stimulus (Boutros et al., 2004; Neuchterlein & Dawson, 2005). AERs are divided into three components depending on the latency i.e. duration of time from the stimulus to the response: (1) a putative brain stem response (responses within the first 10 milliseconds), (2) mid-latency components (from 10-200 ms) and (3) long latency components (responses after 200 ms; Boutros et al., 2004; Neuchterlein & Dawson, 2005). Two particular mid-latency AERs have been studied in a number of studies assessing sensory gating. These two components are the P50 (P1) wave, a positive component seen approximately 50 ms after auditory stimulation, and the N100 (N1), a negative component seen approximately 100 ms after the stimulation (Freedman et al., 1983; Nagamoto et al., 1989; Boutros et al., 1999; Rosberg et al., 2000; Rentzch et al., 2008[a], 2008[b]). Two positive waves have also been described in the long latency phase, P200 (P2) and P300 (P3), occurring after 200 ms and 300 ms, respectively (Boutros et al., 2004; Rentzch et al., 2008[a], 2008[b]).

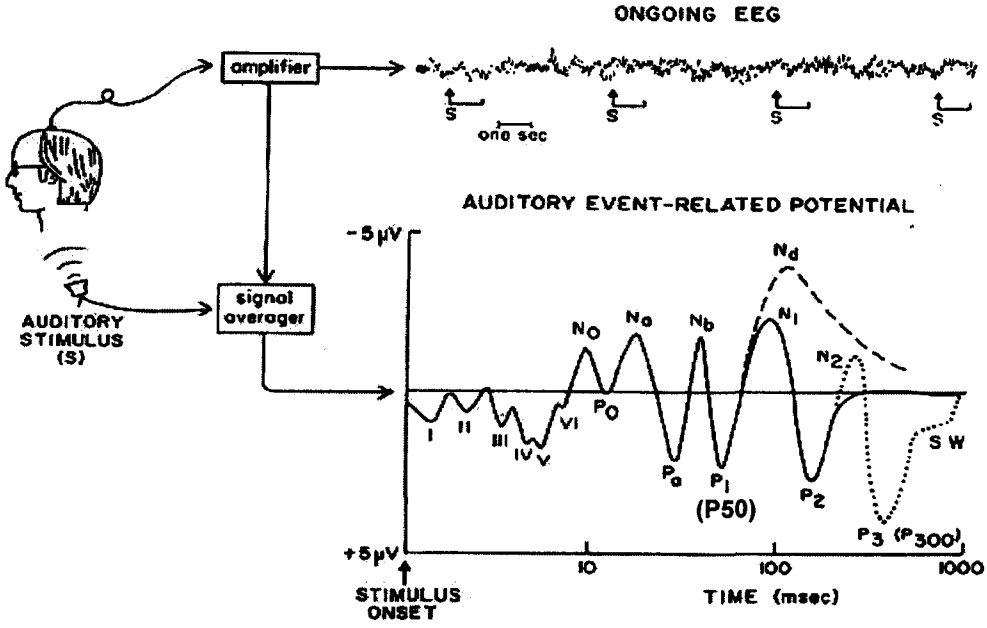


Fig 1.2 Recording of auditory-evoked responses using scalp electrodes. The amplified EEG is averaged to examine AERs, a series of positive and negative waves; P1 (P50), the second positive wave in the mid-latency range is preceded by the Pa and Nb waves and followed by N1, P2, N2 and P3 waves (modified from de Bruin et al., 2003).

The auditory-evoked responses can be better studied by recording from intracranial electrodes, a method more feasible in animals than in humans. Intracranial electrodes not only allow recording from targeted areas in the brain and facilitate examination of the local field potentials (LFPs) but also useful in recording of single-unit activity extracellularly (Bullock, 1997). Local field potentials are vector sums of the intercellular (i.e. synaptic) currents of the nerve cells in the vicinity of the recording electrode tip and single-unit activity represents the discharge of action potentials from post synaptic neurons (Bullock, 1997). A negative auditory-evoked LFP response occurring around 30-80 ms recorded from the skull electrodes and also from the CA3 region of the

hippocampus in rats demonstrate properties similar to the human P50 wave (Adler et., 1986; Bickford et al., 1990). Adler and colleagues named this wave as N50 and later was commonly identified as N40 or N2 by the same research group and other researchers (Adler et., 1986; Bickford et al., 1990; Stevens et al., 1997; Van Luijtelaar et al., 1998, 2001; de Bruin et al., 2001[a]).

1.1.2 Basic properties of auditory gating

Gating of the scalp recorded AERs in both the mid-latency (P50, N100) and long-latency range (P200, P300) has been observed in healthy adult humans with the T/C ratio for the P50 (P1) wave observed to be less than 40% (Adler et al., 1982; Kisley et al., 2001, 2004). The P50 wave is the most commonly used AER in clinical and pre-clinical research since gating of the P50 is unaffected by the level of attention (Adler et al., 1982) and less altered by the behavioural states such as N-REM sleep or passive wakefulness compared to the other AERs (Kisley et al., 2001). Moreover, P50 gating is affected by psychological status such as stress (Jonson and Adler., 1993; Ermutlu et al., 2005), neoropsychiatric diseases (see section 1.1.4) and by some psychoactive drugs (see below) which has led P50 auditory gating to become a reliable and a consistent measure of the intact sensory filtering process. The N40 (N50/N2) wave has also been shown to demonstrate gating in both freely moving and anaesthetized rats (Adler et., 1986; Bickford et al., 1990, 1993; Boutros et al., 1997; Van Luijtelaar et al., 2001). The N40 gating is not altered during N-REM sleep or passive wakefulness (van Luijtelaar et al., 1998) and is sensitive to pharmacological manipulations (Adler et., 1986; Bickford et al., 1990; Miller et

al., 1992; de Bruin et al., 2001; Krause et al., 2003) similar to the finding of the human P50 wave, making it an ideal animal model to examine the auditory gating process.

The effect of age and gender on sensory gating has been the focus of some studies on auditory gating. The gating of the P50 wave has been shown to mature with age with higher T/C ratios in the early childhood and improved gating with age (Freedman et al., 1991; Marshall et al., 2004; Brinkman and Stauder, 2007). The findings of the above studies were correlated to the immaturity of the pre-frontal cortex in early childhood which was proposed to be an important area mediating auditory gating (Freedman et al., 1991; Marshall et al., 2004). Marshall et al (2004) reported a negative correlation between age and the test response amplitude in children between 7-13yrs and adult level gating by 11-13 yrs of age. Kisley et al (2003) studied auditory gating in infants of 1-4 months during REM sleep and found intact gating with improved T/C ratios with age even in infancy. Brinkman and Stauder (2007) demonstrated that sensory gating improves with increases in the response to the conditioning stimulus with age and reaches the adult level at the age of 8yrs. Hetrick et al (1996) reported that women have higher response amplitudes and less sensory gating compared to men. However, later studies found no significant differences in amplitudes or T/C ratios between men and women (Rasco et al., 2000; Brinkman and Stauder, 2007).

Parametric studies examining the effects of stimulus properties on auditory gating are considered useful to establish the consistency, specificity and

stability of this measure. The changes in the intensity of the sound or the duration of the click do not seem to affect gating of the P50 wave in the conditioning-test paradigm (Griffith et al., 1995; Yee and White, 2001; Patterson et al., 2008). Furthermore, studies have shown that the frequency or the type (mixed or pure tone) of the click do not affect the P50 properties and gating (Boutros and Belger., 1998; Ninomiya et al., 2000). Meta analysis of the P50 gating studies by de Wilde et al (2007) and Patterson et al (2008) show that between studies differences in the P50 parameters and associated gating are dependent on the intensity of the auditory stimuli. Based on their findings, Wide et al (2007) suggested the use of stimuli between 85 – 90dB intensity to obtain maximum gating in normal individuals. However, between-groups gating differences were also attributed to other methodological factors such as response filtering parameters (Wilde et al., 2007; Patterson et al., 2008). The N40 auditory evoked responses with changes in the loudness, duration and frequency of the auditory stimuli have been studied in freely moving rats (Boutros et al., 1997; Zhou et al., 2008) and the gating ability has been shown to be persistent irrespective of the differences in the characteristics of the stimuli as long as the second stimulus did not carry any new information (Boutros et al., 1997; Zhou et al., 2008). Studies assessing the reproducibility of the P50 (Boutros et al., 1991; Fuerst et al., 2007; Rentzsch et al., 2008) and N40 waves (Boutros and Kwan, 1998) have indicated the usefulness of studying P50 and N40 gating as measures of intact information processing in the brain.

Several studies over the past two decades have demonstrated that the gating ability of the P50 in humans and N40 (N50/N2) in rats vary with inter-stimulus

interval (ISI). In healthy human subjects, who exhibit normal gating at ISIs of 500-2000ms with maximal gating at 500ms (Adler et al., 1982), show a complete absence of gating with ISIs of 6s (Adler et al., 1982) or 8s (Zouridakis et al., 1997).The N40 response in rats varies with ISI with maximum gating at ISI = 500 ms, correlating with the findings of human studies (Adler et al., 1986; Bickford et al., 1993; Krause et al., 2003). These results indicate that gating occurs only when two identical stimuli are presented within a short time interval.

1.1.3 Neurobiology of auditory gating

The exact neurophysiological mechanism responsible for auditory sensory gating is not fully understood but was initially proposed to be closely linked to habituation, a process involved in synaptic plasticity (Adler et al., 1982; Boutros and Belger, 1999[b]; Rosberg et al., 2000). Habituation is a form of non-associative learning which occurs when a stimulus is repeatedly presented, resulting in a gradual reduction of the response to the stimulus. If habituation is the mechanism behind sensory gating, the process would depend on the development of refractory periods. Furthermore, the amplitude of the test response would depend on the recovery status of the neuronal pool stimulated by the conditioning stimulus. However, refractoriness of sensory neurons is unlikely to be the sole mechanism behind gating of stimuli presented at an inter-stimuli interval of up to 1-2s, since most neurons have refractory periods of only a few milliseconds (Boutros and Belger, 1999[b]).

Recent studies implicate that gating of auditory stimuli involves multiple

neurotransmitter systems and recurrent inhibitory and excitatory circuits with endogenous and exogenous inputs to the brain areas mediating auditory gating (Miller and Freedman 1993, 1995; Flach et al., 1996; Moxon et al., 1999, 2003[a,b]). These studies suggest that the conditioning stimulus activate both inhibitory and excitatory circuits and the inhibitory activity act as comparators to diminish the excitatory response to the identical test stimulus presented while the inhibitory circuits are still active (Miller and Freedman., 1995; Boutros and Belger, 1999). Several neurotransmitter systems have been implicated to play a part in the auditory gating process. Both dopamine and glutamate mechanisms are postulated to produce the excitatory responses to the conditioning stimuli while GABAergic and cholinergic mechanisms modulate the reduction in the excitatory response to the test stimulus (Flach et al., 1996; Moxon et al., 2003). Pharmacological studies suggest further involvement of serotonin (Adler et al., 2005; Mann et al., 2008), noradrenaline (Miller et al., 1992; Stevens et al., 1991, 1993; Adler et al., 1994), adenosine (Ghisolfi et al., 2002), corticosterone (Stevens et al., 2001) and endo-cannabinoids (see 1.5.2) in the auditory gating process highlighting the complexity of the interactions between multiple neurotransmitters and neuro-modulaters in this fundamental sensory processing.

1.1.3 Neural substrates of auditory gating

The exact anatomical sites involved in mediating sensory gating in humans have not yet been established due to the limitations in performing studies using intra-cerebral electrodes. Grunwald et al. (2003), in a study using intracranial

electrodes in epilepsy patients undergoing invasive pre-surgical evaluation, showed that the hippocampus, prefrontal cortex and tempo-parietal cortex contribute to sensory gating. They further suggested that the early phase in sensory gating is subserved by the temporo-parietal and prefrontal cortices, whereas the later phase is mediated by the hippocampus. In another recent study the same group demonstrated gating of auditory responses in the cortical areas responsible for initiating movements (supplementary sensory motor area) and top-down control of sensory input (lateral PFC) and also emphasized that the late responses from hippocampus might represent later components of the multistage sensory gating process (Kurthen et al., 2007). Functional imaging studies have suggested involvement of the hippocampus, thalamus, prefrontal cortex and the enterohinal cortex in the auditory gating process (Tregellas et al., 2007). Higher response amplitudes and abnormal auditory gating were detected in patients who sustained prefrontal lesions (Knight et al., 1989) indicating the importance of the area in the gating process.

Studies on sensory gating in both anaesthetized and freely moving rats have provided more insight to the areas involved in the gating of auditory responses. An early study by Bickford et al (1990), using intra-cerebral electrodes in rats under chloral hydrate anaesthesia, demonstrated that the CA3 region of the hippocampus had the maximal amplitude of N40 wave with an identical latency recorded from the scalp electrodes. Based on findings of the study, they proposed the CA3 region of the hippocampus as the generator of the N40 wave (Bickford et al., 1990). This was confirmed by recording from multiple brain sites including the frontal cortex, auditory cortex, medial geniculate body, medial

septum and brain stem (Bickford et al., 1990). All these areas demonstrated auditory evoked responses: N20 and P30 waves were seen in the auditory cortex and medial geniculate body, a large N20 wave was recorded from the pontine reticular formation and small N40 waves were recorded from the medial septum and the frontal cortex. The largest amplitude N40 wave accompanied by a significant reduction in the test amplitude was recorded only from the CA3 region, emphasizing the dominant role played by the hippocampus in sensory gating. Miller and Freedman (1993) examined the single unit activity in the medial septum in response to the auditory conditioning- test paradigm and demonstrated gating of single-unit responses in the medial septum. They also suggested that the medial septum exerts a direct cholinergic modulatory effect on the auditory gating in the CA3 region of the hippocampus.

Another study by Bickford and colleagues demonstrated sensory gating in the brain stem pontine region, which also exerted a modulatory effect on hippocampal processing (Bickford et al., 1993). However, they failed to detect sensory gating in the inferior colliculus or the thalamus (Bickford et al., 1993). This suggested the presence of a separate auditory pathway (non-leminiscal) of sensory gating as opposed to the direct (leminiscal) auditory pathway (Fig 1.3). Moxon et al (1999) studied auditory gating from the brain stem, medial septum, CA3 and the auditory cortex, brain areas in the leminiscal and non-leminiscal pathways, in freely moving rats and demonstrated that gating ability was confined to the non-leminiscal auditory pathway. It has been proposed that CA3 region integrates the gated information received via the non-leminiscal pathway and the non-gated information received via the leminiscal pathway to mediate

ultimate gated responses (Miller and Freedman, 1993; Moxon et al., 2003)

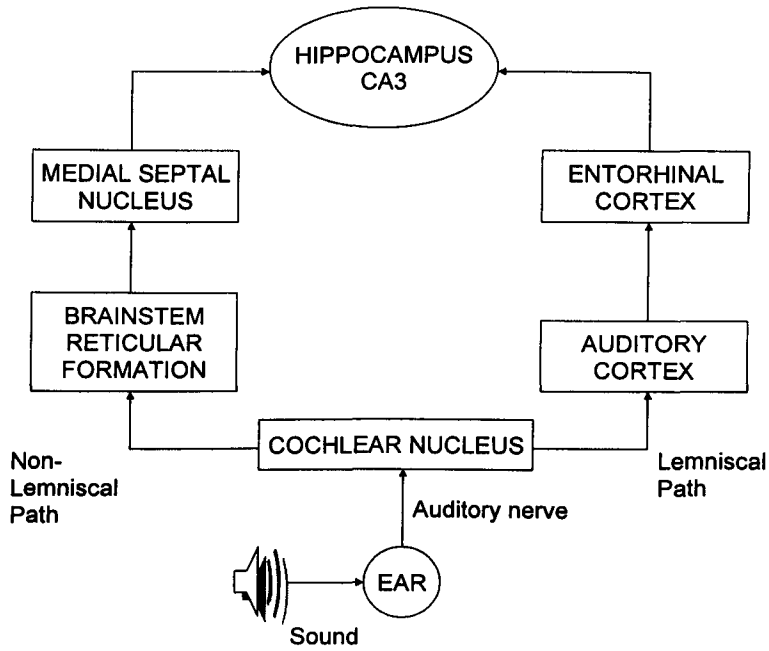


Fig 1.3 Leminiscal and non-leminiscal auditory pathways (modified from Moxon et al., 1999)

Recent studies have found the involvement of other brain areas such as the reticular thalamus (Krause et al., 2003), amygdala (Cromwell et al., 2005), medial prefrontal cortex (Mears et al., 2006), striatum (Cromwell et al., 2007) and midbrain (Anstrom et al., 2007), expanding the auditory sensory gating circuits beyond the conventional non-leminiscal pathway.

The current study examined auditory gating at both LFP and single-unit level in the CA3 and dentate gyrus of hippocampus and the medial prefrontal cortex in the rat brain.

1.2 Hippocampus

The hippocampus is both downstream and upstream of essentially all the cortical association regions and is considered as an essential part of multi-modal sensory integrating system in the CNS (Sweatt, 2004). Both human and rat studies have confirmed the important role played by the hippocampus in mediating the sensory gating process.

The hippocampus is located deep within the temporal lobe of humans and other primates. In rats it is a rostro-caudal structure occupying a large area of the brain providing easy access to electrophysiological recordings.

In both humans and rats, hippocampus is composed of two inter-connected parts known as the dentate gyrus and Ammon's horn. Ammon's horn has four sub-divisions in humans: CA1, CA2, CA3 and CA4 (CA: Cornu Ammonis; Hammond, 2001; Sweatt, 2004). Only two of these sub divisions, CA1 and CA3, are prominent in rats (Fig 1.4A). Both dentate gyrus and Ammons' horn contain two types of neurons which are principle cells and interneurones. Principle cells in Ammons' horn and dentate gyrus are known as pyramidal and granular cells, respectively. Pyramidal cells are located in discrete layers of Ammons' horn and the dentate gyrus and comprise around 90% of the neurons in the CA regions of the hippocampus (Sweatt, 2004). The pyramidal cells in Ammon's horn are located in stratum pyramidale, a discrete layer extending from CA1 to CA3, while interneurons are found in abundance in all layers of the hippocampus (Miller and Freedman, 1995).

The main internal circuit in the hippocampus is the tri-neuronal circuit, involving granular cells in the dentate gyrus and pyramidal cells in CA3 and CA1. The circuit originates from granular cells which send axon collaterals called mossy fibers to synapse with pyramidal cells in CA3 which, in turn, send collaterals called schaffer fibers to CA1 pyramidal cells (Fig 1.4 A). In addition, principle cells in all three regions form local recurrent excitatory circuits between each other and also form connections with interneurons to form recurrent inhibitory circuits (Fig 1.4 B). The hippocampal hemispheres are interconnected via the commissural pathway. Internal circuits connect the CA1 and CA3 with the dentate gyrus and other laminae in the hippocampus to modulate outgoing information according to the incoming signals (Hammond, 2001).

Principle cells are the main projection cells of the hippocampus which form both internal and external communicating channels. Dentate gyrus and CA3 receive efferents from the entorhinal cortex, medial septum and thalamus (Hammond, 2001; Moxon et al., 1999). The CA3 also projects to the medial septum. The CA1 is the main output area sending afferents to the sub cortical areas and prefrontal cortex (Sweatt, 2004; Tierney et al., 2004).

The extrinsic inputs to the hippocampus include projection fibers that are serotonergic, dopaminergic, cholinergic and noradrenergic (Flach et al., 1996; Moxon et al., 2003[a]; Gilbert and Burdette, 1995; Sweatt, 2004).The intrinsic connections between the pyramidal neurons in the hippocampal network are glutamatergic and the inhibitory interneurons are predominantly GABAergic

(Hammond, 2001; Gilbert and Burdette, 1995; Sweatt, 2004). Neuromodulatory peptides such as somatostatin, vasopressin, cholecystokinen, neuropeptide Y, BDNF and NGF are also found within the hippocampus sometimes co-localized with GABA (Gilbert and Burdette, 1995; Sweatt, 2004). The hippocampal interneurons have also been shown to contain a high density of cannabinoid receptors (Wilson and Nicoll, 2002; Iversen, 2003; Howlett et al., 2004; Straiker and Mackie, 2006). Functional significance of some of the neurotransmitters and neuromodulaters in relation to sensory gating in the hippocampus will be discussed later in the thesis; however, it is worth noting that all these neurochemicals are mandatory for hippocampal integrity and attendant cognitive processing.

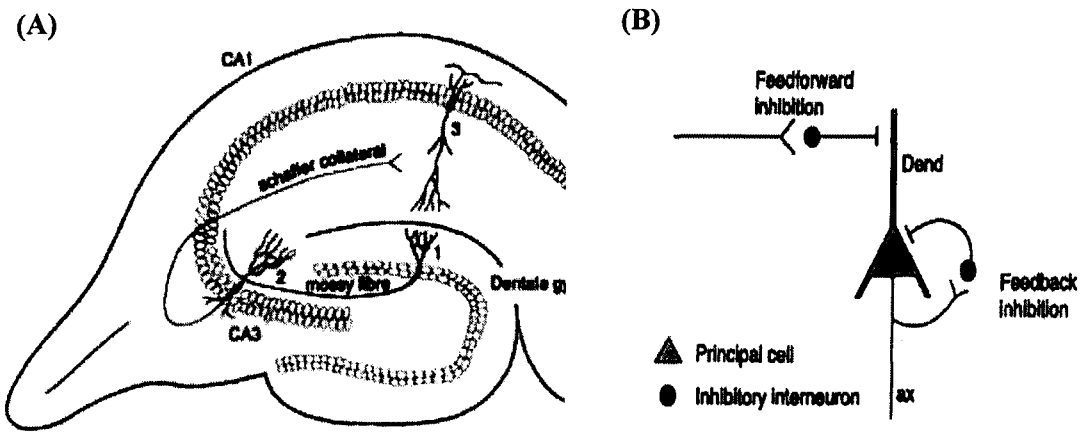


Fig. 1.4 (A) Schematic diagram of a coronal section of the rat hippocampus demonstrating the tri-neuronal circuit (1 = granular cells of DG, 2 = pyramidal cells of CA3, 3 = pyramidal cells of CA1) **(B)** illustration of feed-forward and feed-back inhibitory circuits between principle cells and interneurons (modified from Hammond, 2001).

1.3 Prefrontal cortex

The prefrontal cortex (PFC) is the most anterior area of the frontal lobes and is responsible for mediating executive functions, mainly the orchestration of thoughts and actions in accordance with internal goals. The human PFC consists of cytoarchitecturally and functionally distinct areas out of which the dorsolateral prefrontal cortex is suggested to be involved in the cognitive processes such as attentional tasks and working memory (Postle, 2005; Lewis et al., 2004; Hashimoto et al., 2008). Increased heamodynamic responses in response to the auditory conditioning-test paradigm have been reported in the human dorsolateral PFC indicating the involvement of the area in the auditory gating process (Tregellas et al., 2007).

The rat PFC is classically defined as the part of the frontal lobe with the strongest reciprocal connections with the mediodorsal nucleus of the thalamus (Thierry et al., 2000). According to the findings of some lesion studies, the rat medial prefrontal cortex (mPFC) demonstrates functional similarities to the human dorsolateral prefrontal cortex (Granon et al., 1994; Delatour and Gisquet-Verrier, 2000). These studies have reported deficiencies in cognitive processes involving attention and working memory in the mPFC-lesioned rats (Granon et al., 1994; Delatour and Gisquet-Verrier, 2000; Ostlund and Balleine., 2005). The mPFC of the rat is delineated into sub-regions; anterior cingulate area, pre-limbic area, inferior limbic area and medial orbital areas, (Fig.1.5) based on the architechtonic criteria and the anatomical relationship with distinct cortical and sub-cortical structures (Thierry et al., 2000; Gabbott et al., 2005).

The major neuronal population in the mPFC is the glutamatergic pyramidal cells comprising 70-80% of the neurons and the rest are GABAergic interneurons (Gabbott et al., 2005). Pyramidal cells are the main projection neurons from the mPFC and form main input and out put circuits with other cortical areas and the limbic system (Gabbott et al., 2005).

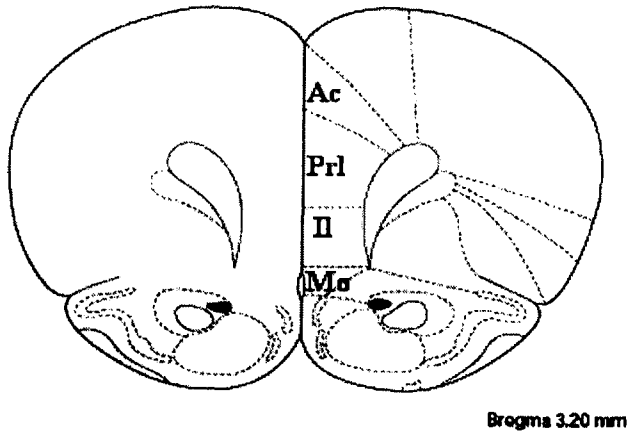


Fig. 1.5 A diagram of the coronal section of the rat brain demonstrating the sub-regions of the mPFC (**Ac** = anterior cingulate area, **Prl** = pre limbic area, **Il** = inferior limbic area, **Mo** = medial orbital area) (modified from Paxinos and Watson, 1997)

The anatomical and functional inter-connections between the hippocampus and the prefrontal cortex are considered to be crucial for higher order cognitive processes such as working memory and organization of behaviors (Floresco et al., 1997; Tiemey et al., 2004). The hippocampo-prefrontal pathway in rats originates from the CA1 and subbiculum of the hippocampus and innervates the pre-limbic and medial-orbital areas of the medial pre-frontal cortex (Jay et al., 1989; Thierry et al., 2000). The hippocampal pyramidal cells have been shown to have direct excitatory effects on both pyramidal cells and interneurons in the medial pre-frontal cortex thus facilitating cortical feedback and feed forward inhibitory and excitatory processes (Thierry et al., 2000; Tiemey et al., 2004,

Lewis et al., 2004). Despite of the direct and very important inputs from hippocampus to mPFC, no direct communications from mPFC to hippocampus have been found (Thierry et al., 2000; Vertes et al., 2007). However, reciprocal connections between the two areas are suggested, with some studies reporting enterohnial cortex, thalamus or the neucleus accumbens as the mediator of information from rat mPFC to the hippocampus (Grace, 2000; Vertes et al., 2007)

Both prelimbic area in the mPFC and the hippocampus have demonstrated auditory gating (see 1.1.3), thus the reciprocal connections between the two areas could be important in filtering out irrelevant information.

1.4 Auditory gating and neuropsychiatric dysfunctions

As mentioned above, normal subjects have a smaller response to the second (test) click compared to the first (conditioning) click, reflecting sensory gating. The test response of normal subjects is often <40% of their conditioning response, whereas the test response of schizophrenic patients is often >85% of their conditioning response (Kisley et al., 2001, 2004). P50 gating defects are also seen in patients with other neuropsychiatric illnesses such as bipolar disorder (Franks et al., 1983; Baker et al., 1987), post traumatic stress disorder (Ghisolfi et al., 2004; Neylan et al., 1999), panic disorder (Ghisolfi et al., 2006) and Alzheimer's disease (Jessen et al., 2001; Cancelli et al., 2006), indicating that a common feature of these disorders is a deficit in information processing. A dysfunction or imbalance in dopaminergic and cholinergic mechanisms is also

suggested as a common defect shared by the diseases with abnormal auditory gating. A sensory gating defect is considered a biological marker for schizophrenia, as studies have shown that P50 sensory gating abnormalities are present not only in patients but also in their first degree relatives (Waldo et al., 1988, 1994) and in people with schizotypal personality (Cadenhead et al., 2000).

1.5 Schizophrenia

Schizophrenia is a chronically disabling neuropsychiatric disorder that affects multiple aspects of cognition and behaviour, including attention, perception, thought process, emotion and volition (Harrison, 1997, 1999; Gainetdinov et al., 2001; Lewis and Levitt, 2002). This disease affects 1% of the world population without ethnic, educational or socioeconomic prejudice (Tamminga, 1997, 2003). The disease typically emerges in late adolescence or early adulthood and leads to lifetime disability in the majority of affected individuals (Harrison, 1997, 1999). Schizophrenia is characterized by positive symptoms, negative symptoms and neurocognitive deficits. Positive symptoms are features additional to normal experience and behaviour, such as delusions, hallucinations, disorganized speech and behaviour. Negative symptoms denote a lack or decline in normal experience and behaviour, such as emotional withdrawals, poor rapport and lack of drive. Neurocognitive deficits refer to cognitive and emotional dysfunction (i.e. memory impairment, attention deficits, impairment in executive functions) and are considered the core abnormalities of the disease which pave the way for the positive and negative symptoms, which,

in turn, are highly likely to reinstate the existing neurocognitive deficits, creating a vicious cycle of disease maintenance (Walters, 2005). The symptoms of the disease are attributed to imbalances or deficits in neurotransmitters such as dopamine, glutamate, serotonin and GABA (see 1.7)

In any disease, diagnosis based on symptoms should ideally be confirmed by using more specific objective means. This is not feasible in many psychiatric diseases, including schizophrenia, for which a globally acceptable standardized tool has yet to be implemented. However, potential tools including evoked potential recording are being used in research and show promise (Walters, 2005; Table1.1).

Table 1.1 Objective diagnostic tools useful for diagnosis of schizophrenia

Type of measurement	Tool used
Psychometric measurements	Thought disorder index in response to questions (TDI) Dysfunction in smooth pursuit eye movement (eye tracker)
Evoked potentials	Failure to inhibit auditory evoked responses to repeated stimuli (measured by electroencephalography (EEG) and magnetoencephalagraphy (MEG) Loss of prepulse inhibition (PPI) to startle reflex (PPI is a measure of inhibitory function, where a weak stimulus inhibits the startle response caused by a sudden intense stimulus) - measured by electromyogram (EMG) which measures the eye blink (muscle activity) component of the startle response
Brain Imaging - measures changes in the structure and function of the brain	Changes in regional blood flow by functional magnetic resonance imaging (fMRI), changes in binding or localization of emitting tracer by positron emission tomography (PET), Changes in structure by computer axial tomography (CT)

Antipsychotic drug treatment accompanied with psychosocial management is the mainstay of effective treatment of schizophrenia. Treatments with anti-psychotics aim to correct the imbalances or defects in the neurotransmitters hypothesized to be involved in the symptomatology of the disease and mainly target to antagonize central dopamine receptors (D1, D2, D3, D4 and D5; see 1.7.2). Typical antipsychotics such as chlorpromazine, fluphanazine, haloperidol, trifluroperazine and thioridazine antagonise dopamine D2 receptors (Tamminga, 1997). These drugs are effective in treating positive but not negative symptoms or neurocognitive deficits and also cause unwanted extrapyramidal side effects by blockade of dopamine receptors in the basal ganglia. Atypical antipsychotics, a group of drugs discovered more recently, are more effective in treating both positive and negative symptoms, are often effective in patients resistant to typical antipsychotics, and cause less extrapyramidal side effects (Tamminga 1997, 2003). Some examples of atypical antipsychotics are clozapine, resperidone, olanzapine, quetiapine and ziprasidone. In this PhD study the effects of clozapine on phencyclidine induced changes in auditory gating in the rat hippocampus and mPFC were examined.

1.5.1 Clozapine

Clozapine is the first of the atypical antipsychotics to be developed and so far is one of the most effective pharmacological treatments for schizophrenia (Tamminga, 1997). However, it is not used as a first line treatment due to the undesirable side-effects such as agranulocytosis, myocarditis, seizures, and cardio-respiratory effects.

Clozapine is a dibenzodiazepine derivative (Fig. 1.6; Tamminga, 1997). It is completely absorbed when used orally, but has a bioavailability of around 47% due to high first pass metabolism (Schaber et al., 1998). The effects of the drug can be observed from around 2 hrs, but takes about 7-10 days to reach a steady state plasma concentration following oral administration (Guitton et al., 1998). Clozapine is extensively metabolised in the liver and has an elimination half life of around 14hrs (Schaber et al., 1998; Guitton et al., 1998). When clozapine was administered systemically in animal studies, effects on the studied parameters were observed 10-15 min following administration (Bakshi et al., 1994; Swerdlow et al., 1998; Joy et al., 2004)

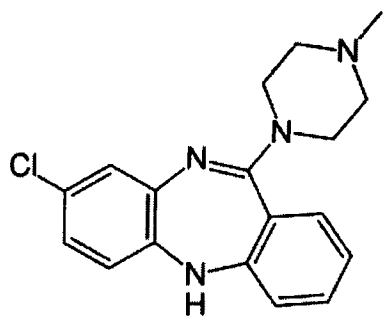


Fig. 1.6 Chemical structure of clozapine- 8-chloro-11-(4-methyl-1-piperazinyl)-5*H*-dibenzo(b,e)(1,4)diazepine

A systemic review by Wahlbeck et al (1999) comparing the effects of clozapine with typical antipsychotic treatments confirms that clozapine is convincingly more effective than typical antipsychotic drugs in reducing symptoms of schizophrenia, producing clinically meaningful improvements and postponing relapse. Another systemic review by Aqid et al (2008) emphasizes that clozapine is superior even to other atypical antipsychotics in treating refractory

schizophrenia. However, Meltzer and McGurk (1999) reviewing 12 clinical studies, reported that clozapine improves some cognitive functions such as attention and verbal fluency, but the effect on some executive functions such as working memory remains inconclusive.

Clozapine has been shown to improve auditory gating deficits in schizophrenic patients with superior efficacy compared to other atypical antipsychotics (Adler et al., 2004; Nagamoto et al., 1996; Light et al., 2000). Typical antipsychotics such as haloperidol do not improve gating deficits in schizophrenic patients (Nagamoto et al., 1996; Becker et al., 2004).

In animal studies clozapine reversed pharmacologically induced (e.g. phencyclidine) behavioural abnormalities resembling schizophrenic symptoms such as PPI abnormalities and deficits in tasks involving working memory (Bakshi et al., 1994; Linn et al., 2003; Grayson et al., 2007; Didrikson et al., 2007). However, the effects of clozapine on pharmacologically-induced deficits in sensory gating has not been examined except for one study in which acute and chronic administration of clozapine failed to prevent amphetamine induced auditory gating deficits in rats (Joy et al., 2004).

Clozapine has a very rich pharmacology with antagonistic effects on dopamine receptors (transient binding to D2 and higher affinity for D4; Seeman et al, 1997; Seeman, 2004), serotonin receptors (e.g. 5-HT2A, 5-HT2C, 5HT1A), noadrenergic receptors (ά1, ά2) and cholinergic receptors (nicotinic and muscarinic) extending throughout cortical and subcortical structures

(Tamminga, 1997; Seeman, 2004). Clozapine is also suggested to have a partial agonist effect on glutamate (NMDA) receptors (Tsai et al., 1999; Gray et al., 2008) and potentiate cortical GABA activity (Bragina et al., 2007; Daskalakis et al., 2008). The actions on D2 and 5-HT2A receptors have received a great deal of attention since some studies suggested the improved cognitive symptoms following clozapine treatment are associated with its greater 5-HT2A binding vs. D2 binding (Meltzer et al., 1996; Remington, 2003). Some authors have attempted to explain the actions of clozapine based only on its selectivity and affinity for dopamine receptors. These reports suggest that transient binding to D2 receptors followed by rapid dissociation to allow normal DA transmission and equal binding to striatal D1 and D2 receptors with possible partial agonist effects on D1 receptors, explain improved cognitive symptoms and lack of extrapyramidal side effects following clozapine (Kapur and Seeman, 2001; Tauscher et al., 2004). Recent studies have suggested the importance of the agonist effects of clozapine on glutamate and GABA receptors in relation to its antipsychotic efficacy (Tsai et al., 1999; Gray et al., 2008; Shim et al., 2008; Bragina et al., 2007; Daskalakis et al., 2008). Based on the findings of the above studies, it is evident that no unitary pharmacological mechanism can account for the wide range of actions of clozapine.

1.6 Auditory gating and schizophrenia

The auditory gating deficit in schizophrenia (Fig. 1.7) is suggested to have a genetic association and considered a potential endophenotype of the disease (Siegel et al., 1984; Clementz et al., 1998; Waldo et al., 2000)

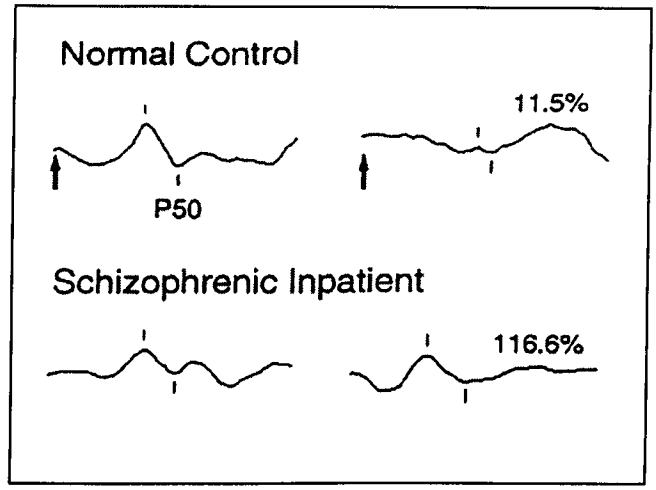


Fig. 1.7 P50 responses to auditory conditioning-test paradigm demonstrating sensory gating in healthy subjects and disruption of gating in schizophrenics. Arrows indicate the stimulus and the waves marked by vertical ticks are P50 responses (modified from Flach et al., 1996).

Research on human P50 recording has failed to establish an exact clinical correlation to the sensory gating defects seen in schizophrenia, with some studies showing no correlation to negative symptoms (Adler et al., 1990) and others showing some correlation to both positive and negative symptoms (Cadenhead et al., 2000; Thoma et al., 2005). However, more recent studies have indicated a significant correlation between neurocognitive deficits and sensory gating defects measured by the auditory conditioning-test paradigm (Potter et al., 2006).

Apart from the gating deficits some studies on schizophrenia show that unmedicated patients exhibit smaller P50 amplitude and shorter P50 latency responses to conditioning stimuli, compared to controls (Adler et al., 1982;

Boutros et al., 2004).The decrease in the conditioning amplitude and latency seen in schizophrenia is hypothesized to result from a synaptic mechanism called occlusion, the decrease in the expected response due to presynaptic fibers sharing post-synaptic neurons. The hypothesis postulates that when a neuronal population is hyperactive, the constant background discharge makes it less likely that the majority of neurons will respond synchronously to the stimulus being studied, leading to smaller amplitudes in response to conditioning stimuli (Adler et al., 1982; de Bruin et al., 2003). This hyperactivity is proposed to be due to the lack of functioning inhibitory inputs in the schizophrenic brain. While treatment with typical antipsychotics (e.g. haloperidol) restores the conditioning amplitude and latency, it does not restore the T/C ratio (Nagamoto et al., 1996; Boutros et al., 1999[b]; Light & Braff, 2003).

Some encouraging results from studies on treatment strategies have shown that the gating deficit can be corrected by some pharmacological and behavioural interventions. Nicotine administration either by smoking or via a nicotine patch has been shown to briefly improve gating in schizophrenic patients (Adler et al., 1993; Griffith et al., 1998; De Luca et al., 2004). Brief improvement in gating deficits has also been detected following a period of N-REM sleep (Griffith et al., 1993). It has also been shown that atypical antipsychotics, especially clozapine, can improve P50 gating in schizophrenic patients (Nagamoto et al., 1996; Adler et al., 2004).

1.7 Auditory gating and animal models of schizophrenia

Although the specific causative factors that give rise to schizophrenia are not fully understood, there are several hypotheses actively being investigated which collectively suggest that schizophrenia is a multi-factorial disorder with genetic and neurodevelopmental components. Detailed study of the aetiology and treatment of schizophrenia necessitated the development of animal models of the disease. Lesion models based on neurodevelopmental hypotheses and pharmacological models, largely based on the theories on neurochemical abnormalities, have been extensively used to study the pathophysiology of schizophrenia. Since auditory gating deficit is considered a biological marker in schizophrenia (see 1.6), presence of gating deficits significantly enhances the validity of an animal model of the disease.

1.7.1 The Neurodevelopmental model

The neurodevelopmental hypothesis of schizophrenia posits that the illness results from either an early (during or after birth) static brain lesion with a longer latency until the appearance of clinical signs and symptoms, or a late (adolescence) brain disturbance of limited duration and short latency (Lewis and Levitt, 2002). This hypothesis is supported by some studies which have found abnormalities in the brain structure and function in the patients with schizophrenia. The enlargement of the ventricles accompanied by increased CSF volume (Lewis and Levitt, 2002; Smythies, 1998) and decreased cortical thickness of certain brain regions such as temporal lobe and prefrontal cortex

(Weinberger, 1987; Smythies, 1998) and disturbed cortical and sub-cortical cytoarchitecture such as maldistribution of cortical neurons, dysplasias of enterohinal cortex and disarray of hippocampal pyramidal neurons (Harrison, 1997,1999) are some of the findings from the postmortem and imaging studies of the schizophrenic brain. Volumetric MRI studies also reported reduction in cortical and hippocampal size in the patients with schizophrenia compared to controls (Nelson et al., 1998; Harrison, 1999). Functional abnormalities such as decreased regional blood flow and metabolic activity in the frontal and temporal lobes have also been detected in schizophrenics (Weinberger, 1987; Lewis and Levitt, 2002). The above studies suggest the involvement of specific brain areas such as the prefrontal cortex, ventral tegmental area, striatum, neucleus accumbens, amygdala, and hippocampus in the pathogenesis of schizophrenia.

Prefrontal cortex, which mediates cognitive and executive functioning, has long been implicated as a main dysfunctional site in the causation of the disease. Many studies have confirmed the involvement of prefrontal cortex in the pathogenesis of schizophrenia (Weinberger et al., 1987; Yu et al., 2004). Humans with prefrontal lesions demonstrate abnormal P50 responses and gating deficits similar to those observed in schizophrenic patients (Knight et al., 1989). Animals with PFC lesions have been shown to exhibit impairments in working memory tasks and learning resembling cognitive deficits in schizophrenia (Granon et al., 1994; Delatour and Gisquet-Verrier, 2000; Ostlund and Balleine., 2005; Kinoshita et al., 2008)

As discussed above some human studies have shown functional and anatomical defects in the hippocampus in schizophrenic patients. Lesion studies in animals have demonstrated behavioural abnormalities and abnormalities in working memory and attention, similar to the deficits seen in schizophrenic patients (Lipska et al., 2002; Adams et al., 2008). Abnormal auditory gating, resistant to typical antipsychotic treatment, was observed in rats with hippocampal lesions induced by kainic acid and the deficits were correlated to the neuronal loss in the CA3 and hilar regions of the hippocampus (Stevens et al., 1998).

Examining the sensory processing in the hippocampus and prefrontal cortex is thus validated not only due to their potential importance in the auditory gating process but also due to the involvement in the pathogenesis of schizophrenia.

1.7.2 The pharmacological model

Neurotransmitters have long been thought to be involved in the development of schizophrenia. It is likely, although not yet certain, that the disorder is associated with some imbalance of the complex, interrelated transmitter systems of the brain involving one or more of dopamine , glutamate, serotonin, acetylcholine and gamma-aminobutyric acid (GABA). Though, all these neurotransmitter systems could equally be important in the pathogenesis of the disease, the dopamine hypothesis and the glutamate hypothesis have received a great deal of attention specially in developing animal models of the disease.

The dopamine hypothesis

The dopamine hypothesis of schizophrenia postulates a dysregulation of dopaminergic neurotransmission in the CNS. The main dopaminergic pathways described in the mammalian brain are the nigrostriatal system which projects from the substantia nigra of the mid brain to the basal ganglia (striatum), the mesolimbic system which projects from the midbrain ventral tengmental area to the nucleus accumbens, the mesocortical system projects from the midbrain ventral tegmental area to the prefrontal cortex and the tubero-infundibular system projects from hypothalamus to the anterior pituitary (Dargham et al., 2003).

According to the dopamine hypothesis, subcortical mesolimbic dopamine projections are hyperactive, resulting in hyperstimulation of D_2 receptors and giving rise to positive symptoms, whereas mesocortical dopamine projections to the prefrontal cortex are hypoactive, resulting in hypostimulation of D_1 receptors and giving rise to negative symptoms and cognitive impairment (Davis et al.,1991; Dargham et al., 2003).

As mentioned above, neuroleptic blockade of central D2 receptors improves symptoms of schizophrenia, whereas psychomimetics (e.g. apomorphine, amphetamine) which potentiate dopamine effects in the CNS, produce positive symptoms and attention deficits similar to those seen in schizophrenia in otherwise healthy individuals (Mcketin et al., 1999; Marcotte et al., 2001; Moxon et al., 2003). However, the ability of dopamine agonists to mimic schizophrenic like behaviour in experimental animals is a subject of debate. Apomorphine

disrupts PPI in animals and the disruption can be reversed by haloperidol (Swerdlow et al., 1998; Linn et al., 2003). Amphetamine has also been shown to produce auditory gating deficits in both freely moving and anesthetized rats (Adler et al., 1986; Bickford et al., 1990; Krause et al., 2003). However, the abnormal T/C ratio following amphetamine was due to the reduction in the conditioning response amplitude only and was considered not to represent gating abnormalities of the test response in schizophrenic patients (Adler et al., 1986). Auditory gating studies on rat genotypes with abnormal dopaminergic properties demonstrated that gating deficits were found only in one of the genotypes (WAG/Rij) which demonstrated high dopamine activity in both the nigrostraital system and the mesolimbic system (de Bruin et al., 2001), while another study found that this genotype also exhibited deficits in cortical GABAergic inhibitory process correlated with the sensory processing deficits (Luhmann et al., 1995) shedding doubts on the correlation between dopamine imbalance and sensory gating deficits in schizophrenia.

Although the dopamine hypothesis is one of the most influential theories, it does not explain all aspects of schizophrenia as either dopamine agonists or antagonists have failed to mimic or restore, respectively, the negative symptoms and cognitive deficits seen in the disease.

The glutamate hypothesis

Glutamate is the major excitatory neurotransmitter in the mammalian brain. It serves as the neurotransmitter of pyramidal cells which are the sources of

efferent interconnecting pathways of the cerebral cortex and the limbic system. Glutamate is also indirectly involved in release and uptake of other neurotransmitters such as dopamine, acetylcholine, serotonin and GABA (Goff and Coyle, 2001; van Berckel, 2003; Coyle, 2006).

The effect of glutamate is mediated by two major classes of receptors: ionotropic (ion channels) and metabotropic (G protein coupled receptors). The ionotropic receptors are ligand gated channels and are divided in to three classes each named for the congeners of glutamate to which they respond in maximum fashion: kainate, AMPA (amino-3-hydroxy-5-methyl-isoxazole-4-proprionic acid) and NMDA (*N*-methyl *D*-aspartate). Kainate and AMPA receptors are found in neurons and glia, the supportive cells of CNS, whereas NMDA receptors are found exclusively in neurons. There is a high expression of NMDA receptors in the hippocampus (Goff and Coyle, 2001; van Berckel, 2003). Kainate and AMPA receptors are simple ion channels which permit Na^+ influx and K^+ efflux in the activated state. The NMDA receptor is also a cation channel but is unique in several ways compared to other ion channels (Fig 1.8). The NMDA receptor permits the passage of Ca^{2+}. It also needs an amino acid, glycine to be bound to a site on the receptor surface for its normal function. When glutamate binds the receptor opens but at the resting membrane potential the channel is blocked by Mg^{2+}. This block is removed only when the membrane is partially depolarized by AMPA or other ion channels (Goff and Coyle, 2001; van Berckel, 2003).

The glutamate hypothesis posits that NMDA receptor hypofunction is

responsible for the emergence of schizophrenia. The hypothesis is based on the findings that NMDA receptor blockers like phencyclidine (PCP) and ketamine produce clinical features including negative symptoms and cognitive deficits identical to those seen in schizophrenia (Krystal et al., 1994; Malhotra et al., 1996; van Berckel., 1998; Oranje et al., 2002).

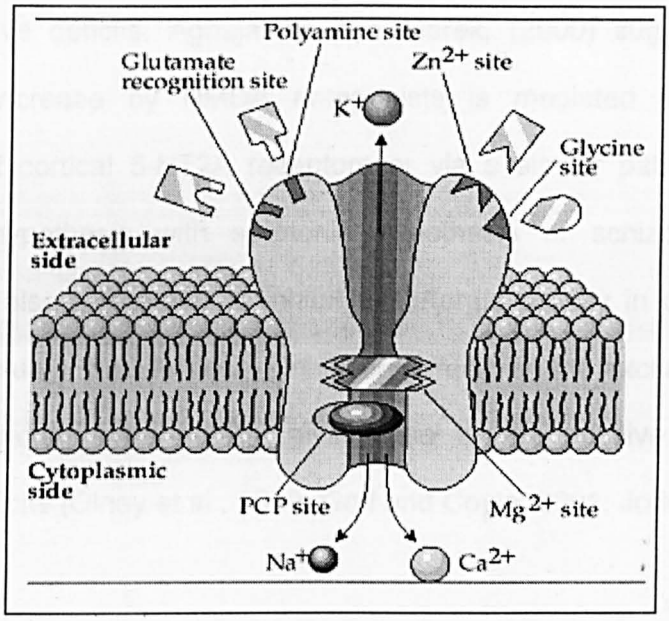


Fig 1.8 Schematic diagram of NMDA receptor showing sites of modulation

It has been suggested that NMDA receptor hypofunction causes excessive release of other central neurotransmitters such as dopamine, giving rise to the positive symptoms of schizophrenia (Jentsch and Roth, 1999; Olney et al., 1999; Svensson, 2000; Tsai and Coyle, 2002). The NMDA antagonists such as PCP and MK-801 have been shown to increase mesocortical dopamine levels and distort the firing patterns of PFC-projecting dopamine neurons, thus impairing information processing between cortical and subcortical structures especially between PFC and VTA (Murase et al., 1993; Moghaddam et al.,

1997; Balla et al., 2001). Some studies have demonstrated that NMDA receptor blockade increases cortical glutamate release (Takahata and Mogaddam, 1998; Olney et al., 1999). This excessively accumulated glutamate leads to excitotoxic cell death by binding to the receptors in the neuronal cell body and increasing intracellular calcium (Olney et al., 1999; van Berckel, 2003). This neurotoxic process is hypothesized to cause more chronic negative symptoms and neurocognitive deficits. Aghajanian and Marek, (2000) suggest that cortical glutamate increase by NMDA antagonists is mediated indirectly by the activation of cortical 5-HT2A receptors or via a similar pathway, linking the glutamate hypothesis with serotonin hypothesis of schizophrenia. NMDA blockade is also suggested to inhibit GABAergic activity in cortical and sub-cortical structures, thus releasing inhibitory effects on the excitatory circuits and causing neuronal hyper-activity giving rise to the positive symptoms and cognitive deficits (Olney et al., 1999; Goff and Coyle, 2001; Jodo et al., 2005).

1.7.2.1 The phencyclidine model of schizophrenia

Phencyclidine (PCP) belongs to the class of non-dissociative anaesthetics, but is no longer used medically due to its disturbing neuropsychiatric side effects during postoperative emergence. However, it has continued to be used illicitly as a recreational drug (e.g. "Angel dust", "crystal"). PCP is an arylcyclohexylamine derivative (Fig. 1.9) and has more than 30 structural analogues used in research and on the street.

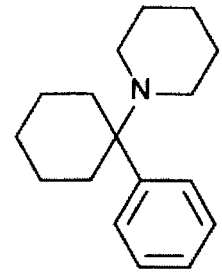


Fig. 1.9 Chemical structure of phencyclidine- 1-(1-phenylcyclohexyl)piperidine

PCP is well absorbed and readily penetrates the central nervous system and its rapidity of action varies with the route of administration. Intravenous (i.v.) and inhalation administration allows for activity within a few minutes whereas intranasal and oral administration takes longer (20-40 minutes; Gorelick & Balster, 2005). The time for the peak effects to occur also varies with the route of administration: 10 min with intravenous, 5-30 min with inhalation and 90 min with oral administration (Gorelick & Balster, 2005). PCP is metabolized in the liver and elimination half life of PCP varies from 7–46hrs and the effects of intoxication last for 4-8hr after recreational doses (Gorelick & Balster, 2005).

Although PCP acts mainly as a non-competitive antagonist at the NMDA glutamate receptor (Fig.1.8), it has effects on a number of other neurotransmitter systems. It has indirect agonist effects on the dopamine and noradrenalin systems by blocking their reuptake. PCP also has a non-competitive inhibitory effect on both nicotinic and muscarinic receptors, in addition to exerting inhibitory effects on voltage-dependent sodium and potassium channels (Morris et al., 2005; Murray, et al., 2002).

As mentioned before, the idea of using PCP to model schizophrenia has recently received a great deal of attention since both PCP and the structurally related drug ketamine produce symptoms that extend beyond psychosis *per se* to include other symptoms associated with schizophrenia.

Jentsch and Roth (1999) reviewed reports on acute and long term PCP/ ketamine exposure in humans and found that both regimens can induce psychosis, thought disorder, delusions, flattened affect and withdrawal, with the effects of chronic exposure being more persistent. Chronic or sub-acute administration of PCP in experimental animals produce not only behavioural and cognitive deficits but also structural abnormalities in the brain resembling the changes observed in schizophrenic patients (Goff and Coyle, 2001; Pietraszek, 2003; Pratt et al., 2008). A recent study by Pratt et al (2008) demonstrated that chronic and sub-chronic PCP administration to rats decreased glucose utilization in the PFC accompanied by a reduction in GABAergic interneuron markers and deficits in attentional set-shifting test (a test to assess executive functions) resembling the changes found in schizophrenic patients.

PCP models using a single dose systemic administration show disrupted performance in attention assessed by PPI, Morris water maze, and working memory tasks (Geyer et al., 1984; Verma and Moghaddam, 1996; Sams-Dodd, 1995; Martinez et al., 1999; Marcotte et al., 2001). Altered social interactions and behavioural disturbances, such as hyperactivity and stereotyped movements, resembling schizophrenia have also been observed following

single dose administration (Sams-Dodd, 1996, 1999; Marcotte et al., 2001; Suzuki et al., 2002). Atypical anti-psychotics have been shown to reverse or prevent acute PCP induced stereotyped movements, social isolation and PPI deficits (Sams-Dodd, 1997; Bruins et al., 2005). PCP disrupts auditory gating in rats under chloral hydrate anaesthesia in a dose dependent manner with peak disruptions observed around 45 min–1hr following i.p. administration (Miller et al., 1992). Findings of these studies validate the acute PCP model to study the neurobiology and pharmacology of schizophrenia.

1.8 Cannabinoids and schizophrenia

Cannabinoids, the active compounds of the plant *Cannabis Sativa* (marijuana, hashish, etc.), have been used medicinally and recreationally for centuries. Cannabis produces alterations in mood, memory and perception and is suggested to be associated with psychotic illnesses. A recent meta-analysis by Moore et al (2007), combining results of 35 population-based longitudinal observational studies of the relationship between the use of cannabis and psychiatric disorders in later life, found an overall increased risk of 41% in the development of any psychosis among individuals who had used marijuana in the past. Some case-control studies of psychotomimteic drug - use among schizophrenic patients, have revealed that schizophrenic patients have a higher incidence of consumption of cannabis, amphetamine, cocaine and other psychoactive drugs compared to the normal controls and patients with other psychiatric illnesses (Hambrecht and Hafner, 1996; Bersani et al., 2002). Prospective and longitudinal studies have suggested that cannabis use

precipitates schizophrenia in vulnerable individuals and exacerbates symptoms in patients already diagnosed with the disease (Hambrecht and Hafner, 2000; Bersani et al., 2002). However, there are studies arguing that cannabis use is not causative, but merely a result of the negative or pro-dromal symptoms of the disease. These studies based on patient communications suggest that cannabis is used for its euphoric effects to relieve negative symptoms of schizophrenia (Hambrecht and Hafner, 2000; Constain, 2008).

The identification of the primary psychoactive compound in cannabis, Δ^9 – tetrahydrocannnabinol (Δ^9-THC), led to the synthesis of high affinity cannabinoid ligands, which also led to the discovery of endogenous cannabinoid receptors in the brain and peripheral tissues (i.e. CB1 and CB2). The CB1 receptor is the most abundant and best characterized receptor found in the brain and is highly expressed in the hippocampus, basal ganglia, cerebellum and cortex (Wilson and Nicoll, 2002; Iversen, 2003; Howlett et al., 2004; Straiker and Mackie, 2006).

CB1 receptors are G-protein coupled receptors and their activation leads to inhibition of adenyl cyclase, inhibition of certain voltage sensitive calcium channels (predominately those found presynaptically), activation of inwardly rectifying potassium channels, and activation of the mitogen-activated protein kinase (Howlett et al., 2004; Biosogno, 2005; Straiker and Mackie, 2006). The identification of CB receptors led to the identification of their endogenous lipid ligands, endocannabnoids (e.g. anandamide, 2-archidonylglycerol). These endocannabinoids are not stored like neurotransmitters, but rapidly synthesized

post synaptically in response to depolarization and act on pre-synaptic CB receptors as a retrograde messenger to mainly inhibit neurotransmitter release (Wilson and Nicoll, 2002; Bisogno, 2005; Di Marzo, 2006). The CB1 receptor mediated inhibition of GABA release from the pre-synaptic terminal is denoted as depolarization- induced suppression of inhibition (DSI) and the same effect on excitatory terminal inhibiting release of glutamate is designated DSE (depolarization- induced suppression of excitation; Howlett et al., 2004; Straiker and Mackie, 2006). The DSE and DSI are transitory effects terminated with rapid degradation of the endocannabinoids by the hydrolyzing enzymes (e.g. fatty acid amide hydrolase, monoacylglycerol). The endocannabinoid system, consisting the CB receptors, endocannabinoids, transporters and the enzymes responsible for production and degradation, is thus functioning as a regulatory apparatus which is activated on demand to re-establish transient perturbations of the homeostasis of the neurotransmitters, which may in turn control the endocannabinoid system in the brain.

Dysregulation of the endocannabinoid system is suggested to be involved in the pathogenesis of schizophrenia. Administration of Δ^9-THC to normal volunteers induced psychotic symptoms and cognitive impairments similar to those observed in schizophrenic patients (Emrich et al., 1997). Postmortem studies have detected increase density of CB1 receptors in the in the prefrontal cortex of the schizophrenic patients compared to controls (Dean et al., 2001; Zavitsanou et al., 2004). Leweke et al (1999) found increased levels of endocannabnoids in the schizophrenic patients CSF. Chronic as well as acute administrations of cannabis have produced P50 gating abnormalities in

otherwise healthy individuals (Patrick et al., 1999, 2000; Rentzsch et al., 2007). The results of these studies suggest a strong link between a dysregulated endocannabnoid system and schizophrenia. Ujike and Morita (2004), reviewing the human studies on cannabis and schizophrenia proposed that the hyperactivity of the endocannabinoid system is involved in the pathogenesis of schizophrenia or the neural mechanisms of the negative symptoms of the disease.

Synthetic agonists (e.g. WIN55,212-2, HU210, C55,940) and antagonists of CB1 receptors (e.g. SR141716A, AM251) are used to examine the neuropsychiatric effects of cannabis in animal studies. Cannabinoid agonists have demonstrated neuroprotective effects probably due to the inhibitory actions on Glutamatergic excitatory transmission and modulation of immune responses along with its anti-oxidant properties, thus considered as important potential therapeutic agents in neurodegenerative diseases such as Huntington's, Alzheimer's and multiple sclerosis (Patcher et al., 2006). However, as mentioned above, cannabis and synthetic CB1 agonists have produced neurocognitive deficits following acute as well as chronic administration in humans as well as in animals with deficits resembling to those observed in schizophrenia (Solowij and Michie, 2007). These deficits are attributed to the inhibition of GABAergic activity and resultant increase in dopamine activity in the meso-cortical areas (hyperdopaminergic state) and also the hypoglutamatergic state produced by the effects of cannabinoids on excitatory neurons (Brodkin and Moerschbaecher, 1997; Pistis et al., 2001; Solowij and Michie, 2007). The current study used WIN55,212-2 (Fig. 1.10) a

non-selective cannabinoid receptor agonist and the CB1 receptor antagonist SR141716A (Fig. 1.11) to study the effects of cannabinoids on auditory gating.

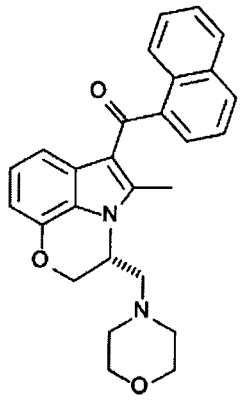


Fig. 1. 10 Chemical structure of WIN55,212-2

(R)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de)-1,4-benzoxazin-6-yl]-1-napthalenylmethanone

WIN55,212-2 is an aminoalkylindole derivative with both CB1 and CB2 receptor agonist properties (Howlett et al., 2004; Pertwee, 2006) . It is metabolised in the liver and produce analgesic, behavioural and cognitive effects lasting for several hrs following single systemic administration (Howlett et al., 2004; Drews et al., 2005). WIN55,212-2, though structurally different, produces CNS effects similar to Δ^9-THC through its CB1 full agonist effects and is preferred in cannabinoid research due to its better solubility and higher potency compared to the highly lipophilic Δ^9-THC. Acute, sub-acute and chronic administration of WIN55,212-2 animals produces behavioural and other CNS effects such as increased locomotor activity at low doses and hypolocomotion at high doses (Schneider and Koch, 2003; Drews et al., 2005), impairment of some tasks involving

memory, attention and sensory processing such as object recognition, progressive ratio test, continuous performance tasks and PPI (Schneider and Koch, 2002, 2003; Drews et al., 2005; Solowij and Michie, 2007). Most of these effects are reversed or prevented by the CB1 antagonist SR141716A (Patcher et al., 2006).

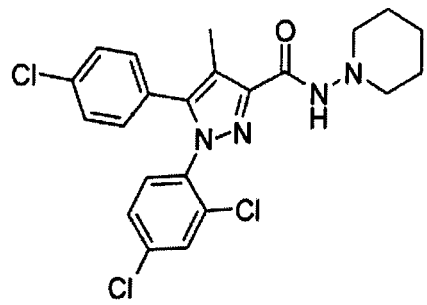


Fig. 1.11 Chemical structure of SR141716A - 5-(4-Chlorophenyl)-1-(2,4-dichloro-phenyl)-4-methyl-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide

SR141716A (rimonabant) exhibits a significant CB1 selectivity and potency and prevents or reverses CB1 mediated effects both *in vivo* and *in vitro* (Brodkin and Moerschbaecher, 1997; Martin et al., 2003; Hajos et al., 2008). Some *in vitro* studies have suggested that SR141716A acts as an inverse agonist at CB1 receptors (Sim-Selly et al., 2001). However, apparent inverse agonist effects are proposed to be due to the inhibitory or antagonist effects on tonically active endocannabinoid system (Crompton et al., 1996; Pattij et al., 2007).

In animal studies SR141716A prevented the deficits in locomotor activity

(Crompton et al., 1996), memory (Mallet and Beninger, 1998), learning (Brodkin and Moerschbaecher, 1997), attention (Arguello and Jentsch, 2004) and sensory processing (Martin et al., 2003) induced by CB agonists (i.e. THC, WIN55,212-2, C55,940) indicating the disruptions were mediated via the CB1 receptors. However, SR141716A did not produce any effects on locomotor activity, learning, memory or attention when administered alone (Brodkin and Moerschbaecher, 1997; Arguello and Jentsch, 2004; Martin et al., 2003) suggesting the tonically active endocannabinoid system was not mandatory in the tested behavioural and cognitive processes.

An interesting study examining the effects of SR141761A on chronic marihuana uses reported that a single dose of SR141761A (administered 2 hrs prior to THC) prevented acute psychological and physiological effects of smoked marijuana (Heustis et al., 2001). They also noted that SR141716A alone did not produce any psychological or physiological effects (Heustis et al., 2001). A placebo-controlled study evaluating the effect of SR141716A on patients with schizophrenia and schizo-affective disorders observed no outcome difference compared to the placebo group (Meltzer et al., 2004). However, limitations of the study such as inadequacy of the dose precluded a definitive conclusion and further studies on SR141716A efficacy in treating schizophrenia were encouraged (Meltzer et al., 2004).

1.9 Aims and Objectives

We had three main objectives in this study of sensory gating in the rat brain. The first and foremost was to study and characterise auditory-evoked LFP responses (AERs) and auditory gating in the CA3 region of the hippocampus under isoflurane anaesthesia. If sensory gating was detected in CA3, we hoped to examine sensory gating in the dentate gyrus, an area involved in the tri-neuronal circuit of the hippocampus. Examining auditory responses in these areas was expected to provide a better understanding of the feed-forward and feedback circuitry which modulates sensory gating mechanism in the hippocampus.

The second objective was to examine AERs and sensory gating in the mPFC while simultaneously recording from hippocampus. We expected that simultaneous recording of LFPs and single-unit responses from both these regions, using auditory conditioning- test paradigm would provide experimental evidence to the role played by the prefrontal cortex and hippocampo-prefrontal pathway in the sensory gating process.

The third objective was to examine auditory gating in hippocampus and mPFC using pharmacological rat models of schizophrenia. It was hypothesised that gating would be disrupted in the hippocampus and mPFC following psychomimetic pharmacological (phencyclidine or WIN55,212-2) manipulations.

Disrupted gating following pharmacological manipulations, in the isoflurane anesthetised rat, would provide a model of information processing dysfunctions, which would allow for further electrophysiological and pharmacological studies

to investigate the pathogenesis and treatment of schizophrenia.

Chapter 3

The main objective of this chapter was to examine AERs and characterize the basic properties of sensory gating in the CA3 region of the hippocampus by analyzing LFP responses to the auditory conditioning-test paradigm. To achieve this objective it was essential to demonstrate AERs and sensory gating in CA3 which would then allow characterisation of auditory- evoked LFPs. Following the detection of AERs and gating we aimed to study the stability/variability of auditory gating in response to different sound pressure levels and at different inter-stimuli intervals. Another goal was to detect AERs and gating in the DG and compare with the findings from CA3.

Chapter 4

The objective of this chapter was to study auditory-evoked LFP responses in the medial prefrontal cortex to characterize the basic properties of sensory gating in this region. The first goal was to demonstrate AERs and sensory gating in mPFC which would then allow characterisation of auditory- evoked LFPs. The next step was to examine the findings from mPFC with simultaneously recorded CA3 and DG responses.

Chapter 5

In this chapter we aimed to examine and compare the effects of phencyclidine

(PCP) on auditory-evoked LFP response gating in the CA3 and DG of hippocampus and mPFC under isoflurane anaesthesia. It was hypothesised that PCP would produce disruption of auditory gating in rat CA3, DG and mPFC with similar changes reported from scalp electrodes in schizophrenic patients. A further goal was to study the effect of clozapine on PCP induced changes with the hypothesis that clozapine would prevent the PCP induced deficits in auditory gating in CA3, DG and mPFC.

Chapter 6

The objective of this chapter was to study and compare the effects of WIN55, 212-2 on auditory-evoked LFP gating in the CA3 and DG of hippocampus and mPFC under isoflurane anaesthesia. We hypothesised that WIN55,212-2 would disrupt sensory gating in rat CA3, DG and mPFC with similar changes reported from scalp electrodes in schizophrenic patients. The next step was to examine the effect of the CB1 antagonist, SR141716 on WIN55,212-2 induced changes with the intention to prevent the WIN55,212-2 induced deficits in auditory gating in CA3, DG and mPFC.

Chapter 7

The aim of this chapter was to evaluate the single-unit responses to the auditory conditioning-test paradigm in the CA3, DG and mPFC and to compare with the simultaneously recorded LFP responses before and after pharmacological interventions, providing important observations and opening pathways for future research.

Chapter Two

Methods

2. Methods

2.1 Animals

Experiments were performed on male Lister hooded rats (n = 42) from the University of Nottingham Biomedical Sciences Services Unit in-house colony, weighing 250-400g at the time of surgery. Animals were group housed in standard plastic cages (3 to 6 animals per cage), 12h:12h light: dark cycle (lights on at 7:00 AM), light intensity 100-200lux at cage bottom, room ambient temp 22±1°C; food and water were available *ad libitum.* Experimental procedures were carried out in accordance to the UK Home Office regulations under project licence 40/2715.

2.2 Surgical procedure

Anaesthesia was induced with 3.5% isoflurane (IVAX Pharmaceuticals, UK) in a 50%:50% N_2O:O_2 mixture (BOC Gases, UK). The isoflurane level was reduced progressively and maintained at 1.5–2% throughout surgery to ensure a state of complete areflexia of the hind paw withdrawal reflex. Rats were mounted in a modified stereotaxic frame (Kopf model 1730) using hollow ear bars adapted with Sony Walkman® stereo-earphones to present auditory stimuli. Core temperature was monitored and maintained at 37-38 °C using a homeothermic heating pad and controller (Harvard Instruments, UK). A scalp incision was made and 5 mm diameter craniotomies were performed separately above the right hippocampus and right mPFC. The dura was reflected and excised from

the cortex and the exposed cortex kept moist with 0.9% (w/v) sodium chloride. Scalp & skin wound edges were treated with lignocaine (2% w/v; Arnolds Veterinary Products, UK). Anaesthetic gases were scavenged using a passive system to Fluosorbers or actively to the external atmosphere.

2.3 Recording procedure

An eight or sixteen channel micro-wire electrode bundle or array (2x4 or 2x8 pattern ~0.25 x 0.7mm; Teflon-coated stainless steel, 50 μm diameter per wire; NB Labs, Texas USA) with a stainless steel ground electrode connected to a unity-gain headstage (Plexon Inc, Tx USA) was used to record local field potentials (LFPs) and simultaneous spike activity from a number of single neurones (Fig 2.1). The impedance of the electrodes was ~100-300 kΩ measured at 1 kHz (Robinson, 1968).

The electrode array / head stage assembly was clamped to a Kopf-type manipulator and the electrodes were stereotactically placed in the CA3 and DG (3.6mm-3.9 mm posterior, 3mm to 3.2 mm lateral and to 3.8 mm ventral from bregma) and mPFC (3.2 mm anterior, 0.5mm to 0.8 mm lateral and 2.5mm ventral from bregma) according to the atlas of Paxinos and Watson (1997). The electrode array and bundle were lowered slowly & progressively through the right cortical hemisphere and positioned in the hippocampus and mPFC via fine micromanipulator control until the areas were identified from the co-ordinates of the rat atlas and the LFP wave form patterns. Unit activity was recorded from all the electrodes and LFPs were recorded from two electrodes in each region.

The headstage was connected to a multichannel PBX preamplifier (Plexon Inc., TX, USA), to amplify and filter extracellular action potential spikes (gain × 1000, band pass filtered for spikes at 250 Hz–9 kHz) and LFPs (gain × 1000, 0.7-170Hz). The preamplifier was then connected to Multichannel Acquisition Processor (MAP) system for further amplification, filtering, A/D conversion and digital signal processing of spikes.

2.4 MAP System

2.4.1 Single- unit activity (spikes)

Analogue output from the pre-amplifier was fed to 32 channel MAP system, which provided computer-controlled amplification (x1,000-32,000; typically the gain was set at x1-2,000 for the experiments), band-pass filtering (100Hz-8kHz; with 50Hz notch if required), A/D conversion (signals were digitised at 40kHz, providing 25μs precision, on each channel at 12-bit resolution), then routed to digital signal processors (DSPs, Motorola 56002), boards running at 40MHz.

The MAP system timing board allowed distribution of digital timing and synchronisation outputs to external devices, while the DSP boards provided inputs for sampling digital or analogue (e.g. auditory stimuli) signals. The MAP box was linked to a PC via an MXI Bus board (National Instruments, UK) within the host PC (Dell 1.5GHz with 1GB RAM and 160GB hard drive) running C++ host software under Windows XP. Recorded files were distributed to a lab-based computer server ("Ramon", a dual Pentium processor PC) and data then

archived to an external 500GB hard drive and CDs.

Commercially available software (RASPUTIN, Plexon Inc, TX) allowed on-line isolation and discrimination using dual voltage-time widows or principal component analysis (Fig. 2.1C; Abeles & Goldstein, 1972) of neuronal spikes from background noise. Firing rate was also monitored aurally with the aid of a loudspeaker. Typically, one to four neurones were observed and discriminated per microware on-line and subsequently validated off-line (Off Line Sorter v2.86; Plexon Inc; see Nicolelis et al, 2003). Activity was also displayed on a Hameg 507 analogue-digital oscilloscope

2.4.2 Local Field Potentials

Local field potentials were recorded in parallel with spikes (Fig. 2.2) from two electrodes per region. Neural signals were split at the Plexon PBX preamplifier, amplified x1000 and filtered (0.7-170Hz). LFP signals were then fed via a National Instruments data acquisition (NIDAQ) card (PCI-6071E 1.25MS/sec, 64 channels 12-bit resolution) in the host PC and digitised at 1 kHz.

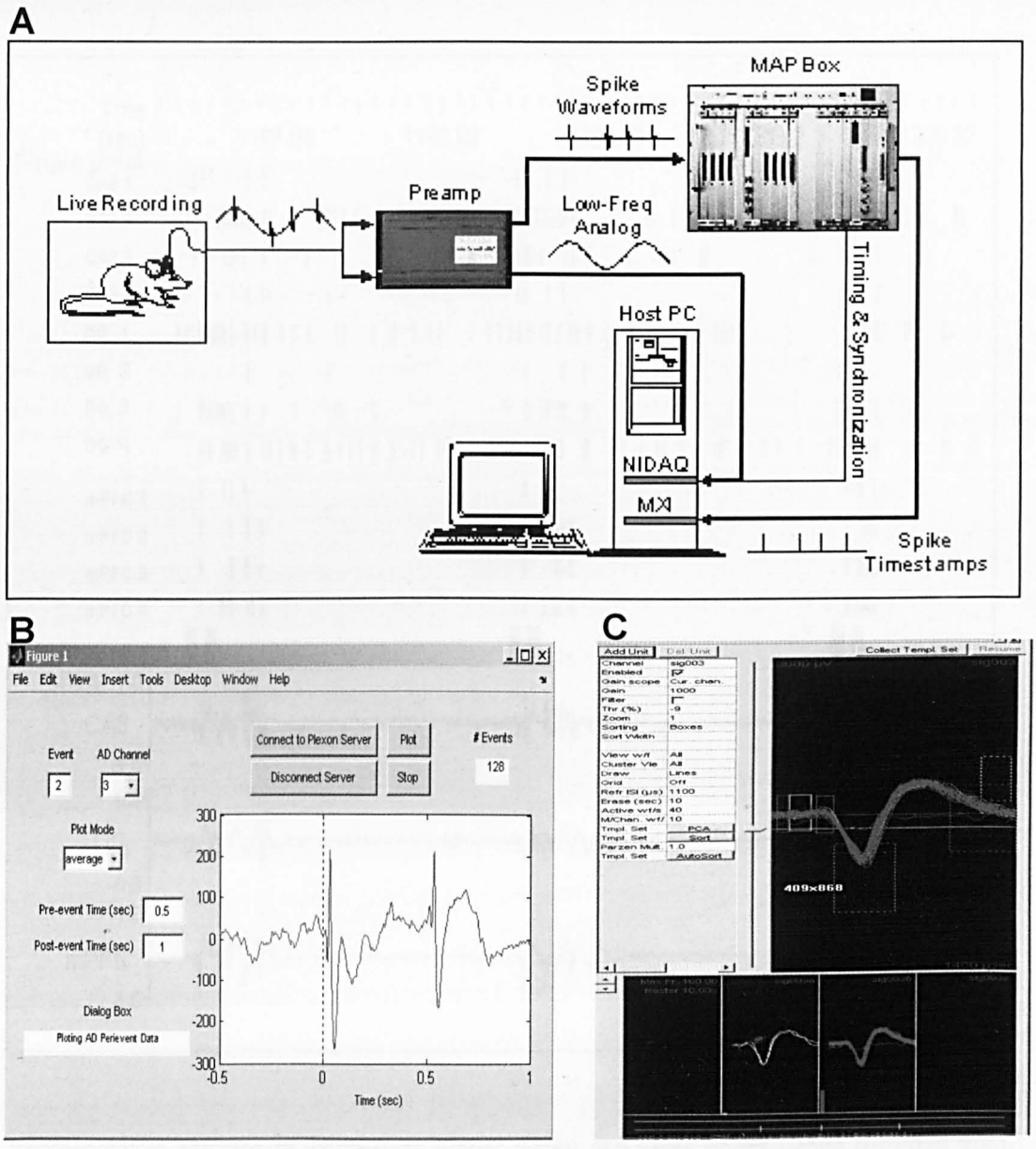
2.5 Auditory sensory gating stimuli

Auditory stimuli were generated using *Cool Edit 96* (Syntrillium Software Corporation, Phoenix, USA) on the host PC. Paired auditory clicks (3kHz 70-90dB (SPL) tones, 10ms duration, with an inter-pair interval 500ms repeated at 10s intervals were supplied binaurally through custom-made (Medical School Workshop) stereotaxic ear bars integrally connected to a pair of Sony Walkman earphones to establish auditory gating (Bickford-Wimer et al 1990; Miller & Freedman, 1995).

The auditory signals were calibrated to decibels sound pressure level, using a Bruel and Kjaer (4138 1/8 inch) condenser microphone and Bruel and Kjaer (2636) measuring amplifier. Calibration was conducted in a plastic coupler designed to generate a cavity approximately the size of the rat's meatus on the end of the hollow ear bar, with the 1/8 inch microphone replacing the tympanic membrane (MRC Institute of Hearing Research, Nottingham).

Stimuli were averaged on-line over 128 stimulus presentations; the number of trials was measured using an online Matlab script (AD Perievent, Plexon Inc.) which also displayed averaged auditory responses (Fig 2.1B).

Fig 2.1 Schematic diagram illustrating the recording procedure

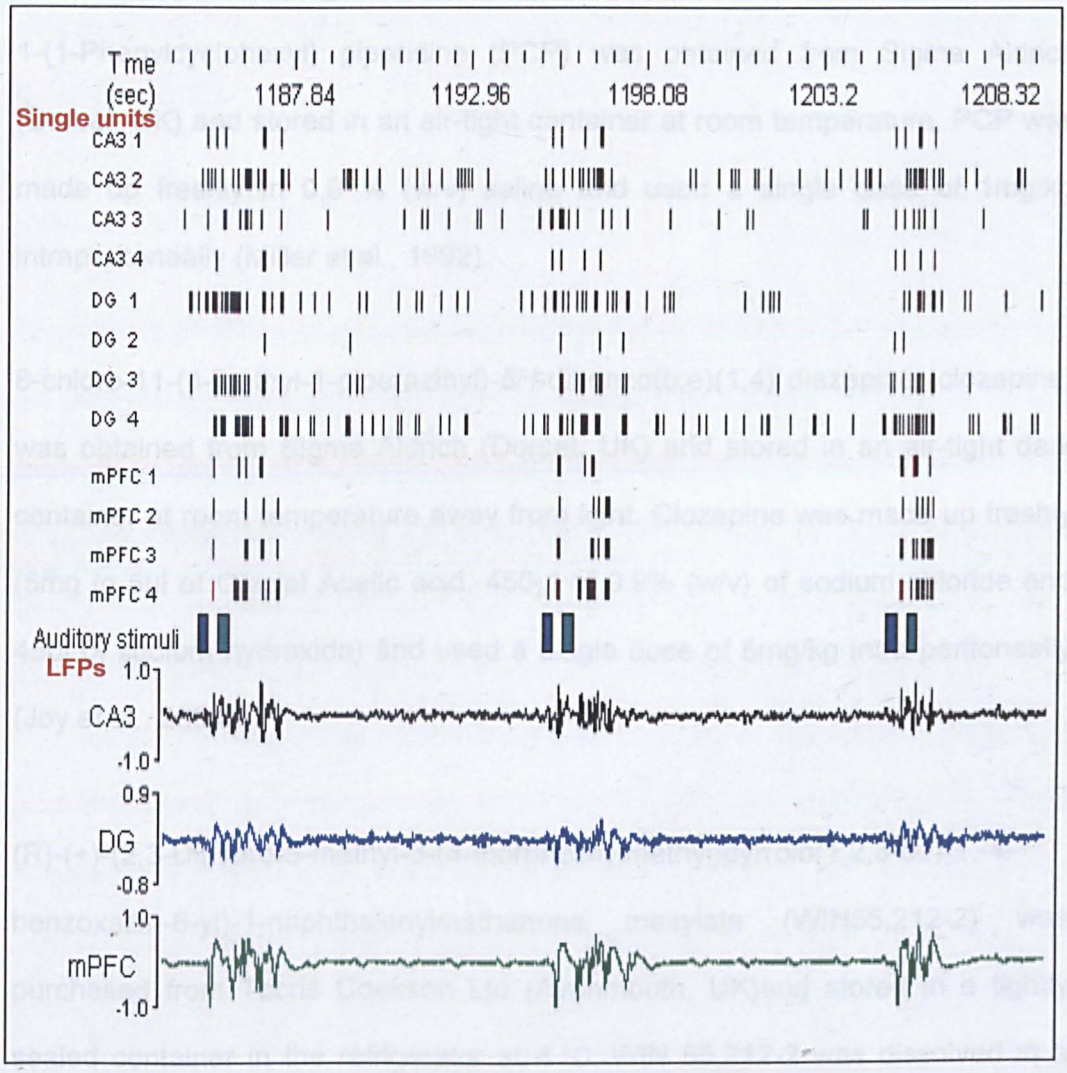


(A) Schematic diagram of the recording system: anaesthetised rat was presented with auditory stimuli and auditory-evoked LFPs and single unit activity was recorded from electrodes placed in brain areas. The information from the headstage was passed to a preamplifier and LFPs and spikes were separated. Further digital processing of spikes was done using the MAP system and the results displayed on the host computer

(B) Averaged auditory-evoked LFPs displayed on screen during a recording session: the figure generated using a Matlab programme allowing to keep a count of the number of events and to look at the averaged response simultaneously

(C) Appearance of action potentials of a single neurone, sorted on-line, as displayed on the monitor during a recording session

Fig. 2.2 Single-unit rasters and LFPs recorded from the CA3, DG and mPFC in response to three successive pairs of auditory stimuli.



Representative samples of single-units (upper panel) and LFPs (lower panel) recorded from CA3, DG and mPFC in a male Lister hooded rat (four representative single units and one LFP recording from each region) demonstrating the appearance of raw data during a recording session (visualized off line using *Nex*). Vertical ticks indicate the time stamps of action potentials of the single-units *(see also section 2.8.2)*. Changes in the single-unit activity in the CA3 and DG were not apparent in the raw data, but increased single-unit activity in the mPFC and increased LFP activity in the CA3, DG and mPFC regions were observed following auditory stimuli.

=Conditioning stimulus (Cs) =Test stimulus (Ts)

2.6 Drug administration

1-(1-Phenylcyclohexyl) piperidine (PCP) was obtained from Sigma Aldrich (Dorset, UK) and stored in an air-tight container at room temperature. PCP was made up freshly in 0.9 % (w/v) saline and used a single dose of 1mg/kg intraperitoneally (Miller et al., 1992).

8-chloro-11-(4-methyl-1-piperazinyl)-*5H*-dibenzo(b,e)(1,4) diazepine (clozapine) was obtained from Sigma Aldrich (Dorset, UK) and stored in an air-tight dark container at room temperature away from light. Clozapine was made up freshly (5mg in 5µl of Glacial Acetic acid, 450µl of 0.9% (w/v) of sodium chloride and 45µl of sodium hydroxide) and used a single dose of 5mg/kg intra peritoneally (Joy et al., 2004).

(R)-(+)-(2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo(1,2,3-de)-1,-4-benzoxazin-6-yl)-1-naphthalenylmethanone mesylate (WIN55,212-2) was purchased from Tocris Cookson Ltd (Avonmouth, UK)and stored in a tightly sealed container in the refrigerator at 4 °C. WIN 55,212-2 was dissolved in a vehicle containing 5% of Propylene glycol, 2% Tween 80 and 9% (w/v) saline, for injection on the day of the experiment and used a single dose of 1.2mg/kg intraperitoneally (Schneider and Koch, 2002; 2003).

N-piperidono-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide (SR141716A) was supplied by the National Institute of Mental Health Chemical Synthesis and Drug Supply Program (contract N01-MH-32005). SR141716A was dissolved in 100% ethanol (4.64mg /1ml) to make a

10μM stock solution and stored at -20 ^{0}C. At the time of the experiment 100μl of stock was dissolved in 364μl of 0.9% saline (w/v) to make up a working concentration of 1mg/ml (Brodkin and Moerschbaecher, 1997; Mallet and Beninger,1998).

Injection volume for all the drugs was 1ml/kg.

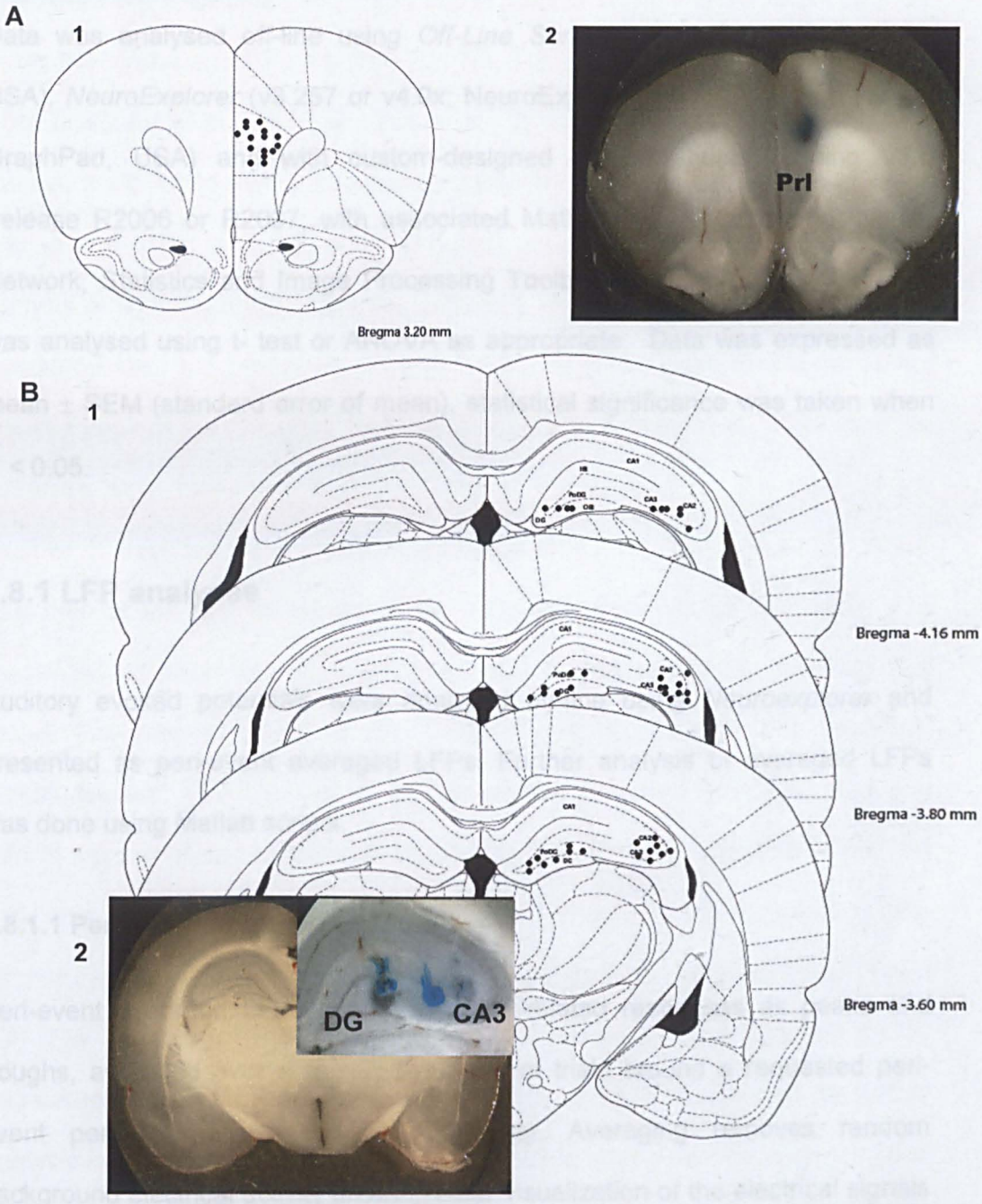
The effects of either phencyclidine (1mg/kg; i.p) or WIN55,212-2 (1.2mg/kg; i.p) on auditory gating were studied by recording the auditory responses to 128 trials of auditory stimuli, 15 and 45 minutes following drug administration. The effects of clozapine (5mg/kg; i.p) or SR141716A (1mg/kg; i.p) alone on auditory gating were examined by recording the responses to 128 trials of auditory stimuli 15minutes after drug administration. Phencyclidine (1mg/kg; i.p) was administered to animals who received a prior injection of clozapine (5mg/kg; i.p) and the effect of clozapine on phencyclidine-induced gating deficits were assessed by recording auditory responses, 15 and 45 minutes after phencyclidine administration. Effects of SR141716A on WIN55, 212-2 induced gating deficits were assessed similarly by administering WIN55,212-2 (1.2mg/kg; i.p) to animals who had already received an injection of SR141716A (1mg/kg; i.p) and recording the auditory responses, 15 and 45 minutes of WIN administration.

Protocols of drug administration for each set of experiments will be explained in detail in the respective results chapters.

2.7 Histological verification of recording sites

At the end of each experiment, 10 μA current was passed for 10 seconds (D.C.constant current lesion marker; Grass instruments, Quincy, MA) through one or more pairs of the stainless-steel microwire electrode to deposit ferric ions at the positive electrode. Rats were perfused transcardially with 0.9% (w/v) saline followed by 4% potassium ferrocyanide. Brains were removed and stored overnight in a 4% paraformaldehyde. Tissue blocks were sectioned transversely at 20 μm on a vibratome (Campden Instruments, UK). Iron deposits at the electrode tips were revealed by the Prussian Blue Reaction. Brain sections were examined under a Olympus stereomicroscope and digital images recorded with a Nikon CoolPix 4500 camera saved as *.jpg files. Images were visualised using *Paint Shop Pro* (v 5.03). Recording sites were identified with reference to the rat brain atlas of Paxinos & Watson (1997) (Fig. 2.3).

Fig 2.3 Histological verification of the recording sites (representative sites from all the animals used for the study) in (A) mPFC and (B) hippocampus



(A) Histological verification of the recording sites in the medial prefrontal cortex in relation to the rat atlas showing electrode localization in the prelimbic area of the mPFC (1) and a photomicrograph showing electrode marking in the prelimbic cortex (2).

(B) Histological verification of recording sites in the hippocampus in relation to the rat atlas demonstrating electrodes being located in CA3 and DG of hippocampus (1) , photomicrographs showing electrode marking in CA3 and DG (2; an enlarged photomicrograph of the right hippocampus is inserted to demonstrate the positioning of electrodes).

2.8 Data analysis & statistics

Data was analysed off-line using *Off-Line Sorter* version 2.86 (Plexon Inc, USA), *NeuroExplorer* (v3.257 or v4.0x; NeuroExplorer Inc USA), *Prism* (v4.03; GraphPad, USA) and with custom-designed *Matlab* scripts running V7.0 (release R2006 or R2007; with associated Matlab Signal Processing, Neural Network, Statistics and Image Processing Toolboxes; The Mathworks). Data was analysed using t- test or ANOVA as appropriate. Data was expressed as mean ± SEM (standard error of mean), statistical significance was taken when $P < 0.05$.

2.8.1 LFP analyses

Auditory evoked potentials were analysed off-line using *Neuroexplorer* and presented as peri-event averaged LFPs. Further analysis of averaged LFPs was done using Matlab scripts.

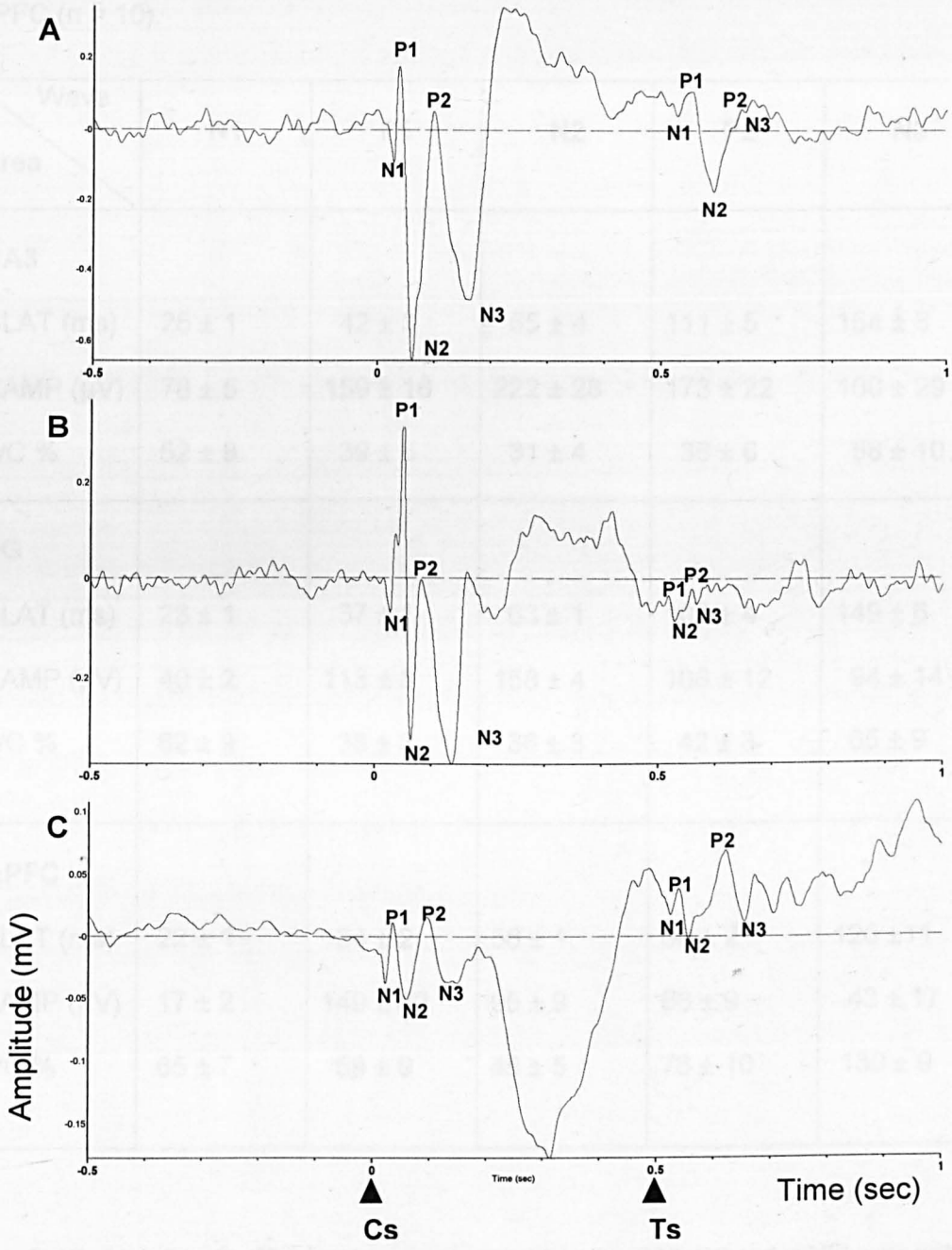
2.8.1.1 Peri-event averaged LFPs

Peri-event averaged LFPs graph the event-related responses as peaks and troughs, averaged over a required number of trials around a requested peri-event period (Y axis-response amplitude). Averaging removes random background electrical activity allowing clear visualization of the electrical signals (Fig 2.4). We averaged auditory–evoked responses (AERs) over 128 trials over a period of 0.5 second before and 1 second after the conditioning stimulus (bin time 1 ms).

2.8.1.1.1 Identification of the auditory-evoked responses

The AERs were identified according to the polarity and the order of occurrence, using the averaged LFPs, i.e. N1, P1, N2, P2, N3 peaks (Fig. 2.4; Table 2.1) (Van Luijtelaar et al., 2001; Boutros et al., 2004). Amplitudes and latencies of the averaged AERs were measured by transferring the NEX developed graph to Matlab and using a Matlab script (Amplat) developed by Margarita Zachariau (School of Mathematical Sciences, University of Nottingham). Amplitudes were measured from the preceding positive peak / negative peak to the negative / positive peak of the response (Boutros et al., 1997; Moxon et al., 1999) and the latencies were measured from the time of the stimulus to the peak of the positive or negative wave response (Boutros et al., 1997; Moxon et al., 1999). The ratio of the test amplitude to the conditioning amplitude (T/C ratio) was calculated for each averaged response and a ratio of ≤ 50% indicated that gating was present (Miller et al., 1992; Joy et al., 2004). The latencies, amplitudes and T/C ratios of all the waveforms recorded from CA3, DG and mPFC were analysed in a group of animals that showed reduction in the test response amplitude on peri-event averaged LFPs (n = 10) to select a mid-latency peak which was consistent and exhibited gating in all three areas (Table 2.1). The mid-latency N2 wave which was consistent in CA3, DG and mPFC and demonstrated maximum gating in all three areas was selected for further analysis in the thesis. The N2 wave was identified as the second negative peak during 100 ms period following the stimulus onset. Identification of the AERs according to the order of occurrence in a specified latency range excluded the error of identifying N1 as N2.

Fig 2.4 Peri-event averaged LFPs recorded from (A) CA3, (B) DG and (C) mPFC in response to 128 auditory stimuli trials.



Representative peri-event averaged LFPs of (A) CA3, (B) DG and (C) mPFC demonstrated positive and negative AER complexes (N1, P1, N2, P2 & N3) in response to the conditioning and test stimuli (timing of the stimuli are indicated by the triangles; Cs = conditioning stimulus, Ts = test stimulus).

Table 2.1 Conditioning response latencies (CLAT), conditioning response amplitudes (CAMP) and T/C ratio (T/C%; mean ± SEM) of the mid-latency auditory evoked responses, i.e. N1, P1, N2, P2, N3 recorded from CA3, DG and mPFC (n = 10).

Wave / Area	N1	P1	N2	P2	N3
CA3					
CLAT (ms)	25 ± 1	42 ± 3	65 ± 4	111 ± 5	154 ± 8
CAMP (μV)	78 ± 5	159 ± 16	222 ± 28	173 ± 22	100 ± 29
T/C %	52 ± 8	39 ± 5	31 ± 4	36 ± 6	58 ± 10
DG					
CLAT (ms)	23 ± 1	37 ± 1	63 ± 1	108 ± 4	149 ± 6
CAMP (μV)	40 ± 2	113 ± 8	158 ± 4	166 ± 12	94 ± 14
T/C %	62 ± 9	38 ± 3	36 ± 3	42 ± 3	65 ± 9
mPFC					
CLAT (ms)	22 ± 1	34 ± 2	58 ± 1	80 ± 2	120 ±11
CAMP (μV)	17 ± 2	149 ± 52	95 ± 9	66 ± 9	43 ± 17
T/C %	65 ± 7	59 ± 9	48 ± 5	78 ± 10	130 ± 9

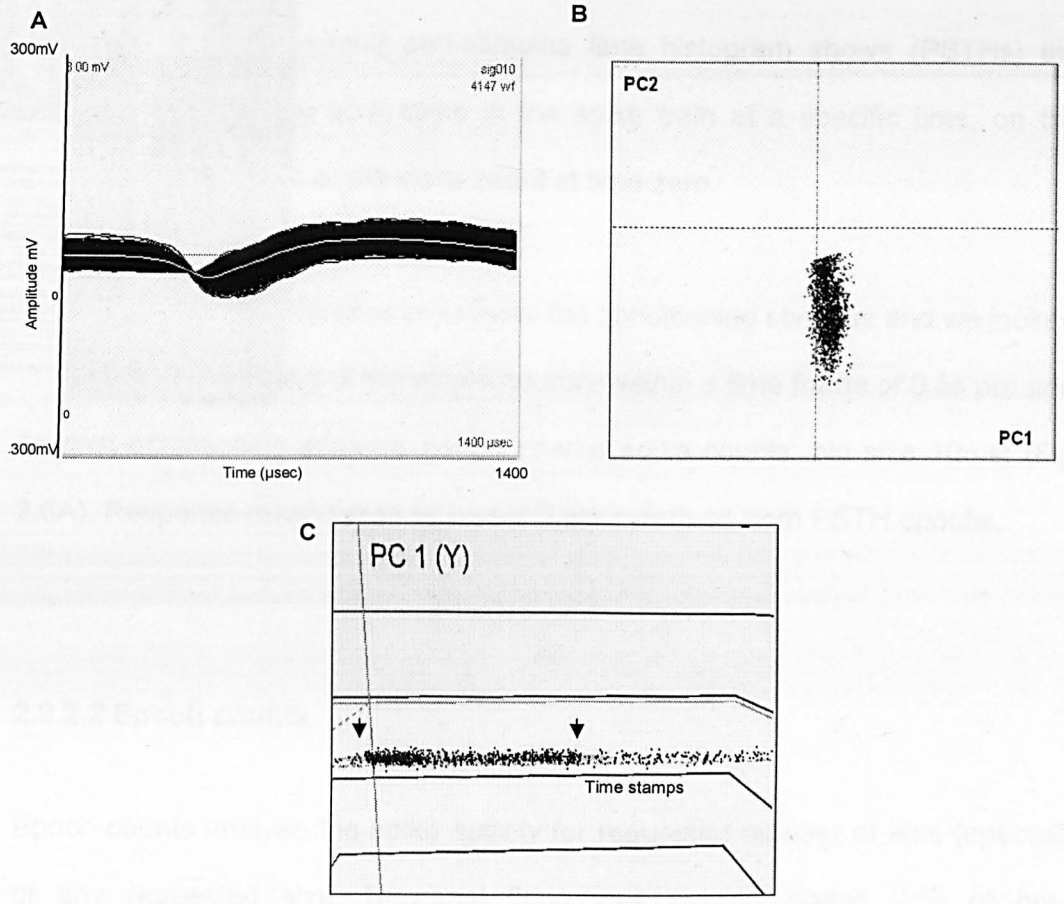
The CLAT of N1, P1, N2, P2 and N3 waves recorded from the CA3, DG and mPFC were within the midlatency range (20 -200 ms). In CA3 and DG only three waves (P1, N2 and P2) had mean T/C ratios ≤ 50% and in mPFC only the N2 wave exhibited a mean T/C ratio ≤ 50%. The N2 wave from all three areas exhibited the lowest mean T/C ratio compared to the other peaks. The N2 wave also demonstrated the highest mean CAMP in CA3 and DG compared to the other AERs.

2.8.2 Single-unit analyses

Neuronal action potentials were validated and sorted off-line with *Off-Line Sorter* using a combination of manual and automatic techniques (including T-Distribution E-M, valley-seeking, k-means clustering methods – see Lewicki, 1998) according to the following criteria: voltage thresholds >2 S.Ds. of amplitude distribution; signal: noise ratio >2.5:1 (verified on oscilloscope screen); <1% interspike intervals <1.2ms; stereotypy of waveform shapes (using waveform algorithm and PCA). Plots of sorted spikes as PC1 v time stamps provided a visual indication of any temporal effects or auditory/drug-evoked changes in spike sorting during the recording session (Fig 2.5).

Within *Neuroexplorer* the discriminated single-unit data was initially visualised off-line as spike rasters which represented time-stamps of neuronal action potential spikes, viewed as vertical ticks, corresponding to the temporal (time stamped) occurrence of the spikes. Data was subsequently analysed as peri-stimulus time histograms and epoch counts.

Fig. 2.5 Sorting single units using Offline sorter



(A) Appearance of an isolated single unit from mPFC as displayed on screen following offline sorting using manual and automatic sorting methods. The graph displays the templates (average waveforms) for the unit, separated from background noise and the action potentials of the other single units recorded from the same electrode.

(B) The same isolated single unit from mPFC displayed as a cluster in the cluster display window of the offline sorter. Cluster display plots all waveforms (as small dots) that crossed a fixed voltage threshold in a X-Y space defined by two principal components (PC1 & PC2). Upon removal of artifacts, well defined clusters are manually separated to contain isolated single units.

(C) A 3D plot from Offline sorter displaying the distribution of spike activity (time stamps = time of occurrence of the action potentials) of the above neurone during the whole recording session (X axis= Time stamps Y &Z axis=PC1). The graph indicates an increased spike activity following auditory stimuli (first arrow) and decreased activity following cannabinoid administration (second arrow).

2.8.2.1 Peri-stimulus time histograms

In general, the peri-event/ peri-stimulus time histogram shows (PSTHs) the conditional probability of a spike in the spike train at a specific time, on the condition that there is a reference event at time zero.

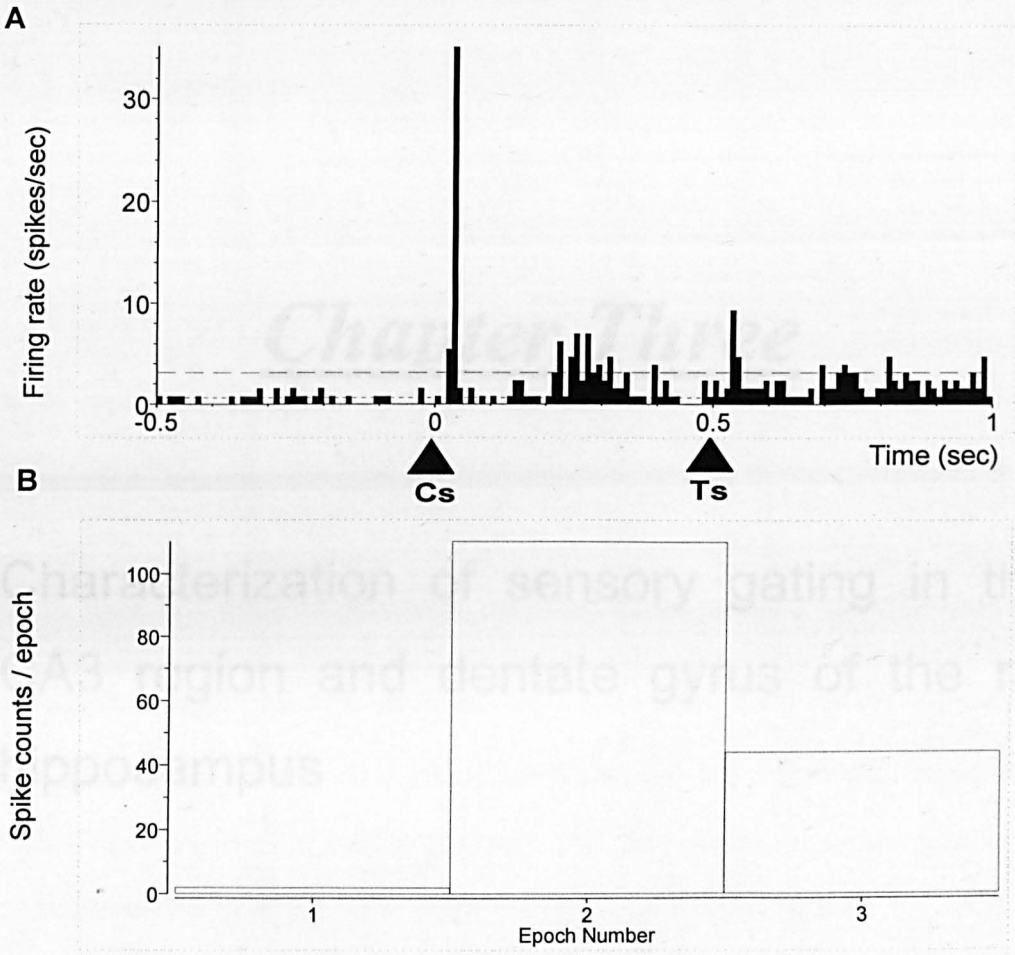
In our analysis the reference event was the conditioning stimulus and we looked at the changes of firing of the single neurons within a time frame of 0.5s pre and 1s post conditioning stimulus period (Y-axis spike counts; bin size 10ms) (Fig 2.6A). Response magnitudes (spikes s^{-1}) were derived from PSTH epochs.

2.8.2.2 Epoch counts

Epoch counts analyse the spike activity for requested number of bins (epochs) of any requested size. Neuronal firing was studied during 0.5s of pre-conditioning stimulus period, 0.5s post conditioning stimulus and 0.5s following the test stimulus to compare the spike activity before and after the conditioning and test stimuli (Fig 2.6 B).The numerical results were then analysed using Prism.

The aim of this chapter was to present a general description of the methodologies used in this in-vivo electrophysiological study of auditory gating. Experimental protocols for each set of experiments will be described in-detail in subsequent results chapters.

Fig 2.6 (A) Peri-stimulus time histogram (bin size = 10 ms) and **(B)** Epoch count analysis of a single unit recorded from the CA3 of hippocampus in response 128 trials of auditory stimuli.



(A) An example of a peri- stimulus time histogram of a single unit recorded from the CA3 region of the hippocampus showing the unit firing rate during 500 ms pre-stimulus period and one second following conditioning stimulus period (the test stimulus presented at 500ms; horizontal dashed lines are the mean firing rate- lower line, 95% confidence limit = upper line). The histogram shows significantly increased single-unit activity (above 95% confidence limit) compared to pre-stimulus period following the conditioning stimulus and reduced activity following test stimulus compared to post conditioning stimulus period.

(B) Epoch count analysis of the same single-unit recorded from CA3 demonstrating the spike activity 100ms before the conditioning stimulus (Epoch Number 1), 100 ms after the conditioning stimulus (Epoch Number 2) and 100 ms period following the test stimulus (Epoch Number 3). Epoch analysis shows increased spike activity following the conditioning stimulus compared to pre-stimulus period and reduced spike activity following test stimulus compared to post conditioning stimulus period.

Chapter Three

Characterization of sensory gating in the CA3 region and dentate gyrus of the rat hippocampus

3. Characterization of sensory gating in the CA3 region and dentate gyrus of the rat hippocampus

3.1 Introduction

The hippocampus plays a major role in modulating the brain's sensitivity to external stimuli by participating in the process of sensory gating. Recent human studies using intracranial electrodes and imaging techniques, to delineate the brain areas responsible for gating of auditory evoked responses (AERs), have indicated the hippocampus as the modulator of sensory gating (Grunwald et al., 2003; Boutros et al., 2005; Tregellas et al., 2007). Gating of the mid latency P50 AER, recorded from the scalp, in response to an auditory conditioning- test paradigm is considered a reliable biological marker of schizophrenia.

The hippocampus is a relatively accessible region in the rat brain for intracranial recordings compared to the human brain and the N40 wave, recorded from the skull as well as from the CA3 region of the hippocampus in anaesthetised and freely moving rats, has similar properties to the human P50 AER (see section 1.1.1; Adler et al., 1986; Bickford et al., 1990; Miller et al., 1992).

The CA3 of the hippocampus receives auditory information via the lemniscal and non-lemniscal pathways (see section 1.1.3). Rat studies have demonstrated auditory gating in areas involved in the non-lemniscal pathway (e.g. brain stem and medial septum) but not in the lemniscal pathway (e.g. auditory cortex and medial geniculate) suggesting that auditory gating is exclusive to the non-leminiscal pathway (Bickford et al., 1990; 1993; Miller &

Freedman., 1993; Moxon et al., 1999). Sensory information from cortical sensory areas is funnelled down to CA3 via the dentate gyrus which receives outputs from perirhinal and entorhinal cortices, which are cortical areas involved in the leminiscal pathway. A recent human study, using intracerebral electrodes, detected auditory gating in the rhinal areas which suggests the possibility of gating in the leminiscal pathway (Boutros et al., 2005). It is important to examine the role played by the dentate gyrus in auditory gating since it is relaying auditory information to CA3 from the surrounding cortical areas.

Previous studies on sensory gating have used either injectable anaesthetics (Bickford et al., 1990; 1993; Krause et al., 2003) or freely moving rats (Adler et al., 1986; Moxon et al., 1999). However no studies have assessed sensory gating under isoflurane anaesthesia. Isoflurane is an easily maintainable gaseous anaesthetic which prevents fluctuations in the level of anaesthesia and also avoids the drawbacks associated with repeated injections of anaesthetics.

The main objective of this chapter was to characterize the basic properties of auditory gating in the CA3 region and dentate gyrus (DG) of the hippocampus by analyzing LFP responses to an auditory conditioning-test paradigm in Lister-hooded rats under isoflurane anaesthesia. We also studied the stability and variability of auditory gating in the CA3 and DG in response to different inter-stimuli intervals (ISIs).

3.2 Methods

3.2.1 Animals

Male Lister hooded rats (n = 42; weight 250g – 400g) were anaesthetised with isoflurane; N_2O:O_2 and all the electrophysiological procedures were carried out as described in chapter 2. Simultaneous recordings from CA3 and DG were carried out using an electrode array (2×8) and only the animals with electrodes placed in CA3 and DG, confirmed by histology, were used for analysis.

3.2.2 Experimental protocol

Basal activity of the two areas was recorded for 5 minutes prior to the auditory stimuli. Auditory stimuli (90dB intensity, 0.5s ISI, 10s inter-trial interval) were presented over 128 trials and auditory evoked LFP and unit responses were recorded simultaneously from the CA3 and DG. Following detection of AERs in the CA3 and DG, responses to five different inter-stimuli intervals (1.5s, 2.5s, 3.5s, 4.5s and 6s; n = 5) over 128 trials were recorded.

3.2.3 Data analysis

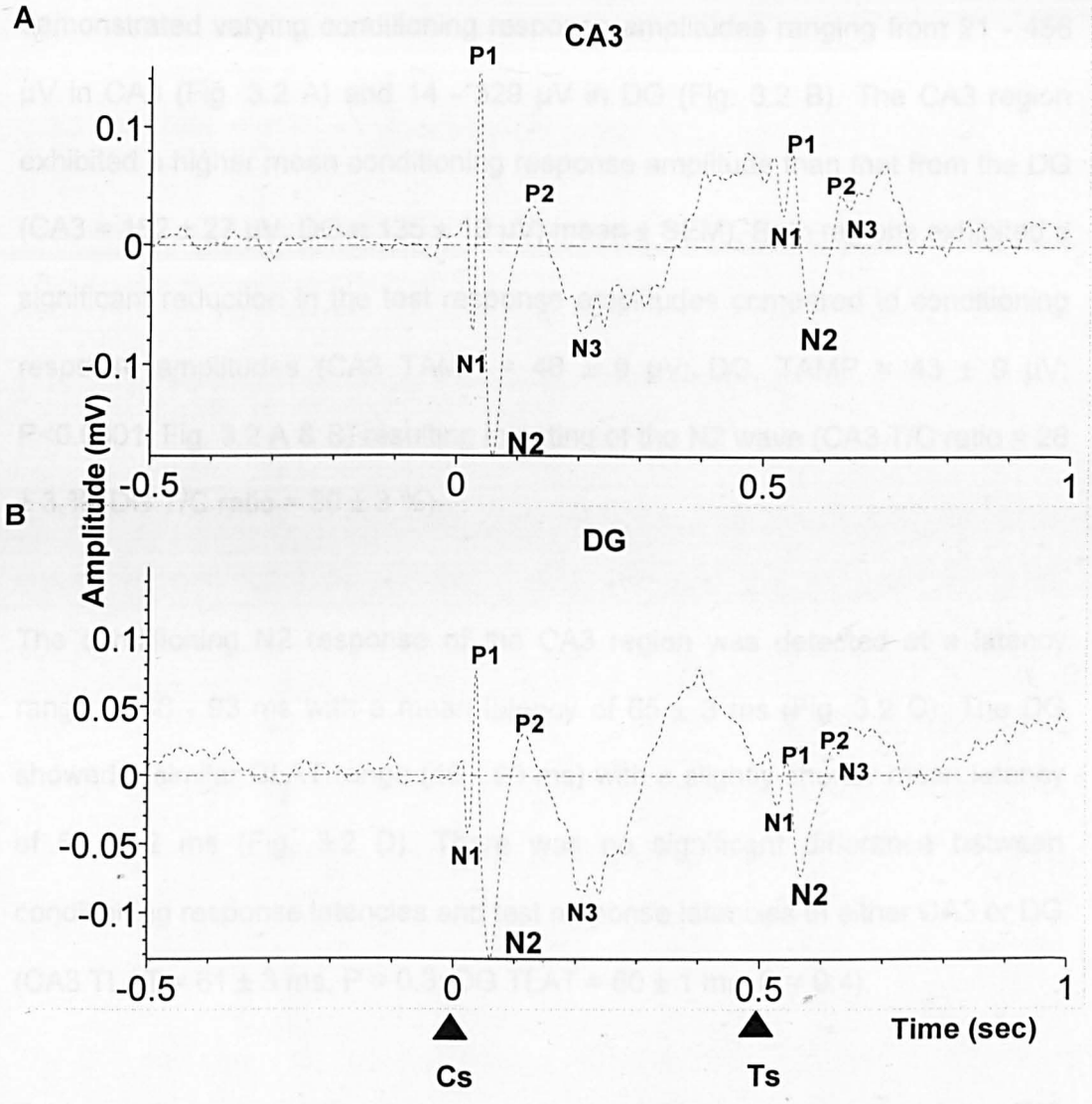
The AERs from the CA3 and DG were initially displayed as peri-event averaged LFPs and the peaks and troughs of LFPs in response to stimuli were identified according to the polarity and the order of occurrence (see chapter 2). Conditioning amplitudes (CAMP), test amplitudes (TAMP), conditioning response latencies (CLAT), test response latencies (TLAT) and T/C ratios of the N2 wave were analysed using a Matlab script (Amplat). An electrode from each

area demonstrating the highest CAMP was selected for further analysis of LFPs. The T/C ratios of the N2 wave at different inter-stimuli intervals were obtained using another Matlab script (Amplatlong) and the T/C ratio of different ISI were compared with each other. Statistical analysis was carried out using Student t test or one way analysis of variance (ANOVA) with post hoc Tukey t test when appropriate. Linear regression coefficient was used to assess the relationship of the amplitude and latency parameters to the T/C ratios. Data are expressed as mean ± SEM (standard error of mean) with $P<0.05$ considered statistically significant.

3.3 Results

The CA3 region of the hippocampus and dentate gyrus demonstrated robust auditory-evoked LFP responses consisting of positive and negative complexes (i.e. N1, P1, N2, P2, N3; Fig. 3.1). The N2 wave, which demonstrated constant gating and occurred within the mid latency range, so comparable to the P50, was used for further analysis (see chapter 2). Some rats (n = 25) showed N2 T/C ratios ≤ 50% in CA3 and DG (CA3 T/C ratio = 28 ± 3 %; DG T/C ratio = 30 ± 3 %) and were identified as gating rats and rats demonstrating N2 T/C ratios >50% (n = 17; CA3 T/C = 106 ± 11 %, DG T/C = 105 ± 13 %) were identified as non-gating rats. The amplitudes (CAMP, TAMP), T/C ratios and latencies (CLAT and TLAT) were analysed in the CA3 and DG of the gating and the non-gating rats separately and then compared between the two groups.

Fig. 3.1 Representative peri-event averaged LFPs from (A) CA3 and (B) DG in a gating rat in response to 128 auditory conditioning-test trials.



(A) The peri-event averaged LFPs from CA3 in response to the conditioning and test stimuli (timing of the stimuli are indicated by the triangles) demonstrated positive and negative AER complexes with gating of the N2 wave (T/C = 44%).

(B) The peri-event averaged LFPs from DG in response to the conditioning and test stimuli demonstrated positive and negative AER complexes with gating of the N2 wave (T/C = 46%).

3.3.1 Auditory evoked N2 responses of the gating rats

The CA3 region and the DG of the hippocampus of the gating rats demonstrated varying conditioning response amplitudes ranging from 21 - 456 μV in CA3 (Fig. 3.2 A) and 14 - 329 μV in DG (Fig. 3.2 B). The CA3 region exhibited a higher mean conditioning response amplitude than that from the DG (CA3 = 182 ± 27 μV; DG = 135 ± 19 μV; mean ± SEM). Both regions exhibited a significant reduction in the test response amplitudes compared to conditioning response amplitudes (CA3 TAMP = 48 ± 9 μV; DG, TAMP = 43 ± 9 μV; $P<0.0001$; Fig. 3.2 A & B) resulting in gating of the N2 wave (CA3 T/C ratio = 28 ± 3 %; DG T/C ratio = 30 ± 3 %).

The conditioning N2 response of the CA3 region was detected at a latency range of 46 - 93 ms with a mean latency of 65 ± 3 ms (Fig. 3.2 C). The DG showed a similar CLAT range (46 - 90 ms) with a slightly shorter mean latency of 63 ± 2 ms (Fig. 3.2 D). There was no significant difference between conditioning response latencies and test response latencies in either CA3 or DG (CA3 TLAT = 61 ± 3 ms, $P = 0.3$; DG TLAT = 60 ± 1 ms, $P = 0.4$).

Evidence for a correlation between either amplitude or latency and the T/C ratios was determined using linear regression analysis in CA3 (Fig. 3.3) and DG (Fig. 3.4). In CA3, the changes in CAMP had no effect on T/C ratio. In contrast a significant positive correlation was detected between the TAMP and T/C ratio ($F = 8$; $P= 0.007$). Neither CLAT nor TLAT showed a significant correlation with the T/C ratio in the CA3. In the DG, no significant correlation was found between T/C ratio and CAMP. But similar to the relationship observed in the

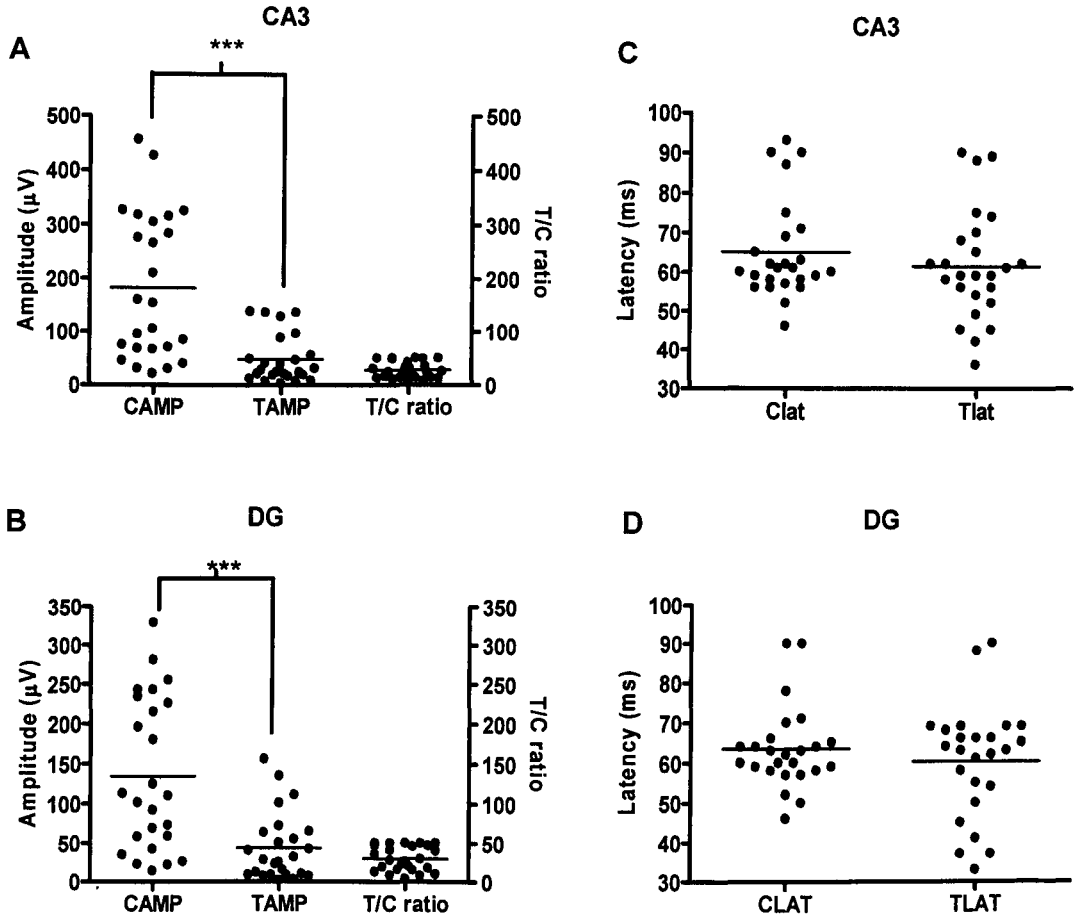
CA3, TAMP of the DG exhibited a significant positive correlation with the T/C ratio (F = 15; P= 0.0007). Neither CLAT nor TLAT showed a significant correlation with the T/C ratio in the DG.

3.3.1.1 Gating at different inter-stimuli intervals

In both CA3 and DG an increase in the T/C ratio was observed with an increase in the inter-stimuli interval (Fig. 3.4). In the CA3, a significant increase in the T/C ratio was detected at all tested inter-stimuli intervals ($F_{5, 35}$ = 45, P<0.0001) and the post hoc t tests revealed the highest significant change in the T/C ratios (P<0.0001) at 1.5s, 2.5s, 3.5s, 4.5s and 6s inter-stimuli intervals when compared with the 0.5s ISI (Fig. 3.4A). However the reduction in TAMP compared to CAMP was maintained even up to 4.5s inter-stimuli intervals resulting in T/C ratios <100%, at 1.5s (T/C ratio = 59 ± 3 %), 2.5s (T/C ratio = 62 ± 2 %), 3.5s (T/C ratio= 77 ± 3 %) and 4.5s (T/C ratio = 93 ± 3 %). Nonetheless, the ability to decrease the TAMP was completely abolished at 6s (T/C = 103 ± 5%).

The DG also demonstrated a significant increase in the T/C ratio at all tested inter-stimuli intervals ($F_{5, 35}$ = 50, P<0.0001) when compared with the T/C ratio at 0.5 s (Fig. 3.4B). However, the reduction in TAMP compared to CAMP was maintained even up to 6s delay from the onset of the conditioning stimulus resulting in T/C ratios <100%, at 1.5s (T/C ratio = 69 ± 3 %), 2.5s (T/C ratio = 65 ± 2 %), 3.5s (T/C ratio = 83 ± 2 %), 4.5s (T/C ratio = 93 ± 2 %) and 6s (T/C ratio = 97 ± 1 %).

Fig. 3.2 Scatter plots demonstrating the distribution of amplitudes and latencies of the N2 wave in (A & C) CA3 and (B & D) DG of the gating rats (n = 25) in response to auditory stimuli.



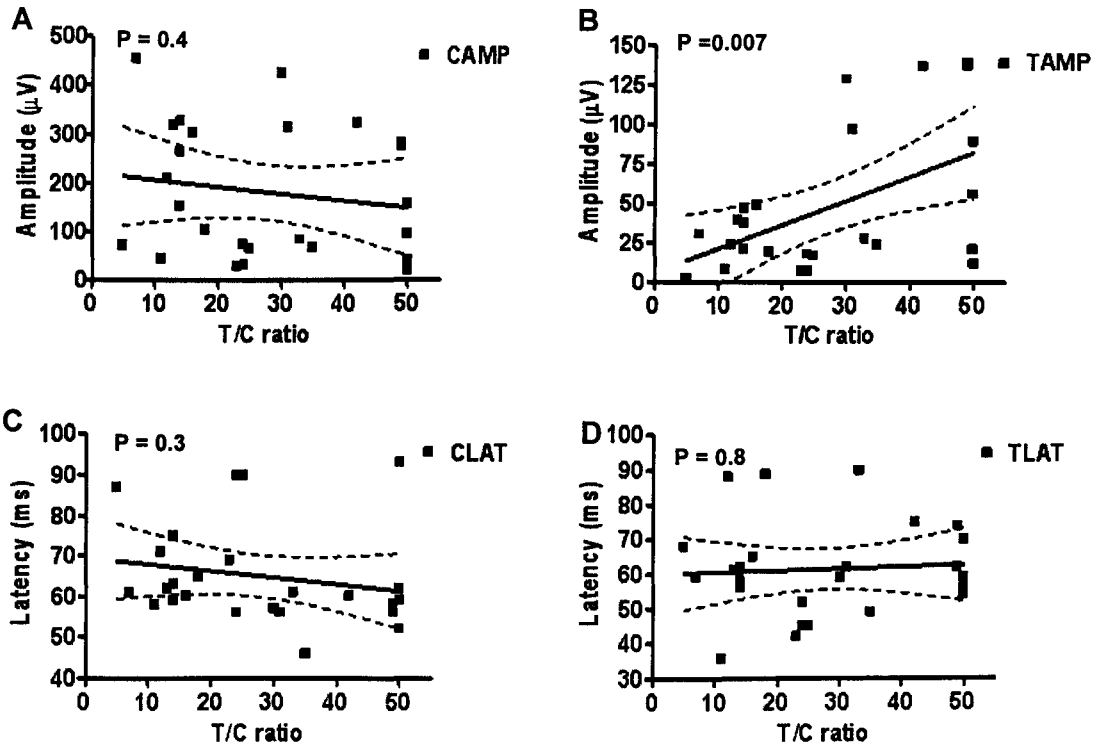
(A) The CA3 region demonstrated a wide variation of CAMP compared to lesser variations in TAMP and T/C ratios. The TAMP was significantly smaller (*** = $P<0.0001$) than CAMP resulting in a mean T/C ratio of 28 ± 3 % (mean ± SEM).

(B) The DG demonstrated a wide variation of CAMP compared to lesser variations in TAMP and T/C ratios. The TAMP was significantly smaller (*** = $P<0.0001$) than CAMP resulting in a mean T/C ratio of 30 ± 3% (mean ± SEM).

(C) The CLAT values varied between 46 – 93 ms while TLAT ranged between 36 – 90 ms. No significant difference was observed between CLAT and TLAT in CA3.

(D) The CLAT values in DG were between 46- 90 ms while TLAT ranged between 33 – 90 ms. No significant difference was observed between CLAT and TLAT in DG.

Fig. 3.3 Linear regression lines (dark lines) with 95% confidence limits (dashed lines) demonstrating the relationship of (A) CAMP, (B) TAMP, (C) CLAT and (D) TLAT with the T/C ratios of the N2 wave in the CA3 region of the gating rats (n = 25).

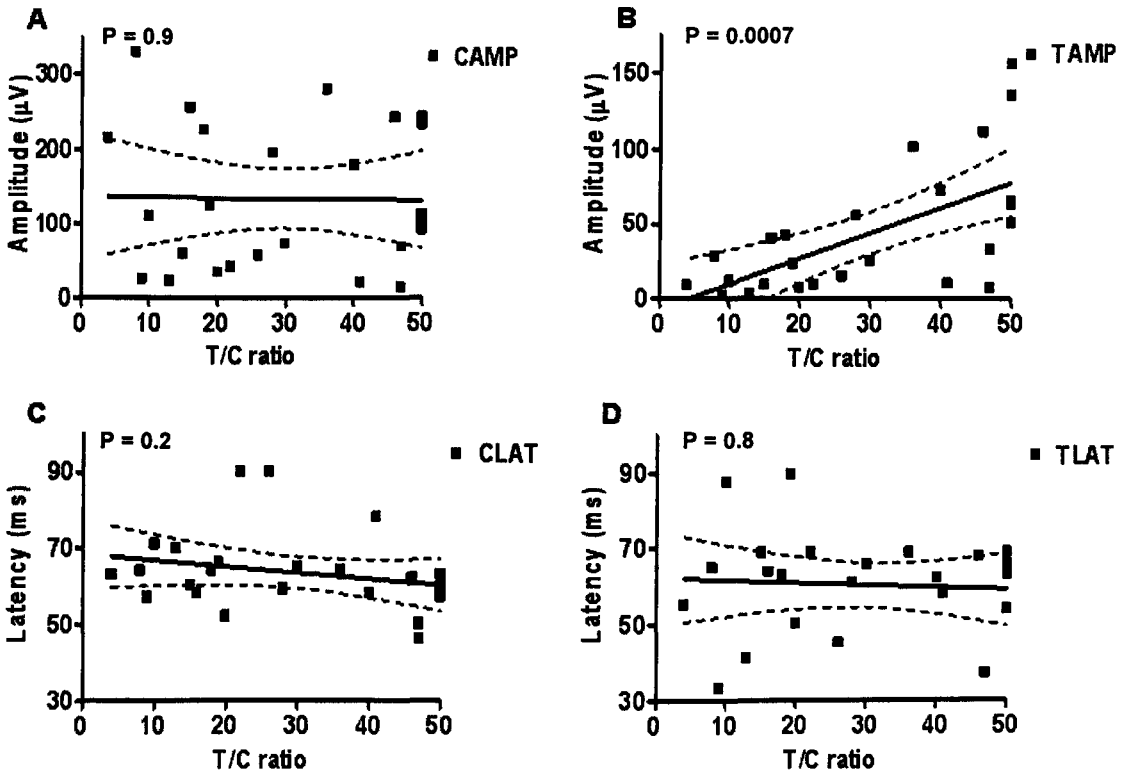


(A) Linear regression analysis examining the relationship between AER parameters with the T/C ratios revealed that changes in CAMP had no effect on the T/C ratios in the CA3.

(B) A significant positive correlation was observed between TAMP and the T/C ratios (P = 0.007).

(C & D) Neither CLAT nor TLAT latency correlated with changes in the T/C ratios.

Fig. 3.4 Linear regression lines (dark lines) with 95% confidence limits (dashed lines) demonstrating the relationship of (A) CAMP, (B) TAMP, (C) CLAT and (D) TLAT with the T/C ratios of the N2 wave in the DG of the gating rats (n = 25).

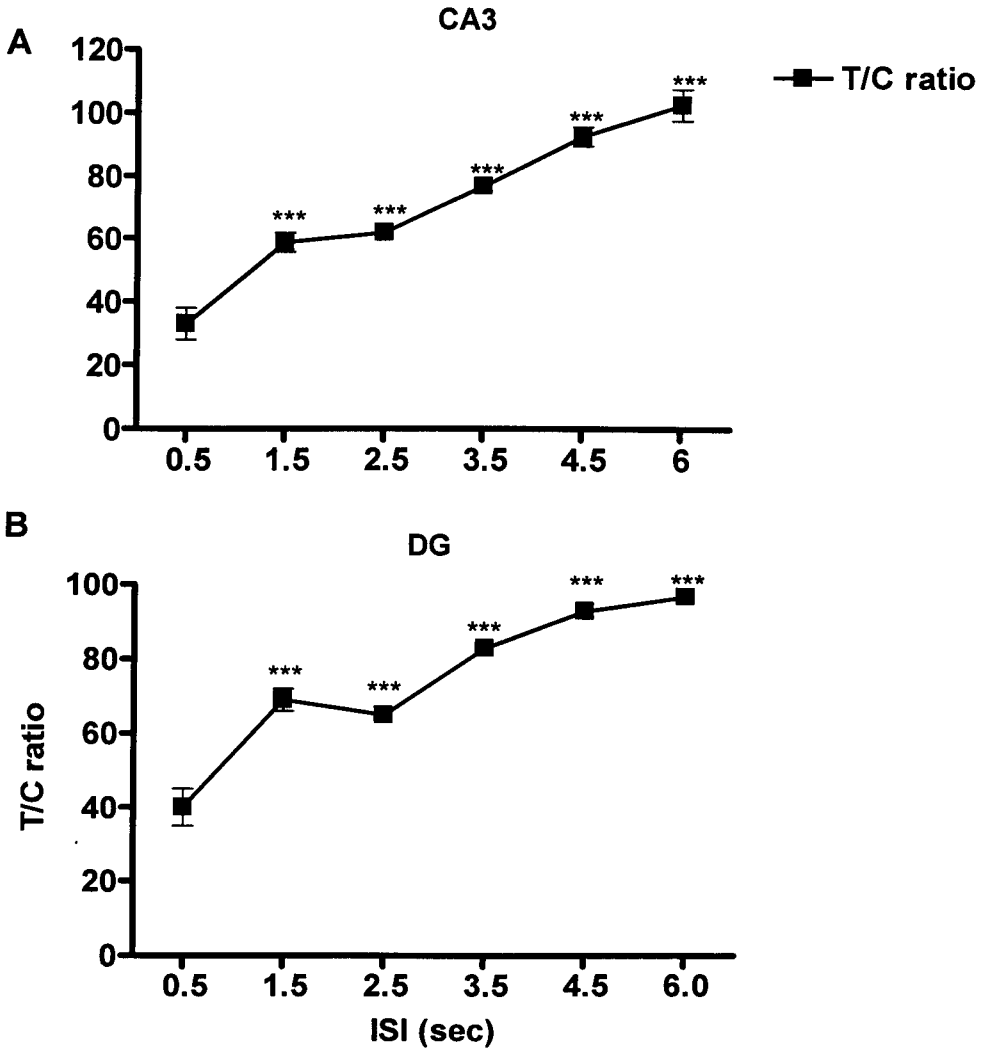


(A) Linear regression analysis revealed that changes in CAMP had no effect on the T/C ratios in the DG.

(B) A significant positive correlation was observed between TAMP and the T/C ratios (P = 0.0007)

(C & D) Neither CLAT nor TLAT latencies correlated with changes in the T/C ratios.

Fig. 3.5 Graphs comparing the T/C ratios of the N2 wave at different inter-stimuli interval (ISI) in (A) CA3 and (B) DG of the gating rats (n = 5; T/C ratios = mean ± SEM).



(A) The CA3 region exhibited significant increases in the T/C ratios at all ISI compared to the 0.5s ISI (••• = $P < 0.001$). The lowest T/C ratio was observed at 0.5s ISI (33 ± 5 %) and the highest at 6s (103 ± 5 %).

(B) The DG region exhibited significant increases in the T/C ratios at all ISI compared to the 0.5s ISI (••• = $P < 0.001$) with the lowest T/C ratio observed at 0.5s ISI (40 ± 3 %) and the highest at 6s (97 ± 1 %).

3.3.2 Auditory evoked N2 responses of the non-gating rats

Large amplitude responses to both the conditioning and test stimuli with absence of gating were detected in the CA3 and DG in the non-gating rats (Fig. 3.5). The CAMP varied from 28 - 343 μV (Fig. 3.6 A) in CA3 and 23 - 292 μV in DG (Fig. 3.6 B). In CA3, no significant difference was detected between the CAMP and TAMP (CAMP = 203 ± 24 μV, TAMP = 185 ± 23 μV; P = 0.2) resulting in a mean T/C ratio of 106 ± 11%. The DG exhibited a similar response to the CA3 with no significant difference between CAMP and TAMP (CAMP = 154 ± 18 μV, TAMP = 140 ± 15 μV; P = 0.2) resulting in a mean T/C ratio of 105 ± 10 %.

The CLAT in CA3 ranged from 47 - 63 ms (Fig 3.6 C).and CLAT of DG was observed from 46 - 65 ms (Fig 3.6 D). The mean CLAT in both CA3 and DG in non-gating rats was 55 ± 1 ms. Interestingly the TLAT of both regions was significantly longer than their CLAT (CA3 TLAT = 59 ± 1 ms; P= 0.0005; DG TLAT = 59 ± 1 ms; P= 0.0004)

Linear regression analysis was used to assess whether there were correlations between either amplitude or latency and the T/C ratio in CA3 (Fig. 3.7) and DG (Fig. 3.8) of the non-gating rats. In the CA3, a significant negative correlation was detected between the T/C ratio and CAMP (F = 9, P = 0.007). However, changes in TAMP did not affect the T/C ratio in CA3. Changes in CLAT or TLAT had no significant effect on the T/C ratio in CA3. The DG of non-gating rats also showed a significant negative correlation between T/C ratio and CAMP (F = 11; P = 0.004). But similar to the relationship observed in the CA3, changes in

TAMP of the DG had no effect on the T/C ratio. The changes in CLAT or TLAT had no significant effect on the T/C ratio in the DG of the non-gating rats.

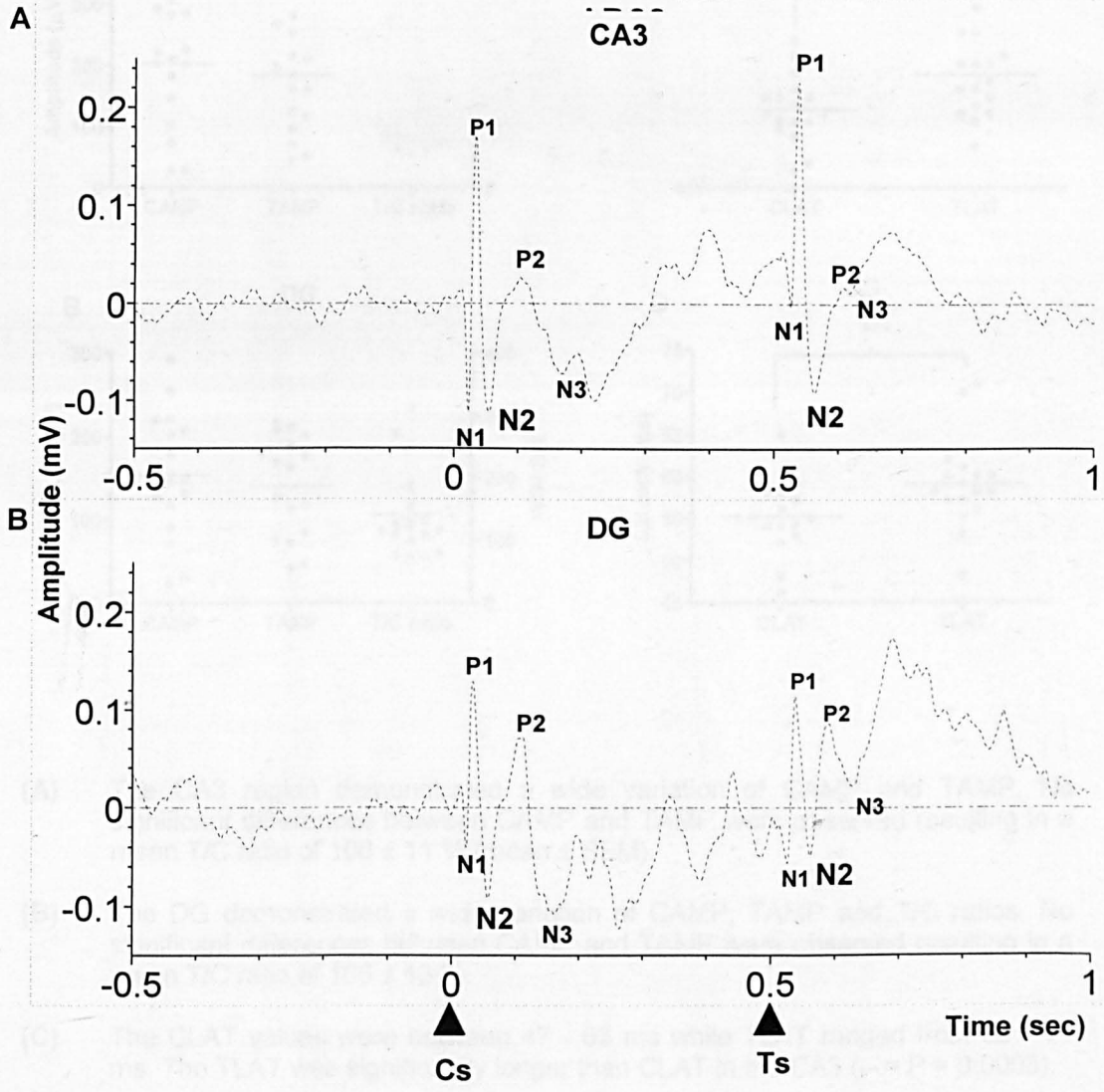
3.3.3 Comparison of LFP parameters of the gating rats and the non-gating rats

Based on the results which showed non-gating rats responding differently to auditory stimuli, we compared the amplitudes, latencies and T/C ratios between the gating rats and non-gating rats in the CA3 and DG to study whether there were significant differences between the two groups to allow us to classify them as two distinct groups with different sensitivities and responses to auditory-conditioning test paradigm (Fig. 3.9).

There were no significant differences in CAMP between gating and non-gating rats in either CA3 ($P = 0.6$) or DG ($P = 0.4$). Non-gating rats demonstrated a significantly higher TAMP in both regions compared to gating rats (CA3 = $P<0.0001$; DG = $P<0.0001$). Significantly higher T/C ratios were also detected in non-gating rats in both areas compared to gating rats (CA3 = $P<0.0001$; DG = $P<0.0001$). According to the above findings higher amplitude responses to the test stimuli appeared to contribute to the higher T/C ratios in non-gating rats compared to gating rats. Non-gating rats also showed significantly shorter CLAT in CA3 and DG compared to those of gating rats (CA3, $P = 0.002$; DG, $P = 0.004$). No significant difference of TLAT was observed between gating and non-gating rats either in the CA3 or DG. In non-gating rats the changes in T/C ratios were negatively correlated with CAMP while in gating rats CAMP had no effect on the T/C ratio. Moreover, the T/C ratio of gating rats increased with

increases in TAMP while TAMP of non-gating rats had no effect on the T/C ratio.

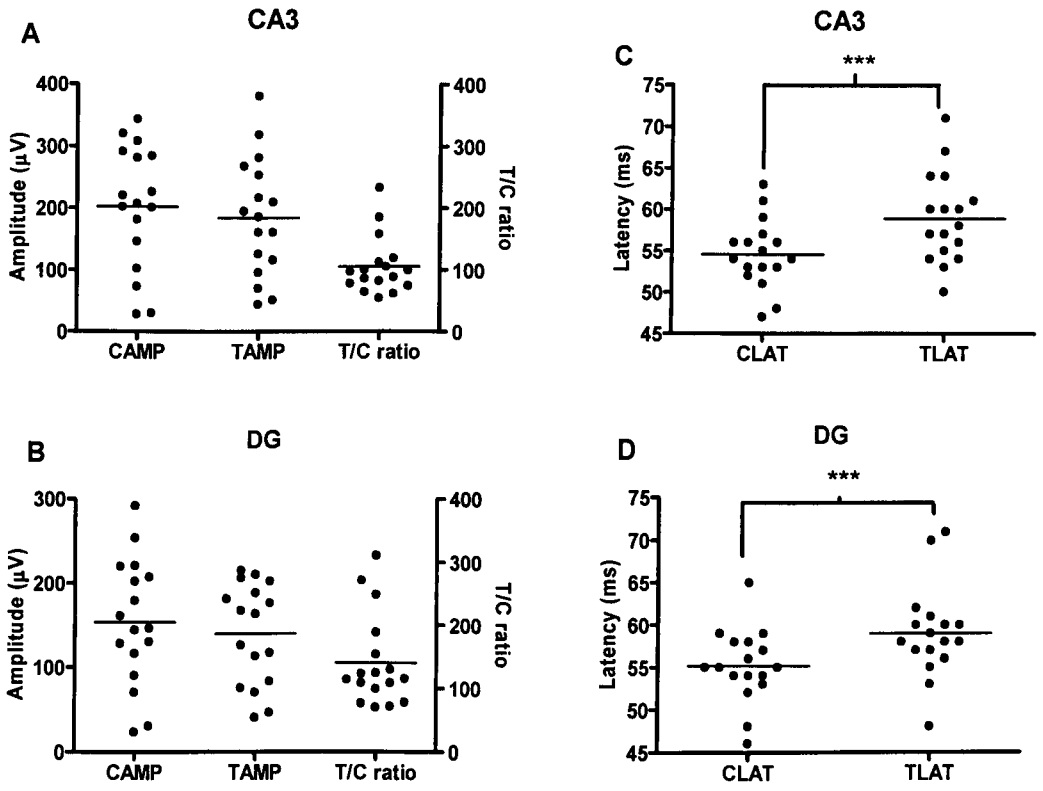
Fig. 3.6 Representative peri-event averaged LFPs from (A) CA3 and (B) DG of a non-gating rat in response to 128 auditory conditioning-test trials.



(A) The peri-event averaged LFPs from CA3 in response to the conditioning and test stimuli (timing of the stimuli are indicated by the triangles) demonstrated positive and negative AER complexes with absence of gating of the N2 wave (T/C = 104%).

(B) The peri-event averaged LFPs from DG demonstrated positive and negative AER complexes with absence of gating of the N2 wave (T/C = 90%).

Fig. 3.7 Scatter plots demonstrating the distribution of amplitudes and latencies of the N2 wave in the non-gating rats in (A & C) CA3 and (B & D) DG in response to auditory stimuli (n = 17).



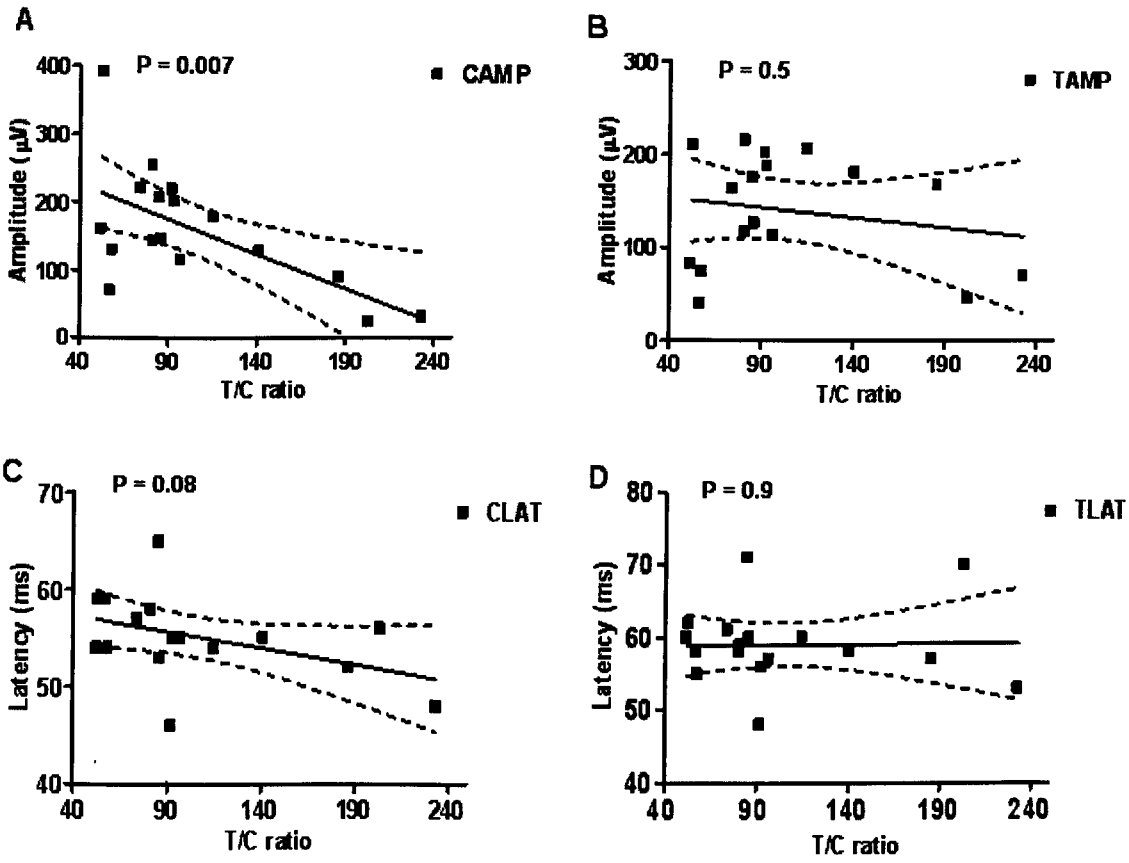
(A) The CA3 region demonstrated a wide variation of CAMP and TAMP. No significant differences between CAMP and TAMP were observed resulting in a mean T/C ratio of 106 ± 11 % (mean ± SEM).

(B) The DG demonstrated a wide variation of CAMP, TAMP and T/C ratios. No significant differences between CAMP and TAMP were observed resulting in a mean T/C ratio of 105 ± 13 %.

(C) The CLAT values were between 47 - 63 ms while TLAT ranged from 50 - 71 ms. The TLAT was significantly longer than CLAT in the CA3 (*** = P = 0.0005).

(D) In the DG, the CLAT values were between 46 – 65 ms while TLAT ranged from 47 – 71 ms. The TLAT was significantly longer than CLAT (*** = P = 0.0004).

Fig. 3.8 Linear regression lines (dark lines) with 95% confidence limits (dashed lines) demonstrating the relationship of (A) CAMP, (B) TAMP, (C) CLAT and (D) TLAT with the T/C ratios of the N2 wave in the CA3 of the non-gating rats (n = 17).

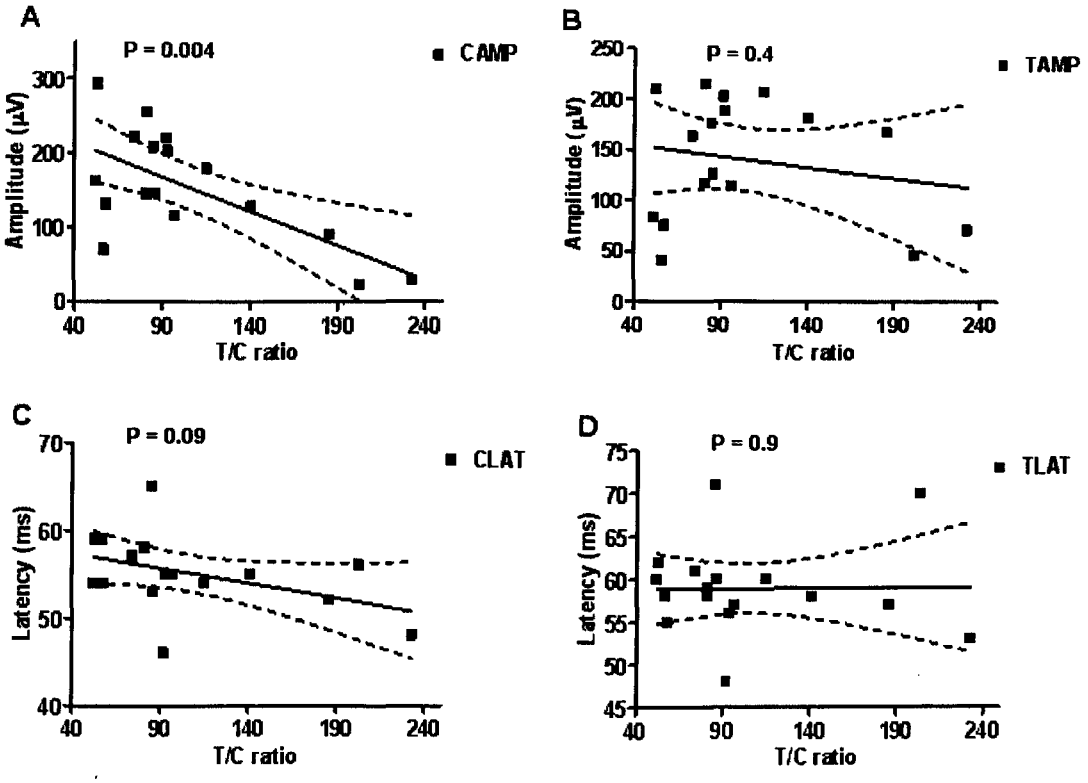


(A) Linear regression analysis demonstrated that CAMP of non-gating rats had a significant negative correlation with the T/C ratios in CA3 (P = 0.007).

(B) The changes in TAMP were not associated with the changes in the T/C ratios.

(C & D) Neither CLAT nor TLAT latencies significantly correlated with changes in the T/C ratios.

Fig. 3.9 Linear regression lines (dark lines) with 95% confidence limits (dashed lines) demonstrating the relationship of (A) CAMP, (B) TAMP, (C) CLAT and (D) TLAT with the T/C ratios of the N2 wave in the DG of the non-gating rats (n = 17).

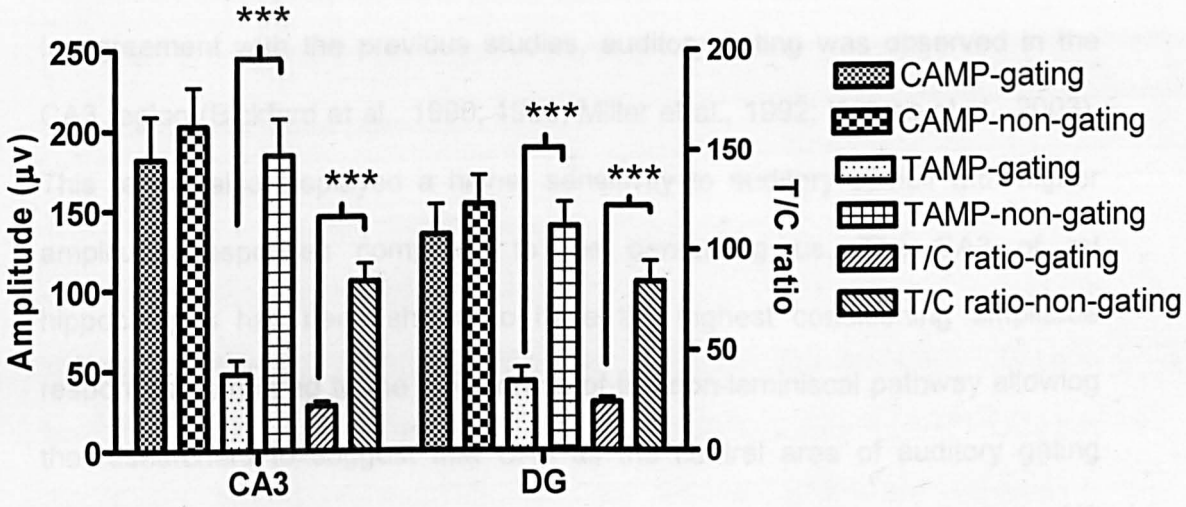


(A) The CAMP demonstrated a significant negative correlation with the T/C ratios in the DG (P = 0.004).

(B) The changes in TAMP were not associated with the changes in the T/C ratios.

(C & D) Neither CLAT nor TLAT latencies significantly correlated with changes in the T/C ratios.

Fig. 3.10 Bar graph depicting CAMP, TAMP and the T/C ratios of the N2 wave in the CA3 and DG of the gating rats (n = 25) compared with the findings of the non-gating rats (n = 17).



There was no significant difference in CAMP ($P > 0.05$) between gating and non-gating rats in either CA3 or DG. The non-gating rats had significantly higher TAMP and T/C ratios compared to the gating rats in both CA3 and DG (*** = $P < 0.0001$; values = mean ± SEM).

3.4 Discussion

Robust auditory evoked potentials were recorded from the CA3 and DG of rat hippocampus with some rats demonstrating auditory gating and some exhibiting absence of gating.

In agreement with the previous studies, auditory gating was observed in the CA3 region (Bickford et al., 1990; 1993; Miller et al., 1992; Krause et al., 2003). This region also displayed a higher sensitivity to auditory stimuli with higher amplitude responses compared to the dentate gyrus. The CA3 of rat hippocampus has been shown to have the highest conditioning amplitude responses compared to the other areas of the non-leminiscal pathway allowing the researchers to suggest that CA3 as the central area of auditory gating (Bickford et al., 1990; Moxon et al., 1999). However, in the current study the N2 wave recorded from the CA3 region had a higher conditioning response latency range compared to the latencies of the "N40" wave recorded from the CA3 region in the previous studies (Adler et al., 1986, CLAT = 50.8 ± 2.8 ms; Bickford-Wimer et al., 1990, CLAT = 41 ± 1 ms; Moxon et al., 1999, CLAT = 33.1 ± 2.2 ms). This disparity could be attributable to the differences in methodology. We used Lister hooded rats under isoflurane anaesthesia, while previous studies used Sprague-Dawley or Wistar rats that were either freely moving with implanted electrodes (Adler et al., 1986; Moxon et al., 1999) or anesthetized with chloral hydrate (Bickford-Wimer et al., 1990). Isoflurane is an easily maintainable gaseous anaesthetic, which minimizes the fluctuation of the level of anaesthesia. Several studies have shown that isoflurane prolongs the latencies of the AERs in both humans and rats and the prolonged latencies

correlate with the depth of anaesthesia (Heneghan et al. 1987; Madler et al. 1991; Santarelli et al. 2003). It is quite likely that the use of isoflurane underlies the latency difference observed in this study compared to other studies.

We observed auditory gating in the DG with similar findings to the CA3 suggesting an important role played by the DG in the auditory gating process. Presence of auditory gating in the DG also suggests the possibility of auditory gating in the lemniscal pathway.

The N2 wave of CA3 and DG regions demonstrated maximum gating at 0.5s ISI with an increase in the T/C ratios with increments in ISI, which is similar to the changes observed in the rat N40 (Bickford et al.,1993; Krause et al., 2003) and human P50 (Freedman et al., 1983; Dolu et al., 2001). However, the findings of the studies, regarding the ISI at which the reduction of the test response is completely abolished, are inconclusive. Human studies have studied ISI up to 8s with some studies showing complete abolition of gating at and above 2s (Freedman et al., 1983), 6s (Adler et al., 1982) or 8s (Fruhstorfer et al., 1970). Some rat studies have shown complete absence of N40 test amplitude reduction at 4s ISI (Adler et al., 1986) while others have shown T/C ratio >100% using an ISI of 5s (Bickford et al., 1993). Different neuronal mechanisms may be responsible for reductions in TAMP at different ISIs and the findings from this and previous studies advocate the use of an ISI of <1s, ideally 0.5s, to obtain maximum gating and a longer inter-trial interval (>8s) to allow the recovery of all the neuronal mechanisms responsible for gating in the conditioning–testing paradigm.

A significant positive relationship was observed between TAMP and T/C ratio

both in the CA3 and DG, suggesting that auditory gating in both areas was dependent on neuronal mechanisms activated following the conditioning stimulus with resultant inhibitory effects on the response to the test stimulus. Moxon et al (1999) observed the same relationship between the T/C ratio and TAMP of N40 in the CA3 of freely moving rats. The same group reported that auditory gating in CA3 was not dependent on CAMP, CLAT or TLAT, which is similar to our findings from both CA3 and DG.

In accordance with previous studies, some rats demonstrated gating of the test response while others demonstrated enhancement of the test response, resulting in non-gating (Adler et al., 1986; Miller & Freedman, 1995; Moxon et al., 1999). No explanation was given to the consistently non-gating state of the rats recorded in previous studies. This effect cannot be attributed to anaesthesia as it has been documented in freely moving rats (Moxon et al., 1999). Some studies have shown gating and non-gating instances in the same rat, both under anaesthesia (Miller and Freedman, 1995) and in freely moving rats (Moxon et al., 1999).

We observed a higher percentage of non-gating rats compared to other studies and this allowed us to characterize their AERs. Non-gating rats had similar amplitude responses to both the conditioning and test stimuli in CA3 and DG indicating the dysfunctions in neuronal mechanisms responsible for gating of the test response. Non-gating rats also had significantly longer TLAT compared to their CLAT highlighting possible derangements in the neuronal circuitry in eliciting the test response.

Interestingly, the T/C ratio of the non-gating rats negatively correlated with CAMP in both CA3 and DG, suggesting that the lack of gating in this rat group is also associated with the inability to respond normally to the first stimulus in the paired stimulus paradigm. This finding with non-gating rats is similar to the non-gating states induced by dopaminergic agonists which disrupt gating by decreasing the CAMP in rats (Adler et al., 1986; de Bruin et al., 2001). Lower CAMP and shorter CLAT have been shown to be associated with higher T/C ratios observed in schizophrenic patients (Adler et al., 1982; Freedman et al., 1983; Siegel et al., 1984), an interesting similarity to our findings with the non-gating rats. However, in the present study, no significant difference in CAMP was observed between gating and non-gating rats in contrast with the findings from human studies which found schizophrenic patients exhibited lower CAMP than controls (Adler et al., 1982; Freedman et al., 1983). Schizophrenic patients have been shown to have shorter CLAT than controls which is similar to the findings of our study between gating and non-gating rats (Adler et al., 1982; Freedman et al., 1983).

Non-gating rats had significantly higher TAMP and T/C ratio than gating rats, highlighting again the deficits in neural inhibitory mechanisms required to gate the test response in the non-gating rat group.

By examining AERs in the CA3 and DG regions of the hippocampus in Lister-hooded rats under isoflurane anaesthesia, we have been able to identify the N2 wave which exhibits auditory gating similar to rat N40 and human P50. We have

also found a group of rats exhibiting non-gating with some AER properties comparable to those recorded from schizophrenic patients. The next step was to examine the AERs and auditory gating in the medial prefrontal cortex, with simultaneous recording from the CA3 and DG of hippocampus, which would expand this model of sensory gating to a brain area that has importantly been implicated in the pathogenesis of schizophrenia (Knight et al., 1989; Tregellas et al., 2007).

Chapter Four

Characterization of sensory gating in the medial prefrontal cortex of the rat

4. Characterization of sensory gating in the medial prefrontal cortex of the rat

4.1 Introduction

Prefrontal cortex (PFC) is a crucial brain area for executive functions, memory and attention. The rat PFC consists of cytoarchitecturally and functionally distinct areas located over the medial, orbital and insular surfaces of the rostral cerebral hemispheres (see chapter 1) and the medial prefrontal cortex (mPFC) is posited to be involved in the cognitive processes such as memory and attention (Gabbott et al., 2005).

Anatomical and functional alterations in the human PFC are implicated in the pathophysiology of schizophrenia. Early imaging studies suggested a decrease in the tonic activity in the PFC (i.e. hypofrontality) and a reduction in the prefrontal cortical volume in patients with schizophrenia compared to controls (Weinberger et al., 1992; Harvey et al., 1993). Some recent studies contradict the state of hypofrontality and suggest that deficits in other cortical regions (e.g. hippocampus, amygdala) interconnecting with the PFC may produce the functional changes elicited in the PFC of schizophrenic patients (Grace et al., 2000).

Recent human studies using intracerebral electrodes have shown gating of auditory evoked responses in the PFC (Knight et al., 1989; Grunwald et al., 2003; Kurthen et al., 2007). Imaging studies have reported prefrontal

involvement in auditory gating with increased activity in the PFC, in response to auditory conditioning-testing paradigm (Tregellas et al., 2007). Knight et al (1989) reported that patients with frontal lobe lesions have larger P50 responses compared to controls, pointing to a frontal modulation of P50 auditory evoked responses.

Two studies have reported auditory gating in the medial prefrontal cortex of freely moving rats with implanted electrodes (Mears et al., 2006; Yang et al., 2006). Mears et al (2006) reported a consistently 'gating' positive wave, occurring around 60 ms following auditory stimuli (P60) in the rat mPFC and Yang et al (2006) observed three AERs (designated P2, N2 & P3) demonstrating gating in the rat mPFC. No studies have examined the AERs and auditory gating in the mPFC of Lister hooded rats under isoflurane anaesthesia, with simultaneous recording from the hippocampus.

The main objective of this chapter was to examine AERs and characterize the basic properties of auditory gating in the medial prefrontal cortex (mPFC) by analyzing the LFP responses to auditory conditioning-test paradigm in Lister hooded rats under isoflurane anaesthesia. It was also aimed to observe the AERs and gating in the mPFC with simultaneously recorded results from CA3 and dentate gyrus (DG) of the hippocampus.

4.2 Methods

4.2.1 Animals

Of the forty two male Lister hooded rats described in chapter 3, twenty five animals (weight 250g – 400g) had electrodes placed in the mPFC and simultaneous recordings from the hippocampus and mPFC were carried out as described in chapter 2. Only the animals with electrodes placed in the mPFC, CA3 and DG, confirmed by histology, were used for analysis.

4.2.2 Experimental protocol

Basal activity of the mPFC was recorded for 5 minutes prior to the auditory stimuli. Auditory stimuli (90dB intensity, 0.5s ISI, 10s inter-trial interval) were presented over 128 trials and auditory-evoked LFP and unit responses were recorded simultaneously. Following detection of auditory gating in the mPFC, responses to five different inter-stimuli intervals (1.5s, 2.5s, 3.5s, 4.5s and 6s; n = 5) over 128 trials were recorded.

4.2.3 Data analysis

The AERs in the mPFC was initially displayed as peri-event averaged LFPs and the peaks and troughs of LFPs in response to stimuli were identified according to the polarity and the order of occurrence i.e. N1, P1, N2, P2, N3 (see chapter 2). Conditioning amplitudes (CAMP), test amplitudes (TAMP), conditioning response latencies (CLAT), test response latencies (TLAT) and T/C ratio of the

N2 wave was analysed using a matlab script (amplat). An electrode from each animal demonstrating the highest conditioning response amplitude was selected for further analysis. The T/C ratios at different inter-stimuli intervals were obtained using another Matlab script (Amplatlong). Statistical analysis was carried out using Student t test or one way analysis of variance (ANOVA) with post hoc Tukey t test when appropriate. Linear regression coefficient was used to assess the relationship of the amplitude and latency parameters to the T/C ratios. The gating and non-gating rats were identified according to the T/C ratio in the simultaneously recorded CA3 region (Moxon et al., 1999). The animals demonstrating T/C ratio $\leq$ 50% in the CA3 were identified as gating rats and animals with T/C ratio > 50% were identified as non-gating rats. Data are expressed as mean $\pm$ SEM (standard error of mean) with $P<0.05$ considered statistically significant.

4.3 Results

The mPFC demonstrated prominent positive and negative waveform complexes (as shown in chapter 2) in response to the auditory stimuli (Fig. 4.1). The amplitudes (CAMP, TAMP), T/C ratios and latencies (CLAT and TLAT) of the N2 wave were analysed in the gating (n = 15) and the non-gating rats (n = 10) and then compared between the two groups.

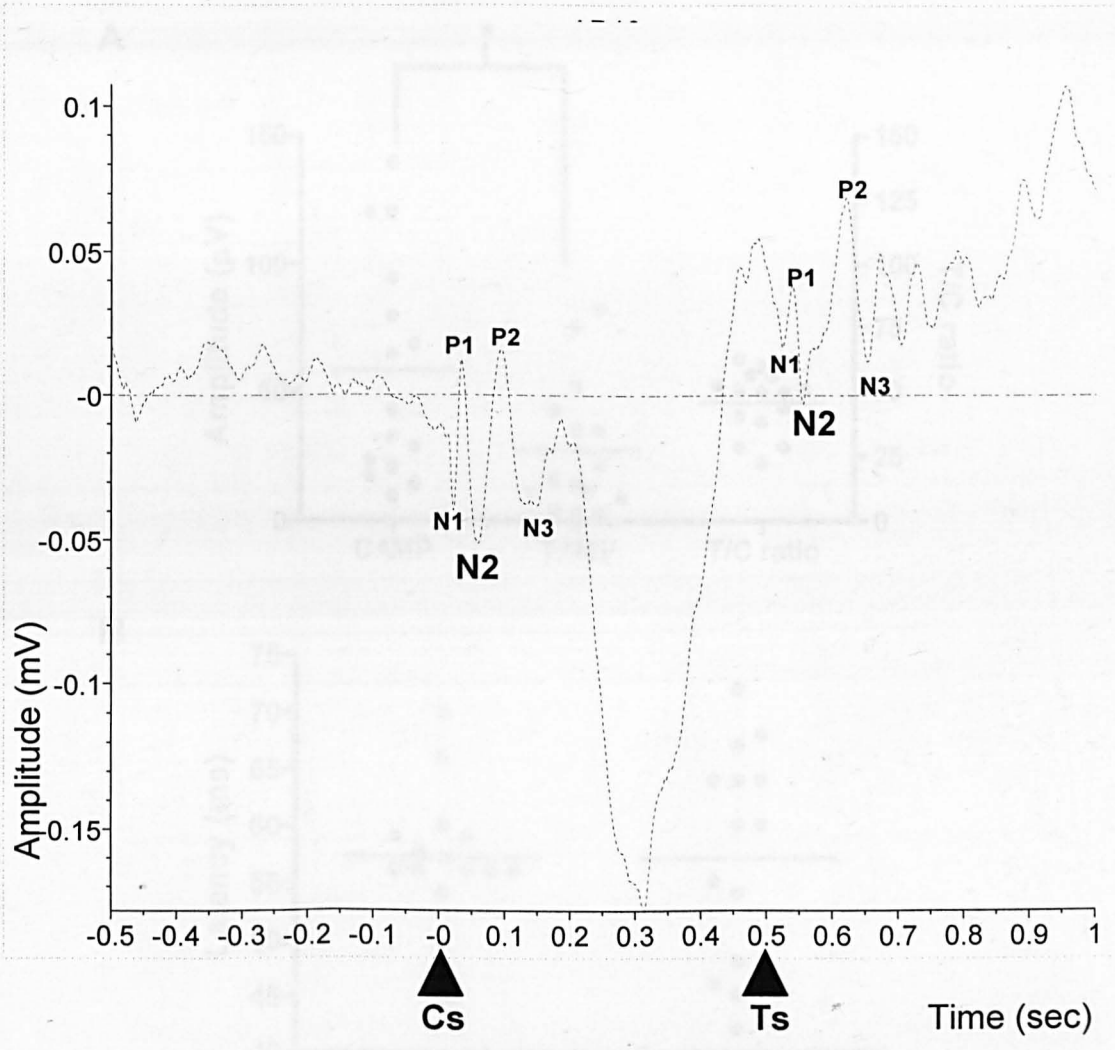
4.3.1 Auditory evoked N2 responses of the gating rats

The N2 wave in the mPFC exhibited widely varying conditioning response amplitudes ranging from 9 - 139 μV (Fig. 4.2A). A significant reduction in the test response amplitudes was observed compared to conditioning response amplitudes (TAMP = 28 ± 5 μV; P= 0.02; Fig. 4.2 A) resulting in gating of the N2 wave (T/C = 45 ± 3%). However, T/C ratio in the mPFC of the gating rats ranged from 22 – 62% with some rats (n = 7) exhibiting T/C ratios between 50 – 62%.

The conditioning response occurred at a latency range of 45 – 70 ms with a mean latency of 57 ± 1 ms (Fig. 4.2 B). There was no significant difference between conditioning response latencies and test response latencies (TLAT = 57 ± 2 ms, P = 0.9).

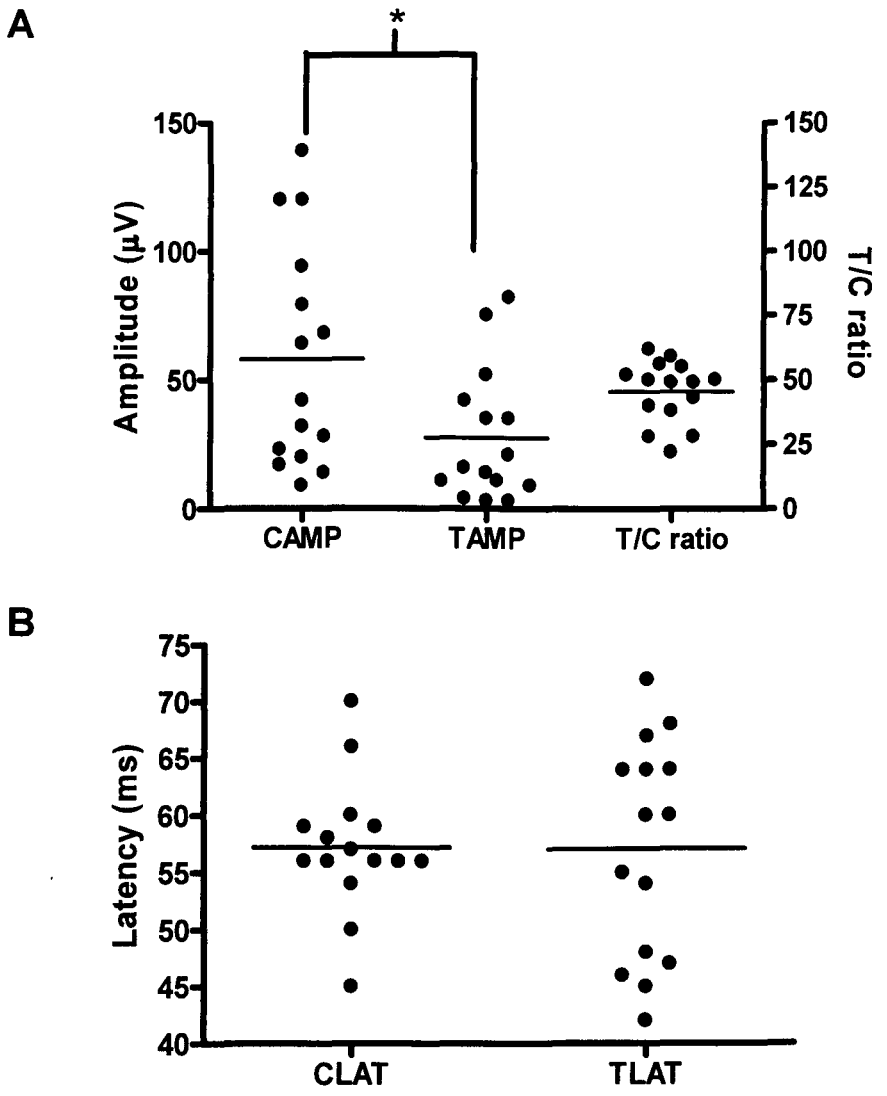
Evidence for a correlation between either amplitude or latency and the T/C ratios was determined using linear regression analysis in the mPFC (Fig. 4.3). The changes in CAMP had no effect on the T/C ratio. In contrast a significant positive correlation was detected between the TAMP and the T/C ratio (F = 4.9; P = 0.04). Neither CLAT nor TLAT showed a significant correlation with the T/C ratios.

Fig. 4.1 Representative peri-event averaged LFPs in the mPFC of a gating rat in response to 128 auditory conditioning-test trials.



The perievent averaged LFPs from the mPFC in response to the conditioning (Cs) and test (Ts) stimuli (timing of the stimuli are indicated by the triangles) demonstrated positive and negative AER complexes (i.e. N1, P1, N2, P2, N3) with gating of the N2 wave (T/C ratio= 48%).

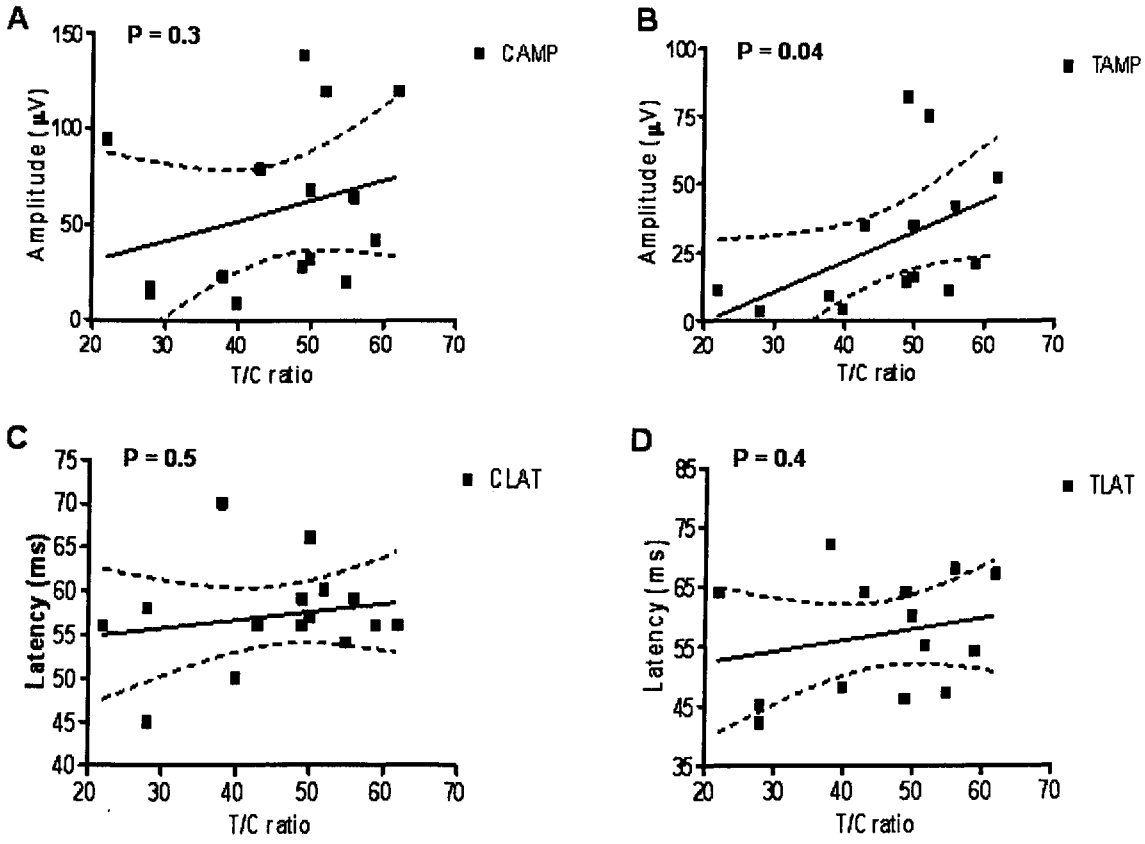
Fig. 4.2 Scatter plots demonstrating the distribution of **(A)** amplitudes, T/C ratios and **(B)** latencies of the N2 wave in the mPFC of the gating rats (n = 15) in response to auditory stimuli.



(A) The mPFC demonstrated a wide variation in the CAMP of N2 compared to lesser variations in TAMP and T/C ratios. The TAMP was significantly smaller (* = P= 0.02) than CAMP resulting in a mean T/C ratio of 45 ± 3 % (mean ± SEM).

(B) The CLAT values of N2 varied between 45 - 70 ms while TLAT ranged between 42 - 72 ms. No significant difference was observed between CLAT and TLAT in the mPFC.

Fig. 4.3 Linear regression lines (dark lines) with 95% confidence limits (dashed lines) demonstrating the relationship of (A) CAMP, (B) TAMP, (C) CLAT and (D) TLAT with the T/C ratios of the N2 wave in the mPFC of the gating rats (n = 15).



(A) The changes in CAMP of the N2 wave had no effect on the T/C ratios in the mPFC.

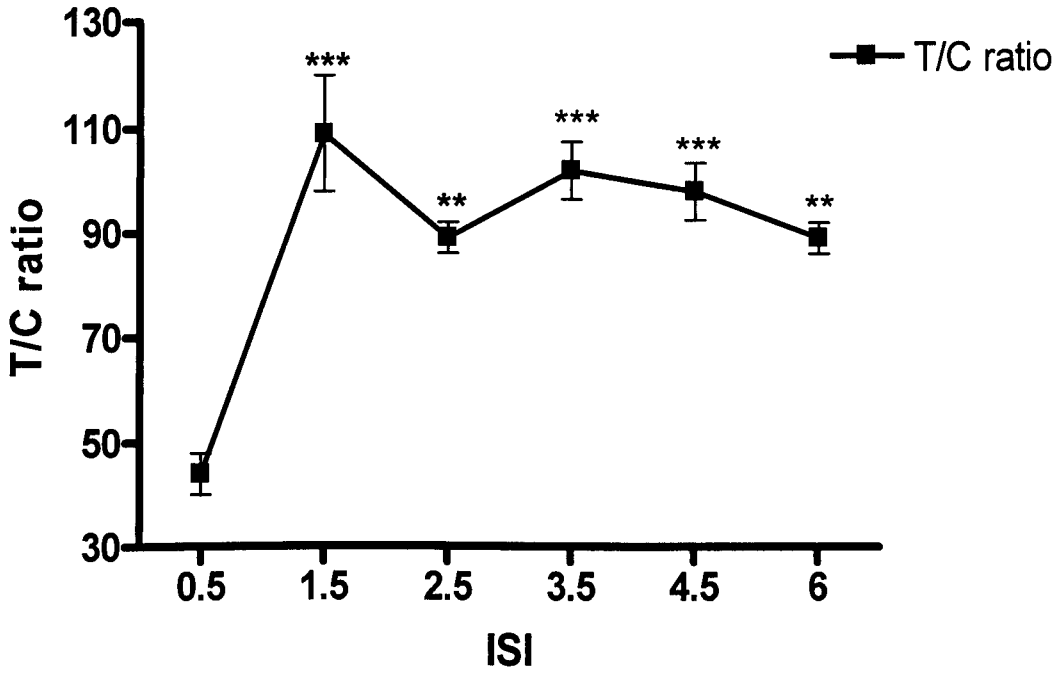
(B) A significant positive correlation was observed between the TAMP and the T/C ratios (P = 0.04).

(C & D) Neither CLAT nor TLAT significantly correlated with changes in the T/C ratios.

4.3.1.1 Gating at different inter-stimuli intervals

The T/C ratio in the mPFC increased with the increases in the inter-stimuli interval (Fig. 4.4). A significant increase in the T/C ratio was detected at all tested inter-stimuli intervals ($F_{5, 35}$ = 7.6, P<0.0001) and the post hoc t tests revealed that the significant change was due to the significant increases in the T/C ratios at 1.5s, 2.5s, 3.5s, 4.5s and 6s inter-stimuli intervals when compared with the 0.5 s inter-stimuli interval. Maximum mean T/C ratio was observed at 1.5s inter-stimuli interval (T/C = 109 ± 22 %). However, there was no significant difference between the T/C value at 1.5s inter-stimuli interval and the T/C ratios at higher inter-stimuli intervals (2.5s, 3.5s, 4.5s, 6s; P>0.05), indicating absence of the ability to decrease the test response amplitude from the 1.5s inter-stimuli interval onwards.

Fig. 4.4 Comparison the T/C ratios of the N2 wave at different inter-stimuli intervals (ISI) with the T/C ratio at 0.5s inter-stimuli interval in the mPFC of the gating rats (n = 5; values = mean ± SEM).



The mPFC exhibited significant increases in the T/C ratios at all ISI compared to the T/C ratio at 0.5s ISI ((.. = P < 0.01, ... = P < 0.001). The lowest T/C ratio was observed at 0.5s ISI (44 ± 8 %; n = 4) and highest T/C ratio at 1.5s (109 ± 22 %).

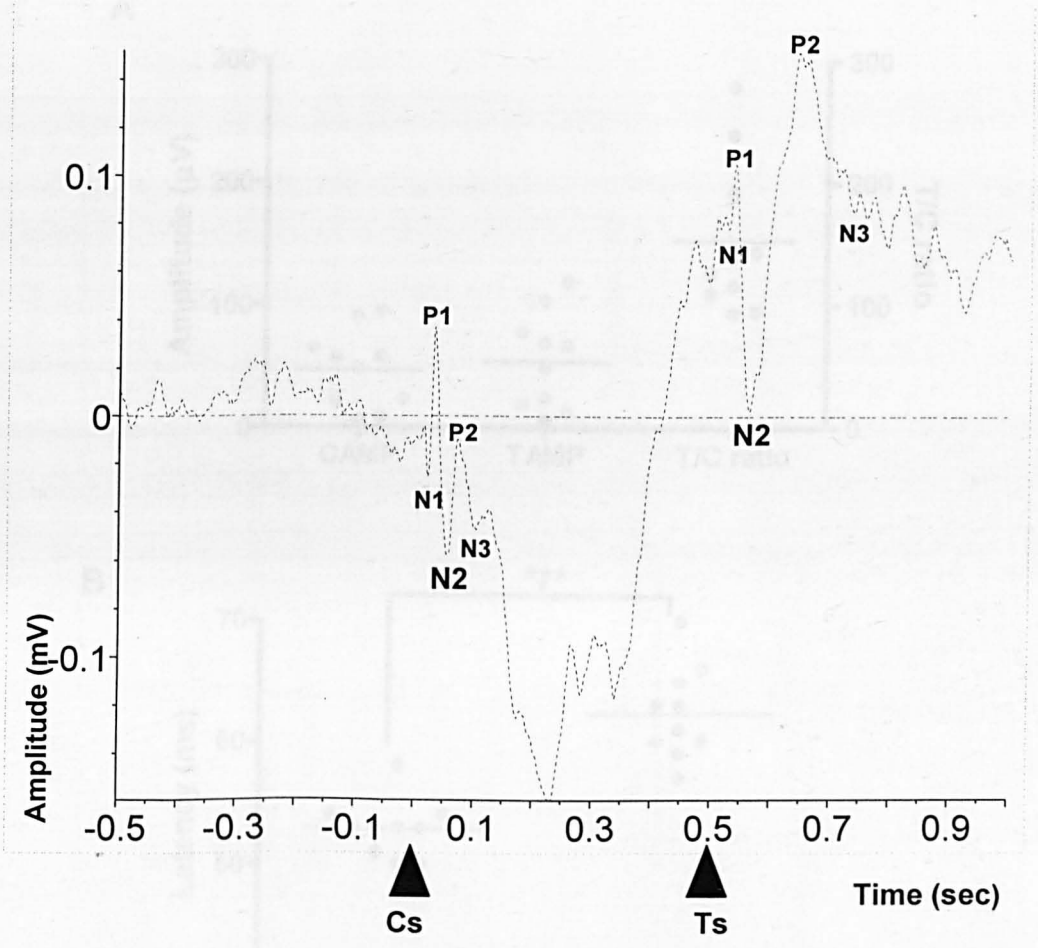
4.3.2 Auditory evoked N2 responses of the non-gating rats

Prominent N2 responses to both the conditioning and test stimuli with absence of gating were detected in the mPFC of the non-gating rats (Fig. 4.5). The CAMP varied from 6 - 95 μV and no significant difference was detected between the CAMP and the TAMP (CAMP = 47 ± 10 μV, TAMP = 55 ± 13 μV; P = 0.4) resulting in a mean T/C ratio of 153 ± 20 % (Fig. 4.6A).

The CLAT in mPFC ranged from 50 - 58 ms (Fig 4.6 B) with a mean CLAT of 53 ± 1 ms. The TLAT was significantly longer compared to CLAT (TLAT = 62 ± 1ms; $P < 0.0001$).

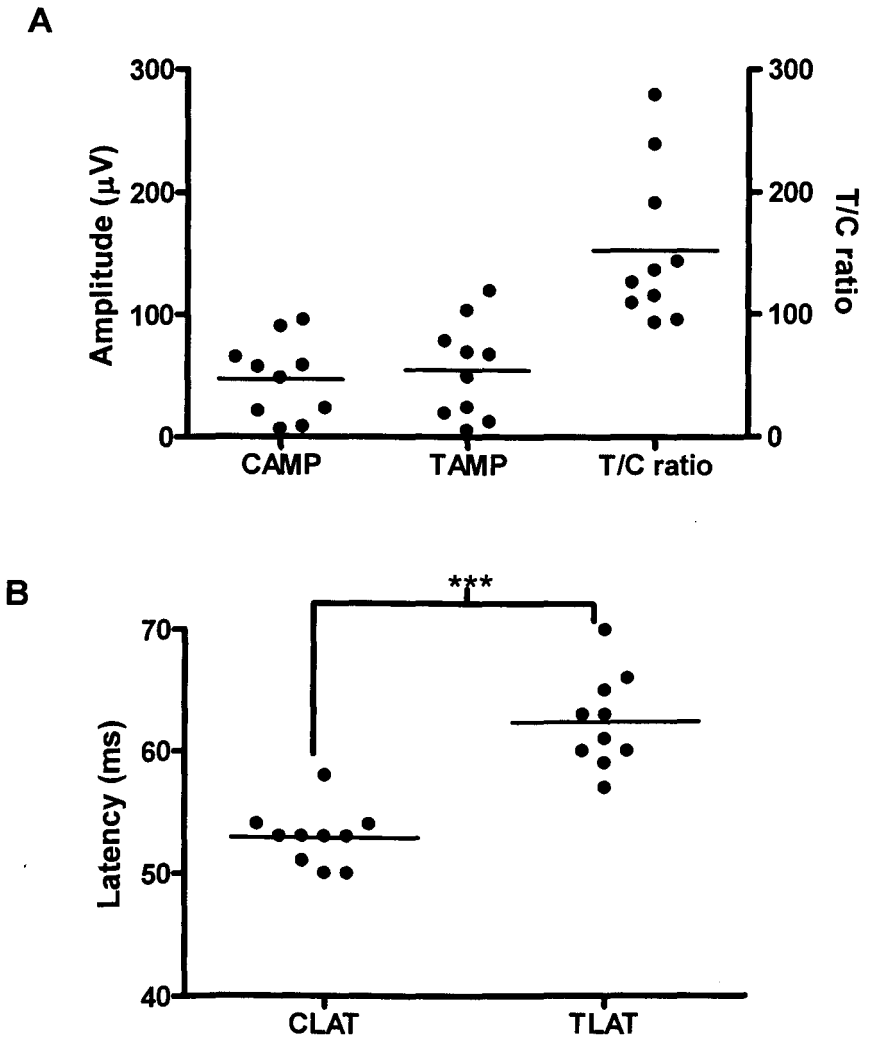
Linear regression analysis in the mPFC of the non-gating rats demonstrated a significant negative correlation between the T/C ratio and CAMP (Fig. 4.7; $F = 8.6$, $P = 0.01$). However, changes in TAMP did not affect the T/C ratio. Neither CLAT nor TLAT showed a significant correlation with the T/C ratios in the non-gating rats.

Fig. 4.5 Representative peri-event averaged LFPs in the mPFC of a non-gating rat in response to 128 auditory conditioning-test trials.



The peri-event averaged LFPs from mPFC in response to the conditioning (Cs) and test (Ts) stimuli (timing of the stimuli are indicated by the triangles) demonstrated positive and negative AER complexes (i.e. N1, P1, N2, P2, N3) with absence of gating of the N2 wave (T/C ratio = 124%).

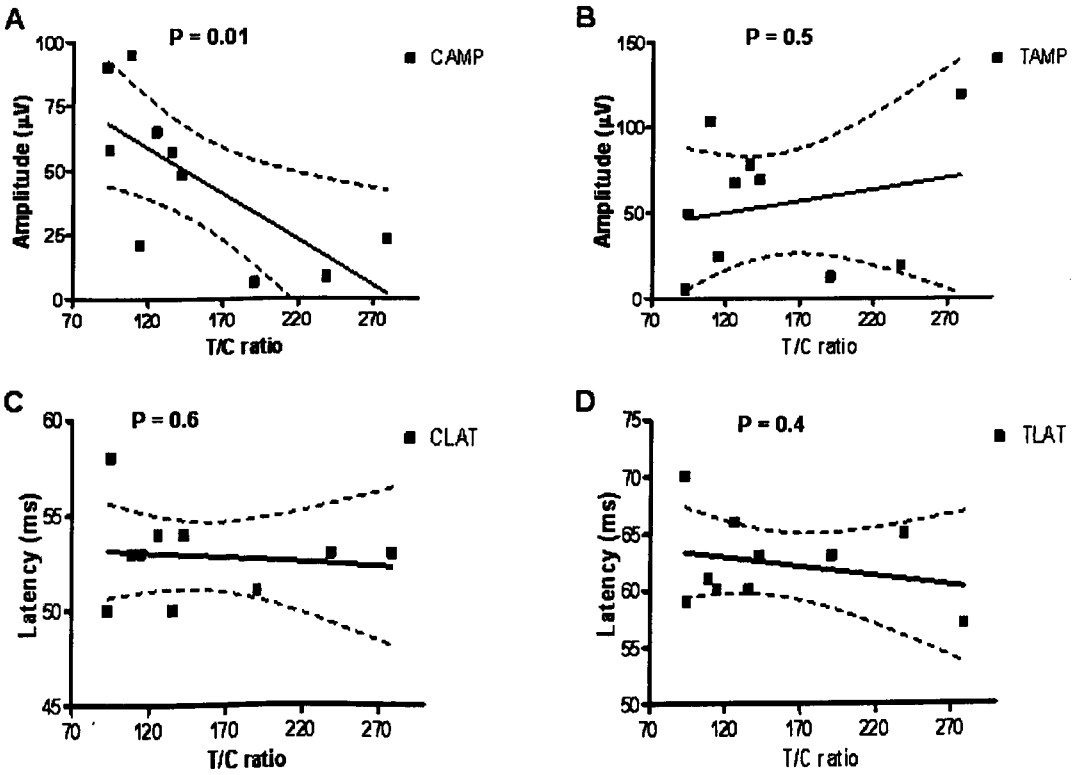
Fig. 4.6 Scatter plots demonstrating the distribution of amplitudes, T/C ratios and latencies of the N2 wave in the mPFC of the non-gating rats (n = 10) in response to auditory stimuli.



(A) The mPFC demonstrated a variation of CAMP, TAMP and T/C ratios of the N2 wave with a few outliers exhibiting TC ratios >200%. No significant difference between CAMP and TAMP was observed resulting in T/C ratio of 107% ± 8 (mean ± SEM).

(B) The CLAT values were between 50 - 58 ms while TLAT ranged from 57 -70ms. The TLAT was significantly longer than the CLAT ($P<0.0001$).

Fig. 4.7 Linear regression lines (dark lines) with 95% confidence limits (dashed lines) demonstrating the relationship of (A) CAMP, (B) TAMP, (C) CLAT and (D) TLAT with T/C ratios of the N2 wave in the mPFC of the non-gating rats (n = 10).



(A) The CAMP of the N2 wave in the non-gating rats had a significant negative correlation with the T/C ratios (P = 0.01).

(B) The changes in TAMP were not associated with the changes in the T/C ratios.

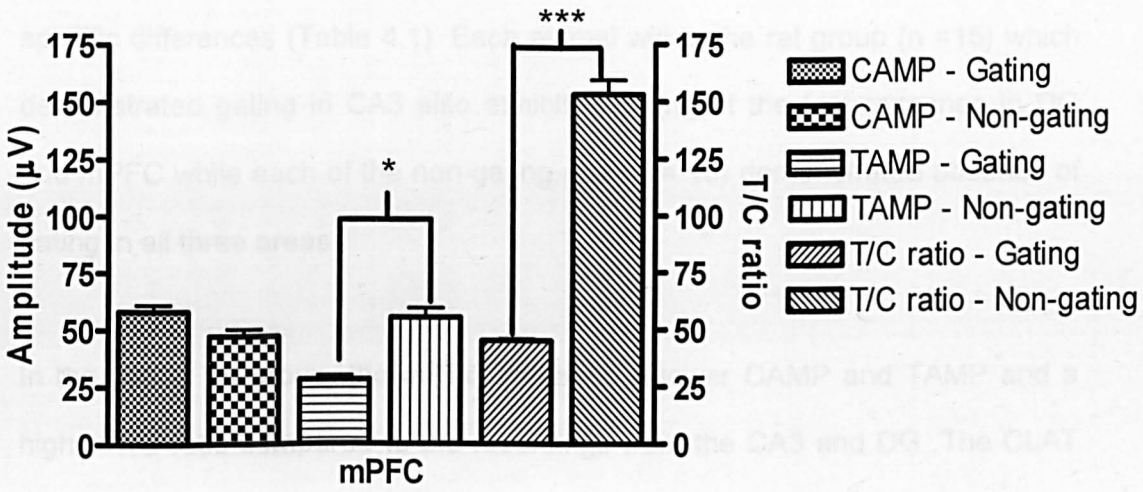
(C) & (D) Neither CLAT nor TLAT significantly correlated with the T/C ratios.

4.3.3 Comparison of the AER parameters of the gating rats and the non-gating rats

The AER parameters in the mPFC between the gating and the non-gating rats were compared (Fig. 4.7) and the differences were examined with the changes observed in the CA3 and DG (Table 4.1).

There were no significant differences in CAMP between the gating and the non-gating rats (Fig. 4.8; Table 4.1). The non-gating rats demonstrated a significantly higher TAMP ($P = 0.04$) resulting in a significantly higher T/C ratio ($P<0.0001$) compared to the gating rats. Non-gating rats also showed significantly shorter CLAT compared to that of gating rats ($P= 0.03$). No significant difference in TLAT was observed between the gating and the non-gating rats. In the non-gating rat group, the changes in the T/C ratios were negatively correlated with CAMP while in the gating rats CAMP had no effect on the T/C ratio. However, the T/C ratio of gating rats increased with increases in TAMP while TAMP of non-gating rats had no effect on the T/C ratio.

Fig. 4.8 Bar graph depicting CAMP, TAMP and T/C ratios (mean ± SEM) of the N2 wave in the mPFC of the gating rats (n = 15) compared with the findings of the non gating rats (n =10).



There was no significant difference in CAMP ($P > 0.05$) between the gating and the non-gating rats. The non-gating rats had significantly higher TAMP and T/C ratios in the mPFC compared to the gating rats (* = P = 01, *** = $P < 0.0001$).

4.3.4 The AER parameters recorded from the CA3, DG and mPFC in the gating and the non-gating rats

The CAMP, TAMP, T/C ratio, CLAT and TLAT in the CA3, DG and mPFC of the gating (n = 15) and the non-gating (n = 10) rats were examined for any region specific differences (Table 4.1). Each animal within the rat group (n =15) which demonstrated gating in CA3 also exhibited gating of the test response in DG and mPFC while each of the non-gating rats (n = 10) demonstrated absence of gating in all three areas.

In the gating rat group, the mPFC exhibited a lower CAMP and TAMP and a higher T/C ratio compared to the recordings from the CA3 and DG .The CLAT and TLAT of the mPFC were shorter than the CLAT and TLAT of the CA3 and DG in the gating rats.

The non-gating rats also had lower CAMP and TAMP with higher T/C ratios in their mPFC compared with the amplitudes and the T/C ratios of CA3 and DG. The mean CLAT was shorter and the mean TLAT was longer in the mPFC of the non-gating rats than those recorded from their CA3 and DG. Neither CLAT nor TLAT showed a significant correlation with the T/C ratios in both groups.

Table 4.1 The summary table of the N2 wave parameters (mean ± SEM) in the CA3, DG and mPFC of the gating (n = 15) and the non-gating (n = 10) rats (gating values were compared to the non-gating values in each region; * = P <0.05; *** = P<0.0001).

Area	CAMP (μV)	TAMP (μV)	T/C ratio	CLAT (ms)	TLAT (ms)
CA3					
Gating	165 ± 33	50 ± 13	28 ± 4	66 ± 3	64 ± 4
Non-gating	203 ± 32	188 ± 26***	103 ± 12***	55 ± 1*	59 ± 2
DG					
Gating	116 ± 23	47 ± 13	35 ± 4	64 ± 3	63 ± 2
Non-gating	154 ± 27	134 ± 21***	100 ± 16***	56 ± 2*	59 ± 1
mPFC					
Gating	58 ± 11	28 ± 7	45 ± 3	57 ± 1	57 ± 2
Non-gating	47 ± 10	55 ± 13*	153 ± 20***	53 ± 1*	62 ± 1

4.4 Discussion

Prominent auditory evoked potentials were recorded from the mPFC in both gating and non-gating rats. The gating rats exhibited a significant reduction in the test response amplitudes compared to the conditioning response amplitudes and the changes in the test response amplitudes were positively correlated with the T/C ratio. The non-gating rats showed a significantly longer test response latencies compared to their conditioning response latencies and their conditioning response amplitude was negatively correlated to the T/C ratio. The mPFC demonstrated lower amplitudes, shorter latencies and higher T/C ratio compared to the CA3 and DG in the gating rats.

The findings of the current study agree with previous human studies which have recorded AERs and auditory gating in the PFC (Grunwald et al., 2003; Kurthen et al., 2007). Studies by Mears et al (2006) and Yang et al (2006) demonstrated AERs and 'gating' in the rat mPFC. Neither of these studies discussed non-gating states of the PFC responses nor used a cut-off T/C value to identify gating states. Both studies used the term 'gating' for the reduction of TAMP and had not observed an enhancement of TAMP (T/C > 100%) which could have been classified as a non-gating state. Mears et al (2006) analysed a positive wave occurring around 60 ms in freely moving rats, which had shown a constant reduction of test response amplitudes, with T/C ratios between 53 – 64%. We observed constant gating of a negative wave occurring at a slightly shorter latency but with lower T/C ratios. However, some gating animals (n = 7)

exhibited T/C ratios comparable to the values observed by the Mears group. Mears et al (2006) also observed an increase in the T/C ratios with increases in the ISI, similar to the observations made in the current study. However, their report does not mention the rat strain or the gender that they have used for their study which, if different to our rats, could account for some of the differences observed.

Yang et al (2006) observed AERs similar to the AERs recorded in the present study and observed constant reduction of the test response amplitude of three waves i.e. P2, N2 and P3. Unfortunately, Yang et al (2006) did not report the basal T/C ratios or latency values (only the percentage changes of post-drug values compared to basal) of the AERs, making it difficult to compare their results with the current findings.

However, based on the present findings and those of Mears and Yang, it could be argued that different criteria (to those used for the hippocampus) to separate gating from non-gating rats need to be employed when assessing auditory gating in the mPFC. This could be also emphasized with the findings in the mPFC of the non-gating rats, which demonstrated a test: conditioning response ratio of 153 ± 20% which was markedly different from those observed in CA3 (T/C ratio = 103 ± 12%) or DG (T/C ratio = 100 ± 16%). Based on the above finding, it is possible to argue that the higher T/C ratios observed in mPFC (45 ± 3%) compared to those in CA3 (28 ± 7%) and DG (35 ± 4%) in the gating rats may not be due to less effective gating in the area, but a result of a higher baseline test response. Thus a higher cut-off T/C ratio may need to be used

when identifying gating and non-gating instances in the mPFC.

Examination of the findings from the mPFC, CA3 and the DG of the current study provides some interesting observations. The mPFC of gating rats demonstrated similar response patterns to those recorded from their CA3 and DG with significant reduction of TAMP compared to CAMP. The results also showed a positive correlation between T/C ratio and TAMP and T/C ratio increased with increases in the inter-stimuli interval, emphasizing that mPFC responds to auditory conditioning-test paradigm in a similar manner to the CA3 and DG in the gating rats.

Similar to the responses observed in the CA3 and DG, the mPFC of the non-gating rats exhibited no significant difference between CAMP and TAMP and a longer TLAT compared to CLAT, highlighting the dysfunctions in the neuronal mechanisms in mediating gating of the test response in this rat group . The mPFC followed the response pattern in the CA3 and DG in the non-gating rats, demonstrating increases in the T/C ratio with decreases in the CAMP, a finding reported in some schizophrenic patients (Adler et al., 1982; Freedman et al., 1983; Siegel et al., 1984). Non-gating rats had higher TAMP and higher T/C ratios in the mPFC compared to the mPFC of the gating rats, indicating a widespread deficits in inhibitory processes mediating the reduction in TAMP, involving both the hippocampus and the mPFC. Schizophrenic patients have shown increased activity in the PFC in response to the auditory conditioning-testing paradigm in a functional magnetic resonance study (fMRI) compared to controls, suggesting diminished inhibitory functions in the PFC in schizophrenia (Tregellas et al., 2007), which could relate to the increased TAMP and T/C

ratios observed in the non-gating rats in the current study.

The mPFC showed the AERs of lowest amplitudes, shortest response latencies and the highest basal T/C ratio compared to the CA3 and DG in the gating rats, suggesting an early phase of auditory gating in the mPFC, which may later be modulated by the hippocampus. This finding parallels that from a human study using intra-cerebral electrodes, which demonstrated a positive AER with gating at a shorter latency value from PFC compared to a negative peak at a longer latency value from hippocampus (Grunwald et al., 2003). Based on their results Grunwald and co-workers suggested that sensory gating is a multi-step process with an initial stage in the PFC and a late stage in the hippocampus. The present study suggests that rats also have different phases of sensory gating with an initial stage in the mPFC and a late modulatory stage in the DG and CA3. Prolonged TLAT in the mPFC in the non-gating rats, compared to their TLAT in the CA3 and DG, suggests the possibility of a dysfunction in the neural circuitry, which may be crucial for the gating of the test response.

The auditory information flow to the mPFC could be via the auditory pathway with the auditory cortex sending projections to the mPFC. However, as mentioned in chapter 3, important structures in the auditory system such as medial geniculate body and the auditory cortex do not demonstrate gating suggesting the possibility of a different pathway mediating auditory gating (Bickford-Wimer et al., 1993; Moxon et al., 1999). Mears et al (2006) suggested that the brain stem reticular region could be sending auditory gating information directly to the mPFC. Auditory gating has been observed in brain stem reticular

region (Bickford-Wimer et al., 1993; Moxon et al., 1999) and the presence of direct connections between the brain stem and the mPFC (Gabbott et al., 2005), supports the role of ascending brainstem inputs to the mPFC to activate the neural circuits mediating auditory gating.

It has been shown that the prelimbic and the medial-orbital areas of the rat PFC receive monosynaptic excitatory inputs from the hippocampus modulating the activity of the mPFC neural circuitry (Thierry et al., 2000; Tierney et al., 2005). Despite the direct innervations of the hippocampus on the mPFC, there is little or no evidence to suggest the presence of direct return projections from the rat mPFC to the hippocampus (Laroche et al., 2000; Vertes et al., 2006). However, the mPFC has efferents to parahippocampal structures including the enterohinal cortex (Laroche et al., 2000; Vertes et al., 2006), which is the likely thoroughfare for gating information from the mPFC to the DG and then to the CA3 of hippocampus.

By examining auditory gating in the mPFC of Lister-hooded rats under isoflurane anaesthesia with simultaneous recordings from the CA3 and DG, we have expanded our animal model, which now includes three crucial areas of the brain for information processing that are implicated in the pathophysiology of schizophrenia. Moreover, we have emphasized the presence of a non-gating rat population which responds differently to the gating rats in response to the auditory conditioning-test paradigm in the hippocampus as well as in the mPFC. The next step was to examine the effects of pharmacological manipulations on auditory gating in the hippocampus and the mPFC in both gating and non-

gating rats.

Chapter Five

Effects of phencyclidine on auditory gating in the rat hippocampus and medial prefrontal cortex

5. Effects of phencyclidine on auditory gating in the rat hippocampus and medial prefrontal cortex

5.1 Introduction

Phencyclidine (PCP) was developed in the 1950s as a surgical anaesthetic. Despite its efficacy, PCP produced some disturbing side effects such as confusion, hallucinations, disordered speech and disoriented behaviour and was discontinued from medical use. However, it has continued to be used illicitly as a recreational drug and marketed as capsules, tablets, powder and in liquid form which is either taken orally or smoked. For smoking, PCP is very commonly spread on to marijuana leaves. PCP has attracted neuroscientists and psychiatrists due to the remarkable resemblance of the symptoms of abusers to those exhibited by patients suffering from schizophrenia.

PCP is a non-competitive antagonist of the N-methyl-D-aspartate (NMDA) glutamate receptors and produces a variety of neurochemical effects in the brain secondary to NMDA dysfunction (see 1. 7.2.1). In addition to the effects on the NMDA receptors, PCP has been found to exert dopaminergic, noradrenergic and serotonergic effects which may explain some of the psychotic and behavioral effects of the drug (see 1.7.2.1; Jentsch & Roth, 1999).

As mentioned before, both PCP and the structurally related drug ketamine, have been shown to have the ability to mirror the symptomatology of schizophrenia including negative symptoms and neurocognitive deficits in otherwise healthy individuals and to exacerbate symptoms in schizophrenic patients (Malhotra et al., 1996; Murray, 2002., Morris et al., 2005;).

Acute or chronic administrations of PCP disrupt performance in attention tasks, working memory tasks and behavioural changes in rats resembling the behaviuoral and cognitive deficits observed in schizophrenia (Bakshi et al., 1994; Sams-Dodd., 1999; Martinez et al., 1999; Marcotte et al., 2001). However, the effects of acute or chronic administration of PCP on auditory gating have not being studied widely. Adler et al (1986) showed that single dose PCP disrupted gating of skull recorded AERs in freely moving rats and Miller et al. (1992) showed that single dose PCP disrupted sensory gating in the CA3 region of the hippocampus under chloral hydrate anesthesia.

In this chapter we sought to determine if sensory gating in the rat CA3, DG and mPFC could be disrupted by a single dose PCP administration and if disrupted whether the atypical antipsychotic clozapine, which is currently one of the most effective drugs in treating schizophrenia, could prevent the disruption.

5.2 Methods

5.2.1 Animals

Male Lister hooded rats (n = 25; weight 250g – 400g) were anaesthetised with isoflurane; N_2O:O_2 and all the electrophysiological procedures were carried out as described in chapter 2. Simultaneous recordings from the CA3, DG and mPFC were carried out and only the animals with electrodes placed in the CA3, DG and mPFC, confirmed by histology, were used for analysis.

5.2.2 Experimental protocol

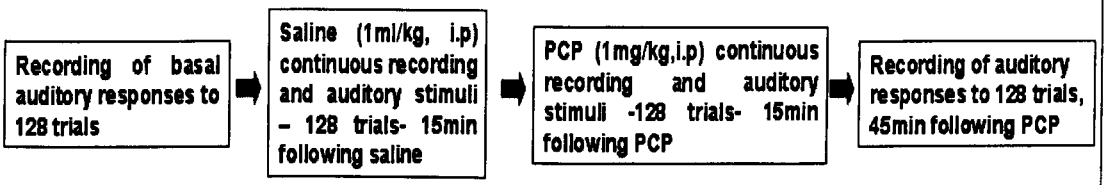
The auditory stimuli (90dB intensity, 0.5s ISI, 10s inter-trial interval) were presented over 128 trials and auditory-evoked LFP and unit responses were recorded simultaneously from the CA3, DG and mPFC using multiple electrode arrays for the CA3 and DG (2× 8) and a multiple electrode bundle for the mPFC (1× 8).

PCP experiments

Following basal recording of the auditory responses to 128 trials of auditory stimuli, the vehicle (saline) was administered (1ml/kg, i.p; n = 14) and the effect on auditory gating assessed by recording the responses to 128 auditory conditioning-test trials 15min after administration (Box 5.1). PCP (1mg/kg, i.p) was administered 40 min after the injection of the vehicle and the effect on

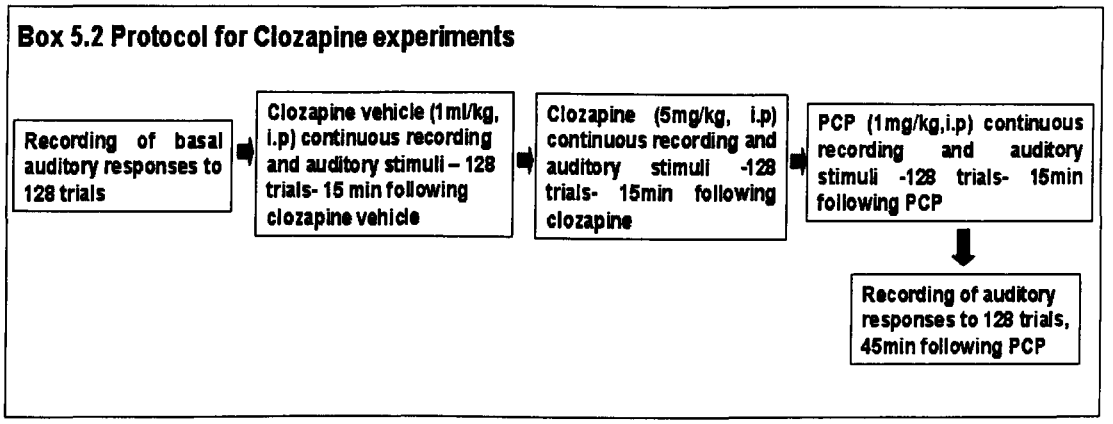
auditory gating assessed by recording auditory responses to 128 stimuli trials, beginning 15 min and 45 min after drug administration.

Box 5.1 Protocol for PCP experiments



Clozapine experiments

Following basal recording of the auditory responses to 128 trials of auditory stimuli, the clozapine vehicle was administered (1ml/kg, i.p; n = 11) and the effect on auditory gating assessed by recording the responses to 128 auditory conditioning-test trials 15min after vehicle administration (Box 5.2). Clozapine was injected (5mg/kg, i.p) 40 min after the vehicle. The effect of clozapine on auditory gating was assessed by recording the responses to 128 auditory conditioning-test trials 15min after administration. Phencyclidine was injected in the same animals 40 min after clozapine injection and the effect on auditory gating was assessed over 128 stimuli trials, 15 and 45 min following PCP administration.



5.2.3 Data analysis

Data were analysed off-line using NeuroExplorer (v3; NEX technologies Inc., USA) with evoked response amplitudes, latencies and T/C ratios computed using a custom-designed Matlab script (v7.0 with associated Matlab Signal Processing, Neural Network, Statistics and Image Processing Toolboxes; The Mathworks). Statistical analysis used Prism (v4.03; GraphPad, USA) and within region changes of all the parameters at different time points were analysed using one-way analysis of variance (ANOVA) for repeated measures with post hoc Tukey t-test. Data are expressed as mean ± SEM (standard error of mean), statistical significance was taken when $P < 0.05$.

5.2.4 LFP analysis

Auditory evoked potentials were initially visualized as peri-event averaged LFPs (Fig. 5.1). The AERs were identified according to the polarity and the order of occurrence, i.e. N1, P1, N2, P2, N3 peaks (Van Luijtelaar et al., 2001; Boutros

et al., 2004). An electrode with the highest N2 wave conditioning amplitude response from each area was selected for further analysis (see chapter 2). The ratio of the test amplitude to the conditioning amplitude (T/C ratio) of the N2 wave was calculated for each averaged response and the T/C ratio in the CA3 was used to separate gating and non-gating rats. A T/C ratio of ≤50% in the CA3 was indicative that gating was present (Joy et al., 2004; Miller et al., 1992).

5.3. Results

5.3.1 Auditory Evoked Responses from CA3, DG and mPFC

Averaged LFPs recorded from the CA3 region, DG and mPFC in response to auditory stimuli exhibited complex AERs (i.e. N1, P1, N2, P2 and N3) with gating (n = 14) and non-gating (n = 11) of the N2 wave. In the PCP group eight animals exhibited gating of the N2 wave (n = 3 in the CA3 & DG, n = 5 in the CA3, DG and mPFC) and six showed non-gating (n = 2 in the CA3 & DG, n = 4 in the CA3, DG and mPFC). In the clozapine group six animals demonstrated gating (n = 3 in the CA3 & DG, n = 3 in the CA3, DG and mPFC) and five showed non-gating (n = 5 in the CA3, DG and mPFC). Neither saline nor clozapine vehicle had any effect on the N2 T/C ratios in any of the three regions in the gating or the non-gating rats (Table 5.1 and Table 5.2).

5.3.2 Effects of PCP on the gating rats

5.3.2.1 Effects of PCP on the conditioning amplitudes

The CA3 region, exhibited an increase in CAMP ($F_{2, 23} = 5.1$, $P = 0.02$) 45 min ($P<0.05$) following PCP administration (Fig. 5.1 & Fig. 5.2). PCP had no significant effect on CAMP in either DG ($P = 0.07$) or mPFC ($P = 0.06$) in the gating rats.

5.3.2.2 Effects of PCP on the test amplitudes

PCP increased the TAMP in all three regions both 15 and 45min following administration (Fig. 5.1 & Fig. 5.2). In the CA3, TAMP was increased significantly ($F_{2, 23} = 10.8$, $P = 0.001$) both 15 ($P<0.05$) and 45 min ($P<0.01$) following PCP administration. The DG demonstrated a similar response with a significant increase in TAMP ($F_{2, 23} = 23.7$, $P = 0.0001$) both 15 ($P<0.01$) and 45 min ($P<0.001$) after PCP. The mPFC also exhibited an increase in TAMP ($F_{2, 14}= 32$, $P = 0.0009$) 15 ($P<0.01$) and 45 min ($P<0.001$) after PCP administration.

5.3.2.3 Effects of PCP on the T/C ratios

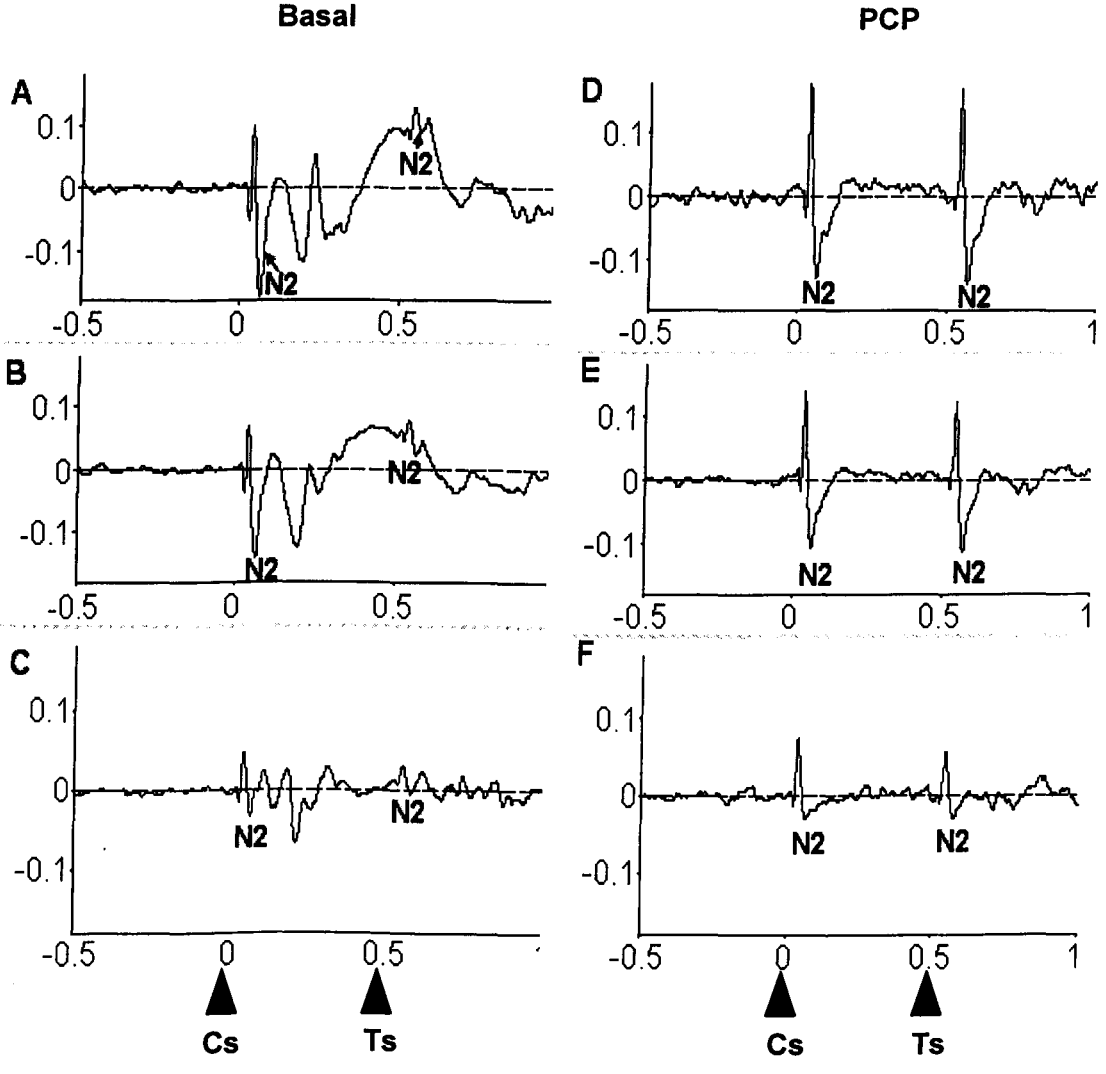
Administration of a single dose of PCP disrupted auditory gating in all three regions both 15 and 45 min after administration when compared to basal values for each region (Fig. 5.1 & Fig 5.2). PCP disrupted gating in the CA3 ($F_{2, 23} =$

14, P = 0.0004), 15 (P<0.01) and 45 min (P<0.001) after administration. In the DG, PCP significantly increased the T/C ratio ($F_{2, 23}$ = 10, P = 0.001) with disruption of gating seen at both 15 (P<0.05) and 45 min (P<0.01) after administration. Disruption of gating was detected in the mPFC following PCP ($F_{2, 14}$= 9.6, P = 0.007), 15 (P<0.05) and 45 min (P<0.01) after drug administration.

5.3.2.4 Effects of PCP on the conditioning and test response latencies

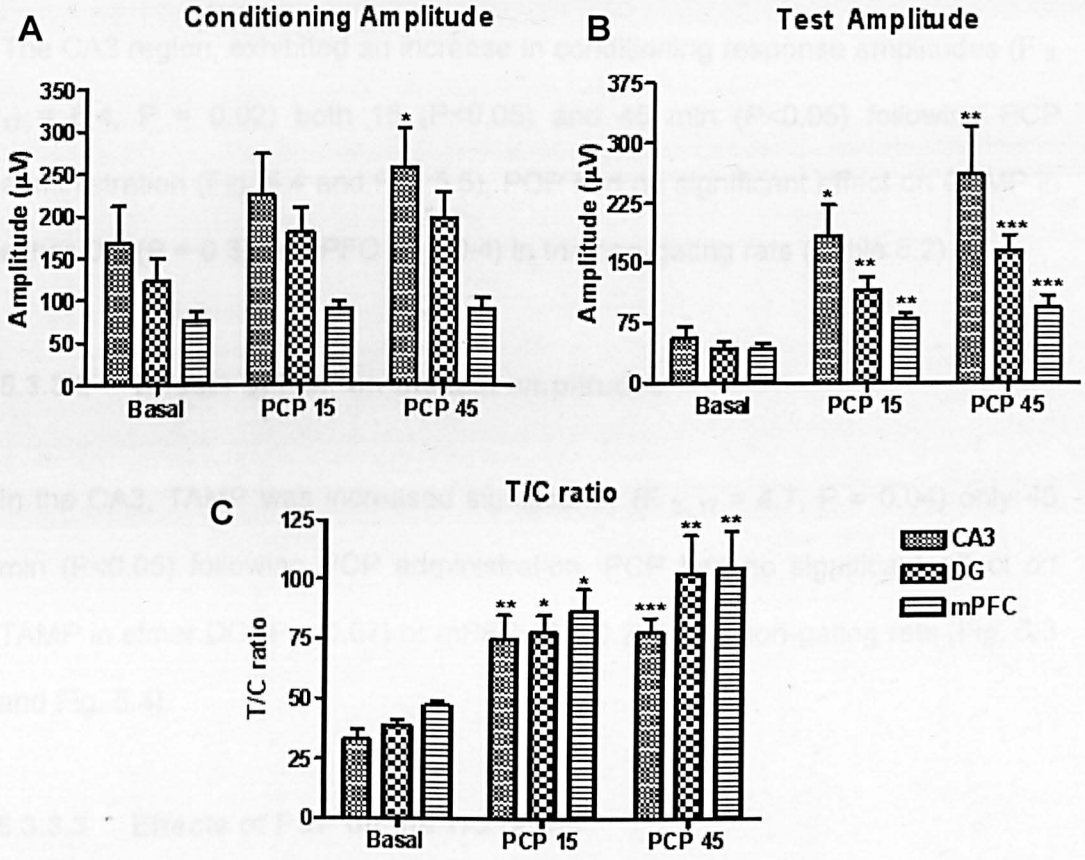
There were no significant changes in CLAT (P>0.05) or TLAT (P>0.05) in the CA3, DG or mPFC following PCP administration (Table 5.1)

Fig. 5.1 Representative peri-event averaged LFPs from (A & D) CA3, (B & E) DG and (C & F) mPFC demonstrating the effects of PCP (45min after administration) on auditory gating compared to basal recordings in a gating rat.



Basal recordings from (A) CA3 (T/C ratio = 14 %), (B) DG (T/C ratio = 25 % and (C) mPFC (41 %) demonstrated gating of the N2 wave. The mPFC demonstrated the lowest basal amplitude responses compared to the response amplitudes in the CA3 and DG. PCP disrupted gating of the N2 wave in (D) CA3 (T/C ratio = 98 %), (E) DG (T/C ratio = 96 %) and (F) mPFC (T/C ratio = 82 %) 45min following drug administration.

Fig. 5.2 Bar graphs demonstrating the effect of PCP on (A) conditioning amplitude (B) test amplitude and (C) T/C ratios of the N2 wave in the gating rats in the CA3 (n = 8), DG (n = 8) and mPFC (n = 5); values = mean ± SEM.



A. PCP increased the CAMP in the CA3 45 min following administration, but had no effect on the CAMP of the DG or mPFC.

B. PCP increased the TAMP in the CA3, DG and mPFC both 15 and 45 min following administration.

C. T/C ratios in the CA3, DG and mPFC increased significantly both 15 and 45 min following PCP administration.

(• = P<0.05, •• = P<0.01, ••• = P<0.001).

5.3.3 Effects of PCP on the non-gating rats

5.3.3.1 Effects of PCP on the conditioning amplitudes

The CA3 region, exhibited an increase in conditioning response amplitudes ($F_{2, 17} = 6.4$, $P = 0.02$) both 15 ($P<0.05$) and 45 min ($P<0.05$) following PCP administration (Fig. 5.4 and Fig. 5.5). PCP had no significant effect on CAMP in either DG ($P = 0.3$) or mPFC ($P = 0.4$) in the non-gating rats (Table 5.2).

5.3.3.2 Effects of PCP on the test amplitudes

In the CA3, TAMP was increased significantly ($F_{2, 17} = 4.7$, $P = 0.04$) only 45 min ($P<0.05$) following PCP administration. PCP had no significant effect on TAMP in either DG ($P = 0.07$) or mPFC ($P = 0.2$) in the non-gating rats (Fig. 5.3 and Fig. 5.4).

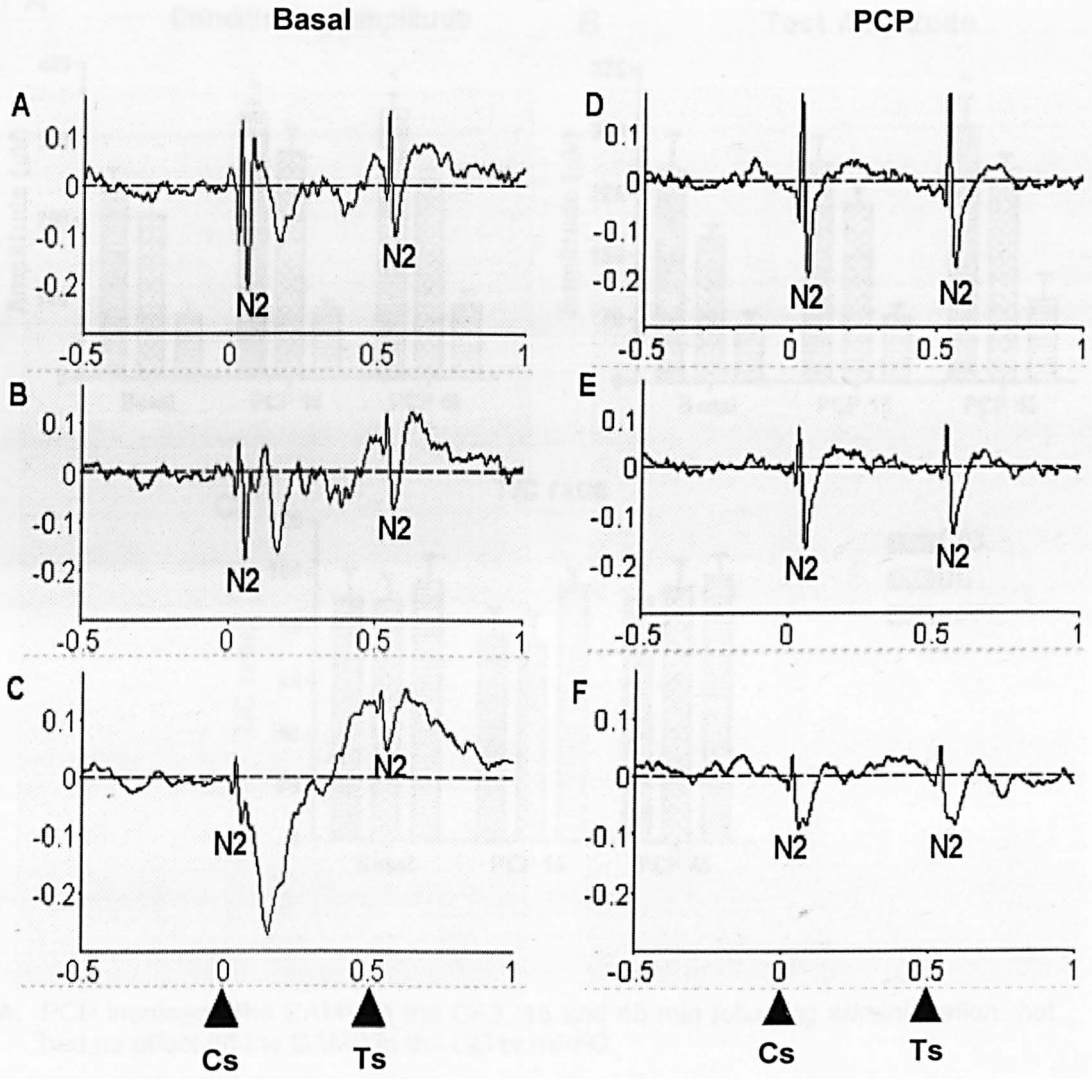
5.3.3.3 Effects of PCP on the T/C ratios

Administration of a single dose of PCP had no significant effect on the T/C ratios in the CA3 ($P = 0.2$), DG ($P = 0.4$) or mPFC (0.9) either 15 or 45 min after administration when compared to basal values for each region in the non-gating rats (Fig. 5.3 and Fig. 5.4).

5.3.3.4 Effects of PCP on the conditioning and test response latencies

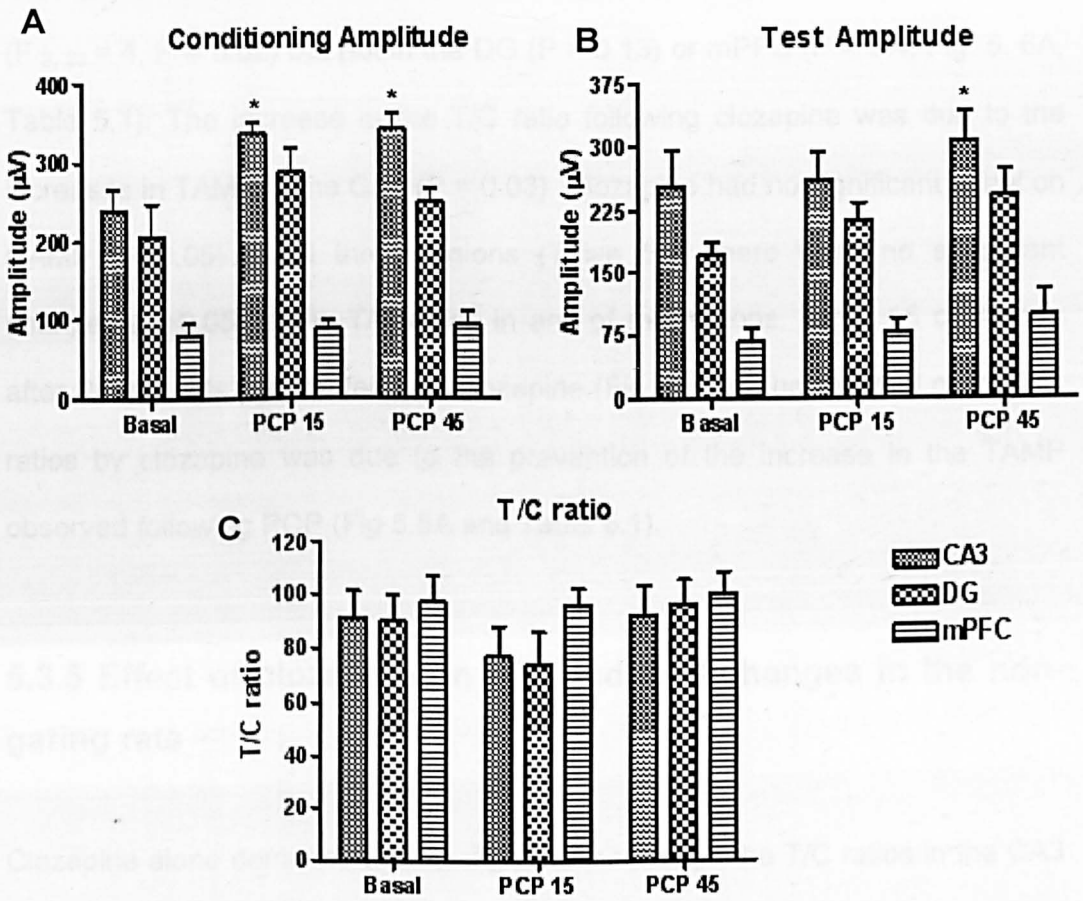
There was no significant changes in CLAT ($P>0.05$) or TLAT ($P>0.05$) in the CA3, DG or mPFC following PCP administration (Table 5.2).

Fig. 5.3 Representative peri-event averaged LFPs from (A & D) CA3, (B & E) DG and (C & F) mPFC demonstrating the effects of PCP (45min after administration) on auditory gating (N2 wave) compared to basal recordings in a non-gating rat.



Basal recordings from (A) CA3 (T/C ratio = 97 %), (B) DG (T/C ratio = 98 %) and (C) mPFC (T/C ratio = 99 %) demonstrated absence of gating of the N2 wave. The mPFC demonstrated the lowest basal amplitude responses compared to the basal response amplitudes in the CA3 and DG. PCP had no significant effect on the T/C ratios ($P>0.05$) in the (D) CA3 (T/C ratio = 95 %), (E) DG (T/C ratio = 100 %) or (F) mPFC (T/C ratio = 125 %) 45 min after administration.

Fig. 5.4 Bar graphs demonstrating the effect of PCP on (A) conditioning amplitude (B) test amplitude and (C) T/C ratios of the N2 wave in non-gating rats in the CA3 (n = 6), DG (n = 6) and mPFC (n = 4); values = mean ± SEM.



A. PCP increased the CAMP in the CA3, 15 and 45 min following administration, but had no effect on the CAMP in the DG or mPFC.

B. PCP increased the TAMP in the CA3, 45 min following administration but had no effect on the TAMP in the DG or mPFC.

C. T/C ratios showed no significant changes (P>0.05) in the CA3, DG or mPFC following PCP administration in the non-gating rats.

(* = P<0.05)

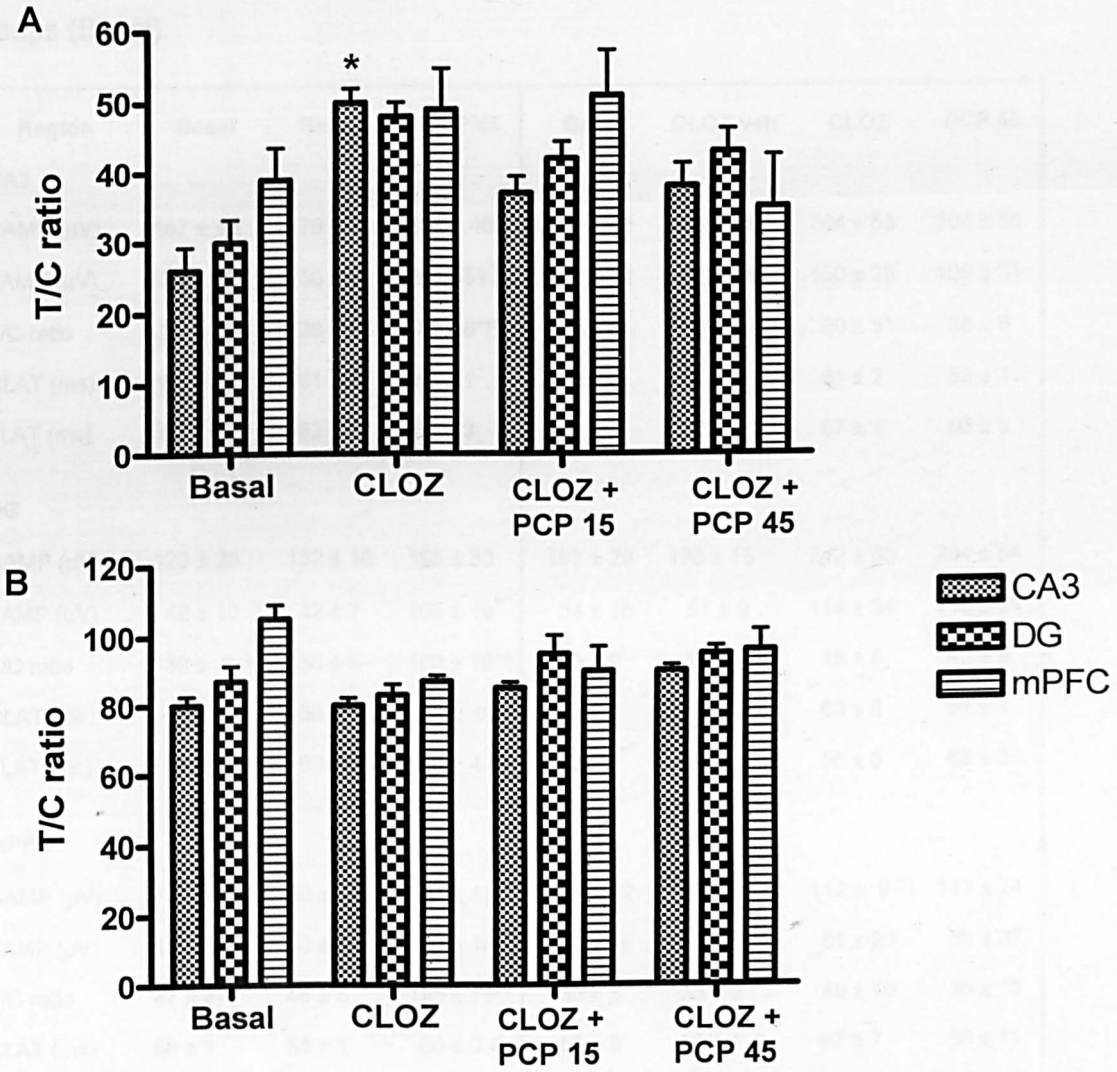
5.3.4 Effect of clozapine on PCP induced changes in the gating rats

Clozapine alone caused a significant enlargement in the T/C ratios in the CA3 ($F_{3, 23} = 4$, $P = 0.02$) but not in the DG ($P = 0.13$) or mPFC ($P = 0.4$; Fig. 5. 6A, Table 5.1). The increase in the T/C ratio following clozapine was due to the increases in TAMP in the CA3 ($P = 0.03$). Clozapine had no significant effect on CAMP ($P>0.05$) in all three regions (Table 5.1).There were no significant changes ($P>0.05$) in the T/C ratios in any of the regions, either 15 or 45 min after PCP in rats pre-treated with clozapine (Fig. 5.5A). The reversal of the T/C ratios by clozapine was due to the prevention of the increase in the TAMP observed following PCP (Fig 5.5A and Table 5.1).

5.3.5 Effect of clozapine on PCP induced changes in the non-gating rats

Clozapine alone demonstrated no significant effect on the T/C ratios in the CA3 ($P = 0.4$), DG ($P = 0.07$) or mPFC ($P = 0.7$) in the non-gating rats (Fig. 5.5B and Table 5.2). The CA3 region exhibited an increase in CAMP ($F_{3, 19} = 4.6$, $P = 0.02$) 45 min ($P<0.05$) after PCP and increase in TAMP ($F_{3, 19} = 10.6$, $P = 0.001$) both 15 ($P<0.01$) and 45 min ($P<0.01$) following PCP in the non-gating rats pre-treated with clozapine. Neither DG nor mPFC demonstrated any significant changes in CAMP ($P>0.05$) or TAMP ($P>0.05$) following PCP. No significant effects on the T/C ratios were observed following PCP in the CA3, DG or mPFC in the non-gating rats pre-treated with clozapine (Fig. 5.5B and Table 5.2).

Fig. 5.5 Bar graphs depicting N2 T/C ratios 15 min (PCP 15) and 45 min (PCP 45) after PCP administration in (A) gating rats (n = 6 in CA3 & DG, n = 3 in mPFC) and (B) non-gating rats (n = 5 in CA3, DG and mPFC) pre-treated with clozapine (CLOZ) compared to basal values (mean ± SEM).



A. The T/C ratio in the CA3 demonstrated a significant increase following clozapine. However, pre-treatment with clozapine prevented the increases in T/C ratios following PCP in the CA3, DG and mPFC in the gating rats (* = $P<0.05$).

B. Neither clozapine nor PCP had any effects on the T/C ratios in the CA3, DG or mPFC in the non-gating rats.

Table 5.1 Summary table of the amplitudes, T/C ratios and latencies 15 min after saline and 45 min after PCP administration (PCP 45) in the gating rats not treated with clozapine (n = 8 in CA3 & DG, n =5 in mPFC) and the gating rats pre-treated with clozapine (n = 6 in CA3 & DG, n = 3 in mPFC; clozapine vehicle = CLOZ veh; clozapine = CLOZ) compared to the basal values of the respective groups (Basal).

Region	Basal	Saline	PCP 45	Basal	CLOZ veh	CLOZ	PCP 45
CA3							
CAMP (μV)	167 ± 45	170 ± 27	258 ± 46 *	251 ± 42	250 ± 34	264 ± 55	304 ± 55
TAMP (μV)	56 ± 15	50 ± 9	261± 59**	56 ± 12	55 ± 18	130 ± 28*	109 ± 31
T/C ratio	33 ± 4	28 ± 6	78 ± 6***	26 ± 8	26 ± 5	50 ± 5*	38 ± 8
CLAT (ms)	61 ± 2	61 ± 1	58 ± 1	66 ± 6	67 ± 4	61 ± 2	59 ± 1
TLAT (ms)	60 ± 5	62 ± 4	62 ± 3	65 ± 7	69 ± 4	67 ± 1	66 ± 3
DG							
CAMP (μV)	123 ± 28	132 ± 18	198 ± 30	198 ± 29	196 ± 15	242 ± 65	264 ± 54
TAMP (μV)	42 ± 10	42 ± 7	165 ± 19***	54 ± 15	51 ± 9	114 ± 34	110 ± 31
T/C ratio	39 ± 2	35 ± 6	103 ± 16**	30 ± 8	28 ± 7	48 ± 5	43 ± 8
CLAT (ms)	60 ± 2	60 ± 3	63 ± 6	62 ± 1	63 ± 1	63 ± 3	61 ± 1
TLAT (ms)	58 ± 5	60 ± 5	66 ± 4	60 ± 7	60 ± 3	66 ± 5	68 ± 3
mPFC							
CAMP (μV)	77 ± 10	80 ± 10	90 ± 13	116 ± 22	118 ± 9	112 ± 9	143 ± 24
TAMP (μV)	42 ± 8	40 ± 6	93 ± 16***	43 ± 16	40 ± 10	61 ± 20	56 ± 27
T/C ratio	47 ± 2	46 ± 5	105 ± 16**	39 ± 8	36 ± 9	49 ± 10	35 ± 13
CLAT (ms)	58 ± 1	58 ± 1	60 ± 0.4	57 ± 3	58 ± 1	67 ± 7	66 ± 11
TLAT (ms)	61 ± 3	60 ± 3	67 ± 3	60 ± 6	60 ± 4	68 ± 1	69 ± 6

(* = P<0.05; ** = P<0.01; *** = P<0.001)

Table 5.2 Summary table of the amplitudes, T/C ratios and latencies, 15 min after saline and 45 min after PCP administration (PCP 45) in the non-gating rats not treated with clozapine (n = 6 in CA3 & DG, n =4 in mPFC) and the non-gating rats pre-treated with clozapine (CLOZ: n = 5 in CA3, DG and mPFC) compared to pre-drug values of the respective groups (Basal).

Region	Basal	Saline	PCP 45	Basal	CLOZ veh	CLOZ	PCP 45
CA3							
CAMP (μV)	239 ± 27	235 ± 49	342 ± 19*	225 ± 31	234 ± 45	326 ± 19*	339 ± 35*
TAMP (μV)	233 ± 43	237 ± 40	306 ± 35*	179 ± 25	170 ± 28	259± 16**	306± 28**
T/C ratio	91 ± 10	95 ± 16	92 ± 11	80 ± 6	77 ± 10	80 ± 5	90 ± 4
CLAT (ms)	56 ± 2	57 ± 1	56 ± 2	53 ± 2	55 ± 2	52 ± 2	59 ± 2
TLAT (ms)	61 ± 2	62 ± 2	61 ± 2	56 ± 2	56 ± 2	55 ± 3	60 ± 8
DG							
CAMP (μV)	207 ± 41	191 ± 30	249 ± 17	184 ± 25	204 ± 35	255 ± 43	232 ± 41
TAMP (μV)	170 ± 15	176 ± 15	165 ± 19	155 ± 24	154 ± 34	193 ± 19	226 ± 24
T/C ratio	90 ± 10	97 ± 8	96 ± 9	87 ± 10	80 ± 8	83 ± 7	95 ± 5
CLAT (ms)	55 ± 2	56 ± 2	54 ± 2	53 ± 2	53 ± 4	52 ± 2	53 ± 2
TLAT (ms)	61 ± 2	63 ± 2	55 ± 2	56 ± 2	56 ± 3	55 ± 1	58 ± 2
mPFC							
CAMP (μV)	79 ± 6	80 ± 10	93 ± 16	65 ± 18	74 ± 12	68 ± 22	87 ± 13
TAMP (μV)	66 ± 15	69 ± 8	96 ± 31	59 ± 15	60 ± 10	58 ± 18	65 ± 29
T/C ratio	97 ± 10	96 ± 6	100 ± 8	105 ± 9	98 ± 8	87 ± 14	96 ± 13
CLAT (ms)	53 ± 2	54 ± 2	52 ± 2	52 ± 1	53 ± 1	58 ± 7	55 ± 2
TLAT (ms)	63 ± 3	62 ± 2	61 ± 5	61 ± 2	64 ± 1	62 ± 7	67 ± 2

(* = P<0.05; ** = P<0.01; *** = P<0.001)

5.4. Discussion

In the present study the NMDA receptor antagonist, PCP (1mg/kg, i.p), disrupted auditory gating of N2 wave in the CA3, DG and mPFC, but had no effect on the T/C ratios in the non-gating rats. Pre-treatment with the atypical antipsychotic, clozapine (5mg/kg, i.p), prevented the disruption of gating induced by PCP in the CA3, DG and mPFC in the gating rats, but showed no effect on the T/C ratios in the non-gating rats.

Our findings in the CA3 region agree with the study by Miller et al (1992) which showed that acute administration of PCP disrupts auditory gating in the CA3. The current study also demonstrates PCP induced disruption of gating in both DG and mPFC, suggesting a widespread alteration of information processing caused by PCP. Our findings also agree with human studies showing ketamine, a structurally similar but low-potent NMDA antagonist, induces schizophrenic symptoms in healthy volunteers and affects auditory gating in a dose dependent manner (Duncan et al., 2001; Oranje et al., 2002). PCP has also been shown to disrupt prepulse inhibition (PPI) in rodents, another unrelated behavioural model of schizophrenia (Bakshi et al., 1994; Martinez et al., 1999; Schwabe et al., 2005). Moreover, the present study demonstrates that PCP disrupts gating in the hippocampus and the mPFC by affecting the ability to suppress the test response amplitude which is similar to the gating deficits found in schizophrenic patients (Boutros et al., 1999; 2004). Adler et al (1986) compared the effect the dopaminergic psychoactive drug amphetamine with PCP on gating, using skull

recorded AERs, and showed that the disruption of gating with PCP by increases in test response amplitude was more representative of human deficits compared to the disruption with amphetamine which was mediated mainly via decreases in CAMP; findings supported by the current study.

The precise mechanism as to how PCP disrupts gating in the hippocampus and mPFC and produce schizophrenic symptoms in humans is yet to be elucidated. Use of NMDA antagonists to model schizophrenic symptoms is based on the glutamate hypothesis of schizophrenia which suggests hypofunction of the glutamatergic neurotransmission in the pathophysiology of the disease. This hypothesis was strengthened by the findings of reduced concentrations of glutamate in the cerebrospinal fluid in patients with schizophrenia (Kim et al., 1980) and decreased concentrations of glutamate in the PFC and hippocampus in postmortem tissues from schizophrenic patients compared to controls (Tsai et al., 1995). Although PCP has a rich pharmacology being a sympathomimetic, a sigma receptor and nicotine acetylcholine receptor antagonist, the most potent effect of clinical and pharmacological doses of the drug has been shown to be the NMDA antagonism (Morris et al., 2005). However, dopaminergic drugs and nicotine receptor antagonists also disrupt auditory gating (Adler et al., 1986; Joy et al., 2004; Martin et al., 2004) and Miller et al (1992) suggested that the PCP induced disruption of gating in CA3 was due to its sympathomimetic properties. Thus sympathomimetic and anti-nicotinic properties of PCP may at least partially contribute to its unique ability to induce schizophrenic symptoms along with the disruption of auditory gating.

The reciprocal connections between GABAergic interneurones and glutamatergic pyramidal neurons in the CA3 of hippocampus are important for auditory gating in CA3 (Flach et al., 1996; Moxon et al., 2003) with GABAergic interneurons mediating the reduction in the test response amplitude and regulating auditory gating (Miller et al., 1995). The NMDA antagonism has been shown to inhibit the activity of GABAergic neurons via these reciprocal connections thus resulting in the release of inhibition on major excitatory pathways leading to hyperstimulation of corticolimbic neurons (Olney et al., 1999). This hyperstimulation may affect information processing and disrupt inhibitory processes such as sensory gating in hippocampus and PFC.

Interestingly in the present study, PCP had no significant effect on the non-gating rats except for the small increases in conditioning response amplitude and test response amplitude in the CA3, which indicates a resistance to NMDA antagonism in this group of rats. It is possible to postulate that the non-gating rats could be having an inherent dysfunction in the glutamatergic neurotransmission accompanied with a functional disturbance of NMDA receptors which may also explain the absence of auditory gating in this rat group.

The atypical antipsychotic, clozapine, prevented the auditory gating deficits induced by PCP in the CA3, DG and mPFC in the gating rats. Several human studies have confirmed the ability of clozapine to reverse auditory gating deficits in schizophrenic patients (Adler et al., 2004; Light et al., 2000; Nagamoto et al., 1996). However, the efficacy of clozapine on reversing gating deficits in animal

models has not been established. Joy et al (2004) reported that chronic and acute clozapine administration failed to reverse auditory gating deficits induced by a single dose of amphetamine suggesting not only that amphetamine may not be useful in modeling gating deficits in schizophrenia but also the reversal of gating deficits by clozapine in humans may not be mediated via dopamine receptors. Clozapine reversed the gating deficits by stimulating nicotine actetylcholine receptors in DBA/2 mice who spontaneously exhibit gating deficits similar to those seen in schizophrenics (Simosky et al., 2003). Clozapine has been shown to have an agonistic effect on NMDA receptors similar to those seen with the agonists of the glycine modulatory site on the receptor (Goff and Joseph, 2001). It is quite likely that the reversal of PCP induced gating deficits by clozapine is mediated via the glycine modulatory site on the NMDA receptor resulting in enhanced glutamatergic neurotransmission and thus preventing PCP induced NMDA antagonism in the CA3, DG and mPFC. Enhanced glutamatergic neurotransmission which probably creates a slight imbalance between glutamatergic and GABAergic activities may explain the increased T/C ratios observed following clozapine in the CA3 in the gating rats. Our findings with clozapine in the gating rats agree with the findings from PPI studies which have shown that clozapine reverses PCP induced PPI deficits in rats (Bakshi et al., 1994) and disrupts PPI when administered alone (Dirks et al., 2003). Clozapine has been reported to worsen PPI when administered to rats pre-treated with PCP (Schwabe et al., 2005), which may be comparable to the increases in TAMP in the non-gating rats following clozapine.

Though clozapine prevented PCP induced deficits in the gating rats it failed to

reverse the auditory gating deficits in the non-gating rats. It could be possible that the non-gating rats are a result of neuro-developmental deficits beyond receptor level, which can not be remedied by altering neurotransmission with exogenous chemicals.

The ability of the NMDA antagonist, PCP, to produce widespread disruption of auditory gating in areas implicated in the pathophysiology of schizophrenia, especially following acute administration under isoflurane anaesthesia, makes it a potential pharmacological model with a high predictive validity, substantiated by the fact that the atypical antipsychotic, clozapine, reversed the gating deficits induced by PCP.

Chapter Six

Effects of WIN55,212-2 on auditory gating in the rat hippocampus and medial prefrontal cortex

6. Effects of WIN55,212-2 on auditory gating in the rat hippocampus and medial prefrontal cortex

6.1. Introduction

Cannabis is one of the most widely abused drugs in the world and is also used commonly with other recreational drugs such as Phencyclidine (PCP). Cannabinoids, including Δ^9-THC, the active compound of the plant *Cannabis sativa*, produce neuropsychiatric effects by binding to specific endogenous receptors (mainly the CB1 receptors) in the central nervous system (see 1.8).

In 1997, Emrich et al proposed that the pathophysiology of schizophrenia may be related to a functional disturbance of the endocannabinoid system. Since then evidence has been accumulating to support the cannabinoid hypothesis of schizophrenia. Several studies have shown that the administration of cannabinoid agonists either acutely (Emrich et al., 1997) or chronically (Patrick et al., 1999) can produce schizophrenic symptoms in healthy individuals and exacerbate symptoms in schizophrenic patients. A recent meta analysis on cannabis and psychosis by Moore et al (2007) reports *'There was an increased risk of any psychotic outcome in individuals who had ever used cannabis (pooled adjusted odds ratio=1·41, 95% CI 1·20–1·65). Findings were consistent with a dose-response effect, with greater risk in people who used cannabis most frequently (2·09, 1·54–2·84)'* highlighting the ominous link between cannabis and schizophrenia.

Patrick et al (1999) and Rentzsch et al (2007) reported that chronic cannabis abuse, in otherwise healthy individuals, disrupts auditory gating in scalp recorded AERs. Recent studies have also shown that cannabinoid agonists disrupt auditory gating in the rat CA3 (Zachariou et al., 2007, 2008; Hajos et al., 2008).

In this chapter we conducted simultaneous recordings from the DG, CA3 and mPFC in rat and sought to determine if auditory gating in these regions could be disrupted by a single administration of the synthetic non-selective cannabinoid agonist WIN55,212-2 and whether any effects observed were reversed by the CB1 receptor antagonist SR141716A.

6.2. Methods

6.2.1 Animals

Experiments were performed on male Lister hooded rats (n = 20) weighing 300-400 g at the time of surgery and anaesthetised with isoflurane; N_2O:O_2 and all the electrophysiological procedures were carried out as described in chapter 2. Simultaneous recordings from the CA3, DG and mPFC were carried out and only the animals with electrodes placed in the CA3, DG and mPFC, confirmed by histology, were used for analysis.

6.2.2 Experimental protocol

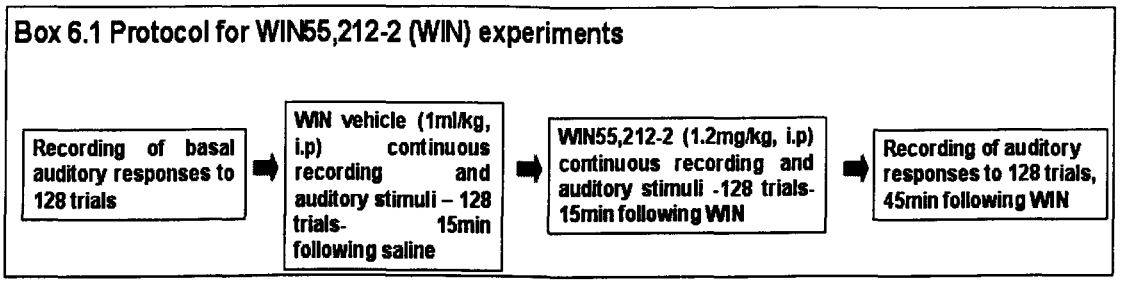
The auditory stimuli (90dB intensity, 0.5s ISI, 10s inter-trial interval) were presented over 128 trials and auditory-evoked LFP and unit responses were recorded simultaneously from the CA3, DG and mPFC.

6.2.3 Drug administration

WIN55,212-2 experiments

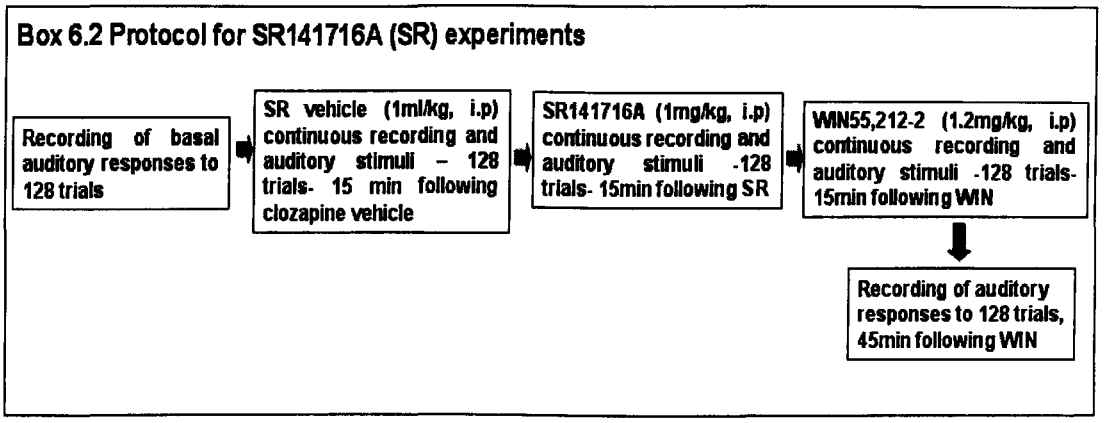
Following basal recording of the auditory responses to 128 trials of auditory stimuli, the vehicle was administered (1ml/kg, i.p; n = 10) and the effect on auditory gating assessed by recording the responses to 128 auditory conditioning-test trials 15min after administration (Box 6.1). WIN55,212-2 was administered to the same animals 40 min after the vehicle and the effect on

auditory gating was again assessed by recording auditory responses to 128 stimuli trials, begining15 and 45 min after drug administration.



SR141716A experiments

Following basal recording of the auditory responses to 128 trials of auditory stimuli, the vehicle for SR141716A was administered (1ml/kg, i.p; n = 10 rats) and the effect on auditory gating assessed by recording the responses to 128 auditory conditioning-test trials 15min after administration (Box 6.2). SR141716A was then administered as a single dose (1mg/kg, i.p) 40 min after the vehicle. The effect of SR141716A on auditory gating was assessed by recording the responses to 128 auditory conditioning-test trials 15min after administration. WIN55,212-2 (1.2mg/kg, i.p) was administered to the same animals 40 min after SR141716A and the effect on auditory gating assessed over 128 stimuli trials, 15 and 45 min following WIN55,212-2 administration.



6.2.4 Data analysis & statistics

Data were analysed off-line using NeuroExplorer (v3; NEX technologies Inc., USA) with evoked response amplitudes, latencies and T/C ratios computed using a custom-designed Matlab script (v7.0 with associated Matlab Signal Processing, Neural Network, Statistics and Image Processing Toolboxes; The Mathworks). Statistical analysis used Prism (v4.03; GraphPad, USA) and within region changes of all the parameters at different time points were analysed using one-way analysis of variance (ANOVA) for repeated measures with post hoc Tukey t-test. Data are expressed as mean ± SEM (standard error of mean), statistical significance was taken when $P < 0.05$.

6.2.5 LFP analysis

Auditory evoked potentials were initially visualized as peri-event averaged LFPs (Fig. 6.1). The AERs were identified according to the polarity and the order of occurrence, i.e. N1, P1, N2, P2, N3 peaks (Van Luijtelaar et al., 2001; Boutros

et al., 2004). An electrode with the highest N2 wave conditioning amplitude response from each area was selected for further analysis (see chapter 2). The ratio of the test amplitude to the conditioning amplitude (T/C ratio) of the N2 wave was calculated for each averaged response and the T/C ratio in the CA3 was used to separate gating and non-gating rats. A T/C ratio of ≤50% in the CA3 was indicative that gating was present (Joy et al., 2004; Miller et al., 1992).

6.3. Results

6.3.1 Auditory Evoked Responses in the CA3, DG and mPFC

Averaged LFPs recorded from the CA3 region, DG and mPFC in response to auditory stimuli exhibited complex AERs responses as discussed in chapter 3 and 4. Evaluation of the T/C ratios of the N2 wave in the CA3 identified the gating rats (T/C ≤ 50%; n = 12) and the non-gating rats (T/C >50%; n = 8). Auditory evoked N2 responses in the CA3, DG and mPFC were studied in gating rats and non-gating rats separately in relation to their conditioning amplitudes (CAMP), test amplitudes (TAMP), T/C ratios, conditioning response latencies (CLAT) and test response latencies (TLAT). In the group of rats given WIN55,212-2, six animals exhibited gating of the N2 wave and four showed non-gating before drug administration. In the group of rats pre-treated with SR141716A, six animals demonstrated gating and four showed non-gating before drug administration. Neither WIN55,212-2 vehicle nor SR141716A vehicle had any effect on the N2 T/C ratios in any of the three regions in the gating or the non-gating rats (Table 6.1 and Table 6.2).

6.3.2 Effects of WIN55,212-2 on the gating rats

6.3.2.1 Effects of WIN55,212-2 on the conditioning amplitudes

WIN55,212-2 increased CAMP in all three areas, 15 and 45 min following drug administration (Fig. 6.1, 6.2 and Table 6.1). In the CA3 an increase in CAMP was observed ($F_{2, 17} = 4.9$, $P = 0.03$) which was significant 15 min post-WIN55,212-2 ($P<0.05$). In the DG there was a significant increase in CAMP ($F_{2, 17} = 7.9$, $P = 0.008$) with increases in amplitudes both 15 ($P<0.05$) and 45 min ($P<0.05$) after WIN55,212-2 administration. A significant increase of CAMP in the mPFC was also found with WIN55,212-2 ($F_{2, 17} = 4.6$, $P = 0.03$) which was significant 15 min post-WIN55,212-2 ($P<0.05$).

6.3.2.2 Effects of WIN55,212-2 on the test amplitudes

There were significant increases in TAMP following WIN55,212-2 administration in the CA3, DG and mPFC (Fig. 6.1, 6.2 and Table 6.1). In the CA3 region TAMP increased ($F_{2, 17} = 9.8$, $P = 0.004$) both 15 ($P<0.01$) and 45 min ($P<0.01$) post-WIN55,212-2. The DG showed a significant increase in TAMP following WIN55,212-2 ($F_{2, 17} = 13$, $P = 0.001$) both 15 ($P<0.01$) and 45 min ($P<0.01$) after administration. Test amplitudes in the mPFC were also increased by WIN55,212-2 ($F_{2, 17} = 18$, $P = 0.0005$), 15 ($P<0.01$) and 45 min ($P<0.001$) following injection.

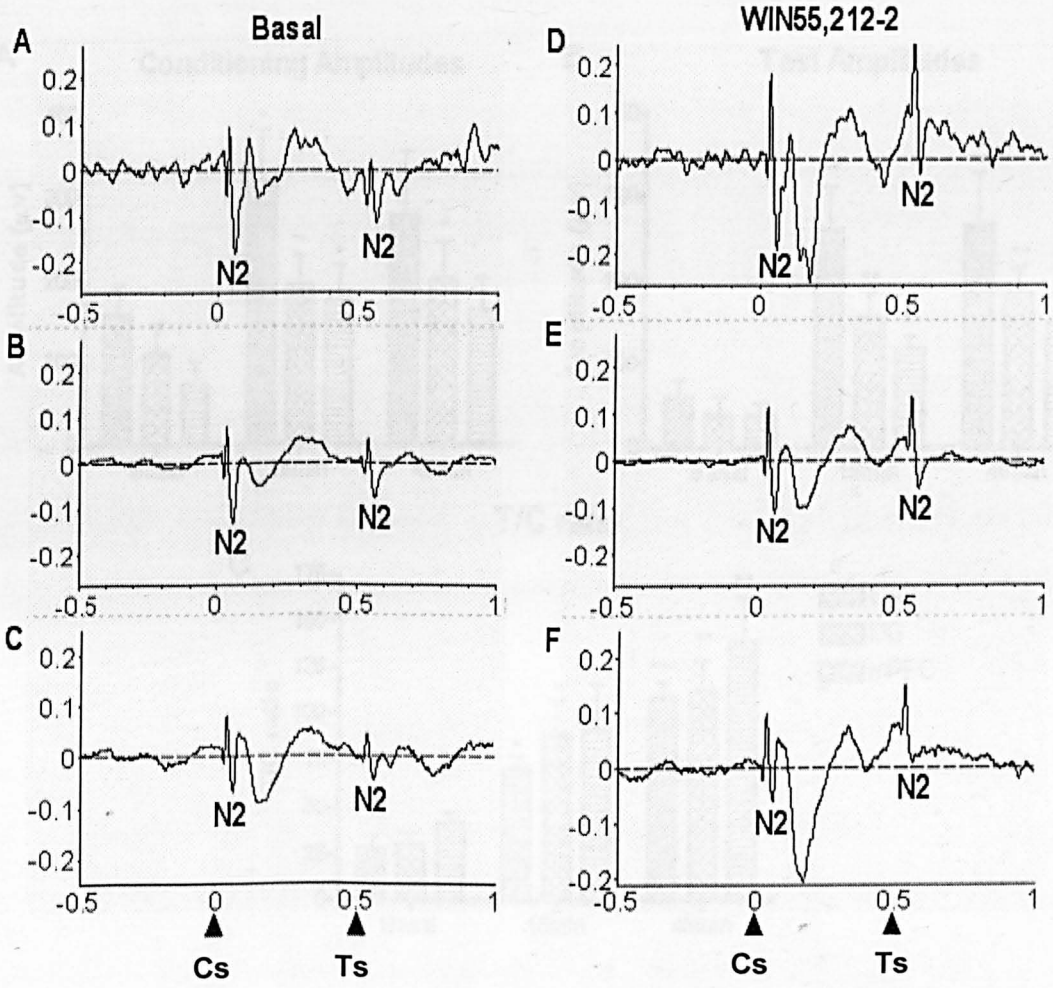
6.3.2.3 Effects of WIN55,212-2 on the T/C ratios

Administration of a single dose of WIN55,212-2 disrupted gating in all three regions both 15 and 45 min after administration when compared to basal values for each region (Fig. 6.1, 6.2 and Table 6.1). WIN55,212-2 disrupted gating in CA3 ($F_{2, 17}$ = 21.5, P = 0.0002), 15 (P<0.05) and 45 min (P<0.001) after injection. In the DG, WIN55,212-2 increased the T/C ratio ($F_{2, 17}$ = 7.7, P = 0.009) with disruption of gating seen at both 15 (P<0.05) and 45 min (P<0.01) after administration. Disruption of gating was detected in the mPFC following WIN55,212-2 ($F_{2, 17}$ = 10.8, P = 0.003) with an increase in T/C ratio at 15 (T/C = 93 ± 23 %; P>0.05) and 45 min (P<0.01) after administration.

6.3.2.4 Effects of WIN55,212-2 on the conditioning latencies and test latencies

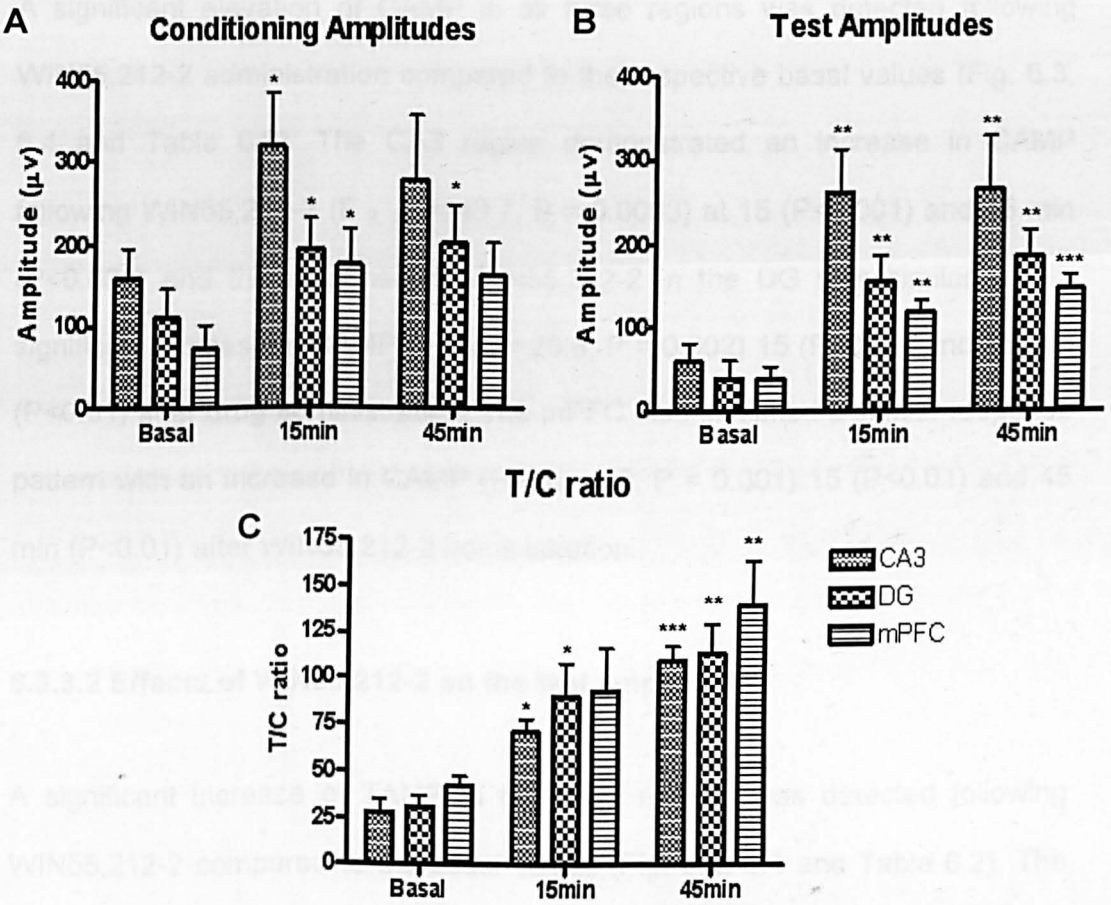
WIN55,212-2 caused a significant shortening of CLAT in the CA3 ($F_{2, 17}$ = 6.8, P = 0.03) and DG ($F_{2, 17}$ = 9.7, P = 0.004). However, WIN55,212-2 had no significant effect on CLAT in the mPFC (P = 0.5). WIN55,212-2 produced no significant changes in TLAT in all three regions (Table 6.1).

Fig. 6.1 Representative peri-event averaged LFPs from (A & D) CA3, (B & E) DG and (C & F) mPFC demonstrating the effects of WIN55,212-2 (45min after administration) on auditory gating compared to basal recordings in a gating rat.



Basal recordings from (A) CA3 (T/C ratio = 38 %), (B) DG (T/C ratio = 40 %) and (C) mPFC (T/C ratio = 48 %) demonstrated gating of the N2 wave. WIN55,212-2 disrupted gating of the N2 wave in (D) CA3 (T/C ratio = 82 %), (E) DG (T/C ratio = 85 %) and (F) mPFC (T/C ratio = 89 %) 45min following drug administration.

Fig. 6.2 Graphs depicting conditioning amplitudes (CAMP), test amplitudes (TAMP) and T/C ratio from the CA3, DG and mPFC of the gating rats (n = 6), 15min and 45min following WIN55,212-2, compared to basal values (mean ± SEM).



A. WI55,212-2 increased the CAMP in the DG, but had no effect on the CAMP of the CA3 or mPFC 45 min following administration.

B. WI55,212-2 increased the TAMP in the CA3, DG and mPFC both 15 and 45 min following administration.

C. T/C ratios in the CA3, DG and mPFC increased significantly both 15 and 45 min following WI55,212-2 administration.

(* = P<0.05, ** = P<0.01, *** = P<0.001)

6.3.3 Effects of WIN55,212-2 on the non-gating rats

6.3.3.1 Effects of WIN55,212-2 on the conditioning amplitudes

A significant elevation of CAMP in all three regions was detected following WIN55,212-2 administration compared to the respective basal values (Fig. 6.3, 6.4 and Table 6.2). The CA3 region demonstrated an increase in CAMP following WIN55,212-2 ($F_{2, 11}$ = 43.7, P = 0.0003) at 15 (P<0.001) and 45 min (P<0.001) and the response to WIN55,212-2 in the DG was similar with a significant increase in CAMP ($F_{2, 11}$ = 20.8, P = 0.002) 15 (P<0.01) and 45 min (P<0.01) after drug administration. The mPFC also exhibited a similar response pattern with an increase in CAMP ($F_{2, 11}$ = 26, P = 0.001) 15 (P<0.01) and 45 min (P<0.01) after WIN55,212-2 administration.

6.3.3.2 Effects of WIN55,212-2 on the test amplitudes

A significant increase of TAMP in the three regions was detected following WIN55,212-2 compared to the basal values (Fig. 6.3, 6.4 and Table 6.2). The CA3 region demonstrated an increase in TAMP following WIN55,212-2 ($F_{2, 11}$ = 18, P = 0.003) 15 (P<0.01) and 45 min (P<0.01) after administration with a similar response in the DG, a significant increase in TAMP ($F_{2, 11}$ = 22, P = 0.002) 15 (P<0.01) and 45 min (P<0.01) post drug administration. The mPFC showed the same response pattern with an increase in TAMP ($F_{2, 11}$ = 11.8, P = 0.008) 15 (P<0.05) and 45 min (P<0.05) after WIN55,212-2.

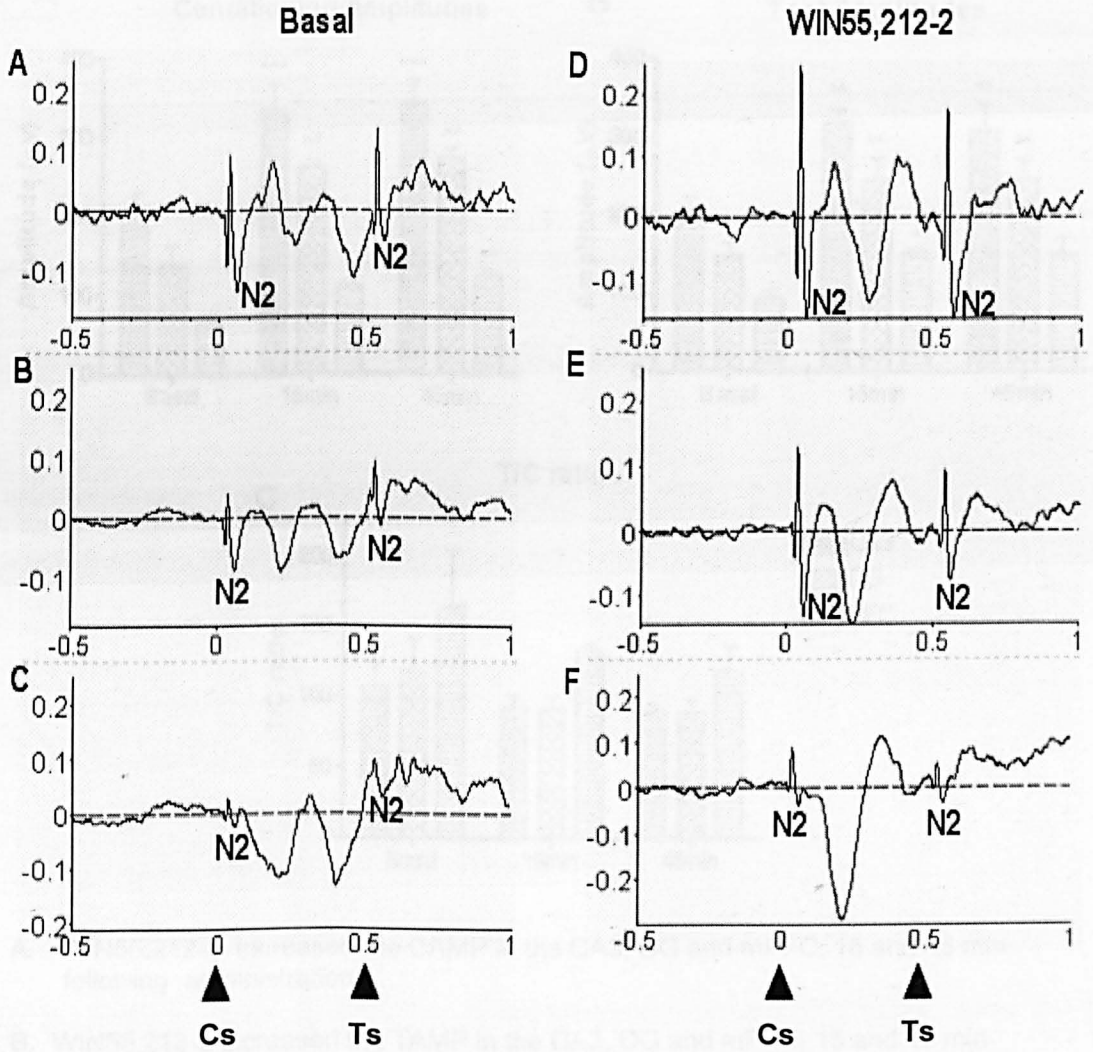
6.3.3.3 Effects of WIN55,212-2 on the T/C ratios

Interestingly administration of WIN55,212-2 had no significant effect on the T/C ratios ($P>0.05$) in the non-gating rats in the CA3, DG or mPFC (Fig. 6. 3 and Table 1). A reduction in T/C ratio was observed in all three regions possibly due the comparatively higher increase in CAMP than TAMP following WIN55,212-2 (Fig. 6.3, Fig. 6.4 and Table 6.2).

6.3.3.4 Effects of WIN55,212-2 on the conditioning latencies and test latencies

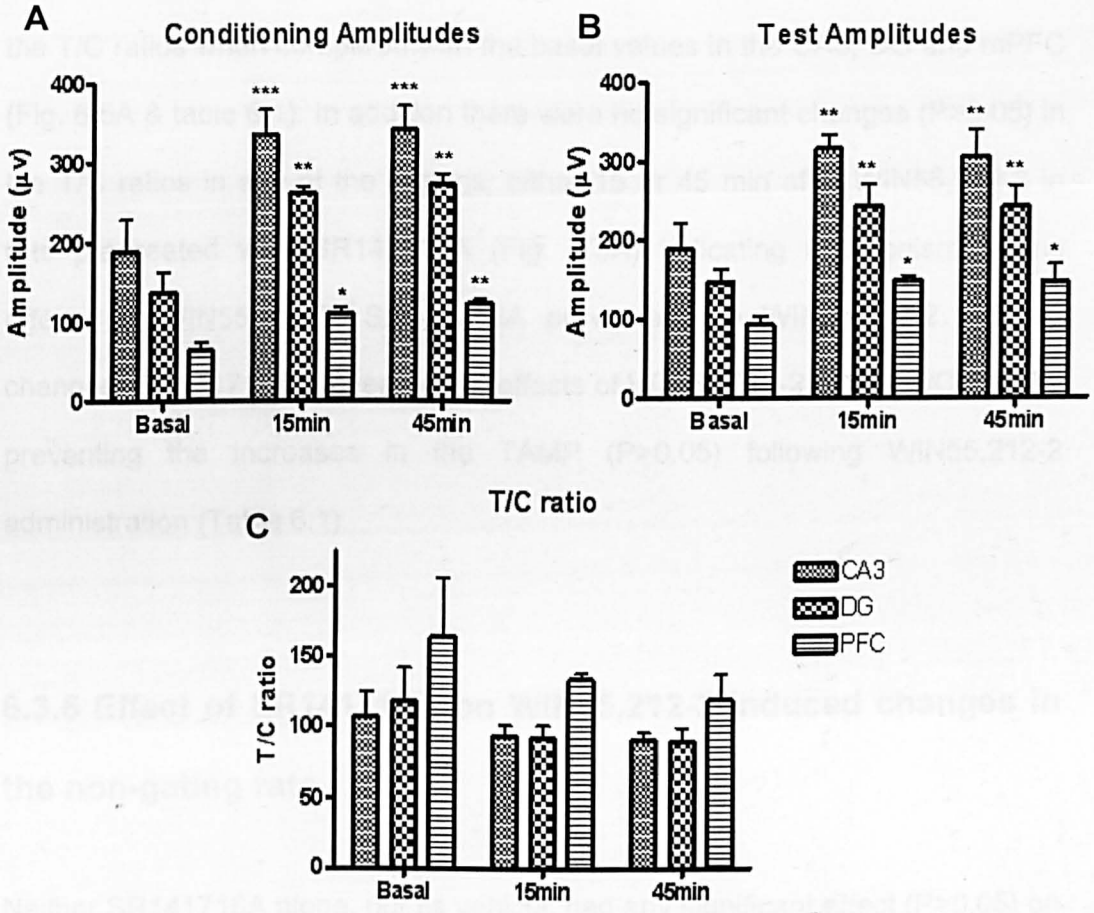
WIN55,212-2 caused a significant increase in CLAT in the DG ($F_{2, 11} = 8.3$, $P = 0.01$) and mPFC ($F_{2, 11} = 5.5$, $P = 0.04$). However, WIN55,212-2 had no significant effect on CA3 conditioning latencies ($P>0.05$). WIN55,212-2 produced no significant changes in TLAT in the three regions ($P>0.05$; Table 6.2).

Fig. 6.3 Representative peri-event averaged LFPs from (A & D) CA3, (B & E) DG and (C & F) mPFC demonstrating the effects of WIN55,212-2 (45min after administration) on auditory gating (N2 wave) compared to basal recordings in a non-gating rat.



Basal recordings from (A) CA3 (T/C ratio = 93 %), (B) DG (T/C ratio = 97 %) and (C) mPFC (T/C ratio = 108 %) demonstrated absence of gating of the N2 wave. WIN55,212-2 had no significant effect on the T/C ratios (P>0.05) in the (D) CA3 (T/C ratio = 82 %), (E) DG (T/C ratio = 83 %) or (F) mPFC (T/C ratio = 80 %) 45 min after administration.

Fig. 6.4 Graphs showing conditioning amplitudes (CAMP), test amplitudes (TAMP) and T/C ratio from CA3, DG and mPFC of the non-gating rats (n = 4), following 15min and 45min of WIN55,212-2 administration, compared to basal (mean ± SEM).



A. WIN55,212-2 increased the CAMP in the CA3, DG and mPFC, 15 and 45 min following administration.

B. WIN55,212-2 increased the TAMP in the CA3, DG and mPFC, 15 and 45 min following administration.

C. T/C ratios showed no significant changes (P>0.05) in the CA3, DG or mPFC following WIN55,212-2 administration in the non-gating rats.

(* = P<0.05, ** = P<0.01, *** = P<0.001)

6.3.4 Effect of SR141716A on WIN55,212-2 induced changes in the gating rats

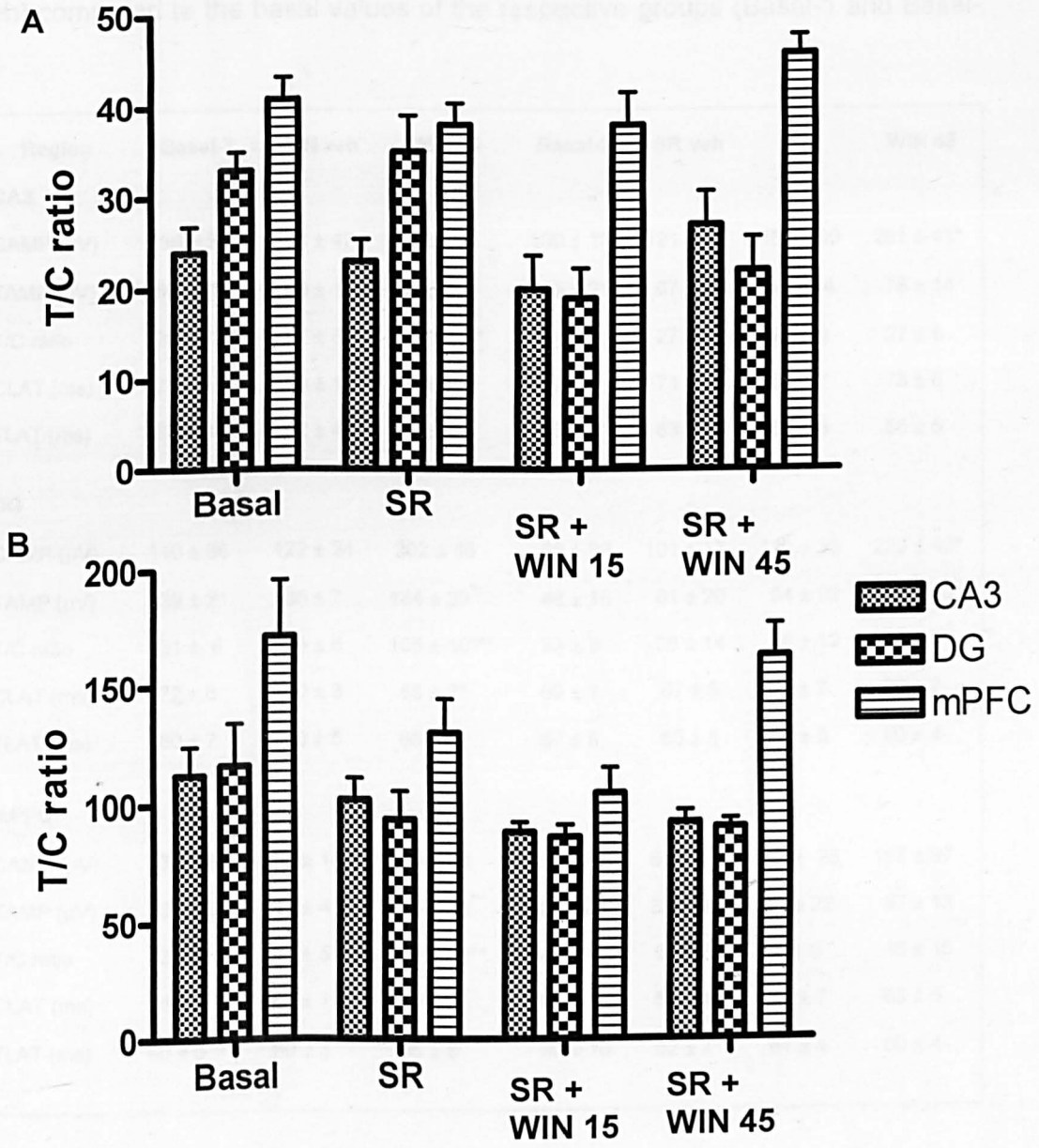
Neither SR141716A alone, nor its vehicle, had any significant effect ($P>0.05$) on the T/C ratios when compared with the basal values in the CA3, DG and mPFC (Fig. 6.5A & table 6.1). In addition there were no significant changes ($P>0.05$) in the T/C ratios in any of the regions, either 15 or 45 min after WIN55,212-2 in rats pre-treated with SR141716A (Fig. 6.5A) indicating antagonism of the effects of WIN55,212-2. SR141716A prevented the WIN55,212-2 induced changes. SR141716A prevented the effects of WIN55,212-2 on the T/C ratio by preventing the increases in the TAMP ($P>0.05$) following WIN55,212-2 administration (Table 6.1).

6.3.5 Effect of SR141716A on WIN55,212-2 induced changes in the non-gating rats

Neither SR141716A alone, nor its vehicle, had any significant effect ($P>0.05$) on the T/C ratios when compared with the basal values in the CA3, DG and mPFC (Fig. 6.5B & Table 6.2) in the non-gating rats. In addition there were no significant changes ($P>0.05$) in the T/C ratios in any of the regions, either 15 or 45 min after WIN55,212-2 in rats pre-treated with SR141716A (Fig. 6.5B). There were significant increases in the CAMP in the CA3 ($F_{4,29}$ = 16.6, $P<0.0001$) and DG ($F_{4,29}$ = 15.7, $P<0.0001$) both 15min ($P<0.001$) and 45min ($P<0.001$) following WIN55,212-2 administration. A significant increase in the CAMP was observed in the mPFC ($F_{4,29}$ = 3.9, P = 0.02) 45min ($P<0.05$)

following WIN55,212-2 in this rat group. TAMP also increased significantly in the CA3 ($F_{4,29}$ = 14, P<0.0001), DG ($F_{4,29}$ = 13.7, P<0.0001) and mPFC ($F_{4,29}$ = 7.7, P= 0.0006) following WIN55,212-2 administration in the non-gating rats pre-treated with SR141716A. There were increases in CLAT in the DG (P<0.05) and mPFC (P<0.05) 45min following WIN55,212-2. The effects of WIN55,212-2 on the AER parameters in the non-gating rats pre-treated with SR141716A were similar to the results obtained when WIN55,212-2 was administered alone (Table 6.2), indicating that SR141716A had no effect on the changes produced by WIN55,212-2 in the non-gating rats.

Fig. 6. 5 Bar graphs depicting N2 T/C ratios 15 min (WIN 15) and 45 min (WIN 45) after WIN55,212-2 administration in (A) gating rats (n = 6) and (B) non-gating rats (n = 4) pre-treated with SR141716A (SR) compared to basal values (mean ± SEM).



A. SR141716A alone had no significant effect on the T/C ratios (P>0.05) in the CA3, DG and mPFC. Pre-treatment with SR141716A prevented the increases in T/C ratios following WIN55,212-2 in the CA3, DG and mPFC in the gating rats.

B. Neither SR141716A nor WIN55,212-2 had any effect on the T/C ratios in the CA3, DG or mPFC in the non-gating rats.

Table 6.1 Summary table of the amplitudes, T/C ratios and latencies 15 min after WIN55,212-2 vehicle (WIN veh) and 45 min after WIN55,212-2 (WIN 45) administration in the gating rats not treated with SR141716A (n = 6) and the gating rats pre-treated with SR141716A (SR; n = 6; SR141716A vehicle = SR veh) compared to the basal values of the respective groups (Basal-1 and Basal-2).

Region	Basal-1	WIN veh	WIN 45	Basal-2	SR veh	SR	WIN 45
CA3							
CAMP (μV)	159 ± 36	164 ± 47	277 ± 79	100 ± 13	121 ± 23	152 ± 60	281 ± 41*
TAMP (μV)	59 ± 22	60 ± 12	264± 24**	45 ± 21	67 ± 22	50 ± 24	78 ± 14
T/C ratio	28 ± 7	27 ± 6	109 ± 8***	24 ± 7	27 ± 9	23 ± 6	27 ± 9
CLAT (ms)	73 ± 6	70 ± 1	58 ± 3*	70 ± 4	71 ± 6	75 ± 7	73 ± 6
TLAT (ms)	67 ± 8	62 ± 4	66 ± 5	66 ± 7	63 ± 5	67 ± 6	56 ± 5
DG							
CAMP (μV)	110 ± 36	122 ± 24	202 ± 46	88 ± 29	101 ± 17	120 ± 33	223 ± 42*
TAMP (μV)	39 ± 21	35 ± 7	184 ± 33**	48 ± 15	61 ± 20	54 ± 22	84 ± 33
T/C ratio	31 ± 6	29 ± 6	105 ± 10***	33 ± 5	38 ± 14	35 ± 12	22 ± 9
CLAT (ms)	72 ± 6	70 ± 3	58 ± 2*	69 ± 1	67 ± 6	65 ± 7	70 ± 7
TLAT (ms)	60 ± 7	60 ± 5	65 ± 5	67 ± 8	65 ± 5	63 ± 8	60 ± 4
mPFC							
CAMP (μV)	73 ± 28	98 ± 15	163 ± 40	50 ± 18	61 ± 17	72 ± 25	157 ± 97
TAMP (μV)	38 ± 15	41 ± 4	145 ± 18***	31 ± 20	32 ± 26	37 ± 22	57 ± 12
T/C ratio	42 ± 5	44 ± 5	139 ± 24**	41 ± 6	28 ± 8	38 ± 6	46 ± 15
CLAT (ms)	65 ± 3	65 ± 1	70 ± 6	63 ± 11	53 ± 10	60 ± 7	63 ± 6
TLAT (ms)	60 ± 5	60 ± 3	65 ± 6	60 ± 10	62 ± 7	61 ± 4	60 ± 4

(* = P<0.05; ** = P<0.01; *** = P<0.001)

Table 6.2 Summary table of the amplitudes, T/C ratios and latencies, 15 min after WIN55,212-2 vehicle (WIN veh) and 45 min after WIN55,212-2 (WIN 45) administration in the non-gating rats not treated with SR141716A (SR; n = 4) and the non-gating rats pre-treated with SR141716A (n = 4) compared to pre-drug values of the respective groups (Basal-1 and Basal-2).

Region	Basal-1	WIN veh	WIN 45	Basal-2	SR veh	SR	WIN 45
CA3							
CAMP (μV)	189 ± 40	194 ± 28	341 ± 30***	138 ± 11	169 ± 27	176 ± 34	341 ± 30***
TAMP (μV)	189 ± 33	190 ± 30	306 ± 34**	134 ± 8	148 ± 14	159 ± 27	331± 44***
T/C ratio	107 ± 18	98 ± 14	90 ± 6	112 ± 24	143 ± 48	102 ± 18	93 ± 10
CLAT (ms)	55 ± 1	55 ± 1	56 ± 1	56 ± 1	56 ± 1	57 ± 2	58 ± 1
TLAT (ms)	58 ± 1	57 ± 2	59 ± 1	60 ± 3	62 ± 2	65 ± 3	60 ± 1
DG							
CAMP (μV)	137 ± 25	141 ± 26	273 ± 13**	133 ± 13	161 ± 23	186 ± 28	283 ± 24***
TAMP (μV)	148 ± 16	145 ± 24	243 ± 27**	132 ± 16	138 ± 10	180 ± 22	268 ± 29***
T/C ratio	118 ± 24	107 ± 18	89 ± 10	116 ± 36	83 ± 11	94 ± 23	91 ± 7
CLAT (ms)	55 ± 1	55 ± 2	58 ± 1*	52 ± 3	53 ± 6	55 ± 1	59 ± 1*
TLAT (ms)	58 ± 1	58 ± 1	60 ± 1	60 ± 2	64 ± 3	65 ± 1	60 ± 1
mPFC							
CAMP (μV)	63 ± 11	70 ± 18	124 ± 5**	70 ± 11	87 ± 16	95 ± 25	126 ± 11*
TAMP (μV)	93 ± 10	90 ± 8	145 ± 22*	76 ± 14	79 ± 12	97 ± 18	170 ± 26***
T/C ratio	164 ± 42	160 ± 15	119 ± 18	172 ± 48	112 ± 23	130 ± 29	163 ± 28
CLAT (ms)	53 ± 0.3	54 ± 2	57 ± 1*	48 ± 1	50 ± 4	50 ± 2	54 ± 1*
TLAT (ms)	61 ± 2	62 ± 1	64 + 2	47 ± 2	48 ± 1	50 ± 7	48 ± 2

(* = P<0.05; ** = P<0.01; *** = P<0.001)

6.4 Discussion

In the present study robust auditory evoked potentials were recorded from the CA3 region, DG and mPFC with some rats showing gating of the N2 wave while others showed a lack of gating. WIN55,212-2 disrupted auditory gating of N2 in the gating rats, but had no effect on the T/C ratios of the non-gating rat group. The CB1 receptor antagonist SR141716A prevented the disruption of gating induced by WIN55,212-2 in the CA3, DG and mPFC.

Studies examining the effect of cannabinoids on rodent behaviours relevant to schizophrenia have produced contrasting results. Schneider and Koch (2002) showed that a single administration of WIN55,212-2 impaired prepulse inhibition (PPI), a paradigm used to model deficits in sensorimotor gating, while in contrast the cannabinoid agonist CP 55,940 augmented PPI (Stanley-Cary et al., 2002). Schneider and Koch (2003) showed that chronic treatment with WIN55,212-2 impaired PPI in pubertal, but not in adult rats while Bortolato et al (2005) showed that acute or chronic administration of WIN55,212-2 failed to change PPI. The same group also found that prenatal exposure to WIN55,212-2 failed to affect the sensory motor gating of adult offspring (Bortolato et al., 2006). The partial cannabinoid agonist Δ^9–Tetrahydrocannabinol (THC) has been shown to disrupt PPI in socially isolated rats, a neurodevelopmental model of schizophrenia, but not in normal rats (Malone and Taylor, 2005).

Our observations with WIN55,212-2 agree with the findings from human and rat studies showing systemic administration of cannabinoid receptor agonists

induce schizophrenic symptoms and disrupt auditory gating (Emrich et al., 1997; Rentzsch et al., 2007; Hajos et al., 2008). The mechanisms by which cannabinoids bring about these effects are unclear though previous studies have shown that endocannabinoids modulate the activity of several neurotransmitters in the brain, mainly via CB1 receptors located presynaptically on GABAergic interneurones (Wilson and Nicoll, 2002). Acute systemic administration of cannabinoid agonists increases extracellular mesolimbic dopamine levels via activation of CB1 receptors on the GABAergic terminals resulting in the disinhibition of the dopamine neurones in the ventral tegmental area (Gardner and Voral, 1998). Dysregulation of several neurotransmitters, including GABA, dopamine and glutamate are implicated in the pathogenesis and symptomatology of schizophrenia, effects that could result from dysregulation of the endocannabinoid system (see 1.8). Lewke et al (1999) found increased levels of endocannabinoids in the CSF of schizophrenic patients which supports the hypothesis of endocannabinoid dysregulation in schizophrenia.

The reciprocal connections between GABAergic interneurones and glutamatergic pyramidal neurons in the CA3 region of the hippocampus and the external inputs from the medial septum (nicotinic and GABAergic) and the entorhinal cortex (glutamatergic) are important for auditory gating in the CA3 (Miller & Freedman., 1993; Flach et al., 1996; Moxon et al., 2003[a]). Either a dysfunction or a blockade of the connections affecting the fine tuning of these neurotransmitters can disrupt gating (Flach et al., 1996; Moxon et al., 2003[a]). A cannabinoid agonist administered systemically may bind to the presynaptic CB1

receptors on the hippocampal and possibly prefrontal interneurones so reducing GABA release and thus GABA inhibition. The absence of GABAergic inputs on excitatory glutamatergic neurons could lead to hyperactivity of the excitatory circuits resulting in deficits in inhibitory processes such as auditory gating.

WIN55,212-2 increased both the conditioning and test amplitudes in the CA3, DG and mPFC in the gating rats, suggesting a hypersensitive/hyperactive state created by cannabinoids in the three regions either by activating or recruiting more stimuli sensitive neurons. The increase in auditory test response amplitudes were relatively greater than that of the conditioning amplitudes resulting in disruption of gating following WIN55,212-2. This finding is similar to the effect of phencyclidine (as observed in chapter 5), which disrupts gating by increasing response amplitudes (Miller et al., 1992). The fact the disruption of gating by WIN55,212-2 is brought about by the inability to suppress the test responses and not by reduction in conditioning responses makes it a potential pharmacological agent to model the information processing deficits in schizophrenia. WIN55,212-2 also shortened the response latencies in the CA3 and DG in the gating rats which could indicate disrupted information processing following cannabinoid administration.

WIN55,212-2 increased the conditioning amplitudes and test amplitudes in the non-gating rats in the CA3, DG and mPFC but the T/C ratios were smaller possibly due to comparatively higher increases in CAMP than TAMP. It is interesting that WIN55,212-2 had a differential selectivity on the response amplitudes in non-gating rats compared to the gating rats, due possibly to an

action of the cannabinoid agonist on the dysfunctional neural circuits in the non-gating rats. WIN55,212-2 also delayed the response latencies of the non-gating rats in the CA3, DG and mPFC, opposite to the effect it had on the gating rats, which further illustrates the innate neurophysiological differences between the two groups. Interestingly WIN55,212-2 produced opposite effects on these parameters in the gating and non-gating rats for which an explanation is required from future studies.

The potent and selective CB1 receptor antagonist SR141716A prevented the WIN55,212-2 induced disruption of gating in the CA3, DG and mPFC, suggesting the disruption of auditory gating by WIN55,212-2 was mediated via CB1 receptors. The results from the CA3 measurements agree with the study by Hajos et al (2008) showing that disruption of gating in the CA3 region by the CB1 agonist CP-55940 was reversed by the CB1 receptor antagonist AM-251. Considering the endocannabinoid hypothesis of schizophrenia and that the cannabinoid agonists produce gating deficits similar to those seen in schizophrenics, the prevention of gating deficits by SR141716A makes the endocannabinoid system a potential target for future clinical research in the treatment of schizophrenia.

Chapter Seven

Auditory-evoked single-unit responses in the rat hippocampus and the medial prefrontal cortex

7. Auditory-evoked single-unit responses in the rat hippocampus and the medial prefrontal cortex

7.1 Introduction

In the previous chapters the LFP activity in response to auditory conditioning-test paradigm indicated that the neural processes mediating auditory gating in the CA3, DG and mPFC are inter-related and can be disturbed by NMDA antagonists and CB1 agonists. However, the neuronal mechanisms underlying the sensory gating process are still unclear. Auditory-evoked LFP responses, the primary measure of sensory gating, pool information from a large number of neural elements and the results obtained can not be directly extrapolated to the cellular level. Studying the gating mechanism at the single-unit level not only improves the understanding of the underlying neural mechanisms crucial for sensory gating but provides a valuable tool to study the neurobiology of schizophrenia and other neurological diseases in which sensory gating process is dysfunctional.

Single-unit responses to auditory gating paradigm have been examined in the CA3 region of the rat hippocampus (Bickford et al., 1990; Miller et al., 1992; Miller and Freedman., 1995; Moxon et al., 1999). These studies have demonstrated robust single-unit responses to auditory conditioning-test paradigm with gating of the unit responses in the CA3 under chloral hydrate anaesthesia (Bickford et al., 1990; Miller et al., 1992; Miller and Freedman.,

1995) and in freely moving rats (Moxon et al., 1999).

Mears et al (2006) examined the single-unit responses in the mPFC to the auditory conditioning-test paradigm in freely moving rats and observed three different types of tone responses to the conditioning stimulus (excitatory short duration, excitatory long duration and inhibitory) which were gated following the test stimulus. They also reported that the gating of the unit responses were stable between different recording sessions.

Auditory gating at the single-unit level has been observed in several other regions in the rat CNS including the medial septum (Miller and Freedman., 1993; Moxon et al., 1999), brain stem (Moxon et al., 1999), reticular nucleus of the thalamus (Krause et al., 2003), amygdala (Cromwell et al., 2005), striatum (Cromwell et al., 2007) and the midbrain (Anstrom et al., 2007). However, the dentate gyrus of the hippocampus has not yet been explored in relation to auditory gating at the single-unit level.

The effects of pharmacological manipulations on the single-unit gating responses have not been studied widely. Miller et al (1992) demonstrated that PCP disrupts auditory gating of the single-unit responses in the CA3 of rat hippocampus under chloral hydrate anaesthesia. Krause et al (2003) demonstrated that amphetamine (a dopamine agonist) disrupts gating of the reticular thalamic neurons in chloral hydrate anaesthetised rats. We have shown that WIN55,212-2 disrupts gating at the single-unit level in the CA3 of the rat hippocampus under isoflurane anaesthesia (Zachariou et al 2007, 2008).

Cromwell and Woodward (2007) examined the effects of ketamine (a NMDA antagonist), haloperidol (a dopamine antagonist) and nicotine on the single-unit gating responses in the amygdala in freely moving rats and showed that these three different drugs affected gating in partially over-lapping ways, suggesting the existence of multiple and diverse neural circuits mediating auditory gating.

In this chapter the single-unit responses were analysed and compared with simultaneously recorded LFP responses to the auditory conditioning-test paradigm in both gating and non-gating rats in the CA3, DG and mPFC under isoflurane anaesthesia. We also studied the effects of PCP and WIN55,212-2 on the single-unit responses to the auditory conditioning-test paradigm in the gating rats with the aim of correlating the results with those obtained with LFPs.

7.2 Methods

Single-unit activity was recorded simultaneously with LFPs in the CA3, DG and mPFC in the male Lister hooded rats (n = 21) in response to the auditory conditioning-test paradigm, before and after pharmacological manipulations. Neuronal action potential activity recorded from the three areas were validated and sorted off-line (see chapter 2). Within *Neuroexplorer* data were subsequently analysed as peri-stimulus time histograms and epoch counts.

7.2.1 Single-unit analysis

Single-units recorded from the three regions were examined separately for auditory-evoked responses using peri-event / peri-stimulus time histograms (10ms bin; ± 500ms peri-stimuli period). Only the neurons with significant responses (above 95% confidence limit from the mean basal firing rate in the peri-stimulus time histograms; $P<0.05$) to the auditory stimuli (conditioning / test / both) were considered as auditory-responsive (see chapter 2, Fig. 2.10).

Auditory-responsive single-units in the CA3, DG and mPFC were grouped in to two main types according to their stimuli-related responses using peri-stimulus time histograms. Single-units that exhibited the highest peak activity within 100ms of the conditioning stimulus delivery were classified as early excitatory (Fig. 7.1) and the units which demonstrated highest peak activity following 100 ms of the conditioning stimulus delivery were classified as late excitatory (Fig. 7.2). Epoch count analysis of the auditory responsive cells was used to obtain

the average unit activity (spike count (number of action potentials) / epoch) during specified time periods (epochs) and the values obtained were used to calculate the T/C ratios of the units. The T/C ratio for the early excitatory cells was calculated as the ratio of the average unit activity during 100ms following the onset of the test stimulus to the average unit activity during 100 ms following the onset of the conditioning stimulus. For late excitatory cells, the T/C ratio was calculated as the ratio of the average unit activity during 500ms following the onset of the test stimulus to the average unit activity during 500 ms following the onset of the conditioning stimulus. A Matlab script (TCpsth.m) was used to obtain the latencies (from the timing of the stimulus to the peak firing in the peri-stimulus histogram) of the peak activities.

T/C ratios of the unit responses were compared to the T/C ratios of the N2 LFPs recorded from the same electrode where possible. The T/C ratios of the LFPs were obtained using a Matlab script as described in the previous chapters. The N2 LFP T/C ratio in the CA3 was used to separate gating and non-gating rats. A T/C ratio of ≤50% in the CA3 was indicative that gating was present (Joy et al., 2004; Miller et al., 1992). Single-unit activity in the gating (n = 16) and the non-gating rats (n = 5) were analysed separately.

Since the auditory gating process at the LFP level (i.e. the N2 T/C ratio) in the gating rats appeared to be more sensitive to pharmacological manipulations compared to the non-gating rats, the single-unit spike activity and the T/C ratios of the gating rats were analysed further to examine the drug related changes in the unit activity in response to the auditory conditioning-test paradigm. The pre-

drug unit activity following auditory stimuli (basal) were compared to the activity 45 min after PCP (1mg/kg, i.p) or 45 min after WIN55,212-2 (1.2mg/kg, i.p) administration. The effects of clozapine (5mg/kg, i.p) on the basal unit activity 15 min after administration and also the effects of pre-administration of clozapine (5mg/kg, i.p) on PCP (1mg/kg, i.p) induced changes in the unit activity were examined. The effects of SR141716A (1mg/kg, i.p) on the basal unit activity 15 min after administration and the effects of pre-administration of SR141716A (1mg/kg, i.p) on WIN55,212-2 (1.2 mg/kg, i.p) induced changes in the unit activity were also examined.

7.2.2 Statistical analysis

Statistical analysis used Prism (v4.03; GraphPad, USA) and within region changes in the T/C ratios and latencies were analysed using Student t-test or one way analysis of variance (ANOVA) for repeated measures with post hoc Tukey t-test when appropriate. Data are expressed as mean ± SEM (standard error of mean), statistical significance was taken when $P < 0.05$.

7.3 Results

A total of 704 single-units were recorded from both gating rats (n = 16) and non-gating rats (n = 5). Of the units recorded from the gating rats (n = 527; CA3, n = 202; DG, n = 178; mPFC, n = 147), in the CA3, 52% (105/202), in the DG, 46% (82/178) and in the mPFC, 78% (115/147) demonstrated significant responses to auditory stimuli. Of the units from the non-gating rats (n = 177; CA3, n = 78, DG, n = 33; mPFC = 66), in the CA3, 45% (35/78), in the DG, 70% (23/33) and in the mPFC, 53% (35/66) responded to auditory stimuli. Auditory responsive cells from the gating and the non-gating rats were examined separately.

7.3.1 Auditory-evoked single-unit activity in the gating rats

Of the auditory-responsive units recorded in the CA3 of the gating rats 43% (n = 45) were early excitatory units (Fig. 7.1A) and 57% (n = 60) were late excitatory units (Fig. 7.2A). In the DG, 34% (n = 28) were early excitatory (Fig. 7.1B) while 66% were (n = 54) late excitatory units (Fig. 7.2B). The auditory responsive cells in the mPFC demonstrated a high percentage of late excitatory units (77%, n = 88; Fig. 7.1C) compared to early excitatory units 18% (n = 27; Fig. 7.2C).

7.3.1.1 The early excitatory cells in the gating rats

The T/C ratios of the early excitatory cells in the gating rats

The majority of early excitatory cells in the gating rats (CA3, 87%, n = 39; DG, 93%, n =26; mPFC, 86%, n = 23) exhibited a significant decrease in unit activity (spike count / epoch) following the test stimulus compared to the activity following the conditioning stimulus in the CA3 ($P<0.001$), DG ($P<0.001$) and mPFC ($P<0.01$; Fig.7.1). All the early excitatory regions had a small percentage of early excitatory units with increased activity following the test stimulus compared to the conditioning stimulus (T/C >100%; CA3, 13%, n = 6; DG, 7%, n = 2; mPFC, 14%, n = 4) and they were excluded from further analysis due to the small (n) number.

The early excitatory cells in the CA3 of the gating rats had a lower T/C ratio (45 ± 4%) compared to the T/C ratios in the DG (50 ± 5 %) and mPFC (54 ± 5%). There were no significant differences between the early excitatory single unit T/C ratios and the N2 LFP T/C ratios recorded from the same recording electrodes in the CA3 (36 ± 3%; P= 0.1), DG (38 ± 6%; P = 0.06) or mPFC (43 ± 5%;P = 0.2; Fig. 7.1).

The peak latencies of the early excitatory cells in the gating rats

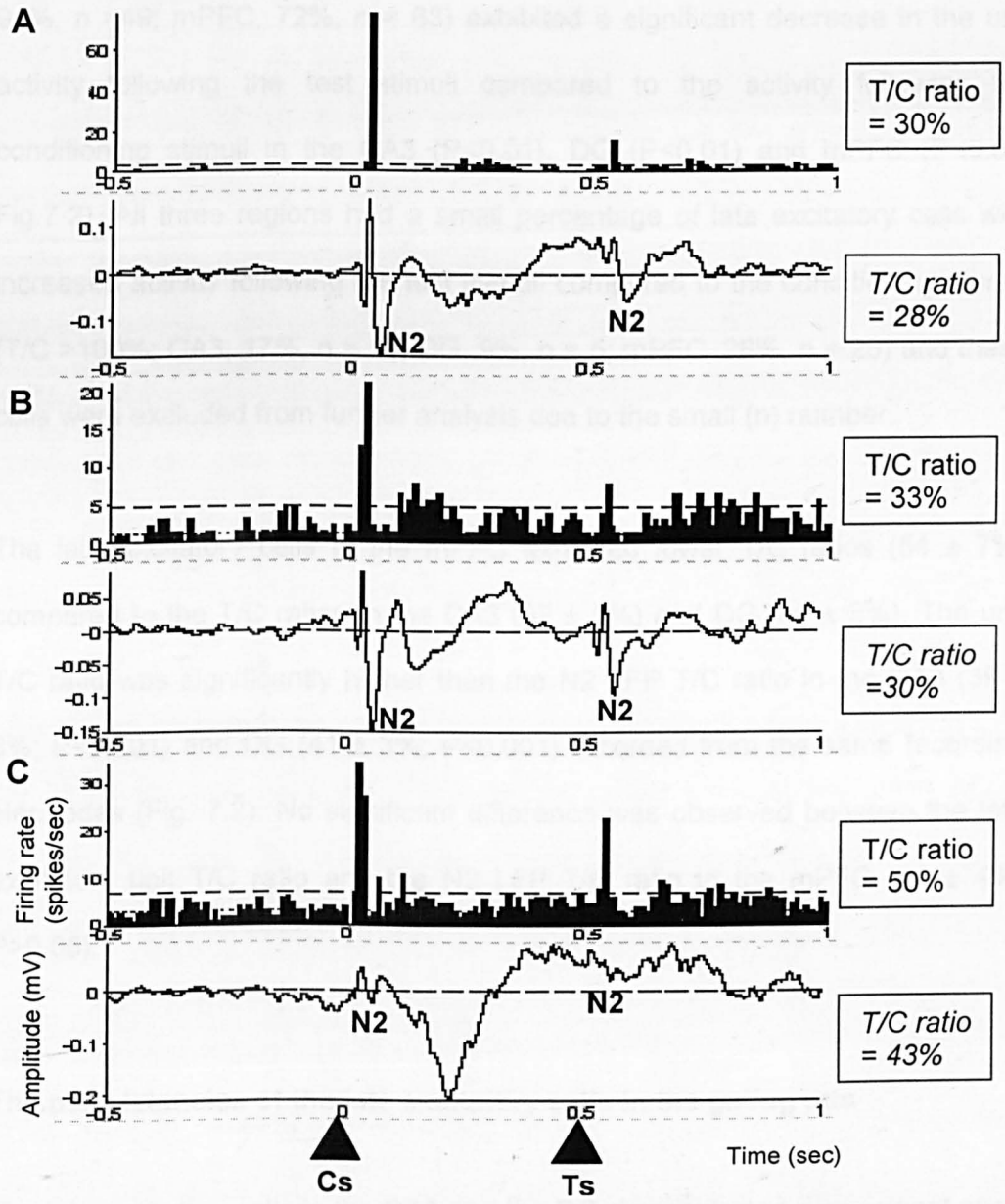
The peak unit activity of the early excitatory cells in the CA3 started at 27 ± 2 ms and peaked at 33 ± 1 ms following the conditioning stimuli (Fig. 7.1). The DG demonstrated peak activity with the onset at 27 ± 3 ms and peaking at 32 ±

0.4 ms following the conditioning stimuli. In the mPFC, the onset of the significant activity following the conditioning stimuli was at 26 ± 1 ms and demonstrated the highest peak at 30 ± 1 ms. The peak latency of the early excitatory units following the conditioning stimuli in the CA3, DG and mPFC were significantly shorter than the N2 LFP conditioning response latencies (Fig. 7.1; CA3, N2 CLAT = 66 ± 2 ms, $P<0.001$; DG, N2 CLAT = 64 ± 1 ms, $P<0.001$; mPFC, N2 CLAT = 59 ± 1 ms ,$P<0.01$). However, the peak unit latencies were comparable to the P1 LFP latencies recorded from the three regions (CA3, P1 CLAT = 40 ± 6 ms, $P>0.05$; DG P1 CLAT = 35 ± 2 ms, $P>0.05$; mPFC P1 CLAT = 32 ± 2 ms, $P>0.05$).

The peak early excitatory unit activity following the test stimuli, started at 29 ± 2 ms and peaked at 33 ± 1 ms in the CA3, started at 27 ± 1 ms and peaked at 32 ± 0.4 ms in the DG and started at 26 ± 2 ms and peaked at 31 ± 1 ms in the mPFC.

Very small peak activities were observed in the early excitatory cells during the 100ms – 300ms periods following the conditioning stimulus in the CA3 and the DG and between the 100ms-500ms in the mPFC (Fig. 7.1). These peaks were less prominent following the test stimuli in the three areas.

Fig. 7.1 Representative peri-stimulus time histograms (upper panels; 10ms bin, ± 500ms peri-stimuli period) of the early excitatory cells and averaged LFPs (lower panels) obtained from the same recording electrodes used to record cells in **(A)** CA3, **(B)** DG and **(C)** mPFC of a gating rat. The dashed lines in the histograms indicate the mean firing rate (smaller lower line) and the 95% confidence limit (bigger-upper line).



The peri-stimulus histograms of the early excitatory cells in the **(A)** CA3 **(B)** DG and **(C)** mPFC demonstrated an early peak activity following the conditioning stimulus which was decreased following the test stimulus. The unit T/C ratios were comparable to the T/C ratios of the N2 wave in the averaged LFPs. The latencies of the peak unit activity following stimuli (CA3 peak =33 ms; DG peak = 32 ms; mPFC peak = 29 ms) were shorter than the N2 LFP latencies (CA3 CLAT = 58 ms; DG CLAT = 56 ms; mPFC CLAT=49 ms).

7.3.1.2 The late excitatory cells in the gating rats

The T/C ratios of the late excitatory cells in the gating rats

The majority of late excitatory cells in the gating rats (CA3, 83%, n = 50; DG, 91%, n =49; mPFC, 72%, n = 63) exhibited a significant decrease in the unit activity following the test stimuli compared to the activity following the conditioning stimuli in the CA3 ($P<0.01$), DG ($P<0.01$) and mPFC ($P<0.01$; Fig.7.2). All three regions had a small percentage of late excitatory cells with increased activity following the test stimuli compared to the conditioning stimuli (T/C >100%; CA3, 17%, n = 10; DG, 9%, n = 5; mPFC, 28%, n = 25) and these cells were excluded from further analysis due to the small (n) number.

The late excitatory cells in the mPFC exhibited lower T/C ratios (54 ± 7%) compared to the T/C ratios in the CA3 (57 ± 5%) and DG (60 ± 3%). The unit T/C ratio was significantly higher than the N2 LFP T/C ratio in the CA3 (39 ± 3%; $P<0.001$) and DG (41 ± 3%; $P<0.001$) recorded from the same recording electrodes (Fig. 7.2). No significant difference was observed between the late excitatory unit T/C ratio and the N2 LFP T/C ratio in the mPFC (44 ± 4%; $P>0.05$).

The peak latencies of the late excitatory cells in the gating rats

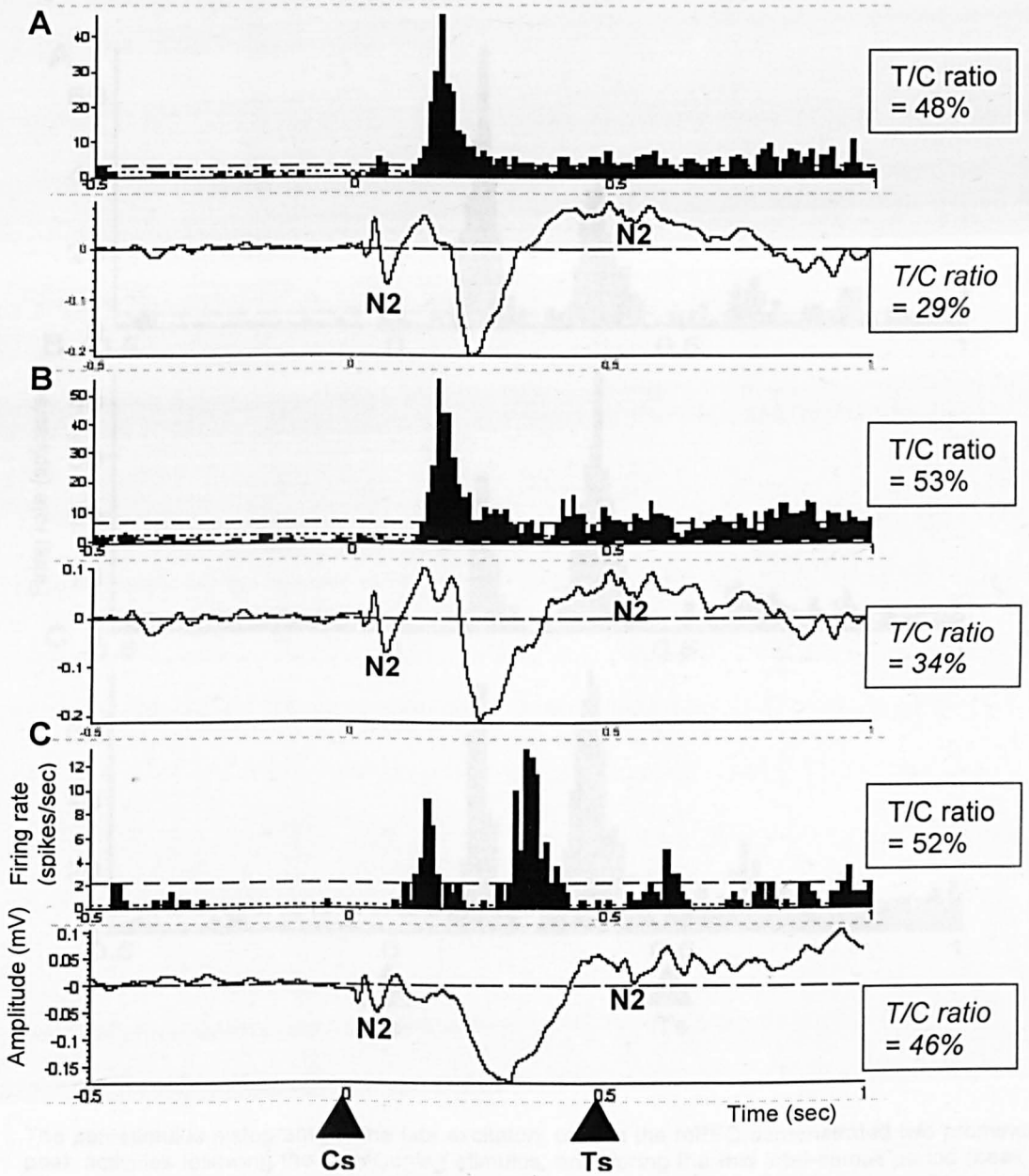
The late excitatory cells in the CA3 and the DG demonstrated a prominent peak activity in the 100 – 250 ms period following the onset of the conditioning stimuli with no prominent peak activities following the test stimuli (Fig. 7.2). The onset

of the peak activity in the CA3 was at 114 ± 3 ms which peaked at 151 ± 6 ms and in the DG the peak activity occurred later than the CA3 (DG onset = 131 ± 2 ms, peak = 175 ± 3 ms). In the mPFC two prominent peak activities were observed following the conditioning stimuli which showed a significant decrease in activity following the test stimuli. The first peak started at 115 ± 7 ms and peaked at 152 ± 2 ms coinciding with the peak LE activity in the CA3. The second LE peak started at 252 ± 44 ms and peaked at 334 ± 7 ms. In some of the late excitatory cells recorded from the mPFC the first peak demonstrated a higher firing rate than the second peak (Fig. 7.3A; 63%; n = 38) and in another group of cells the second peak was higher than the first (Fig. 7.3B; 24%; n = 15) while in some cells both peak activities were similar (Fig. 7.3C; 13%; n = 10). However, there were no significant differences between the T/C ratios of the three different LE cell response types ($P>0.05$) and were analysed together for basal and pharmacologically induced changes.

There were no prominent peak activities in late excitatory cells coinciding with the P1 or N2 LFP peaks within the 100 ms period following the onset of the conditioning stimuli. The late excitatory peaks appeared to coincide with the P2 LFP peak in the CA3 and DG and P2 and N3 wave in the mPFC (Fig. 7.2 and see chapters 2, 3 &4).

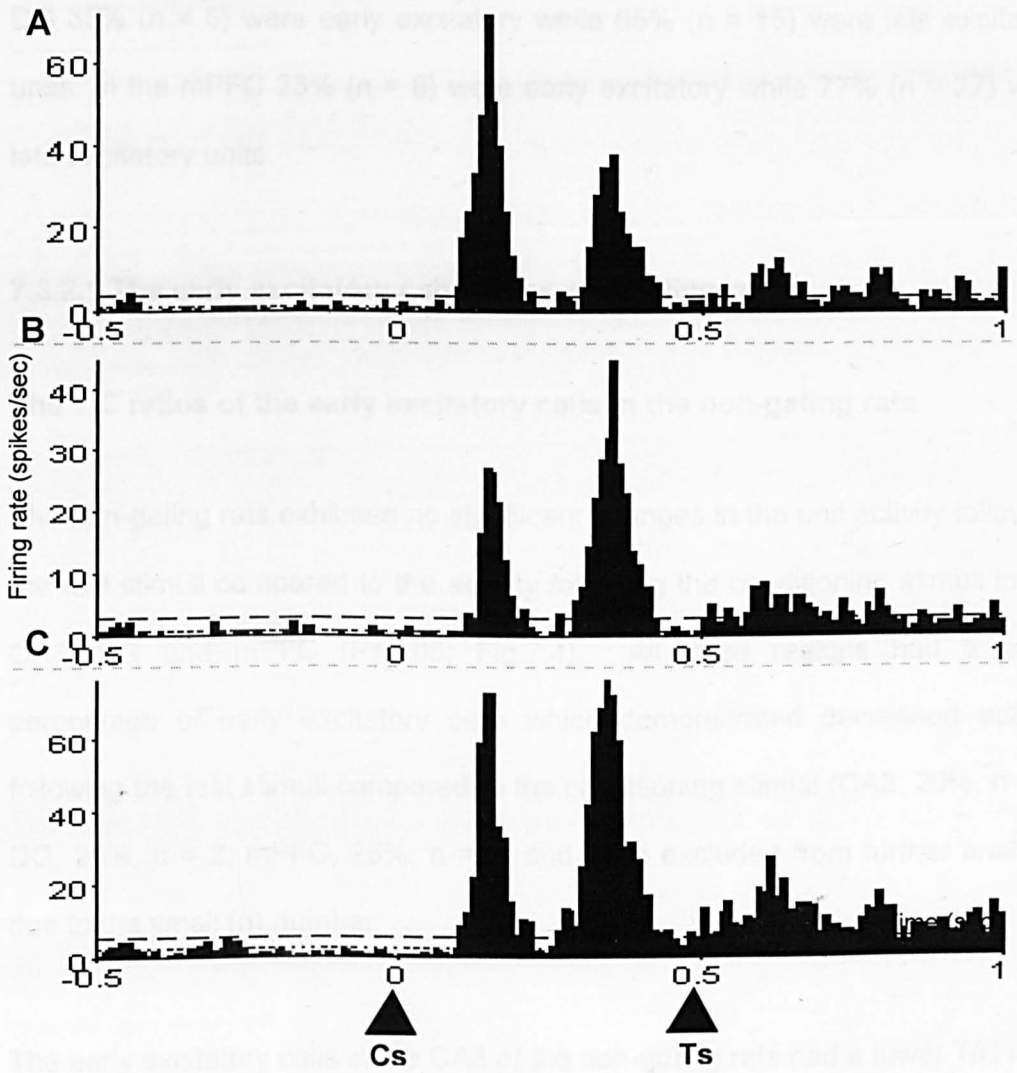
There were no prominent peaks in late excitatory unit activity following the test stimuli in the CA3 and DG (Fig 7.2). In the mPFC a small increase in the unit activity was observed following the test stimuli, starting at 100 ± 2 ms with peak activity at 142 ± 24 ms.

Fig. 7.2 Representative peri-stimulus time histograms (upper panels; 10ms bin, ± 500ms peri-stimuli period) of the late excitatory cells and averaged LFPs (lower panels) obtained from the same recording electrodes used to record cells in **(A)** CA3, **(B)** DG and **(C)** mPFC in a gating rat.



The peri-stimulus histograms of the late excitatory cells in the (A) CA3 (B) DG demonstrated a prominent peak activity during the mid inter-stimuli period (CA3 peak = 150 ms; DG peak = 168 ms) which was decreased following the test stimulus. The T/C ratios in the CA3 and DG were higher than the T/C ratios of the N2 wave. The mPFC demonstrated two main peak activities, one during the mid inter-stimuli period (peak at 150 ms) and the other just before the test stimulus (peak at 340 ms). No prominent peaks were observed following the test stimulus. The unit T/C ratio was similar to the N2 LFP T/C ratio. The dashed lines in the histograms indicate the mean firing rate (small lower line) and the 95% confidence limit (bigger upper line).

Fig. 7.3 Representative peri-stimulus time histograms (upper panels; 10ms bin, ± 500ms peri-stimuli period) demonstrating different response types of late excitatory cells **(A)** high-low peaks **(B)** Low-high peaks **(C)** equal peaks in the mPFC of gating rats.



The peri-stimulus histograms of the late excitatory cells in the mPFC demonstrated two prominent peak activities following the conditioning stimulus; one during the mid inter-stimuli period (peak = 152 ± 3 ms) and the other just before the test stimulus (peak 334 ± 7 ms). Some cells (n = 38) exhibited the highest peak activity during the mid inter-stimuli period (A), some had the highest peak just before the test stimulus (n = 15; B) while other exhibited equal peak responses at both time points following the conditioning stimulus (n = 10; C). There were no significant differences in the T/C ratios ($P>0.05$). The dashed lines in the histograms indicate the mean firing rate (small dashes) and the 95% confidence limit (bigger- upper line).

7.3.2 Auditory-evoked single unit activity in the non-gating rats

Of the auditory-responsive units recorded in the CA3 of the non-gating rats 43% (n = 15) were early excitatory and 57% (n = 20) were late excitatory units. In the DG 35% (n = 8) were early excitatory while 65% (n = 15) were late excitatory units. In the mPFC 23% (n = 8) were early excitatory while 77% (n = 27) were late excitatory units.

7.3.2.1 The early excitatory cells in the non-gating rats

The T/C ratios of the early excitatory cells in the non-gating rats

The non-gating rats exhibited no significant changes in the unit activity following the test stimuli compared to the activity following the conditioning stimuli in the CA3, DG and mPFC ($P>0.05$; Fig.7.4). All three regions had a small percentage of early excitatory cells which demonstrated decreased activity following the test stimuli compared to the conditioning stimuli (CA3, 20%, n = 3; DG, 25%, n = 2; mPFC, 25%, n = 2) and were excluded from further analysis due to the small (n) number.

The early excitatory cells in the CA3 of the non-gating rats had a lower T/C ratio (117 ± 6%) compared to the T/C ratios in the DG (138 ± 15%) and mPFC (125 ± 35%). There were no significant differences between the single unit T/C ratios and the LFP T/C ratios (Fig. 7.4) recorded from the same recording electrodes in the CA3 (98 ± 10%; $P = 0.07$), DG (99 ± 38%; $P = 0.09$) or mPFC (118 ± 20%; $P = 0.7$).

The peak latencies of the early excitatory cells in the non-gating rats

The peak unit activity of the early excitatory cells in the CA3 started at 24 ± 2 ms following the conditioning stimuli and peaked at 29 ± 2 ms. The DG demonstrated peak activity with the onset at 20 ± 0.3 ms and peaking at 29 ± 0.3 ms. In the mPFC the onset of the significant activity following the conditioning stimuli was at 29 ± 0.4 ms and demonstrated the highest peak at 34 ± 1 ms. The peak latency of the early excitatory units following the conditioning stimuli in the CA3, DG and mPFC were significantly shorter than the N2 LFP conditioning response latencies in the non-gating rats (CA3, N2 CLAT = 55 ± 1 ms, $P<0.001$; DG, N2 CLAT = 55 ± 1 ms ,$P<0.001$; mPFC N2 CLAT = 53 ± 1 ms, $P<0.001$). However, the peak unit latencies were comparable to the P1 LFP latencies recorded from the three regions (CA3, P1 CLAT = 33 ± 5 ms, $P>0.05$, DG P1 CLAT = 32 ± 3 ms, $P>0.05$; mPFC, P1 CLAT = 30 ± 4 ms, $P>0.05$).

The peak early excitatory unit activity following the test stimuli, started at 20 ± 1 ms and peaked at 32 ± 0.4 ms in the CA3, started at 23 ± 3 ms and peaked at 31 ± 1 ms in the DG and started at 32 ± 0.3 ms and peaked at 33 ± 0.6 ms in the mPFC.

There was an increased firing rate during 200 - 500ms inter-stimuli period which extended following the test stimuli in the CA3 and DG (Fig. 7.4 A&B). No increased unit activity was detected in the mPFC in the 200ms-500ms inter-stimuli period (Fig. 7.4C).

7.3.2.2 The late excitatory cells in the non-gating rats

The T/C ratios of the late excitatory cells in the non-gating rats

The late excitatory cells (n = 62; CA3, n = 20; DG, n = 15; mPFC, n = 27) in the non-gating rats exhibited multiple peak activities following both the conditioning and the test stimuli. There was no significant change in the unit activity following the test stimuli compared to the activity following the conditioning stimuli in the CA3, DG or mPFC ($P>0.05$; Fig.7.5). All three regions had a small percentage of late excitatory cells with decreased unit activity following the test stimuli compared to the conditioning stimuli (CA3, 25%, n = 4; DG, 20%, n = 3; mPFC, 30%, n = 8) and were excluded from further analysis due to the small (n) n umber.

The late excitatory cells in the DG exhibited lower mean T/C ratio (92 ± 6%) compared to the T/C ratios in the CA3 (98 ± 6%) and mPFC (97 ± 5%). There were no significant differences between the single unit T/C ratios and the LFP T/C ratios ($P>0.05$) recorded from the same recording electrodes in the CA3 (94 ± 6%; $P = 0.3$), DG (90 ± 8%; $P = 0.8$) or mPFC (104 ± 14%; $P = 0.1$) (Fig. 7.5).

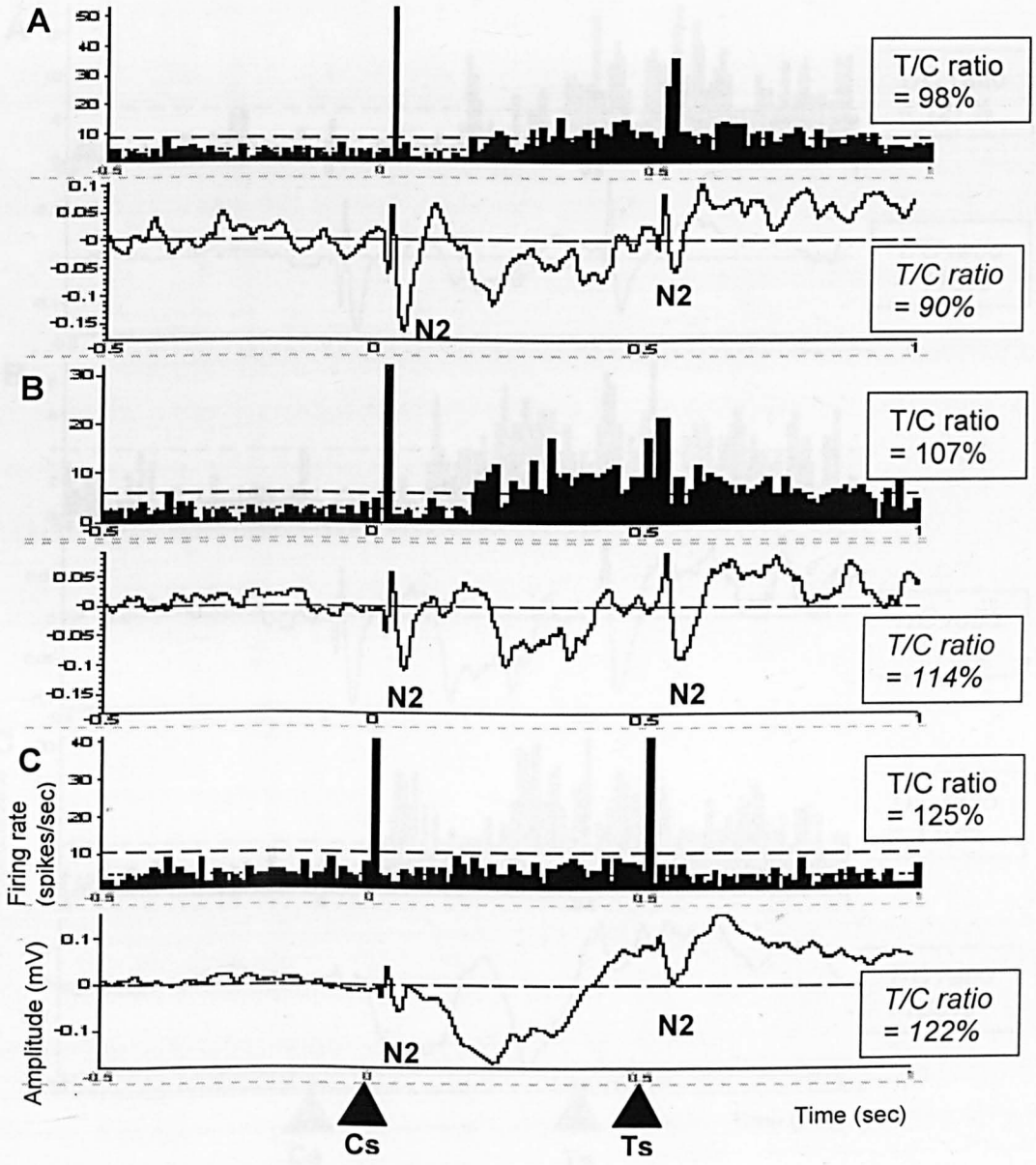
The peak latencies of the late excitatory cells in the non-gating rats

The late excitatory cells in the CA3, DG and mPFC of the non-gating rats demonstrated multiple peak activities following the onset of the conditioning stimulus and the test stimulus with the highest peak activities observed during

the 200 -500ms inter-stimuli period and/ or during 150 ms period following the onset of the test stimulus (Fig. 7.5). In the CA3 the peak activity started at 109 ± 5 ms and demonstrated the highest peak at 241 ± 22 ms following the conditioning stimulus and the second peak at 41 ± 9 ms following the test stimulus. In the DG a significant firing rate of the late excitatory cells started at 143 ± 26 ms and peaked at 337 ± 47 ms following the conditioning stimulus and the second peak at 44 ± 5 ms following the test stimulus. In the mPFC the peak activity had a late onset compared to the CA3 and DG with a significant increase in the firing rate starting at 182 ± 25 ms and peaking at 408 ± 15 ms and 75 ± 19 ms following conditioning and test stimuli respectively.

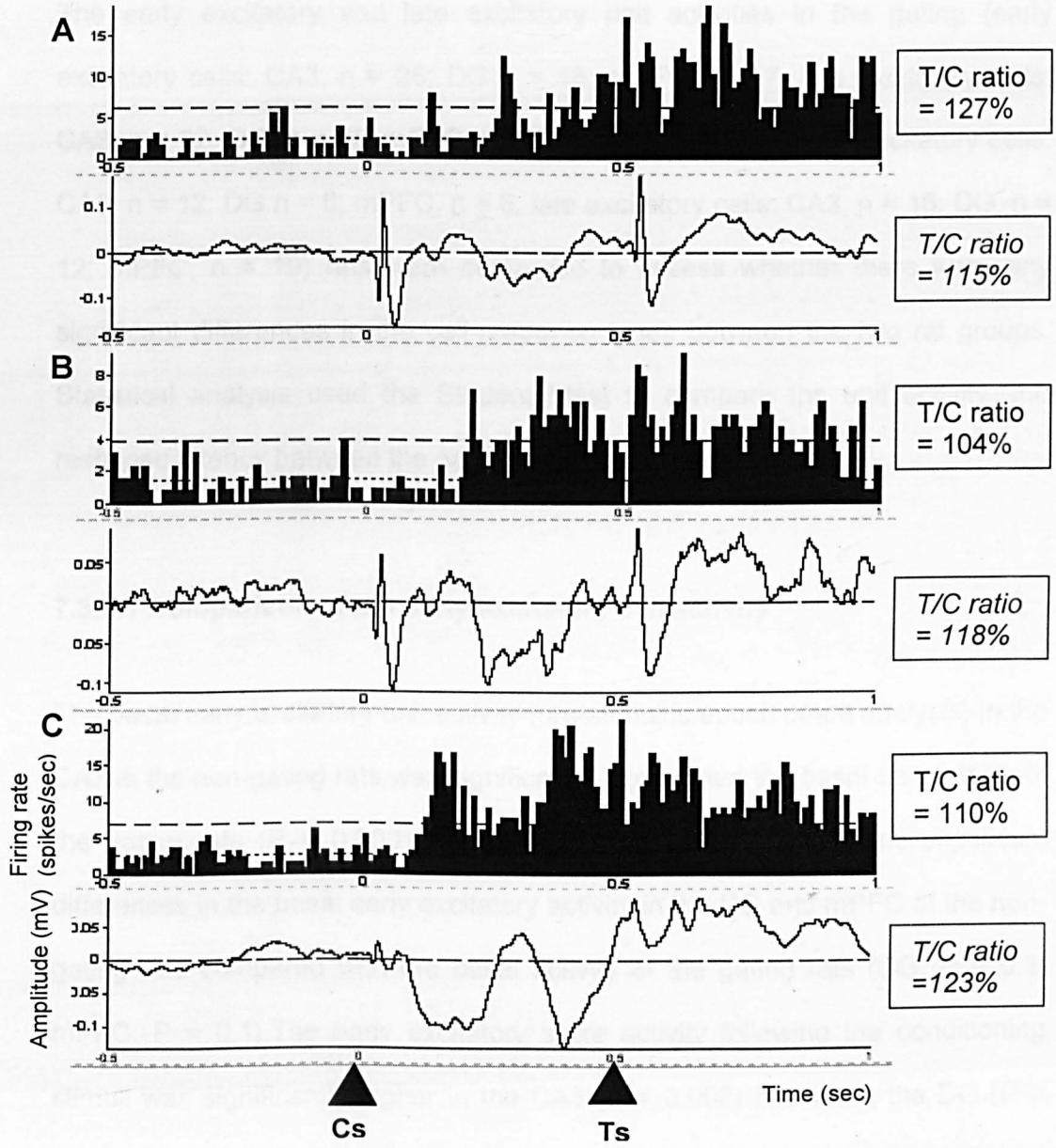
The peak unit activity did not appear to coincide with the LFP peaks in the CA3, DG or mPFC in the non-gating rats.

Fig. 7.4 Representative peri-stimulus histograms (upper panels; 10ms bin, ± 500ms peri-stimuli period) of the early excitatory cells and averaged LFPs (lower panels) obtained from the same recording electrodes used to record cells in **(A)** CA3, **(B)** DG and **(C)** mPFC in a non-gating rat.



The peri-stimulus histograms of the (A) CA3 (B) DG and (C) mPFC demonstrated prominent peak activities following both conditioning and test stimuli. The unit T/C ratios were comparable to the T/C ratios of the N2 wave in the averaged LFPs. The latencies of the peak activity following stimuli (CA3 peak = 28 ms; DG peak = 30 ms; mPFC peak = 35 ms) were shorter than the N2 LFP latencies (CA3 CLAT = 54 ms; DG CLAT = 56 ms; mPFC CLAT= 53 ms). Increased peak activities were observed in the CA3 and DG during the later part of the inter-stimuli period. No increased neuronal firing compared to basal was observed during the inter-stimuli period following the initial peak response to the conditioning and test stimuli in the mPFC. The dashed lines in the histograms indicate the mean firing rate (small dashes) and the 95% confidence limit (bigger-upper line)

Fig. 7.5 Representative peri-stimulus histograms (upper panels; 10ms bin, ± 500ms peri-stimuli period) of the late excitatory cells and averaged LFPs (lower panels) obtained from the same recording electrode used to record cells in **(A)** CA3, **(B)** DG and **(C)** mPFC in a non-gating rat.



The peri-stimulus histograms of the (A) CA3 (B) DG and (C) mPFC demonstrated multiple peak activities during the inter-stimuli period which continued with increased peak activity following the test stimulus. The T/C ratios were comparable to the T/C ratios of the N2 wave in the averaged LFPs. Very little peak unit activities were observed during the 200ms period following the conditioning stimuli but there were multiple peaks during 200ms period following the test stimuli in the CA3, DG and mPFC. Increased spike activity was also detected just before the onset of the test stimulus in the CA3, DG and mPFC of the non-gating rats. The dashed lines in the histograms indicate the mean firing rate (small dashes) and the 95% confidence limit (bigger- upper line)

7.3.3 Comparison of single-unit activity between gating and non gating rats

The early excitatory and late excitatory unit activities in the gating (early excitatory cells: CA3, n = 26; DG n = 16; mPFC, n = 7; late excitatory cells: CA3, n = 22; DG, n = 25; mPFC, n = 21) and non-gating (early excitatory cells: CA3, n = 12; DG n = 6; mPFC, n = 6; late excitatory cells: CA3, n = 16; DG, n = 12; mPFC, n = 19) rats were compared to assess whether there were any significant differences in the cell response types between the two rat groups. Statistical analysis used the Student t-test to compare the unit activity and response latency between the gating and the non-gating rats.

7.3.3.1 Comparison of the early excitatory cell activity

The basal early excitatory unit activity (pre-stimulus epoch count analysis) in the CA3 in the non-gating rats was significantly higher than the basal unit activity of the gating rats ($P = 0.0001$; Fig. 7.6A). However, there were no significant differences in the basal early excitatory activity in the DG and mPFC of the non-gating rats compared with the basal activity of the gating rats (DG, $P = 0.3$; mPFC, $P = 0.1$).The early excitatory spike activity following the conditioning stimuli was significantly higher in the CA3 ($P = 0.002$) but not in the DG ($P = 0.12$) or mPFC ($P = 0.9$) of the non-gating rats compared to the early excitatory unit activity following the conditioning stimulus in the gating rats. Non-gating rats demonstrated significantly higher early excitatory unit activities following the test stimuli in all three areas (CA3, $P<0.0001$; DG, $P = 0.007$; mPFC, $P = 0.02$)

compared to the test stimuli-evoked activities in the gating rats. Moreover, the T/C ratios of the early excitatory unit activities were significantly higher in the CA3 (gating T/C = 46 ± 4%, non-gating T/C = 117 ± 6%; $P<0.0001$), DG (gating T/C = 59 ± 5%, non-gating T/C = 180 ± 14%; $P<0.0001$) and mPFC (gating T/C = 62 ± 25%, non-gating T/C = 79 ± 26%; $P = 0.01$) of the non-gating rats compared to the T/C ratios of the gating rats (Fig. 7. 6A).

Comparison of the response latencies revealed that the peak of the spike activity following the conditioning stimulus in the non-gating rats occurred significantly earlier in the CA3 ($P = 0.001$) and DG ($P = 0.01$) compared to the gating rats. However, the conditioning peak latency in the mPFC of the non-gating rats was significantly delayed than that of the gating rats ($P = 0.001$). The onset of the peak activity following the conditioning stimulus was not significantly different in the CA3 ($P =0.2$), but was significantly earlier in the DG ($P = 0.01$) and significantly delayed in the mPFC ($P = 0.03$) in the non-gating rats compared to the gating rats.

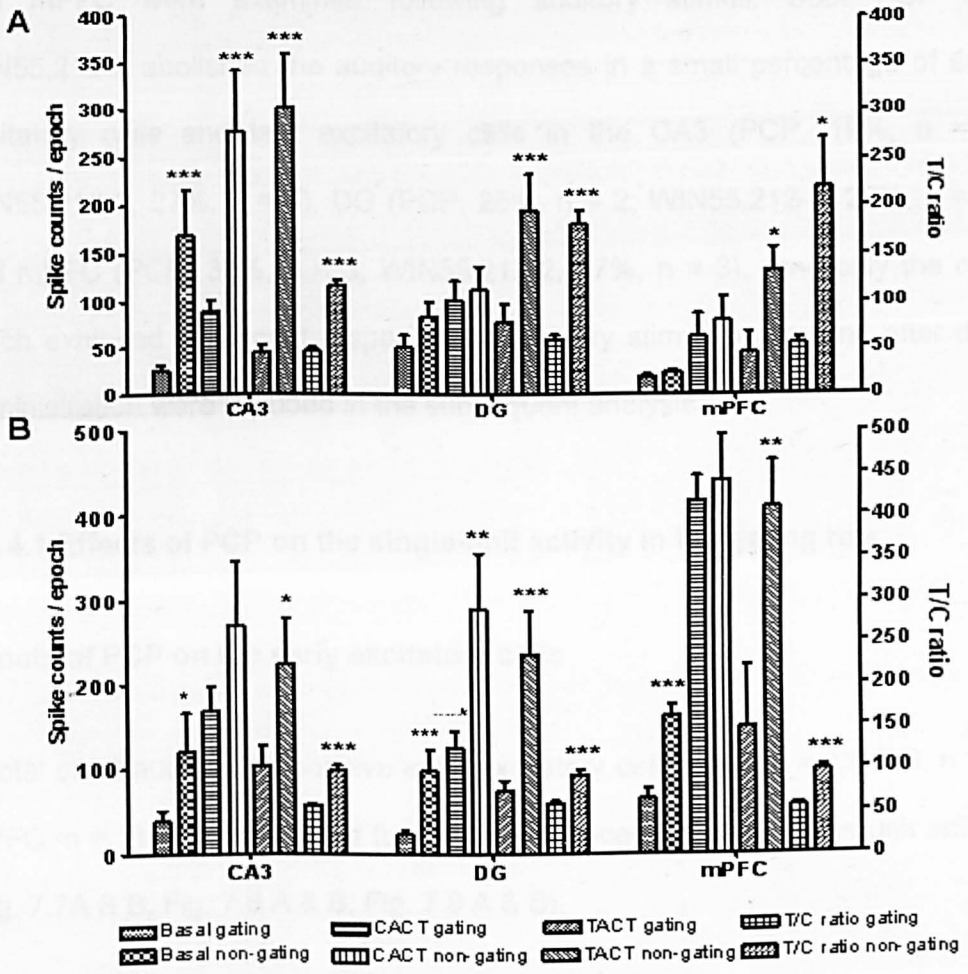
The onset of the peak activity following the test stimuli was earlier in the CA3 ($P = 0.002$), delayed in the mPFC ($P = 0.002$) and not significantly different in the DG (0.2) in the non-gating rats compared to the gating rats. The latency of the peak activity following the test stimulus was not significantly different in the CA3 ($P = 0.4$) or DG ($P = 0.07$) but was significantly delayed in the mPFC ($P = 0.02$) in the non-gating rats compared to the gating rats.

7.3.3.2 Comparison of the late excitatory cell activity

The basal late excitatory unit activity (prior to auditory stimulation) in the non-gating rats was significantly higher than the basal unit activity of the gating rats in the CA3 ($P = 0.04$), DG ($P = 0.0005$) and mPFC ($P<0.0001$; Fig. 7. 6B). The LE spike activity following the conditioning stimuli was significantly higher in the DG ($P = 0.003$) but not in the CA3 ($P = 0.2$) and mPFC ($P = 0.8$) of the non-gating rats compared to the late excitatory unit activity following the conditioning stimulus in the gating rats. Non-gating rats demonstrated significantly higher late excitatory unit activities following the test stimuli in all three areas (CA3, $P= 0.02$; DG, $P = 0.002$; mPFC, $P = 0.007$) compared to the test stimuli-evoked activities in the gating rats resulting in significantly higher T/C ratios in the CA3 (gating T/C = 57 ± 2%, non-gating T/C = 98 ± 6%; $P<0.0001$), DG (gating T/C = 61 ± 3%, non-gating T/C = 92 ± 6%; $P<0.0001$) and mPFC (gating T/C = 56 ± 3%, non-gating T/C = 97 ± 5%; $P < 0.0001$) of the non-gating rats compared to the T/C ratios of the gating rats (Fig. 7. 6B).

Comparison of the response latencies revealed that the peak latency of the spike activity following the conditioning stimulus in the non-gating rats was significantly delayed in the CA3 ($P = 0.001$), DG ($P = 0.004$) and mPFC ($P = 0.02$) compared to the gating rats. The peak activity following the **test** stimulus was not significantly different in the CA3 ($P =0.6$) and DG ($P = 0.3$) but was significantly delayed in the mPFC ($P<0.001$) in the non-gating rats compared to the gating rats.

Fig. 7.6 Pooled epoch count data depicting the changes in the **(A)** early excitatory unit and **(B)** late excitatory unit activities (spike counts / epoch) in the non-gating rats (early excitatory cells: CA3, n = 12; DG n = 6; mPFC, n = 6; late excitatory cells: CA3, n = 16; DG, n = 12; mPFC, n = 19) compared to the gating rats (early excitatory cells: CA3, n = 26; DG n = 16; mPFC, n = 7; late excitatory cells: CA3, n = 22; DG, n = 25; mPFC, n = 21). Values = mean ± SEM. CACT = unit activity following the conditioning stimulus; TACT = unit activity following the test stimulus.



A. The basal activity and CACT of the early excitatory cells were significantly higher in the CA3, but not in the DG or mPFC in the non-gating rats compared to the gating rats. The TACT and the T/C ratio were significantly higher in the CA3, DG and mPFC of the non-gating rats compared to the gating rats (* = P<0.01; ** = P<0.001, *** = P<0.0001).

B. The basal activity of the late excitatory cells were significantly higher in the CA3, DG and mPFC, in the non-gating rats compared to the gating rats. The CACT in the DG, (but not in the CA3 or mPFC) was significantly higher in the non-gating rats compared to the gating rats. The TACT and the T/C ratio were significantly higher in the CA3, DG and mPFC of the non-gating rats compared to the gating rats (* = P<0.01; ** = P<0.001, *** = P<0.0001).

7.3.4 Effects of pharmacological manipulations on the auditory-evoked single unit activity in the gating rats

Effects of PCP (1mg/kg, i.p) and WIN55,212-2 (1.2mg/kg, i.p) on the early excitatory and late excitatory unit activity (spike count / epoch) in the CA3, DG and mPFC were examined following auditory stimuli. Both PCP and WIN55,212-2 abolished the auditory responses in a small percentage of early excitatory cells and late excitatory cells in the CA3 (PCP, 19%, n = 3; WIN55,212-2, 27%, n = 4), DG (PCP, 25%, n = 2; WIN55,212-2, 22%, n = 2), and mPFC (PCP, 30%, n = 3; WIN55,212-2, 17%, n = 3), and only the cells which exhibited significant responses to auditory stimuli before and after drug administration were included in the subsequent analysis.

7.3.4.1 Effects of PCP on the single-unit activity in the gating rats

Effects of PCP on the early excitatory cells

A total of 26 auditory responsive early excitatory cells (CA3, n = 13; DG, n = 6; mPFC, n = 7) were examined for the PCP induced changes in the unit activity (Fig. 7.7A & B, Fig. 7.8 A & B, Fig. 7.9 A & B).

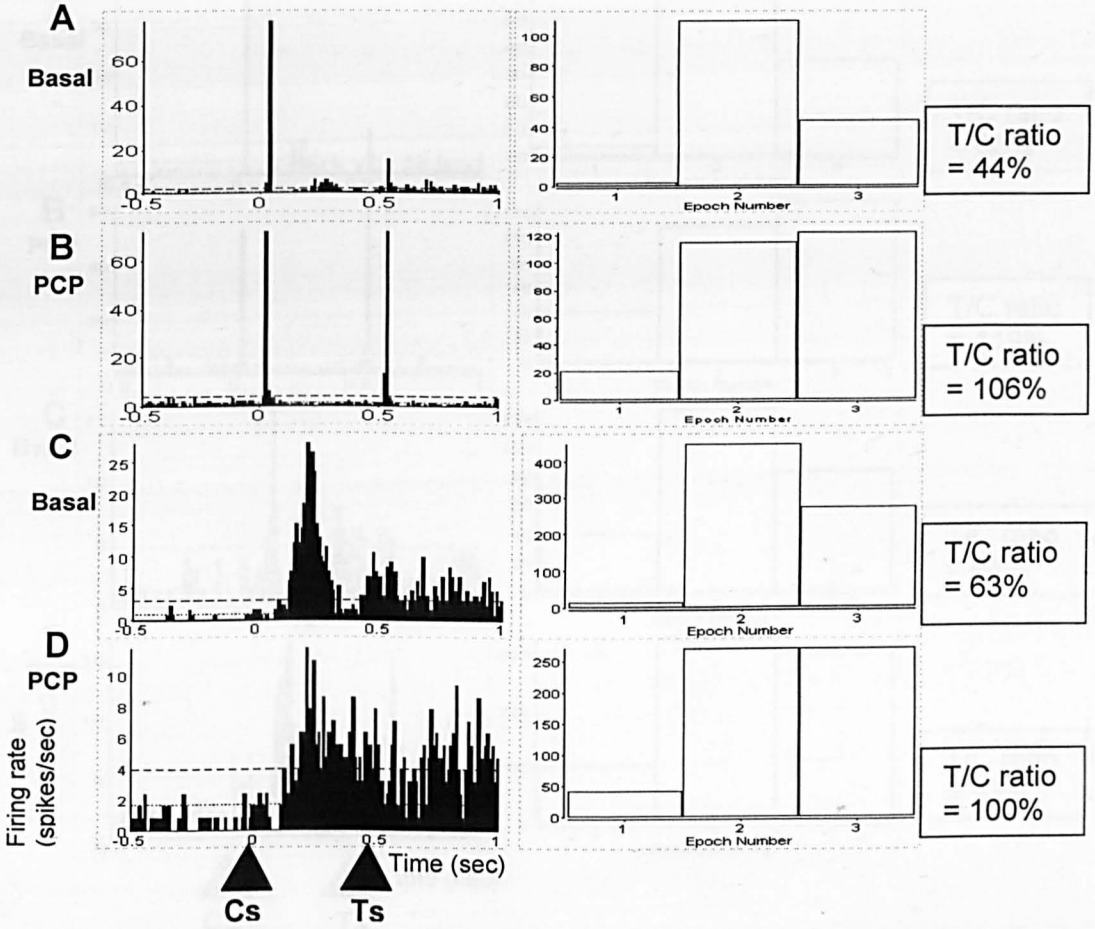
The early excitatory units exhibited no significant changes in activity following conditioning stimulus (CA3, P = 0.06; DG, P = 0.6; mPFC, P = 0.7) with PCP. However, PCP increased spike activity following the test stimulus in the CA3 ($P<0.0001$; Fig. 7.7 A & B), DG (P = 0.003: 7.8 A & B) and mPFC (P = 0.004; 7.9 A & B) resulting in significantly increased T/C ratios in the CA3 (Basal T/C =

46 ± 4%; PCP T/C = 105 ± 7%; P<0.0001), DG (Basal T/C = 44 ± 2%; PCP T/C = 102 ± 2%; P<0.0001) and mPFC (Basal T/C = 63 ± 7%; PCP T/C = 144 ± 22%; P = 0.004) 45 min following drug administration (Table 7.1).

Effects of PCP on the late excitatory cells

Thirty six auditory responsive late excitatory cells (CA3, n = 11; DG, n = 10; mPFC, n = 15) were studied for the changes in auditory-evoked responses following PCP (Fig. 7.7 C & D, Fig. 7.8 C & D, Fig. 7.9 C & D and Table 7.1). PCP had no significant effects on the unit activity following the conditioning stimulus in the CA3 (P = 0.2) or DG (P = 0.08), but increased the activity following the test stimulus (CA3, P = 0.009; DG, P = 0.04) resulting in significant increases in the T/C ratios in the CA3 (basal T/C = 60 ± 3%; PCP T/C = 115 ± 8%; P<0.0001: Fig. 7.7 C & D) and DG (basal T/C = 62 ± 5%; PCP T/C = 97 ± 7%; P = 0.0007; Fig. 7.8 C & D). In the mPFC, late excitatory unit activity following the test stimulus was not altered significantly (P = 0.6) following PCP. However, PCP decreased the unit activity following the conditioning stimulus (P = 0.03) resulting in a significant increase in the late excitatory unit T/C ratio (Basal T/C = 59 ± 3%; PCP T/C = 97 ± 6%; P<0.0001) in the mPFC (Fig. 7.9 C & D and Table 7.1).

Fig. 7.7 Effects of PCP on the single-unit activity in the CA3 of a gating rat; representative peri-stimulus histograms (left panels; 10ms bin, ± 500ms peri-stimuli period) and the epoch counts (right panels; 10ms bin, peri-stimuli period ± 100ms for early excitatory and ± 500ms for late excitatory cells; y axis = spike counts per epoch) of the (A & B) early excitatory and (C & D) late excitatory cells, basal activity compared to the activity 45 min following PCP.



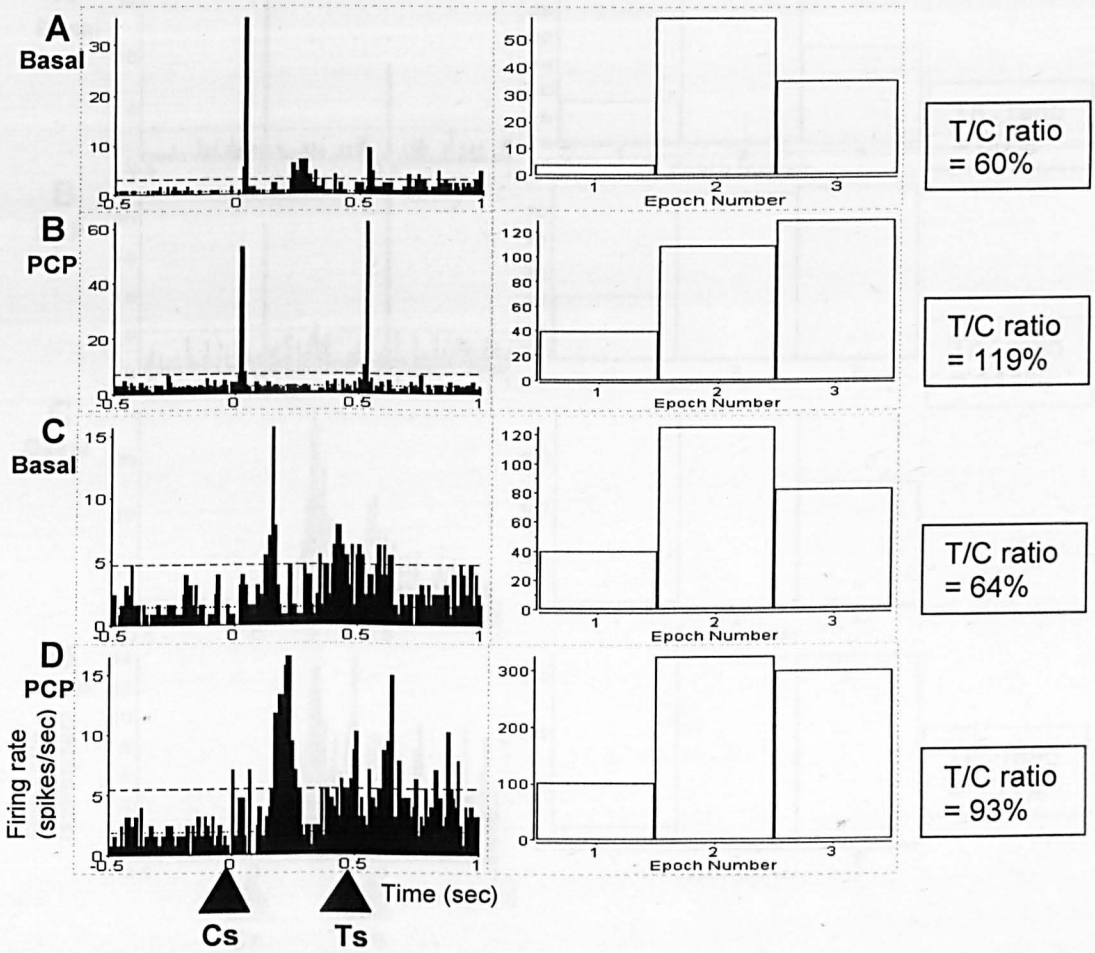
A. Increased unit activity (spike counts/epoch) was observed in the early excitatory cells during the 100ms period following the conditioning stimulus (Epoch Number 2) compared to the 100ms pre-stimulus period (Epoch Number 1) with a reduction in peak response following the test stimulus (Epoch Number 3).

B. The early excitatory peak activity following the test stimulus increased resulting in an increase in the T/C ratio following PCP (1mg/kg, i.p) administration.

C. Late excitatory cells exhibited peak activity in the mid inter-stimuli period in the peri-stimulus histogram with increased activity in the 500ms period following the conditioning stimulus (Epoch 2) compared to the 500ms pre-stimulus period (Epoch 1). There was a reduction in the peak activity following the test stimulus (Epoch 3).

D. The peak activity following the test stimulus increased resulting in an increase in the T/C ratio following PCP (1mg/kg, i.p) administration. There was also an increase in activity in the late inter-stimuli period (300 – 500 ms) in the histograms of the late excitatory cells following PCP.

Fig. 7.8 Effects of PCP on the single-unit activity in the DG of a gating rat; representative peri-stimulus histograms (left panels; 10ms bin, ± 500ms peri-stimuli period) and the epoch counts (right panels; 10ms bin, peri-stimuli period ± 100ms for early excitatory and ± 500ms for late excitatory cells; y axis =spike counts per epoch) of the (A & B) early excitatory and (C & D) late excitatory cells, basal activity compared to the activity 45 min following PCP.



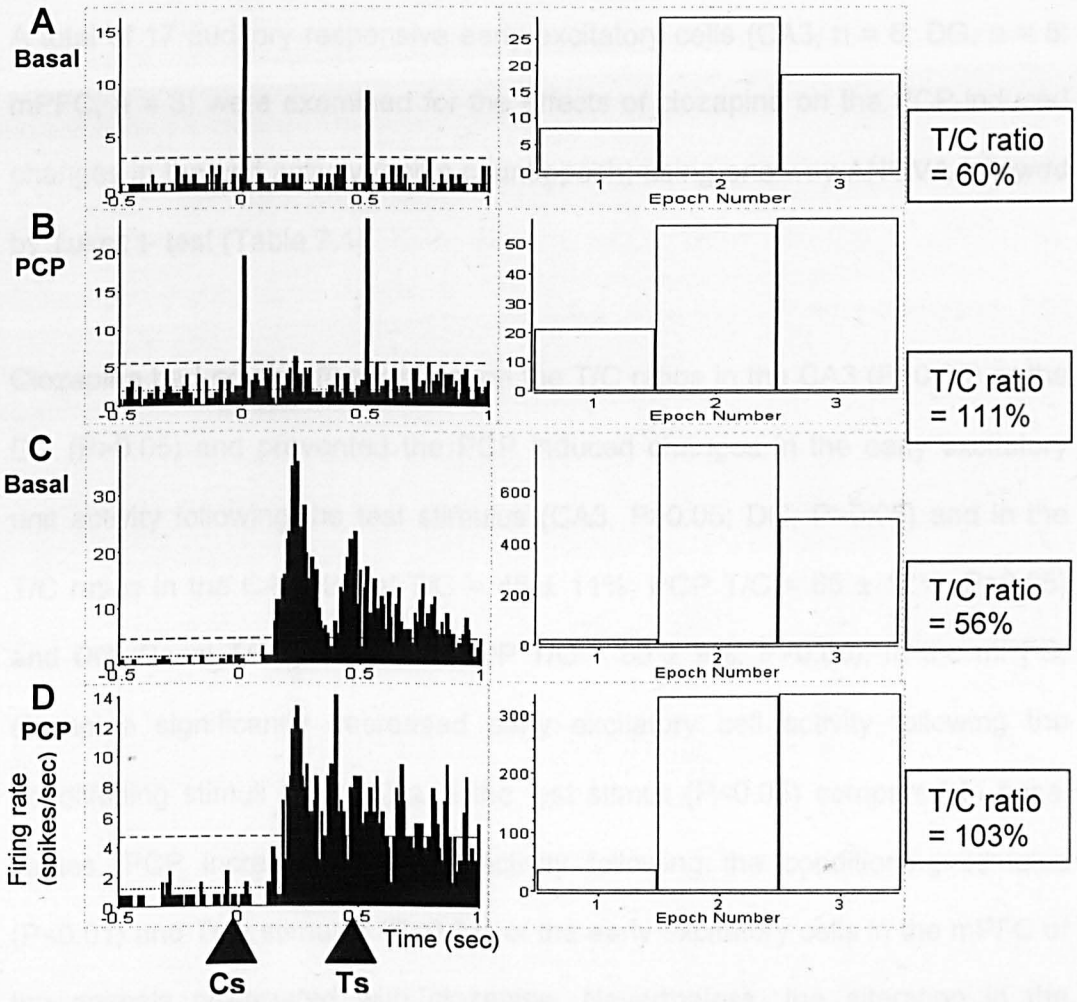
A. Increased unit activity was observed in the early excitatory cells during the 100ms period following the conditioning stimulus (Epoch Number 2) compared to the 100ms pre-stimulus period (Epoch Number 1) with a reduction in peak response following the test stimulus (Epoch Number 3).

B. The peak activity following the test stimulus increased resulting in an increase in the T/C ratio following PCP (1mg/kg, i.p) administration.

C. Late excitatory cells exhibited peak activity in the mid inter-stimuli period in the peri-stimulus histogram and increased activity in the 500ms period following the conditioning stimulus (Epoch 2) compared to the 500ms pre-stimuli period (Epoch 1). There was a reduction in the spike counts following the test stimulus (Epoch 3).

D. The spike count following the test stimulus increased resulting in an increase in the T/C ratio following PCP (1mg/kg, i.p) administration.

Fig. 7.9 Effects of PCP on the single-unit activity in the mPFC of a gating rat; representative peri-stimulus histograms (left panels; 10ms bin, ± 500ms peri-stimuli period) and the epoch counts (right panels; 10ms bin, peri-stimuli period ± 100ms for early excitatory and ± 500ms for late excitatory cells; y axis = spike counts per bin) of the (A & B) early excitatory and (C & D) late excitatory cells, basal activity compared to the activity 45 min following PCP.



A. Increased unit activity was observed in the early excitatory cells during the 100ms period following the conditioning stimulus (Epoch Number 2) compared to the 100ms pre-stimulus period (Epoch Number 1) with a reduction in peak response following the test stimulus (Epoch Number 3).

B. The peak activity following the test stimulus increased resulting in an increase in the T/C ratio following PCP (1mg/kg, i.p) administration.

C. Late excitatory cells exhibited peak activity in the mid inter-stimuli period in the peri-stimulus histogram and increased activity in the 500ms period following the conditioning stimulus (Epoch 2) compared to the 500ms pre-stimuli period (Epoch 1). There was a reduction in the peak activity following the test stimulus (Epoch 3).

D. The peak activity following the conditioning stimulus decreased resulting in an increase in the T/C ratio following PCP (1mg/kg, i.p) administration.

7.3.4.2 Effects of clozapine on the PCP induced changes in the single-unit activity of the gating rats

Effects of clozapine on the early excitatory cells

A total of 17 auditory responsive early excitatory cells (CA3, n = 6; DG, n = 8; mPFC, n = 3) were examined for the effects of clozapine on the PCP-induced changes in the unit activity (spike count/epoch) using one way ANOVA followed by Tukey t- test (Table 7.1).

Clozapine had no significant effect on the T/C ratios in the CA3 ($P>0.05$) or the DG ($P>0.05$) and prevented the PCP induced changes in the early excitatory unit activity following the test stimulus (CA3, $P>0.05$; DG, $P>0.05$) and in the T/C ratios in the CA3 (Basal T/C = 45 ± 11%; PCP T/C = 65 ± 12%; $P>0.05$) and DG (Basal T/C = 58 ± 3%; PCP T/C = 60 ± 9%; $P>0.05$). In the mPFC, clozapine significantly decreased early excitatory cell activity following the conditioning stimuli ($P<0.05$) and the test stimuli ($P<0.05$) compared to basal values. PCP increased the unit activity following the conditioning stimulus ($P<0.01$) and Test stimulus ($P<0.01$) of the early excitatory cells in the mPFC of the animals pre-treated with clozapine. Nevertheless, the alteration in the activities in response to both conditioning and test stimuli following clozapine resulted in no significant change in the early excitatory unit T/C ratio (Basal T/C = 58 ± 6%; CLOZ T/C = 77 ± 17%; $P>0.05$) and prevented the PCP induced changes in the T/C ratios (Basal T/C = 58 ± 6%; PCP T/C = 71 ± 15%; $P>0.05$) compared to basal values.

Effects of clozapine on the late excitatory cells

A total of 56 auditory-responsive late excitatory cells (CA3, n = 19; DG, n = 17; mPFC, n = 20) were examined for the effects of clozapine on the PCP induced changes in the unit activities using one-way ANOVA followed by Tukey t-test (Table 7.1). The late excitatory units in the CA3 and DG exhibited a decrease in the activities following the conditioning stimulus (CA3, $P<0.001$; DG, $P>0.05$) and Test stimulus (CA3, $P<0.01$; DG, $P>0.05$) resulting in a significant increase in the T/C ratio (CA3, Basal T/C = 58± 3%; CLOZ T/C = 73 ± 2%; $P<0.001$; DG, Basal T/C = 64 ± 3%; CLOZ T/C = 82 ± 6%; $P<0.01$) following clozapine. The late excitatory cells in the mPFC exhibited a significant reduction in the unit activities following the conditioning stimulus ($P<0.001$) and test stimulus ($P<0.001$) following clozapine administration, but no significant changes in the T/C ratios (Basal T/C = 59 ± 2%; CLOZ T/C = 62 ± 4%; $P>0.05$). However, PCP had no significant effects on the activities following the conditioning stimulus ($P>0.05$), the test stimulus ($P>0.05$) or the unit T/C ratio in the CA3 (Basal T/C = 58 ± 3%; PCP T/C = 64 ± 3%; $P>0.05$), DG (Basal T/C = 64 ± 3%; PCP T/C = 69 ± 3%; $P>0.05$) or mPFC (Basal T/C = 59 ± 2%; PCP T/C = 62 ± 2%; $P>0.05$) in the rats pre-treated with clozapine (Table 7.1) suggesting that clozapine prevented the PCP induced changes in the auditory-evoked late excitatory unit responses.

Table 7.1 Summary table of the unit activities (spikes count/epoch; epoch = 100ms for early excitatory cells and 500ms for late excitatory cells) following conditioning stimulus (CACT) and test stimulus (TACT) with the T/C ratios, 45 min after PCP administration (1mg/kg; i.p; PCP 45) in the gating rats not treated with clozapine and the gating rats pre-treated with clozapine (5mg/kg, i.p; CLOZ) compared to the basal values of the respective groups (Basal-1 and Basal-2; * = P<0.05, ** = P<0.01, *** = P<0.001).

Region	Basal-1	PCP 45	Basal-2	CLOZ	PCP 45
CA3					
Early excitatory cells (n = 13)			**(n = 6)**		
CACT (spikes/epoch)	98 ± 15	122 ± 23	144 ± 27	142 ± 82	169 ± 65
TACT (spikes/epoch)	46 ± 10	112 ± 23***	78 ± 30	113 ± 68	125 ± 61
T/C ratio (%)	46 ± 4	105 ± 7***	45 ± 10	43 ± 15	65 ± 11
Late excitatory cells (n = 11)			**(n = 19)**		
CACT	183 ± 25	231 ± 29	263 ± 55	133 ± 32***	197 ± 25
TACT	92 ± 9	201 ± 37**	166 ± 39	98 ± 24***	133 ± 20
T/C ratio	60 ± 3	115 ± 8***	58 ± 3	73 ± 2**	68 ± 2
DG					
Early excitatory cells (n = 6)			**(n = 8)**		
CACT	148 ± 39	170 ± 26	159 ± 36	119 ± 42	78 ± 23
TACT	62 ± 15	171 ± 26**	97 ± 24	81 ± 30	45 ± 13
T/C ratio	44 ± 2	102 ± 2***	58 ± 3	60 ± 9	62 ± 3
Late excitatory cells (n = 10)			**(n = 17)**		
CACT	182 ± 32	379 ± 103	147 ± 35	79 ± 27	63 ± 3
TACT	122 ± 24	410 ± 131*	98 ± 28	55 ± 14	66 ± 5
T/C ratio	62 ± 5	92 ± 5***	64 ± 3	82 ± 6*	69 ± 3
mPFC					
Early excitatory cells (n = 7)			**(n = 3)**		
CACT	98 ± 18	109 ± 23	159 ± 27	64 ± 1**	216 ± 3*
TACT	64 ± 16	150 ± 28*	90 ± 10	48 ± 12*	158 ± 5**
T/C ratio	63 ± 7	144 ± 22**	58 ± 6	77 ± 17	71 ± 3
Late excitatory cells (n = 15)			**(n = 20)**		
CACT	497 ± 84	277 ± 47*	476 ± 57	173 ± 22***	506 ± 65
TACT	305 ± 59	263 ± 56	341 ± 48	123 ± 15***	372 ± 53
T/C ratio	59 ± 3	97 ± 6***	59 ± 2	62 ± 4	62 ± 2

7.3.4.3 Effects of WIN55,212-2 on the single-unit activity in the gating rats

Effects of WIN55,212-2 on the early excitatory cells

A total of 30 auditory responsive early excitatory cells (CA3, n = 14; DG, n = 7; mPFC, n = 9) were examined for the WIN55,212-2 induced changes in the unit activities (Fig. 7.10 A & B, Fig. 7.11 A & B, Fig 7.12 A & B).

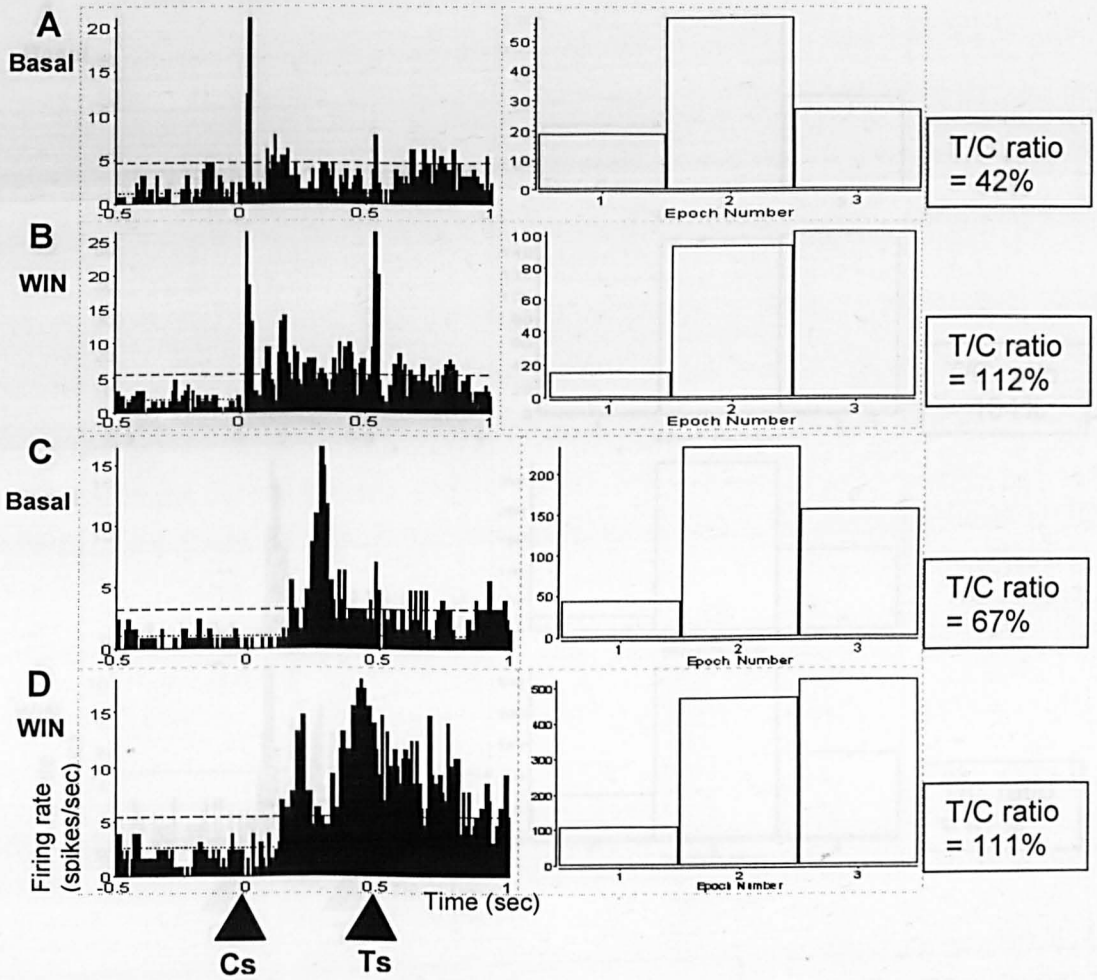
The early excitatory units demonstrated no significant changes in the activity following the conditioning stimulus (CA3, P = 0.2; DG, P = 0.3; mPFC, P = 0.8) 45min following WIN55,212-2 administration in the CA3, DG and mPFC (Table 7.2). However, WIN55,212-2 increased unit activity following the test stimulus in the CA3 (P= 0.04), DG (P = 0.03) and mPFC (P = 0.01) resulting in significantly increased T/C ratios in the CA3 (Basal T/C = 41 ± 5%; WIN T/C = 75 ± 11%; P = 0.009; Fig. 7.10 A & B), DG Basal T/C = 41 ± 10%; WIN T/C = 106 ± 9%; P < 0.0001; Fig. 7.11 A & B) and mPFC (Basal T/C = 54 ± 2%; WIN T/C = 141± 17%; P < 0.0001; Fig 7.12 A & B) 45 min following drug administration (Table 7.2).

Effects of WIN55,212-2 on the late excitatory cells

Thirty two auditory responsive late excitatory cells (CA3, n = 6; DG, n = 9; mPFC, n = 17) were studied for the changes in basal and auditory-evoked responses following WIN55,212-2 (Fig. 7.10 C & D, Fig. 7.11 C & D, Fig 7.12 C & D).

WIN55,212-2 administration had no significant effect on the activity following the conditioning stimulus ($P>0.05$) or the test stimulus ($P>0.05$) in the CA3, DG and mPFC. However, there were significant increases in the T/C ratios of the late excitatory cells in the CA3 (Basal T/C = 51 ± 5%; WIN T/C = 73 ± 4%; $P = 0.02$; Fig. 7.10 C & D) and mPFC (Basal T/C = 52 ± 13%; WIN T/C = 71 ± 4%; $P = 0.009$; Fig 7.12 C & D) but not in DG (Basal T/C = 53 ± 2%; WIN T/C = 57 ± 3%; $P = 0.2$; Fig. 7.11 C & D) following WIN55,212-2 (Table 7.2).

Fig. 7.10 Effects of WIN55,212-2 on the single unit activity in the CA3 of a gating rat; representative peri-stimulus histograms (left panels; 10ms bin, ± 500ms peri-stimuli period) and the epoch counts (right panels; 10ms bin, peri-stimuli period ± 100ms for early excitatory and ± 500ms for late excitatory cells; y axis = spike counts per bin) of the (A & B) early excitatory and (C & D) late excitatory cells, basal activity compared to the activity 45min following WIN55,212-2 (WIN).



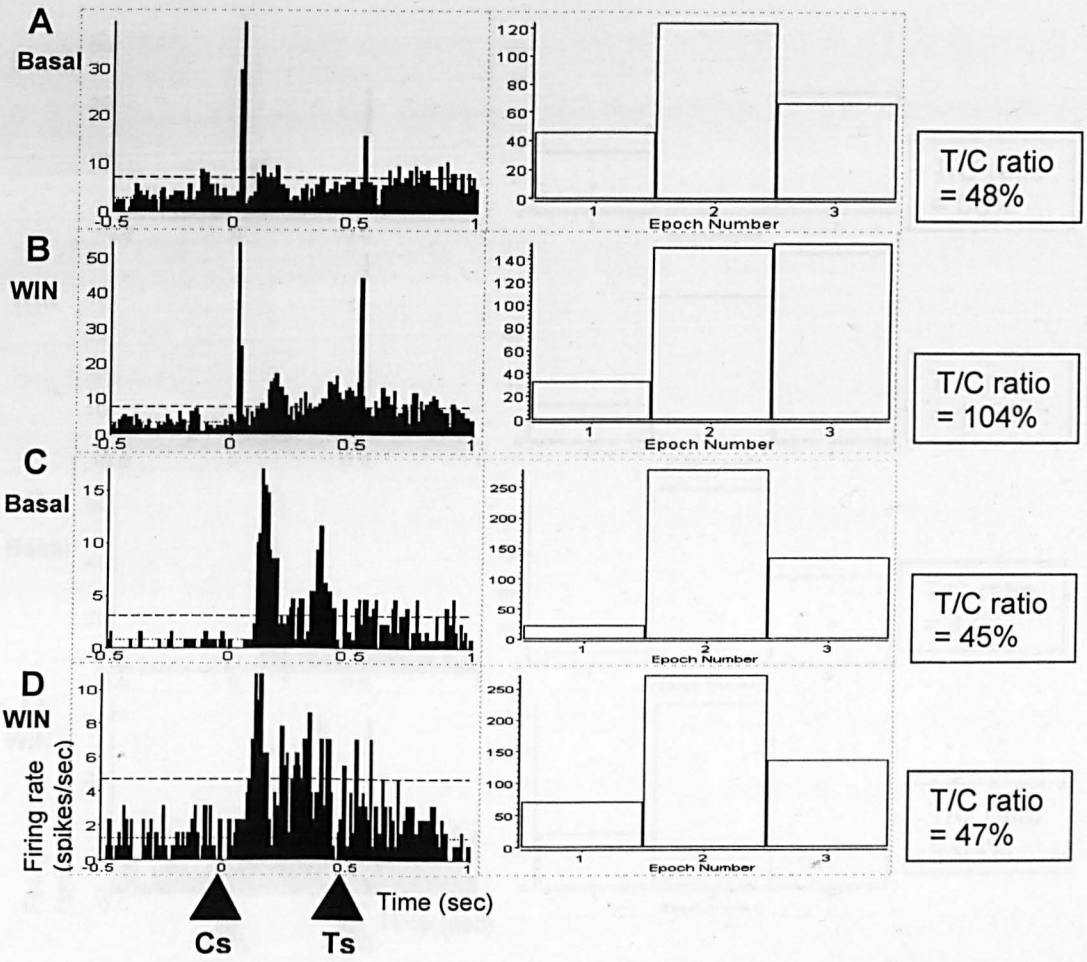
A. Increased unit activity was observed in the early excitatory cells during the 100ms period following the conditioning stimulus (Epoch Number 2) compared to the 100ms pre-stimulus period (Epoch Number 1) with a reduction in peak response following the test stimulus (Epoch Number 3).

B. The peak activity following the test stimulus increased resulting in an increase in the T/C ratio following WIN55,212-2 (1.2mg/kg, i.p) administration.

C. Late excitatory cells exhibited peak activity in the mid inter-stimuli period in the peri-stimulus histogram and increased activity in the 500ms period following the conditioning stimulus (Epoch 2) compared to the 500ms pre-stimuli period (Epoch 1). There was a reduction in the peak activity following the test stimulus (Epoch 3).

D. The peak activity following the test stimulus increased resulting in an increase in the T/C ratio following WIN55,212-2 (1.2mg/kg, i.p) administration.

Fig. 7.11 Effects of WIN55,212-2 on the single unit activity in the DG of a gating rat; representative peri-stimulus histograms (left panels; 10ms bin, ± 500ms peri-stimuli period) and the epoch counts (right panels; 10ms bin, peri-stimuli period ± 100ms for early excitatory and ± 500ms for late excitatory cells; y axis = spike counts per bin) of the (A & B) early excitatory and (C & D) late excitatory cells, basal activity compared to the activity 45min following WIN55,212-2 (WIN).



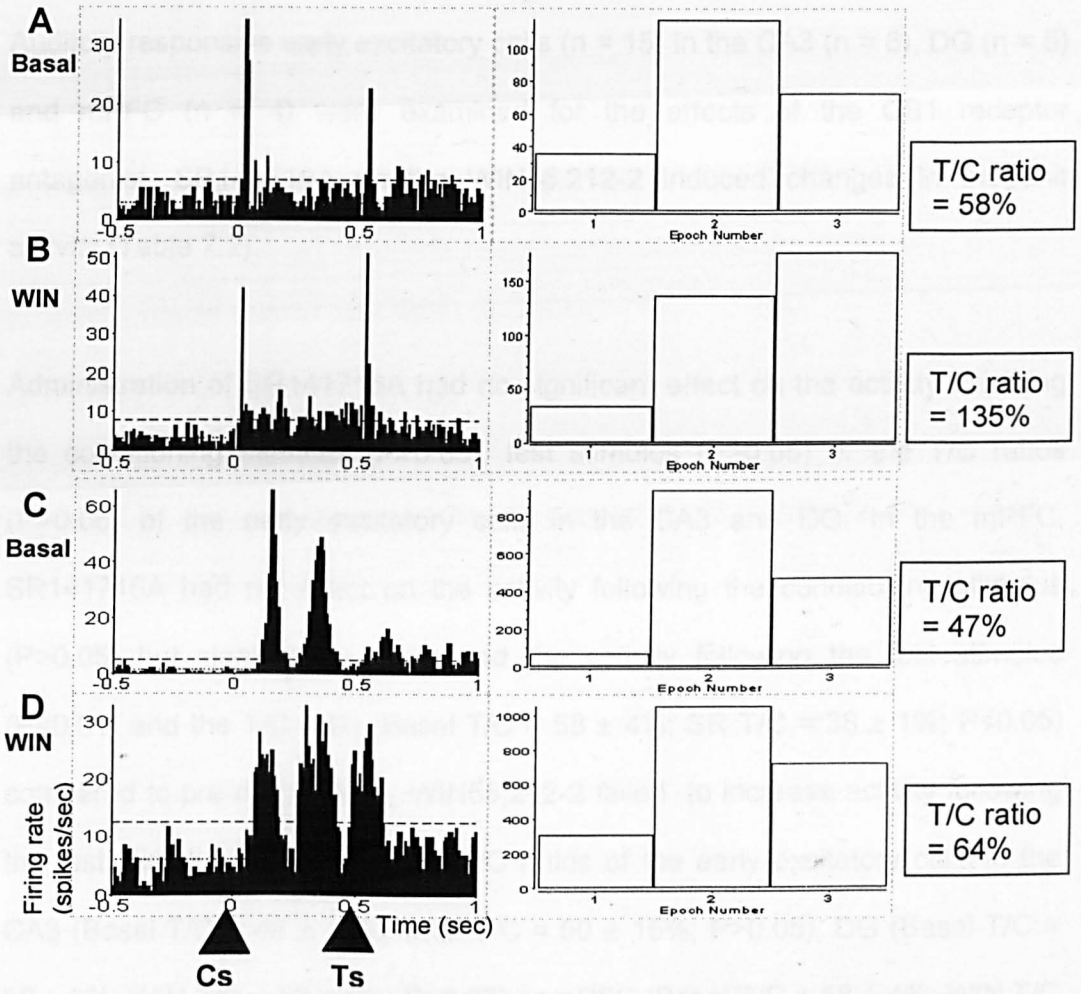
A. Increased unit activity was observed in the early excitatory cells during the 100ms period following the conditioning stimulus (Epoch Number 2) compared to the 100ms pre-stimulus period (Epoch Number 1) with a reduction in spike activity following the test stimulus (Epoch Number 3).

B. The spike activity following the test stimulus increased resulting in an increase in the T/C ratio following WIN55,212-2 (1.2mg/kg, i.p) administration.

C. Late excitatory cells exhibited increased firing in the mid inter-stimuli period in the peri-stimulus histogram and increased activity in the 500ms period following the conditioning stimulus (Epoch 2) compared to the 500ms pre-stimuli period (Epoch 1). There was a reduction in the spike activity following the test stimulus (Epoch 3).

D. There was no change in the peak activity following the conditioning stimulus or the test stimulus resulting in no change in the T/C ratio following WIN55,212-2 (1.2mg/kg, i.p) administration.

Fig. 7.12 Effects of WIN55,212-2 on the single unit activity in the mPFC of a gating rat; representative peri-stimulus histograms (left panels; 10ms bin, ± 500ms peri-stimuli period) and the epoch counts (right panels; 10ms bin, peri-stimuli period ± 100ms for early excitatory cells and ± 500ms for late excitatory cells; y axis = counts per bin) of the (A & B) early excitatory and (C & D) late excitatory cells, basal activity compared to the activity 45min following WIN55,212-2 (WIN).



A. Increased unit activity was observed in the early excitatory cells during the 100ms period following the conditioning stimulus (Epoch Number 2) compared to the 100ms before the onset of the conditioning stimulus (Epoch Number 1) with a reduction in peak response following the test stimulus (Epoch Number 3).

B. The peak activity following the test stimulus increased resulting in an increase in the T/C ratio following WIN55,212-2 (1.2mg/kg, i.p) administration.

C. Late excitatory cells exhibited peak activity in the mid inter-stimuli period in the peri-stimulus histogram and increased activity in the 500ms period following the conditioning stimulus (Epoch 2) compared to the 500ms pre-stimuli period (Epoch 1). There was a reduction in the peak activity following the test stimulus (Epoch 3).

D. The spike activity of the late excitatory cells increased following the test stimulus resulting in an increase in the T/C ratio following WIN55,212-2 (1.2mg/kg, i.p) administration.

7.3.4.4 Effects of SR141716A on the WIN55,212-2 induced changes in the single-unit activity of the gating rats

Effects of SR141716A on the early excitatory cells

Auditory responsive early excitatory cells (n = 15) in the CA3 (n = 6), DG (n = 5) and mPFC (n = 4) were examined for the effects of the CB1 receptor antagonist, SR141716A on the WIN55,212-2 induced changes in the unit activity (Table 7.2).

Administration of SR141716A had no significant effect on the activity following the conditioning stimulus ($P>0.05$), test stimulus ($P>0.05$) or the T/C ratios ($P>0.05$) of the early excitatory cells in the CA3 and DG. In the mPFC, SR141716A had no effect on the activity following the conditioning stimulus ($P>0.05$) but significantly decreased the activity following the test stimulus ($P<0.01$) and the T/C ratio (Basal T/C = 58 ± 4%; SR T/C = 38 ± 1%; $P<0.05$) compared to pre-drug values. WIN55,212-2 failed to increase activity following the test stimulus ($P>0.05$) or the T/C ratios of the early excitatory cells in the CA3 (Basal T/C = 46 ± 16%; WIN T/C = 50 ± 15%; $P>0.05$), DG (Basal T/C = 59 ± 6%; WIN T/C = 52 ± 5%; $P>0.05$) or mPFC (Basal T/C = 58 ± 4%; WIN T/C = 50 ± 5%; $P>0.05$) in the gating rats pre-treated with SR141716A.

Effects of SR141716A on the late excitatory cells

Thirty eight auditory responsive late excitatory cells (CA3, n = 14; DG, n = 13; mPFC, n = 11) were examined for the effects of SR141716A on the WIN55,212-2 induced changes in the unit activity (Table 7.2).

The late excitatory cells in the CA3 and DG demonstrated no change in the activity following the conditioning stimulus (CA3, $P>0.05$; DG, $P>0.05$) but a significant decrease in the activity following the test stimulus (CA3, $P<0.01$; DG, $P<0.001$) resulting in a significant decrease in the T/C ratio (CA3, T/C basal = 55 ± 3%, T/C SR = 32± 9%, $P<0.001$; DG, T/C basal = 56 ± 3%, SR T/C ratio = 27 ± 7%, $P<0.001$) following SR141716A. The late excitatory cells in the mPFC exhibited a significant increase in the activity following the conditioning stimulus ($P<0.001$) and the test stimulus ($P<0.001$) resulting in a significant increase in the T/C ratios (T/C basal = 49 ± 5%, T/C SR = 78 ± 9%, $P<0.01$) following SR141716A. WIN55,212-2 increased the activity following the conditioning stimulus in the CA3 ($P<0.01$) and DG ($P<0.05$), but had no effect on the unit activity following the test stimulus ($P>0.05$) or the T/C ratios in the CA3 (basal T/C = 55 ± 3%; WIN T/C = 57 ± 5%; $P>0.05$) or DG (basal T/C = 56 ± 5%; WIN T/C = 55 ± 4%; $P>0.05$) with pre-treatment of SR141716A. In the mPFC, WIN55,212-2 increased the unit activity following the conditioning stimulus ($P<0.001$) and the test stimulus ($P<0.01$) resulting in a significant increase in the late unit T/C ratio (T/C basal = 49 ± 5%, T/C WIN = 79 ± 3%, $P<0.01$) in the rats pre-treated with SR141716A (Table 7.2).

Table 7.2 Summary table of the unit activities (spikes counts/epoch; epoch =100ms for early excitatory cells; 500ms for late excitatory cells) following conditioning stimulus (CACT) and test stimulus (TACT) with the T/C ratios, 45 min after WIN55,212-2 (1.2mg/kg, i.p; WIN 45) in the gating rats not treated with SR141716A and the gating rats pre-treated with SR141716A (1mg/kg, i.p; SR) compared to the basal values of the respective groups (Basal-1 and Basal-2; * = P<0.05, ** = P<0.01, *** = P<0.001).

Region	Basal-1	WIN 45	Basal-2	SR	WIN 45
CA3					
Early excitatory cells (n = 14)			**(n = 6)**		
CACT (spikes/epoch)	172 ± 14	167 ± 74	122 ± 17	95 ± 16	110 ± 41
TACT (spikes/epoch)	81 ± 6	147 ± 58*	74 ± 13	45 ± 12	48 ± 12
T/C ratio (%)	41 ± 5	75 ± 11**	46 ± 16	50 ± 15	57 ± 27
Late excitatory cells (n = 6)			**(n = 14)**		
CACT	216 ± 45	274 ± 54	209 ± 43	308 ± 56	368 ± 65**
TACT	126 ± 25	177 ± 35	155 ± 36	114 ± 32	257 ± 56
T/C ratio	51 ± 5	73 ± 4*	55 ± 3	32 ± 9***	57 ± 5
DG					
Early excitatory cells (n = 7)			**(n = 5)**		
CACT	131 ± 10	140 ± 14	108 ± 15	96 ± 6	81 ± 7*
TACT	64 ± 15	142 ± 14*	66 ± 5	59 ± 11	45 ± 6
T/C ratio	41 ± 10	106 ± 9***	59 ± 5	57 ± 10	52 ± 5
Late excitatory cells (n = 9)			**(n = 13)**		
CACT	162 ± 31	195 ± 37	157 ± 28	193 ± 38	197 ± 34
TACT	90 ± 19	108 ± 20	103 ± 18	35 ± 7***	119 ± 27
T/C ratio	53 ± 2	57 ± 3	56 ± 5	27 ± 7***	55 ± 4
mPFC					
Early excitatory cells (n = 9)			**(n = 4)**		
CACT	89 ± 11	86 ± 15	144 ± 25	123 ± 12	107 ± 24
TACT	51 ± 4	93 ± 12*	82 ± 8	47 ± 5**	50 ± 6*
T/C ratio	54 ± 2	141 ± 17***	58 ± 4	38 ± 1*	50 ± 5
Late excitatory cells (n = 17)			**(n = 11)**		
CACT	465 ± 96	401 ± 47	288 ± 58	499 ± 66***	538 ± 54***
TACT	196 ± 52	227 ± 56	109 ± 51	275 ± 44*	348 ± 42**
T/C ratio	50 ± 3	66 ± 4**	49 ± 5	78 ± 9**	79 ± 3**

7.4 Discussion

The current study demonstrated two different single-unit response types (early excitatory and late excitatory) in response to the conditioning stimulus with a significant reduction in the activity following the test stimulus in the CA3, DG and mPFC of the gating rats under isoflurane anaesthesia. Non-gating rats demonstrated higher test response activity measured as spike counts/ epoch and higher T/C ratios of both early excitatory and late excitatory units compared to the gating rats. Administration of PCP or WIN55,212-2 increased the T/C ratios of one or both cell types in the CA3, DG and mPFC in the gating rats. Pre-administration of clozapine prevented the unit activity changes induced by PCP while SR141716A prevented the unit activity changes induced by WIN55,212-2.

The auditory-evoked unit responses recorded from the CA3 region of the current study agree with some of the findings of previous studies which have detected prominent single unit responses to auditory conditioning-test paradigm in the CA3. In parallel to previous reports, the present study recorded a high percentage of auditory-responsive cells in the CA3 under anaesthesia (52%; Bickford et al., 1990; Miller and Freedman, 1993, 1995) and contrasts the finding of Moxon et al (1999) who reported only 15% percent of auditory-responsive cells in the CA3 of freely moving rats. It is possible that anaesthesia improves the responsiveness to auditory stimuli by controlling other sensory inputs and preventing animal movements both of which can influence auditory responses in the brain.

The previous studies have detected early excitatory cells in the CA3 similar to those observed in the current study both under anaesthesia (Bickford-wimer et al., 1990; Miller et al., 1992; Miller and Freedman., 1995) and in freely moving rats (Moxon., et al., 1999). However, the current early excitatory peak occurred later (33ms) than the latencies reported in the previous studies (Bickford-Wimer et al., 1990, peak latency = 27 ms; Miller and Freedman, 1995, peak latency = 28 ms; Moxon et al., 1999, peak latency = 20 ms) an effect, which could possibly be attributable to isoflurane anaesthesia, a drug that delays sensory-evoked neuronal responses in the rat brain (Detsch et al., 2002). Similar to the finding of the other studies, the present study shows that the early excitatory peak occurred before the N2/N40 LFP peak in the CA3 (Bickford-Wimer et al., 1990; Miller et al., 1992; Miller and Freeman., 1995; Moxon et al.,1999) suggesting the possibility that neuronal firing could be responsible for the subsequent evoked potential generation. However, we have found that the P1 LFP latency corresponds to the peak unit activity of the early excitatory cells, suggesting that early unit excitation may be responsible for an early LFP activity which may be important to prime the N2 response and gating in the auditory conditioning-test paradigm. The unit T/C ratios were similar to the N2 LFP T/C ratio indicating that the early excitatory activity was correlated with N2 response gating.

Miller and Freedman (1995) identified the early excitatory cells in the CA3 as pyramidal cells and also detected late excitatory cells similar to those observed in the current study, which were classified as interneurons, in the CA3 of both

the gating and the non-gating rats. This putative interneuron activity demonstrated multiple peaks during the inter-stimuli period and also demonstrated an inverse relationship with the putative pyramidal cells indicating inhibition of pyramidal cell activity by the interneurons in the gating rats (Miller and Freedman, 1995). The late excitatory cells in the current study also exhibited increased activity during the inter-stimuli period when the activity of the early excitatory cells were diminished suggesting the possibility of inhibitory control on early excitatory cells by the late excitatory cells in the gating rats. In contrast, the present study failed to detect correlated activity between pyramidal and interneurons within the first 100ms following the condition stimulus reported by Miller and Freedman (1995). Miller and Freedman suggested that the conditioning stimulus activates both pyramidal cells and interneurons simultaneously and continuous activation of the interneurons, especially during the 250ms inter-stimuli period, mediates a reduction of the test response of the pyramidal cells. According to the current findings, it appears that early excitatory cells are initially activated by the conditioning stimulus which activates the firing of the late excitatory cells which in turn inhibit the activity of the early excitatory cells to mediate gating of the test response. These recurrent inhibitory and excitatory relationships are consistent with intrinsic mechanisms between pyramidal cells and the interneurons in the CA3 of the hippocampus (see 1.2). However, auditory gating in the CA3 can not be solely attributable to the intrinsic recurrent inhibitory mechanisms, since the input from the areas such as the medial septum (Miller and Freedman, 1993), the brain stem (Bickford et al.,1993) and dentate gyrus (Moxon et al., 2003) have been shown to modulate the single-unit gating in the CA3.

The early excitatory and late excitatory unit responses in the dentate gyrus were similar to those recorded from the CA3, and the peak activity occurred earlier compared to the peaks of the CA3, suggesting that DG neurons are activated by the auditory stimuli prior to the activation of CA3 neurons. This observation also agrees with the latency measurements seen in the LFPs (see 3.3.1) indicating that DG functions as the preceding step to the CA3 in the multi step process of sensory gating and may also serve as a thoroughfare of gating information from cortical areas to CA3.

The current study observed a similar percentage of auditory-responsive cells (78%) to the percentage reported by Mears et al (2006; 75%) in the mPFC in the freely moving rats suggesting that the anaesthetic effects may not have greatly influenced the auditory responsiveness in the mPFC. The early excitatory activity recorded from the mPFC in the current study is also comparable to the excitatory short duration units recorded from the mPFC by Mears et al (2003). Moreover, the peak latency of the early excitatory cells of the current study (30 ± 1 ms) is similar the peak latency they have observed (28 ± 6 ms) in the excitatory short duration cells. Mears and colleagues also observed gating of the peak activity similar to the gating of the early excitatory cells recorded in this study. However, they reported significantly higher LFP T/C ratios compared to the unit T/C ratios of the excitatory cells in the mPFC and suggested that these two levels of neuronal information (i.e. LFPs and unit activity) might represent distinct sources or influences (Mears et al., 2006). We have not observed a significant difference between the T/C ratios of LFPs and

excitatory units possibly because we have selected the N2 wave for LFP analysis, while the Mears group selected the P60 wave which is of slightly prolonged latency compared to the N2 wave. We have not observed the excitatory long duration cells or the inhibitory cells reported by Mears et al (2003), but observed late excitatory cells with two prominent peaks in the inter-stimuli period. The peaks appeared to coincide with the LFPs of longer latency (i.e. P2, N3). It is possible that behavioural status or anaesthesia could alter the response patterns of auditory responsive neurons giving rise to the differences observed between the two studies. Nevertheless, Cromwell and his colleagues observed similar single-unit response types to the auditory conditioning-test paradigm in the mPFC (Mears et al.,2006) , amygdala (Cromwell et al., 2005), striatum (Anstrom et al., 2007) and the midbrain (Cromwell et al., 2007) in freely moving rats and the current study observed similar response types in the CA3, DG and mPFC under isoflurane anesthesia indicating that single-unit gating responses are constant and pervasive across the areas mediating the auditory gating mechanism irrespective of the effects of anaesthesia.

The non-gating rats demonstrated significant increases in the test response unit activity resulting in significantly higher T/C ratios compared to the gating rats in the CA3, DG and mPFC. These findings are similar to the results obtained with LFP responses (see chapter 3 and 4) confirming the possibility of neuronal dysfunctions mediating the gating process in the non-gating rat population. Miller and Freedman (1995) reported a lower percentage of auditory responsive cells in the CA3 region of the non-gating rats compared to the gating rats. We have observed a markedly lower percentage of auditory responsive cells in the

mPFC (53%) but not in the CA3 (45%) or DG (70%) in the non-gating rats compared to the gating rats. Non-gating rats also showed marked increase in the pre-stimulus firing rate of the interneurons in the CA3 (Miller and Freedman., 1995) which is similar to that observed with early excitatory cells in the CA3 and late excitatory cells in the DG and mPFC. Miller and Freedman (1995) failed to observe auditory responsive pyramidal cells in the CA3, but reported lower firing rate in pyramidal cells during the pre-stimulus period in the CA1 region of the hippocampus in the non-gating rats.

We have observed similar response patterns in the early excitatory cells with increased early peak activities following both conditioning and test stimuli in the CA3, DG and mPFC, to the pyramidal cells recorded from the CA1 region of the non-gating rats by Miller and Freedman (1995). Late excitatory cells in the CA3, DG and mPFC also demonstrated a comparable response pattern to the interneurons in the CA3 with delayed peak activities in the inter-stimuli period compared to the gating rats suggesting a selective loss or alteration in late excitatory /interneuron response patterns in the non-gating rats (Miller and Freedman., 1995). Based on the findings of the current study and the study by Miller and Freedman (1995), it is possible to conclude that the activity of the late excitatory cells during the inter-stimuli period is crucial for normal gating and this activity is altered in the non-gating rats in the CA3, DG and mPFC.

Supporting the finding of Miller et al (1992), which demonstrated disruption of pyramidal cell gating following local administration of phencyclidine, the current study demonstrated increased T/C ratios of the early excitatory and late

excitatory cells in the CA3, DG and mPFC following intra-peritoneal administration of PCP. These findings are similar to the changes in auditory-evoked LFP responses recorded from the CA3, DG and mPFC in the gating rats following PCP (see chapter 5). The response patterns of late excitatory cells during the inter-stimuli period also changed with increased spike activity just before and after the test stimuli following PCP in all three regions (Fig. 7.7, Fig. 7.8, Fig. 7.9), which support our view that the inter-stimuli activity of the late excitatory cells is a key factor in gating mechanisms at cellular level. As discussed in chapter 5, NMDA antagonists can reduce recurrent inhibition in neuronal networks, presumably by blocking the NMDA receptor dependent excitatory drive on the inhibitory interneurons which reduces the GABAergic inhibition on pyramidal cell activity causing hyper-excitation of the neuronal circuits (Jodo et al., 2005). The change in response pattern of the late excitatory cells with increased activity following the test stimulus in both cell types after PCP administration, indicate the disruptive effects of the NMDA antagonist on the intrinsic inhibitory pathways in the CA3, DG and mPFC which also explains the disruption of LFP gating following PCP observed in chapter 5. Systemic administration of NMDA antagonists have also been shown to disturb several other neurochemical systems including a profound dysregulation of the mesocorticolimbic dopamine system resulting in a severe impairment of the dynamic physiological response range of the neurons (Svensson, 2000). NMDA antagonists such as PCP has been shown to disrupt hippocampal function at the cellular level either following local application or following systemic administration (Jodo et al., 2005). However, mPFC neurons are not affected by local application of NMDA antagonists, but demonstrate tonic long lasting

hyper-activation following local application to the hippocampus, suggesting a hippocampal regulation of the mPFC neuronal activity (Jodo et al., 2005). The effects of PCP on auditory gating at cellular level in the CA3, DG and mPFC could therefore involve dysregulation of multiple neurotransmitter systems and hyper-activation or inhibition of reciprocal connections between the regions involved in the process.

Clozapine, the atypical anti-psychotic drug which antagonises the noradrenergic, dopaminergic and serotoninergic transmission (see 1.5.1), prevented the PCP induced changes in the single-unit gating correlating with the changes observed with the LFP activity in chapter 5, emphasising its ability to act on PCP targets. Furthermore, clozapine decreased the single unit responses to the conditioning and test stimuli especially of the late excitatory cell types in the CA3, DG and mPFC suggesting the possibility that clozapine decreases the cell sensitivity to auditory stimuli probably by hyperpolarising some auditory responsive cells which in turn could increase the threshold of the PCP targets.

The cannabinoid agonist, WIN55,212-2, disrupted single-unit gating in the CA3, DG and mPFC with changes comparable to the LFP changes recorded in the regions and also similar to the changes in the unit activities observed with PCP. The effect of WIN55,212-2 were prevented by the CB1 antagonist, SR141716A, suggesting that the WIN55,212-2 induced changes in the single-unit activities were mediated via the CB1 receptors. As discussed in chapter 6 the CB1 receptors are highly localized in the inhibitory neurons in the PFC and

hippocampus (Wilson and Nicoll, 2002) and activation of these receptors causes a reduction in release of the inhibitory neurotransmitter GABA (DSI; Wilson and Nicoll, 2002). Lack of GABAergic inhibition results in hyperactivity of the corticolimbic neurons, increases distractibility and disrupt sensory gating (Freedman, 2008). However, the effects of WIN55,212-2 were not as robust as the effects of PCP on the gating at single-unit level and did not affect gating of the late excitatory cells in the DG. It is possible that the effect of WIN55,212-2 on sensory gating could be dose-dependent and administration of higher doses might have produced more disruptive effects on the two cell types. Nevertheless, the dose of WIN55,212-2 used (1.2mg/kg, i.p) exhibited profound effects on gating at LFP level which highlights the distinct pharmacological effects by the CB1 agonists on these two types of neuronal recordings.

SR141716A decreased the T/C ratios of the late excitatory cells in the CA3 and DG (Table 7.2) producing an enhancement or improved gating in the areas. Apart from the effect on the GABAergic neurons CB1 receptor activation has been shown to activate firing of the mesoprefrontocortical dopamine neurons and release dopamine in the pre-frontal cortex (Pistis et al., 2001). When administered alone SR141716A has been shown to inhibit a percentage of these dopaminergic neurons thus decreasing the excitability of the areas (Pistis et al., 2001). Increased GABAergic inhibition and lack of dopaminergic drive could explain the enhanced gating observed following SR141716A. However, SR141716A on its own had no effect on the LFP gating in any of the areas. Based on the definition of LFP activity as the vector sum of all the synaptic responses in the vicinity of the recording electrodes, it could be argued that

SR141716A may not have a total effect on the neural responses to the conditioning-test paradigm. However, similar to the finding with WIN55,212-2 it is also possible that CB1 antagonists have differential effects on LFP and single-unit activity.

Studying the gating properties of neurons in the CA3, DG and mPFC in the Lister hooded rats under isoflurane anesthesia extended the results obtained from LFP recordings, to infer the cellular mechanisms underlying the auditory gating process. The results of the pharmacological effects on the neurons in the CA3, DG and mPFC broadened the understanding of the effects of the NMDA antagonist, PCP, and the cannabinoid agonist, WIN55,212-2 on the sensory gating process at the single-unit level and also opened doors for future electrophysiological and pharmacological research on sensory gating and schizophrenia.

Chapter Eight

General Discussion

8. General Discussion

The previous chapters of the thesis discussed the findings of the current study in relation to the presence or absence of auditory gating in the CA3, DG and mPFC and the effects of pharmacological manipulations on the gating processes in the three areas. The general discussion of this thesis focuses on three important aspects of the auditory gating process, expanded and improved through findings of the current study; (1) the putative pathway for auditory gating in the CNS, (2) the putative neuronal mechanisms of auditory gating and (3) modelling schizophrenia using auditory gating deficits.

8.1 Auditory gating pathway in the CNS

In the current study, gating of the auditory responses was observed in the CA3, DG and the mPFC. Based on the latency differences and also the findings from other studies (Grunwald et al., 2003; Kurthen et al., 2007) it is possible to suggest that the mPFC region participates in the gating process prior to the hippocampus and that the CA3 region is the ultimate mediator of the process (see chapter 4). This is more evident with the CA3 region demonstrating lower T/C ratios compared to the mPFC (see chapter 4). The DG is quite likely the thoroughfare of gating information from the mPFC to the CA3. As mentioned in chapter 1, auditory gating has been detected in several other brain areas including the brain stem reticular formation (Bickford et al., 1990, 1993; Moxon et al., 1999; Anstrom et al., 2007), medial septum (Miller and Freedman, 1993; Moxon et al., 1999), reticular thalamus (Krause et al., 2003), amygdala

(Cromwell et al., 2005) and striatum (Cromwell et al., 2007). Thus it is obvious that the gating mechanism is more widespread than the leminiscal pathway suggested by Moxon et al (1999; see Fig. 1.3). Recent human studies also suggest the existence of a pathway mediating the auditory gating process distinct from the well established auditory pathway which runs from the cochlea to the auditory cortex (Leavitt et al., 2007). Combining the finding of the current study with those of the previous studies a more complex auditory gating pathway propagating information to the CA3 is proposed. The figure 8.1 highlights the putative stages of auditory gating process subserved by the mPFC, DG and CA3. As discussed in chapter 4, the projection of gating information to the mPFC is likely to be from the brain stem reticular areas which receive auditory inputs from the cochlear nucleus. Brain stem reticular region has been suggested to project gating information to the amygdala (Cromwell et al., 2005) and striatum (Cromwell et al., 2007). Krause et al (2003) suggested that the auditory gating information to the reticular thalamus arrives via the cholinergic afferents from the pons. Miller and Freedman (1993) reported that the medial septul neurons acquire auditory gating information from the brain stem reticular nucleus and proposed that the gating process originates from the brain stem. Thus brain stem has adequate evidence to be pronounced as the main distributor of the auditory gating information to the cortical and subcortical areas postulated in the gating pathway (Fig. 8.1). The CA3 of hippocampus receives the auditory gating information via the medial septum, enterohinal cortex and the dentate gyrus. The auditory gating process thus culminates in the CA3 and in turn assists the CA3 to modulate information processing in the PFC and the subcortical areas via its reciprocal connections. Two other areas

worth exploring in relation to the auditory gating are the nucleus accumbens and the ventral tegmental area which are important structures in the dopaminergic pathways in the CNS and influences the mPFC and hippocampal responses in crucial cognitive processes such as memory and learning (Goto and Grace, 2008). Yang et al (2006) suggested the presence of auditory gating in the ventral tegmental area and the neucleus accumbens in their multiple site recording study. However, neither characterization of the responses nor any relationship between areas was reported in the study as the focus was to demonstrate the effects of pharmacological manipulations (Yang et al., 2006).

The results of the current study strengthen the findings of the previous studies and provide further evidence to a potential complex neural circuitry that regulates early attentive sensory processing reflected by the gating of the mid-latency auditory responses. However, though the present findings favour the possibility, it is too early to argue that auditory gating is sharpened or improved by the subsequent components of the postulated circuits without recording from more areas in the circuitry simultaneously. Moxon et al (1999) found that the brain stem had the lowest T/C ratio compared to the medial septum and the CA3 and doubted that the gating process improved in the subsequent stages in the non-leminiscal pathway. Future work can build on the current findings in the search for the neural circuitry behind auditory gating and would be beneficial to perform simultaneous multiple site recordings including the CA3 and brainstem to assist comparing and correlating regional responses.

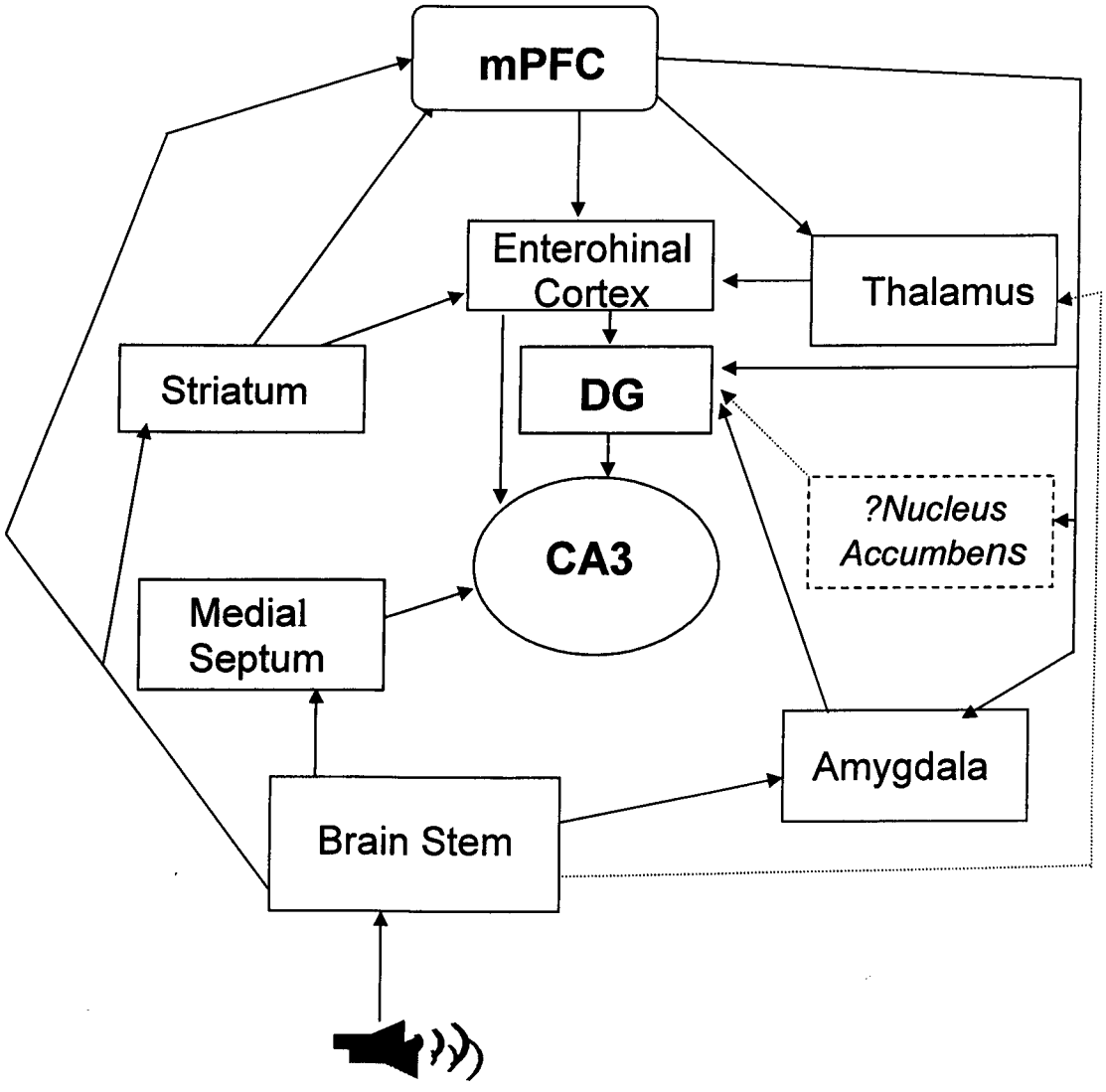


Fig. 8.1 Putative neural circuitry of auditory gating information flow to the CA3 of hippocampus from the brain stem. Brain stem receives auditory inputs via the cochlear nerve and subserves as the distributor of gating information to the cortical and subcortical structures. The CA3 region functions as the culminating point of auditory gating process.

8.2 Neural mechanisms of auditory gating

Unravelling the exact neuronal mechanisms involved in sensory gating will advance the research on mechanisms behind cognitive brain functions and improve the diagnostic and therapeutic approaches to schizophrenia. Studies on auditory gating postulate that the decrement of the test response amplitude is mediated via the inhibitory mechanisms activated by the conditioning stimulus (Adler et al., 1982).

In the previous chapters the importance of the reciprocal connections between the GABAergic inhibitory interneurons and the glutamertigic excitatory pyramidal cells in mediating the auditory gating process was highlighted. According to the current findings we proposed that the early excitatory cells activate the late excitatory cells in the CA3, DG and mPFC following the conditioning stimulus and the late excitatory cells attenuated the test response of the early excitatory cells. Thus analysis of both the LFP activity and the single-unit activity in response to the auditory conditioning-test paradigm demonstrated the importance of inhibitory drive on the excitatory responses during the inter-stimuli period to suppress the test response amplitude.

The current study emphasizes the importance of the integrity of glutamatergic and GABAergic neurotransmission for auditory gating by demonstrating the disruption of gating by NMDA antagonist, PCP and CB1 agonist, WIN55, 212-2 (Fig. 8.2). Depending on the duration of the gating mechanism auditory gating is postulated to be mediated by long-acting $GABA_B$ mechanisms (Hershman et al., 1995; Moxon et al., 2003). Experimental evidence for this view was provided by

Hershman et al (1995) who demonstrated that the $GABA_B$ antagonists disrupt auditory gating in the rat CA3. Disruption of gating by the cannabinoid agonists has been postulated to be mediated via the suppression of pre-synaptic $GABA_B$ activity (Zachariou et al., 2008). Since the experimental evidences are not sufficient enough to pinpoint to $GABA_B$ receptor activity as the neurochemical mechanism of gating in the neural pathway proposed in Fig. 8.1, it is an interesting avenue for future experiments on neurophysiology of auditory gating and its' deficits in neuropsychiatric diseases .

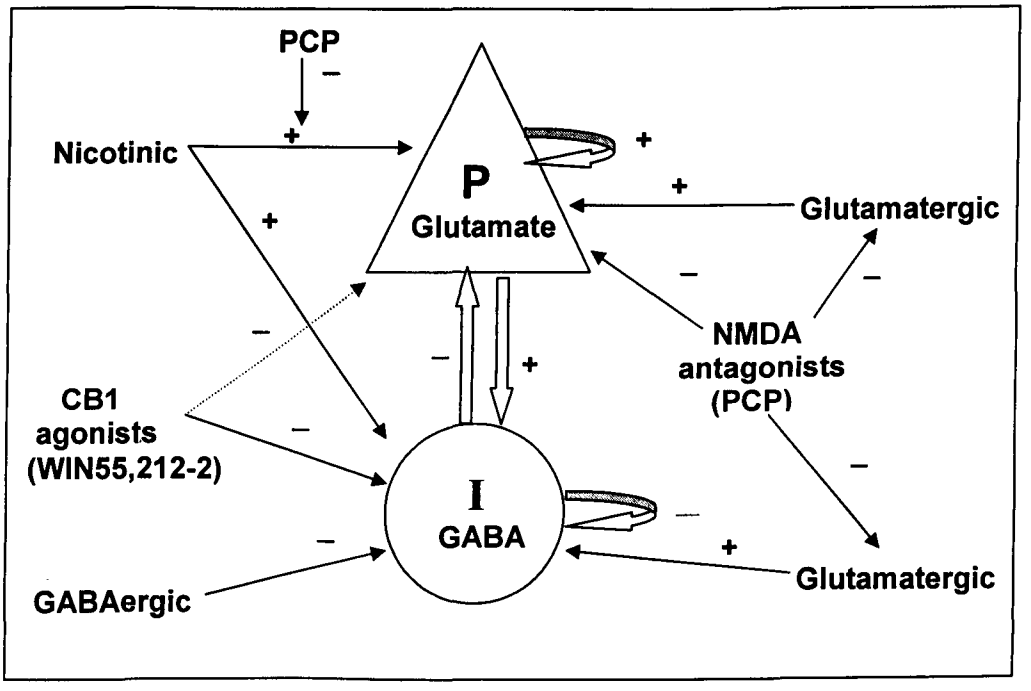


Figure 8.2 Schematic representations of reciprocal connections between pyramidal cells (P) and interneurons (I) within a neural substrate mediating gating accompanied with the postulated extrinsic inputs i.e. GABAergic, glutamatergic and nicotinic, and the effects of PCP and WIN55,212-2 (- = inhibition; + = excitation).

The multiplicity of the pharmacological actions of PCP and the reversal of its effects by clozapine indicated the involvement of the dopaminergic, serotonergic, noradrenergic and cholinergic mechanisms in mediating the auditory gating process (see chapter 5 and 7).

The nicotinic cholinergic input to the neural substrates mediating auditory gating, though not discussed in detail in the thesis, is considered an important mechanism for normal gating (Fig. 8.2). Separation of medial septal cholinergic inputs has been shown to disrupt auditory gating in the CA3 which was corrected by nicotinic agonists (Miller and Freedman, 1993). The nicotinic receptor agonists have been shown to improve gating in schizophrenic patients (Martin et al., 2004) as well as the animal models (Stevens et al., 1998). It has also been suggested that clozapine improves auditory gating deficits by stimulating alpha-7 nicotinic receptors in the brain (Simosky et al., 2003). Adler et al (1998) in their review on sensory gating, nicotinic receptors and schizophrenia, proposed that in the paired stimulus paradigm, the first auditory stimulus activates the pyramidal cells in the CA3 through the perforant path input from the entorhinal cortex and the granule cell input from the dentate gyrus. They suggested that the nicotinic cholinergic input from the medial septum is also activated following the conditioning stimulus so that there is a burst of activity in the pyramidal cells as their entire network excites itself (Adler et al., 1998). The excitatory postsynaptic potentials associated with this massive excitation were suggested to give rise to the P50 wave observed on the scalp surface of humans and the N2 wave in the rat CA3 (Adler et al., 1998). The inhibitory neurons, especially those that likely activate $GABA_B$ receptors, were

also posited to be activated by the first stimulus (Adler et al., 1998). These neurons with high concentration of alpha7-nicotinic receptors were suggested to form channels that admit large amounts of calcium into the cells, which sustain a long-lasting excitation (Adler et al., 1998). The simultaneous activation of the NMDA receptors, which have a similar ability to admit calcium, was also suggested to join in to produce a long-lasting excitation of inhibitory neurons in the hippocampus. Adler et al (1998) proposed $GABA_B$ receptor activation inhibits release of glutamate from the granule cell mossy fibre synapses and from the recurrent pyramidal cell synapses but not the perforant path input. Thus, the second stimulus can engender only a modest excitation of the pyramidal cells and not the burst of recurrent excitation elicited by the first stimulus thereby diminishing the P50/N2 response to the second stimulus (Adler et al., 1998). So it is worth exploring experimentally whether the simultaneous nicotinic inputs on the excitatory glutamatergic neurons and the $GABA_B$ interneurons can mediate auditory gating in the neural substrates suggested in Fig.8.1 and participate in the neural mechanisms as postulated in Fig.8.2.

The present findings demonstrated that the exogenous CB1 agonists disrupt gating. However, the role of the endocannabnoid system in the auditory gating process is still unclear. Though the CB1 antagonist, SR141716A alone had no effect on the auditory-evoked LFP responses, it improved gating of the early excitatory and late excitatory cells in the CA3 and DG and had the opposite effects in the mPFC. Related to the current findings it could be argued that the inhibition of a tonically active endocannabnoid system in the brain may not produce effects on the cumulative neuronal responses related to gating

represented by LFPs but can have differential effects on the underlying specific neural responses. Both WIN55,212-2 and SR141716A had been shown to exhibit affinity towards a 'non-CB1, non-CB2' (some times referred to as CB3) cannabinoid receptor type suggested to be found in the CNS (Wilson and Nicoll, 2002) and further studies on these effects may explain some of the contradictory effects observed in the different neuronal responses.

The analysis of the single-unit activity in the current study focussed mainly to examine the stimuli-locked activities in response to auditory stimuli in the gating and the non-gating rats and to correlate the changes with the LFP activity and also to examine the effects of pharmacological manipulations on the unit activity of the gating rats in the CA3, DG and mPFC. Attempts were not made to examine the basic properties (i.e. wave form analysis, basal firing rate, burst activity) of neurons to identify the cell types (e.g. pyramidal cells or interneurons). If not for the time constraints, these analyses would have provided more information regarding the observations made in the current study. However, further analysis of the current data with respect to the identification of the cell types and also assessing neuronal correlations between regions during the gating process and following pharmacological manipulations would enhance the understanding of the neuronal mechanisms responsible for auditory gating.

Future studies examining the neuronal mechanisms could advance the current finding by examining the role played by the serotonergic agents in the gating process and also by examining whether $GABA_B$ agonists could prevent or

reverse the gating deficits induced by PCP or WIN55,212-2, which will not only confirm the role played by $GABA_B$ receptors in the process but could suggest a therapeutic target for gating deficits observed in neuropsychiatric diseases.

8.3 Modelling auditory gating deficits in schizophrenia

There are several potential difficulties in modeling schizophrenia in animals, including the caveat of reproducing an essentially cognitive disorder in less cognitively developed animals. Accordingly, current animal models of schizophrenia are not intended to serve as the complete animal equivalent of the human disorder, but are often designed to test causative and mechanistic hypotheses (Marcotte et al., 2001).

As mentioned in chapter 3, comparison of our findings with previous rat studies on auditory gating requires the consideration of the methodological differences. Previous studies have used either Sprague-Dawley or Wistar rats who were either freely moving or under injectable anaesthetics (Bickford et al., 1990; Moxon et al., 1999; Krause et al., 2003). We have used Lister-hooded rats under isoflurane anaesthesia. Lister hooded rats have been shown to be more sensitive to pre-pulse inhibition paradigm especially with pharmacological manipulations (Varty & Higgins, 1994; Kalnichev and Pemberton, 2005) and it was postulated that they would be more sensitive to the auditory-conditioning test paradigm compared to other rat strains. Isoflurane is an easily maintainable gaseous anesthetic which minimises the fluctuation of the level of anaesthesia. The advantage of using anaesthetised rats is the ability to control sensory inputs and movements, both of which can influence auditory responses in

polysensory areas (Miller & Freedman, 1995). Adopting a change in the methodology was expected to produce an animal model of auditory gating with a better predictive validity and an easier approach to invasive recording.

As reviewed by de Bruin et al (2003), one of the main problems in comparing rat studies on sensory gating aiming to model human deficits, is the lack of standardized methodology as opposed to human recordings, resulting in different studies describing different components of rat AERs as the P50 equivalent. Some studies propose an early vertex component (P13) as the rat equivalent of P50 (Miyazato et al., 1995; 1996; 1999) while others suggest the vertex N40 (Boutros et al., 1997; Boutros and Kwan, 1998) or N50/N2 (Adler et al., 1986; De Bruin et al., 2001) as the P50 equivalent. Studies recording AERs from CA3 region of the hippocampus proposed N35 (De Bruin et al., 1999; 2001) or N40 (Bickford et al., 1990; 1993; Miller et al., 1992) wave as the rat homologues of P50. Drug-induced changes observed with both vertex N40/N50 components and hippocampal N35/N40 components which are comparable to P50 changes are strong arguments in favour of these negative waves, since such changes have not been reported with P13 (de Bruin et al., 2003). The different methods adopted by different groups in identification of N40 could lead to misidentification of early negative components N25/ N20/N1 as N40, giving rise to differences in latency, amplitude and gating values.

Human studies have attempted to correct the error of identifying P30 as P50 by having a higher latency value for the lower limit of the latency range (e.g. 40 ms) and identifying P50 as the second positive wave in the AER complex (Nagamoto et al., 1999; Boutros et al., 1999; 2004). Identification of AER

according to the polarity and order of occurrence has been adopted by some rodent studies (Van Luijtelaar et al., 2001). However, this method of identification of AER requires the elicitation of wave form complexes in rats comparable to human AER complexes which is not commonly reported in hippocampal CA3 recordings. Adler et al (1986) reported positive and negative waveform complexes within 100ms from the onset of the conditioning stimulus, which are similar to the AERs recorded in the present study. However, the same study reported morphological differences (e.g. absence of N1) observed in Sprague-Dawley rats obtained from different breeding sources which suggests the possibility of environmental factors affecting the morphology of rat AERs.

The waveforms recorded after 100 ms in the present study (P2, N3) have not been reported from rat hippocampus so far. However, as described in chapter 1 human studies have consistently shown scalp recorded waveforms of similar latencies which are representative of higher cognitive stages of information processing (Cadenhead et al., 2000; Boutros et al., 2004). Thus, the P2 and N3 recorded from this study could be homologues to human N100 and P200 recordings, respectively. Post-100 ms waveforms demonstrated apparent gating in response to the test stimulus which is also in accordance with the findings of human studies (Boutros et al., 2004). Here we have focused on the N2 mid latency wave which is occurring in a latency range comparable to P50 and also has the same order of occurrence as the P50 in the AER wave form complexes.

Based on the findings of the current study, it is quite likely that strain differences

also affect the AER morphology and gating with Lister-hooded rats demonstrating AER complexes and gating which are more equivalent to human AERs compared to the other rat strains.

8.3.1 The non-gating rats as a model of auditory gating deficits in schizophrenia

The non-gating rats exhibited some changes such as shorter response latencies and the negative correlation between the conditioning amplitude and the T/C ratio similar to those reported in schizophrenic patients (Adler et al., 1982). Related to the findings of the present study, we suggested that the non-gating rats could be harbouring neurodevelopmental or neurochemical abnormalities comparable to some of the deficits observed in schizophrenia (see chapter 3 & 4). It could also be suggested that this rat group could be sharing some genetic mutations similar to the rat genotypes (WAG/Rij) or mice genotypes (DBA/2) with gating difficulties and would be a cheaper source for future experiments. However, there are obvious limitations in relation to the usage of this rat group for future research. One is that the non-gating rats can not be separately identified from the gating rats prior to electrophysiological experiments. Furthermore, the T/C ratios of the non-gating rats were resistant to PCP and WIN55,212-2 treatment and did not improve with clozapine treatment (see chapter 5 and 6) , thus shedding doubts whether the gating deficits in this rat group represent gating deficits in schizophrenia. However, the single-unit analysis of the non-gating rats revealed alternation in the response patterns of the inhibitory inputs on the excitatory responses which might

represent the deficits in the GABAergic inhibitory processes in schizophrenia. Thus non-gating rats may have a place in future studies on sensory gating deficits in schizophrenia despite the considerable limitations.

8.3.2 Single dose PCP treatment to model auditory gating deficits in schizophrenia

Reviewing the studies on PCP, Morris et al (2005) suggested that "*PCP can be used in rodents to produce a pattern of metabolic, neurochemical and behavioural changes that reproduces almost exactly those seen in patients with schizophrenia*". Some researchers prefer sub-acute and chronic administration of PCP to acute/ single administration as the chronic PCP has been shown to produce long-lasting cognitive and structural deficits resembling those seen in schizophrenia and is considered more representative of the deficits than those created following single administration. However, Jentsch and Roth (1999) reported that both acute and chronic administration of PCP produce similar behavioural and cognitive deficits in both human and animals apart from the differences in the duration of the effects and some associated structural changes. Though the deficits are short-term and reversible, the acute administration of PCP holds an important place in modelling cognitive and behavioural abnormalities of schizophrenia in animal models (Jentsch and Roth, 1999).

In the current study we used single dose PCP under isoflurane anesthesia to model auditory gating deficits in Lister-hooded rats. The anaesthetic properties

of isoflurane are mediated mainly via the $GABA_A$ agonist effects. In adult humans $GABA_A$ activation has been shown to enhance the voltage-dependent Mg^{2+} block of NMDA channels and decrease the Ca^{2+} influx through NMDA channels thus decreasing glutamatergic neural transmission (Xiang et al., 1996). The GABAergic enhancement would be expected to improve auditory gating and the glutamate inhibition may be expected to disrupt gating. However, the robust gating of auditory responses under isoflurane anaesthesia suggested that the hypoglutamatergic effect of isoflurane did not disrupt gating. Furthermore, observation of consistently non-gating animals, which have been reported in previous studies of freely moving animals, suggest the $GABA_A$ enhancement had no effect on any deficits in the inhibitory mechanism of this rat group. This again brings out the possibility that $GABA_B$ activation is the inhibitory process behind the neural mechanism of gating. Based on the current findings it is possible to argue that isoflurane neither had an effect on the gating properties of the Lister hooded rats nor altered the effects of PCP on their gating parameters.

Previous studies examining the dose-response of PCP and clozapine were used to identify the minimum effective dose of the drugs when given intraperitoneally (Miller et al., 1992; Joy et al., 2004). The decision was made only to do studies with higher or lower doses if the dose selected failed to have an effect on gating or if there were side effects affecting the results. This approach was taken to minimise the number of animals used for the study. However, further studies on the dose response relationship of PCP and the effects of clozapine on PCP-induced effects would significantly enhance the

findings of the current study towards developing an animal model of gating deficits.

As discussed in chapter 5 and 7, the acute administration of PCP disrupted auditory gating in the CA3, DG and mPFC and the PCP induced disruption was prevented by clozapine, resembling the gating deficits observed in the schizophrenic patients. According to Geyer and Markou (1994) a valid animal model of schizophrenia should exhibit some symptomatic isomorphism with the disease, presents a robust and a reproducible measure that can be objectively quantified and yield predictive insight to novel treatment strategies. Findings of the current study indicate that auditory gating deficits following a single systemic administration of PCP to Lister hooded rats under isoflurane anaesthesia possess the qualities of a valid animal model of gating deficits observed in schizophrenia. This study could be considered as an open door for the future studies to examine the effects of chronic PCP in anesthetised and freely moving rats to improve and produce an animal model with a high predictive validity to advance research on gating abnormalities and treatment of schizophrenia.

8.3.2 Single dose WIN55,212-2 treatment to model auditory gating deficits in schizophrenia

As discussed in chapter 1 and 6 previous research has provided evidence to link cannabis and schizophrenia. The findings of the present study added more evidence to the proposed link. The effect of single dose WIN55,212-2 on

auditory gating was similar to those observed following acute PCP administration suggesting the possibility of using auditory gating deficits following WIN55,212-2 to pharmacologically model information processing deficits observed in schizophrenic patients. It would be interesting to find out whether clozapine could prevent or reverse the gating deficits induced by WIN55,212-2; if the deficits are prevented the findings would improve the predictive validity of the acute WIN55,212-2 model and if not prevented it could be argued that the WIN55,212-2 induced deficits may represent the cognitive deficits that are resistant to clozapine treatment. Clozapine has been shown to alter the CB1 receptor activity in rats and attenuated the maximal stimulation of CB1 receptors in the PFC by Δ^9-THC (Wiley et al., 2008). Clozapine also decreases binding of the CB1 receptor agonist, C55,940 in the nucleus accumbens (Sundram et al., 2005). Above findings indicate that some of the 'atypical' effects of clozapine could be mediated via the CB1 receptors and future research would improve the understanding of the neurochemical properties of clozapine and its relationship with the endocannabnoid system.

Interestingly a recent study showed that the chronic intermittent PCP treatment altered CB1 receptor functions and elevated endocannabinoid levels in the rat PFC suggesting that PCP induced deficits could be related to the imbalance of the endocannabnoid system (Vigano et al., 2008). Furthermore, Tzavara et al (2003) demonstrated that SR141716A reverses the PCP induced hyperlocomotion in mice and also has procholinergic properties and mild stimulatory effects on serotonin and noradrenaline, suggesting it as a unique candidate for treating cognitive disorders. The above studies implicate a link

between the effects observed with PCP and WIN55,212-2 in the current study suggesting auditory gating deficits observed with them could indicate a link between the NMDA hypofunction and endocannabnoid dysregulation.

8.4 Conclusion

The current study is the first to show that CB1 receptor agonist, WIN55,212-2 disrupts auditory gating in the CA3, DG and mPFC by affecting the inhibitory mechanisms attenuating the test response, thus providing evidence to a potential role played by the endocannabnoid system in the neuronal mechanisms behind auditory gating. The present study is also the first to demonstrate that clozapine reverses the auditory gating deficits induced by PCP, which apart from the clinical implications discussed in 8.3.2, highlights the complexity of the neurophysiology of auditory gating. The current study expanded the studies linking NMDA hypofunction and cannabis with auditory gating deficits in schizophrenia and suggested pharmacological models using PCP and WIN55,212-2 for further studies on these deficits. Furthermore the current findings with SR141716A also suggest the endocannabnoid system as a possible therapeutic target for schizophrenia.

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